# THE ROLE OF PENTRAXINS: FROM INFLAMMATION, TISSUE REPAIR AND IMMUNITY TO BIOMARKERS

EDITED BY: Barbara Bottazzi, Cecilia Garlanda and Mauro Martins Teixeira
PUBLISHED IN: Frontiers in Immunology

IUIS

frontiers Research Topics

# frontiers



ISSN 1664-8714
ISBN 978-2-88963-387-6
DOI 10.3389/978-2-88963-387-6

## About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

## Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

## Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

## What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

# THE ROLE OF PENTRAXINS: FROM INFLAMMATION, TISSUE REPAIR AND IMMUNITY TO BIOMARKERS

Topic Editors:
**Barbara Bottazzi,** Humanitas Clinical and Research Center, Italy
**Cecilia Garlanda,** Humanitas Clinical and Research Center, Humanitas University, Italy
**Mauro Martins Teixeira,** Federal University of Minas Gerais, Brazil

We acknowledge the initiation and support of this Research Topic by the International Union of Immunological Societies (IUIS). We hereby state publicly that the IUIS has had no editorial input in articles included in this Research Topic, thus ensuring that all aspects of this Research Topic are evaluated objectively, unbiased by any specific policy or opinion of the IUIS.

**Citation:** Bottazzi, B., Garlanda, C., Teixeira, M. M., eds. (2020). The Role of Pentraxins: From Inflammation, Tissue Repair and Immunity to Biomarkers. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-387-6

# Table of Contents

frontiers in Immunology

EDITORIAL
published: 03 December 2019
doi: 10.3389/fimmu.2019.02817

# Editorial: The Role of Pentraxins: From Inflammation, Tissue Repair and Immunity to Biomarkers

*Barbara Bottazzi*[1]*, *Cecilia Garlanda*[1,2] *and Mauro Martins Teixeira*[3]

[1] Humanitas Research Hospital, Rozzano, Italy, [2] Department of Biomedical Sciences, Humanitas University, Rozzano, Italy, [3] Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

**Keywords: pentraxins, inflammation, CRP—C-reactive protein, PTX3, SAP, acute phase protein**

## Editorial on the Research Topic

## The Role of Pentraxins: From Inflammation, Tissue Repair and Immunity to Biomarkers

Pentraxins are a superfamily of highly conserved molecules characterized by a common structural motif, the so-called "pentraxin domain" (1, 2). C reactive protein (CRP), originally identified for its ability to bind the C-polysaccharide of *Streptococcus pneumoniae*, and serum amyloid P component (SAP) are the prototypes of the family and constitute the short pentraxin arm. The latter are 25 kDa secreted proteins characterized by a structural organization in which five (CRP) or ten (SAP) identical protomers are assembled in a pentameric symmetry. The long pentraxin PTX3, first identified in the early 90's as prototype of the long pentraxin family, is characterized by the presence of a long N-terminal domain unrelated to other proteins. Short and long pentraxins diverged from a common ancestor of all pentraxins, an events that occurs very early in evolution, given that members of the long pentraxin superfamily where identified in the most ancient vertrebate *Takifugu rubripes* (1).

CRP, SAP and PTX3 are multifunctional molecules mainly produced by inflammatory mediators and tissue injury. CRP is the most important acute phase protein in humans and is routinely measured to monitor human diseases. SAP contributes to amyloid formation and is possibly a therapeutic target. PTX3 is an essential mediator of innate resistance to selected pathogens of fungal, bacterial and viral origin, and is involved in regulation of inflammation, tissue remodeling and cancer.

This Research Topic, carried out with the support of the International Union of Immunological Societies (IUIS), wants to offer an overview of the main biological characteristics of these proteins, pointing to their essential role as regulators of the innate immune response and the possible translational implications.

The review from Pepys can be considered the grand opening of the Research Topic (Pepys). Pepys trace the history of short pentraxins from the discovery to their structural characterization, from the biological properties to the translational potential. In particular, it is shown how both CRP and SAP have become extremely useful as biomarker of human disease and as possible therapeutic targets in different pathological conditions, including amyloidosis and Alzheimer's disease (SAP), or myocardial and cerebral infarction (CRP).

The essential role that the members of the pentraxin superfamily exert in the innate immune response fully accounts for the strong evolutionarily pressure observed. Pathak and Agrawal describe the organisms where CRP has been found and the evolution of CRP from a constitutively expressed protein in arthropods to an acute phase molecule in humans. They also report the structural and biological similarities and differences among CRPs from different

OPEN ACCESS

***Approved by:***
*Francesca Granucci, University of Milano Bicocca, Italy*

***Correspondence:**
*Barbara Bottazzi*
*barbara.bottazzi@humanitasresearch.it*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *12 November 2019*
***Accepted:*** *15 November 2019*
***Published:*** *03 December 2019*

***Citation:***
*Bottazzi B, Garlanda C and Teixeira MM (2019) Editorial: The Role of Pentraxins: From Inflammation, Tissue Repair and Immunity to Biomarkers. Front. Immunol. 10:2817. doi: 10.3389/fimmu.2019.02817*

animals, while Ngwa and Agrawal describe the relationship between structure and function, in particular in relation to the anti-bacterial effect of CRP. Different structures are reported for CRP, with a native or a non-native pentameric protein and a monomeric molecule. Singh and Agrawal investigate the contribution of the different structural arrangements of CRP in relation to the atheroprotective role of the protein.

A main property of pentraxins is represented by the regulation of Complement activation. Two papers in this Research Topic are dealing with this important aspect of the regulation of inflammation (Ma and Garred, Haapasalo and Meri). The classical, the alternative and the lectin pathway of complement activation are all affected by interaction of members of the pentraxin family with their main initiating molecules (C1q, Ficolin-2, Mannose Binding lectin, CL-12) or regulators (Factor H and C4-binding protein). Ma and Garred underline the role of pentraxins in complement activation via crosstalk with both initiators and regulators of the classical and lectin pathways, while Haapasalo and Meri focus their review on the regulation of the alternative pathway. In both reviews, it is evidenced how the regulation of complement activity is an essential component of the role of CRP, SAP and PTX3 in immunosurveillance, anti-microbial immune response and immunologic homeostasis. In line with the general cross-talk between pentraxins and complement molecules, PTX3 has been shown to interact with C1q through the globular recognition domains (gC1q) modulating complement activity via the classical complement pathway. Bally et al. dissect the molecular determinants of this interaction, showing a key contribution of the B chain Arg residues that line the side of the gC1q heterotrimer, supporting the hypothesis that binding of C1q to targets through this region triggers efficient activation of the C1 complex.

Pentraxins can also directly bind to selected pathogens to act as opsonins and promote the removal of recognized microorganisms through phagocytosis. Lu et al. coworkers review the interaction of CRP, SAP and PTX3 with Fc receptors and describe the structural and functional characteristics of this interaction. The interaction of pentraxins with Fc receptors results in activation of cellular immune functions, similarly to Fc receptor activation by immune-complexes.

A unique characteristic of SAP is its ability to be deposited on amyloid fibrils, contributing to amyloid formation. Based on this observation, Pilling and Gomer describe in their review how SAP has been developed as possible therapeutic. SAP administration can inhibit fibrosis, an effect observed in preclinical studies as well as in small clinical trials with myelofibrosis patients. On the other hand, SAP depletion has a therapeutic potential for amyloidosis and can result in unleashing the innate immune system (Pilling and Gomer).

Doni et al. give a general overview on the structure and function of PTX3 and focus on the involvement of the molecule in sterile conditions of tissue damage and cancer, providing evidence that microbial and matrix recognition are evolutionarily conserved properties shared by humoral innate immunity molecules. They report that in models of tissue damage, PTX3 promotes tissue remodeling repair by interacting with fibrinogen/fibrin, as well as plasminogen (Plg), and favoring pericellular fibrinolysis. They also discuss the complexity of the roles of PTX3 in cancer, suggesting that PTX3 may have different functions on carcinogenesis depending on the tissue and cancer type. PTX3 is involved in tuning carcinogenesis through the modulation of cancer-related inflammation or angiogenesis or has a pro-tumorigenic function, by promoting tumor cell migration and invasion and macrophage infiltration (Doni et al.). One of the mechanisms underlying the involvement of PTX3 in tissue remodeling and cancer stems for its interaction with FGF2 and other members of the FGF family via its N-terminal domain, leading to inhibition of FGF-mediated angiogenic responses, in particular in FGF-dependent tumors and FGF2-mediated smooth muscle cell proliferation and artery restenosis. Presta and Foglio discuss this property of PTX3 and present the first low molecular weight pan-FGF trap able to inhibit FGF-dependent tumor growth and neovascularization, identified based on the FGF2/PTX3 interaction, and the implications for its development in FGF-mediated clinical conditions. de Oliveira et al. discuss the role of PTX3 in ischemia and reperfusion injury (IRI), a condition associated with increased expression of this pentraxin in response to DAMPS and inflammatory cytokines. In condition of sterile IRI, such as acute myocardial infarction or kidney, lung and brain IRI, PTX3 deficiency results in worse outcome. Regulation of P-selectin-dependent neutrophil recruitment in damaged tissues and tuning of complement activation and inflammation by PTX3 are among the most relevant mechanisms proposed. On the contrary, PTX3 was shown to have a clear deleterious role in intestinal IRI, a condition associated with significantly more systemic inflammation and remote damage than in the other models of IRI, potential loss of the intestinal barrier and bacterial translocation (de Oliveira et al.).

The generation of PTX3-deficient mice provided the first evidence that this molecule plays a non-redundant role in female fertility. Camaioni et al. discuss the studies performed in this field demonstrating that PTX3 is synthesized before ovulation by cells surrounding the oocyte and actively participates in the organization of the hyaluronan-rich provisional matrix required for successful fertilization. These results are relevant in humans since PTX3 polymorphisms have been associated with female fertility, in terms of dizygotic twinning and number of children given birth during the lifetime (3). It has been proposed that PTX3 may act as a biomarker of oocyte quality, and its systemic levels, determined by genetic variations and/or low-grade chronic inflammation, may affect the growth and development of the follicle and affect the incidence of ovarian disorders (Camaioni et al.).

In line with the role of the short pentraxin CRP as a systemic biomarker and independent predictor of adverse cardiovascular events, such as acute myocardial infarction, stroke, and peripheral artery disease, the involvement of PTX3 in cardiovascular diseases (CVD) has been investigated in mice and humans. Ristagno et al. discuss data on animal CVD models indicating that PTX3 can have cardioprotective and atheroprotective roles by regulating inflammation. In addition, data collected in several clinical settings indicate

that PTX3 is a potential biomarker of CVD. PTX3 plasma levels rise rapidly in acute myocardial infarction, heart failure and cardiac arrest, reflecting the extent of tissue damage and predicting the risk of mortality. Along the same line and based on the expression of PTX3 by endothelial cells (Ramirez et al.), discuss the association between PTX3 concentration and autoimmune vasculitis, showing that systemic lupus erythematosus (SLE), ANCA-associated systemic small vessel vasculitides, giant cell arteritis and Takayasu's arteritis were all associated with increased PTX3 plasma concentration, which correlated with disease activity, acute phase reactants and prednisone treatment. Their study suggests that high levels of PTX3 in the systemic circulation can be used to identify the risk of vascular involvement in systemic immune-mediated diseases. It has been shown that SLE patients display high frequencies and titers of anti-PTX3 antibodies, which are inversely correlated with Lupus nephritis (LN) occurrence, suggesting an immunomodulatory capacity of anti-PTX3 antibodies. Gatto et al. describe the identification and characterization of peripheral B cells recognized by PTX3 present in SLE patients and healthy donors, but absent in LN patients, and suggest a potential immune regulatory role or protective function of these B cells.

Finally, Trojnar et al. investigated the involvement of PTX3 and CRP in thrombotic microangiopathies, such as typical and atypical hemolytic uremic syndrome, secondary thrombotic microangiopathies and thrombotic thrombocytopenic purpura. They found that both PTX3 and CRP levels were elevated in the acute phase of thrombotic microangiopathies. In contrast with CRP, PTX3 levels were associated with patient survival, and signs of complement consumption.

Identification of sepsis biomarkers allowing early stratification and recognition of patients at higher risk of death is crucial. PTX3 has been proposed as a promising biomarker candidate in sepsis patients since PTX3 plasma concentration increase and persistence has been positively associated with severity and mortality. Albert Vega et al. elucidated that despite their immune dysfunctions, circulating cells were responsible for the maintenance of PTX3 concentration in the blood of severe sepsis patients.

PTX3 has been previously described to bind both human and murine cytomegalovirus (CMV) and mediate several host antiviral mechanisms. Campos et al. show the contribution of genetic variation in donor PTX3 to the risk of CMV reactivation in patients undergoing allogeneic hematopoietic stem-cell transplantation. This result suggests that donor PTX3 allelic variants can predict the risk of CMV reactivation in this clinical setting, similarly to what reported on invasive aspergillosis in hematopoietic stem-cell transplanted patients (4).

This Research Topic describes the pleiotropic functions of pentraxin family members and suggests the complexity of their involvement in modulating innate and inflammatory responses. The potential contradictory roles of these molecules in health and disease depends on the disease context, the cellular source, or the levels of protein released. Deciphering more clearly the multifaceted functional roles of pentraxins, and in particular PTX3, in physiology and disease may facilitate the development of targeted therapeutic approaches in various clinical conditions.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

This Research Topic has been supported by the International Union of Immunological Societies (IUIS). The activities in CG and BB laboratories are funded by Ministero della Salute (RF-2013-02355470); the European Research Council (ERC–No. 669415); and the Italian Association for Cancer Research [AIRC, IG and 5x1000 (contract 21147)]. Work in MT lab was funded by the program grant National Institute in Science and Technoloy in Dengue and Host Pathogen Interactions FAPEMIG/CNPQ/CAPES 465425/2014-3).

## REFERENCES

1. Garlanda, C, Bottazzi, B, Bastone, A, Mantovani, A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol*. (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
2. Casas, JP, Shah, T, Hingorani, AD, Danesh, J, Pepys, MB. C-reactive protein and coronary heart disease: a critical review. *J Intern Med*. (2008) 264:295–314. doi: 10.1111/j.1365-2796.2008.02015.x
3. Sirugo, G, Edwards, DR, Ryckman, KK, Bisseye, C, White, MJ, Kebbeh, B, et al. PTX3 genetic variation and dizygotic twinning in the Gambia: could pleiotropy with innate immunity explain common dizygotic twinning in Africa? *Ann Hum Genet*. (2012) 76:454–63. doi: 10.1111/j.1469-1809.2012.00723.x
4. Cunha, C, Aversa, F, Lacerda, JF, Busca, A, Kurzai, O, Grube, M, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med*. (2014) 370:421–32. doi: 10.1056/NEJMoa1211161

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 08 October 2018
doi: 10.3389/fimmu.2018.02327

# Long Pentraxin-3 Modulates the Angiogenic Activity of Fibroblast Growth Factor-2

*Marco Presta*, Eleonora Foglio, Ander Churruca Schuind and Roberto Ronca**

*Department of Molecular and Translational Medicine, School of Medicine, University of Brescia, Brescia, Italy*

Angiogenesis, the process of new blood vessel formation from pre-existing ones, plays a key role in various physiological and pathological conditions. Alteration of the angiogenic balance, consequent to the deranged production of angiogenic growth factors and/or natural angiogenic inhibitors, is responsible for angiogenesis-dependent diseases, including cancer. Fibroblast growth factor-2 (FGF2) represents the prototypic member of the FGF family, able to induce a complex "angiogenic phenotype" in endothelial cells *in vitro* and a potent neovascular response *in vivo* as the consequence of a tight cross talk between pro-inflammatory and angiogenic signals. The soluble pattern recognition receptor long pentraxin-3 (PTX3) is a member of the pentraxin family produced locally in response to inflammatory stimuli. Besides binding features related to its role in innate immunity, PTX3 interacts with FGF2 and other members of the FGF family via its N-terminal extension, thus inhibiting FGF-mediated angiogenic responses *in vitro* and *in vivo*. Accordingly, PTX3 inhibits the growth and vascularization of FGF-dependent tumors and FGF2-mediated smooth muscle cell proliferation and artery restenosis. Recently, the characterization of the molecular bases of FGF2/PTX3 interaction has allowed the identification of NSC12, the first low molecular weight pan-FGF trap able to inhibit FGF-dependent tumor growth and neovascularization. The aim of this review is to provide an overview of the impact of PTX3 and PTX3-derived molecules on the angiogenic, inflammatory, and tumorigenic activity of FGF2 and their potential implications for the development of more efficacious anti-FGF therapeutic agents to be used in those clinical settings in which FGFs play a pathogenic role.

**Keywords: angiogenesis, FGF, inflammation, PTX3, endothelium, cancer**

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Humanitas Clinical and Research Center, Italy*

***Reviewed by:***
*Andreas Bikfalvi, Université de Bordeaux, France*
*Angelo A. Manfredi, Università Vita-Salute San Raffaele, Italy*

****Correspondence:***
*Marco Presta*
*marco.presta@unibs.it*
*Roberto Ronca*
*roberto.ronca@unibs.it*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *22 August 2018*
***Accepted:*** *19 September 2018*
***Published:*** *08 October 2018*

***Citation:***
*Presta M, Foglio E, Churruca Schuind A and Ronca R (2018) Long Pentraxin-3 Modulates the Angiogenic Activity of Fibroblast Growth Factor-2. Front. Immunol. 9:2327. doi: 10.3389/fimmu.2018.02327*

## FGF2 AS AN ANGIOGENIC GROWTH FACTOR

Angiogenesis is a multistep process leading to the formation of new blood vessels from pre-existing ones. It occurs in different physiological and pathological settings, including embryonic development, wound repair, inflammation, and cancer. During the "angiogenic switch," activated endothelial cells (ECs) degrade the basement membrane and start migrating (tip cells) and proliferating (stalk cells) to form EC sprouts that will originate vascular loops and capillary tubes with formation of tight junctions, deposition of a new basement membrane and pericyte recruitment (1, 2). The activation of ECs results from the balance between pro-angiogenic growth factors and anti-angiogenic players released by different perivascular cell types (2). A plethora of molecules have been described to regulate angiogenesis, including Fibroblast Growth

Factor-2 (FGF2) that, together with FGF1, was first identified in the 1980s as a heparin-binding angiogenic factor (3, 4).

FGF2 exerts pleiotropic activities on target cells, including ECs, by interacting with cell surface heparan-sulfate proteoglycans (HSPGs) and high affinity tyrosine kinase receptors (FGFRs) (5). FGF2/FGFR interaction fosters the dimerization of the receptor and the autophosphorylation of its intracellular tyrosine kinase domain that, in turn, leads to the activation of complex signal transduction pathways (6).

Among the 23 members of the FGF family (5), FGF2 represents the most characterized and potent pro-angiogenic mediator *in vitro* and *in vivo* (7), even though a significant pro-angiogenic activity has been demonstrated also for FGF4 and FGF8 whereas it remains debated for other FGFs (including FGF5, FGF7, FGF9, FGF16, and FGF18) (8). *In vitro*, FGF2 induces EC proliferation and migration, promotes the production of proteases and expression of integrin and cadherin receptors (9).

*In vivo*, FGF2 stimulates the neovascularization process in different experimental models, including the chick embryo chorioallantoic membrane (CAM) (10), rabbit/mouse cornea (11, 12), zebrafish yolk membrane (ZFYM) (13), and murine subcutaneous Matrigel plug (14) assays. Conversely, loss of FGF signaling in ECs results in augmented vascular permeability and loss of vessel integrity (15). Notably, the pro-angiogenic function of FGF2 is mostly mediated by FGFR1, that represents the main FGFR expressed by activated ECs (9), and less frequently by FGFR2 (16), whereas FGFR3 and FGFR4 do not appear to be expressed in ECs.

Usually, the biological effect exerted by FGF2 on ECs is the consequence of a paracrine stimulation due to its release by inflammatory cells, stromal components or tumor cells, as well as by its mobilization from FGF-binding components that are present in the extracellular matrix (ECM) (6, 7, 17). Moreover, ECs can undergo autocrine or intracrine stimulation due to the self-production of FGF2 (18).

Finally, FGF2 stimulates lymphangiogenesis by direct and indirect (often vascular endothelial growth factor (VEGF)-C mediated) action on lymphatic endothelial cells (LECs), where it promotes proliferation, migration, and survival (19, 20). Recent observations have shown that FGF2 controls the glycolytic metabolism in ECs and LECs through a FGFR/MYC/Hexokinase 2-mediated pathway (21).

## FGF2-DEPENDENT ANGIOGENESIS AND INFLAMMATION

Emerging evidence supports a role for inflammation in angiogenesis and suggests mutual dependency of the two processes in several physiological and pathological conditions (22, 23) due to common signaling pathways and mediators (24). During inflammatory reactions, the immune infiltrate may produce pro-inflammatory cytokines with pro-angiogenic properties, together with growth factors and proteases that contribute to the formation of new vascular structures (25, 26). The newly formed vasculature, in turn, sustains inflammation by facilitating the recruitment of inflammatory cells to the site of inflammation (27–29).

Noteworthy, elevated levels of FGF2 have been implicated in the pathogenesis of several diseases characterized by a deregulated angiogenic/inflammatory response, including cancer (7).

### Contribution of Inflammatory Cells in Promoting FGF2-Dependent Angiogenesis

In response to phlogistic stimuli, inflammatory cells provide key cytokines and growth factors to the angiogenic vascular network and interact with endothelial surface adhesion molecules, affecting vascular permeability and inducing EC migration and proliferation (30–32). These cells can produce pro-angiogenic factors, including FGF2, that stimulate the proliferation and migration of hypoxic ECs, supporting a paracrine model for the modulation of EC growth at the inflammatory site. Thus, various cell types known to play a pivotal role in the initiation and progression of inflammation have been considered active players in angiogenesis (33–36). In this context, monocytes/macrophages (MCs/MPHs) (37, 38), T lymphocytes (34, 39) and mast cells (40) express FGF2 and their homing to inflammatory sites can impact the neovascular response associated to inflammation (41). In addition, platelet alpha granules represent a source of various angiogenic factors, including FGF2, that are released during physiological and pathological conditions and may contribute to angiogenic responses (42).

The involvement of MCs/MPHs in inflammatory angiogenesis has been reported in a variety of experimental settings (43). For instance, Polverini and colleagues found that activated MPHs and their cell culture media were able to induce neovascularization in the cornea assay, thus relating the angiogenic activity of macrophages with their secretome (44). MCs/MPHs are frequently associated with proliferating blood vessels where they accumulate and provide angiogenic growth factors, including FGF2, as is the case for coronary collaterals where the rapid vessel growth correlates with MC adhesion to the intima (45, 46).

Factors released by MCs/MPHs alter the tissue microenvironment, promoting EC migration, proliferation and new vessel formation (47, 48) and stimulate the migration of other accessory cells, in particular mast cells, able to potentiate the angiogenic response (29, 49). The early recruitment of MCs/MPHs (within 2–3days after implantation) precedes blood vessel formation in a FGF2-driven Matrigel plug angiogenesis assay (23). Accordingly, a significant reduction of the angiogenic response elicited by FGF2 and other angiogenic factors has been demonstrated following MC/MPH depletion induced by intraperitoneal pretreatment with clodronate liposomes (Clodrolip) (50, 51). Notably, MPHs may facilitate FGF signaling by producing heparinases and plasmin that degrade the ECM, thus disengaging ECM-bound FGF molecules that eventually will activate FGFRs in ECs, and create "guiding paths" for proliferating and migrating ECs (35, 43). Accordingly, long-term treatment with FGF2 stimulates ECM degradation by MCs/MPHs to facilitate the invasion of $Tie2^{+}$ EC precursors and blood vessel formation in Matrigel implants (48).

The significant inhibition of the angiogenic response to FGF2 observed in neutropenic mice suggests that, similar to MCs/MPHs, neutrophils may play a key role in FGF2-mediated angiogenesis (32), most likely by producing additional pro-angiogenic cytokines and ECM-degrading proteases (52–54). On the other hand, neutrophil-derived elastase may favor FGF2 degradation, thus counteracting its angiogenic activity (55, 56).

The tissue density of mast cells is highly correlated with the extent of normal and pathologic angiogenesis (57). Mast cells are recruited by FGF2 (58) and, in turn, may release FGF2, as well as other pro-angiogenic factors, leading to EC activation (59, 60). Accordingly, mast cells and their isolated secretory granules induce an angiogenic response in the chick embryo CAM assay (61) that is inhibited by neutralizing anti-FGF2 antibodies (40).

More recently, it has been demonstrated that dendritic cells may sustain inflammatory neovascularization through the expression of a wide array of pro-angiogenic mediators (including FGF2, VEGF, and ETS-1) (62–66). In addition, similar to MCs, DCs may contribute to neovessel formation by differentiating into endothelial-like cells following treatment with FGF2, VEGF-A, and IGF-1 (67).

## FGF2 Amplifies the EC Response to Inflammatory Stimuli

ECs themselves may play important autocrine, intracrine, or paracrine roles in angiogenesis *via* FGF2 production (18), thus inducing a pro-angiogenic status in the endothelium that creates a favorable environment for vascular growth. FGF2 production and release from ECs can be triggered by inflammatory mediators such as IL-1β (68), nitric oxide (NO) (69), prostaglandin E2 ($PGE_2$) (70), and IL-2 upon exposure of ECs to interferon-α (IFN-α) (71).

The observation that angiogenesis is accompanied by vasodilation prompted studies aimed to assess the involvement of vasodilators, like NO and $PGE_2$, in the angiogenic activity of FGF2. Even though FGF2-induced angiogenesis can occur independently from NO production (72), elevation of NO levels in ECs increases their FGF2 production (72). Similarly, $PGE_2$ exerts its pro-angiogenic action through paracrine activation of endothelial FGFR1 following mobilization of FGF2 sequestered in the ECM (70). Conversely, FGF2 and VEGF-A induce angiogenesis by increasing cyclooxygenase and $PGE_2$ production (73, 74).

A transcriptome study on murine microvascular ECs demonstrated that FGF2-driven neovascularization induces a complex pro-inflammatory signature in the endothelium, with early upregulation of several inflammation-related genes (23). Even though also VEGF-A may upregulate the expression of inflammation-related genes in ECs (75–77), it remains unclear whether the two angiogenic mediators utilize distinct or common molecular pathways to exert their biological effects on ECs. Indeed, although an intimate cross-talk between FGF2 and VEGF-A during angiogenesis may exist (78), FGF2 appears to be responsible for the early induction of inflammation-related genes independently from VEGF expression, that represents a later event (23).

FGF2 amplifies the EC response to inflammatory stimuli by vasoactive effects and recruitment of a consistent inflammatory infiltrate. Besides inducing vasodilation of coronary arterioles through endothelial NO production (79), FGF2 increases vascular permeability *via* VEGF-A and protease upregulation (80). Moreover, FGF2 enhances the recruitment of MCs, T cells, and neutrophils (25) by increasing their adhesion and trans-endothelial migration *via* the upregulation/expression of the cell adhesion molecules ICAM-1 and VCAM-1 in ECs (81, 82).

Notably, studies from different groups suggest that FGF2 might have a context-dependent pro- or anti-inflammatory activity. While a rapid, transient exposure to FGF2 induces the upregulation of endothelial adhesion molecules that contribute to immune infiltrate recruitment, a prolonged exposure to FGF2 may result in a marked down-regulation of ICAM-1, VCAM-1, and E-selectin expression on ECs, accompanied by a strong reduction of adhesion and transmigration of monocytes, neutrophils and $CD4^+$ T lymphocytes even in response to potent chemotactic factors (83–85). This biphasic effect of FGF2 might be one of the mechanisms utilized by cancer cells to escape from host immune reactions during the angiogenic stage of tumor development (86).

Finally, inflammation may also impair the angiogenic effects mediated by FGF2 *via* the production of molecules that sequester FGF2. For instance, the C-X-C chemokine platelet factor 4, a well-known inhibitor of angiogenesis released from alpha-granules of activated platelets, is able to bind FGF2, thus preventing FGFR activation and proliferation in ECs (87). A further, remarkable example is represented by long pentraxin-3 (PTX3), a member of the innate immunity with relevant functions in inflammatory responses and pathogen recognition, whose FGF2 antagonist activity will be discussed in details here below.

# PTX3/FGF INTERACTION

## Biochemical Interactions

The pentraxin family is a highly conserved group of pattern recognition glycoproteins implicated in innate immunity. PTX3, a prototypic member of the long pentraxin subfamily, is a 340 kDa octamer in which up to 92% of the amino acid sequence (each subunit being formed by 389 residues) is common between mouse and human proteins (88).

The roles played by PTX3 in innate immunity, wound healing/tissue remodeling, cardiovascular diseases, fertility, and infectious diseases span, among others, from opsonization to apoptotic cell clearance, extracellular matrix formation and FGF2 inhibition in tissue homeostasis (89). This functional variety is due to the complex structure of the protein. PTX3 has a unique N-terminal domain with non-redundant functions, whereas its C-terminal domain is common to all pentraxins and contains the "pentraxin signature" (89, 90). PTX3 contains an *N*-glycosylation site in Asn220 that contributes to the fine tuning of ligand binding (91).

The *N*-terminal domain of PTX3 binds FGF2 with high affinity (Kd ∼ 30–300 nM) (92–94) and one octameric PTX3 molecule binds FGF2 in a 1 to 2 stoichiometric ratio (95).

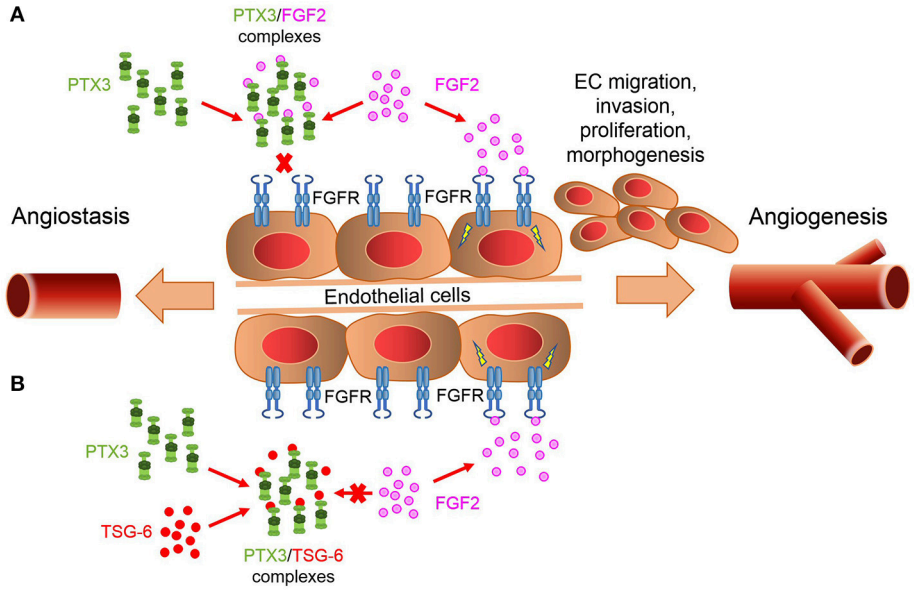


**FIGURE 1 |** PTX3/TSG-6 interaction modulates FGF2-mediated angiogenesis. **(A)** PTX3 acts as a natural FGF trap, thus inhibiting FGF2/FGFR1 complex formation and angiogenesis. **(B)** TSG-6 binds PTX3 and prevents PTX3/FGF2 interaction. This abrogates the inhibitory effect exerted by PTX3 on FGF2 activity.

Using various biochemical approaches, the *N*-terminal amino acidic sequence 97–110 was recognized as responsible for FGF2 binding. Later, the acetylated pentapeptide Ac-ARPCA, corresponding to amino acids 100–104, was identified as the minimal sequence of PTX3 able to bind FGF2 (93, 96). Of note, PTX3 can interact *via* its *N*-terminal also with FGF8b, another member of the FGF family endowed with pro-angiogenic properties (97), and other family members, like FGF6, FGF10, and FGF17 (92).

An important player in modulating PTX3/FGF2 interaction is represented by the tumor necrosis factor-stimulated gene-6 (TSG-6) protein. TSG-6 is expressed in inflamed and neovascularization sites by lymphocytes, smooth muscle cells, and ECs in response to inflammatory stimuli (98). TSG-6 binds PTX3 and other ECM components, like hyaluronic acid and the heavy chains of inter-α-inhibitor, thus allowing the formation of intricate molecular webs in the ECM (99, 100). TSG-6 binds the PTX3 *N*-terminus and prevents its interaction with FGF2, thus reverting the inhibition exerted by PTX3 on FGF2 activity. This may provide a mechanism to control angiogenesis in those inflammatory conditions characterized by the co-expression of TSG-6 and PTX3, in which the relative levels of these proteins may act as a biological rheostat to fine-tune the angiogenic activity of FGF2 (101) (**Figure 1**).

## Biological Implications

PTX3/FGF2 interaction prevents the formation of the biologically active HSPG/FGF2/FGFR ternary complex, thus inhibiting FGF2-dependent EC activation and angiogenesis (94, 102). *In vitro* experiments demonstrated that the *N*-terminal domain of PTX3 and the PTX3-derived ARPCA pentapeptide impair the proliferation/activation of ECs in response to FGF2 but not to VEGF-A, thus confirming the specificity of the effect (94, 96). *In vivo*, PTX3 significantly hampers the angiogenic response triggered by alginate beads adsorbed with FGF2 and implanted on the chick embryo CAM (**Figures 2Aa**) (96). Similar results were obtained in a zebrafish/tumor xenograft model (103) where the angiogenic response to FGF2-overexpressing tumor cells was strongly impaired by the co-injection of PTX3 or ARPCA (**Figures 2Ab**) (96). Accordingly, overexpression of PTX3 by tumor cells of different origin (including melanoma, prostate, and breast cancer cells) causes a significant inhibition of tumor-associated neovascularization and FGF-dependent tumor growth (92, 104, 105).

The effect of PTX3 overexpression on ECs was assessed in a transgenic mouse model where the human *Ptx3* gene was under the control of endothelial-specific Tie2 promoter [TgN(Tie2-hPTX3) mice] (106). When isolated from the lung of TgN(Tie2-hPTX3) animals, PTX3-overexpressing ECs showed a reduced capacity to respond to exogenous FGF2 in terms of cell proliferation and 3D-sprouting when compared to ECs isolated from wild type animals (106). This was accompanied by a significant reduction of endothelial FGFR1 activation/phosphorylation following stimulation with FGF2. In agreement with these observations, the overexpression of PTX3 by the endothelium of transgenic animals caused a significant inhibition of the angiogenic response triggered by FGF2 in an *ex vivo* murine aorta ring assay and *in vivo* when TgN(Tie2-hPTX3) mice were tested in a Matrigel plug assay (**Figures 2Acd**). No inhibitory effect was observed when VEGF-A was used an angiogenic stimulus, thus confirming that the anti-angiogenic activity of PTX3 was directly mediated by the impairment of the FGF2/FGFR1 axis. As a consequence of the anti-FGF2/anti-angiogenic activity of PTX3, FGF2-dependent syngeneic tumor grafts of different origin were characterized by impaired FGFR1 activation and reduced $CD31^{+}$ vascularization

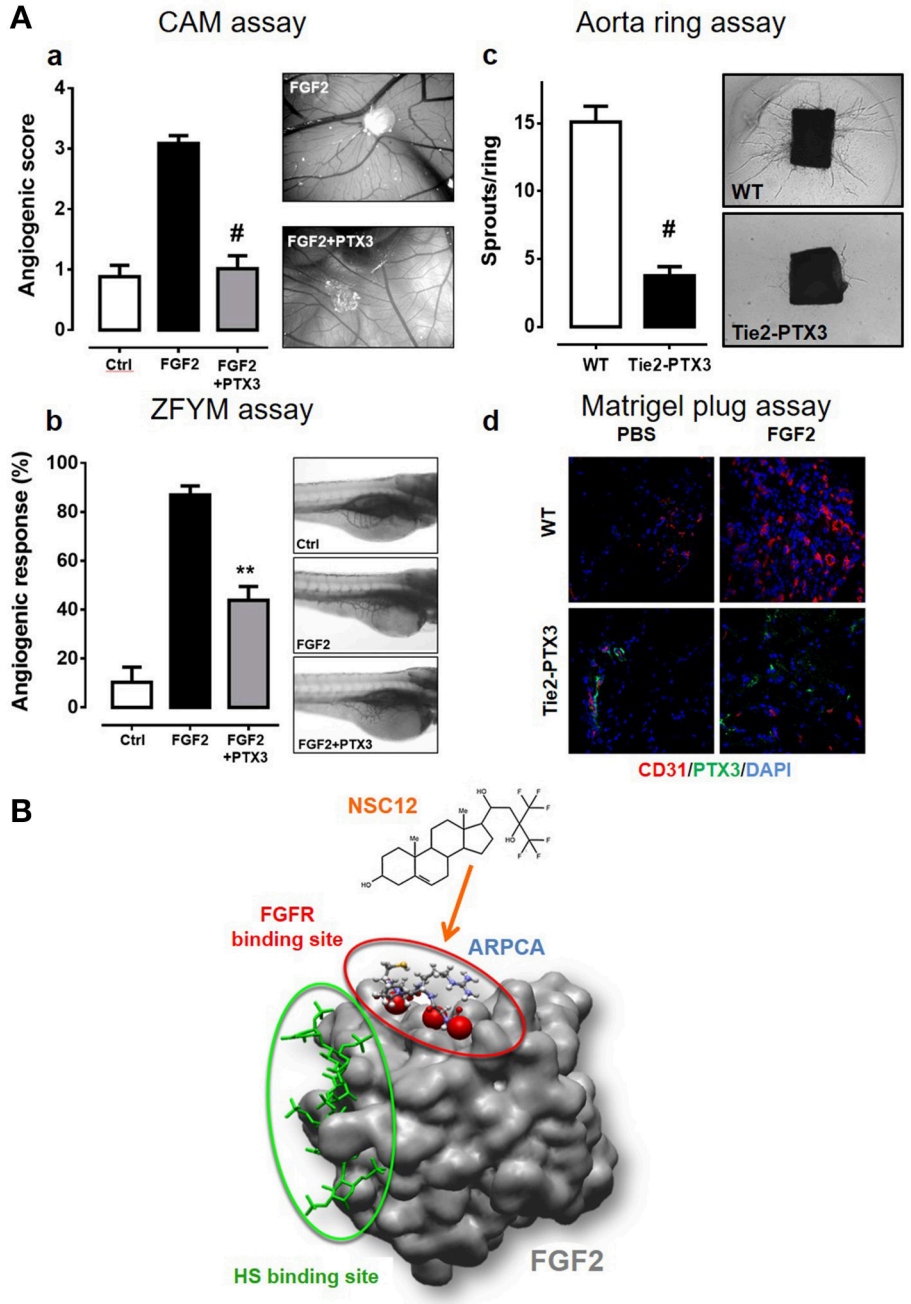


**FIGURE 2 |** PTX3 inhibits the angiogenic activity of FGF2. **(A)** When tested in different angiogenesis models, a molar excess of purified PTX3 protein **(a,b)** or its transgenic endothelial overexpression **(c,d)** inhibits the neovascular response triggered by an optimal dose of recombinant FGF2 [see references (13), (94), (106) for details] $^{**}p < 0.01$; $^{\#}p < 0.001$. **(B)** The PTX3-derived pentapeptide ARPCA (ball and stick representation) interacts with the FGFR-binding domain of FGF2 (red circle) without affecting its heparin-binding region (green circle). A similar mechanism of action is hypothesized for the FGF trap small molecule NSC12.

and tumor growth when injected in TgN(Tie2-hPTX3) mice (106). Notably, the TRAMP-C2 prostate adenocarcinoma cell grafts generated in TgN(Tie2-hPTX3) mice were characterized also by a significant decrease of the mast cell infiltrate into the lesion (58). These data, in keeping with previous observations about the capacity of mast cells to respond chemotactically to FGF2, provide evidence about a relationship among FGF2-dependent mast cell recruitment, angiogenesis, and tumor growth in prostate adenocarcinoma, all hampered by PTX3.

Moreover, when considering the role of FGF2 in the formation and maintenance of lymphatic vessels (19, 20), it is possible to hypothesize that PTX3 may inhibit FGF2-mediated lymphangiogenesis and its associated events, including tumor

metastatic dissemination (107). Further experiments are required to assess this hypothesis.

The anti-angiogenic/anti-tumor activity of PTX3 was not restricted to FGF2. Indeed, due to its capacity to bind FGF8b, PTX3 prevents the interaction of this FGF family member with FGFR1 and blocks FGF8b-induced EC proliferation and chemotaxis *in vitro* and angiogenesis *in vivo*, causing a significant inhibition of tumor growth and vascularization when transduced in androgen-regulated Shionogi 115 mouse breast tumor cells (97) that express both FGF2 and FGF8b following stimulation with dihydrotestosterone (105).

PTX3 binds extracellular matrix component of the vessel wall, including collagen and fibrinogen, thus affecting platelet aggregation (108), In addition, it can bind activated circulating platelets and dampen their proinflammatory and prothrombotic action (109). It will be of interest to assess whether such interactions may result in the sequestration of platelet-released FGF2, with a consequent modulation of its bioavailability and biological activity in different thrombosis-prone conditions, including tissue ischemia, wound healing, atherosclerosis, and cancer.

## Therapeutic Implications

When considering its FGF2 antagonist activity, PTX3 might be regarded as a potential therapeutic agent in those pathological settings in which FGF2 exerts a driving role. Endovascular injection of adeno-associated virus harboring the *PTX3* cDNA was used to block FGF2-mediated intimal thickening after balloon injury in the rat carotid artery (110) whereas its retroviral/lentiviral transduction has been exploited to inhibit FGF activity in different tumor models (102). However, due to its size (340 kDa), complex quaternary structure (homo-octamer), and proteinaceous nature, any pharmacological application of PTX3 protein appears unrealistic unless functional "shuttles" can be identified for this "cargo." One possibility for a direct therapeutic exploitation of the PTX3 protein has been shown by using "tumor targeting" $Tie2^{+}$ monocytes (TEMs) (111) derived from the bone marrow of TgN(Tie2-hPTX3) mice (106). In this experimental model, PTX3-expressing TEMs were able to efficiently deliver the PTX3 protein to the tumor site in a syngeneic FGF2-dependent model of prostate cancer, causing a significant reduction of the growth of the tumor grafts (106).

In order to set the basis for the development of novel PTX3-derived FGF2 antagonists with potential therapeutic implications, the PTX3-derived pentapeptide ARPCA was characterized in preclinical models of FGF-dependent angiogenesis and cancer. Acetylated ARPCA appears to bind the FGF2 protein in a region responsible for its interaction with the D2-D3 linker and D3 domain of FGFR1 (**Figure 2B**) and inhibits the angiogenic activity exerted by FGF2/FGF8, as well as the FGF-dependent growth of prostate and androgen-dependent breast tumors (96, 105). More recently, based on the analysis of ARPCA/FGF2 interaction, molecular modeling and small molecule library screening, a PTX3-derived 480 Da compound (named NSC12, **Figure 2B**) was identified as the first small molecule to function as a pan FGF2 trap (106, 112). Indeed, NSC12 binds and impairs the biological activity of all the canonical FGF family members and displays significant anti-angiogenic activities *in vitro*, *ex vivo* and *in vivo* in a series of FGF2-dependent angiogenesis assays, with no effect on VEGF-dependent EC activation (106). In addition, *in vivo* experiments performed on FGF-dependent models of prostate and lung cancer confirmed the capacity of NSC12 to inhibit FGFR1 activation and to reduce tumor growth and tumor-associated angiogenesis (26, 74). The non-aminoacidic structure of NSC12 makes this molecule a promising candidate for the development of more efficacious anti-FGF therapeutic agents to be used in clinical settings.

It must be pointed out that, at variance with tyrosine kinase FGFR inhibitors, FGF trapping following PTX3 overexpression in transgenic mice, as well as long-term NSC12 administration (106) or treatment with the FGFR-derived decoy molecule FP-1039 (113), are all devoid of significant toxic effects. This appears to be in contrast with the alterations of vascular integrity observed after systemic overexpression of soluble FGFRs in transgenic mice (15) and calls for further experiments aimed at assessing the therapeutic window of FGF trapping agents.

In conclusion, FGF2/PTX3 interaction may exert a deep impact on the angiogenesis process during inflammation and tumor growth. The balance among these interactors and other FGF and/or PTX3 binding molecules (e.g., TSG-6, ECM components and HSPGs) may further modulate neovessel formation under different physio/pathological conditions. A better understanding of these interactions may provide valuable insights into the pathogenesis of angiogenesis-dependent diseases and will set the basis for the development of novel therapeutic agents.

## AUTHOR CONTRIBUTIONS

All the authors contributed to the writing of the manuscript, MP and RR revised the final version.

## FUNDING

This work was supported by IG 18943 and MFAG 18459 grants from Associazione Italiana per la Ricerca sul Cancro (AIRC) to MP and RR, respectively.

## REFERENCES

1. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med.* (2000) 6:389–95. doi: 10.1038/74651
2. Ronca R, Benkheil M, Mitola S, Struyf S, Liekens S. Tumor angiogenesis revisited: regulators and clinical implications. *Med Res Rev.* (2017) 37:1231–74. doi: 10.1002/med.21452
3. Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. *Science* (1984) 223: 1296–9.
4. Maciag T, Mehlman T, Friesel R, Schreiber AB. Heparin binds endothelial cell growth factor, the principal endothelial cell mitogen in bovine brain. *Science* (1984) 225:932–5.

5. Katoh M, Nakagama H. FGF receptors: cancer biology and therapeutics. *Med Res Rev*. (2014) 34:280–300. doi: 10.1002/med.21288
6. Giacomini A, Chiodelli P, Matarazzo S, Rusnati M, Presta M, Ronca R. Blocking the FGF/FGFR system as a "two-compartment" antiangiogenic/antitumor approach in cancer therapy. *Pharmacol Res*. (2016) 107:172–85. doi: 10.1016/j.phrs.2016.03.024
7. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev*. (2005) 16:159–78. doi: 10.1016/j.cytogfr.2005.01.004
8. Ronca R, Giacomini A, Rusnati M, Presta M. The potential of fibroblast growth factor/fibroblast growth factor receptor signaling as a therapeutic target in tumor angiogenesis. *Expert Opin Ther Targets* (2015) 19:1361–77. doi: 10.1517/14728222.2015.1062475
9. Javerzat S, Auguste P, Bikfalvi A. The role of fibroblast growth factors in vascular development. *Trends Mol Med*. (2002) 8:483–9. doi: 10.1016/S1471-4914(02)02394-8
10. Ribatti D, Vacca A, Roncali L, Dammacco F. The chick embryo chorioallantoic membrane as a model for *in vivo* research on anti-angiogenesis. *Curr Pharm Biotechnol*. (2000) 1:73–82. doi: 10.2174/1389201003379040
11. Herbert JM, Laplace MC, Maffrand JP. Effect of heparin on the angiogenic potency of basic and acidic fibroblast growth factors in the rabbit cornea assay. *Int J Tissue React*. (1988) 10:133–9.
12. Seghezzi G, Patel S, Ren CJ, Gualandris A, Pintucci G, Robbins ES, et al. Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis. *J Cell Biol*. (1998) 141:1659–73.
13. Nicoli S, De Sena G, Presta M. Fibroblast growth factor 2-induced angiogenesis in zebrafish: the zebrafish yolk membrane (ZFYM) angiogenesis assay. *J Cell Mol Med*. (2009) 13:2061–8. doi: 10.1111/j.1582-4934.2008.00432.x
14. Coltrini D, Di Salle E, Ronca R, Belleri M, Testini C, Presta M. Matrigel plug assay: evaluation of the angiogenic response by reverse transcription-quantitative PCR. *Angiogenesis* (2013) 16:469–77. doi: 10.1007/s10456-012-9324-7
15. Murakami M, Nguyen LT, Zhuang ZW, Moodie KL, Carmeliet P, Stan RV, et al. The FGF system has a key role in regulating vascular integrity. *J Clin Invest*. (2008) 118:3355–66. doi: 10.1172/JCI35298
16. Dell'Era P, Belleri M, Stabile H, Massardi ML, Ribatti D, Presta M. Paracrine and autocrine effects of fibroblast growth factor-4 in endothelial cells. *Oncogene* (2001) 20:2655–63. doi: 10.1038/sj.onc.1204368
17. Ronca R, Van Ginderachter JA, Turtoi A. Paracrine interactions of cancer-associated fibroblasts, macrophages and endothelial cells: tumor allies and foes. *Curr Opin Oncol*. (2018) 30:45–53. doi: 10.1097/CCO.0000000000000420
18. Gualandris A, Rusnati M, Belleri M, Nelli EE, Bastaki M, Molinari-Tosatti MP, et al. Basic fibroblast growth factor overexpression in endothelial cells: an autocrine mechanism for angiogenesis and angioproliferative diseases. *Cell Growth Differ*. (1996) 7:147–60.
19. Chang LK, Garcia-Cardena G, Farnebo F, Fannon M, Chen EJ, Butterfield C, et al. Dose-dependent response of FGF-2 for lymphangiogenesis. *Proc Natl Acad Sci USA*. (2004) 101:11658–63. doi: 10.1073/pnas.0404272101
20. Shin JW, Min M, Larrieu-Lahargue F, Canron X, Kunstfeld R, Nguyen L, et al. Prox1 promotes lineage-specific expression of fibroblast growth factor (FGF) receptor-3 in lymphatic endothelium: a role for FGF signaling in lymphangiogenesis. *Mol Biol Cell* (2006) 17:576–84. doi: 10.1091/mbc.e05-04-0368
21. Yu P, Wilhelm K, Dubrac A, Tung JK, Alves TC, Fang JS, et al. FGF-dependent metabolic control of vascular development. *Nature* (2017) 545:224–8. doi: 10.1038/nature22322
22. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* (2000) 407:249–57. doi: 10.1038/35025220
23. Andres G, Leali D, Mitola S, Coltrini D, Camozzi M, Corsini M, et al. A pro-inflammatory signature mediates FGF2-induced angiogenesis. *J Cell Mol Med*. (2009) 13:2083–108. doi: 10.1111/j.1582-4934.2008.00415.x
24. Szade A, Grochot-Przeczek A, Florczyk U, Jozkowicz A, Dulak J. Cellular and molecular mechanisms of inflammation-induced angiogenesis. *IUBMB Life* (2015) 67:145–59. doi: 10.1002/iub.1358
25. Presta M, Andres G, Leali D, Dell'era P, Ronca R. Inflammatory cells and chemokines sustain FGF2-induced angiogenesis. *Eur Cytokine Netw*. (2009) 20:39–50. doi: 10.1684/ecn.2009.0155
26. Zijlstra A, Seandel M, Kupriyanova TA, Partridge JJ, Madsen MA, Hahn-Dantona EA, et al. Proangiogenic role of neutrophil-like inflammatory heterophils during neovascularization induced by growth factors and human tumor cells. *Blood* (2006) 107:317–27. doi: 10.1182/blood-2005-04-1458
27. Aplin AC, Gelati M, Fogel E, Carnevale E, Nicosia RF. Angiopoietin-1 and vascular endothelial growth factor induce expression of inflammatory cytokines before angiogenesis. *Physiol Genomics* (2006) 27:20–8. doi: 10.1152/physiolgenomics.00048.2006
28. Danese S, Dejana E, Fiocchi C. Immune regulation by microvascular endothelial cells: directing innate and adaptive immunity, coagulation, and inflammation. *J Immunol*. (2007) 178:6017–22. doi: 10.4049/jimmunol.178.10.6017
29. Ribatti D, Crivellato E. Immune cells and angiogenesis. *J Cell Mol Med*. (2009) 13:2822–33. doi: 10.1111/j.1582-4934.2009.00810.x
30. Bussolino F, Mantovani A, Persico G. Molecular mechanisms of blood vessel formation. *Trends Biochem Sci*. (1997) 22:251–6.
31. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* (1996) 86:353–64.
32. Shaw JP, Chuang N, Yee H, Shamamian P. Polymorphonuclear neutrophils promote rFGF-2-induced angiogenesis *in vivo*. *J Surg Res*. (2003) 109:37–42. doi: 10.1016/S0022-4804(02)00020-3
33. Mor F, Quintana FJ, Cohen IR. Angiogenesis-inflammation cross-talk: vascular endothelial growth factor is secreted by activated T cells and induces Th1 polarization. *J Immunol*. (2004) 172:4618–23. doi: 10.4049/jimmunol.172.7.4618
34. Blotnick S, Peoples GE, Freeman MR, Eberlein TJ, Klagsbrun M. T lymphocytes synthesize and export heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by CD4+ and CD8+ T cells. *Proc Natl Acad Sci USA*. (1994) 91:2890–94.
35. Moldovan NI, Goldschmidt-Clermont PJ, Parker-Thornburg J, Shapiro SD, Kolattukudy PE. Contribution of monocytes/macrophages to compensatory neovascularization: the drilling of metalloelastase-positive tunnels in ischemic myocardium. *Circ Res*. (2000) 87:378–84. doi: 10.1161/01.RES.87.5.378
36. Sunderkotter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. *J Leukoc Biol*. (1994) 55:410–22.
37. Kuwabara K, Ogawa S, Matsumoto M, Koga S, Clauss M, Pinsky DJ, et al. Hypoxia-mediated induction of acidic/basic fibroblast growth factor and platelet-derived growth factor in mononuclear phagocytes stimulates growth of hypoxic endothelial cells. *Proc Natl Acad Sci USA*. (1995) 92:4606–10.
38. Baird A, Mormede P, Bohlen P. Immunoreactive fibroblast growth factor in cells of peritoneal exudate suggests its identity with macrophage-derived growth factor. *Biochem Biophys Res Commun*. (1985) 126:358–64.
39. Peoples GE, Blotnick S, Takahashi K, Freeman MR, Klagsbrun M, Eberlein TJ. T lymphocytes that infiltrate tumors and atherosclerotic plaques produce heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor: a potential pathologic role. *Proc Natl Acad Sci USA*. (1995) 92:6547–51.
40. Ribatti D, Crivellato E, Candussio L, Vacca A, Nico B, Benagiano V, et al. Angiogenic activity of rat mast cells in the chick embryo chorioallantoic membrane is down-regulated by treatment with recombinant human alpha-2a interferon and partly mediated by fibroblast growth factor-2. *Haematologica* (2002) 87:465–71.
41. Anderton SM, Wraith DC. Selection and fine-tuning of the autoimmune T-cell repertoire. *Nat Rev Immunol*. (2002) 2:487–98. doi: 10.1038/nri842
42. Bertrand-Duchesne MP, Grenier D, Gagnon G. Epidermal growth factor released from platelet-rich plasma promotes endothelial cell proliferation *in vitro*. *J Periodontal Res*. (2010) 45:87–93. doi: 10.1111/j.1600-0765.2009.01205.x

43. Sunderkotter C, Goebeler M, Schulze-Osthoff K, Bhardwaj R, Sorg C. Macrophage-derived angiogenesis factors. *Pharmacol Ther.* (1991) 51:195–216.
44. Polverini PJ, Cotran PS, Gimbrone MA, Jr., Unanue ER. Activated macrophages induce vascular proliferation. *Nature* (1977) 269:804–6.
45. Schaper J, Konig R, Franz D, Schaper W. The endothelial surface of growing coronary collateral arteries. Intimal margination and diapedesis of monocytes. A combined SEM and TEM study. *Virchows Arch A Pathol Anat Histol.* (1976) 370:193–205.
46. Arras M, Ito WD, Scholz D, Winkler B, Schaper J, Schaper W. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. *J Clin Invest.* (1998) 101:40–50. doi: 10.1172/JCI119877
47. Crosby JR, Kaminski WE, Schatteman G, Martin PJ, Raines EW, Seifert RA, et al. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. *Circ Res.* (2000) 87:728–30. doi: 10.1161/01.RES.87.9.728
48. Anghelina M, Krishnan P, Moldovan L, Moldovan NI. Monocytes/macrophages cooperate with progenitor cells during neovascularization and tissue repair: conversion of cell columns into fibrovascular bundles. *Am J Pathol.* (2006) 168:529–41. doi: 10.2353/ajpath.2006.050255
49. Gruber BL, Marchese MJ, Kew R. Angiogenic factors stimulate mast-cell migration. *Blood* (1995) 86:2488–93.
50. Sakurai E, Anand A, Ambati BK, van Rooijen N, Ambati J. Macrophage depletion inhibits experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci.* (2003) 44:3578–85. doi: 10.1167/iovs.03-0097
51. Nakao S, Kuwano T, Tsutsumi-Miyahara C, Ueda S, Kimura YN, Hamano S, et al. Infiltration of COX-2-expressing macrophages is a prerequisite for IL-1 beta-induced neovascularization and tumor growth. *J Clin Invest.* (2005) 115:2979–91. doi: 10.1172/JCI23298
52. Tazzyman S, Lewis CE, Murdoch C. Neutrophils: key mediators of tumour angiogenesis. *Int J Exp Pathol.* (2009) 90:222–31. doi: 10.1111/j.1365-2613.2009.00641.x
53. McCourt M, Wang JH, Sookhai S, Redmond HP. Proinflammatory mediators stimulate neutrophil-directed angiogenesis. *Arch Surg.* (1999) 134:1325–31; discussion 31–2.
54. Ericson SG, Zhao Y, Gao H, Miller KL, Gibson LF, Lynch JP, et al. Interleukin-6 production by human neutrophils after Fc-receptor cross-linking or exposure to granulocyte colony-stimulating factor. *Blood* (1998) 91:2099–107.
55. Ai S, Cheng XW, Inoue A, Nakamura K, Okumura K, Iguchi A, et al. Angiogenic activity of bFGF and VEGF suppressed by proteolytic cleavage by neutrophil elastase. *Biochem Biophys Res Commun.* (2007) 364:395–401. doi: 10.1016/j.bbrc.2007.10.027
56. Tecchio C, Cassatella MA. Neutrophil-derived cytokines involved in physiological and pathological angiogenesis. *Chem Immunol Allergy* (2014) 99:123–37. doi: 10.1159/000353358
57. Meininger CJ. Mast cells and tumor-associated angiogenesis. *Chem Immunol.* (1995) 62:239–57.
58. Ronca R, Tamma R, Coltrini D, Ruggieri S, Presta M, Ribatti D. Fibroblast growth factor modulates mast cell recruitment in a murine model of prostate cancer. *Oncotarget* (2017) 8:82583–92. doi: 10.18632/oncotarget.19773
59. Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* (1999) 13:1382–97.
60. Meininger CJ, Zetter BR. Mast cells and angiogenesis. *Semin Cancer Biol.* (1992) 3:73–9.
61. Mignatti P, Morimoto T, Rifkin DB. Basic fibroblast growth factor, a protein devoid of secretory signal sequence, is released by cells via a pathway independent of the endoplasmic reticulum-Golgi complex. *J Cell Physiol.* (1992) 151:81–93. doi: 10.1002/jcp.1041510113
62. Conejo-Garcia JR, Benencia F, Courreges MC, Kang E, Mohamed-Hadley A, Buckanovich RJ, et al. Tumor-infiltrating dendritic cell precursors recruited by a beta-defensin contribute to vasculogenesis under the influence of Vegf-A. *Nat Med.* (2004) 10:950–8. doi: 10.1038/nm1097
63. Coukos G, Benencia F, Buckanovich RJ, Conejo-Garcia JR. The role of dendritic cell precursors in tumour vasculogenesis. *Br J Cancer* (2005) 92:1182–7. doi: 10.1038/sj.bjc.6602476
64. Bosisio D, Ronca R, Salvi V, Presta M, Sozzani S. Dendritic cells in inflammatory angiogenesis and lymphangiogenesis. *Curr Opin Immunol.* (2018) 53:180–6. doi: 10.1016/j.coi.2018.05.011
65. Presta M, Chiodelli P, Giacomini A, Rusnati M, Ronca R. Fibroblast growth factors (FGFs) in cancer: FGF traps as a new therapeutic approach. *Pharmacol Ther.* (2017) 179:171–87. doi: 10.1016/j.pharmthera.2017.05.013
66. Riboldi E, Musso T, Moroni E, Urbinati C, Bernasconi S, Rusnati M, et al. Cutting edge: proangiogenic properties of alternatively activated dendritic cells. *J Immunol.* (2005) 175:2788–92. doi: 10.4049/jimmunol.175.5.2788
67. Fernandez Pujol B, Lucibello FC, Zuzarte M, Lutjens P, Muller R, Havemann K. Dendritic cells derived from peripheral monocytes express endothelial markers and in the presence of angiogenic growth factors differentiate into endothelial-like cells. *Eur J Cell Biol.* (2001) 80:99–110. doi: 10.1078/0171-9335-00136
68. Lee HT, Lee JG, Na M, Kay EP. FGF-2 induced by interleukin-1 beta through the action of phosphatidylinositol 3-kinase mediates endothelial mesenchymal transformation in corneal endothelial cells. *J Biol Chem.* (2004) 279:32325–32. doi: 10.1074/jbc.M405208200
69. Walford G, Loscalzo J. Nitric oxide in vascular biology. *J Thromb Haemost.* (2003) 1:2112–8. doi: 10.1046/j.1538-7836.2003.00345.x
70. Finetti F, Solito R, Morbidelli L, Giachetti A, Ziche M, Donnini S. Prostaglandin E2 regulates angiogenesis via activation of fibroblast growth factor receptor-1. *J Biol Chem.* (2008) 283:2139–46. doi: 10.1074/jbc.M703090200
71. Cozzolino F, Torcia M, Lucibello M, Morbidelli L, Ziche M, Platt J, et al. Interferon-alpha and interleukin 2 synergistically enhance basic fibroblast growth factor synthesis and induce release, promoting endothelial cell growth. *J Clin Invest.* (1993) 91:2504–12.
72. Ziche M, Morbidelli L, Choudhuri R, Zhang HT, Donnini S, Granger HJ, et al. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J Clin Invest.* (1997) 99:2625–34. doi: 10.1172/JCI119451
73. Salcedo R, Zhang X, Young HA, Michael N, Wasserman K, Ma WH, et al. Angiogenic effects of prostaglandin E2 are mediated by up-regulation of CXCR4 on human microvascular endothelial cells. *Blood* (2003) 102:1966–77. doi: 10.1182/blood-2002-11-3400
74. Hernandez GL, Volpert OV, Iniguez MA, Lorenzo E, Martinez-Martinez S, Grau R, et al. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J Exp Med.* (2001) 193:607–20. doi: 10.1084/jem.193.5.607
75. Reinders ME, Sho M, Izawa A, Wang P, Mukhopadhyay D, Koss KE, et al. Proinflammatory functions of vascular endothelial growth factor in alloimmunity. *J Clin Invest.* (2003) 112:1655–65. doi: 10.1172/JCI17712
76. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. *J Biol Chem.* (2001) 276:7614–20. doi: 10.1074/jbc.M009705200
77. Abe M, Sato Y. cDNA microarray analysis of the gene expression profile of VEGF-activated human umbilical vein endothelial cells. *Angiogenesis* (2001) 4:289–98. doi: 10.1023/A:1016018617152
78. Jih YJ, Lien WH, Tsai WC, Yang GW, Li C, Wu LW. Distinct regulation of genes by bFGF and VEGF-A in endothelial cells. *Angiogenesis* (2001) 4:313–21. doi: 10.1023/A:1016080321956
79. Tiefenbacher CP, Chilian WM. Basic fibroblast growth factor and heparin influence coronary arteriolar tone by causing endothelium-dependent dilation. *Cardiovasc Res.* (1997) 34:411–7.
80. Reuss B, Dono R, Unsicker K. Functions of fibroblast growth factor (FGF)-2 and FGF-5 in astroglial differentiation and blood-brain barrier permeability: evidence from mouse mutants. *J Neurosci.* (2003) 23:6404–12. doi: 10.1523/JNEUROSCI.23-16-06404.2003
81. Zittermann SI, Issekutz AC. Endothelial growth factors VEGF and bFGF differentially enhance monocyte and neutrophil recruitment to inflammation. *J Leukoc Biol.* (2006) 80:247–57. doi: 10.1189/jlb.1205718

82. Zittermann SI, Issekutz AC. Basic fibroblast growth factor (bFGF, FGF-2) potentiates leukocyte recruitment to inflammation by enhancing endothelial adhesion molecule expression. *Am J Pathol.* (2006) 168:835–46. doi: 10.2353/ajpath.2006.050479
83. Zhang H, Issekutz AC. Growth factor regulation of neutrophil-endothelial cell interactions. *J Leukoc Biol.* (2001) 70:225–32. doi: 10.1189/jlb.70.2.225
84. Zhang H, Issekutz AC. Down-modulation of monocyte transendothelial migration and endothelial adhesion molecule expression by fibroblast growth factor: reversal by the anti-angiogenic agent SU6668. *Am J Pathol.* (2002) 160:2219–30. doi: 10.1016/S0002-9440(10)61169-8
85. Griffioen AW, Damen CA, Martinotti S, Blijham GH, Groenewegen G. Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. *Cancer Res.* (1996) 56:1111–17.
86. Kitayama J, Nagawa H, Yasuhara H, Tsuno N, Kimura W, Shibata Y, et al. Suppressive effect of basic fibroblast growth factor on transendothelial emigration of CD4(+) T-lymphocyte. *Cancer Res.* (1994) 54:4729–33.
87. Perollet C, Han ZC, Savona C, Caen JP, Bikfalvi A. Platelet factor 4 modulates fibroblast growth factor 2 (FGF-2) activity and inhibits FGF-2 dimerization. *Blood* (1998) 91:3289–99.
88. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
89. Presta M, Camozzi M, Salvatori G, Rusnati M. Role of the soluble pattern recognition receptor PTX3 in vascular biology. *J Cell Mol Med.* (2007) 11:723–38. doi: 10.1111/j.1582-4934.2007.00061.x
90. Breviario F, d'Aniello EM, Golay J, Peri G, Bottazzi B, Bairoch A, et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem.* (1992) 267:22190–7.
91. Inforzato A, Peri G, Doni A, Garlanda C, Mantovani A, Bastone A, et al. Structure and function of the long pentraxin PTX3 glycosidic moiety: fine-tuning of the interaction with C1q and complement activation. *Biochemistry* (2006) 45:11540–51. doi: 10.1021/bi0607453
92. Ronca R, Alessi P, Coltrini D, Di Salle E, Giacomini A, Leali D, et al. Long pentraxin-3 as an epithelial-stromal fibroblast growth factor-targeting inhibitor in prostate cancer. *J Pathol.* (2013) 230:228–38. doi: 10.1002/path.4181
93. Camozzi M, Rusnati M, Bugatti A, Bottazzi B, Mantovani A, Bastone A, et al. Identification of an antiangiogenic FGF2-binding site in the N terminus of the soluble pattern recognition receptor PTX3. *J Biol Chem.* (2006) 281:22605–13. doi: 10.1074/jbc.M601023200
94. Rusnati M, Camozzi M, Moroni E, Bottazzi B, Peri G, Indraccolo S, et al. Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood* (2004) 104:92-9. doi: 10.1182/blood-2003-10-3433
95. Inforzato A, Baldock C, Jowitt TA, Holmes DF, Lindstedt R, Marcellini M, et al. The angiogenic inhibitor long pentraxin PTX3 forms an asymmetric octamer with two binding sites for FGF2. *J Biol Chem.* (2010) 285:17681–92. doi: 10.1074/jbc.M109.085639
96. Leali D, Bianchi R, Bugatti A, Nicoli S, Mitola S, Ragona L, et al. Fibroblast growth factor 2-antagonist activity of a long-pentraxin 3-derived anti-angiogenic pentapeptide. . *J Cell Mol Med.* (2010) 14:2109–21. doi: 10.1111/j.1582-4934.2009.00855.x
97. Leali D, Alessi P, Coltrini D, Ronca R, Corsini M, Nardo G, et al. Long pentraxin-3 inhibits FGF8b-dependent angiogenesis and growth of steroid hormone-regulated tumors. *Mol Cancer Ther.* (2011) 10:1600–10. doi: 10.1158/1535-7163.MCT-11-0286
98. Bayliss MT, Howat SL, Dudhia J, Murphy JM, Barry FP, Edwards JC, et al. Up-regulation and differential expression of the hyaluronan-binding protein TSG-6 in cartilage and synovium in rheumatoid arthritis and osteoarthritis. *Osteoarthritis Cartilage* (2001) 9:42–8. doi: 10.1053/joca.2000.0348
99. Kohda D, Morton CJ, Parkar AA, Hatanaka H, Inagaki FM, Campbell ID, et al. Solution structure of the link module: a hyaluronan-binding domain involved in extracellular matrix stability and cell migration. *Cell* (1996) 86:767–75.
100. Baranova NS, Nileback E, Haller FM, Briggs DC, Svedhem S, Day AJ, et als. The inflammation-associated protein TSG-6 cross-links hyaluronan via hyaluronan-induced TSG-6 oligomers. *J Biol Chem.* (2011) 286:25675–86. doi: 10.1074/jbc.M111.247395
101. Leali D, Inforzato A, Ronca R, Bianchi R, Belleri M, Coltrini D, et al. Long pentraxin 3/tumor necrosis factor-stimulated gene-6 interaction: a biological rheostat for fibroblast growth factor 2-mediated angiogenesis. *Arterioscler Thromb Vasc Biol.* (2012) 32:696–703. doi: 10.1161/ATVBAHA.111.243998
102. Giacomini A, Ghedini GC, Presta M, Ronca R. Long pentraxin 3: a novel multifaceted player in cancer. *BBA* Rev *Cancer* (2018) 1869:53–63. doi: 10.1016/j.bbcan.2017.11.004
103. Nicoli S, Presta M. The zebrafish/tumor xenograft angiogenesis assay. *Nat Protoc.* (2007) 2:2918–23. doi: 10.1038/nprot.2007.412
104. Ronca R, Di Salle E, Giacomini A, Leali D, Alessi P, Coltrini D, et al. Long pentraxin-3 inhibits epithelial-mesenchymal transition in melanoma cells. *Mol Cancer Ther.* (2013) 12:2760–71. doi: 10.1158/1535-7163.MCT-13-0487
105. Giacomini A, Matarazzo S, Pagano K, Ragona L, Rezzola S, Corsini M, et al. A long pentraxin-3-derived pentapeptide for the therapy of FGF8b-driven steroid hormone-regulated cancers. *Oncotarget* (2015) 6:13790–802. doi: 10.18632/oncotarget.3831
106. Ronca R, Giacomini A, Di Salle E, Coltrini D, Pagano K, Ragona L, et al. Long-Pentraxin 3 Derivative as a small-molecule FGF trap for cancer therapy. *Cancer Cell* (2015) 28:225–39. doi: 10.1016/j.ccell.2015.07.002
107. Cao R, Ji H, Feng N, Zhang Y, Yang X, Andersson P, et al. Collaborative interplay between FGF-2 and VEGF-C promotes lymphangiogenesis and metastasis. *Proc Natl Acad Sci USA*. (2012) 109:15894–9. doi: 10.1073/pnas.1208324109
108. Bonacina F, Barbieri SS, Cutuli L, Amadio P, Doni A, Sironi M, et al. Vascular pentraxin 3 controls arterial thrombosis by targeting collagen and fibrinogen induced platelets aggregation. *Biochim Biophys Acta* (2016) 1862:1182–90. doi: 10.1016/j.bbadis.2016.03.007
109. Maugeri N, Rovere-Querini P, Slavich M, Coppi G, Doni A, Bottazzi B, et al. Early and transient release of leukocyte pentraxin 3 during acute myocardial infarction. *J Immunol.* (2011) 187:970–9. doi: 10.4049/jimmunol.1100261
110. Camozzi M, Zacchigna S, Rusnati M, Coltrini D, Ramirez-Correa G, Bottazzi B, et al. Pentraxin 3 inhibits fibroblast growth factor 2-dependent activation of smooth muscle cells *in vitro* and neointima formation *in vivo*. *Arterioscler Thromb Vasc Biol.* (2005) 25:1837–42. doi: 10.1161/01.ATV.0000177807.54959.7d
111. De Palma M, Murdoch C, Venneri MA, Naldini L, Lewis CE. Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications. *Trends Immunol.* (2007) 28:519–24. doi: 10.1016/j.it.2007.09.004
112. Castelli R, Giacomini A, Anselmi M, Bozza N, Vacondio F, Rivara S, et al. Synthesis, structural elucidation, and biological evaluation of NSC12, an orally available fibroblast growth factor (FGF) ligand trap for the treatment of FGF-dependent lung tumors. *J Med Chem.* (2016) 59:4651–63. doi: 10.1021/acs.jmedchem.5b02021
113. Harding TC, Long L, Palencia S, Zhang H, Sadra A, Hestir K, et al. Blockade of nonhormonal fibroblast growth factors by FP-1039 inhibits growth of multiple types of cancer. *Sci Transl Med.* (2013) 5:178ra39. doi: 10.1126/scitranslmed.3005414


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 16 October 2018
doi: 10.3389/fimmu.2018.02328

# The Development of Serum Amyloid P as a Possible Therapeutic

*Darrell Pilling* and Richard H. Gomer**

*Department of Biology, Texas A&M University, College Station, TX, United States*

Pentraxins such as serum amyloid P (SAP; also known as PTX2) regulate several aspects of the innate immune system. SAP inhibits the differentiation of monocyte-derived fibroblast-like cells called fibrocytes, promotes the formation of immuno-regulatory macrophages, and inhibits neutrophil adhesion to extracellular matrix proteins. In this minireview, we describe how these effects of SAP have led to its possible use as a therapeutic, and how modulating SAP effects might be used for other therapeutics. Fibrosing diseases such as pulmonary fibrosis, cardiac fibrosis, liver fibrosis, and renal fibrosis are associated with 30–45% of deaths in the US. Fibrosis involves both fibrocyte differentiation and profibrotic macrophage differentiation, and possibly because SAP inhibits both of these processes, in 9 different animal models, SAP inhibited fibrosis. In Phase 1B and Phase 2 clinical trials, SAP injections reduced the decline in lung function in pulmonary fibrosis patients, and in a small Phase 2 trial SAP injections reduced fibrosis in myelofibrosis patients. Acute respiratory distress syndrome/ acute lung injury (ARDS/ALI) involves the accumulation of neutrophils in the lungs, and possibly because SAP inhibits neutrophil adhesion, SAP injections reduced the severity of ARDS in an animal model. Conversely, depleting SAP is a potential therapeutic for amyloidosis, topically removing SAP from wound fluid speeds wound healing in animal models, and blocking SAP binding to one of its receptors makes cultured macrophages more aggressive toward tuberculosis bacteria. These results suggest that modulating pentraxin signaling might be useful for a variety of diseases.

**Keywords: pentraxin, serum amyloid P component (SAP), fibrosis, macrophage, fibrocyte, pulmonary fbrosis**

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Humanitas Clinical and Research Center, Italy*

***Reviewed by:***
*Amrita Dosanjh, Pediatric Pulmonologist, San Diego, CA, United States*
*Alberto Mantovani, Humanitas Research Hospital, Italy*

****Correspondence:***
*Darrell Pilling*
*dpilling@bio.tamu.edu*
*Richard H. Gomer*
*rgomer@tamu.edu*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *27 July 2018*
***Accepted:*** *19 September 2018*
***Published:*** *16 October 2018*

***Citation:***
*Pilling D and Gomer RH (2018) The Development of Serum Amyloid P as a Possible Therapeutic. Front. Immunol. 9:2328. doi: 10.3389/fimmu.2018.02328*

## INTRODUCTION: SAP AND DEBRIS CLEARANCE

SAP (PTX2) is a member of the pentraxin family of proteins that includes C-reactive protein (CRP; PTX1) and pentraxin-3 (PTX3). SAP is made by hepatocytes and secreted into the blood (1, 2). Searches of proteomics and RNA-seq databases suggests that the liver is the major source of SAP. In humans and most mammals, the levels of SAP in the plasma are maintained at relatively constant levels, between 20 and 50 μg/ml (3–5). There is little evidence for sequence variation of SAP at the genomic or amino acid level. In mice, SAP acts as an acute phase protein, with levels rising up to 20-fold following an inflammatory insult (6, 7). SAP is a pentameric protein with sequence and structural similarity to CRP (8–10). The structure of SAP (and CRP) pentamers is a flat disk with a hole in the middle (11, 12). The crystal structure of PTX3 has yet to be determined, but models based on site-directed mutagenesis, electron microscopy, and small-angle X-ray scattering data suggests that PTX3 is an octamer of two tetramers (13). Each SAP molecule has two $Ca^{++}$ atoms bound to it, and the pentamer thus has 10 $Ca^{++}$ atoms on one side of the disk. With the

help of the bound $Ca^{++}$, this side of the disk binds to a variety of molecules including apoptotic debris, bacterial polysaccharides, amyloid deposits, and bacterial toxins (1, 14, 15). Phagocytic cells such as monocytes and macrophages then bind the SAP, CRP, or PTX3, and engulf the debris or other material the pentraxin has bound (16). CRP and PTX3 can similarly bind a variety of debris molecules (17, 18). Proteins with strong similarity to SAP (and CRP and PTX3) are present in the hemolymph of horseshoe crabs (17, 19), so this debris clearance mechanism appears to have evolved during the early evolution of animals (**Figure 1**).

## REMOVING STUCK SAP AS A POSSIBLE THERAPEUTIC FOR AMYLOIDOSIS

Amyloidosis is a disease where misfolded proteins aggregate and form large deposits in a tissue, leading to organ dysfunction (20, 21). SAP was originally isolated as a serum-derived protein found in all types of amyloid deposits (hence the serum amyloid part of its name) (22, 23). SAP was found to be a pentameric protein, hence the P part of its name (24–27). SAP is also easily purified by incubating serum with certain types of agarose in the presence of calcium, washing unbound protein off, and then eluting fairly pure bound SAP with a calcium chelator (28, 29). One possibility is that the SAP in the amyloid deposits binds to the misfolded proteins in an attempt to opsonize them for phagocytosis, but cannot pull proteins out of the deposit, and the SAP then gets stuck in the deposits. SAP knockout mice have reduced severity of experimentally-induced amyloidosis, suggesting that the stuck SAP exacerbates the amyloid deposit formation and/or hinders the ability of other opsins to pull the amyloid complexes apart (30). The Pepys group found a small molecule compound that causes two human SAP pentamers to stick to each other, and this complex is then quickly cleared from the circulation (31). In SAP knockout mice expressing human SAP, the compound decreased serum SAP levels but did not reduce the severity of experimentally-induced amyloidosis (31). Adding anti-SAP antibodies to this treatment however did reduce experimentally-induced amyloidosis, suggesting that reducing SAP levels is a possible therapeutic for amyloidosis (32–35).

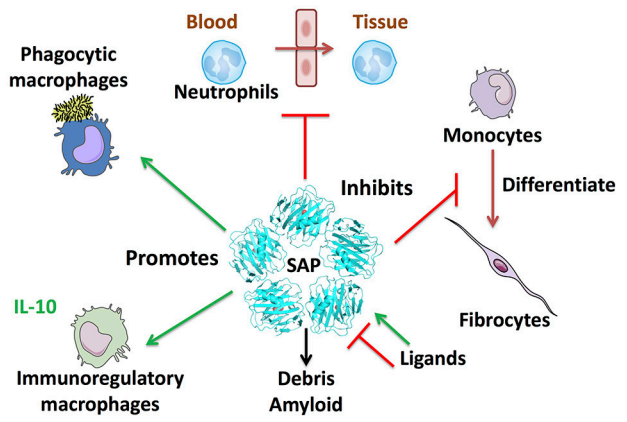


**FIGURE 1 |** SAP regulates multiple aspects of immune responses. Some of the known effects of SAP are shown clockwise from top: SAP inhibits neutrophil adhesion to extracellular matrix and inhibits neutrophil movement into tissues. SAP binds to FcγR and DC-SIGN to inhibit monocyte to fibrocyte differentiation. SAP also binds multiple plasma proteins such as the complement component C1q and mannose-binding lectin (MBL) to promote phagocytosis of bacteria and regulate macrophage differentiation. SAP opsonizes bacteria and cell debris to promote removal by macrophages, and binds amyloid deposits. Finally, SAP promotes immuno-regulatory, and M1 phagocytic macrophages.

## INTERMEZZO 1: WOUND HEALING AND FIBROSIS

Most plant and animal tissues have a remarkable ability to heal mechanical wounds, indicating a strong evolutionary pressure for wound healing (36). In vertebrates, a typical dermal wound fills with scar tissue consisting of fibroblasts, connective tissue, and a capillary bed, and then is covered with an epithelium (37). Unfortunately, inappropriate wound healing responses to perceived wounds cause fibrosing diseases, where scar tissue forms in an internal organ, leading to organ dysfunction. There are at least 62 different fibrosing diseases, and these are associated with 30–45% of deaths in the US (38, 39). Examples of fibrosing diseases include cardiac fibrosis, probably triggered by reduced blood flow to part of the heart, and this fibrosis accounts for a significant fraction of the 450,000 deaths per year from cardiovascular disease in the US (40, 41). Other fibrosing diseases are cirrhosis of the liver, triggered by damage from viral infections, alcohol, or other chemical insults (39), end-stage kidney disease in diabetics, where the scar tissue formation is probably triggered by damage from high glucose levels (42), and pulmonary fibrosis, where particulate matter such as coal dust, and other unknown factors, triggers the progressive formation of scar tissue in the lungs (43). The only FDA-approved therapeutics for fibrosis are two drugs which slow, but do not stop, the progression of pulmonary fibrosis (44).

## INTERMEZZO 2: FIBROCYTES

In the 1850's, James Paget examined healing wounds and observed that cells from the blood enter the wound and then differentiate into elongated cells with an oval nucleus [see Figure 14, page 127 in (45)]. Bucala et al. found that these cells originate from bone marrow derived circulating $CD14^{+}$ monocytes and express markers such as such as CD34 and CD45 that identify them as bone marrow-derived cells, as well as markers such as collagen that identify them as fibroblast-like cells (46–49). They named the cells fibrocytes. Although fibrocytes are rarely observed in normal tissues, they are present in high numbers in healing wounds (46, 50, 51) and fibrotic lesions in pulmonary fibrosis (51–58), keloid scars (59, 60), asthma (52, 61, 62), chronic kidney disease (63–65), and nephrogenic systemic fibrosis (66). Fibrocytes are also present in the fibrotic lesions in animal models of pulmonary fibrosis (53, 67–75), liver fibrosis (71) and renal fibrosis (73, 76). In addition to contributing to the mass of fibrotic

lesions, fibrocytes promote angiogenesis (77), which can then promote the growth of the scar tissue, and secrete TGF-β (78), which causes resident fibroblasts to proliferate and increase their collagen production. Fibroblasts thus have a multiplicative effect on scar tissue formation. At the time we started working on fibrocytes, nothing was known about extracellular factors that regulate their differentiation, and thus why fibrocytes are present in wounds but not normal tissues, and how to control them.

## OUR ENTRY INTO THE SAP, FIBROCYTES, WOUND HEALING, AND FIBROSIS FIELDS

Our lab had been studying the ability of diffusible secreted factors to indicate the local density and composition of cells in a tissue, using the eukaryotic amoeba *Dictyostelium discoideum* as a model system (79, 80). We decided to try to look for cell density sensing factors secreted by human white blood cells. To simplify the purification of any such factors from the extracellular medium, human peripheral blood mononuclear cells (PBMCs) were cultured in serum-free medium. Some of the cells became long, spindle-shaped cells after 3–5 days (81, 82). Videomicroscopy indicated that these spindle shaped cells were quite motile, and they stained for fibrocyte markers (81). Fibrocytes did not appear during this timeframe when serum was present, indicating that something in serum inhibits fibrocyte differentiation.

Since removing something that inhibits fibrocyte differentiation might potentiate wound healing, and conversely adding something that inhibits fibrocyte differentiation might inhibit fibrosis, we abandoned the search for human density sensing factors and purified the fibrocyte differentiation inhibitor from human serum. It turned out to be SAP (81). SAP also inhibits the differentiation of mouse, rat, and dog PBMCs into fibrocytes (83–86). CRP has no significant effect on, and PTX3 potentiates, fibrocyte differentiation, indicating that the three pentraxins differentially affect fibrocytes (81, 87). When PBMCs were cultured in serum that was depleted of SAP, fibrocytes rapidly appeared, indicating that SAP is the main endogenous inhibitor of fibrocyte differentiation in the blood (81).

Pentraxins also regulate macrophages (17, 39, 88–95). In addition to inhibiting fibrocyte differentiation, SAP inhibits pro-fibrotic macrophages, and promotes the formation of immuno-regulatory macrophages (84, 95–105). Although SAP can bind complement component C1q and mannose-binding lectin, these proteins have very modest effects on the ability of SAP to affect macrophage phenotypes (95, 106–108). We refer the reader to the above references and reviews for information on the complexity of pentraxin (including SAP) regulation of macrophages that is beyond the simplicity of this minireview (**Figure 1**).

## REMOVING SAP AS A POSSIBLE THERAPEUTIC FOR WOUND HEALING

Since after blood clots, a wound is covered with serum, and serum contains SAP, and SAP inhibits fibrocyte differentiation and thus wound healing, an intriguing possibility is that removing SAP from wound fluid might potentiate fibrocyte differentiation and wound healing. Wound dressings with $Ca^{++}$ and the type of agarose originally used to purify SAP from serum were tested on full thickness dermal wounds in rats. These dressings speeded healing of these wounds, as well as partial thickness dermal wounds in pigs (109). In the pig wounds, the agarose/ $Ca^{++}$ dressings caused wound to heal faster than wounds treated with commercial dressings such as Tielle, Intrasite, and Xeroform. Although SAP levels in humans are unaffected by inflammation, serum SAP levels in the general population range from 20 to 60 μg/ml (3, 5, 110). Compared to controls, patients with low levels of SAP have better survival of skin grafts, supporting the idea that reducing SAP levels might help wound healing (111). In part because the wound dressing market is basically saturated, efforts to fund clinical tests of this SAP-depleting dressing have been unsuccessful.

## ADDING SAP AS A POSSIBLE THERAPEUTIC FOR FIBROSING DISEASES

A simple non-surgical animal model of a fibrosing disease is pulmonary fibrosis in mice and rats, where a drug called bleomycin can be pipetted through the mouth into the airway, and within 14 days causes pulmonary fibrosis (112, 113). In the bleomycin model, SAP injections led to reduced numbers of fibrocytes in the lungs and reduced fibrosis in rats and mice, and delaying SAP injections until inflammation and fibrosis was already apparent (therapeutic dosing) also reduced symptoms (84). SAP injections have now been shown to inhibit inflammation and fibrosis in other models of pulmonary fibrosis (102, 103), cardiac fibrosis (96, 97), radiation-induced oral mucositis (101), allergic airway disease (100), autoimmune encephalomyelitis (114), corneal wound healing (75), and two models of renal fibrosis/ end stage kidney disease (98). An obvious question about using SAP as a therapeutic for fibrosis is that this might block wound healing. We found that SAP injections slow, but do not stop, dermal wound healing in mice (115).

## SAP EFFICACY AS AN ANTIFIBROTIC IN CLINICAL TRIALS

Compared to control mice, mice lacking SAP have strongly increased pulmonary fibrosis in response to bleomycin, indicating that an endogenous function of SAP is to reduce fibrosis (116). Compared to controls, patients with renal fibrosis, pulmonary fibrosis, scleroderma, myelofibrosis, rheumatoid arthritis, and mixed connective tissue disease tend to have low levels of SAP, supporting the idea that fibrosis might in part involve a SAP deficiency (81, 98, 103, 117). One initial problem obtaining NIH funding to study SAP and fibrosis was that people confused SAP with serum amyloid A (SAA; a completely different, and probably not beneficial protein). We encountered this with a grant application where a reviewer denounced our efforts to inject animals with SAA. After politely explaining

that SAP was not SAA, the grant was funded. An early worker in the SAP field encountered this too, and published a letter in Nature entitled "Serum Amyloid P component (not Serum Amyloid Protein)" (118). For this and other reasons, colleagues used the alternative SAP nomenclature Pentraxin 2 (PTX2) (26, 119), and called the recombinant SAP used for clinical trials PRM-151 (120). Compared to standard of care, injections of recombinant human SAP/PTX2 improved lung function in a Phase 1b trial and a Phase 2 trial in pulmonary fibrosis patients (5, 121). In the 28-weeks Phase 2 trial, SAP injections slowed the decline in forced exhalation volume (FEV), and essentially stopped the decline in the distance patients could walk in 6 min. 61 of the patients receiving SAP in this trial were also taking either pirfenidone or nintedanib, FDA-approved drugs that slow the decline in lung function in pulmonary fibrosis, while 16 other patients treated with SAP were not taking these drugs. Intriguingly, the 16 patients not taking these drugs who were treated with SAP appeared to show on average a very slight improvement in forced exhalation volume and an improvement in how far they could walk in 6 min, suggesting the exciting possibility that SAP might be able to partially reverse pulmonary fibrosis. Myelofibrosis is a fibrosis of the bone marrow (122). SAP injections also reduced fibrosis and improved bone marrow function in a 27-patient Phase 2 trial on myelofibrosis patients (123).

## SAP PHARMACOLOGY

The plasma clearance rates for patients treated with SAP is ∼24 to 30 h (5, 124). In the Phase 2 clinical trial, and in earlier trials, efficacy was observed with monthly dosing. The apparent paradox of how something with a short plasma half-life could show efficacy with monthly dosing can be answered by looking at the tissue half-life, which for healthy volunteers is 7.2 days (124). SAP, as well as CRP and PTX3, have long been known as opsonins that helps phagocytic cells ingest debris (16, 125–129). In fibrosis, debris and other tissue insults are thought to both initiate as well as potentiate fibrosis (39). Clearing debris from the vicinity of a fibrotic lesion is very likely one mechanism whereby SAP inhibits fibrosis. The debris is not detectable in the circulation, rather it is localized to the vicinity of the fibrotic lesion. Thus this beneficial effect of SAP occurs in the tissue rather than in the circulation. Although SAP has some modest effects on macrophage differentiation from monocytes (changes in the expression of a small number of surface markers in some but not all of the macrophages) (95, 99), macrophage polarization from one macrophage phenotype to another macrophage phenotype (again, even more subtle changes in the expression of a small number of surface markers) (95), and neutrophil adhesion to tissue extracellular matrix components (see below), the most obvious effect of SAP on innate immune cells is its ability to completely inhibit the differentiation of monocytes into fibrocytes. All of these effects on innate immune cells affect what the cells do after they have entered a tissue. A reasonable assumption is thus that the SAP effects occur in the tissue, specifically in the vicinity of the fibrotic lesion, rather than in the circulation, and thus that the key half-life is the tissue rather than plasma half-life.

Two observations suggest that the half-life of SAP in a fibrotic lesion may be considerably longer than 7.2 days. First, amyloid deposits resemble in many ways fibrotic lesions, and the half-life of SAP in amyloid deposits is 24–27 days (124, 130, 131). Second, in mice where fibrosis was induced in one kidney by obstructing the ureter, injected SAP localized to the fibrotic kidney, with much less localization to the non-injured contralateral kidney (98). Together, these arguments and results support the idea that even with a short plasma half-life, monthly injections of SAP can be efficacious.

## SAP INHIBITION OF FIBROCYTES ALLOWS AN ASSESSMENT OF THE POSSIBLE EFFECT OF FACTORS SUCH AS DIETARY SALT ON FIBROSIS

Human PBMC cultured in serum-free medium differentiate into easily identifiable (by microscopy) fibrocytes, and adding different concentrations of SAP to inhibit this generates a standard curve of SAP effects. This allows a simple assay to look at the effects of various conditions or compounds on this process. For instance, ELISA assays of sera from keloid patients (these patients form greatly exaggerated dermal scars) showed normal levels of SAP, but the fibrocyte assay on keloid patient PBMC showed that these cells are relatively insensitive to SAP (132). A variety of compounds affect fibrocyte differentiation and/or the ability of SAP to inhibit fibrocyte differentiation (57, 63, 82, 87, 117, 133–142). One compound that may be clinically relevant is NaCl, which when added to increase the medium NaCl concentration by 25 mM (this level of increase can be seen in the serum after a very salty meal) potentiates fibrocyte differentiation and inhibits the SAP effect, possibly explaining why high salt intake is associated with a propensity for cardiac fibrosis (137). Peritoneal dialysis can lead to peritoneal fibrosis, and we found that peritoneal dialysis fluid and dialysis fluid components such as NaCl also promote fibrocyte differentiation and impede SAP (139). In support of this connection between salt and fibrocytes, low dietary salt reduces the severity of bleomycin-induced pulmonary fibrosis in mice, suggesting that low salt diets may be beneficial for fibrosis patients (143).

## SAP CAN OVERRIDE OTHER PROFIBROTIC FACTORS

A variety of signals promote wound healing and fibrosis. For instance, TGF-β1 is an extracellular signal that drives fibrosis (144, 145), and in mice, conditional expression of TGF-β1 in the lungs causes pulmonary fibrosis (103, 146). In this model, SAP injections stopped and reversed fibrosis (103). We found that although quiescent fibroblasts secrete the protein Slit2 to inhibit fibrocyte differentiation (essentially telling incoming monocytes that no more fibroblast-cells are needed) (138),

fibroblasts activated by the pro-fibrotic signal TNF-α secrete the protein lumican, which promotes fibrocyte differentiation (142). Thankfully, SAP can override the effect of lumican on fibrocytes (142). Other signals that promote fibrocyte differentiation and profibrotic macrophage differentiation include thrombin activated during blood clotting (this may thus initiate the fibrocyte component of wound healing), and tryptase released from mast cells (147, 148). SAP also competes with these signals to inhibit fibrocytes and macrophages (148, 149). In addition, SAP inhibits fibrocyte differentiation induced by IL-4, IL-13, high molecular weight hyaluronic acid, and PTX3 (82, 87, 136, 141). Together, these results suggest that one reason SAP appears to be effective in the clinic as an anti-fibrotic is a fortunate dominance of SAP over these signals.

## ELUCIDATING SAP RECEPTORS LED TO SMALL-MOLECULE SAP MIMETICS

Fcγ receptors (FcγRs) bind the Fc domain of IgG immunoglobulins (150). Once aggregated IgG cross-links multiple FcγRs (this prevents monomeric IgGs from activating FcγRs), a signaling cascade is activated through tyrosine kinases to initiate an immune response (151). Phagocytic cells, such as monocytes, bind SAP, CRP, and PTX3 using different combinations of FcγRs (16, 86, 98, 152–156), and the structure of SAP bound to FcγRIIa and modeling of SAP binding to other Fc receptors has been published (157, 158). In support of the hypothesis that SAP inhibits fibrocyte differentiation by binding to FcγRs, we found that cross-linked but not monomeric IgG also inhibits fibrocyte differentiation (159). Mouse monocytes lacking FcγRI, or human monocytes with siRNA-reduced FcγRI, had a reduced sensitivity to SAP, while mouse cells lacking other FcγRs had normal or enhances sensitivity to SAP, indicating that FcγRI mediates SAP signaling (86). Surprisingly, monocytes from cells lacking all four known FcγRs still responded to SAP, indicating that a different receptor also mediates SAP signaling (156, 160).

To help elucidate SAP signaling, we mutated SAP protein surface amino acids that were different from CRP, and the mutant SAPs were assayed for their ability to inhibit fibrocyte differentiation (SAP and CRP are have highly similar amino acid sequences and structures, but CRP does not inhibit fibrocyte differentiation) (81, 87)). None of the mutant SAPs completely abrogated SAP activity (86, 156). One amino acid initially overlooked was a glycosylated asparagine on SAP that is a non-glycosylated alanine on CRP, and when SAP was desialylated, the SAP largely lost its ability to inhibit fibrocyte differentiation; conversely when the CRP alanine was mutated to an asparagine, the asparagine became glycosylated and the glycosylated CRP inhibited fibrocyte differentiation (160). This suggested that a polysaccharide receptor might help to sense SAP, and we found that the C-type lectin DC-SIGN mediated SAP effects on monocytes (160). Other workers found a variety of compounds that block the ability of polysaccharides to bind DC-SIGN, and three of these potently inhibited fibrocyte differentiation. One of the DC-SIGN-binding molecules showed efficacy in a mouse pulmonary fibrosis model at 0.001 mg/kg (160). These results suggest that small molecules that mimic SAP might be useful as therapeutics for fibrosing diseases.

## ADDING SAP AS A POSSIBLE THERAPEUTIC FOR NEUTROPHIL-DRIVEN DISEASES

Inflammatory lesions recruit neutrophils to the site of damage (161, 162). This however can sometimes be counterproductive; for instance some patients with damaged lungs develop acute respiratory distress syndrome/ acute lung injury (ARDS/ ALI), where neutrophils enter the lungs and release proteases and reactive oxygen species. This causes further damage and further neutrophil recruitment and subsequent damage, and this vicious cycle results in the ∼40% mortality seen in the ∼200,000 ARDS patients each year in the US (163). SAP decreases neutrophil binding to extracellular matrix components (164–166), and in a mouse model of ARDS, SAP injections starting 24 h after injury reduced the number of neutrophils in the lungs (166). The small-molecule SAP mimetic discussed above also showed efficacy in this ARDS model (160). These results suggest that SAP and SAP mimetics might be useful as therapeutics for neutrophil-driven diseases such as ARDS/ ALI.

## BLOCKING SAP SIGNALING AS A POSSIBLE THERAPEUTIC FOR DISEASES SUCH AS TUBERCULOSIS

M1 macrophages are highly aggressive against bacteria and other pathogens, but SAP, which is a constitutive component of the blood, pushes macrophages toward an anti-inflammatory/anti-fibrotic phenotype (90, 95, 99, 100, 103, 156, 167, 168). Tuberculosis bacteria can live inside macrophages, where they also push the host macrophage away from a M1 phenotype to help the survival of the parasitic bacteria (169). To test the hypothesis that blocking SAP signaling to macrophages would reduce regulatory macrophages and increase M1 macrophages, we screened 3,000 compounds for the ability to inhibit the binding of SAP to FcγRI, and found 12 that reduced this binding (170). In support of the hypothesis, SAP potentiated the proliferation of *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* in human macrophages, and in the presence of SAP, 2 of the compounds reduced the intra-macrophage proliferation of these bacteria (170).

## CONCLUSION

Pentraxins are ancient and fascinating molecules. Increasing levels of SAP either locally or systemically is showing promise as a therapeutic for a variety of diseases where the ability of SAP to help clear debris and calm the innate immune system is beneficial. Conversely, decreasing levels of SAP, or decreasing SAP effects, shows promise as potential therapeutics

where unleashing the innate immune system is beneficial. An intriguing possibility is that altering levels of other pentraxins might similarly be useful as stand-alone therapeutics or in combination with manipulations of SAP levels for even more diseases.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

This work was funded by NIH R01 HL132919 and NIH R01 HL118507.

## ACKNOWLEDGMENTS

We thank Tejas Karhadkar and Kristen Consalvo for comments on the manuscript. Some components of the figure were adjusted from originals generated by Servier Medical Art, under a Creative Commons License.

## REFERENCES

1. Pepys MB, Booth DR, Hutchinson WL, Gallimore JR, Collins PM, Hohenester E. Amyloid P component. A critical review. *Amyloid* (1997) 4:274–95. doi: 10.3109/13506129709003838
2. Hutchinson WL, Hohenester E, Pepys MB. Human serum amyloid P component is a single uncomplexed pentamer in whole serum. *Mol Med.* (2000) 6:482–93. doi: 10.1007/BF03401789
3. Nelson SR, Tennent GA, Sethi D, Gower PE, Ballardie FW, Amatayakul-Chantler S, et al. Serum amyloid P component in chronic renal failure and dialysis. *Clin Chim Acta* (1991) 200:191–9. doi: 10.1016/0009-8981(91)90090-Y
4. Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today* (1994) 15:81–8. doi: 10.1016/0167-5699(94)90138-4
5. Dillingh MR, van den Blink B, Moerland M, van Dongen MGJ, Levi M, Kleinjan A, et al. Recombinant human serum amyloid P in healthy volunteers and patients with pulmonary fibrosis. *Pulmonary Pharmacol Therapeut.* (2013) 26:672–6. doi: 10.1016/j.pupt.2013.01.008
6. Pepys MB, Baltz M, Gomer K, Davies AJ, Doenhoff M. Serum amyloid P-component is an acute-phase reactant in the mouse. *Nature* (1979) 278:259–61. doi: 10.1038/278259a0
7. Le PT, Muller MT, Mortensen RF. Acute phase reactants of mice. I Isolation of serum amyloid P- component (SAP) and its induction by a monokine. *J Immunol.* (1982) 129:665–72.
8. Du Clos TW. The interaction of C-reactive protein and serum amyloid P component with nuclear antigens. *Mol Biol Rep.* (1996) 23:253–60. doi: 10.1007/BF00351177
9. Kravitz M, Pitashny M, Shoenfeld Y. Protective Molecules–C-Reactive Protein (CRP), Serum Amyloid P (SAP), Pentraxin3 (PTX3), Mannose-Binding Lectin (MBL), and Apolipoprotein A1 (Apo A1), and Their Autoantibodies: Prevalence and Clinical Significance in Autoimmunity. *J Clin Immunol.* (2005) 25:582–91. doi: 10.1007/s10875-005-7828-2
10. Agrawal A, Singh PP, Bottazzi B, Garlanda C, Mantovani A. Pattern recognition by pentraxins. *Adv Exp Med Biol.* (2009) 653:98–116. doi: 10.1007/978-1-4419-0901-5_7
11. Emsley J, White HE, O'Hara BP, Oliva G, Srinivasan N, Tickle IJ, et al. Structure of pentameric human serum amyloid P component. *Nature* (1994) 367:338–45. doi: 10.1038/367338a0
12. Shrive AK, Cheetham GM, Holden D, Myles DA, Turnell WG, Volanakis JE, et al. Three dimensional structure of human C-reactive protein. *Nat Struct Biol.* (1996) 3:346–54. doi: 10.1038/nsb0496-346
13. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. PTX3, a Humoral Pattern Recognition Molecule, in Innate Immunity, Tissue Repair, and Cancer. *Physiol Rev.* (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
14. Pepys MB, Dyck RF, de Beer FC, Skinner M, Cohen AS. Binding of serum amyloid P-component (SAP) by amyloid fibrils. *Clin ExpImmunol.* (1979) 38:284–93.
15. Hamazaki H. Ca(2+)-dependent binding of human serum amyloid P component to Alzheimer's beta-amyloid peptide. *J Biol Chem.* (1995) 270:10392–4. doi: 10.1074/jbc.270.18.10392
16. Bharadwaj D, Mold C, Markham E, Du Clos TW. Serum amyloid P component binds to Fc gamma receptors and opsonizes particles for phagocytosis. *J Immunol.* (2001) 166:6735–41. doi: 10.4049/jimmunol.166.11.6735
17. Du Clos TW. Pentraxins: structure, function, and role in inflammation. *ISRN Inflamm.* (2013) 2013:379040. doi: 10.1155/2013/379040
18. Bottazzi B, Inforzato A, Messa M, Barbagallo M, Magrini E, Garlanda C, et al. The pentraxins PTX3 and SAP in innate immunity, regulation of inflammation and tissue remodelling. *J Hepatol.* (2016) 64:1416–27. doi: 10.1016/j.jhep.2016.02.029
19. Iwanaga S. The molecular basis of innate immunity in the horseshoe crab. *Curr Opin Immunol.* (2002) 14:87–95. doi: 10.1016/S0952-7915(01)00302-8
20. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Eng J Med.* (2003) 349:583–96. doi: 10.1056/NEJMra023144
21. Sipe JD, Benson MD, Buxbaum JN, Ikeda SI, Merlini G, Saraiva MJM, et al. Nomenclature 2014: *Amyloid* fibril proteins and clinical classification of the amyloidosis. *Amyloid* (2014) 21:221–4. doi: 10.3109/13506129.2014.964858
22. Cathcart ES, Comerford FR, Cohen AS. Immunologic studies on a protein extracted from human secondary amyloid. *N Engl J Med.* (1965) 273:143–6. doi: 10.1056/NEJM196507152730306
23. Cathcart ES, Wollheim FA, Cohen AS. Plasma protein constituents of amyloid fibrils. *J Immunol.* (1967) 99:376–85.
24. Bladen HA, Nylen MU, Glenner GG. The ultrastructure of human amyloid as revealed by the negative staining technique. *J Ultrastruct Res.* (1966) 14:449–59. doi: 10.1016/S0022-5320(66)80075-8
25. Benson Merrill D, Skinner M, Shirahama T, Cohen Alan S. P-component of amyloid. Isolation from human serum by affinity chromatography. *Arthr Rheumat.* (1976) 19:749–54. doi: 10.1002/1529-0131(197607/08)19:4<749::AID-ART1780190415>3.0.CO;2-C
26. Osmand AP, Friedenson B, Gewurz H, Painter RH, Hofmann T, Shelton E. Characterization of C-reactive protein and the complement subcomponent C1t as homologous proteins displaying cyclic pentameric symmetry (pentraxins). *Proc Natl Acad Sci USA.* (1977) 74:739–43. doi: 10.1073/pnas.74.2.739
27. Pepys MB. Amyloidosis. *Annu Rev Med.* (2006) 57:223–41. doi: 10.1146/annurev.med.57.121304.131243
28. Pepys MB, Dash AC, Munn EA, Feinstein A, Skinner M, Cohen AS, et al. Isolation of amyloid p component (protein ap) from normal serum as a calcium-dependent binding protein. *Lancet* (1977) 309:1029–31. doi: 10.1016/S0140-6736(77)91260-0
29. Painter RH, De Escallon I, Massey A, Pinteric L, Stern SB. The structure and binding characteristics of serum amyloid protein (9.5S alpha 1-glycoprotein). *Ann N Y Acad Sci.* (1982) 389:199–215. doi: 10.1111/j.1749-6632.1982.tb22138.x
30. Botto M, Hawkins PN, Bickerstaff MC, Herbert J, Bygrave AE, McBride A, et al. Amyloid deposition is delayed in mice with targeted deletion of the serum amyloid P component gene. *Nat Med.* (1997) 3:855–9. doi: 10.1038/nm0897-855
31. Pepys MB, Herbert J, Hutchinson WL, Tennent GA, Lachmann HJ, Gallimore JR, et al. Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature* (2002) 417:254–9. doi: 10.1038/417254a

32. Bodin K, Ellmerich S, Kahan MC, Tennent GA, Loesch A, Gilbertson JA, et al. Antibodies to human serum amyloid P component eliminate visceral amyloid deposits. *Nature* (2010) 468:93–7. doi: 10.1038/nature09494
33. Richards DB, Cookson LM, Berges AC, Barton SV, Lane T, Ritter JM, et al. Therapeutic clearance of amyloid by antibodies to serum amyloid P component. *N Engl J Med.* (2015) 373:1106–14. doi: 10.1056/NEJMoa1504942
34. Nuvolone M, Merlini G. Systemic amyloidosis: novel therapies and role of biomarkers. *Nephrol Dialysis Trans.* (2017) 32:770–80. doi: 10.1093/ndt/gfw305
35. Richards DB, Cookson LM, Barton SV, Liefaard L, Lane T, Hutt DF, et al. Repeat doses of antibody to serum amyloid P component clear amyloid deposits in patients with systemic amyloidosis. *Sci Transl Med.* (2018) 10:eaan3128. doi: 10.1126/scitranslmed.aan3128
36. Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. *Science* (2017) 356:1026–30. doi: 10.1126/science.aam7928
37. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci Trans Med.* (2014) 6:265sr266. doi: 10.1126/scitranslmed.3009337
38. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med.* (2012) 18:1028–40. doi: 10.1038/nm.2807
39. Duffield JS, Lupher M, Thannickal VJ, Wynn TA. Host responses in tissue repair and fibrosis. *Ann Rev Pathol.* (2013) 8:241–76. doi: 10.1146/annurev-pathol-020712-163930
40. Assayag P, Carre F, Chevalier B, Delcayre C, Mansier P, Swynghedauw B. Compensated cardiac hypertrophy: arrhythmogenicity and the new myocardial phenotype. *I Fibr Cardiovas Res.* (1997) 34:439–44. doi: 10.1016/S0008-6363(97)00073-4
41. Haider AW, Larson MG, Benjamin EJ, Levy D. Increased left ventricular mass and hypertrophy are associated with increased risk for sudden death. *J Am Coll Cardiol.* (1998) 32:1454–9. doi: 10.1016/S0735-1097(98)00407-0
42. Reidy K, Kang HM, Hostetter T, Susztak K. Molecular mechanisms of diabetic kidney disease. *J Clin Invest.* (2014) 124:2333–40. doi: 10.1172/JCI72271
43. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Res Crit Care Med.* (2011) 183:788–824. doi: 10.1164/rccm.2009-040GL
44. Lederer DJ, Martinez FJ. Idiopathic pulmonary fibrosis. *N Eng J Med.* (2018) 378:1811–23. doi: 10.1056/NEJMra1705751
45. Paget J. *Lectures on surgical pathology: delivered at the Royal College of Surgeons of England*. Philadelphia: Lindsay & Blakiston (1854).
46. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med.* (1994) 1:71–81.
47. Chesney J, Bacher M, Bender A, Bucala R. The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. *Proc Natl Acad Sci USA.* (1997) 94:6307–12. doi: 10.1073/pnas.94.12.6307
48. Chesney J, Metz C, Stavitsky AB, Bacher M, Bucala R. Regulated production of type I collagen and inflammatory cytokines by peripheral blood fibrocytes. *J Immunol.* (1998) 160:419–25.
49. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol.* (2001) 166:7556–62. doi: 10.4049/jimmunol.166.12.7556
50. Yang L, Scott PG, Giuffre J, Shankowsky HA, Ghahary A, Tredget EE. Peripheral blood fibrocytes from burn patients: identification and quantification of fibrocytes in adherent cells cultured from peripheral blood mononuclear cells. *Lab Invest.* (2002) 82:1183–92. doi: 10.1097/01.LAB.0000027841.50269.61
51. Mori L, Bellini A, Stacey MA, Schmidt M, Mattoli S. Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. *Exp Cell Res.* (2005) 304:81–90. doi: 10.1016/j.yexcr.2004.11.011
52. Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J Immunol.* (2003) 171:380–9. doi: 10.4049/jimmunol.171.1.380
53. Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R. Circulating fibrocytes: collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol.* (2004) 36:598–606. doi: 10.1016/j.biocel.2003.10.005
54. Yang L, Scott PG, Dodd C, Medina A, Jiao H, Shankowsky HA, et al. Identification of fibrocytes in postburn hypertrophic scar. *Wound Repair Regener.* (2005) 13:398–404. doi: 10.1111/j.1067-1927.2005.130407.x
55. Mehrad B, Burdick MD, Zisman DA, Keane MP, Belperio JA, Strieter RM. Circulating peripheral blood fibrocytes in human fibrotic interstitial lung disease. *Biochem Biophys Res Commun.* (2007) 353:104–8. doi: 10.1016/j.bbrc.2006.11.149
56. Andersson-Sjöland A, de Alba CG, Nihlberg K, Becerril C, Ramírez R, Pardo A, et al. Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol.* (2008) 40:2129–40. doi: 10.1016/j.biocel.2008.02.012
57. Reilkoff RA, Bucala R, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. *Nat Rev Immunol.* (2011) 11:427–35. doi: 10.1038/nri2990
58. Just SA, Lindegaard H, Hejbøl EK, Davidsen JR, Bjerring N, Hansen SWK, et al. Fibrocyte measurement in peripheral blood correlates with number of cultured mature fibrocytes *in vitro* and is a potential biomarker for interstitial lung disease in Rheumatoid Arthritis. *Respirat Res.* (2017) 18:141. doi: 10.1186/s12931-017-0623-9
59. Aiba S, Tagami H. Inverse correlation between CD34 expression and proline-4-hydroxylase immunoreactivity on spindle cells noted in hypertrophic scars and keloids. *J Cutan Pathol.* (1997) 24:65–9. doi: 10.1111/j.1600-0560.1997.tb01098.x
60. Shin JU, Kim SH, Kim H, Noh JY, Jin S, Park CO, et al. TSLP is a potential initiator of collagen synthesis and an activator of CXCR4/SDF-1 axis in keloid pathogenesis. *J Invest Dermatol.* (2016) 136:507–15. doi: 10.1016/j.jid.2015.11.008
61. Isgr,ò M, Bianchetti L, Marini MA, Mattoli S. Involvement of fibrocytes in allergen-induced T cell responses and rhinovirus infections in asthma. *Biochem Biophys Res Commun.* (2013) 437:446–51. doi: 10.1016/j.bbrc.2013.06.099
62. Mattoli S. Involvement of fibrocytes in asthma and clinical implications. *Clin Exp Allergy* (2015) 45:1497–509. doi: 10.1111/cea.12525
63. Niedermeier M, Reich B, Rodriguez Gomez M, Denzel A, Schmidbauer K, Gobel N, et al. CD4+ T cells control the differentiation of Gr1+ monocytes into fibrocytes. *Proc Natl Acad Sci USA.* (2009) 106:17892–7. doi: 10.1073/pnas.0906070106
64. Sakai N, Furuichi K, Shinozaki Y, Yamauchi H, Toyama T, Kitajima S, et al. Fibrocytes are involved in the pathogenesis of human chronic kidney disease. *Hum Pathol.* (2010) 41:672–8. doi: 10.1016/j.humpath.2009.10.008
65. Wada T, Sakai N, Sakai Y, Matsushima K, Kaneko S, Furuichi K. Involvement of bone-marrow-derived cells in kidney fibrosis. *Clin Exp Nephrol.* (2011) 15:8–13. doi: 10.1007/s10157-010-0372-2
66. Cowper SE. Nephrogenic fibrosing dermopathy: the first 6 years. *Curr Opin Rheumatol.* (2003) 15:785–90. doi: 10.1097/00002281-200311000-00017
67. Direkze NC, Forbes SJ, Brittan M, Hunt T, Jeffery R, Preston SL, et al. Multiple organ engraftment by bone-marrow-derived myofibroblasts and fibroblasts in bone-marrow-transplanted mice. *Stem Cells* (2003) 21:514–20. doi: 10.1634/stemcells.21-5-514
68. Epperly MW, Guo H, Gretton JE, Greenberger JS. Bone marrow origin of myofibroblasts in irradiation pulmonary fibrosis. *Am J Res Cell Mol Biol.* (2003) 29:213–24. doi: 10.1165/rcmb.2002-0069OC
69. Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest.* (2004) 113:243–52. doi: 10.1172/JCI200418847
70. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest.* (2004) 114:438–46. doi: 10.1172/JCI200420997
71. Kisseleva T, Uchinami H, Feirt N, Quintana-Bustamante O, Segovia JC, Schwabe RF, et al. Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. *J Hepatol.* (2006) 45:429–38. doi: 10.1016/j.jhep.2006.04.014
72. Moore BB, Murray L, Das A, Wilke CA, Herrygers AB, Toews GB. The Role of CCL12 in the Recruitment of Fibrocytes and Lung Fibrosis. *Am J Respirat Cell Mol Biol.* (2006) 35:175–81. doi: 10.1165/rcmb.2005-0239OC

73. Sakai N, Wada T, Yokoyama H, Lipp M, Ueha S, Matsushima K, et al. Secondary lymphoid tissue chemokine (SLC/CCL21)/CCR7 signaling regulates fibrocytes in renal fibrosis. *Proc Natl Acad Sci.* (2006) 103:14098–103. doi: 10.1073/pnas.0511200103
74. Varcoe RL, Mikhail M, Guiffre AK, Pennings G, Vicaretti M, Hawthorne WJ, et al. The role of the fibrocyte in intimal hyperplasia. *J Thromb Haemost.* (2006) 4:1125–33. doi: 10.1111/j.1538-7836.2006.01924.x
75. Santhiago MR, Singh V, Barbosa FL, Agrawal V, Wilson SE. Monocyte development inhibitor PRM-151 decreases corneal myofibroblast generation in rabbits. *Exp Eye Res.* (2011) 93:810–7. doi: 10.1016/j.exer.2011.08.015
76. Sakai N, Wada T, Matsushima K, Bucala R, Iwai M, Horiuchi M, et al. The renin-angiotensin system contributes to renal fibrosis through regulation of fibrocytes. *J Hypert.* (2008) 26:780–90. doi: 10.1097/HJH.0b013e3282f3e9e6
77. Hartlapp I, Abe R, Saeed RW, Peng T, Voelter W, Bucala R, et al. Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis *in vivo*. *FASEB J.* (2001) 15:2215–24. doi: 10.1096/fj.01-0049com
78. Wang JF, Jiao H, Stewart TL, Shankowsky HA, Scott PG, Tredget EE. Fibrocytes from burn patients regulate the activities of fibroblasts. *Wound Repair Regen.* (2007) 15:113–21. doi: 10.1111/j.1524-475X.2006.00192.x
79. Yuen IS, Jain R, Bishop JD, Lindsey DF, Deery WJ, Van Haastert PJ, et al. A density-sensing factor regulates signal transduction in Dictyostelium. *J Cell Biol.* (1995) 129:1251–62. doi: 10.1083/jcb.129.5.1251
80. Brock DA, Gomer RH. A cell-counting factor regulating structure size in Dictyostelium. *Genes Dev.* (1999) 13:1960–9. doi: 10.1101/gad.13.15.1960
81. Pilling D, Buckley CD, Salmon M, Gomer RH. Inhibition of fibrocyte differentiation by serum amyloid P. *J Immunol.* (2003) 17:5537–46. doi: 10.4049/jimmunol.171.10.5537
82. Shao DD, Suresh R, Vakil V, Gomer RH, Pilling D. Pivotal Advance: Th-1 cytokines inhibit, and Th-2 cytokines promote fibrocyte differentiation. *J Leukocyte Biol.* (2008) 83:1323–33. doi: 10.1189/jlb.1107782
83. Pilling D, and Gomer RH. Regulatory pathways for fibrocyte differentiation. In: Bucala, R, editor. *Fibrocytes-New Insights into Tissue Repair and Systemic Fibroses* (2007). p. 37–60. doi: 10.1142/9789812706690_0003
84. Pilling D, Roife D, Wang M, Ronkainen SD, Crawford JR, Travis EL, et al. Reduction of bleomycin-induced pulmonary fibrosis by serum amyloid P. *J Immunol.* (2007) 179:4035–44. doi: 10.4049/jimmunol.179.6.4035
85. Crawford JR, Pilling D, Gomer RH. Improved serum-free culture conditions for spleen-derived murine fibrocytes. *J Immunol Methods* (2010) 363:9–20. doi: 10.1016/j.jim.2010.09.025
86. Crawford JR, Pilling D, Gomer RH. FcγRI mediates serum amyloid P inhibition of fibrocyte differentiation. *J Leukocyte Biol.* (2012) 92:699–711. doi: 10.1189/jlb.0112033
87. Pilling D, Cox N, Vakil V, Verbeek JS, Gomer RH. The long pentraxin PTX3 promotes fibrocyte differentiation. *PLoS ONE* (2015) 10:e0119709. doi: 10.1371/journal.pone.0119709
88. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Ann Rev Immunol.* (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
89. Devaraj S, Jialal I. C-reactive protein polarizes human macrophages to an M1 phenotype and inhibits transformation to the M2 phenotype. *Arterioscler Thromb Vas Biol.* (2011) 31:1397–402. doi: 10.1161/ATVBAHA.111.225508
90. Zhang W, Xu W, Xiong S. Macrophage differentiation and polarization via phosphatidylinositol 3-Kinase/Akt–ERK signaling pathway conferred by serum amyloid P component. *J Immunol.* (2011a) 187:1764–77. doi: 10.4049/jimmunol.1002315
91. Zhao XQ, Zhang MW, Wang F, Zhao YX, Li JJ, Wang XP, et al. CRP enhances soluble LOX-1 release from macrophages by activating TNF-α converting enzyme. *J Lipid Res.* (2011) 52:923–33. doi: 10.1194/jlr.M015156
92. Lu J, Marjon KD, Mold C, Du Clos TW, Sun PD. Pentraxins and Fc receptors. *Immunol Rev.* (2012) 250:230–8. doi: 10.1111/j.1600-065X.2012.01162.x
93. Cox N, Pilling D, Gomer RH. Serum amyloid P: a systemic regulator of the innate immune response. *J Leukocyte Biol.* (2014) 96:739–43. doi: 10.1189/jlb.1MR0114-068R
94. Pegues MA, McWilliams IL, Szalai AJ. C-reactive protein exacerbates renal ischemia-reperfusion injury: are myeloid derived suppressor cells to blame? *Am J Physiol Renal Physiol.* (2016) 311:F176–81. doi: 10.1152/ajprenal.00107.2016
95. Pilling D, Galvis-Carvajal E, Karhadkar TR, Cox N, Gomer RH. Monocyte differentiation and macrophage priming are regulated differentially by pentraxins and their ligands. *BMC Immunol.* (2017) 18:30. doi: 10.1186/s12865-017-0214-z
96. Haudek SB, Xia Y, Huebener P, Lee JM, Carlson S, Crawford JR, et al. Bone marrow-derived fibroblast precursors mediate ischemic cardiomyopathy in mice. *Proc Natl Acad Sci USA.* (2006) 103:18284–9. doi: 10.1073/pnas.0608799103
97. Haudek SB, Trial J, Xia Y, Gupta D, Pilling D, Entman ML. Fc receptor engagement mediates differentiation of cardiac fibroblast precursor cells. *Proc Natl Acad Sci USA.* (2008) 105:10179–84. doi: 10.1073/pnas.0804910105
98. Castano AP, Lin SL, Surowy T, Nowlin BT, Turlapati SA, Patel T, et al. Serum amyloid P inhibits fibrosis through Fc gamma R-dependent monocyte-macrophage regulation *in vivo*. *Sci Trans Med.* (2009) 1:5ra13. doi: 10.1126/scitranslmed.3000111
99. Pilling D, Fan T, Huang D, Kaul B, Gomer RH. Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS ONE* (2009) 4:e7475. doi: 10.1371/journal.pone.0007475
100. Moreira AP, Cavassani KA, Hullinger R, Rosada RS, Fong DJ, Murray L, et al. Serum amyloid P attenuates M2 macrophage activation and protects against fungal spore-induced allergic airway disease. *J Allergy Clin Immunol.* (2010) 126:712–721 e717. doi: 10.1016/j.jaci.2010.06.010
101. Murray L, Kramer M, Hesson D, Watkins B, Fey E, Argentieri R, et al. Serum amyloid P ameliorates radiation-induced oral mucositis and fibrosis. *Fibrogen Tissue Repair* (2010) 3:11. doi: 10.1186/1755-1536-3-11
102. Murray LA, Rosada R, Moreira AP, Joshi A, Kramer MS, Hesson DP, et al. Serum amyloid P therapeutically attenuates murine bleomycin-induced pulmonary fibrosis via its effects on macrophages. *PLoS ONE* (2010) 5:e9683. doi: 10.1371/journal.pone.0009683
103. Murray LA, Chen Q, Kramer MS, Hesson DP, Argentieri RL, Peng X, et al. TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. *Int J Biochem Cell Biol.* (2011) 43:154–62. doi: 10.1016/j.biocel.2010.10.013
104. Nakagawa N, Barron L, Gomez IG, Johnson BG, Roach AM, Kameoka S, et al. Pentraxin-2 suppresses c-Jun/AP-1 signaling to inhibit progressive fibrotic disease. *JCI Insight*. (2016) 1:e87446. doi: 10.1172/jci.insight.87446
105. Chen W, Pilling D, Gomer RH. C-reactive protein (CRP) but not the related pentraxins serum amyloid P and PTX3 inhibits the proliferation and induces apoptosis of the leukemia cell line Mono Mac 6. *BMC Immunol.* (2017) 18:47. doi: 10.1186/s12865-017-0230-z
106. Sorensen IJ, Nielsen EH, Andersen O, Danielsen B, Svehag SE. Binding of complement proteins C1q and C4bp to serum amyloid P component (SAP) in solid contra liquid phase. *Scand J Immunol.* (1996) 44:401–7. doi: 10.1046/j.1365-3083.1996.d01-326.x
107. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem.* (1997) 272:32817–23. doi: 10.1074/jbc.272.52.32817
108. Ma YJ, Doni A, Skjoedt MO, Honore C, Arendrup M, Mantovani A, et al. Heterocomplexes of mannose-binding lectin and the pentraxins PTX3 or serum amyloid P component trigger cross-activation of the complement system. *J Biol Chem.* (2011) 286:3405–17. doi: 10.1074/jbc.M110.190637
109. Gomer RH, Pilling D, Kauvar L, Ellsworth S, Pissani S, Real L, et al. A Serum Amyloid P-binding hydrogel speeds healing of partial thickness wounds in pigs. *Wound Repair Regen.* (2009) 17:397–404. doi: 10.1111/j.1524-475X.2009.00482.x
110. Sen JW, Recke C, Rahbek L, Skogstrand K, Heegaard NH. Structural, quantitative and functional comparison of amyloid P component in sera from patients with systemic lupus erythematosus and healthy donors. *Scand J Immunol.* (2002) 56:645–51. doi: 10.1046/j.1365-3083.2002.01178.x
111. Zhang YM, Fang YD, Wang YC, Wang SL, Lei ZY, Liu XW, et al. Role of serum amyloid P in skin graft survival and wound healing in burned patients receiving skin grafts. *Clin Chim Acta* (2011) 412:227–9. doi: 10.1016/j.cca.2010.09.036
112. Moore BB, Lawson WE, Oury TD, Sisson TH, Raghavendran K, Hogaboam CM. Animal models of fibrotic lung disease. *Am J Respirat Cell Mol Biol.* (2013) 49:167–79. doi: 10.1165/rcmb.2013-0094TR

113. Tashiro J, Rubio GA, Limper AH, Williams K, Elliot SJ, Ninou I, et al. Exploring animal models that resemble idiopathic pulmonary fibrosis. *Front Med.* (2017) 4:118. doi: 10.3389/fmed.2017.00118
114. Ji Z, Ke ZJ, Geng JG. SAP suppresses the development of experimental autoimmune encephalomyelitis in C57BL/6 mice. *Immunol Cell Biol.* (2011) 90:388–95. doi: 10.1038/icb.2011.51
115. Naik-Mathuria B, Pilling D, Crawford JR, Gay AN, Smith CW, Gomer RH, et al. Serum amyloid P inhibits dermal wound healing. *Wound Repair Regen.* (2008) 16:266–73. doi: 10.1111/j.1524-475X.2008.00366.x
116. Pilling D, Gomer RH. Persistent lung inflammation and fibrosis in serum amyloid P component (Apcs -/-) knockout mice. *PLoS ONE* (2014) 9:e93730. doi: 10.1371/journal.pone.0093730
117. Verstovsek S, Manshouri T, Pilling D, Bueso-Ramos CE, Newberry KJ, Prijic S, et al. Role of neoplastic monocyte-derived fibrocytes in primary myelofibrosis. *J Exp Med.* (2016) 213:1723–40. doi: 10.1084/jem.20160283
118. Pepys MB. Serum amyloid P component (not serum amyloid protein). *Nat Med.* (1999) 5:852–3. doi: 10.1038/11272
119. Pontet M, D'Asnieres M, Gache D, Escaig J, Engler R. A new pentraxin (serum amyloid P-component) in the rat: evidence for two quaternary structures and effect of ligands on self-association. *Biochim Biophys Acta* (1981) 671:202–10. doi: 10.1016/0005-2795(81)90135-5
120. Duffield JS, Lupher ML Jr, PRM-151 (recombinant human serum amyloid P/pentraxin 2) for the treatment of fibrosis. *Drug News Perspect.* (2010) 23:305–15. doi: 10.1358/dnp.2010.23.5.1444206
121. Raghu G, van den Blink B, Hamblin MJ, Brown AW, Golden JA, Ho LA, et al. Effect of recombinant human pentraxin 2 vs placebo on change in forced vital capacity in patients with idiopathic pulmonary fibrosis: a randomized clinical trial. *JAMA* (2018) 319:2299–307. doi: 10.1001/jama.2018.6129
122. Barbui T, Thiele J, Gisslinger H, Kvasnicka HM, Vannucchi AM, Guglielmelli P, et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. *Blood Cancer J.* (2018) 8:15. doi: 10.1038/s41408-018-0054-y
123. Verstovsek S, Mesa RA, Foltz LM, Gupta V, Mascarenhas JO, Ritchie EK, et al. PRM-151 in myelofibrosis: durable efficacy and safety at 72 Weeks. *Blood* (2015) 126:56.
124. Hawkins PN, Wootton R, Pepys MB. Metabolic studies of radioiodinated serum amyloid P component in normal subjects and patients with systemic amyloidosis. *J Clin Invest.* (1990) 86:1862–9. doi: 10.1172/JCI114917
125. Mortensen RF, Osmand AP, Lint TF, Gewurz H. Interaction of C-reactive protein with lymphocytes and monocytes: complement-dependent adherence and phagocytosis. *J Immunol.* (1976) 117:774–81.
126. Singh PP, Gervais F, Skamene E, Mortensen RF. Serum amyloid P-component-induced enhancement of macrophage listericidal activity. *InfectImmun* (1986) 52:688–94.
127. de Haas CJ, van Leeuwen EM, van Bommel T, Verhoef J, van Kessel KP, van Strijp JA. Serum amyloid P component bound to gram-negative bacteria prevents lipopolysaccharide-mediated classical pathway complement activation. *InfectImmun* (2000) 68:1753–9. doi: 10.1128/IAI.68.4.1753-1759.2000
128. Noursadeghi M, Bickerstaff MC, Gallimore JR, Herbert J, Cohen J, Pepys MB. Role of serum amyloid P component in bacterial infection: protection of the host or protection of the pathogen. *Proc Natl Acad Sci USA.* (2000) 97:14584–9. doi: 10.1073/pnas.97.26.14584
129. Diniz SN, Nomizo R, Cisalpino PS, Teixeira MM, Brown GD, Mantovani A, et al. PTX3 function as an opsonin for the dectin-1-dependent internalization of zymosan by macrophages. *J Leukocyte Biol.* (2004) 75:649–56. doi: 10.1189/jlb.0803371
130. Hawkins PN, Myers MJ, Lavender JP, Pepys MB. Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component. *Lancet* (1988) 1:1413–8. doi: 10.1016/S0140-6736(88)92235-0
131. Hawkins PN, Aprile C, Capri G, Vigano L, Munzone E, Gianni L, et al. Scintigraphic imaging and turnover studies with iodine-131 labelled serum amyloid P component in systemic amyloidosis. *Eur J Nucl Med.* (1998) 25:701–8. doi: 10.1007/s002590050272
132. Naylor MC, Lazar DA, Zamora IJ, Mushin OP, Yu L, Brissett AE, et al. Increased *in vitro* differentiation of fibrocytes from keloid patients is inhibited by serum amyloid P. *Wound Repair Regen.* (2012) 20:277–83. doi: 10.1111/j.1524-475X.2012.00782.x
133. Wang J, Jiao H, Stewart TL, Shankowsky HA, Scott PG, Tredget EE. Improvement in postburn hypertrophic scar after treatment with IFN-alpha2b is associated with decreased fibrocytes. *J Interferon Cytokine Res.* (2007) 27:921–30. doi: 10.1089/jir.2007.0008
134. Maharjan AS, Pilling D, Gomer RH. Toll-like receptor 2 agonists inhibit human fibrocyte differentiation. *Fibrogenesis Tissue Repair* (2010) 3:23. doi: 10.1186/1755-1536-3-23
135. Gan Y, Reilkoff R, Peng X, Russell T, Chen Q, Mathai SK, et al. Role of semaphorin 7a signaling in transforming growth factor β1–induced lung fibrosis and scleroderma-related interstitial lung disease. *Arthrit Rheumat.* (2011) 63:2484–94. doi: 10.1002/art.30386
136. Maharjan AS, Pilling D, Gomer RH. High and low molecular weight hyaluronic acid differentially regulate human fibrocyte differentiation. *PLoS ONE* (2011) 6:e26078. doi: 10.1371/journal.pone.0026078
137. Cox N, Pilling D, Gomer RH. NaCl potentiates human fibrocyte differentiation. *PLoS ONE* (2012) 7:e45674. doi: 10.1371/journal.pone.0045674
138. Pilling D, Zheng Z, Vakil V, Gomer RH. Fibroblasts secrete Slit2 to inhibit fibrocyte differentiation and fibrosis. *Proc Natl Acad Sci USA.* (2014) 111:18291–6. doi: 10.1073/pnas.1417426112
139. Herlihy SE, Starke HE, Lopez-Anton M, Cox N, Keyhanian K, Fraser DJ, et al. Peritoneal dialysis fluid and some of its components potentiate fibrocyte differentiation. *Perit Dial Int.* (2016) 36:367–73. doi: 10.3747/pdi.2014.00284
140. Karhadkar TR, Pilling D, Cox N, Gomer RH. Sialidase inhibitors attenuate pulmonary fibrosis in a mouse model. *Sci Rep.* (2017) 7:15069. doi: 10.1038/s41598-017-15198-8
141. Zhong JN, Lan L, Chen YF, Huang G, He GZ, Yang J, et al. IL-4 and serum amyloid P inversely regulate fibrocyte differentiation by targeting store-operated Ca2+ channels. *Pharmacol Rep.* (2018) 70:22–8. doi: 10.1016/j.pharep.2017.07.005
142. Pilling D, Vakil V, Cox N, Gomer RH. TNF-α-stimulated fibroblasts secrete lumican to promote fibrocyte differentiation. *Proc Natl Acad Sci USA.* (2015) 112:11929–34. doi: 10.1073/pnas.1507387112
143. Chen W, Pilling D, Gomer RH. Dietary NaCl affects bleomycin-induced lung fibrosis in mice. *Exp Lung Res.* (2017) 43:395–406. doi: 10.1080/01902148.2017.1385110
144. Wolters PJ, Collard HR, Jones KD. Pathogenesis of idiopathic pulmonary fibrosis. *Ann Rev Pathol.* (2014) 9:157–79. doi: 10.1146/annurev-pathol-012513-104706
145. Kim KK, Sheppard D, Chapman HA. TGF-beta1 Signaling and Tissue Fibrosis. *Cold Spring Harb Perspect Biol.* (2017) 10:a022293. doi: 10.1101/cshperspect.a022293
146. Lee CG, Cho SJ, Kang MJ, Chapoval SP, Lee PJ, Noble PW, et al. Early growth response gene 1-mediated apoptosis is essential for transforming growth factor beta1-induced pulmonary fibrosis. *J Exp Med.* (2004) 200:377–89. doi: 10.1084/jem.20040104
147. White MJV, Glenn M, Gomer RH. Trypsin potentiates human fibrocyte differentiation. *PLoS ONE* (2013) 8:e70795. doi: 10.1371/journal.pone.0070795
148. White MJ, Roife D, Gomer RH. Galectin-3 binding protein secreted by breast cancer cells inhibits monocyte-derived fibrocyte differentiation. *J Immunol.* (2015) 195:1858–67. doi: 10.4049/jimmunol.1500365
149. White MJ, Gomer RH. Trypsin, tryptase, and thrombin polarize macrophages towards a pro-fibrotic M2a phenotype. *PLoS ONE* (2015) 10:e0138748. doi: 10.1371/journal.pone.0138748
150. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol.* (2008) 8:34–47. doi: 10.1038/nri2206
151. Daeron M. Fc Receptor Biology. *Ann Rev Immunol.* (1997) 15:203–34. doi: 10.1146/annurev.immunol.15.1.203
152. Mortensen RF, Zhong W. Regulation of phagocytic leukocyte activities by C-reactive protein. *J Leukoc Biol.* (2000) 67:495–500. doi: 10.1002/jlb.67.4.495
153. Mold C, Gresham HD, Du Clos TW. Serum amyloid P component and C-reactive protein mediate phagocytosis through murine Fc gamma Rs. *J Immunol.* (2001) 166:1200–5. doi: 10.4049/jimmunol.166.2.1200

154. Bodman-Smith KB, Gregory RE, Harrison PT, Raynes JG. FcgammaRIIa expression with FcgammaRI results in C-reactive protein- and IgG-mediated phagocytosis. *J Leukocyte Biol.* (2004) 75:1029–35. doi: 10.1189/jlb.0703306
155. Moalli F, Doni A, Deban L, Zelante T, Zagarella S, Bottazzi B, et al. Role of complement and Fc{gamma} receptors in the protective activity of the long pentraxin PTX3 against Aspergillus fumigatus. *Blood* (2010) 116:5170–80. doi: 10.1182/blood-2009-12-258376
156. Cox N, Pilling D, Gomer RH. Distinct Fcγ receptors mediate the effect of serum amyloid p on neutrophil adhesion and fibrocyte differentiation. *J Immunol.* (2014) 193:1701–8. doi: 10.4049/jimmunol.1400281
157. Lu J, Marnell LL, Marjon KD, Mold C, Du Clos TW, Sun PD. Structural recognition and functional activation of FcgR by innate pentraxins. *Nature* (2008) 456:989–92. doi: 10.1038/nature07468
158. Lu J, Marjon KD, Mold C, Marnell L, Du Clos TW, Sun P. Pentraxins and IgA share a binding hot-spot on FcαRI. *Prot Sci.* (2014) 23:378–86. doi: 10.1002/pro.2419
159. Pilling D, Tucker NM, Gomer RH. Aggregated IgG inhibits the differentiation of human fibrocytes. *J Leukocyte Biol.* (2006) 79:1242–51. doi: 10.1189/jlb.0805456
160. Cox N, Pilling D, Gomer RH. DC-SIGN activation mediates the differential effects of SAP and CRP on the innate immune system and inhibits fibrosis in mice *Proc Natl Acad Sci USA*. (2015) 112:8385–90. doi: 10.1073/pnas.1500956112
161. Nourshargh S, Hordijk PL, Sixt M. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat Rev Mol Cell Biol.* (2010) 11:366–78. doi: 10.1038/nrm2889
162. Phillipson M, Kubes P. The neutrophil in vascular inflammation. *Nat Med.* (2011) 17:1381–90. doi: 10.1038/nm.2514
163. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. *Mol Med.* (2011) 17:293–307. doi: 10.2119/molmed.2010.00138
164. Galkina EV, Nazarov PG, Polevschikov AV, Berestovaya LK, Galkin VE, Bychkova NV. Interactions of C-reactive protein and serum amyloid P component with interleukin-8 and their role in regulation of neutrophil functions. *Russ J Immunol* .(2000) 5:363–74.
165. Stibenz D, Grafe M, Debus N, Hasbach M, Bahr I, Graf K, et al. Binding of human serum amyloid P component to L-selectin. *Eur J Immunol.* (2006) 36:446–56. doi: 10.1002/eji.200425360
166. Maharjan AS, Roife D, Brazill D, Gomer RH. Serum amyloid P inhibits granulocyte adhesion. *Fibrogenesis Tissue Repair* (2013) 6:2. doi: 10.1186/1755-1536-6-2
167. Wang Y, Guo Y, Wang X, Huang J, Shang J, Sun S. Human serum amyloid P functions as a negative regulator of the innate and adaptive immune responses to DNA vaccines. *The J Immunol.* (2011) 186:2860–70. doi: 10.4049/jimmunol.1003641
168. Sato T, Kameyama T, Noto T, Ueno H, Inoue H. Enhanced expression of hemoglobin scavenger receptor CD163 in accumulated macrophages within filtered debris between acute coronary syndromes and stable angina pectoris. *Int Heart J.* (2015) 56:150–6. doi: 10.1536/ihj.14-224
169. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* (2016) 44:450–62. doi: 10.1016/j.immuni.2016.02.015
170. Xiang W, Cox N, Gomer RH. Identification of compounds that decrease numbers of Mycobacteria in human macrophages in the presence of serum amyloid P. *J Leukoc Biol.* (2017) 102:857–69. doi: 10.1189/jlb.1A0317-118RR


**Conflict of Interest Statement:** DP and RG are inventors on patents for the use of SAP as a therapeutic for fibrosing diseases, and patents for the use of SAP-depleting materials to enhance wound healing. DP and RG are members of the Science Advisory Board of, and have stock options from, Promedior, a start-up company that is developing SAP as a therapeutic for fibrosing diseases, and receive a share of milestone payments made by Promedior to Rice University.

The reviewer AM and handling Editor declared their shared affiliation.

frontiers in Immunology

HYPOTHESIS AND THEORY
published: 16 October 2018
doi: 10.3389/fimmu.2018.02382

# The Pentraxins 1975–2018: Serendipity, Diagnostics and Drugs

*Mark. B. Pepys* [1,2]*

[1] Wolfson Drug Discovery Unit, Centre for Amyloidosis and Acute Phase Proteins, University College London, London, United Kingdom, [2] National Institute for Health Research University College London Hospitals Biomedical Research Centre, London, United Kingdom

The phylogenetically ancient, pentraxin family of plasma proteins, comprises C-reactive protein (CRP) and serum amyloid P component (SAP) in humans and the homologous proteins in other species. They are composed of five, identical, non-covalently associated protomers arranged with cyclic pentameric symmetry in a disc-like configuration. Each protomer has a calcium dependent site that mediates the particular specific ligand binding responsible for all the rigorously established functional properties of these proteins. No genetic deficiency of either human CRP or SAP has been reported, nor even any sequence polymorphism in the proteins themselves. Although their actual functions in humans are therefore unknown, gene deletion studies in mice demonstrate that both proteins can contribute to innate immunity. CRP is the classical human acute phase protein, routinely measured in clinical practice worldwide to monitor disease activity. Human SAP, which is not an acute phase protein, is a universal constituent of all human amyloid deposits as a result of its avid specific binding to amyloid fibrils of all types. SAP thereby contributes to amyloid formation and persistence *in vivo*. Whole body radiolabelled SAP scintigraphy safely and non-invasively localizes and quantifies systemic amyloid deposits, and has transformed understanding of the natural history of amyloidosis and its response to treatment. Human SAP is also a therapeutic target, both in amyloidosis and Alzheimer's disease. Our drug, miridesap, depletes SAP from the blood and the brain and is currently being tested in the DESPIAD clinical trial in Alzheimer's disease. Meanwhile, the obligate therapeutic partnership of miridesap, to deplete circulating SAP, and dezamizumab, a humanized monoclonal anti-SAP antibody that targets residual SAP in amyloid deposits, produces unprecedented removal of amyloid from the tissues and improves organ function. Human CRP binds to dead and damaged cells *in vivo* and activates complement and this can exacerbate pre-existing tissue damage. The adverse effects of CRP are completely abrogated by compounds that block its binding to autologous ligands and we are developing CRP inhibitor drugs. The present personal and critical perspective on the pentraxins reports, for the first time, the key role of serendipity in our work since 1975. (345 words)

**Keywords: pentraxin, C-reactive protein, serum amyloid P component, amyloidosis, drugs, miridesap, dezamizumab, complement**

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Humanitas Clinical and Research Center, Italy*

***Reviewed by:***
*Seppo Meri, University of Helsinki, Finland*
*Alberto Mantovani, Humanitas Research Hospital, Italy*

***Correspondence:**
*Mark. B. Pepys*
*m.pepys@ucl.ac.uk*

***Specialty section:***
*This article was submitted to Inflammation, a section of the journal Frontiers in Immunology*

***Received:*** *02 September 2018*
***Accepted:*** *25 September 2018*
***Published:*** *16 October 2018*

***Citation:***
*Pepys MB (2018) The Pentraxins 1975–2018: Serendipity, Diagnostics and Drugs. Front. Immunol. 9:2382. doi: 10.3389/fimmu.2018.02382*

## DISCOVERY OF THE PENTRAXINS

When I returned to clinical training at the Royal Postgraduate Medical School in London in 1973, after my PhD discovery of the role of complement in induction of antibody formation (1–5), the Head of Medicine, Professor (later Sir) Christopher Booth, advised to me to start a more clinical research project. He suggested that I should "crack Crohn's disease." This led me serendipitously[1] to the pentraxins.

In the early 1970s, reduced numbers of circulating T cells had been reported in many chronic inflammatory diseases of unknown etiology, including Crohn's disease. Although this was actually an artifact caused by differential loss of T cells during isolation of peripheral blood lymphocytes (6), T cell function in Crohn's disease was still of interest in 1975 when Henry Gewurz reported that C-reactive protein (CRP) bound to antigen activated T cells and suppressed their functions (7). I assumed that CRP production would be increased in active Crohn's disease and speculated that it could be responsible for suppression of T cell function. However, to my surprise, in 1975, CRP measurements had not been reported in either Crohn's disease or ulcerative colitis and I set out to do this for the first time.

There were no commercial quantitative immunoassays for CRP at that time. I therefore isolated some human CRP and immunized a rabbit to raise my own anti-CRP antiserum. This "famous" rabbit, known only as R1032, produced strong precipitating antibodies to CRP, which were excellent for electroimmunoassay. But it also produced precipitating antibodies against another, immunochemically distinct, normal trace plasma protein with fast α-mobility, which was not an acute phase reactant. None of the available antisera to known human plasma proteins reacted with this unknown protein; which I designated "protein X." I had neither the resources nor the motivation to attempt amino acid sequencing and, since many plasma proteins had not been sequenced, it might not have helped. Indeed, as it transpired, if we had sequenced it then it would have been the first time for that protein! Meanwhile I used the antiserum to assay CRP concentrations in clinical samples using electroimmunoassay (8), and made important new observations (see section Routine clinical measurement of CRP), whilst ignoring the immunoprecipitates produced by the contaminating antibodies to protein X.

My CRP antigen preparation had obviously been contaminated with protein X and I therefore sought to improve the isolation procedure. CRP was named for its calcium dependent binding to pneumococcal C-polysaccharide so calcium dependent affinity chromatography was an obvious and attractive possibility (9). CRP from whole serum bound efficiently, in the presence of calcium, to suitable ligands that had been covalently immobilized on Sepharose, commercial beaded agarose, and other serum proteins were then washed away. The CRP could then be eluted by calcium chelation but, regardless of the immobilized ligand, protein X was still present. The obvious control experiment showed that, unlike CRP, protein X underwent avid calcium dependent binding to plain unsubstituted Sepharose, and was eluted by calcium chelation. This simple one step isolation in pure form of a trace plasma protein was unique and demanded identification of protein X. In collaboration with Arnold Feinstein and Ed Munn, who had first reported negative staining electron microscopy (EM) of IgM, EM of isolated protein X instantly identified it as amyloid P component (AP) (10–14) (**Figure 1**). Unexpectedly, isolated CRP had a remarkably similar appearance (**Figure 1**). Both these homopentameric, calcium dependent, ligand binding, plasma proteins were composed of globular subunits arranged with cyclic symmetry in a disc like configuration.

At the same time, two other groups were working on these two proteins. Robert Painter isolated the C1 component of complement from whole serum by calcium dependent affinity chromatography on IgG covalently immobilized on Sepharose (16). In addition to the known subcomponents, C1q, C1r, and C1s, he always found a fourth protein that he designated C1t (17) and which he soon found to resemble AP (18) in the EM. Meanwhile Alex Osmand and Henry Gewurz observed marked *N*-terminal sequence homology between CRP and C1t (AP) and, together with Painter noted their highly characteristic, similar EM appearances (19). Osmand coined the name "pentraxin" for this newly recognized protein family, derived from the Greek words "penta" (five) and "ragos" (berries), representing the EM appearance of the molecules[2]. We confirmed immunochemically that our protein X was serum amyloid P component (SAP) (21), and my discovery of its calcium dependent binding to unsubstituted Sepharose explained its presence in Painter's C1 preparations, showing that it had nothing to do with C1.

Work on the pentraxins, CRP and SAP, then proceeded energetically in various directions, albeit with some false starts. The claims for binding and effects of CRP on lymphocytes, that had serendipitously introduced me to the field, proved not to be reproducible. Indeed there have been, and still are, a number of highly controversial claims about properties, functions and effects of CRP and SAP. However, the early discovery of classical pathway complement activation by CRP following its binding to macromolecular ligands (22, 23) withstood the test of time and it is unequivocally crucial for the role of CRP in exacerbation of tissue damage (24).

## WHAT ARE PENTRAXINS?

> "What's in a name? That which we call a rose
> By any other name would smell as sweet."
> *William Shakespeare. Romeo and Juliet (II, ii, 1-2)*

The question is both scientific and semantic. The neologism, pentraxin, was invented by Alex Osmand (19) from the Greek words meaning five berries, to describe the unique cyclic pentameric symmetrical appearance of the molecules of human

[1]Serendipity: "making discoveries, by accident and sagacity, of things not sought" *Horace Walpole, 28 Jan 1745.*

[2]Pentraxin is a splendid name but I confess to having misleadingly tried to change it. In 1983, based on advice by Greek colleague who wrongly thought that the etymology was from "*penta*" and "*axin,*" I suggested, incorrectly, that the name should be "pentaxin" (20). Alex Osmand corrected me - *mea culpa*.

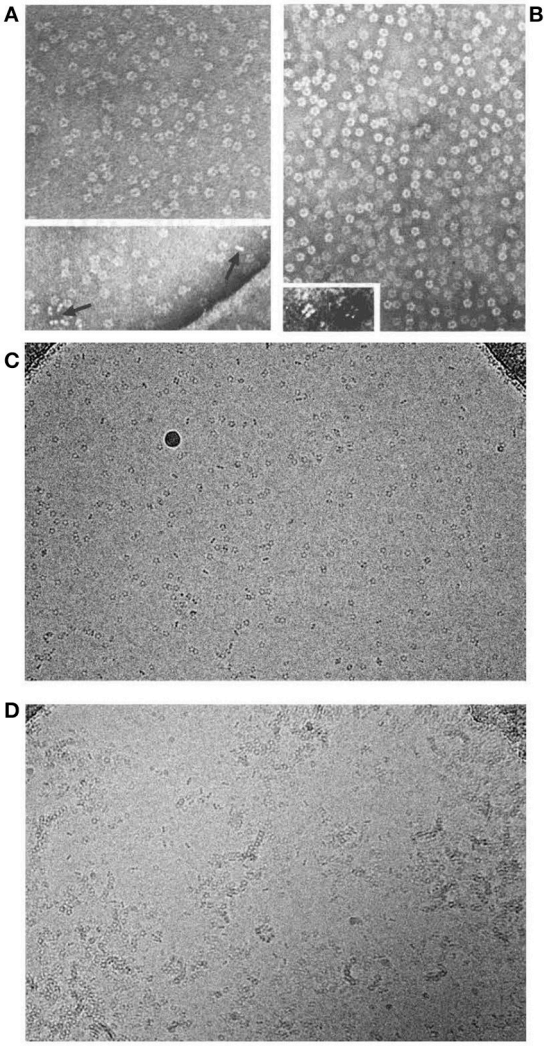


**FIGURE 1 |** Molecular appearance of the pentraxins. **(A)** Negatively stained electron microscopic image of human CRP with the characteristic symmetrical pentameric ring viewed face on. Inset shows disc-like appearance of single molecules side on. **(B)** Negatively stained electron microscopic image of human SAP with the characteristic symmetrical pentameric ring viewed face on. Inset shows typical face to face double pentamer forming the decameric assembly present in calcium free conditions. This was thought to be the normal assembly of human SAP until the actual physiological native pentameric structure was demonstrated (15). **(C)** Cryoelectron microscope image of our preparations of human CRP (mass 115,135) and of **(D)** human SAP (mass 127,310) showing the actual native pentraxin structure with no staining or artefactual enhancement (courtesy of Dr Richard Henderson).

CRP and SAP. The appearance is shared by the pentraxins from all the different species that have been visualized, apart from the hexameric CRP homolog of the horseshoe crab, *Limulus polyphemus* (25) and other multimeric invertebrate homologs. In addition to the numbers of subunits there are other differences between species. For example, rat CRP differs from the human CRP in being glycosylated and in having a covalent disulphide bond between one pair of protomers in each pentameric molecule (26). Nevertheless the very high degree of sequence homology, together with the instantly recognizable pentraxin molecular appearance, demonstrate that all the different plasma proteins characterized by calcium dependent binding to the classical pentraxin ligands are unequivocally members of the same family. The "long pentraxins" (27) do not have the pentraxin appearance although they contain a domain with modest sequence homology to pentraxins. Also calcium binding, which is required for stability of the secondary, tertiary and quaternary structures of most actual pentraxins, and is essential for the specific ligand binding that underlies all robustly reproducible pentraxin functions, is not a feature of the "long pentraxins." An analogous situation exists in relation to the many diverse non-immunoglobulin proteins which contain immunoglobulin sequence homology domains but do not share antibody-like specific epitope binding. They are, accordingly, not called antibodies but the well-established "long pentraxin" names are evidently not going to change.

## PENTRAXIN STRUCTURE

In 1994, we reported the first pentraxin structure: the 3D X-ray crystal structure of human SAP alone and of its calcium dependent complex with the cyclic pyruvate acetal of galactose (28) (**Figure 2**). SAP crystallized easily but it followed nearly 17 years of failure to grow reproducible crystals of human CRP suitable for X-ray crystallography. Eventually I thought of lowering the calcium concentration to reduce the solubility of human CRP as it starts to denature. This yielded a batch of poor and fragile crystals that nonetheless provided a low resolution structure of partly calcified CRP (30). Then my serendipitous, inadvertent, "overconcentration" of a batch of isolated human CRP to more than 20 mg/ml in the presence of physiological calcium, caused sudden, concentration dependent, reversible precipitation of the protein that pointed the way to effective crystallization conditions. Finally, the full physiological structure of human CRP alone and with bound phosphocholine was solved (31) (**Figure 3**).

The tertiary fold of the two human pentraxins is closely similar, with the main chain forming a flattened β-jellyroll with closely tethered loops between the antiparallel strands. There is a short α-helix on one face, the "A" face (29), of each protomer and calcium tethered loops on the opposite, binding, "B," face, forming the shallow ligand binding pocket. Although there is only about 11% amino acid sequence homology with the human pentraxins, the proteins with the most similar β-jellyroll tertiary fold are the legume lectins, pea lectin and concanavalin A (28). This architecture is apparently an effective support for proteins that provide calcium dependent binding of carbohydrate and other non-protein ligands.

The extensively hydrogen bonded antiparallel β-strands and tightly bound loops make the pentraxins rather resistant to proteolysis but, in the absence of calcium, the calcium coordinating loops are disorganized and readily cleaved (33, 34). Calcium is obviously always present at ∼2 mM in the extracellular environment *in vivo* but the normally very

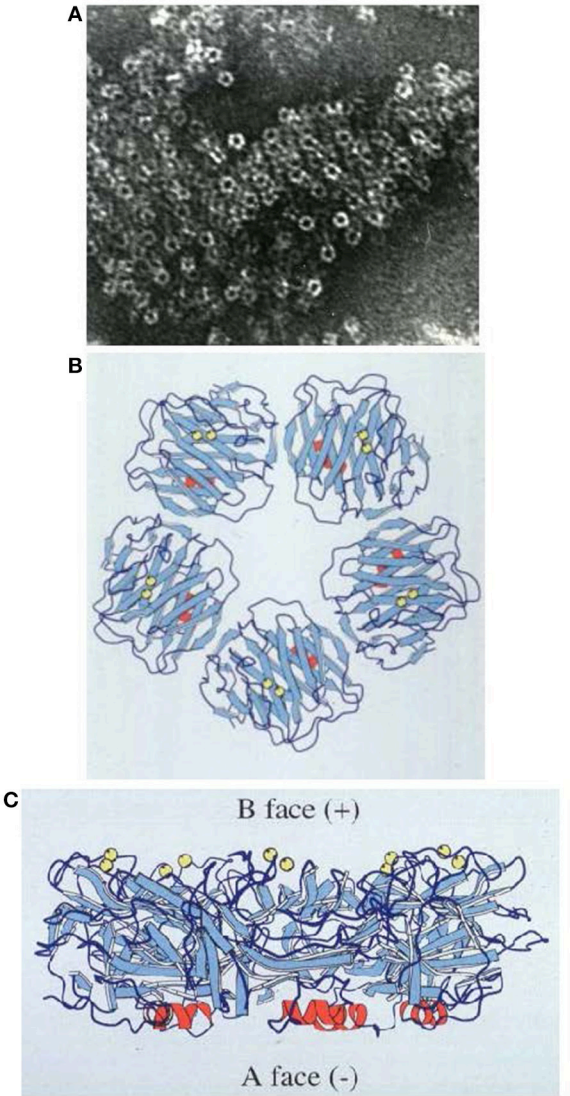


**FIGURE 2 |** Structure of human SAP. **(A)** Electron micrograph of negatively stained human SAP molecules. **(B)** Ribbon diagram of the 3D X-ray crystal structure of human SAP face on ("B" face uppermost). **(C)** Ribbon diagram of the 3D X-ray crystal structure of human SAP side on. Calcium atoms are represented as yellow spheres located on the binding, "B" face; the single small α-helix of each protomer is shown in red, located on the "A" face (29); β-sheets are in pale blue and loops in dark blue.

stable, albeit non-covalent, native pentameric assembly of human CRP is notably destabilized in the absence of calcium. Free protomers are released and the protein readily aggregates. Use of non-physiological experimental conditions, leading to artefactual properties of human CRP, has produced misleading conclusions about properties and effects of the protein. Even worse, effects caused by sodium azide preservative in CRP preparations (35) and contamination of recombinant CRP by bacterial products (36) have been misleadingly attributed to CRP itself.

Human SAP is also more susceptible to proteolytic cleavage in the calcium coordinating loops when calcium is absent (34) but, unlike human CRP, under these non-physiological conditions human SAP forms stable decameric assemblies of pairs of pentameric SAP molecules interacting "B" face to "B" face (37). The interaction is mediated by displacement of the loop comprising residues 134–151 in each protomer and then binding of the loop in the inter-subunit groove in the "B" face of the apposed pentameric ring (37). For a number of years we believed that this double pentamer was the native state of the SAP molecule [see for example (28)], in contrast to the single pentamer of human CRP. However, careful characterization of the molecular form of native SAP within the milieu of whole serum showed that human SAP is actually a single pentamer that is not complexed with any other plasma constituent (15). These studies are challenging because, as we had discovered very early on, exposure of isolated pure human SAP to calcium leads to rapid autoaggregation (38). Aggregated human SAP acquires novel ligand binding and other properties (39), unfamiliarity with which produced a number of misleading reports on possible functions of SAP. We eventually showed that human SAP autoaggregation is mediated by binding of the exposed γ-carboxylate of residue Glu167 on one SAP molecule in the calcium dependent ligand pocket on another (40). This is prevented by the presence of physiological concentrations of serum albumin (15), probably, at least in part, by virtue of calcium binding by the albumin, critically lowering the free ionisable calcium concentration. In any case, in the presence of the calcium, that is required for its ligand binding, isolated human SAP must be stabilized by a sufficient concentration of serum albumin.

## FUNCTIONAL ROLES OF THE PENTRAXINS *in vivo*?

Identification of the roles of human CRP and SAP is complicated by the failure so far to detect any genetic deficiency of either protein: the ultimately informative "experiment of Nature" has not been seen. There are also no common structural variants. Although some extremely rare coding polymorphisms of the *CRP* and *SAP* genes have been noted in genomic studies, the variant proteins that they might encode have not yet been reported. This remarkable conservation suggests that both proteins may have important functions, necessary for survival, presumably in relation to host defense, since this is a major driver of natural selection. However, given the ancient phylogeny of the pentraxins, long antedating acquired immunity, some of these primitive putative "survival" functions are now likely to be redundant.

Our early original studies of pentraxins in other species (25, 26, 41–55) showed that the pentraxin family is phylogenetically ancient with highly conserved sequence homology, secondary, tertiary and quaternary structure as well as calcium dependent ligand binding. Nonetheless, there are major differences between family members in different, even closely related, species. For example, rat SAP (26) has a similar abundance to human

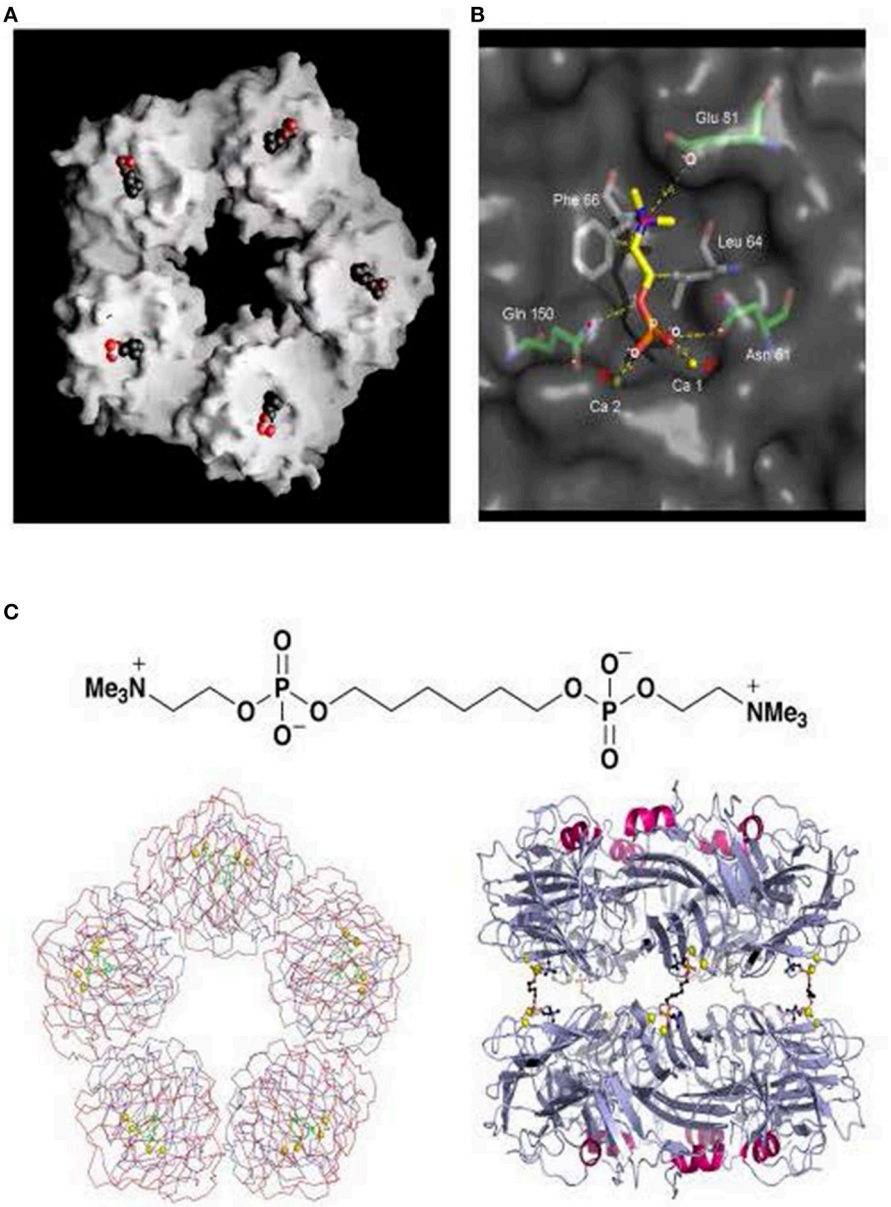


**FIGURE 3 |** Structure of human CRP with bound phosphocholine and bis(phosphocholine)-hexane. **(A)** Space filling model of "B" face of human CRP with phosphocholine bound in each of the protomer binding sites. **(B)** 3D X-ray crystal structure of phosphocholine in the binding pocket of a single CRP protomer within the native molecule, showing the ligand interactions with calcium and the CRP residues responsible for binding. **(C)** The structure of bis(phosphocholine)-hexane (above) and the structure of the complex formed by two CRP molecules cross linked by five bis(phosphocholine)-hexane molecules; face on (left) and side on (right) [From reference (32) with permission of Macmillan Publishers Ltd].

SAP (56) (mean (SD, range) concentration, women: 21 mg/l (8. 8-5-5); men: 32 mg/l (7, 12–19, 21–31, 33–52), and neither is an acute phase protein (57). In contrast, mouse SAP baseline concentrations are strain dependent with a ∼50-fold range between C57BL/6 (∼3–5 mg/l) and DBA (>150 mg/l), and it is a major acute phase reactant rising to >300 mg/l (42). On the other hand mice have low baseline CRP concentrations, ∼5–9 mg/l, which rise only twofold in the acute phase response (58). Meanwhile rats have baseline CRP concentrations of ∼300–500 mg/l rising 3- to 4-fold in the acute phase response (26). In humans, the median baseline CRP concentration is 0.8 mg/l, with 90% of healthy subjects below 3 mg/l and 99% below 10 mg/l (59). But the concentration can be as low as 50 μg/l (59) and can rise to >500 mg/l at the peak of the acute phase response (60). There are many other variations between species, including behavior as acute phase reactants, precise ligand specificity and the secondary effects of ligand binding: precipitation, agglutination and complement activation. In some

species, the hallmark properties of human CRP and SAP are variably distributed between the two pentraxins while neither the dog nor the rabbit even have an SAP gene, although their respective CRP molecules behave rather similarly to human CRP. These findings suggest that the various pentraxins may have different functions in different species and they make it impossible to extrapolate reliably from experimental animal studies to possible functional roles of the pentraxins in humans.

## THE CHALLENGE OF IDENTIFYING PHYSIOLOGICAL FUNCTIONS OF THE PENTRAXINS

There have been wide ranging claims, speculations and many evidence-free assertions about pentraxin functions. There are very few robustly definitive observations or experiments. A major weakness in most studies of putative pentraxin functions has been lack of information about the provenance, purity and functional integrity of the CRP and SAP preparations that have been used. Isolation of structurally and functionally intact preparations of these trace plasma proteins, and rigorous demonstration of their quality, are challenging. It is not adequate to use a commercial product or in house preparation without comprehensive characterization. For example, among the many claimed activities is the assertion that the human pentraxins trigger production and secretion of pro-inflammatory cytokines. We have never been able to replicate these reports (36). In order to make definitive observations, we isolated sterile, endotoxin-free, structurally and functionally intact, clinical Good Manufacturing Practice (cGMP) grade human CRP and SAP from pooled normal human plasma of healthy, pathogen free US donors (61). We showed that neither protein had inherent pro-inflammatory effects, either on human peripheral blood mononuclear cells *in vitro* or when administered parenterally to mice or healthy human volunteers *in vivo* (61, 62).

## FUNCTIONS OF HUMAN C-REACTIVE PROTEIN

Human CRP binds avidly to exposed phosphocholine residues on macromolecules of both autologous and extrinsic origin (22, 63). It then aggregates particulate ligands and precipitates soluble ligands and also triggers classical complement pathway activation (22, 64). Beneficial effects of some of these phenomena may thus underlie the evolutionary persistence of the protein and the highly adaptive regulation of its production in response to injury, infection and inflammation. CRP binds selectively to dead and damaged cells but not to healthy living cells. Phospholipase action on plasma membranes of damaged cells disrupts the normal lipid bilayer, exposing the phosphocholine head groups recognized by CRP. Co-localization of CRP with fixed complement in areas of tissue damage suggests a possible role for CRP in removal of cellular debris from the tissues. However, there is no direct evidence that this function actually operates.

Injection of human CRP into mice at the time of inoculation with virulent pneumococci confers efficient protection against sepsis (65–67). Administration of human CRP after inoculation of the bacteria does not protect. Indeed, all patients with active pneumococcal infections have greatly increased plasma CRP concentrations and abundant circulating human CRP so CRP evidently does not control established pneumococcal sepsis.

In order to study this question further we created pure-line *Crp* gene-deleted C57BL/6 mice using C57BL/6 embryonic stem cells (58). Normally housed CRP deficient mice had normal growth, development, fertility and life span. They did not develop anti-nuclear autoimmunity and responded normally to endotoxin challenge, two processes in which roles for CRP had been proposed (68). However, the CRP-deficient mice were remarkably susceptible to *Streptococcus pneumoniae* infection and were protected by reconstitution with isolated pure human CRP, or by anti-pneumococcal antibodies (58). Autologous mouse CRP is evidently essential for innate resistance to pneumococcal infection before antibodies are produced, probably by clumping the bacteria, limiting their spread and promoting their phagocytosis and destruction by neutrophils. Our findings are consistent with the significant association between clinical pneumococcal infection and non-coding human *CRP* gene polymorphisms which reduce CRP expression (69–71). Deficiency or loss of function variation in CRP may therefore be lethal at the first early-life encounter with this ubiquitous virulent pathogen, explaining the invariant presence and structure of CRP in human adults. Meanwhile, the protective function of mouse CRP against pneumococcal infection is the only function of any CRP to be firmly established so far in the same species.

## FUNCTIONS OF HUMAN SERUM AMYLOID P COMPONENT

Continuous treatment for up to several years with the drug, CPHPC (72) (now called miridesap, see below), that persistently depletes circulating SAP by over 90% for as long as the drug is taken, has had no adverse effects (73). Thus, despite its invariant presence, human SAP probably does not have a necessary function in adults.

Our discovery of the avid specific binding of human SAP to DNA (74) and to chromatin (75), where it displaces H1-type histones, thereby solubilizing native long chromatin under physiological conditions, strongly suggested a possible function of human SAP in the *in vivo* handling of exposed DNA and chromatin. Indeed, in *ex vivo* human tissues, both apoptotic cells, which always bear chromatin fragments on their surface, and nuclear debris are always coated with SAP (76, 77). However, our early observation of increased spontaneous anti-nuclear autoimmunity in SAP knockout mice (78) turned out to be limited to the autoimmunity susceptible C57BL/6 strain and not a general effect of SAP deficiency (79). There was no increased autoimmunity, even after autoantigen challenge, with SAP knockout in different mouse strain backgrounds (79). Furthermore, there has been no increased autoantibody production in patients with SAP depletion produced by miridesap (73).

An intriguing possibility is that the avid binding of human SAP to DNA may be the mechanism responsible for the failure of DNA vaccination to be immunogenic in humans. We discovered that there is complete concordance among species tested so far (sub-human primates, dog, rabbit, horse, cow, sheep, pig, goat) between the effectiveness of DNA vaccination and the absence of SAP binding strongly to DNA (unpublished observations). In particular, mice respond well to DNA vaccination and mouse SAP binds DNA very weakly (79). Also transgenic expression of human SAP in mice blocks immune responses to DNA vaccination (80) and this inhibition is completely abrogated by administration of my SAP-depleting drug, CPHPC (miridesap) (72, 81). We therefore lately conducted a preliminary clinical trial, HIV-CORE 003, of SAP depletion by CPHPC (miridesap) in healthy volunteers receiving a DNA vaccine against HIV (82). The results were largely negative although, compared to placebo treated controls, the SAP depleted subjects mounted significantly broader immune responses (82). Further studies of this important question are needed.

SAP is inherently resistant to proteolysis (34) and is also a potent anti-opsonin (83). Its binding therefore "protects" its macromolecular ligands from degradation, whether these are the amyloid fibrils in local or systemic amyloid deposits, or pathogenic bacteria. Indeed those bacterial pathogens to which SAP binds (84), use the bound SAP to shield themselves from the host's phagocytic defenses (83). Thus, for example, SAP knockout mice are more resistant than wild type mice to lethal infection with *Strep. pyogenes* and rough Gram negative bacteria (83). In contrast, SAP deficient mice are more susceptible than wild type controls to lethal infection with smooth Gram negative bacteria, to which SAP does not bind (83). Mouse SAP therefore contributes to innate immunity to some bacterial infections and, although the mechanism is unknown, this is so far the only definite *in vivo* function identified for an autologous SAP.

A host defense role for SAP is potentially consistent with the fact that human SAP binds avidly to Shiga toxin 2 and neutralizes it *in vitro* (85, 86), which led to our demonstration that human SAP protects against cytotoxicity of *E. coli* Shiga toxin 2 for podocytes *in vitro* (87) and against lethality in mice *in vivo* (88). However, we did not find any association between human SAP concentrations and haemolytic uraemic syndrome or antibody titres against toxigenic *E. coli* lipopolysaccharide (88). Although SAP binds many lipopolysaccharides, there is no reproducible evidence that either SAP (83) or CRP (68) protect against their *in vivo* toxicity in mice.

Interestingly, binding of human SAP to the lipopolysaccharide of rough Gram negative bacteria blocks classical complement pathway activation by the endotoxin (89). We had previously discovered (39) that pairs of aggregated SAP molecules, but not single soluble SAP molecules, calcium dependently bind C4-binding protein, a negative regulator protein of the classical cascade. On the other hand, supraphysiological concentrations of human SAP, which undergo calcium dependent autoaggregation, do activate complement. However, the abundant coating of amyloid fibrils with SAP clearly does not activate complement and the *in vitro* observation is therefore probably not relevant *in vivo*.

How the anti-opsonin and "ligand protective" properties of SAP contribute to beneficial functions of the protein remains a matter for speculation. However, in addition to being a circulating plasma protein, human SAP is also a normal constituent of the extracellular matrix; and aggregated human SAP has a highly specific binding interaction with fibronectin (39), another universal matrix glycoprotein. Human SAP is an integral component of the glomerular basement membrane (90) and of the microfibrillar mantle present on elastic fibers throughout the body (91). It is therefore conceivable that the SAP helps to protect the integrity of the structures with which it is associated. Experimental investigation of this concept is challenging. Mouse SAP is not detected in the extracellular matrix of normal mouse tissues and SAP evidently does not have a specific obligatory function since neither dogs nor rabbits have an SAP gene, while horses, which do have an SAP gene, do not express a protein with the same calcium dependent ligand binding specificity as SAP of other species (unpublished observations). Our SAP deficient, gene deleted mice have no phenotype when unchallenged (92), supporting the view that, despite the evolutionary conservation of SAP, its functions may well be redundant in normal health.

## SAP AND AMYLOIDOSIS

My discovery of calcium dependent ligand binding by SAP to agarose (9, 21) enabled our demonstration that the analogous binding of SAP to amyloid fibrils is responsible for the universal presence of SAP in all amyloid deposits of all types in humans (93). We formally demonstrated that the circulating SAP is the precursor of amyloid P component (AP) in amyloid deposits (94). This led directly to my use of radiolabelled SAP as an amyloid specific tracer *in vivo* (95, 96) and the invention of SAP scintigraphy and metabolic studies (97–99). The ability to image amyloid throughout the whole body in patients with systemic amyloidosis and thus, safely and non-invasively, localize and quantify amyloid deposits, has made major contributions to understanding the natural history of amyloidosis and its response to therapy (100) (**Figure 4**). Once the scan became available, the Immunological Medicine Unit at the Royal Postgraduate Medical School soon became the *de facto* national referral center for amyloidosis patients in the UK. In 1999, when I moved with my team to the Royal Free Campus of University College Hospital, the UK Department of Health funded us as the NHS National Amyloidosis Centre to provide diagnostic and management advice for the whole national caseload (www.ucl.ac.uk/amyloidosis/ and www.amyloidosis.org.uk). The Centre now sees over 4,000 amyloidosis patients per year, follows the world's largest and most diverse cohort of such patients and has conducted about 40,000 SAP scintigraphy studies since 1988 with no adverse effects.

The observation of calcium dependent ligand binding by SAP (9, 21) also led toward potential new treatments. My serendipitous finding in 1983 that widely differing amounts of SAP bound to different batches of Sepharose led to the discovery that SAP binding correlated precisely with the

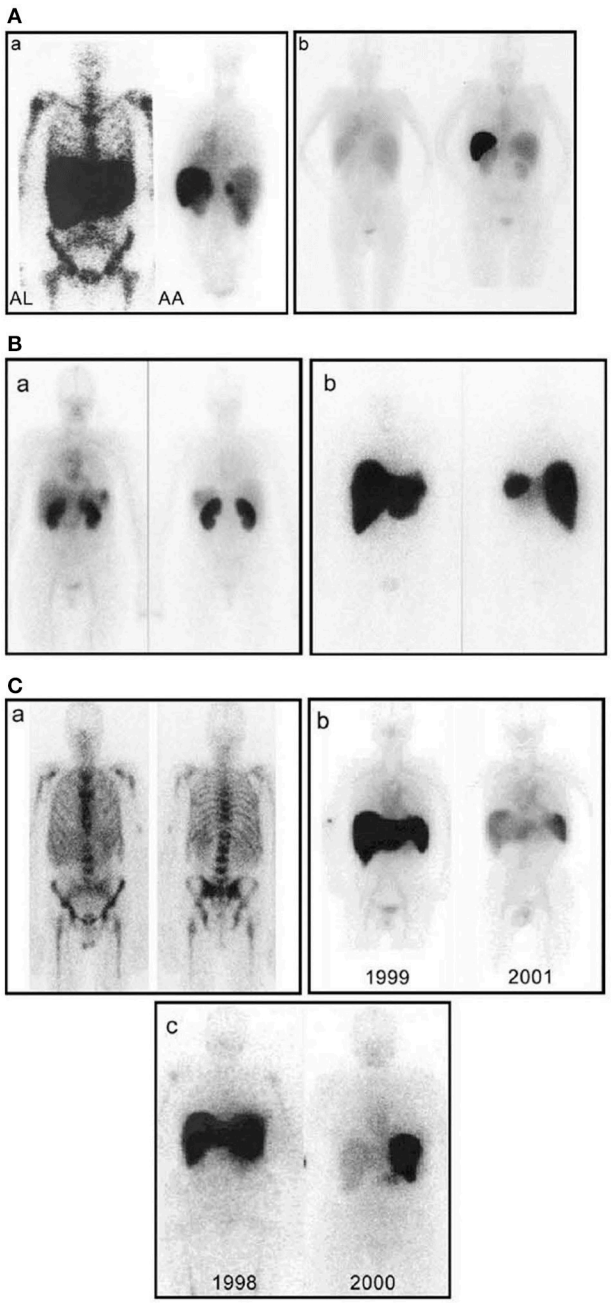


**FIGURE 4 |** Whole-body scintigraphy with $^{123}$I-labeled serum amyloid P component in systemic amyloidosis. **(A) (a)** Left: Anterior view of a typical patient with AL amyloidosis showing massive liver and spleen amyloid and the pathognomonic deposits throughout the bone marrow that are not seen in any other type of amyloidosis. Right: Posterior view of a typical patient with AA amyloidosis showing amyloid in the spleen, kidneys, and adrenal. The left adrenal is obscured by the overlying spleen, but the right is clearly visible above the kidney. **(b)** Posterior scans taken a year apart in a patient with longstanding rheumatoid arthritis who suddenly developed AA amyloidosis. The earlier scan (left) is normal; the later one (right) shows heavy splenic and significant renal amyloidosis. **(B) (a)** Anterior (left) and posterior (right) views of a patient with AL amyloid who presented with minor proteinuria and no other clinical or investigational evidence of disease. There is substantial renal

*(Continued)*

**FIGURE 4 |** amyloid but no scintigraphically detectable de- posits elsewhere. **(b)** Anterior (left) and posterior (right) views of a different patient with AL amyloid who also presented with minor proteinuria and no other clinical or investigational evidence of disease. There is massive amyloid deposition in the liver and spleen. The kidneys are not visualized, probably because the tracer, which distributes according to the amount of amyloid, is all taken up elsewhere. Note that, in contrast to **(a)**, there is no residual tracer in the circulation, indicating a heavy whole-body amyloid load. This patient did not tolerate intensive chemotherapy and developed liver failure. **(C) (a)** Anterior (left) and posterior (right) views of a patient with AL amyloid who presented with multiple fractures over 4 years. X-ray and bone scan were normal but bone biopsy unexpectedly revealed amyloid. No monoclonal gammopathy was identifiable at that time, but bone amyloid is frequent in AL and may be the main clinical feature. **(b)** Serial anterior views showing regression of AA amyloidosis in a juvenile rheumatoid arthritis patient treated with chlorambucil, in whom the SAA concentration was suppressed to <10 mg/l. **(c)** Serial anterior views showing regression of AL amyloidosis in a patient treated with high-dose melphalan and stem cell rescue. [From Pepys (100) with permission of Annual Reviews].

pyruvate content of the agarose. Pyruvate is a variable trace component present as the cyclic acetal of β-D-galactopyranose in agarobiose (101). We synthesized the monosaccharide, methyl 4,6-*O*- (1-carboxyethylidene)-β-D-galactopyranoside (MOβDG) and showed that it completely blocked and reversed the binding of SAP to all its known ligands, crucially including pure protein ligands and amyloid fibrils with no carbohydrate present (101). These seminal results enabled localisation of the calcium dependent ligand binding site in SAP when we solved its 3D X-ray crystal structure (28), and also led to a new therapeutic approach. Although we did not then know the role, if any, of SAP in pathogenesis of amyloidosis, the finding that MOβDG could remove all the SAP bound in amyloid deposits suggested an approach to disrupting the deposits and promoting their clearance (102).

We subsequently showed that AP in amyloid is identical to its SAP precursor in the plasma and remains completely intact despite very prolonged residence in the tissue deposits (103). The plasma half-life of SAP in normal healthy subjects is ∼24 h whilst the half-life of SAP in visceral amyloid deposits is ∼30 days (99). Furthermore, binding of SAP to amyloid fibrils *in vitro* mutually protects the fibrils and the SAP from degradation by proteases and phagocytic cells (104). SAP, although itself rather resistant to proteolysis, is not a protease inhibitor. It protects the fibrils only when it is actually bound to them (104).

Amyloid fibrils are readily digested by proteases and ingested and degraded by phagocytic cells *in vitro*. In contrast, *in vivo*, systemic amyloid deposits are almost entirely ignored by the normally highly efficient cellular and molecular mechanisms for clearance of extracellular debris from the tissues. The reasons for this are unknown but, in view of our discovery that bound SAP protects amyloid fibrils from degradation *in vitro*, I proposed that it might do the same thing *in vivo*. I hypothesized that the universal, ubiquitous coating of SAP on amyloid fibrils *in vivo* protects them from clearance and removal (104). I claimed that stripping of bound SAP, and prevention of SAP binding, would enable amyloid deposits to be recognized as abnormal and therefore phagocytosed and degraded, leading to amyloid

removal (105). We then went on to create the first SAP knockout mice and to show that, although it was possible to induce systemic AA amyloidosis in them, it took longer than in wild type mice and the deposits were smaller (92). SAP was thus validated as a therapeutic target. Meanwhile SAP was also shown to promote amyloid fibril formation from soluble precursors *in vitro*[3], apparently by binding to and stabilizing protofibrillar aggregates (109–111).

I invented a high throughput screen for inhibitors of SAP binding to amyloid fibrils (105) and in the late 1990s, I persuaded Roche to use it to explore their compound library. With the help of some fortuitous serendipity, this swiftly led to the creation of a drug candidate, (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid, which I abbreviated as CPHPC, a palindromic acronym for a palindromic molecule[4] (72). Binding of SAP to amyloid fibrils and all its other known ligands is inhibited by CPHPC because SAP binds to the drug in a complex composed of two pentameric SAP molecules cross linked face to face by five of these bivalent hexanoyl bis(D-proline) molecules (72) (**Figure 5**). Each D-proline head group is located in the calcium dependent ligand binding pocket of a protomer. Although N-acetyl D-proline is only weakly bound by SAP, with Kd ∼15 μM, the cross linking of pairs of SAP molecules by five CPHPC molecules forms a very stable complex with Kd ∼10 nM due to the avidity gain of multivalency. In the SAP-CPHPC complex, all the calcium dependent ligand binding sites are occupied and the ligand binding "B" face of the disc-like SAP molecules is also occluded (72, 112) (**Figure 5**).

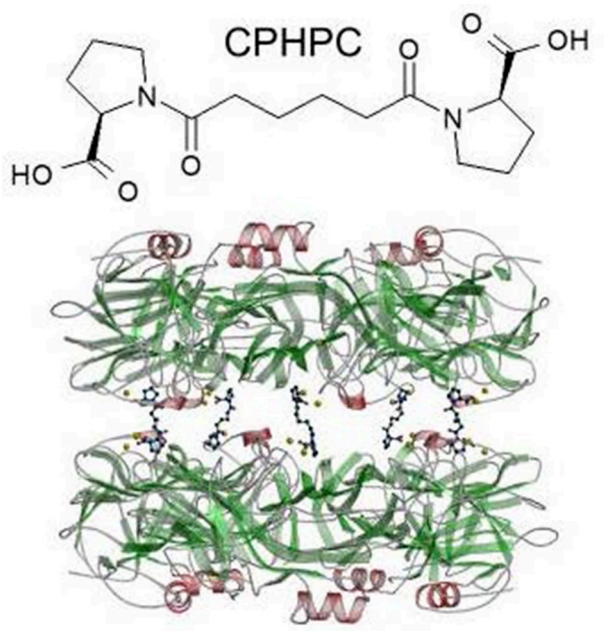


**FIGURE 5 |** Structure of CPHPC (miridesap) and its complex with human SAP. The palindromic bivalent structure of (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl] -6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC), now known by its WHO INN, miridesap, is shown above. Below is the 3D X-ray crystal structure of the SAP-drug complex which is also the structure of the complex in solution (112). [From Pepys et al. (72) with permission of Macmillan Publishers Ltd].

Work toward clinical testing in humans proceeded rapidly but, shortly before the first in human study, Roche stopped their development and handed the project over to us. Our first administration of CPHPC to humans immediately revealed that the drug produced very rapid and almost complete depletion of SAP from the circulation that persisted for as long as the drug was given (72, 73) (**Figure 6**). We showed that this resulted from the instant clearance of the SAP-CPHPC complex by the liver (72), where the SAP was promptly destroyed whilst the CPHPC, which is not metabolized at all, is released and swiftly excreted, mainly in the urine and to a smaller extent in the bile. The invention of CPHPC and the novel, and so far unique, pharmacological mechanism, by which a small molecule drug produces a targeted knockout of a pathogenic plasma protein, was recognized by the American Chemical Society as one of the medicinal chemistry highlights of 2002. CPHPC itself and prolonged SAP depletion were both well tolerated with no adverse effects other than mild transient stinging at sites of subcutaneous injection of the drug (73). However, the treatment did not promote regression of amyloid deposits from the tissues of patients with systemic amyloidosis. Depletion of circulating SAP removed much but never all SAP from its binding to amyloid, despite months of CPHPC treatment (73). This reflects a combination of factors that cannot be overcome: the avidity of binding of SAP to amyloid fibrils, the continuous production of new SAP by the liver, and the rapid excretion of CPHPC. In addition, crucially, the avid binding of SAP to CPHPC requires simultaneous binding of multiple D-proline head groups by pairs of SAP molecules. Complete elution of SAP from amyloid deposits therefore requires the presence of ∼1 mM CPHPC, an extremely high concentration that is not attainable *in vivo* despite the excellent tolerability of the drug. Something more was required to clear amyloid.

Phagocytosis and degradation by macrophages is the most important mechanism for removal of autologous debris and extrinsic materials from the extracellular space of the tissues. It is

[3]In the artefactual, non-physiological, absence of calcium, SAP inhibits the formation of Aβ amyloid fibrils *in vitro* (106). Indeed, we subsequently found that human SAP has classical chaperone properties in protein refolding assays (37, 107). The activity is calcium independent, does not involve ligand binding by the SAP and is apparently mediated by the "A" face of the molecule (37) but it is not clear whether and how it might operate *in vivo*.

[4]The high throughput screen of 100,000 compounds identified a small number of hits, the most attractive of which was one of the four disastereoisomers of captopril. Captopril itself and a third isomer were inactive but the fourth was more active than the original hit. When the actual material being tested was analyzed, it was found that the active substance was no longer the compound itself, with a free sulphydryl group, but a disulphide bonded covalent dimer. We had then lately reported the 3D X-ray crystal structure of the complex of SAP with dAMP, in which pairs of SAP molecules were cross linked face to face by hydrogen bonding between each dAMP molecules held in the calcium dependent ligand binding pockets of each SAP protomer (108). It was therefore obvious that similar cross linking of SAP molecules by a palindromic covalent structure would be a more potent inhibitor of SAP binding to other ligands than just single univalent ligands potentially occupying individual protomer binding sites. The disulphide dimer of the original hit was then synthesized and, as we had predicted, it was the most potent inhibitor. Further medicinal chemistry to replace the sulfur atoms with the hexanoyl chain yielded (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC).

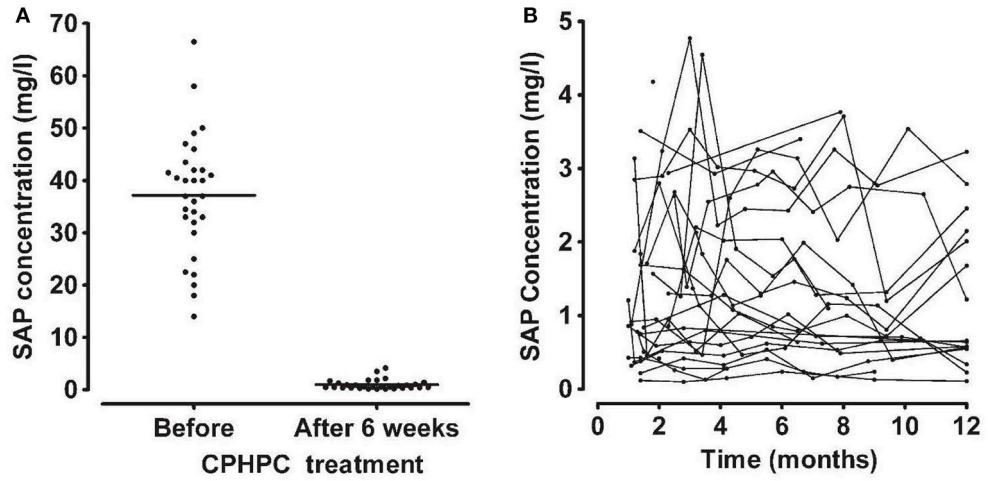


**FIGURE 6 |** Depletion of circulating SAP by CPHPC (miridesap) in patients with systemic amyloidosis. **(A)** Serum concentration of SAP immediately before and 6 weeks after starting daily treatment with CPHPC. **(B)** Sustained depletion of SAP throughout CPHPC treatment. Each line shows the results of serial measurements in an individual patient. Note different scale for SAP concentration compared to **(A)**. From Gillmore et al. (73) with permission of Blackwell Publishing Ltd). In patients without systemic amyloidosis and the associated massive extracellular load of SAP (98), CPHPC (miridesap) treatment reduces plasma SAP concentration to much lower values, for example, mean (SD) 0.25 (0.16) mg/l, in our 5 patients with Alzheimer's disease (112).

potently engaged by antibody mediated complement activation. In 2005, I realized that the residual SAP left in amyloid deposits, after depletion of the circulating SAP by CPHPC, could be used as a target for anti-SAP antibodies that would trigger amyloid removal (113). We tested the idea in human SAP transgenic mice in which we had induced systemic AA amyloidosis (114). Circulating human SAP was depleted with CPHPC and the mice then received a single dose of either sheep polyclonal anti-human SAP antibody or of normal control sheep IgG. There were no discernible adverse effects. Within 2 weeks almost no amyloid was detectable in the anti-SAP treated animals, compared to the unchanged massive amyloid load in the controls (114). Both classical complement pathway activation and macrophages were necessary and amyloid clearance was effected by multinucleated, macrophage derived, giant cells which surrounded, engulfed and destroyed the amyloid within days of antibody administration (114) (**Figure 7**). Depletion of SAP from the plasma and extracellular fluid is obviously essential, before administration of the anti-SAP antibody, so the proposed treatment is an obligate therapeutic partnership, not just a combination of two different drugs. Suitable avid, complement activating mouse monoclonal anti-human SAP antibodies were as effective as the xenogeneic polyclonal antibody (113), enabling potential clinical implementation with humanized antibody. In 2009, the invention was licensed to GlaxoSmithKline (GSK) for clinical drug development.

GSK fully humanized our optimal mouse monoclonal antibody and the first in human phase 1 study in patients with different types of systemic amyloidosis, starting in 2013, demonstrated unprecedented removal of visceral amyloid, with progressive removal after serial antibody doses (115, 116) (**Figure 8**). The antibody caused moderate infusion reactions and higher antibody doses produced skin rashes but there was no disturbance of organ function, even in heavily amyloidotic organs. Indeed abnormal liver function tests returned toward normal in all patients as their amyloid load was reduced (115, 116). All amyloid reducing doses of anti-SAP antibody produced transient early acute phase responses and dramatic depletion of plasma complement C3 concentration, consistent with activation of the same mechanism as we characterized in mice (115, 116). In 2017, the two drugs received their WHO International Non-proprietary Names (INN), miridesap for CPHPC and dezamizumab for the humanized monoclonal anti-SAP antibody, and the encouraging phase 1 results led to the current GSK phase 2 trial in patients with cardiac amyloidosis.

## SAP, ALZHEIMER'S DISEASE AND CEREBRAL AMYLOID ANGIOPATHY

Miridesap was intended from the outset to target SAP associated with the Aβ amyloid deposits in the brain and cerebral vasculature in Alzheimer's disease, as well as for systemic amyloidosis. Human SAP is synthesized only by the liver. As we had predicted, our initial, preliminary, clinical study in Alzheimer's disease confirmed that depletion of circulating SAP also completely removed SAP from the cerebrospinal fluid (112). Our subsequent study in a triple transgenic, human SAP expressing, mouse model of human Alzheimer's disease, confirmed that miridesap does indeed achieve the desired "molecular dissection" of Alzheimer's disease neuropathology by removing all SAP from cerebral amyloid deposits (117). This contrasts with the failure of miridesap to removal all SAP from the enormously more abundant visceral amyloid deposits in systemic amyloidosis (73), and, encouragingly, should enable the original SAP removal hypothesis to be tested with respect

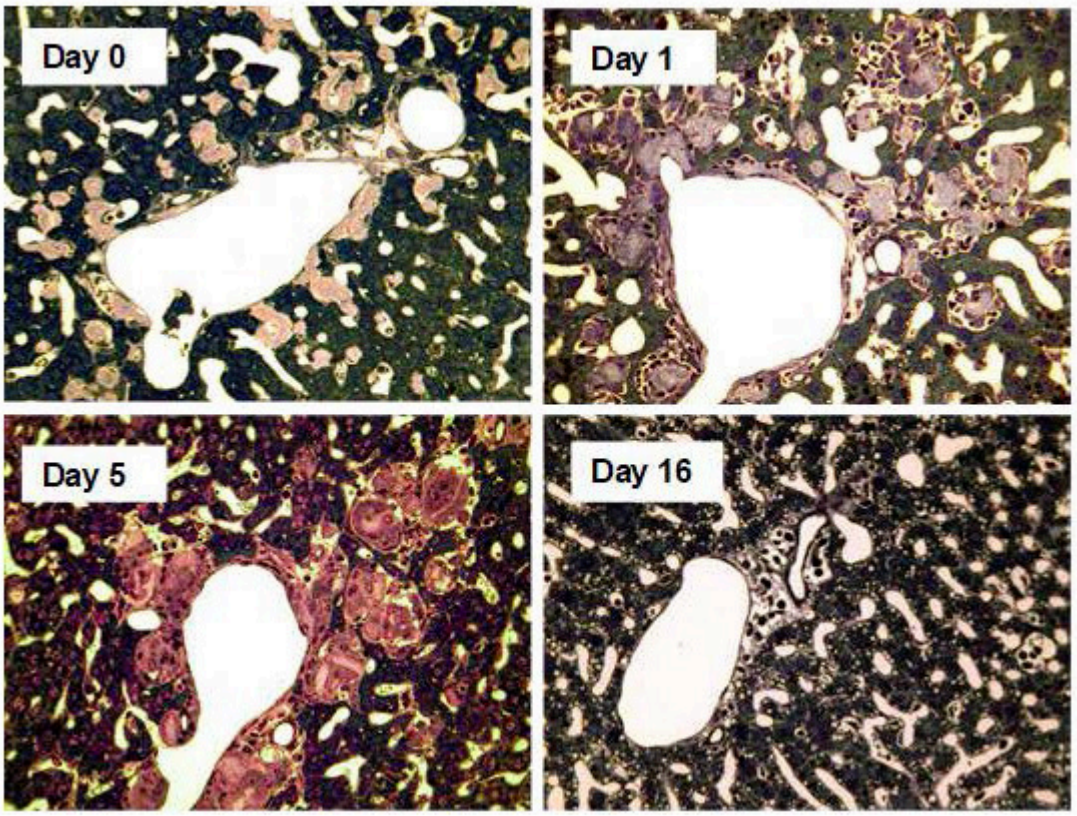


**FIGURE 7 |** Amyloid clearance mediated by macrophage derived multinucleated giant cells after depletion of circulating SAP followed by treatment with anti-SAP antibody. Thin sections of liver stained with toluidine blue from AA amyloidotic human SAP transgenic mice treated with CPHPC (miridesap) to deplete circulating SAP followed by anti-SAP antibody to target residual SAP in the amyloid deposits. Control mouse, not treated with anti-SAP antibody, show abundant amorphous pink-stained amyloid deposits, with the characteristic absence of any surrounding inflammatory reaction or cellular infiltrate. One day after anti-SAP antibody treatment there is intense, predominantly mononuclear cell infiltration in and around the amyloid. Five days after anti-SAP-antibody treatment there is fusion of macrophages to form multinucleated giant cells surrounding and infiltrating the deposits and containing large masses of ingested amyloid undergoing degradation. At 16 days there is complete elimination of amyloid deposits with no residual cellular infiltrate and restoration of normal tissue architecture. [From Bodin et al. (114) with permission of Macmillan Publishers Ltd].

to cerebral amyloid. This is one of the goals for our current "Depletion of serum amyloid P component in Alzheimer's disease" (DESPIAD) phase 2b clinical trial of miridesap. We also hope to study SAP depletion in cerebral amyloid angiopathy (118), the most prevalent form of clinical amyloidosis.

However, there is another rationale for SAP depletion in these brain diseases. Human SAP is directly cytotoxic for cerebral neurones, *in vitro* and *in vivo*, causing death by apoptosis (119–123). The SAP enters the cells, tracks to the nucleus, presumably via the nuclear localisation sequence present in pentraxins (124), enters the nucleus and then binds to chromatin, as we first demonstrated (75, 125). We have lately confirmed and extended (unpublished observations) an original preliminary report (126) that individuals with dementia have a higher brain content of SAP than individuals without dementia, regardless of the presence of Alzheimer's disease neuropathology. The results are consistent with a possible direct pathogenetic role of SAP in dementia, unrelated to the role of SAP in amyloid. Detection of potential benefit from abrogation of direct SAP neurotoxicity is the other major goal of the DESPIAD trial.

## ROUTINE CLINICAL MEASUREMENT OF CRP

My initial measurements of serum CRP concentration in 1975 swiftly showed that CRP was an excellent marker of Crohn's disease, closely reflecting extent and activity much better that any other single measurement (127). Our subsequent work confirmed and extended the results (128–130). I also made the striking discovery that the CRP response in ulcerative colitis, which, in 1975, also had not previously been reported, was completely different from Crohn's disease. Despite even severe, extensive, active ulcerative colitis, the circulating CRP concentration was generally modestly increased if at all (127, 128, 130). The unexpected, surprising, original observation of a marked difference in the CRP response to two rather similar disease processes, both with extensive inflammatory activity and tissue damage, initiated my lifelong interest in the clinical significance and utility of CRP assays.

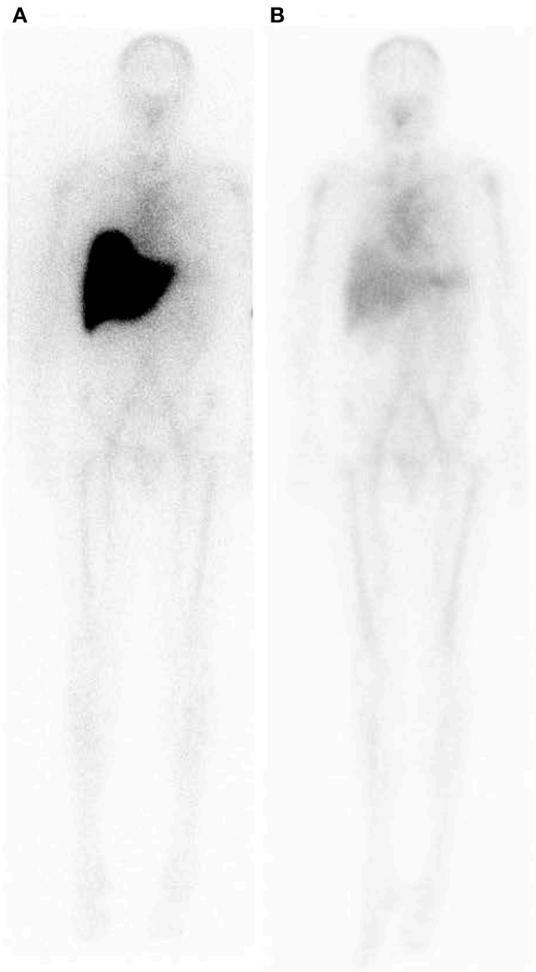


**FIGURE 8 |** Whole body scintigraphy with $^{123}$I-labeled serum amyloid P component in a patient with systemic amyloidosis before and after depletion of circulating SAP followed by treatment with anti-SAP antibody. **(A)** Scan immediately before treatment. **(B)** Scan 42 days after single dose of dezamizumab (fully humanized monoclonal anti-human SAP antibody) infused following depletion of circulating SAP with miridesap. The heavy load of amyloid in the liver has been dramatically reduced. [From Richards et al. (115) with permission of Massachusetts Medical Society].

**TABLE 1 |** Human CRP responses in different diseases.

| | |
|---|---|
| **Major CRP acute-phase response** | |
| Infections | Bacterial |
| | Systemic/Severe fungal, mycobacterial, viral |
| Allergic complications of infection | Rheumatic fever |
| | Erythema nodosum |
| Inflammatory disease | Rheumatoid arthritis |
| | Juvenile chronic arthritis |
| | Ankylosing spondylitis |
| | Psoriatic arthritis |
| | Systemic vasculitides |
| | Polymyalgia rheumatica |
| | Crohn's disease |
| | Familial Mediterranean fever |
| | Cryopyrin-associated periodic syndromes |
| Necrosis | Myocardial infarction |
| | Stroke |
| | Tumor embolisation |
| | Acute pancreatitis |
| Trauma | Surgery |
| | Burns |
| | Fractures |
| Malignancy | Lymphoma |
| | Carcinoma |
| | Sarcoma |
| **Modest or absent CRP acute-phase response** | |
| | Systemic lupus erythematosus |
| | Scleroderma |
| | Dermatomyositis |
| | Ulcerative colitis |
| | leukemia |
| | Graft-vs.-host disease |

Initially we studied the behavior of CRP as an acute phase reactant in a very broad range of different conditions, in well characterized series of patient, thereby establishing the optimal use of CRP in routine clinical practice (**Tables 1**, **2**). Commercial instrument based, rapid quantitative CRP immunoassays emerged in the early 1980s and modern high throughput automatic clinical chemistry analysers followed. In 1983, the World Health Organization invited me to create the First International Reference Standard for Immunoassay of C-reactive protein 84/506 (131). It remains the primary standard for all commercial clinical measurement of CRP. I also provided all the CRP for the major international secondary standards, the IFCC CRM470 and the ERM DA470 and ERM DA472. By virtue of my uniquely broad clinical experience with CRP measurement, and the expertise I had acquired in very large scale isolation and purification of human CRP to provide standards and calibrators, I played a substantial role in development of routine clinical CRP testing worldwide, working closely with major diagnostics companies. As recently noted by the EU SCIENCE HUB, the European Commission's science and knowledge service, "C-reactive protein (CRP) is one of the most important analytes in clinical chemistry." I have comprehensively reviewed elsewhere the scientific and clinical basis for routine use of CRP measurements (60, 132) (**Tables 1**, **2**).

## CRP AS A THERAPEUTIC TARGET

Our original 1994 report identified for the first time the association between acute phase responses and adverse prognosis in acute coronary syndromes (133). Our 1997 epidemiological work on CRP in patients with angina (134), and studies by others in general populations, identified an association between increased baseline values of CRP and future incidence

**TABLE 2 |** Routine clinical uses of CRP measurement.

| |
|---|
| **Screening test for organic disease** |
| **Assessment of disease activity in inflammatory conditions** |
| Juvenile chronic (rheumatoid) arthritis |
| Rheumatoid arthritis |
| Ankylosing spondylitis |
| Psoriatic arthropathy |
| Systemic vasculitides |
| Polymyalgia rheumatica |
| Crohn's disease |
| Rheumatic fever |
| Familial Mediterranean fever |
| Cryopyrin-associated periodic syndromes |
| Acute pancreatitis |
| **Diagnosis and management of infection** |
| Most systemic/severe bacterial, mycobacterial, viral and fungal infections |
| Response to antimicrobial treatment |
| Bacterial endocarditis |
| Neonatal septicaemia and meningitis |
| Intercurrent infection in systemic lupus erythematosus |
| Intercurrent infection in leukemia and its treatment |
| Postoperative complications including infection and thromboembolism |
| **Differential diagnosis/classification of inflammatory disease** |
| Systemic lupus erythematosus vs. rheumatoid arthritis |
| Crohn's disease vs. ulcerative colitis |

of cardiovascular disease. The association initially seemed potentially consistent with a pathogenic role for CRP in atherosclerosis and stimulated very widespread clinical interest in CRP, particularly as it was so easy to measure. An avalanche of epidemiological and experimental observations followed, purporting to show that CRP is a pro-atherogenic risk factor for cardiovascular disease. We were initially enthusiastic but it was soon clear that the early observational epidemiology cohorts had grossly overestimated the significance of the association. They included large total numbers of subjects but only small numbers of cardiovascular disease events, and their interpretation was then flawed by remorseless conflation of the overestimated association with causality. Poorly controlled experimental work purporting to show atherogenic activities of CRP was also badly flawed by use of uncharacterized and often contaminated CRP preparations. It soon became clear there was no evidence for causality of CRP in cardiovascular disease, as detailed in our extensive critical reviews (35, 135, 136). Appropriately large scale observational epidemiology firmly established that baseline CRP values are actually only a very modest risk marker for cardiovascular disease (137, 138) and Mendelian randomization studies proved that CRP itself is definitely not a causative risk factor (139). In addition to many unequivocally negative experimental *in vitro* and *in vivo* studies (35, 135, 136), we finally showed that direct infusion of pharmaceutical grade authentic cGMP human CRP had no pro-inflammatory effects in healthy volunteers (62) in contrast to the pro-inflammatory effect of recombinant CRP made in *E. coli*!

In contrast to the now discredited idea that CRP is pro-atherogenic, the evidence for a role of CRP in exacerbation of pre-existing ischemic and other tissue injury is robust. Complement has long been known to be responsible for the inflammatory neutrophil infiltrate that characterizes experimental acute myocardial infarction (140) and it had been speculated that CRP, via its capacity to activate complement after binding to its ligands *in vivo*, might exacerbate tissue damage (141–145). In 1999 we were the first to actually demonstrate this *in vivo*, using the rat acute myocardial infarction model (24). Although rat CRP circulates at very high concentration in normal healthy animals, rat CRP does not activate rat complement whereas human CRP activates both human and rat complement (26). Rat thus provide an excellent model for investigation of the effects of human CRP in humans. Administration of isolated pure human CRP to rats following ligation of the coronary artery substantially increased the size of the resulting myocardial infarct and human CRP was co-deposited with rat complement on and around the infarcted tissue (24). Crucially, the exacerbation of injury by human CRP was completely abrogated by prior depletion of C3 using cobra venom factor (24). The CRP effect was thus totally complement dependent. We subsequently showed that human CRP also increased cerebral infarct size in the rat middle cerebral artery occlusion model (146).

Having identified and validated human CRP as a therapeutic target, we designed novel bis(phosphocholine)-alkanes as inhibitors of ligand binding by human CRP *in vivo*. These ligands for CRP were based on our knowledge of the 3D X-ray crystal structure of the CRP-phosphocholine complex (31) and our experience with miridesap, hexanoyl-bis(D-proline), the SAP inhibitor drug (72). We showed that bis(phosphocholine)-hexane (32) and bis(phosphocholine)-octane (unpublished) completely abrogated the enhancement of tissue damage caused by human CRP in the rat acute myocardial infarction model. Binding of human CRP to these compounds inhibits CRP binding to other ligands, though it does not accelerate clearance of CRP from the circulation as miridesap does with human SAP.

Exacerbation by CRP of ischemic and inflammatory tissue injury in various different animal models has been independently confirmed by other groups. Abrogation of the pathogenic CRP effect has also been replicated with our compound, bis(phosphocholine)-hexane, and by suppression of CRP production with antisense oligonucleotides, and by using CRP apheresis to remove circulating CRP (147–154).

The cross linking of pairs of CRP molecules by five bis(phosphocholine)-alkane molecules markedly stabilizes the non-covalent homopentameric assembly of native human CRP, preventing denaturation and the release of protomers. However, in the absence of calcium or of calcium dependent ligand binding, denatured CRP can dissociate *in vitro* to release free protomers, so-called monomeric or "mCRP," that bear specific neoepitopes. Based on *ex vivo* immunohistochemical detection of these epitopes, it has been asserted that mCRP products of CRP denaturation mediate the pro-inflammatory effects of CRP *in vivo* (155). Inhibition by bis(phosphocholine)-hexane of CRP-mediated inflammation has then been attributed exclusively to stabilization of native CRP (155), curiously ignoring

our unequivocal demonstration of the absolute complement dependence of the pro-inflammatory actions of human CRP *in vivo* (24). Fortunately this oversight and mechanistic disagreement have no practical importance as the avid binding of our palindromic CRP inhibitor ligands, designed to prevent CRP-mediated complement activation *in vivo*, inevitably also robustly stabilizes the native pentameric CRP structure.

The bis(phosphocholine)-alkanes are well tolerated and would have been suitable for development as infusional drugs but it was not possible to synthesize and purify them at scale. We have therefore designed new, different and more potent inhibitors of CRP binding and are currently working toward candidate selection for clinical development. Clinical observations are consistent with the experimental evidence that high circulating CRP concentrations exacerbate pre-existing tissue damage. For example, higher CRP values during and after acute myocardial infarction are strongly associated with poor prognosis overall, including more extensive myocardial injury, impaired cardiac function and progression to heart failure (156). The same is true in a wide range of other tissue damaging ischaemic, inflammatory, infective, traumatic and malignant conditions. There are thus likely to be many indications for therapeutic use of CRP inhibitor drugs.

## CONCLUSIONS

The range of physiological and pathophysiological roles of the pentraxins remains incompletely understood. Their gene and amino acid sequences, and very characteristic molecular assembly, are highly conserved in phylogeny and there are no human genetic deficiencies or even isoforms, and yet there are major differences in behavior and properties between even closely related species. The pentraxins thus display a fascinating, and so far unexplained, mixture of conservation and plasticity in a single protein family. However, regardless of their normal roles, both human CRP and SAP have become extremely useful in clinical diagnosis and monitoring of disease. CRP assay is one of the most widely used clinical chemistry tests and SAP scintigraphy has transformed understanding and optimal management of systemic amyloidosis. Importantly, human CRP and SAP are also therapeutic targets for which the design and development of potential new medicines are making exciting progress.

## ETHICS STATEMENT

No new work on human or animal subjects is included in the article. All work on human and animal subjects that is mentioned in the article was conducted with full ethical approval current at the time of publication, as detailed in the original source literature which is cited in the references.

## AUTHOR CONTRIBUTIONS

MP conceived and wrote the paper and takes full responsibility for all observations, results and opinions expressed therein.

## ACKNOWLEDGMENTS

The invaluable contributions of the co-authors of my cited work, and of the colleagues and collaborators on pentraxin studies since 1975, are gratefully acknowledged. In particular, all the X-ray crystallographic studies were directed by Professor Steve Wood while Professor Philip Hawkins made seminal contributions to the development and clinical implementation of SAP scintigraphy. The work was funded by the UK Medical Research Council without interruption from 1969 to 2016 and also generously supported by The Wellcome Trust, The British Heart Foundation and other UK Medical Charities. The Wolfson Foundation contributed to establishment of the Centre for Amyloidosis and Acute Phase Proteins and subsequently funded creation of the Wolfson Drug Discovery Unit. Core support for the Wolfson Drug Discovery Unit has been provided since 2012 by the UK National Institute for Health Research Biomedical Research Centre and Unit Funding Scheme via the UCLH/UCL Biomedical Research Centre, which also funds the DESPIAD trial. DESPIAD is also supported by contributions from the Dana Foundation, New York and by Alzheimer Research UK. MP's invention of SAP depletion followed by anti-SAP antibody to remove systemic amyloid deposits is licensed to GlaxoSmithKline for clinical development. GSK also greatly assisted preparations for the DESPIAD trial.

## REFERENCES

1. Pepys MB. Role of complement in induction of the allergic response. *Nature New Biol.* (1972) 237:157–9. doi: 10.1038/newbio237157a0
2. Feldmann M, Pepys MB. Role of C3 in *in vitro* lymphocyte cooperation. *Nature* (1974) 249:159–61. doi: 10.1038/249159a0
3. Pepys MB. Role of complement in induction of antibody production *in vivo*. Effect of cobra factor and other C3-reactive agents on thymus-dependent and thymus-independent antibody responses. *J Exp Med.* (1974) 140:126–45.
4. Pepys MB. Complement-mediated mixed aggregation of murine spleen cells. *Nature* (1974) 249:51–3. doi: 10.1038/249051a0
5. Pepys MB. Role of complement in the induction of immunological responses. *Transplant Rev.* (1976) 32:93–120.
6. Brown G, Greaves MF. Enumeration of absolute numbers of T and B lymphocytes in human blood. *Scand J Immunol.* (1974) 3:161–72. doi: 10.1111/j.1365-3083.1974.tb01244.x
7. Mortensen RF, Osmand AP, Gewurz H. Effects of C-reactive protein on the lymphoid system. I. Binding to thymus-dependent lymphocytes and alteration of their functions. *J Exp Med.* (1975) 141:821–39.
8. Laurell C-B. Electroimmunoassay. *Scand J Clin Lab Invest.* (1972) 29:21–37. doi: 10.3109/00365517209102748
9. Pepys MB, Dash AC, Ashley J. Isolation of C-reactive protein by affinity chromatography. *Clin Exp Immunol.* (1977) 30:32–7.
10. Cathcart ES, Comerford FR, Cohen AS. Immunologic studies on a protein extracted from human secondary amyloid. *N Engl J Med.* (1965) 273:143–6. doi: 10.1056/NEJM196507152730306
11. Bladen HA, Nylen MU, Glenner GG. The ultrastructure of human amyloid as revealed by the negative staining technique. *J Ultrastruct Res.* (1966) 14:449–59. doi: 10.1016/S0022-5320(66)80075-8
12. Cathcart ES, Shirahama T, Cohen AS. Isolation and identification of a plasma component of amyloid. *Biochim Biophys Acta* (1967) 147:392–3. doi: 10.1016/0005-2795(67)90420-5

13. Cathcart ES, Wollheim FA, Cohen AS. Plasma protein constituents of amyloid fibrils. *J Immunol.* (1967) 99:376–85.
14. Cathcart ES, Skinner M, Cohen AS, Lawless OJ, Benson MD. Antigenic determinants in amyloid deposits. *Nature* (1970) 228:1090–1. doi: 10.1038/2281090b0
15. Hutchinson WL, Hohenester E, Pepys MB. Human serum amyloid P component is a single uncomplexed pentamer in whole serum. *Mol Med.* (2000) 6:482–93. doi: 10.1007/BF03401789
16. Assimeh SN, Bing DH, Painter RH. A simple method for the isolation of the subcomponents of the first component of complement by affinity chromatography. *J Immunol.* (1974) 113:225–34.
17. Assimeh SN, Painter RH. The identification of a previously unrecognized subcomponent of the first component of complement. *J Immunol.* (1975) 115:482–7.
18. Painter RH, Pinteric L, Hoffman T, Kells DIC, Katz A. Ultrastructure and chemistry of Clt subcomponent of Cl: similarities to amyloid P component. *J Immunol.* (1976) 116:1745.
19. Osmand AP, Friedenson B, Gewurz H, Painter RH, Hofmann T, Shelton E. Characterisation of C-reactive protein and the complement subcomponent Clt as homologous proteins displaying cyclic pentameric symmetry (pentraxins). *Proc Natl Acad Sci USA.* (1977) 74:739–43. doi: 10.1073/pnas.74.2.739
20. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv Immunol.* (1983) 34:141–212. doi: 10.1016/S0065-2776(08)60379-X
21. Pepys MB, Dash AC, Munn EA, Feinstein A, Skinner M, Cohen AS, et al. Isolation of amyloid P component (protein AP) from normal serum as a calcium-dependent binding protein. *Lancet* (1977) 1:1029–31.
22. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. *J Immunol.* (1974) 112:2135–47.
23. Siegel J, Rent R, Gewurz H. Interactions of C-reactive protein with the complement system. I. Protamine-induced consumption of complement in acute phase sera. *J Exp Med.* (1974) 140:631–47.
24. Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, et al. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med.* (1999) 190:1733–9. doi: 10.1084/jem.190.12.1733
25. Tennent GA, Butler PJG, Hutton T, Woolfit AR, Harvey DJ, Rademacher TW, et al. Molecular characterization of *Limulus polyphemus* C-reactive protein. I. Subunit composition. *Eur J Biochem.* (1993) 214:91–7.
26. de Beer FC, Baltz ML, Munn EA, Feinstein A, Taylor J, Bruton C, et al. Isolation and characterisation of C-reactive protein and serum amyloid P component in the rat. *Immunology* (1982) 45:55–70.
27. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. PTX3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiol Rev.* (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
28. Emsley J, White HE, O'Hara BP, Oliva G, Srinivasan N, Tickle IJ, et al. Structure of pentameric human serum amyloid P component. *Nature* (1994) 367:338–45. doi: 10.1038/367338a0
29. Ashton AW, Boehm MK, Gallimore JR, Pepys MB, Perkins SJ. Pentameric and decameric structures in solution of serum amyloid P component by X-ray and neutron scattering and molecular modelling analyses. *J Mol Biol.* (1997) 272:408–22. doi: 10.1006/jmbi.1997.1271
30. Shrive AK, Cheetham GMT, Holden D, Myles DAA, Turnell WG, Volanakis JE, et al. Three-dimensional structure of human C-reactive protein. *Nat Struct Biol.* (1996) 3:346–54. doi: 10.1038/nsb0496-346
31. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* (1999) 7:169–77. doi: 10.1016/S0969-2126(99)80023-9
32. Pepys MB, Hirschfield GM, Tennent GA, Gallimore JR, Kahan MC, Bellotti V, et al. Targeting C-reactive protein for the treatment of cardiovascular disease. *Nature* (2006) 440:1217–21. doi: 10.1038/nature04672
33. Kinoshita CM, Ying S-C, Hugli TE, Siegel JN, Potempa LA, Jiang H, et al. Elucidation of a protease-sensitive site involved in the binding of calcium to C-reactive protein. *Biochemistry* (1989) 28:9840–8. doi: 10.1021/bi00451a044
34. Kinoshita CM, Gewurz AT, Siegel JN, Ying S-C, Hugli TE, Coe JE, et al. A protease-sensitive site in the proposed $Ca^{2+}$-binding region of human serum amyloid P component and other pentraxins. *Protein Sc* (1992) 1:700–9. doi: 10.1002/pro.5560010602
35. Pepys MB. CRP or not CRP? That is the question. *Arterioscler Thromb Vasc Biol.* (2005) 25:1091–4. doi: 10.1161/01.ATV.0000169644.88847.28
36. Pepys MB, Hawkins PN, Kahan MC, Tennent GA, Gallimore JR, Graham D, et al. Proinflammatory effects of bacterial recombinant human C-reactive protein are caused by contamination with bacterial products, not by C-reactive protein itself. *Circ Res.* (2005) 97:e97–103. doi: 10.1161/01.RES.0000193595.03608.08
37. Coker AR, Coker S-F, Coker R, Pye VE, Pepys MB, Wood SP. The crystal structure of the calcium-free serum amyloid P component decamer. In: Hazenberg BPC, Bijzet J, editors. *From Misfolded Proteins to Well-Designed Treatment XIIth International Symposiumon Amyloidosis*. Groningen: GUARD (2012). p. 64–6.
38. Baltz ML, de Beer FC, Feinstein A, Pepys MB. Calcium-dependent aggregation of human serum amyloid P component. *Biochim Biophys Acta* (1982) 701:229–36. doi: 10.1016/0167-4838(82)90118-2
39. de Beer FC, Baltz M, Holford S, Feinstein A, Pepys MB. Fibronectin and C4-binding protein are selectively bound by aggregated amyloid P component. *J Exp Med.* (1981) 154:1134–49. doi: 10.1084/jem.154.4.1134
40. Booth DR, Gallimore JR, Hutchinson WL, Hohenester E, Thompson D, Wood S, et al. Analysis of autoaggregation and ligand binding sites of serum amyloid P component by *in vitro* mutagenesis. In: Kyle RA, Gertz MA, editors. *Amyloid and Amyloidosis 1998*. Pearl River, NY: Parthenon Publishing (1999). p. 23–5.
41. Pepys MB, Dash AC, Fletcher TC, Richardson N, Munn EA, Feinstein A. Analogues in other mammals and in fish of human plasma proteins C-reactive protein and amyloid P component. *Nature* (1978) 273:168–70. doi: 10.1038/273168a0
42. Pepys MB, Baltz M, Gomer K, Davies AJS, Doenhoff M. Serum amyloid P-component is an acute-phase reactant in the mouse. *Nature* (1979) 278:259–61. doi: 10.1038/278259a0
43. White A, Fletcher TC, Pepys MB, Baldo BA. The effect of inflammatory agents on C-reactive protein and serum amyloid P component levels in plaice (*Pleuronectes platessa L.*) serum. *Comp Biochem Physiol.* (1981) 69C:325–9.
44. Baltz ML, de Beer FC, Feinstein A, Munn EA, Milstein CP, Fletcher TC, et al. Phylogenetic aspects of C-reactive protein and related proteins. *Ann NY Acad Sci.* (1982) 389:49–75. doi: 10.1111/j.1749-6632.1982.tb22125.x
45. Pepys MB, de Beer FC, Milstein CP, March JF, Feinstein A, Buttress N, et al. C-reactive protein and serum amyloid P component in the plaice *(Pleuronectes platessa L.)*, a marine teleost, are homologous with their human counterparts. *Biochim Biophys Acta* (1982) 704:123–33.
46. Taylor J, Bruton C, Clamp JR, de Beer FC, Baltz ML, Pepys MB. Studies of the primary structure of mouse serum amyloid P component (SAP). *Ann NY Acad Sci.* (1982) 389:471. doi: 10.1111/j.1749-6632.1982.tb22177.x
47. Caspi D, Baltz ML, Snel F, Gruys E, Niv D, Batt RM, et al. Isolation and characterisation of C-reactive protein from the dog. *Immunology* (1984) 53:307–13.
48. Taylor JA, Burton CJ, Anderson JK, Mole JE, de Beer FC, Baltz ML, et al. Amino acid sequence homology between rat and human C-reactive protein. *Biochem J.* (1984) 221:903–6. doi: 10.1042/bj2210903
49. Maudsley S, Hind CRK, Munn EA, Buttress N, Pepys MB. Isolation and characterization of guinea-pig serum amyloid P component. *Immunology* (1986) 59:317–22.
50. Caspi D, Snel FWJJ, Batt RM, Bennett D, Rutteman GR, Hartman EG, et al. C-reactive protein in dogs. *Am J Vet Res.* (1987) 48:919–21.
51. Maudsley S, Baltz ML, Munn EA, Buttress N, Herbert J, Feinstein A, et al. Isolation and characterization of goat C-reactive protein. *Biochim Biophys Acta* (1987) 924:75–80. doi: 10.1016/0304-4165(87)90072-9
52. Maudsley S, Pepys MB. Immunochemical cross-reactions between pentraxins of different species. *Immunology* (1987) 62:17–22.

53. Maudsley S, Rowe IF, de Beer FC, Munn EA, Herbert J, Feinstein A, et al. Identification and isolation of two pentraxins from bovine serum. *Clin Exp Immunol.* (1987) 67:662–73.
54. Amatayakul-Chantler S, Dwek RA, Tennent GA, Pepys MB, Rademacher TW. Molecular characterization of *Limulus polyphemus* C-reactive protein. II. Asparagine-linked oligosaccharides. *Eur J Biochem.* (1993) 214:99–110.
55. Tennent GA, Baltz ML, Osborn GD, Butler PJG, Noble GE, Hawkins PN, et al. Studies of the structure and binding properties of hamster female protein. *Immunology* (1993) 80:645–51.
56. Nelson SR, Tennent GA, Sethi D, Gower PE, Ballardie FW, Amatayakul-Chantler S, et al. Serum amyloid P component in chronic renal failure and dialysis. *Clin Chim Acta* (1991) 200:191–200. doi: 10.1016/0009-8981(91)90090-Y
57. Pepys MB, Dash AC, Markham RE, Thomas HC, Williams BD, Petrie A. Comparative clinical study of protein SAP (amyloid P component) and C-reactive protein in serum. *Clin Exp Immunol.* (1978) 32:119–24.
58. Simons JP, Loeffler JM, Al-Shawi R, Ellmerich S, Hutchinson WL, Tennent GA, et al. C-reactive protein is essential for innate resistance to pneumococcal infection. *Immunology* (2014) 142:414–20. doi: 10.1111/imm.12266
59. Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta* (1981) 117:13–23. doi: 10.1016/0009-8981(81)90005-X
60. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest.* (2003) 111:1805–12. doi: 10.1172/JCI200318921
61. Pepys MB, Gallimore JR, Lloyd J, Li Z, Graham D, Taylor GW, et al. Isolation and characterization of pharmaceutical grade human pentraxins, serum amyloid P component and C-reactive protein, for clinical use. *J Immunol Methods* (2012) 384:92–102. doi: 10.1016/j.jim.2012.07.013
62. Lane T, Wassef N, Poole S, Mistry Y, Lachmann HJ, Gillmore JD, et al. Infusion of pharmaceutical-grade natural human C-reactive protein is not pro-inflammatory in healthy adult human volunteers. *Circ Res.* (2014) 114:672–6. doi: 10.1161/CIRCRESAHA.114.302770
63. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med.* (1971) 136:612–4. doi: 10.3181/00379727-136-35323
64. Volanakis JE. Complement activation by C-reactive protein complexes. *Ann NY Acad Sci.* (1982) 389:235–50. doi: 10.1111/j.1749-6632.1982.tb22140.x
65. Mold C, Nakayama S, Holzer TJ, Gewurz H, Du Clos TW. C-reactive protein is protective against *Streptococcus pneumoniae* infection in mice. *J Exp Med.* (1981) 154:1703–8. doi: 10.1084/jem.154.5.1703
66. Yother J, Volanakis JE, Briles DE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in mice. *J Immunol.* (1982) 128:2374–6.
67. Szalai AJ, Briles DE, Volanakis JE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in transgenic mice. *J Immunol.* (1995) 155:2557–63.
68. Hirschfield GM, Herbert J, Kahan MC, Pepys MB. Human C-reactive protein does not protect against acute lipopolysaccharide challenge in mice. *J Immunol.* (2003) 171:6046–51. doi: 10.4049/jimmunol.171.11.6046
69. Roy S, Hill AV, Knox K, Griffiths D, Crook D. Research pointers: association of common genetic variant with susceptibility to invasive pneumococcal disease. *BMJ* (2002) 324:1369. doi: 10.1136/bmj.324.7350.1369
70. Eklund C, Huttunen R, Syrjänen J, Laine J, Vuento R, Hurme M. Polymorphism of the C-reactive protein gene is associated with mortality in bacteraemia. *Scand J Infect Dis.* (2006) 38:1069–73. doi: 10.1080/00365540600978922
71. Mukamal KJ, Pai JK, O'Meara ES, Tracy RP, Psaty BM, Kuller LH, et al. *CRP* gene variation and risk of community-acquired pneumonia. *Respirology* (2010) 15:160–4. doi: 10.1111/j.1440-1843.2009.01661.x
72. Pepys MB, Herbert J, Hutchinson WL, Tennent GA, Lachmann HJ, Gallimore JR, et al. Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature* (2002) 417:254–9. doi: 10.1038/417254a
73. Gillmore JD, Tennent GA, Hutchinson WL, Gallimore JR, Lachmann HJ, Goodman HJB, et al. Sustained pharmacological depletion of serum amyloid P component in patients with systemic amyloidosis. *Br J Haematol.* (2010) 148:760–7. doi: 10.1111/j.1365-2141.2009.08036.x
74. Pepys MB, Butler PJG. Serum amyloid P component is the major calcium-dependent specific DNA binding protein of the serum. *Biochem Biophys Res Commun.* (1987) 148:308–13. doi: 10.1016/0006-291X(87)91111-9
75. Butler PJG, Tennent GA, Pepys MB. Pentraxin-chromatin interactions: serum amyloid P component specifically displaces H1-type histones and solubilizes native long chromatin. *J Exp Med.* (1990) 172:13–8. doi: 10.1084/jem.172.1.13
76. Hintner H, Booker J, Ashworth J, Auböck J, Pepys MB, Breathnach SM. Amyloid P component binds to keratin bodies in human skin and to isolated keratin filament aggregates *in vitro*. *J Invest Dermatol.* (1988) 91:22–8.
77. Breathnach SM, Kofler H, Sepp N, Ashworth J, Woodrow D, Pepys MB, et al. Serum amyloid P component binds to cell nuclei *in vitro* and to *in vivo* deposits of extracellular chromatin in systemic lupus erythematosus. *J Exp Med.* (1989) 170:1433–8. doi: 10.1084/jem.170.4.1433
78. Bickerstaff MCM, Botto M, Hutchinson WL, Herbert J, Tennent GA, Bybee A, et al. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. *Nature Med.* (1999) 5:694–7. doi: 10.1038/9544
79. Gillmore JD, Hutchinson WL, Herbert J, Bybee A, Mitchell DA, Hasserjian RP, et al. Autoimmunity and glomerulonephritis in mice with targeted deletion of the serum amyloid P component gene: SAP deficiency or strain combination? *Immunology* (2004) 112:255–64. doi: 10.1111/j.1365-2567.2004.01860.x
80. Wang Y, Guo Y, Wang X, Huang J, Shang J, Sun S. Human serum amyloid P functions as a negative regulator of the innate and adaptive immune responses to DNA vaccines. *J Immunol.* (2011) 186:2860–70. doi: 10.4049/jimmunol.1003641
81. Wang Y, Guo Y, Wang X, Huang J, Shang J, Sun S. Serum amyloid P component facilitates DNA clearance and inhibits plasmid transfection: implications for human DNA vaccine. *Gene Ther.* (2011) 19:70–7. doi: 10.1038/gt.2011.67
82. Borthwick NJ, Lane T, Moyo N, Crook A, Shim JM, Baines I, et al. Randomized phase I trial HIV-CORE 003: Depletion of serum amyloid P component and immunogenicity of DNA vaccination against HIV-1. *PLoS ONE* (2018) 13:e0197299. doi: 10.1371/journal.pone.0197299
83. Noursadeghi M, Bickerstaff MCM, Gallimore JR, Herbert J, Cohen J, Pepys MB. Role of serum amyloid P component in bacterial infection: protection of the host or protection of the pathogen. *Proc Natl Acad Sci USA.* (2000) 97:14584–9. doi: 10.1073/pnas.97.26.14584
84. Hind CRK, Collins PM, Baltz ML, Pepys MB. Human serum amyloid P component, a circulating lectin with specificity for the cyclic 4,6-pyruvate acetal of galactose. Interactions with various bacteria. *Biochem J.* (1985) 225:107–11.
85. Kimura T, Tani S, Matsumoto Y, Takeda T. Serum amyloid P component is the Shiga toxin 2-neutralizing factor in human blood. *J Biol Chem.* (2001) 276:41576–9. doi: 10.1074/jbc.M107819200
86. Marcato P, Vander Helm K, Mulvey GL, Armstrong GD. Serum amyloid P component binding to Shiga toxin 2 requires both a subunit and B pentamer. *Infect Immun.* (2003) 71:6075–8. doi: 10.1128/IAI.71.10.6075-6078.2003
87. Dettmar AK, Binder E, Greiner FR, Liebau MC, Kurschat CE, Jungraithmayr TC, et al. Protection of human podocytes from Shiga toxin 2-induced phosphorylation of mitogen-activated protein kinases and apoptosis by human serum amyloid P component. *Infect Immun.* (2014) 82:1872–9. doi: 10.1128/IAI.01591-14
88. Armstrong GD, Mulvey GL, Marcato P, Griener TP, Kahan MC, Tennent GA, et al. Human serum amyloid P component protects against *Escherichia coli* O157:H7 Shiga toxin 2 *in vivo*: therapeutic implications for hemolytic-uremic syndrome. *J Infect Dis.* (2006) 193:1120–4. doi: 10.1086/501472
89. de Haas CJC, van Leeuwen EMM, van Bommel T, Verhoef J, van Kessel KPM, van Strijp JAG. Serum amyloid P component bound to gram-negative bacteria prevents lipopolysaccharide-mediated classical pathway complement activation. *Infect Immun.* (2000) 68:1753–9. doi: 10.1128/IAI.68.4.1753-1759.2000
90. Dyck RF, Lockwood M, Kershaw M, McHugh N, Duance V, Baltz ML, et al. Amyloid P-component is a constituent of normal human glomerular basement membrane. *J Exp Med.* (1980) 152:1162–74. doi: 10.1084/jem.152.5.1162

91. Breathnach SM, Melrose SM, Bhogal B, de Beer FC, Dyck RF, Tennent G, et al. Amyloid P component is located on elastic fibre microfibrils of normal human tissues. *Nature* (1981) 293:652–4. doi: 10.1038/293652a0
92. Botto M, Hawkins PN, Bickerstaff MCM, Herbert J, Bygrave AE, McBride A, et al. Amyloid deposition is delayed in mice with targeted deletion of the serum amyloid P component gene. *Nature Med.* (1997) 3:855–9. doi: 10.1038/nm0897-855
93. Pepys MB, Dyck RF, de Beer FC, Skinner M, Cohen AS. Binding of serum amyloid P component (SAP) by amyloid fibrils. *Clin Exp Immunol.* (1979) 38:284–93.
94. Baltz ML, Caspi D, Evans DJ, Rowe IF, Hind CRK, Pepys MB. Circulating serum amyloid P component is the precursor of amyloid P component in tissue amyloid deposits. *Clin Exp Immunol.* (1986) 66:691–700.
95. Caspi D, Zalzman S, Baratz M, Teitelbaum Z, Yaron M, Pras M, et al. Imaging of experimental amyloidosis with $^{131}$I-serum amyloid P component. *Arthritis Rheum.* (1987) 30:1303–6. doi: 10.1002/art.1780301115
96. Hawkins PN, Myers MJ, Epenetos AA, Caspi D, Pepys MB. Specific localization and imaging of amyloid deposits *in vivo* using $^{123}$I-labeled serum amyloid P component. *J Exp Med.* (1988) 167:903–13. doi: 10.1084/jem.167.3.903
97. Hawkins PN, Myers MJ, Lavender JP, Pepys MB. Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component. *Lancet* (1988) 1:1413–8.
98. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with $^{123}$I-labeled serum amyloid P component. *N Engl J Med.* (1990) 323:508–13. doi: 10.1056/NEJM199008233230803
99. Hawkins PN, Wootton R, Pepys MB. Metabolic studies of radioiodinated serum amyloid P component in normal subjects and patients with systemic amyloidosis. *J Clin Invest.* (1990) 86:1862–9. doi: 10.1172/JCI114917
100. Pepys MB. Amyloidosis. *Annu Rev Med.* (2006) 57:223–41. doi: 10.1146/annurev.med.57.121304.131243
101. Hind CRK, Collins PM, Renn D, Cook RB, Caspi D, Baltz ML, et al. Binding specificity of serum amyloid P component for the pyruvate acetal of galactose. *J Exp Med.* (1984) 159:1058–69. doi: 10.1084/jem.159.4.1058
102. Hind CRK, Collins PM, Caspi D, Baltz ML, Pepys MB. Specific chemical dissociation of fibrillar and non-fibrillar components of amyloid deposits. *Lancet* (1984) 2:376–8.
103. Pepys MB, Rademacher TW, Amatayakul-Chantler S, Williams P, Noble GE, Hutchinson WL, et al. Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. *Proc Natl Acad Sci USA.* (1994) 91:5602–6. doi: 10.1073/pnas.91.12.5602
104. Tennent GA, Lovat LB, Pepys MB. Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer's disease and systemic amyloidosis. *Proc Natl Acad Sci USA.* (1995) 92:4299–303. doi: 10.1073/pnas.92.10.4299
105. Pepys MB, Blundell TL, inventors; Pentraxin Therapeutics Limited, assignee. *Screening Assays to Identify Therapeutic Agents for Amyloidosis.* US Patent No. 6,126,918 (2000).
106. Janciauskiene S, García de Frutos P, Carlemalm E, Dahlbäck B, Eriksson S. Inhibition of Alzheimer β-peptide fibril formation by serum amyloid P component. *J Biol Chem.* (1995) 270:26041–4.
107. Coker AR, Purvis A, Baker D, Pepys MB, Wood SP. Molecular chaperone properties of serum amyloid P component. *FEBS Lett.* (2000) 473:199–202. doi: 10.1016/S0014-5793(00)01530-1
108. Hohenester E, Hutchinson WL, Pepys MB, Wood SP. Crystal structure of a decameric complex of human serum amyloid P component with bound dAMP. *J Mol Biol.* (1997) 269:570–8. doi: 10.1006/jmbi.1997.1075
109. Hamazaki H. Amyloid P component promotes aggregation of Alzheimer's β-amyloid peptide. *Biochem Biophys Res Commun.* (1995) 211:349–53. doi: 10.1006/bbrc.1995.1819
110. Myers SL, Jones S, Jahn TR, Morten IJ, Tennent GA, Hewitt EW, et al. A systematic study of the effect of physiological factors on $\beta_2$-microglobulin amyloid formation at neutral pH. *Biochemistry* (2006) 45:2311–21. doi: 10.1021/bi052434i
111. Mold M, Shrive AK, Exley C. Serum amyloid P component accelerates the formation and enhances the stability of amyloid fibrils in a physiologically significant under-saturated solution of amyloid- $\beta_{42}$. *J Alzheimers Dis.* (2012) 29:875–81. doi: 10.3233/JAD-2012-120076
112. Kolstoe SE, Ridha BH, Bellotti V, Wang N, Robinson CV, Crutch SJ, et al. Molecular dissection of Alzheimer's disease neuropathology by depletion of serum amyloid P component. *Proc Natl Acad Sci USA.* (2009) 106:7619–23. doi: 10.1073/pnas.0902640106
113. Pepys MB, inventor; Pentraxin Therapeutics Limited, assignee. *Combinations of SAP Depleting Agents and Anti-SAP Antibodies.* US Patent No. 7,910,106 (2011).
114. Bodin K, Ellmerich S, Kahan MC, Tennent GA, Loesch A, Gilbertson JA, et al. Antibodies to human serum amyloid P component eliminate visceral amyloid deposits. *Nature* (2010) 468:93–7. doi: 10.1038/nature09494
115. Richards DB, Cookson LM, Berges AC, Barton SV, Lane T, Ritter JM, et al. Therapeutic clearance of amyloid by antibodies to serum amyloid P component. *N Engl J Med.* (2015) 373:1106–14. doi: 10.1056/NEJMoa1504942
116. Richards DB, Cookson LM, Barton SV, Liefaard L, Lane L, Hutt DF, et al. Repeat doses of antibody to serum amyloid P component clear amyloid deposits in patients with systemic amyloidosis. *Sci Transl Med.* (2018) 10:eaan3128. doi: 10.1126/scitranslmed.aan3128
117. Al-Shawi R, Tennent GA, Millar DJ, Richard-Londt A, Brandner S, Werring DJ, et al. Pharmacological removal of serum amyloid P component from intracerebral plaques and cerebrovascular Aβ amyloid deposits *in vivo*. *Open Biol.* (2016) 6:150202. doi: 10.1098/rsob.150202
118. Charidimou A, Gang Q, Werring DJ. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. *J Neurol Neurosurg Psychiatry* (2012) 83:124–37. doi: 10.1136/jnnp-2011-301308
119. Urbányi Z, Lakics V, Erdó SL. Serum amyloid P component-induced cell death in primary cultures of rat cerebral cortex. *Eur J Pharmacol.* (1994) 270:375–88.
120. Duong T, Acton PJ, Johnson RA. The *in vitro* neuronal toxicity of pentraxins associated with Alzheimer's disease brain lesions. *Brain Res.* (1998) 813:303–12. doi: 10.1016/S0006-8993(98)00966-4
121. Urbányi Z, Laszlo L, Tomasi TB, Toth E, Mekes E, Sass M, et al. Serum amyloid P component induces neuronal apoptosis and beta-amyloid immunoreactivity. *Brain Res.* (2003) 988:69–77. doi: 10.1016/S0006-8993(03)03345-6
122. Urbányi Z, Sass M, Laszy J, Takacs V, Gyertyan I, Pazmany T. Serum amyloid P component induces TUNEL-positive nuclei in rat brain after intrahippocampal administration. *Brain Res.* (2007) 1145:221–6. doi: 10.1016/j.brainres.2007.01.132
123. Pisalyaput K, Tenner AJ. Complement component C1q inhibits b-amyloid- and serum amyloid P-induced neurotoxicity via caspase- and calpain-independent mechanisms. *J Neurochem.* (2008) 104:696–707. doi: 10.1111/j.1471-4159.2007.05012.x
124. Du Clos TW, Mold C, Stump RF. Identification of a polypeptide sequence that mediates nuclear localization of the acute phase protein C-reactive protein. *J Immunol.* (1990) 145:3869–75.
125. Pepys MB, Booth SE, Tennent GA, Butler PJG, Williams DG. Binding of pentraxins to different nuclear structures: C-reactive protein binds to small nuclear ribonucleoprotein particles, serum amyloid P component binds to chromatin and nucleoli. *Clin Exp Immunol.* (1994) 97:152–7. doi: 10.1111/j.1365-2249.1994.tb06594.x
126. Crawford JR, Bjorklund NL, Taglialatela G, Gomer RH. Brain serum amyloid P levels are reduced in individuals that lack dementia while having Alzheimer's disease neuropathology. *Neurochem Res.* (2012) 37:795–801. doi: 10.1007/s11064-011-0674-0
127. Pepys MB, Druguet M, Klass HJ, Dash AC, Mirjah DD, Petrie A. Immunological studies in inflammatory bowel disease. In: Knight J, Porter R, editors. *Immunology of the Gut, Ciba Foundation Symposium 46 (new series).* Amsterdam: Elsevier/Excerpta Medica/North Holland (1977). p. 283–97.
128. Fagan EA, Dyck RF, Maton PN, Hodgson HJF, Chadwick VS, Pepys MB. Serum levels of C-reactive protein in Crohn's disease and ulcerative colitis. *Eur J Clin Invest.* (1982) 12:351–60. doi: 10.1111/j.1365-2362.1982.tb02244.x

129. Saverymuttu SH, Peters AM, Lavender JP, Pepys MB, Hodgson HJF, Chadwick VS. Quantitative fecal indium III-labeled leukocyte excretion in the assessment of disease in Crohn's disease. *Gastroenterology* (1983) 85:1333–9.
130. Saverymuttu SH, Hodgson HJF, Chadwick VS, Pepys MB. Differing acute phase responses in Crohn's disease and ulcerative colitis. *Gut* (1986) 27:809–13. doi: 10.1136/gut.27.7.809
131. WHO Expert Committee on Biological Standardization 37th Report. *WHO Technical Report Series 760*. Geneva (1987). 21–2.
132. Pepys MB. The acute phase response and C-reactive protein. In: Warrell DA, Cox TM, Firth JD, editors. *Oxford Textbook of Medicine, 5th Edn*. Oxford: Oxford University Press (2014).
133. Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB, et al. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med.* (1994) 331:417–24. doi: 10.1056/NEJM199408183310701
134. Haverkate F, Thompson SG, Pyke SDM, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet* (1997) 349:462–6. doi: 10.1016/S0140-6736(96)07591-5
135. Lowe GDO, Pepys MB. C-reactive protein and cardiovascular disease: weighing the evidence. *Curr Cardiovasc Risk Rep*. (2007) 1:72–9. doi: 10.1007/s12170-007-0012-x
136. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: a critical review. *J Int Med.* (2008) 264:295–314. doi: 10.1111/j.1365-2796.2008.02015.x
137. Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* (2010) 375:132–40. doi: 10.1016/S0140-6736(09)61717-7
138. Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med.* (2012) 367:1310–20. doi: 10.1056/NEJMoa1107477
139. C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC), Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, et al. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. *BMJ* (2011) 342:d548. doi: 10.1136/bmj.d548
140. Hill JH, Ward PA. The phlogistic role of C3 leukotactic fragments in myocardial infarcts of rats. *J Exp Med.* (1971) 133:885–900. doi: 10.1084/jem.133.4.885
141. Pepys MB. C-reactive protein fifty years on. *Lancet* (1981) 1:653–6.
142. Pepys MB. C-reactive protein, amyloidosis and the acute phase response. The Goulstonian Lecture. In: Sarner M, editor. *Advanced Medicine*. Tunbridge Wells: Pitman Books Ltd (1982). p. 208–30.
143. Beranek JT. C-reactive protein and complement in myocardial infarction and postinfarction heart failure. *Eur Heart J.* (1997) 18:1834–5. doi: 10.1093/oxfordjournals.eurheartj.a015180
144. Hack CE, Wolbink G-J, Schalkwijk C, Speijer H, Hermens WT, van den Bosch H. A role for secretory phospholipase $A_2$ and C-reactive protein in the removal of injured cells. *Immunol Today* (1997) 18:111–5. doi: 10.1016/S0167-5699(97)01002-5
145. Beranek JT. C-reactive protein in postinfarction heart rupture. *Am Heart J.* (1998) 136:563–4. doi: 10.1016/S0002-8703(98)70237-6
146. Gill R, Kemp JA, Sabin C, Pepys MB. Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats. *J Cereb Blood Flow Metab.* (2004) 24:1214–8. doi: 10.1097/01.WCB.0000136517.61642.99
147. Lu XY, Peckham R, Falabella M, Tsokos GC. Human C-reactive protein augments the gut ischemia/reperfusion injury in mice. *Clin Immunol.* (2007) 123(Suppl. 1):S185–6.
148. Lu X, Li Y, Simovic MO, Peckham R, Wang Y, Tsokos GC, et al. Decay-accelerating factor attenuates C-reactive protein-potentiated tissue injury after mesenteric ischemia/reperfusion. *J Surg Res.* (2011) 167:e103–15. doi: 10.1016/j.jss.2009.10.021
149. Slagman AC, Bock C, Abdel-Aty H, Vogt B, Gebauer F, Janelt G, et al. Specific removal of C-reactive protein by apheresis in a porcine cardiac infarction model. *Blood Purif.* (2011) 31:9–17. doi: 10.1159/000320763
150. Jones NR, Pegues MA, McCrory MA, Singleton W, Bethune C, Baker BF, et al. A selective inhibitor of human C-reactive protein translation is efficacious *in vitro* and in C-reactive protein transgenic mice and humans. *Mol Ther Nucleic Acids* (2012) 1:e52. doi: 10.1038/mtna.2012.44
151. Pegues MA, McCrory MA, Zarjou A, Szalai AJ. C-reactive protein exacerbates renal ischemia-reperfusion injury. *Am J Physiol Renal Physiol.* (2013) 304:F1358–65. doi: 10.1152/ajprenal.00476.2012
152. Szalai AJ, McCrory MA, Xing D, Hage FG, Miller A, Oparil S, et al. Inhibiting C-reactive protein for the treatment of cardiovascular disease: promising evidence from rodent models. *Mediators Inflamm.* (2014) 2014:353614. doi: 10.1155/2014/353614
153. Sheriff A, Schindler R, Vogt B, Abdel-Aty H, Unger JK, Bock C, et al. Selective apheresis of C-reactive protein: a new therapeutic option in myocardial infarction? *J Clin Apher.* (2015) 30:15–21. doi: 10.1002/jca.21344
154. Gao R, Wang L, Bai T, Zhang Y, Bo H, Shu Y. C-reactive protein mediating immunopathological lesions: a potential treatment option for severe influenza A diseases. *EBioMedicine* (2017) 22:133–42. doi: 10.1016/j.ebiom.2017.07.010
155. Thiele JR, Zeller J, Bannasch H, Stark GB, Peter K, Eisenhardt SU. Targeting C-Reactive protein in inflammatory disease by preventing conformational changes. *Mediators Inflamm.* (2015) 2015:372432. doi: 10.1155/2015/372432
156. Bursi F, Weston SA, Killian JM, Gabriel SE, Jacobsen SJ, Roger VL. C-reactive protein and heart failure after myocardial infarction in the community. *Am J Med.* (2007) 120:616–22. doi: 10.1016/j.amjmed.2006.07.039


**Conflict of Interest Statement:** MP is the inventor on patents for miridesap (CPHPC) and miridesap plus anti-SAP antibody: WO 03/013508 A1, "Therapeutic agent for depletion of an unwanted protein population from the plasma"; WO/2009/155962, "Use"; and WO/2009/000926, US7910106 B2, and US9192668 B2, "Combinations of SAP depleting agents and anti-SAP antibodies." MP founded and owns shares in Pentraxin Therapeutics Ltd, the University College London spinout company that owns these patents and EP 0915088B1 A1, "D-proline derivatives."

frontiers in Immunology

REVIEW
published: 13 November 2018
doi: 10.3389/fimmu.2018.02607

# Pentraxins and Fc Receptor-Mediated Immune Responses

*Jinghua Lu*[1], *Carolyn Mold*[2,3], *Terry W. Du Clos*[4] *and Peter D. Sun*[1]*

[1] Structural Immunology Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2] Department of Molecular Genetics and Microbiology, Albuquerque, NM, United States, [3] Department of Internal Medicine, University of New Mexico, Albuquerque, NM, United States, [4] VA Medical Center, Albuquerque, NM, United States

C-reactive protein (CRP) is a member of the pentraxin family of proteins. These proteins are highly conserved over the course of evolution being present as far back as 250 million years ago. Mammalian pentraxins are characterized by the presence of five identical non-covalently linked subunits. Each subunit has a structurally conserved site for calcium-dependent ligand binding. The biological activities of the pentraxins established over many years include the ability to mediate opsonization for phagocytosis and complement activation. Pentraxins have an important role in protection from infection from pathogenic bacteria, and regulation of the inflammatory response. It was recognized early on that some of these functions are mediated by activation of the classical complement pathway through C1q. However, experimental evidence suggested that cellular receptors for pentraxins also play a role in phagocytosis. More recent experimental evidence indicates a direct link between pentraxins and Fc receptors. The Fc receptors were first identified as the major receptors for immunoglobulins. The avidity of the interaction between IgG complexes and Fc receptors is greatly enhanced when multivalent ligands interact with the IgG binding sites and activation of signaling pathways requires Fc receptor crosslinking. Human pentraxins bind and activate human and mouse IgG receptors, FcγRI and FcγRII, and the human IgA receptor, FcαRI. The affinities of the interactions between Fc receptors and pentraxins in solution and on cell surfaces are similar to antibody binding to low affinity Fc receptors. Crystallographic and mutagenesis studies have defined the structural features of these interactions and determined the stoichiometry of binding as one-to-one. Pentraxin aggregation or binding to multivalent ligands increases the avidity of binding and results in activation of these receptors for phagocytosis and cytokine synthesis. This review will discuss the structural and functional characteristics of pentraxin Fc receptor interactions and their implications for host defense and inflammation.

**Keywords: pentraxin, CRP, SAP, Fc receptor activation by pentraxin, structure and function**

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Humanitas Clinical and Research Center, Italy*

***Reviewed by:***
*Annette Karen Shrive, Keele University, United Kingdom*
*Martyn Andrew French, University of Western Australia, Australia*

***Correspondence:**
*Peter D. Sun*
*psun@nih.gov*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *11 September 2018*
***Accepted:*** *23 October 2018*
***Published:*** *13 November 2018*

***Citation:***
*Lu J, Mold C, Du Clos TW and Sun PD (2018) Pentraxins and Fc Receptor-Mediated Immune Responses. Front. Immunol. 9:2607. doi: 10.3389/fimmu.2018.02607*

## INTRODUCTION

Pentraxins are an ancient family of serum proteins that are part of the innate immune system. Pentraxins are defined by a homologous pentraxin (PTX) domain of ∼200 amino acids that contains a calcium-dependent ligand-binding site. The two classical pentraxins, C-reactive protein (CRP) and serum amyloid P component (SAP) are composed of non-covalently linked subunits arranged in a planar cyclic pentamer or hexamer (**Figure 1**) (1). Both CRP and SAP are present in most mammalian species and are represented in evolution as early as the horseshoe crab. A group of proteins designated "long" pentraxins contains the PTX domain along with an additional N-terminal domain (2, 3). This review will focus on the classical or "short" pentraxins, CRP, and SAP, and more specifically the mouse and human proteins and receptors, where the most complete functional studies of CRP and SAP have been done (4, 5).

Pentraxins are pattern-recognition molecules with specificity for damaged cell membranes, nuclear components and microbial antigens (**Table 1** comparing CRP and SAP) (6–9). The prototypic ligand for CRP is phosphocholine (PC) (**Figure 1**). SAP binds phosphoethanolamine (PE) and a number of other ligands, including microbial polysaccharides. CRP was first characterized and named for its binding to the cell wall C-polysaccharide of *Streptococcus pneumoniae*. SAP was initially identified as the precursor for a shared component of amyloid. Each subunit has a single ligand binding site allowing multivalent binding on one face of the pentamer. Multivalent binding by pentraxins initiates complement activation through the classical pathway (10) and promotes recognition and activation of cellular receptors.

CRP and SAP are serum proteins, synthesized in the liver, but differ from each other in expression as well as binding specificity. In humans, SAP is expressed constitutively at moderate concentrations (30 μg/ml) whereas CRP is an acute phase protein that increases dramatically in concentration from <1 μg/ml to several hundred μg/ml during acute inflammation. In the mouse CRP is only found at low concentrations and SAP is an acute phase reactant (11, 12) (**Table 1**).

Immunoglobulin Fc receptors (FcRs) expressed primarily on hematopoietic cells provide essential links between antibody and cellular responses (13, 14). FcR are named for their specificity for different isotypes of immunoglobulins. Structurally IgG receptors, FcγR, as well as the IgE receptor, FcεRI, and the IgA receptor, FcαRI, are members of the immunoglobulin superfamily with two or three C2-type immunoglobulin-like extracellular domains (**Figure 2**). There are multiple human FcγR including the high affinity receptor FcγRI, and several low affinity receptors, FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb, that differ in cell expression and associated signaling pathways. Despite a high degree of sequence identify in their extracellular domains, FcγR have distinct IgG subtype specificities as well as differences in affinity for IgG. FcγR crosslinking is required for signaling through either activating motifs (immunoreceptor tyrosine-based activation motifs, ITAM) or inhibitory motifs (immunoreceptor tyrosine-based inhibitory motifs, ITIM) found in receptor cytoplasmic domains or associated signaling chains (**Figure 2**). This restricts cellular responses such as phagocytosis, cytokine synthesis, and cytolysis to IgG in complex with multivalent antigen. Several structures of the extracellular portions of FcR have been published and similar modes of binding to immunoglobulin Fc domains have been defined (15–19). In all cases, one Fc receptor interacts with both heavy chains of Fc asymmetrically in the lower hinge region of IgG between CH1 and CH2 domains (**Figure 3**). This Fc receptor binding induces a conformational change in the relative orientation of the two antibody heavy chain CH2 domains, such that the two-fold symmetrically positioned CH2 domains observed in the receptor-free antibody structures become asymmetrically positioned to the bound Fc receptor. This obligates immune complex formation as the means for antigen aggregation.

## IDENTIFICATION OF FCγ RECEPTORS AS PENTRAXIN RECEPTORS

Opsonization of bacteria and complement activation were the earliest recognized activities of CRP (10, 20). Using C-polysaccharide-coated erythrocytes as targets, CRP was shown to promote phagocytosis both directly and via complement activation (21). CRP-dependent phagocytosis was inhibited by aggregated IgG. Despite these functional data, identification of the cellular receptors for CRP was controversial for many years. These studies were complicated by the expression of multiple FcγR on different hematopoietic cells. A comparison of the presence of different FcγR on cells with CRP receptors suggested that human FcγRIIa was a major CRP receptor. The recognition between pentraxins and FcγR were established using COS cells transfected with FcγRI and FcγRIIa (22), demonstrating FcγRIIa as the primary CRP receptor on human monocytes (23). Additional studies found that SAP also bound to FcγR to promote phagocytosis (24). In subsequent studies both human CRP and SAP bound and activated human and mouse FcγR on monocytes, macrophages and neutrophils (4, 25). Interestingly, CRP binding to FcγRIIa, the predominant receptor on neutrophils and monocytes, was found to be allele-specific (26). FcγRIIa is found in two allelic variants, which differ in a single amino acid (R or H) at position 131. CRP binding is specific for the R variant whereas IgG binds with higher affinity to the H allelic form.

## STRUCTURAL RECOGNITION OF PENTRAXINS BY FCγ RECEPTORS

Using a solution BIAcore-based binding assay, the binding affinities between CRP, SAP, and the long pentraxin PTX3 and FcγR were examined systematically (**Table 2**). The results showed both a general ∼μM binding affinity between pentraxins and FcγR as well as isoform dependent differences (25). Solution studies using isothermal calorimetry established the affinity for the CRP-FcγRIIa interaction as 4 μM with a stoichiometry of 1:1. As previously seen in experiments

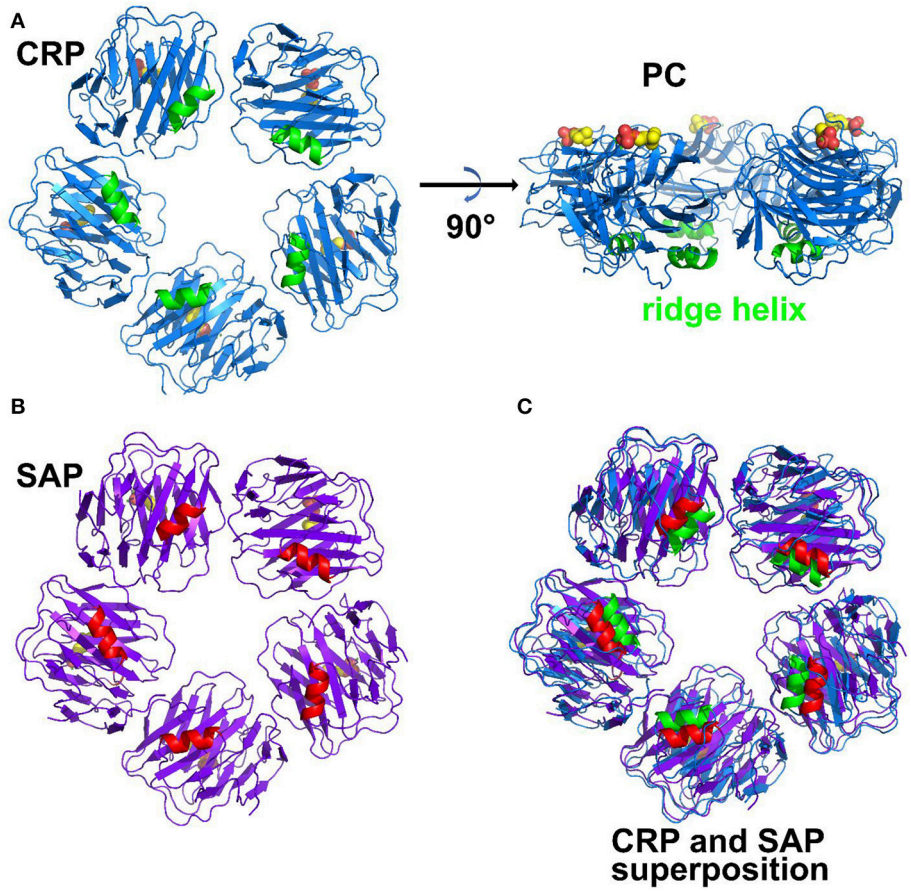


**FIGURE 1 |** Structure of pentraxins. **(A)** Pentameric structure of CRP. The ridge helix is highlighted in green and the bound phosphocholine molecules are shown in bold sticks. **(B)** Pentameric structure of SAP with ridge helix highlighted in red. **(C)** structural superposition of CRP and SAP (Protein Data Bank ID: 1B09, 1SAC).

with peripheral blood cells from FcγRIIa-typed individuals, CRP recognition of FcγRIIa was limited to the R allelic variant.

Structural characteristics of the interaction between pentraxins and FcγRs were determined from the crystal structure of the complex between human SAP and FcγRIIa (**Figure 3**) (25). FcγRIIa docks across the face of SAP opposite the ligand binding face contacting two of the five pentraxin subunits. The diagonal docking structure of FcR on SAP precludes additional receptor from binding to the pentraxin and ensures a 1:1 stoichiometry between the pentraxin and the receptor despite the presence of five identical receptor binding epitopes. There are no significant conformational changes in either SAP or the receptor. The contact area of the two SAP subunits is approximately equal and similar residues are involved, including Tyr 173 and Gln 174 from the ridge helix and residues 200–204 from the C-terminus. Since Fc receptors have a shared structural fold consisting of two tandem Ig-like domains and CRP and SAP share a cyclic pentameric structure, it is likely that the characteristics of the SAP-FcγRIIa co-crystal apply to other pentraxin-FcγR interactions. This mode of binding for CRP to FcγR is consistent with mutations of the putative interface residues Tyr 175 and Leu 176 of CRP impaired FcR binding (27).

The pentraxin binding partially overlap with the IgG binding sites on the receptor. This is consistent with cellular studies showing the inhibition of CRP and SAP function by IgG.

## ACTIVATION OF FCγR BY PENTRAXINS

Pentraxin binding to peripheral blood cells leads to signaling, opsonization and cytokine production. The opsonic activity of CRP was recognized soon after its discovery as a pneumococcal binding protein. CRP increased phagocytosis of C-polysaccharide coated erythrocytes by both complement-dependent and complement-independent mechanisms (21). Aggregated IgG inhibited CRP-dependent phagocytosis. More recently, the direct participation of FcγR in pentraxin-mediated phagocytosis was shown by the co-localization of FcγRIIa with CRP and SAP-opsonized zymosan during phagocytosis by human macrophages. The pentraxin-opsonized zymosan uptake was inhibited by human IgG (25).

**TABLE 1 |** Comparison between CRP and SAP in human and mouse.

| | Human CRP | Human SAP | Mouse CRP | Mouse SAP |
|---|---|---|---|---|
| Pentameric structure | Yes | Yes | Yes | Yes |
| Calcium-dependent ligand binding | Yes | Yes | Yes | Yes |
| Ligands | PC, C-polysaccharide | PE, LPS | PC, C-polysaccharide | PE, LPS |
| Nuclear antigen binding | snRNP, histones | DNA, chromatin | Unknown | Unknown |
| Found in amyloid | No | Yes | No | Yes |
| Acute phase reactant | Yes | No | No | Yes |
| Baseline concentration | <1 μg/ml | 30 μg/ml | <1 μg/ml | 10–100 μg/ml varies by strain |
| FcγR binding | Yes | Yes | Not measured | Not measured |
| FcαR binding | Yes | Yes | Not measured | Not measured |

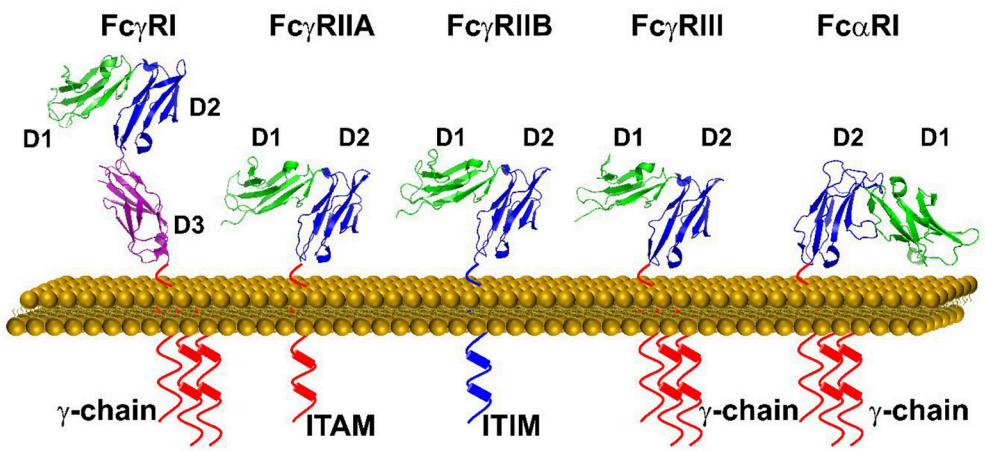


**FIGURE 2 |** A schematic representation of FcγRs and FcαRI on the cell surface. Each tyrosine residue on the cytoplasmic immuno-tyrosine activating motif (ITAM) or immune-tyrosine inhibitory motif (ITIM) are represented by a cylinder. The orientation of each Ig domain in different Fc receptors is based on the structural superposition of the second Ig domain (D2) (Protein Data Bank ID: 3RJD, 1FCG, 2FCB,3AY4, and 1UCT).

Pentraxins also affect leukocyte cytokine production. Many of the ligands for CRP and SAP directly activated toll-like receptors (TLR), and CRP enhanced proinflammatory cytokine production by human peripheral blood mononuclear cells (PBMC) responding to *S. pneumonia* (28). Cytokine responses of PBMC from individuals homozygous for the R-131 allele of FcγRIIa were more affected by CRP than responses of PBMC from individuals homozygous for the H-131 allele. The ability of SAP to induce cytokines (IL-6, IL-8, IL-10) independently of TLR activation was shown using macrophages from mice genetically deficient in Myd88 or RIP2 to prevent TLR and NOD pathway signaling (25).

Additional *in vitro* activities of pentraxins mediated through FcγR are under further investigation. A limiting role for CRP in dendritic cell maturation and T cell activation in a mouse model of experimental autoimmune encephalomyelitis (EAE) was shown to require the inhibitory receptor FcγRIIb (29). Anti-inflammatory and anti-fibrotic activities of SAP on neutrophils and monocyte/macrophage differentiation mediated through FcγR have been reviewed recently (30). The development of SAP as a therapeutic agent for renal and pulmonary fibrosis is discussed further below.

## BINDING AND ACTIVATION OF FCαR BY PENTRAXINS

While FcγRs mediate cellular function of IgG, FcαRI, and FcεRI are high affinity receptors for IgA and IgE, respectively. Both FcαRI and FcεRI consist of two tandem C2-type Ig domains that structurally resemble members of FcγRs (**Figure 2**). The conserved structures of the pentraxins and the shared structural folds of FcR raise the possibility of a broad recognition between pentraxins and FcR. Indeed, FcαRI but not FcεRI showed binding to CRP and SAP in solution (**Table 2**) (31). Although the *in vivo* relevance remains to be established, much of the functional evidence for pentraxin interaction with FcαRI were shown using FcαRI transfected RBL (Rat Basophilic Leukemia) cells. CRP not only bound FcαRI on transfected cells, crosslinking by CRP induced ERK (extracellular signal-regulated kinase) phosphorylation, degranulation, and cytokine production in transfected RBL cells. In addition, CRP induced surface expression of FcαRI on neutrophils, resulting in phagocytosis and TNF-α production (31). Interestingly, FcαRI is structurally more similar to members of inhibitory NK receptor (KIRs) rather than FcγRs. In particular, the juxtaposition of the

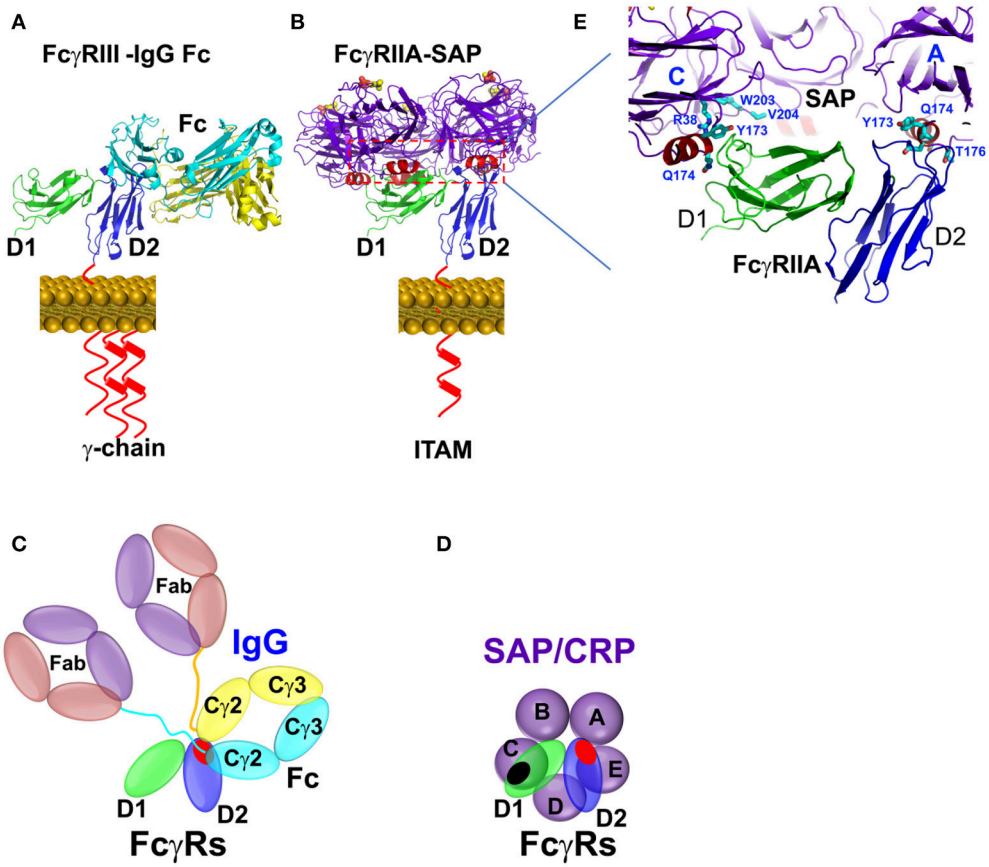


**FIGURE 3 |** Binding mode between Fcγ receptors and pentraxins or IgG antibody. **(A)** The FcγR-IgG Fc interaction was represented by FcγRIIIA-IgG1 Fc complex structure (PDB ID: 1T83). **(B)** The complex structure between SAP and FcγRIIA(PDB ID: 3D5O). **(C,D)** Schematic representation of the interaction between pentraxin-FcγRIIa, IgG1 Fc-FcγRIIa, or FcγRIII. The interfaces are highlighted in shaded red or black circles. **(E)** Binding sites of FcγRIIA on SAP in crystal structure (PDB ID: 3D5O).

two Ig domains on FcαRI is opposite to that of FcγRs (**Figure 2**). In addition, the IgA binding by FcαRI is also distinct from that of IgG binding by FcγRs. Unlike FcγRs which bind to the lower hinge region of IgG, FcαRI recognizes the membrane proximal region of IgA CH3 domain (**Figure 4**) (32). The IgA binding epitope on FcαRI involves the receptor N-terminal D1 domain rather than the IgG recognition by the D2 domain of FcγRs. As a result of the structural difference, CRP binding site on FcαRI appears distinct to that of CRP binding site on FcγRs. Subsequent alanine mutations on the receptor have indicated a number of FcαRI residues important for the pentraxin binding. They include Y35, R48, E49, and R82 on the receptor D1 domain, that partially overlaps with the receptor IgA binding site (**Figure 4**) (33).

**TABLE 2 |** Binding affinity between pentraxins and human Fc receptors in solution (25).

| | Dissociation constants Kd (μM) | | | | |
|---|---|---|---|---|---|
| | **CRP** | **SAP** | **PTX3** | **IgG1** | **IgA** |
| FcγRI | 3.2 | 0.5 | n.d. | 0.03 | – |
| FcγRIIa | 1.9 | 1.4 | 19 | 0.32 | – |
| FcγRIIb | 4.1 | 1.2 | n.d. | 0.64 | – |
| FcγRIII | 4.1 | 2.9 | 1.6 | 0.38 | – |
| FcαRI | 2.8 | 3.2 | n.d. | – | 0.12 |

*n.d. means Not detectable.*

## PENTRAXIN ACTIVATION OF FCγR *IN VIVO*

*In vivo* functions of pentraxins have primarily been studied in mouse models using human injected or transgenic human CRP. Mice genetically deficient in individual FcγR or complement components have been used to delineate the role of FcγR in these functional settings. Human CRP and human SAP were shown to mediate phagocytosis through mouse FcγRs (34). In these studies CRP opsonization was mediated by mouse FcγRI. SAP opsonization was mediated by mouse FcγRI and FcγRIII. CRP effects in the mouse may also be mediated by the inhibitory receptor FcγRIIb as described below. Mouse pentraxin binding to mouse FcγRs has not been studied due to the very low levels of expression of CRP in the mouse.

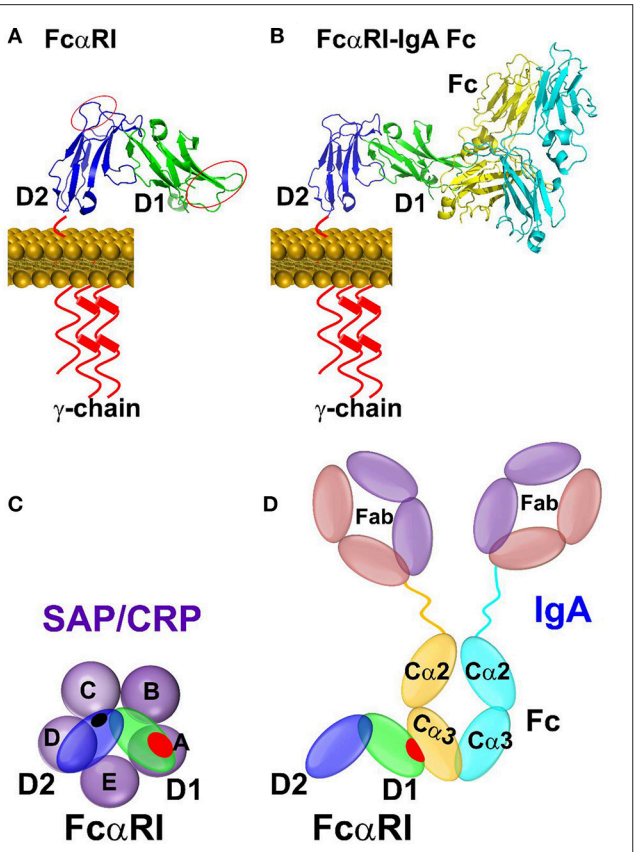


**FIGURE 4 |** Binding mode between FcαRI and pentraxins or IgA antibody. **(A)** The binding sites identified by site-directed mutagenesis between CRP and FcαRI were indicated by red circles on D1 and D2 domain of FcαRI. **(B)** The complex structure between FcαRI and IgA Fc(PDB ID: 1OW0). **(C)** Schematic representation of the interaction between pentraxin-FcαRI based on site-directed mutagenesis and docking studies. **(D)** Schematic representation of the interaction between IgA Fc-FcαRI in crystal structure (PDB ID: 1OW0). The interfaces are highlighted in shaded red or black circles.

The protective role of CRP in pneumococcal Infection was first described in 1989 (35). Mice injected with human CRP were protected from lethal infection with type III or type IV *S. pneumoniae*. These findings have been reproduced using mice transgenic for human CRP. More recently, the relative contributions of complement and FcγR were investigated in CRP-mediated protection from pneumococcal infection (36). CRP was protective in mice genetically deficient in individual or multiple FcγR. However, CRP did not protect C3 or C4-deficient mice from *S. pneumoniae*.

In contrast to the results in pneumococcal infection, CRP protection in a mouse model of endotoxin shock was completely dependent on FcγR (37). It had previously been shown by others that mice transgenic for rabbit CRP or injected with human CRP, but not SAP were resistant to high dose endotoxin lethality (38). In our studies CRP protected wild type mice, but not mice genetically deficient in the FcR γ-chain. Injection of CRP increased serum levels of the anti-inflammatory cytokine IL-10 in wild type, but not γ-chain deficient mice. Mice deficient in FcR γ-chain lack expression of all activating FcγRs, including FcγRI, FcγRIII, and FcγRIV. An additional role for the regulatory FcγRIIb was found in these studies. Mice deficient in FcγRIIb had similar sensitivity to endotoxin shock as wild type mice. However, CRP treatment of FcγRIIb-deficient mice increased their sensitivity to endotoxin shock that was associated with greatly increased serum levels of the pro-inflammatory cytokines TNF-α and IL-12. These results suggest a complex interaction between CRP and FcγR in the regulation of the inflammatory response generated through TLR in response to endotoxin.

A possible clinical correlate of these experiments was found in a study of patients who were hospitalized following severe trauma (39). Patients expressing the CRP-binding allele of FcγRIIa (R-131) were at decreased risk of sepsis and maintained greater MHC class II expression on monocytes in the period following traumatic injury. Decreased class II expression is associated with poor monocyte activation and increased susceptibility to infection in patients following traumatic injury.

Mouse models of immune thrombocytopenic purpura (ITP) have been used extensively to study IgG-FcγR interactions in immune complex and autoimmune disease. Injection of anti-platelet antibodies (rat monoclonal anti-mouse CD41) induces platelet clearance over a period of 24 h with recovery by 48 h. Injection of either human CRP or human IgG (IVIG) prevented platelet depletion in this model and protection was also seen following transfer of CRP-treated spleen cells (40). The transfer ITP model was used to identify the cells and receptors required for CRP effects on immune complex disease. The results showed that CRP treated splenic or bone marrow-derived macrophages transferred suppression and that FcγRI and *syk* activation were required in the donor cells. The protective effect of CRP and CRP-treated macrophages required FcγRIIb in the recipient mice similar to IVIG-mediated suppression of ITP (41).

CRP injection was also protective in immune complex mediated-nephritis (42). Nephrotoxic nephritis (NTN) was induced by immunizing mice with rabbit IgG followed by injection of rabbit antibody to mouse glomerular basement membrane. CRP was protective in this model by an FcγRI, macrophage, and IL-10 dependent pathway, similar to what was found in the endotoxin shock model.

Together these studies demonstrate that CRP can activate macrophages through FcγR to regulate inflammatory responses. In contrast CRP protection against *S. pneumoniae* infection is primarily mediated by complement activation.

Effects of either injected or transgenic CRP have also been seen in more complex autoimmune mouse models (43–45). Injected CRP prevented and treated renal disease in two mouse models of SLE (NZBxNZW F1, and MRL/lpr) similar to its effects in NTN. NZBxNZW F1 mice expressing CRP from a transgene showed delayed development of disease. In these cases, the mechanisms of protection are likely to be complex and have not been fully determined. Overproduction of type I interferon by plasmacytoid dendritic cells in response to immune complexes containing autoantibodies and nuclear antigens is an important contributor to SLE. We recently showed that CRP inhibits interferon production by purified human pDC responding to immune complexes containing lupus autoantibodies and nuclear

antigens (46). pDC express FcγRIIa which mediates uptake of immune complexes that stimulate the type I IFN response through intracellular TLR (47).

Experimental autoimmune encephalomyelitis (EAE) is a model for multiple sclerosis and has been studied extensively as a T cell-mediated autoimmune disease. CRP expressed from a transgene is protective in mouse EAE by shifting the phenotype of autoimmune T cells from $T_H1$ to $T_H2$ (48). More recently, CRP was found to inhibit dendritic cell maturation thereby decreasing antigen-driven T cell activation. The effect of CRP on dendritic cells and its effect on EAE *in vivo* required the inhibitory receptor, FcγRIIb (29).

SAP also regulates the inflammatory response by binding to FcγR. SAP promotes phagocytosis and enhances cytokine production by monocytes and macrophages through activating FcγR as described above. In addition, SAP was identified as a serum factor responsible for inhibiting the differentiation PBMC into fibrocytes, and this effect was inhibited by aggregated IgG (49). *In vivo* SAP attenuated fibrosis in both renal and pulmonary models by regulating macrophage polarization (30, 50). Recombinant human SAP is currently in phase II clinical trials for the treatment of myelofibrosis and idiopathic pulmonary fibrosis (51).

## CONCLUSION

Pentraxins are serum acute phase proteins conserved throughout the animal kingdom. While they are known to activate complement to provide innate immunity against infections, recent work established their role in activating cellular immune functions. Members of the pentraxin family can crosslink and activate a subgroup of Fc receptors upon opsonization, similar to Fc receptor activation by immune-complexes. Because of their ability to activate both soluble and cellular immune responses and their inflammatory nature, pentraxins interface innate, and adaptive immunity. These features are essentially shared with antibodies. However, while antibodies are highly restricted to individual antigenic epitopes, pentraxins are pattern recognition receptors with broad specificity for microbial glycans. They are therefore not redundant but complementary to each other in providing host immune surveillance.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## ACKNOWLEDGMENTS

The funding of this work is provided by the Intramural Research Program (IRP) of National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, and by the Department of Internal Medicine and Department of Molecular Genetics and Microbiology, University of New Mexico.

## REFERENCES

1. Shrive AK, Cheetham GM, Holden D, Myles DA, Turnell WG, Volanakis JE, et al. Three dimensional structure of human C-reactive protein. *Nat Struct Biol.* (1996) 3:346–54. doi: 10.1038/nsb0496-346
2. Alles VV, Bottazzi B, Peri G, Golay J, Introna M, Mantovani A. Inducible expression of PTX3, a new member of the pentraxin family, in human mononuclear phagocytes. *Blood* (1994) 84:3483–93.
3. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
4. Lu J, Marjon KD, Mold C, Du Clos TW, Sun PD. Pentraxins and Fc receptors. *Immunol Rev.* (2012) 250:230–8. doi: 10.1111/j.1600-065X.2012.01162.x
5. Peisajovich A, Marnell L, Mold C, Du Clos TW. C-reactive protein at the interface between innate immunity and inflammation. *Expert Rev Clin Immunol.* (2008) 4:379–90. doi: 10.1586/1744666X.4.3.379
6. Mikolajek H, Kolstoe SE, Pye VE, Mangione P, Pepys MB, Wood SP. Structural basis of ligand specificity in the human pentraxins, C-reactive protein and serum amyloid P component. *J Mol Recogn.* (2011) 24:371–7. doi: 10.1002/jmr.1090
7. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med.* (1971) 136:612–4.
8. Du Clos TW. The interaction of C-reactive protein and serum amyloid P component with nuclear antigens. *Mol Biol Rep.* (1996) 23:253–60.
9. Schwalbe RA, Dahlback B, Coe JE, Nelsestuen GL. Pentraxin family of proteins interact specifically with phosphorylcholine and/or phosphorylethanolamine. *Biochemistry* (1992) 31:4907–15. doi: 10.1021/bi00135a023
10. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. *J Immunol.* (1974) 112:2135–47.
11. Ganapathi MK, Schultz D, Mackiewicz A, Samols D, Hu SI, Brabenec A, et al. Heterogeneous nature of the acute phase response. Differential regulation of human serum amyloid A, C-reactive protein, and other acute phase proteins by cytokines in Hep 3B cells. *J Immunol.* (1988) 141:564–9.
12. Mortensen RF, Beisel K, Zeleznik NJ, Le PT. Acute-phase reactants of mice. II. Strain dependence of serum amyloid P-component (SAP) levels and response to inflammation. *J Immunol.* (1983) 130:885–9.
13. Ravetch JV, Bolland S. IgG Fc receptors. *Annu Rev Immunol.* (2001) 19:275–90. doi: 10.1146/annurev.immunol.19.1.275
14. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol.* (2008) 8:34–47. doi: 10.1038/nri2206
15. Tamm A, Schmidt RE. IgG binding sites on human Fc gamma receptors. *Int Rev Immunol.* (1997) 16:57–85. doi: 10.3109/08830189709045703
16. Maxwell KF, Powell MS, Hulett MD, Barton PA, McKenzie IF, Garrett TP, et al. Crystal structure of the human leukocyte Fc receptor, Fc gammaRIIa. *Nat Struct Biol.* (1999) 6:437–42.
17. Sondermann P, Huber R, Jacob U. Crystal structure of the soluble form of the human fcgamma-receptor IIb: a new member of the immunoglobulin superfamily at 1.7 A resolution. *EMBO J.* (1999) 18:1095–103. doi: 10.1093/emboj/18.5.1095
18. Zhang Y, Boesen CC, Radaev S, Brooks AG, Fridman WH, Sautes-Fridman C, et al. Crystal structure of the extracellular domain of a human Fc gamma RIII. *Immunity* (2000) 13:387–95. doi: 10.1016/S1074-7613(00)00038-8
19. Garman SC, Wurzburg BA, Tarchevskaya SS, Kinet JP, Jardetzky TS. Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcŒRIa. *Nature* (2000) 406:259–66. doi: 10.1038/35018500

20. Kindmark CO. Stimulating effect of C-reactive protein on phagocytosis of various species of pathogenic bacteria. *Clin Exp Immunol.* (1971) 8:941–8.
21. Mortensen RF, Osmand AP, Lint TF, Gewurz H. Interaction of C-reactive protein with lymphocytes and monocytes: complement-dependent adherence and phagocytosis. *J Immunol.* (1976) 117:774–81.
22. Marnell LL, Mold C, Volzer MA, Burlingame RW, Du Clos TW. C-reactive protein binds to Fc gamma RI in transfected COS cells. *J Immunol.* (1995) 155:2185–93.
23. Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW. The major receptor for C-reactive protein on leukocytes is Fcγ receptor II. *J Exp Med.* (1999) 190:585–90. doi: 10.1084/jem.190.4.585
24. Bharadwaj D, Mold C, Markham E, Du Clos TW. Serum amyloid P component binds to Fc gamma receptors and opsonizes particles for phagocytosis. *J Immunol.* (2001) 166:6735–41. doi: 10.4049/jimmunol.166.11.6735
25. Lu J, Marnell LL, Marjon KD, Mold C, Du Clos TW, Sun PD. Structural recognition and functional activation of FcgammaR by innate pentraxins. *Nature* (2008) 456:989–92. doi: 10.1038/nature07468
26. Stein MP, Edberg JC, Kimberly RP, Mangan EK, Bharadwaj D, Mold C, et al. C-reactive protein binding to FcγRIIa on human monocytes and neutrophils is allele-specific. *J Clin Invest.* (2000) 105:369–76. doi: 10.1172/JCI7817
27. Bang R, Marnell L, Mold C, Stein MP, Clos KT, Chivington-Buck C, et al. Analysis of binding sites in human C-reactive protein for FcγRI, FcγRIIA, and C1q by site-directed mutagenesis. *J Biol Chem.* (2005) 280:25095–102. doi: 10.1074/jbc.M504782200
28. Mold C, Du Clos TW. C-reactive protein increases cytokine responses to *Streptococcus pneumoniae* through interactions with Fc gamma receptors. *J Immunol.* (2006) 176:7598–604. doi: 10.4049/jimmunol.176.12.7598
29. Jimenez RV, Wright TT, Jones NR, Wu J, Gibson AW, Szalai AJ. C-reactive protein impairs dendritic cell development, maturation, and function: implications for peripheral tolerance. *Front Immunol.* (2018) 9:372. doi: 10.3389/fimmu.2018.00372
30. Cox N, Pilling D, Gomer RH. Serum amyloid P: a systemic regulator of the innate immune response. *J Leukoc Biol.* (2014) 96:739–43. doi: 10.1189/jlb.1MR0114-068R
31. Lu J, Marjon KD, Marnell LL, Wang R, Mold C, Du Clos TW, et al. Recognition and functional activation of the human IgA receptor (FcalphaRI) by C-reactive protein. *Proc Natl Acad Sci USA.* (2011) 108:4974–9. doi: 10.1073/pnas.1018369108
32. Herr AB, Ballister ER, Bjorkman PJ. Insights into IgA-mediated immune responses from the crystal structures of human FcalphaRI and its complex with IgA1-Fc. *Nature* (2003) 423:614–20. doi: 10.1038/nature01685
33. Lu J, Marjon KD, Mold C, Marnell L, Du Clos TW, Sun P. Pentraxins and IgA share a binding hot-spot on FcalphaRI. *Protein Sci.* (2014) 23:378–86. doi: 10.1002/pro.2419
34. Mold C, Gresham HD, Du Clos TW. Serum amyloid P component and C-reactive protein mediate phagocytosis through murine Fc gamma Rs. *J Immunol.* (2001) 166:1200–5. doi: 10.4049/jimmunol.166.2.1200
35. Mold C, Nakayama S, Holzer TJ, Gewurz H, Du Clos TW. C-reactive protein is protective against *Streptococcus pneumoniae* infection in mice. *J Exp Med.* (1981) 154:1703–8. doi: 10.1084/jem.154.5.1703
36. Mold C, Rodic-Polic B, Du Clos TW. Protection from *Streptococcus pneumoniae* infection by C-reactive protein and natural antibody requires complement but not Fc gamma receptors. *J Immunol.* (2002) 168:6375–81. doi: 10.4049/jimmunol.168.12.6375
37. Mold C, Rodriguez W, Rodic-Polic B, Du Clos TW. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. *J Immunol.* (2002) 169:7019–25. doi: 10.4049/jimmunol.169.12.7019
38. Xia D, Samols D. Transgenic mice expressing rabbit C-reactive protein are resistant to endotoxemia. *Proc Natl Acad Sci USA.* (1997) 94:2575–80. doi: 10.1073/pnas.94.6.2575
39. West SD, Ziegler A, Brooks T, Krencicki M, Myers O, Mold C. An FcgammaRIIa polymorphism with decreased C-reactive protein binding is associated with sepsis and decreased monocyte HLA-DR expression in trauma patients. *J Trauma Acute Care Surg.* (2015) 79:773–81. doi: 10.1097/TA.0000000000000837
40. Marjon KD, Marnell LL, Mold C, Du Clos TW. Macrophages activated by C-reactive protein through Fc gamma RI transfer suppression of immune thrombocytopenia. *J Immunol.* (2009) 182:1397–403. doi: 10.4049/jimmunol.182.3.1397
41. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* (2001) 291:484–6. doi: 10.1126/science.291.5503.484
42. Rodriguez W, Mold C, Kataranovski M, Hutt JA, Marnell LL, Verbeek JS, et al. C-reactive protein-mediated suppression of nephrotoxic nephritis: role of macrophages, complement, and Fcgamma receptors. *J Immunol.* (2007) 178:530–8. doi: 10.4049/jimmunol.178.1.530
43. Rodriguez W, Mold C, Marnell LL, Hutt J, Silverman GJ, Tran D, et al. Prevention and reversal of nephritis in MRL/lpr mice with a single injection of C-reactive protein. *Arthritis Rheum.* (2006) 54:325–35. doi: 10.1002/art.21556
44. Rodriguez W, Mold C, Kataranovski M, Hutt J, Marnell LL, Du Clos TW. Reversal of ongoing proteinuria in autoimmune mice by treatment with C-reactive protein. *Arthritis Rheum.* (2005) 52:642–50. doi: 10.1002/art.20846
45. Szalai AJ, Weaver CT, McCrory MA, van Ginkel FW, Reiman RM, Kearney JF, et al. Delayed lupus onset in (NZB x NZW)F1 mice expressing a human C-reactive protein transgene. *Arthritis Rheum.* (2003) 48:1602–11. doi: 10.1002/art.11026
46. Mold C, Du Clos TW. C-reactive protein inhibits plasmacytoid dendritic cell interferon responses to autoantibody immune complexes. *Arthritis Rheum.* (2013) 65:1891–901. doi: 10.1002/art.37968
47. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol.* (2008) 8:594–606. doi: 10.1038/nri2358
48. Szalai AJ, Nataf S, Hu X-Z, Barnum SR. Experimental allergic encephalomyelitis is inhibited in transgenic mice expressing human C-reactive protein. *J Immunol.* (2002) 168:5792–7. doi: 10.4049/jimmunol.168.11.5792
49. Pilling D, Buckley CD, Salmon M, Gomer RH. Inhibition of fibrocyte differentiation by serum amyloid P. *J Immunol.* (2003) 171:5537–46. doi: 10.4049/jimmunol.171.10.5537
50. Castano AP, Lin SL, Surowy T, Nowlin BT, Turlapati SA, Patel T, et al. Serum amyloid P inhibits fibrosis through Fc gamma R-dependent monocyte-macrophage regulation *in vivo*. *Sci Transl Med.* (2009) 1:5ra13. doi: 10.1126/scitranslmed.3000111
51. Raghu G, van den Blink B, Hamblin MJ, Brown AW, Golden JA, Ho LA, et al. Effect of recombinant human pentraxin 2 vs placebo on change in forced vital capacity in patients with idiopathic pulmonary fibrosis: a randomized clinical trial. *JAMA.* (2018) 319:2299–307. doi: 10.1001/jama.2018.6129


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 29 November 2018
doi: 10.3389/fimmu.2018.02808

# The Influence of Pentraxin 3 on the Ovarian Function and Its Impact on Fertility

*Antonella Camaioni, Francesca Gioia Klinger, Luisa Campagnolo and Antonietta Salustri**

*Histology and Embryology Section, Department of Biomedicine and Prevention, University of Rome "Tor Vergata," Rome, Italy*

Follicular development is a highly coordinated process that in humans takes more than 6 months. Pituitary gonadotropins and a variety of locally produced growth factors and cytokines are involved in determining a precise sequence of changes in cell metabolism, proliferation, vascularization, and matrix remodeling in order to obtain a follicle with full ovulatory and steroidogenic capability. A low-grade inflammation can alter such processes leading to premature arrest of follicular growth and female reproductive failure. On the other hand, factors that are involved in inflammatory response as well as in innate immunity are physiologically upregulated in the follicle at the final stage of maturation and play an essential role in ovulation and fertilization. The generation of pentraxin 3 (PTX3) deficient mice provided the first evidence that this humoral pattern recognition molecule of the innate immunity has a non-redundant role in female fertility. The expression, localization, and molecular interactions of PTX3 in the periovulatory follicle have been extensively studied in the last 10 years. In this review, we summarize findings demonstrating that PTX3 is synthesized before ovulation by cells surrounding the oocyte and actively participates in the organization of the hyaluronan-rich provisional matrix required for successful fertilization. Data in humans tend to confirm these findings, indicating PTX3 as a biomarker of oocyte quality. Moreover, we discuss the emerging evidence that in humans altered PTX3 systemic levels, determined by genetic variations and/or low-grade chronic inflammation, can also impact the growth and development of the follicle and affect the incidence of ovarian disorders.

**Keywords: PTX3, fertility, PCOS, ovarian disorders, theca cells, follicle growth, cumulus matrix**

OPEN ACCESS

***Edited by:***
*Cecilia Garlanda, Humanitas Università, Italy*

***Reviewed by:***
*Chiara Agostinis, University of Trieste, Italy*
*Simon John Clark, University of Manchester, United Kingdom*

***Correspondence:**
*Antonietta Salustri*
*salustri@med.uniroma2.it*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *17 September 2018*
***Accepted:*** *14 November 2018*
***Published:*** *29 November 2018*

***Citation:***
*Camaioni A, Klinger FG, Campagnolo L and Salustri A (2018) The Influence of Pentraxin 3 on the Ovarian Function and Its Impact on Fertility. Front. Immunol. 9:2808. doi: 10.3389/fimmu.2018.02808*

## INTRODUCTION

The ovary is the organ assigned to the cyclic production of a mature egg as well as of steroid hormones that, acting locally and systemically, influence female fertility and metabolic activity of many tissues. These functions are accomplished by ovarian follicles in which growth and maturation of the oocyte occur in parallel with proliferation and differentiation of epithelial somatic cells, named granulosa cells. During its development, the follicle induces the formation of a specialized connective tissue, the theca layer, which organizes an extended network of blood vessels supplying the avascular multilayered follicle cells with nutrients, oxygen, and pituitary gonadotropins. Theca cells are also directly involved in the ovarian endocrine function producing androgens that granulosa cells convert to estrogens. As expected in a connective tissue, immune cells are present in the theca layer of the follicle and their number and type change at different follicle stages. Strong evidences

indicate that the immune system plays an important role in the physiology of the ovary (1). Depletion of macrophages/dendritic cells in CD11c-diptheria toxin receptor transgenic mice resulted in loss of ovarian vascular integrity, reduction in mature follicles and impaired ovulation (2–4). Immune cells are also essential for vasculature invasion of luteinized theca and granulosa cells and for the formation of the corpus luteum (2, 3, 5, 6). Of note, before ovulation also granulosa cells acquire an inflammatory and immune–like phenotype producing prostaglandins, inflammatory cytokines, chemokines, and innate immune components that play an essential role in ovulation and fertilization (7–9). Uncontrolled systemic pro-inflammatory conditions alter ovarian homeostasis and have a negative impact on follicular dynamics. Indeed, it has been proposed that even a low-grade chronic inflammation and a small imbalance between pro- and anti-inflammatory cytokines play a role in the pathogenesis of polycystic ovarian syndrome (PCOS) (10, 11), characterized by follicle growth alteration and oligo or anovulatory cycles.

Here we will review the ovarian expression and biological function of pentraxin 3 (PTX3), a multifunctional protein implicated in innate immunity response, regulation of inflammation, angiogenesis and formation and remodeling of the extracellular matrix.

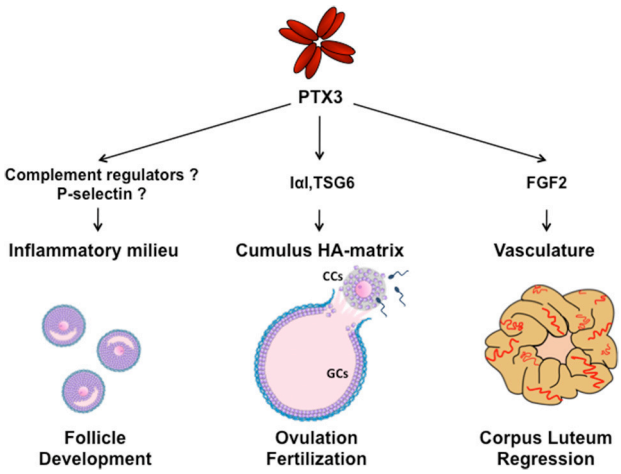


**FIGURE 1 |** Schematic representation of molecular interactions and functions of PTX3 during folliculogenesis. SNP haplotypes of PTX3 and altered circulating PTX3 levels in reproductive disorders suggest a role of PTX3 in the control of ovarian immune milieu during follicle development. In the preovulatory follicle, PTX3 is expressed by CCs and interacts with IαI and TSG6 for organizing the HA matrix, which is essential for oocyte ovulation and fertilization. After ovulation, PTX3 is expressed by stromal and endothelial cells of the corpus luteum and, by sequestering FGF2, is involved in vasculature involution during its regression. (CCs, cumulus cells; GCs, granulosa cells).

## THE LONG PENTRAXIN PTX3: GENE, STRUCTURE AND LIGANDS

PTX3 is a glycoprotein assembled to form an octameric complex stabilized by intermolecular disulfide bonds (12–14). The primary sequence of PTX3 is highly conserved among species and consists of two structural domains: a C-terminal region showing homology with the classical short pentraxin C-reactive protein (CRP) and serum amyloid P component (SAP), and a unique N-terminal domain that has no homology with any other known protein (15). The PTX3 gene is arranged in three exons, with the first and second coding the signal peptide and the N-terminal domain, and the third exon coding the C-terminal pentraxin domain. PTX3 is released by peripheral blood leukocytes and myeloid dendritic cells following stimulation with pro-inflammatory cytokines (IL-1 and TNF-α), agonists of TLR or microbial components (16). PTX3 production is also stimulated in myeloid cells by the anti-inflammatory cytokine IL-10, which is essential for damping inflammation and preventing tissue damage (17). Human neutrophils store PTX3 in lactoferrin-positive granules and rapidly release it at the inflammatory site (18). Other cell types produce PTX3 locally in response to inflammatory conditions and appropriate stimuli: smooth muscle cells, fibroblasts, adipocytes, chondrocytes, mesangial, endothelial, mesenchymal stroma cells, and ovarian cells (19). PTX3 has multifunctional properties for its capacity to interact with different types of ligands (19). In particular, PTX3 plays a non-redundant role in innate immunity by opsonizing selected pathogens and binding and facilitating clearance of apoptotic cells (20, 21). PTX3 modulates the inflammatory reaction by binding elements of the complement cascade and regulating complement activation. It interacts with surface-bound C1q, ficolin 1, ficolin 2 and mannose binding lectin and activates the classical and lectin complement pathways (22–26). On the other hand, PTX3 modulates the alternative complement pathway by recruiting the factor H and enhancing the inactivation of C3b to iC3b, both recognized by the leukocyte receptor CD11/CD18 (27, 28). Coating of microbes and apoptotic cells by PTX3 would help phagocytosis during infection and sterile inflammation without inducing excessive complement activation and tissue harm. PTX3 also binds Fibroblast Growth Factor-2 (FGF2) and sequesters the growth factor in an inactive form, thus modulating angiogenesis in various physio-pathological conditions (29). Interaction of PTX3 with plasminogen and fibrin in wounding or injured tissue matrix has been recently demonstrated. It allows migration of macrophages and mesenchymal stroma cells by promoting pericellular fibrinolysis (30). PTX3 is expressed at specific sites and times during the ovarian cycle and play different roles (**Figure 1**).

## PTX3 EXPRESSION IN THE OVARY

PTX3 is specifically expressed by a small group of granulosa cells surrounding the oocyte, namely cumulus cells, following LH or hCG stimulation of preovulatory follicles. This cell subpopulation differs in many aspects from the majority of granulosa cells and have different fate. Granulosa cells produce PGE2 and a series of EGF-like growth factors and cytokines under the LH/hCG stimulation. They upregulate proteolytic activity in the theca, leading to matrix degradation, cell death and break at

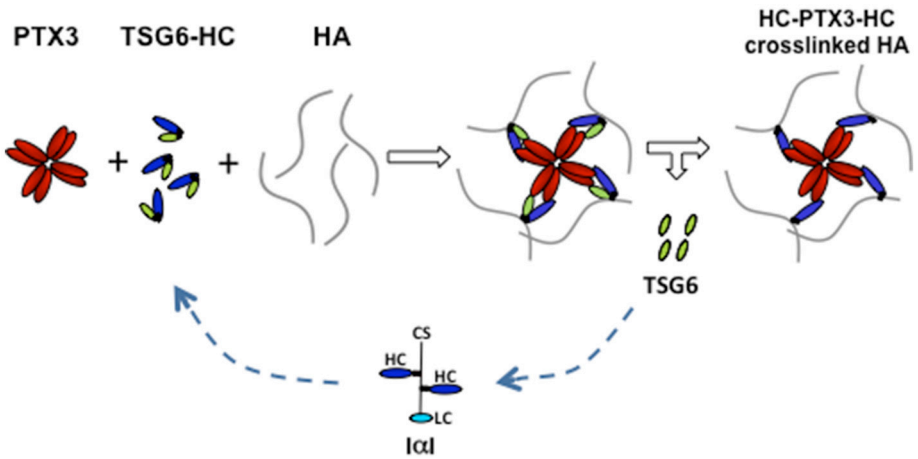


**FIGURE 2 |** Proposed model of PTX3/HC/TSG6/HA interactions in cumulus matrix. During cumulus matrix formation, the binding of HA-linked HCs to the multimeric PTX3 molecule allows the crosslinking of several HA strands. TSG6, that catalyzes the transfer of HCs from CS of IαI to HA through the formation of an intermediate complex (TSG6-HC), also binds to PTX3. These interactions facilitate the simultaneous and coordinated integration of HCs and PTX3 within the cumulus matrix. (CS, chondroitin sulfate; LC, light chain; HC, heavy chain; HA, hyaluronan).

the site of follicle wall facing the ovarian surface (9). Conversely, the oocyte modulates the response of cumulus cells to granulosa cell-derived factors mentioned above thereby inducing these cells to synthesize an extensive extracellular matrix, which facilitates the oocyte release and *in vivo* fertilization (31–33) (**Figure 1**). Mechanical analysis of the rheological properties of this matrix by colloidal-probe atomic force microscopy showed that it is extremely soft and with mucoelastic characteristics (34). The main component of such peculiar matrix is hyaluronan (HA), a long polysaccharide synthesized by HAS2 and organized by proteins in a highly hydrated mesh-like structure, which increases the space among the cells and consequently the overall volume of the cumulus cell oocyte complex (COC) (35, 36). For this characteristic, the process is named cumulus expansion.

PTX3 is one of the most upregulated genes by the oocyte in the mouse cumulus cells before ovulation and is involved in cumulus matrix formation (37). Deletion of *Ptx3* gene in mice results in female infertility for the failure of oocyte fertilization due to ovulation of abnormal COCs. In COC ovulated by *Ptx3* deficient mice cumulus cells appear to form a uniform unstable mass, rather than surrounding a central positioned oocyte, and quickly disperse in the oviduct (38). *In vitro* studies demonstrated that $Ptx3^{-/-}$ COC induced to expand *in vitro* is able to synthesize HA at the normal rate but this polymer is released into the medium, instead being organized in a matrix. The normal matrix phenotype can be restored *in vitro* by stimulating $Ptx3^{-/-}$ COCs in the presence of the recombinant full length PTX3 (rhPTX3) or the recombinant N-terminal region (rhNter-PTX3), but not by the C-terminal fragment of the protein (39). Therefore, although short pentraxins have the ability to bind to some matrix components (40), the action of PTX3 is distinct being fully exerted through the unique sequence of the molecule, then assigning a specific role to PTX3 in HA matrix organization (**Figure 1**).

PTX3 does not bind to HA but can bind to inter-α-trypsin inhibitor (IαI) proteoglycan and tumor necrosis factor-inducible gene 6 (TSG-6) protein (38, 39). The former is mainly synthesized by the liver and circulating in the blood (41), while the latter is synthesized by granulosa cells and cumulus cells concomitantly to HA and PTX3 (38). IαI is a peculiar proteoglycan consisting in a protein carrying one chondroitin sulfate (CS) chain, called bikunin, to which two homologous proteins, named heavy chains (HCs), are linked to the CS in the Golgi through an ester bond (41). The increased vessel permeability in the periovulatory follicle facilitates the diffusion of IαI in the follicular fluid (42, 43) and the HCs are translocated from the CS to the elongating HA polymers by TSG-6, which catalyzes the transfer via a transesterification reaction. Blocking HC integration in the cumulus matrix by the deletion of *bikunin* (which prevents the assembly of intact IαI) or *Tsg6* gene in mice produces female sterility and cumulus matrix instability, as in *Ptx3* null mice (38, 44, 45). PTX3 does not influence the transfer of HCs to HA, but it interacts with HCs in biological context as assessed by co-localization and co-precipitation from COC matrix extracts (39). In addition, the HC binding site resides in the PTX3 N-terminal domain and a monoclonal antibody inhibiting their interaction neutralizes full-length rhPTX3 in restoring normal phenotype in *Ptx3* deficient COCs (39). Site direct mutagenesis of cysteines forming disulphide bonds revealed the relevance of PTX3 multimeric state in matrix formation and suggested that its octameric structure provides at least four binding sites for HCs (12, 46). Thus, it has been hypothesized that multimeric PTX3 might stabilize the HA network by binding several HCs covalently linked to distinct HA molecules, acting as a "node" (39, 46) (**Figure 2**). TSG-6 has an HA binding capacity and PTX3 has multiple binding sites for this protein, as found for HCs (46, 47). However, several lines of evidence do not support the possibility that TSG-6 directly participates in crosslinking HA.

First, matrix formation is not inhibited by HA hexasaccharides competing with the TSG-6 binding to HA (48). In agreement, mutants of TSG-6 with highly reduced HA binding capacity do support matrix assembly of *Tsg-6* deficient COC *in vitro* (49). Finally, during expansion, all TSG-6 molecules form covalent complexes with individual HCs that act as intermediates in the transfer reaction. On these bases, it has been proposed that "the binding of TSG-6 to PTX3 might favor the interaction of PTX3 with HCs committed to link with HA (those in TSG6-HC complexes), leading to the integration of PTX3 into the matrix at the same time as, and in coordinate fashion to, HCs" (39) (**Figure 2**). This hypothesis found a strong support in an *in vitro* binding assay where PTX3, IαI, and TSG-6 are added to an immobilized HA film in a controlled sequence. The results demonstrated that PTX3 can be incorporated into the HA film only if it is pre-mixed with IαI and TSG-6 (47).

Interestingly, HA-HC-PTX3 complex is also formed in human amniotic membrane and is reported to exert anti-inflammatory and anti-scarring actions in inflamed tissues by inhibiting M1 macrophages infiltration and further polarizing them toward M2 phenotype (50). Then, it is possible that HC-PTX3 interaction in the cumulus matrix, besides having a structural role, could also protect the oocyte by dampening the activity of leukocytes in the reproductive tracts and preserving the microenvironment for optimal fertilization. The presence of PTX3 in the cumulus matrix and in the follicular fluids aspirated from patients undergoing IVF suggest that this molecule might have the same role in human female fertility (38).

Following the expulsion of the COC, theca cells, fibroblasts, and endothelial cells invade the granulosa cell layers by initiating the formation of the corpus luteum. Luteal theca and granulosa cells produce progesterone, which sustains embryo implantation. An intensive formation of blood vessels occurs during the maturation of the corpus luteum producing one of the greatest rates of blood flow of any tissue in the body (51). In the absence of pregnancy, this gland loses its function and structure and undergo to regression. Transcriptome analysis of the bovine corpus luteum isolated from animals treated with prostaglandin F2alpha, the physiological inducer of luteolysis in most domestic animals and likely in primates, showed a significant upregulation of PTX3 expression at the early stage of luteal regression, concomitant with FGF2 expression. Either luteal endothelial and steroidogenic cells showed this ability *in vitro* (52, 53). It has been then suggested that PTX3 might participate in the involution of microvasculature during corpus luteum regression by sequestering FGF2 and preventing its pro-angiogenic activity (53, 54) (**Figure 1**). In contrast, one study performed in sheep reported that PTX3 expression is downregulated during physiological regression of corpus luteum while it is maintained in gravidic corpus luteum (55). If these conflicting results depend on differences among species or between physiological and experimental-induced luteolysis remains to be determined. In any event, the evidence that PTX3 is expressed and modulated in the corpus luteum urges further studies.

## IMMUNE CELL-DERIVED PTX3 AND HUMAN OVARIAN DISORDERS

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting 5-10% of premenopausal women. It is characterized by hyperandrogenism, oligo- anovulation and polycystic ovary, often associated with obesity and other metabolic dysfunctions (56). The syndrome is caused by the pronounced increase in the number of small-mid antral follicles (2–9 mm) unable to complete the growth (17–20 mm) and proceed to maturation. Theca layer is thicker and the cells produce an excess of androgens. Such alteration in the development of ovarian follicles is associated to low-grade inflammation (10, 11) and local infiltration of immune cells in the theca layer (57–61). Of note, it has been reported that the short CRP and classical pro-inflammatory cytokines levels are slightly but significantly elevated while the long PTX3 level is reduced in the blood of PCOS and overweight women (62–67). Based on the protective and anti-inflammatory role recently assigned to PTX3 (68), it is likely that reduced PTX3 levels would increase the sensitivity of the ovary to the inflammatory status. In agreement, a lower level of circulating IL-10 was found in PCOS patients and linked to higher risk to develop the ovarian hyperstimulation syndrome (69), an exacerbated reaction to hormone stimulation in assisted reproductive programs characterized by local and generalized increased capillary permeability and enhanced production of inflammatory cytokines by immune cells. These findings further support the importance of appropriate balance of immune cell types in controlling and promoting follicle development (**Figure 1**).

The altered mechanisms underlying the excessive follicle formation in PCOS has not been clearly understood. The overexpression of LH receptor mRNA in granulosa cells and under-expression of GDF9 by the oocyte in PCOS follicles compared to normal follicles of the same size suggest a premature terminal differentiation (70, 71) and closely resemble the conditions promoting polyovulation and dizygotic twinning in sheep (72, 73). Noteworthy, a study on PTX3 single-nucleotide polymorphisms (SNPs) performed in Gambia, where the dizygotic twinning incidence is the highest worldwide (74), showed that a specific three SNP haplotype GAG (at positions rs2305619, rs3816527 and rs1840680) is more frequent in mothers of dizygotic twins compared to women without a history of twinning (75). In addition, in another study performed on Ghanaian women, the same haplotype positively correlates with the number of children given birth during the lifetime (76). Unfortunately, the twinning frequency was not analyzed in this study, but Ghana is another African state with high twinning incidence (77). Moreover, the GAG haplotype confers resistance to *Mycobacterium tuberculosis* and decreased the risk of pulmonary tuberculosis (78), indicating that the protein is functional.

All together, these data indicate that altered expression of PTX3 can influence the ovarian microenvironment and alter

folliculogenesis, likely deregulating the fine-tuned inflammatory milieu of the follicle (**Figure 1**).

## AUTHOR CONTRIBUTIONS

AS prepared the manuscript draft in consultation with AC. FK and LC participated in revising it critically for important intellectual content. All authors contributed to and approved the final version of the manuscript.

## ACKNOWLEDGMENTS

Thanks to Serena Marcozzi for graphical assistance.

## REFERENCES


1. Wu R, Van der Hoek KH, Ryan NK, Norman RJ, Robker RL. Macrophage contributions to ovarian function. *Hum Reprod Update* (2004) 10:119–33. doi: 10.1093/humupd/dmh011
2. Turner EC, Hughes J, Wilson H, Clay M, Mylonas KJ, Kipari T, et al. Conditional ablation of macrophages disrupts ovarian vasculature. *Reprod* (2011) 141:821–31. doi: 10.1530/REP-10-0327
3. Cohen-Fredarow A, Tadmor A, Raz T, Meterani N, Addadi Y, Nevo N, et al. Ovarian dendritic cells act as a double-edged pro-ovulatory and anti-inflammatory sword. *Mol Endocrinol.* (2014) 28:1039–54. doi: 10.1210/me.2013-1400
4. Ono Y, Nagai M, Yoshino O, Koga K, Nawaz A, Hatta H, et al. CD11c+ M1-like macrophages (MΦs) but not CD206+ M2-like MΦ are involved in folliculogenesis in mice ovary. *Sci Rep*. (2018) 8:8171. doi: 10.1038/s41598-018-25837-3
5. Połeć A, Ráki M, Åbyholm T, Tanbo TG, Fedorcsák P. Interaction between granulosa-lutein cells and monocytes regulates secretion of angiogenic factors *in vitro*. *Hum Reprod*. (2011) 26:2819–29. doi: 10.1093/humrep/der216
6. Care AS, Diener KR, Jasper MJ, Brown HM, Ingman WV, Robertson SA. Macrophages regulate corpus luteum development during embryo implantation in mice. *J Clin Invest.* (2013) 123:3472–87. doi: 10.1172/JCI60561
7. Espey LL, Richards JS. Ovulation. In: Knobil E and Neill JD, editors. *The Physiology of Reproduction.* New York, NY: Raven Press (2006). p. 425–500.
8. Richards JS, Liu Z, Shimada M. Immune-like mechanisms in ovulation. *Trends Endocrinol Metab.* (2008) 19:191–6. doi: 10.1016/j.tem.2008.03.001
9. Akison LK, Robertson SA, Gonzalez MB, Richards JS, Smith CW, Russell DL, et al. Regulation of the ovarian inflammatory response at ovulation by nuclear progesterone receptor. *Am J Reprod Immunol.* (2018) 79:e12835. doi: 10.1111/aji.12835
10. Repaci A, Gambineri A, Pasquali R. The role of low-grade inflammation in the polycystic ovary syndrome. *Mol Cell Endocrinol.* (2011) 335:30–41. doi: 10.1016/j.mce.2010.08.002
11. Boots CE, Jungheim ES. Inflammation and human ovarian follicular dynamics. *Semin Reprod Med.* (2015) 33:270–5. doi: 10.1055/s-0035-1554928
12. Inforzato A, Rivieccio V, Morreale AP, Bastone A, Salustri A, Scarchilli L, et al. Structural characterization of PTX3 disulfide bond network and its multimeric status in cumulus matrix organization. *J Biol Chem.* (2008) 283:10147–61. doi: 10.1074/jbc.M708535200
13. Inforzato A, Baldock C, Jowitt TA, Holmes DF, Lindstedt R, Marcellini M, et al. The angiogenic inhibitor long pentraxin PTX3 forms an asymmetric octamer with two binding sites for FGF2. *J Biol Chem.* (2010) 285:17681–92. doi: 10.1074/jbc.M109.085639
14. Inforzato A, Reading PC, Barbati E, Bottazzi B, Garlanda C, Mantovani A. The "sweet" side of a long pentraxin: how glycosylation affects PTX3 functions in innate immunity and inflammation. *Front Immunol.* (2013) 3:407. doi: 10.3389/fimmu.2012.00407
15. Introna M, Alles VV, Castellano M, Picardi G, De Gioia L, Bottazzi B, et al. Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites. *Blood* (1996) 87:1862–72.
16. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol.* (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
17. Doni A, Michela M, Bottazzi B, Peri G, Valentino S, Polentarutti N, et al. Regulation of PTX3, a key component of humoral innate immunity in human dendritic cells: stimulation by IL-10 and inhibition by IFN-gamma. *J Leukoc Biol.* (2006) 79:797–802. doi: 10.1189/jlb.0905493
18. Jaillon S, Jeannin P, Hamon Y, Frémaux I, Doni A, Bottazzi B, et al. Endogenous PTX3 translocates at the membrane of late apoptotic human neutrophils and is involved in their engulfment by macrophages. *Cell Death Differ.* (2009) 163:465–74. doi: 10.1038/cdd.2008.173
19. Mantovani A, Valentino S, Gentile S, Inforzato A, Bottazzi B, Garlanda C. The long pentraxin PTX3: a paradigm for humoral pattern recognition molecules. *Ann N Y Acad Sci.* (2013) 1285:1–14. doi: 10.1111/nyas.12043
20. Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, Nota R, et al. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature* (2002) 420:182–6. doi: 10.1038/nature01195
21. Daigo K, Inforzato A, Barajon I, Garlanda C, Bottazzi B, Meri S, et al. Pentraxins in the activation and regulation of innate immunity. *Immunol Rev.* (2016) 274:202–17. doi: 10.1111/imr.12476.
22. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem.* (1997) 272:32817–23.
23. Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, et al. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. *Eur J Immunol.* (2003) 33:465–73. doi: 10.1002/immu.200310022
24. Ma YJ, Doni A, Romani L, Jürgensen HJ, Behrendt N, Mantovani A, et al. Ficolin1-PTX3 complex formation promotes clearance of altered self-cells and modulates IL-8 production. *J Immunol.* (2013) 191:1324–33. doi: 10.4049/jimmunol.1300382
25. Ma YJ, Doni A, Hummelshøj T, Honoré C, Bastone A, Mantovani A, et al. Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. *J Biol Chem.* (2009) 284: 28263–75. doi: 10.1074/jbc.M109.009225
26. Ma YJ, Doni A, Skjoedt MO, Honoré C, Arendrup M, Mantovani A, et al. Heterocomplexes of mannose-binding lectin and the pentraxins PTX3 or serum amyloid P component trigger cross-activation of the complement system. *J Biol Chem.* (2011) 286:3405–17. doi: 10.1074/jbc.M110.190637
27. Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, et al. Binding of the long pentraxin PTX3 to factor H: interacting domains and function in the regulation of complement activation. *J Immunol.* (2008) 181:8433–40. doi: 10.4049/jimmunol.181.12.8433
28. Moalli F, Doni A, Deban L, Zelante T, Zagarella S, Bottazzi B, et al. Role of complement and Fcgamma receptors in the protective activity of the long pentraxin PTX3 against *Aspergillus fumigatus*. *Blood* (2010) 116: 5170–80. doi: 10.1182/blood-2009-12-258376
29. Rusnati M, Camozzi M, Moroni E, Bottazzi B, Peri G, Indraccolo S, et al. Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood* (2004) 104:92–9. doi: 10.1182/blood-2003-10-3433
30. Doni A, Musso T, Morone D, Bastone A, Zambelli V, Sironi M, et al. An acidic microenvironment sets the humoral pattern recognition molecule PTX3 in a tissue repair mode. *J Exp Med.* (2015) 212:905–25. doi: 10.1084/jem.20141268
31. Su YQ, Sugiura K, Eppig JJ. Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. *Semin Reprod Med.* (2009) 27:32–42. doi: 10.1055/s-0028-1108008
32. Di Giacomo M, Camaioni A, Klinger FG, Bonfiglio R, Salustri A. Cyclic AMP-elevating agents promote cumulus cell survival and hyaluronan matrix stability, thereby prolonging the time of mouse oocyte fertilizability. *J Biol Chem.* (2016) 291:3821–36. doi: 10.1074/jbc.M115.680983

33. Salustri A, Campagnolo L, Klinger FG, Camaioni A. Molecular organization and mechanical properties of the hyaluronan matrix surrounding the mammalian oocyte. *Matrix Biol.* (2018). doi: 10.1016/j.matbio.2018.02.002. [Epub ahead of print].
34. Chen X, Bonfiglio R, Banerji S, Jackson DG, Salustri A, Richter RP. Micromechanical analysis of the hyaluronan-rich matrix surrounding the oocyte reveals a uniquely soft and elastic composition *Biophys J.* (2016) 110:2779–89. doi: 10.1016/j.bpj.2016.03.023
35. Salustri A, Yanagishita M, Underhill C, Laurent TC, Hascall VC. Localization and synthesis of hyaluronic acid in the cumulus cells and mural granulosa cells of the preovulatory follicles. *Dev Biol.* (1992) 151:541–51.
36. Fulop C, Salustri A, Hascall VC. Coding sequence of a hyaluronan synthase homologue expressed during expansion of the mouse cumulus-oocyte complex. *Arch Biochem Biophys.* (1997) 337:261–6. doi: 10.1006/abbi.1996.97931-6
37. Varani S, Elvin JA, Yan C, DeMayo J, DeMayo FJ, Horton HF, et al. Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. *Mol Endocrinol.* (2002) 16:1154–67. doi: 10.1210/mend.16.6.0859
38. Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, et al., PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in *in vivo* fertilization. *Development* (2004) 131:1577–86. doi: 10.1242/dev.01056
39. Scarchilli L, Camaioni A, Bottazzi B, Negri V, Doni A, Deban L, et al. PTX3 interacts with inter-alpha-trypsin inhibitor: implications for hyaluronan organization and cumulus oophorus expansion. *J Biol Chem.* (2007) 282:30161–70. doi: 10.1074/jbc.M703738200
40. Doni A, D'Amico G, Morone D, Mantovani A, Garlanda C. Humoral innate immunity at the crossroad between microbe and matrix recognition: the role of PTX3 in tissue damage. *Semin Cell Dev Biol.* (2017) 61:31–40. doi: 10.1016/j.semcdb.2016.07.026
41. Zhuo L, Salustri A, Kimata K. A physiological function of serum proteoglycan bikunin: the chondroitin sulfate moiety plays a central role. *Glycoconj J.* (2002) 19:241–7. doi: 10.1023/A:1025331929373
42. Powers RW, Chen L, Russell PT, Larsen WJ. Gonadotropin-stimulated regulation of blood-follicle barrier is mediated by nitric oxide. *Am J Physiol.* (1995) 269:E290–8.
43. Nagyova E, Camaioni A, Prochazka R, Salustri A. Covalent transfer of heavy chains of inter-alpha-trypsin inhibitor family proteins to hyaluronan in *in vivo* and *in vitro* expanded porcine oocyte-cumulus complexes. *Biol Reprod.* (2004) 71:1838-43. doi: 10.1095/biolreprod.104.029595
44. Zhuo L, Yoneda M, Zhao M, Yingsung W, Yoshida N, Kitagawa Y, et al. Defect in SHAP-hyaluronan complex causes severe female infertility. A study by inactivation of the bikunin gene in mice. *J Biol Chem.* (2001) 276:7693–6. doi: 10.1074/jbc.C000899200
45. Fulop C, Szanto S, Mukhopadhyay D, Bardos T, Kamath RV, Rugg MS, et al. Impaired cumulus mucification and female sterility in tumor necrosis factor-induced protein-6 deficient mice. *Development* (2003) 130:2253–61. doi: 10.1242/dev.00422
46. Ievoli E, Lindstedt R, Inforzato A, Camaioni A, Palone F, Day AJ, et al. Implication of the oligomeric state of the N-terminal PTX3 domain in cumulus matrix assembly. *Matrix Biol.* (2011) 30:330–7. doi: 10.1016/j.matbio.2011.05.002
47. Baranova NS, Inforzato A, Briggs DC, Tilakaratna V, Enghild JJ, Thakar D, et al. Incorporation of pentraxin 3 into hyaluronan matrices is tightly regulated and promotes matrix cross-linking. *J Biol Chem.* (2014) 289:30481–98. doi: 10.1074/jbc.M114.568154
48. Mukhopadhyay D, Asari A, Rugg MS, Day AJ, Fülöp C. Specificity of the tumor necrosis factor-induced protein 6-mediated heavy chain transfer from inter-alpha-trypsin inhibitor to hyaluronan: implications for the assembly of the cumulus extracellular matrix. *J Biol Chem.* (2004) 279:11119–28. doi: 10.1074/jbc.M313471200
49. Briggs DC, Birchenough HL, Ali T, Rugg MS, Waltho JP, Ievoli E, et al. Metal ion-dependent heavy chain transfer activity of TSG-6 mediates assembly of the cumulus-oocyte matrix. *J Biol Chem.* (2015) 290:28708–23. doi: 10.1074/jbc.M115.669838
50. Ogawa Y, He H, Mukai S, Imada T, Nakamura S, Su CW, et al. Heavy chain-hyaluronan/pentraxin 3 from amniotic membrane suppresses inflammation and scarring in murine lacrimal gland and conjunctiva of chronic graft-versus-host disease. (2017) *Sci Rep*. 7:42195. doi: 10.1038/srep42195
51. Reynolds LP, Grazul-Bilska AT, Redmer DA. Angiogenesis in the corpus luteum. *Endocrine* (2000) 12:1–9. doi: 10.1385/ENDO:12:1:1
52. Mondal M, Schilling B, Folger J, Steibel JP, Buchnick H, Zalman Y, et al. Deciphering the luteal transcriptome: potential mechanisms mediating stage-specific luteolytic response of the corpus luteum to prostaglandin F2α. *Physiol Genomics* (2011) 43:447–56. doi: 10.1152/physiolgenomics.00155.2010
53. Zalman Y, Klipper E, Farberov S, Mondal M, Wee G, Folger JK, et al. Regulation of angiogenesis-related prostaglandin f2alpha-induced genes in the bovine corpus luteum. *Biol Reprod.* (2012) 86:92. doi: 10.1095/biolreprod.111.095067
54. Meidan R, Girsh E, Mamluk R, Levy N, Farberov S. Luteolysis in ruminants: past concepts, new insights, and persisting challenges. In: Meidan R, editor. *The Life Cycle of the Corpus Luteum.* Basel: Springer International Publishing Switzerland (2017). p. 159–82.
55. Romero JJ, Antoniazzi AQ, Smirnova NP, Webb BT, Yu F, Davis JS, et al. Pregnancy-associated genes contribute to antiluteolytic mechanisms in ovine corpus luteum. *Physiol Genomics* (2013) 45:1095–108. doi: 10.1152/physiolgenomics.00082.2013
56. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol.* (2011) 7:219–31. doi: 10.1038/nrendo.2010.217
57. Figueroa F, Davicino R, Micalizzi B, Oliveros L, Forneris M. Macrophage secretions modulate the steroidogenesis of polycystic ovary in rats: effect of testosterone on macrophage pro-inflammatory cytokines. *Life Sci.* (2012) 90:733–9. doi: 10.1016/j.lfs.2012.03.019
58. Dumesic DA, Richards JS. Ontogeny of the ovary in polycystic ovary syndrome. *Fertil Steril.* (2013) 100:23–38. doi: 10.1016/j.fertnstert.2013.02.011
59. Adams J, Liu Z, Ren YA, Wun WS, Zhou W, Kenigsberg S, et al. Enhanced inflammatory transcriptome in the granulosa cells of women with polycystic ovarian syndrome. *J Clin Endocrinol Metab.* (2016) 101:3459–68. doi: 10.1210/jc.2015-4275
60. Lima PDA, Nivet AL, Wang Q, Chen YA, Leader A, Cheung A, et al. Polycystic ovary syndrome: possible involvement of androgen-induced, chemerin-mediated ovarian recruitment of monocytes/macrophages. *Biol Reprod.* (2018) 99:838–52. doi: 10.1093/biolre/ioy096
61. Richards JS, Ren YA, Candelaria N, Adams JE, Rajkovic A. Ovarian follicular theca cell recruitment, differentiation, and impact on fertility: 2017 update. *Endocr Rev.* (2018) 39:1–20. doi: 10.1210/er.2017-00164
62. Yamasaki K, Kurimura M, Kasai T, Sagara M, Kodama T, Inoue K. Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations. *Clin Chem Lab Med.* (2009) 47:471–7. doi: 10.1515/CCLM.2009.110
63. Barazzoni R, Aleksova A, Carriere C, Cattin MR, Zanetti M, Vinci P, et al. Obesity and high waist circumference are associated with low circulating pentraxin-3 in acute coronary syndrome. *Cardiovasc Diabetol.* (2013) 12:167. doi: 10.1186/1475-2840-12-167
64. Miyaki A, Maeda S, Choi Y, Akazawa N, Eto M, Tanaka K, et al. Association of plasma pentraxin 3 with arterial stiffness in overweight and obese individuals. *Am J Hypertens.* (2013) 26:1250–5. doi: 10.1093/ajh/hpt103
65. Sahin FK, Sahin SB, Balik G, Ural UM, Tekin YB, Cure MC, et al. Does low pentraxin-3 levels associate with polycystic ovary syndrome and obesity? *Int J Clin Exp Med.* (2014) 7:3512–9.
66. Tosi F, Di Sarra D, Bonin C, Zambotti F, Dall'Alda M, Fiers T, et al. Plasma levels of pentraxin-3, an inflammatory protein involved in fertility, are reduced in women with polycystic ovary syndrome. *Eur J Endocrinol.* (2014) 170:401–9. doi: 10.1530/EJE-13-0761
67. Witasp A, Carrero JJ, Michaëlsson K, Ahlström H, Kullberg J, Adamsson V, et al. Inflammatory biomarker pentraxin 3 (PTX3) in relation to obesity, body fat depots and weight loss. *Obesity* (2014) 22:1373–9. doi: 10.1002/oby.20695
68. Daigo K, Mantovani A, Bottazzi B. The yin-yang of long pentraxin PTX3 in inflammation and immunity. *Immunol Lett.* (2014) 161:38–43. doi: 10.1016/j.imlet.2014.04.012
69. Enskog A, Nilsson L, Brännström M. Low peripheral blood levels of the immunosuppressive cytokine interleukin 10 (IL-10) at the start of gonadotrophin stimulation indicates increased risk for development of

ovarian hyperstimulation syndrome (OHSS). *J Reprod Immunol.* (2001) 49:71–85. doi: 10.1016/S0165-0378(00)00075-9

70. Jakimiuk AJ, Weitsman SR, Navab A, Magoffin DA. Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries. *J Clin Endocrinol Metab.* (2001) 86:1318–23. doi: 10.1210/jcem.86.3.7318
71. Teixeira Filho FL, Baracat EC, Lee TH, Suh CS, Matsui M, Chang RJ, et al. Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* (2002) 87:1337–44. doi: 10.1210/jcem.87.3.8316
72. McNatty KP, Smith P, Moore LG, Reader K, Lun S, Hanrahan JP, et al. Oocyte-expressed genes affecting ovulation rate. *Mol Cell Endocrinol.* (2005) 234:57–66. doi: 10.1016/j.mce.2004.08.013
73. Monestier O, Servin B, Auclair S, Bourquard T, Poupon A, Pascal G, et al. Evolutionary origin of bone morphogenetic protein 15 and growth and differentiation factor 9 and differential selective pressure between mono- and poly-ovulating species. *Biol Reprod.* (2014) 91:83. doi: 10.1095/biolreprod.114.119735
74. Hoekstra C, Zhao ZZ, Lambalk CB, Willemsen G, Martin NG, Boomsma DI, et al. Dizygotic twinning. *Hum Reprod Update* (2008) 14:37–47. doi: 10.1093/humupd/dmm036
75. Sirugo G, Edwards DR, Ryckman KK, Bisseye C, White MJ, Kebbeh B, et al. PTX3 genetic variation and dizygotic twinning in theGambia: could pleiotropy with innate immunity explain common dizygotic twinning in Africa? *Ann Hum Genet.* (2012) 76:454–63. doi: 10.1111/j.1469-1809.2012.00723.x
76. May L, Kuningas M, van Bodegom D, Meij HJ, Frolich M, Slagboom PE, et al. Genetic variation in pentraxin (PTX) 3 gene associates with PTX3 production and fertility in women. *Biol Reprod.* (2010) 82:299–304. doi: 10.1095/biolreprod.109.079111
77. Smits J, Monden C. Twinning across the developing world. *PLoS ONE* (2011) 6:e25239. doi: 10.1371/journal.pone.0025239
78. Olesen R, Wejse C, Velez DR, Bisseye C, Sodemann M, Aaby P, et al. DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. *Genes Immun.* (2007) 8:456–67. doi: 10.1038/sj.gene.6364410

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 19 December 2018
doi: 10.3389/fimmu.2018.03046

# Pentraxins in Complement Activation and Regulation

*Ying Jie Ma* and Peter Garred**

*The Laboratory of Molecular Medicine, Department of Clinical Immunology, Section 7631, Rigshospitalet, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

The complement is the first line of immune defense system involved in elimination of invading pathogens and dying host cells. Its activation is mainly triggered by immune complexes or pattern recognition molecules (PRMs) upon recognition against non-self or altered self-cells, such as C1q, collectins, ficolins, and properdin. Recent findings have interestingly shown that the pentraxins (C-reactive protein, CRP; serum-amyloid P component, SAP; long pentraxin 3, PTX3) are involved in complement activation and amplification via communication with complement initiation PRMs, but also complement regulation via recruitment of complement regulators, for instance C4b binding protein (C4BP) and factor H (fH). This review addresses the potential roles of the pentraxins in the complement system during infection and inflammation, and emphasizes the underlining implications of the pentraxins in the context of complement activation and regulation both under physiological and pathological conditions.

**Keywords: pentraxins, PTX3, CRP, SAP, collectin, the ficolins, complement activation, complement regulation**

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Humanitas Clinical and Research Center, Italy*

***Reviewed by:***
*Uday Kishore, Brunel University London, United Kingdom*
*Cordula M. Stover, University of Leicester, United Kingdom*

****Correspondence:***
*Ying Jie Ma*
*ying.jie.ma@regionh.dk*
*Peter Garred*
*peter.garred@regionh.dk*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *11 October 2018*
***Accepted:*** *10 December 2018*
***Published:*** *19 December 2018*

***Citation:***
*Ma YJ and Garred P (2018) Pentraxins in Complement Activation and Regulation. Front. Immunol. 9:3046. doi: 10.3389/fimmu.2018.03046*

## INTRODUCTION

The complement system is one of the ancient innate immune defense system, and can evolutionarily be traced back from the sea urchins (1). In humans, the complement system was initially discovered in 1895 as a heat-labile effector of antibody-mediated immunity. Since then, complement has experienced more than 100 years to unveil its authentic features (2). Today, complement is not only a driver of innate immunity, its functions even extend to additional physiological and/or pathophysiological roles in immune surveillance and homeostasis far beyond simple antimicrobial effector functions (3). Complement exerts its functions through effective rules of activation and regulation under precise control of balance. The part of activation comprises three routes: the classical pathway (CP), the lectin pathway (LP) and the alternative pathway (AP) (**Figure 1**). However, the complement system has the potential to harm the host if it is not properly controlled and regulated. Therefore, complement activation is precisely modulated in the circulation and on the healthy host cells by exclusive fluid-phase and cell-bound regulators, which are crucial in protecting host cells from complement over-activation (**Figure 1**).

## PENTRAXINS: CRP, SAP, AND PTX3

Pentraxins are conserved multifunctional soluble pattern recognition molecules (PRMs) characterized by a C-terminal pentraxin signature containing a conserved eight amino acid sequence (13). Proteins of pentraxin family comprise three major members, C-reactive protein (CRP), serum-amyloid P component (SAP), and pentraxin 3 (PTX3). Based on the primary

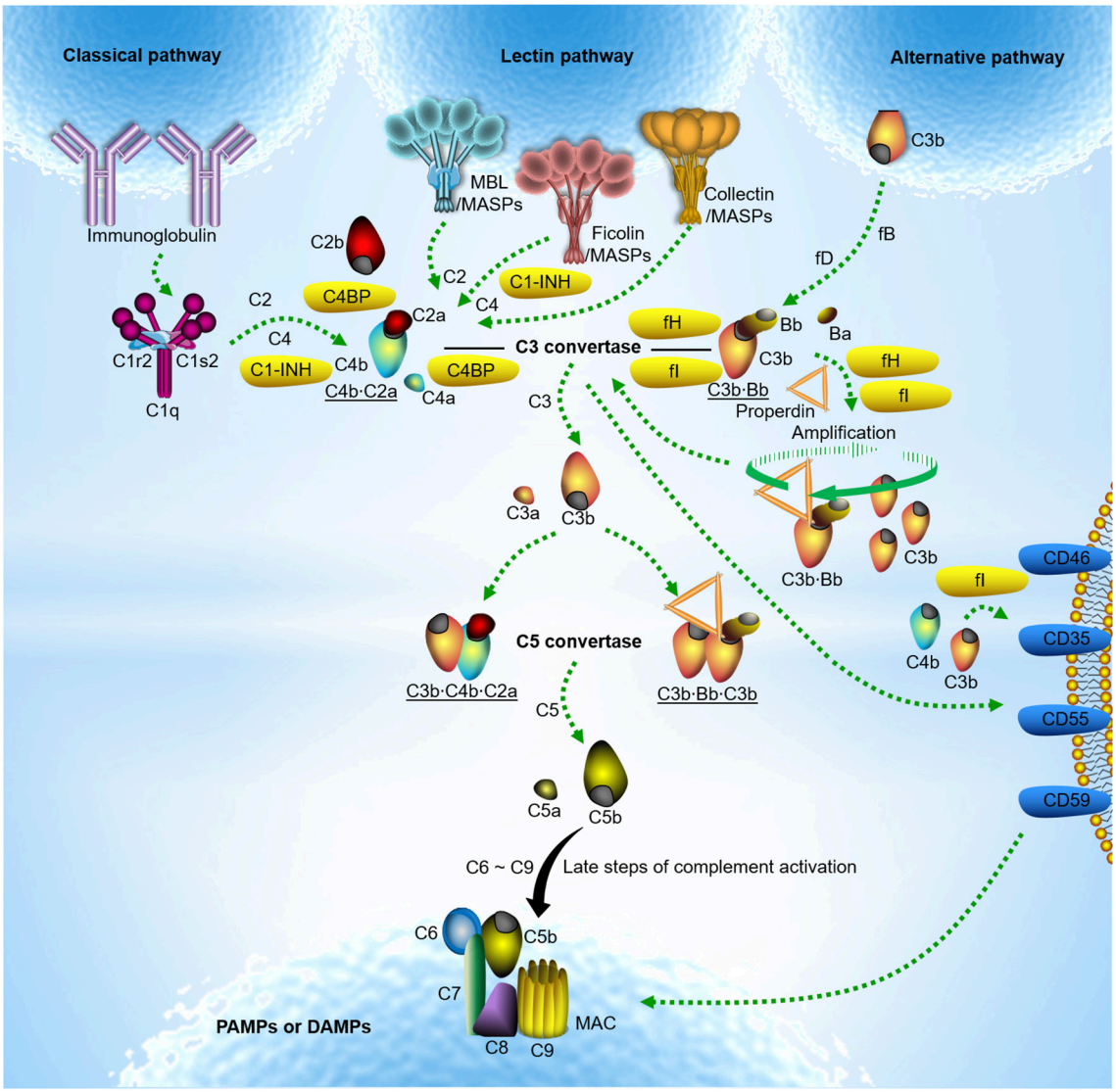


**FIGURE 1 |** Classical model of complement activation and regulation. Complement activation occurs typically through three routes. Classical pathway activation is triggered by the C1 complex comprising C1q, C1s, and C1r upon binding to IgM or clusters of IgG against pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) (4). Lectin pathway activation is induced by soluble pattern recognition molecules (PRMs) mannose-binding lectin (MBL) upon binding to PAMPs or DAMPs. It is now apparent that the ficolins (ficolin −1,−2, or−3) or collectins (collectin-10, collectin-11 or a heteromeric complex of collectin-10 and collectin-11) are also involved in lectin pathway activation (5–9). Unlike C1q complexed with the serine proteases C1r and C1s, PRMs involving lectin pathway activation are often associated with the mannose-binding lectin-associated serine proteases (MASPs). Upon classical and lectin pathway activation, the serine proteases cleave C4 and C2 to form C3 convertase (C4b·C2a). In contrast, alternative pathway activation occurs by direct tick-over activation of C3 thioester in solution regardless of trigger, and creates its own C3 convertase (C3b·Bb) when activated C3b covalently bind to the target surfaces in contact with factor B (fB) and the enzyme factor D (fD) (10, 11). The alternative C3 convertase is highly stabilized when properdin is associated. With release of anaphylatoxin C3a, surface-bound C3 convertases generate more opsonin C3b, leading to the formation of the classical and lectin pathway C5 convertase (C3b·C4b·C2a) and the alternative C5 convertase (C3b·Bb·C3b). The C5 convertase in turn cleaves C5 into another anaphylatoxin C5a and C5b. Surface-deposited C5b sequentially recruits complement subunits C6, C7, C8, and C9 on target surface and initiates formation of C5b-9 membrane attack complex (MAC) also named the terminal complement complex (TCC) which may lead to target lysis (3, 12). The alternative pathway also serves to amplify classical and lectin pathway activation. Fluid-phase and cell-bound regulators help to modulate complement over-activation; C1 inhibitor (C1-INH) controls the functions of C1r, C1s and MASP-2; C3b and C4b are inactivated by either fluid-phase factor H (fH)/C4b-binding protein (C4BP) or cell-bound complement receptor type 1 (CR1)/CD46 as cofactors for factor I (fI). Fluid-phase fH/C4BP or cell-bound CR1/CD55 regulate convertase activity by disassembly through decay-accelerating activity of the regulators. The formation of MAC is controlled by CD59 (3).

structure of the protomer, CRP and SAP are distinguished as the short pentraxins from the main long pentraxin PTX3 (14). The genes encoding the short pentraxins (CRP/SAP) and the long pentraxin (PTX3) are located on chromosome 1q23 and 3q25, respectively. However, in contrast to the short pentraxins, which are expressed in liver upon stimulation of inflammatory cytokine,

the long pentraxin PTX3 is produced by diverse immune cells, such as macrophages, neutrophils, dendritic cells, and endothelial cells (15). CRP is one of the major acute phase proteins in human, and its level in plasma elevates by 1,000-fold via stimulation of hepatocytes by acute phase stimulus. By contrast, PTX3 is barely detectable in human blood circulation (< 2 ng/ml) under physiological conditions, while the serum level drastically increases (200–800 ng/ml) within 6–8 h during infection and inflammation (15). The pentraxins share the conserved C-terminal pentraxin domain, whereas PTX3 harbors a unique N-terminal region without sequence homology to any known proteins so far (15).

The pentraxins recognize wide spectrum of microbial moieties to mediate opsonophargocytosis, but also interact with extracellular matrix (ECM) proteins to stabilize ECM deposition and modified self-structures on dying host cells to keep homeostasis (14). Concerning the antibody-like common features conserved in evolution, the pentraxins mediate phagocytosis and inflammation by macrophages via interaction with the surface Fcγ receptors upon target opsonization (16–18). Thus, the pentraxins are important for both innate immune defense and tissue homeostasis (13, 14, 19).

## PENTRAXINS-MEDIATED COMPLEMENT CROSS-ACTIVATION

Decades of initial research have solidified complement activation by three separate and autonomous routes as described in **Figure 1**. The CP and LP activation are mainly mediated by exclusive PRMs upon opsonization. Apart from immunoglobulins and several other ligands the CP is also activated via the antibody-like common features of the pentraxins, where CRP, SAP, and PTX3 instead of immunoglobulins recruit C1 complexes upon binding to target surfaces (16, 20, 21). Following antigen stimulation, antibody production often experiences complexity of multiple process by B-cells via differentiation and proliferation and antibody interaction is highly dependent on its antigen specificity. In contrast, the pentraxins are either acute phase reactants under infectious and inflammatory conditions or present constitutively with invariant and high level, and broadly recognize the common pattern moieties arising from microorganisms. Therefore, pentraxins-mediated immune responses are often mobilized rapidly and drastically in resistant to microbial invasion and tissue damage, acting at systemic and local tissue level. Although the antibody-like features have been endowed with the pentraxins in evolution, it is still deficient in antigen-exclusive antibody specificity. Considering complement activation and the underlying functional consequences, pentraxin-mediated initiation is much more rapid and efficient than antibody-dependent responses during infection at early stage (14).

As has been shown for C1q it has recently been shown that the pentraxins interact with some of the PRMs from LP, thus allowing CRP, SAP, and PTX3 to effectively dock at certain bacteria through sensory inputs due to their spontaneous defect in opsonization toward it (22). The interaction has been shown to specifically elevate host immune defense via the LP of complement activation against various microorganism including bacteria and fungi, thus adapting to tricky pathogenic conditions (22). More importantly, the CP and LP, which were previously defined separate and autonomous, have been demonstrated to establish cross-communication through the interaction between the pentraxins and LP PRMs, enabling amplification of complement activation and its concomitant anti-microbial activity (23). In this respect, it should be emphasized that the interaction between the pentraxins and LP PRMs not only serves to boost complement activation, may also result in broadening repertoire of pattern recognition and complement-mediated effector functions via such synergistic effects (24).

The pentraxins are capable of selectively opsonizing certain bacteria, fungi, and viruses (25), but not *Candida albicans* and *Burkholderia cepacia* (26). However, the purified pentraxins (PTX3 and SAP in particular, but not CRP) could bind to *Candida albicans* only in the premise of presence of human serum (27), implying that the presence of certain human serum factor might enable anchorage of the pentraxins on *Candida albicans* indirectly. Recent findings have shown that serum MBL docks both PTX3 and SAP to *Candida albicans*, and that this interaction enhances complement activation and the subsequent opsonophagocytosis by polymorphonuclear leukocytes (PMN) (27). Interaction of PTX3 with MBL leads to communication between the LP and CP via C1q, whereas it is still enigma how SAP:MBL complexes boost complement activation. These findings suggest that crosstalk between the pentraxins and MBL provides two potential complement amplification mechanisms via cross-activation within the complement system (**Figure 2**).

It has been well documented that the complement system plays a non-redundant role against *Aspergillus fumigatus* infection (29). The state of complement deficiency is highly susceptible to *Aspergillus fumigatus* infection and mice deficient in C5 has been shown to be hardly survived (30, 31). Ficolin-2 has been recently suggested to serve as a particular inducer of anti-fungal activity through provoking the LP of complement activation and/or regulating pro- and anti-inflammatory airway immune responses in treatment of *Aspergillus fumigatus* challenge (24, 32, 33). Ficolin-2 has been shown to recognize *Aspergillus fumigatus* in a $Ca^{2+}$-insensitive manner with stronger binding at acidic pH (34, 35), which typically prevails in a local infectious and inflammatory condition (36) and is required to boost complement activity (37). Zhang et al. previously reported that interaction of CRP with ficolin-2 is elevated in an acidic pH-sensitive manner (23). These data suggest that local prevalence of acidic circumstances may be essential to trigger reciprocal interaction between ficolin-2 and CRP to combat *Aspergillus fumigatus* at the early stage of infection. Consistent with the previous reports, analysis of bronchoalveolar lavage (BAL) fluid has attested presence of ficolin-2 in invasive aspergillosis (IA)-suffering patients, and demonstrated a notable roles of ficolin-2 in modulation of alveolar immune responses against infection of *Aspergillus fumigatus* (35). In agreement with those reports, Genster et al. recently found that mice are vulnerable to fungal infection under the ficolin-deficient

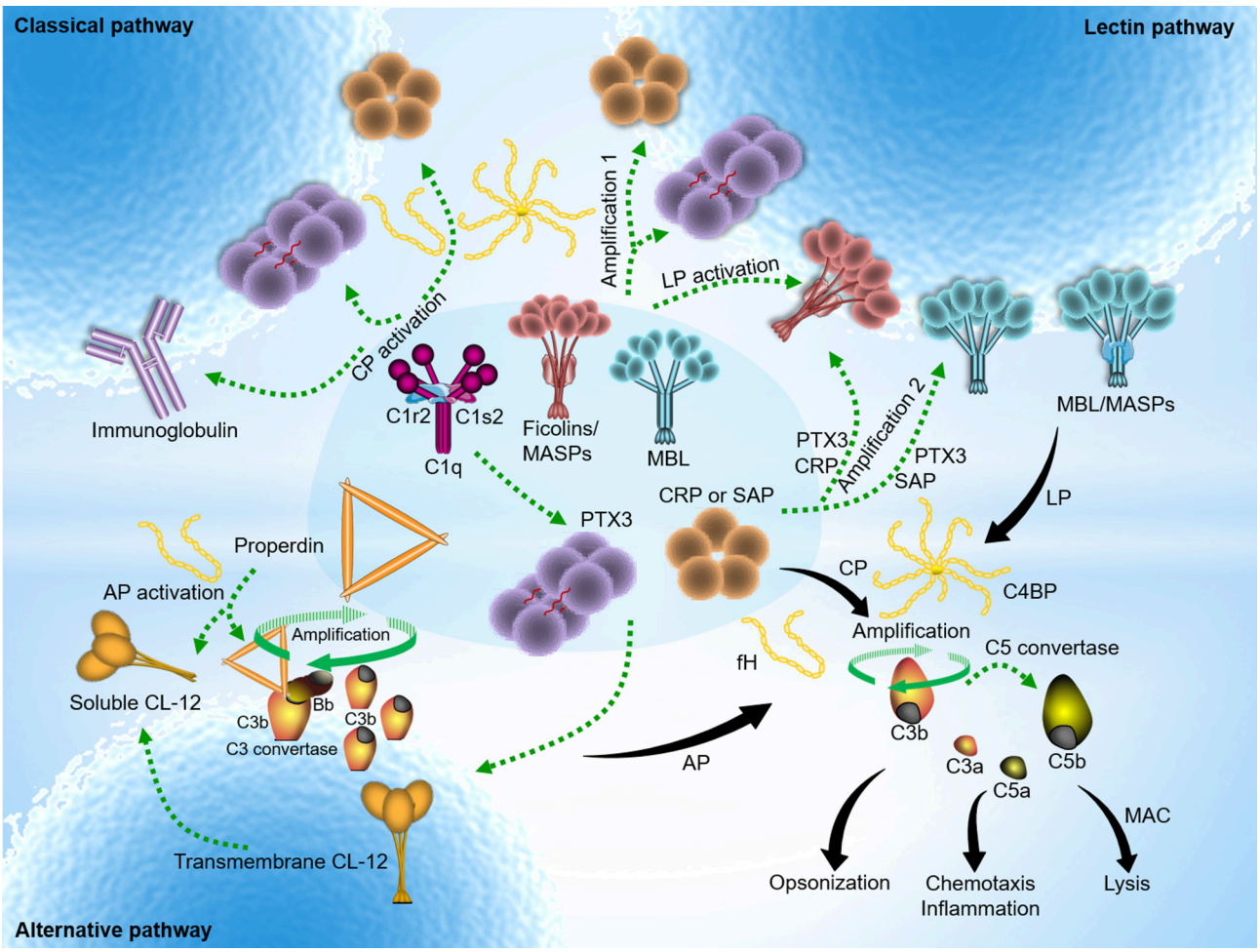


**FIGURE 2 |** Pentraxins-expanded network of complement activation and regulation. Classical pathway (CP) activation mainly occurs by immune complexes, but also mediated through antibody-like features of the pentraxins in complex with C1q. In addition to the traditional activating fashion as depicted above, lectin pathway (LP) is also indirectly activated by PTX3, CRP, or SAP upon recruitment of MBL or the ficolins. Cross-activation of the CP and LP initiates when heterocomplexes are created on target surfaces between PTX3 (or CRP) and MBL (or ficolin-2) and C1q. Two additional activation pathways emerge to boost complement amplification. The amplification pathways comprise the avenue open to the sequence below: target→ PTX3 (or CRP)→ ficolin-2/MASPs→ C4→ C3→ formation of membrane attach complex (MAC) (amplification 1); target→ MBL (or ficolin-2/MASPs)→ PTX3 (or CRP) → C1q→ C4→ C3→ formation of MAC (amplification 2) (3). Alternative pathway (AP) activation often occurs independently regardless of trigger by spontaneous C3 activation in solution, and rapidly propels nascent C3b binding to nearby target surfaces covalently. In addition, it is hypothesized that surface-bound collectin-12 (CL-12) mediates CP activation by crosstalk with PTX3, CRP or SAP. A recent emerging activation avenue follows relatively specific sequence that involves pattern recognition and opsonization by soluble CL-12→ recruitment of properdin→ *de novo* C3 convertase assembly→ C3 amplification loop→ generation of downstream effector molecules→ induction of immune signaling (28). Despite its diverse avenues, overarching concept of complement activation focuses on sensing and eliminating potential danger signals through immunesurveillance and immune effector mechanisms. The pentraxins recognize major fluid-phase complement regulators, C4BP and fH, resulting in down-regulation of complement-mediated inflammation.

condition (38). These results suggest that serum ficolin-2 may facilitate to elevate host immune responses at local sites of pulmonary fungal infection via transmigration to alveoli and thus play a crucial role in lung infection (35). Mice deficient in PTX3 have shown increased susceptibility to invasive pulmonary aspergillosis (IPA) and accelerated death compared with mock control, which was attributed to the defect in fungicidal activities against IPA in regard to opsonophagocytosis and activation of an adaptive type 2 responses (39). In parallel with those evidences, Cunha et al. intriguingly found a PTX3 single-nucleotide polymorphisms (SNPs) in donors with a homozygous haplotype, leading to increased vulnerability to invasive aspergillosis when patients receives hematopoietic stem-cell transplantation from such donors (39). These results suggest that PTX3 plays non-redundant roles in antifungal immunity. In this respect, it should be noted that collaboration of liver-derived CRP and immune cell-expressed PTX3 with migratory ficolin-2 might also further boost the microbicidal immune responses at systemic and local tissue level, respectively, for instance through the complement cross-activation and amplification mechanisms.

Recently, the pentraxins have been shown to interact with CL-12, a newly identified scavenger receptor C-type

lectin (SRCL) (40, 41). By using ELISA and CHO/ldlA7 cell lines expressing transmembrane CL-12 as platform, the interaction of the pentraxins with transmembrane CL-12 has been visualized. It was shown a propensity of the pentraxins including CRP, SAP, and PTX3 for direct binding of CL-12, and demonstrated that the interaction is able to result in the CP of complement activation via recruitment of C1q on HEK293 cell lines expressing transmembrane CL-12 (42). However, whether this is indeed a case on native CL-12-expressing primary cells, for instance HUVEC or HUAEC, is still awaiting further clarification. Nevertheless, a soluble form of CL-12 has recently been identified and revealed to trigger AP of complement activation through direct contact with properdin (28). Whether the pentraxins interact with soluble form of CL-12 and expand a novel complement crosstalk against invading pathogens is still unknown.

## PENTRAXINS IN COMPLEMENT REGULATION

In general, complement activation is precisely modulated in the circulation and on healthy host cells by exclusive soluble and cell-bound regulators as described in **Figure 1**. Recent data show that the pentraxins surveil and modulate the action of complement to avoid overwhelming activation via interaction with complement negative regulators. Like CRP and SAP, PTX3 is able to bind the main fluid-phase regulator of the CP and LP C4BP. Similar to the short pentraxins, PTX3 in solution preserve the cofactor activity of C4BP for fI upon complex formation (43). When anchored on apoptotic/necrotic cells and extracellular matrix (ECM), PTX3 is capable of recruiting functionally active C4BP, leading to complement C4b inactivation and reduced terminal complement complex (TCC) deposition on the surfaces. Both CRP and PTX3 have been observed to interact with fH, the main soluble regulator of the AP, and the two PTX3 binding sites on fH were defined to be located on fH short consensus repeat (SCR) 7 and SCR19-20 (44), of which the former is also employed for CRP binding in addition to SCR8-11 (45, 46). fH was found to remain its C3 inhibitory activity upon binding to the pentraxins, thus preventing exaggerated AP mediated complement activation on CRP or PTX3-immobilized surfaces. Therefore, the pentraxins may target all the complement pathways by interaction with C4BP and fH and they may assist in regulation of complement activation to avoid the adverse effect of complement in tissues.

Mutations or polymorphisms of fH are associated with the pathogenesis of various inflammatory human diseases, for instance atypical hemolytic uremic syndrome (aHUS) and age-related macular degeneration (AMD) due to dysregulation of the AP. Interestingly, Tyr402His (a polymorphic amino acid variant in SCR7 of fH), which is linked to high risk of aHUS and AMD, influences the binding of CRP, but not PTX3-fH interaction. Therefore, reduced CRP- fH Tyr402His interaction might be involved in pathogenesis of the diseases due to the complement-mediated increased inflammation, where PTX3 could compensate for these changes at the site of inflammation. Kelly et al. previously showed that heparin sulfate inhibits the AP through interaction with fH, and found that fH-mediated C3b inactivation is highly dependent on heparin sulfate and the degree of sulphation in the Bruch's membrane (BrM) (47). Thus, BrM/choroid, a site of tissue damage expressing abundant heparin sulfate glycosaminoglycans in AMD, might be protected by recruitment of fH under normal conditions. In addition, PTX3 stored inside BrM might also act as a second line protection for mobilization of fH and modulate complement activation. Given the following facts that (1) the binding site for heparin on fH is overlapped with PTX3 to SCR19-20; (2) fH Tyr402His influences the binding of heparin as compared with its wild type; (3) PTX3 binds equally to both the variant and the wild type, the state of PTX3 deficiency would impact on the patients more with fH Tyr402His than its wild type, since the decline in fH binding to heparin would not be supplied by reserved PTX3. In this respect, it is worthy to note that since PTX3 is rapidly and dramatically expressed as an acute phase reactant in the retinal pigmented epithelium (RPE) in response to inflammatory stimuli, the manifestations of fH Tyr402His might be veiled in AMD. Interestingly, it has been recently substantiated that PTX3 serves to brake the complement and the subsequent NLRP3 inflammasome activation through regulation of fH in the RPE (48).

Malignant cells have been previously shown to be monitored and recognized by the complement system and the concomitant complement activation often occurs in many cancers upon recognition (49). However, the complement system has also been suggested to have a role in the development of tumor promoting inflammation and the intermediate product of complement activation C5a has been shown to play a tumor-exacerbating role via elevation of T cell suppression effect and CCL-2 production attracting tumor-associated macrophages (TAMs) and favoring M2-like polarization by bone marrow-derived suppressor cells (50, 51). PTX3 has recently been shown to contribute in regulation of tumor growth, which was attributed to its capacity in control of tumor promoting inflammation through coordination with complement regulator fH (52). As such PTX3 does seem to have a direct effect on tumor cell growth. However, PTX3 deficient animals were more susceptible to chemically induced mesenchymal and epithelial carcinogenesis than control animals (52). PTX3 deficient tumors showed enhanced complement C3 deposition and C5a levels, CCL2 production, and tumor-promoting macrophage recruitment, which was attributed to dysregulated complement activation since C3-genetic inactivation and CCL2 inhibition reverted the phenotype and the increased susceptibility to mesenchymal carcinogenesis in PTX3-deficient mice. These findings suggest a crucial role of PTX3 in complement-dependent tumor-related inflammation probably via fH recruitment. Lack of the functional PTX3 and/or fH might exacerbate pathophysiological consequences during tumor growth and development due to complement dysregulation.

Apart from being a serum marker of systemic inflammation CRP is also suggested to have a direct pathological role in tissues in diseases, such as AMD (53, 54). CRP exists primarily in two forms with distinct structure and biological activity, the native pentameric CRP (pCRP) and monomeric CRP (mCRP),

and circulates in the blood stream as a non-covalent, anti-inflammatory pCRP, however when dissociated into mCRP upon binding to certain cell and matrix surfaces, it becomes rather pro-inflammatory (55). Given the findings that the majority of CRP found in BrM is in monomeric form, although pCRP will still be present in the blood vessels of the choroid *in vivo* (53), mCRP is likely a tissue-associated form derived from circulating pCRP. Accumulating evidences show that if fH is dysfunctional mCRP activates the complement at retina/choroid interface and leads to exacerbate chronic inflammation and subsequent tissue damage (56), indicating the pivotal role of pro-inflammatory mCRP-mediated complement activation and regulation in normal conditions. In addition, fH has been shown to interact with mCRP through its binding sites located in SCR 6-8 and SCR 19-20 regions, but not with pCRP (46, 57–61).

## CONCLUDING REMARKS

The complement system is a double-edged sword, one toward to eliminate danger signals and hostile intruders, while the other is toward healthy self, which may lead to pathological situations if it is not controlled properly. The pentraxins are involved in both complement activation and regulation via crosstalk with major complement initiating molecules (C1q, ficolin-2, MBL, and CL-12) as well as complement regulators (C4BP and fH) (**Figure 2**). The crosstalk events between the pentraxins and the CP and LP PRMs have been recently highlighted for their amplifying roles in immunosurveillance, anti-microbial immune responses, and immunologic homeostasis. However, evidence suggests immunopathogenicity of those cooperative events in infectious inflammation if activated inappropriately, and also emphasizes the potential deleterious impact on pathogen immune evasion and development of complement-related diseases. The pathogenic side of the potential functional roles of PRM heterocomplexes and their involvement in complement activation need to be further explored: whether PRM heterocomplexes are involved in potent inducers of immunopathology during infection and inflammation and how it exacerbates disease severity are both intriguing unanswered questions. Furthermore, recent work has focused on how the pentraxins-mediated complement activation and regulation influences and thus contributes to chronic inflammation, which likely constructs a microenvironment for initiation and development of complement-mediated pathology. Emerging evidences discussed above reinforce the importance of the pentraxins (CRP and PTX3 in particular) in complement activation and regulation in the pathogenesis of age-associated diseases and complement-dependent tumor-promoting inflammation. Given the recent findings that novel collectin 11 or properdin-directed complement activation triggers acute kidney injury, it is also interesting to determine whether and how the pentraxins regulate or exacerbate complement-involved renal injury upon complex formation with the inducers of renal injury. After many years' success of complement drug applications in the treatment of complement-related diseases (ex. PNH and aHUS), the future of the complement field becomes much brighter and many of complement drugs are being processed in preclinical and clinical stages of development in current years. Pentraxins are recently positioning with renewed focus as a novel therapeutic targets being explored in the community of drug development. Importantly, anti-pentraxins drugs (CPHPC and dezamizumab) targeting human SAP have recently entered in clinical phase 2 trial for treatment of amyloidosis and Alzheimer's disease, and human CRP inhibitor targeting CRP-driven complement activation-mediated tissue damage is also being developed (62). Taken together, a better understanding of the complex roles of the pentraxins in the complement system and its involvement in human inflammatory diseases will direct more promising options of therapy against the consequences of certain pathogen infection, and possibly certain complement-related inflammatory diseases.

## AUTHOR CONTRIBUTIONS

YM and PG conceived, designed and wrote the review article. YM and PG revised the review article and approved the final manuscript.

## FUNDING

This work was supported by grants from the Kirsten og Freddy Johansens Fond, the Weimann and Seedorffs Foundation, the Benzon Foundation and the Research Foundation of the Capital Regions of Denmark. The Sven Andersen Research Foundation and the Danish Research Foundation of Independent Research (DFF-6110-00489).

## REFERENCES

1. Nonaka M, and Yoshizaki F. Evolution of the complement system. *Mol Immunol.* (2004) 40:897–902. doi: 10.1007/978-94-017-8881-6_3
2. Walport MJ. Complement. First of two parts. *N Engl J Med.* (2001) 344:1058–66. doi: 10.1056/NEJM200104053441406
3. Ricklin D, Hajishengallis G, Yang K, and Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol.* (2010) 11:785–97. doi: 10.1038/ni.1923
4. Gaboriaud C, Thielens NM, Gregory LA, Rossi V, Fontecilla-Camps JC, Arlaud GJ. Structure and activation of the C1 complex of complement: unraveling the puzzle. *Trends Immunol.* (2004) 25:368–73. doi: 10.1016/j.it.2004.04.008
5. Garred P, Honore C, Ma YJ, Munthe-Fog L, Hummelshoj T. MBL2, FCN1, FCN2 and FCN3-The genes behind the initiation of the lectin pathway of complement. *Mol Immunol.* (2009) 46:2737–44. doi: 10.1016/j.molimm.2009.05.005
6. Henriksen ML, Brandt J, Andrieu JP, Nielsen C, Jensen PH, Holmskov U, et al. Heteromeric complexes of native collectin kidney 1 and collectin liver 1 are found in the circulation with MASPs and activate the complement system. *J Immunol.* (2013) 191:6117–27. doi: 10.4049/jimmunol.1302121
7. Axelgaard E, Jensen L, Dyrlund TF, Nielsen HJ, Enghild JJ, Thiel S, et al. Investigations on collectin liver 1. *J Biol Chem.* (2013) 288:23407–20. doi: 10.1074/jbc.M113.492603
8. Ma YJ, Skjoedt MO, Garred P. Collectin-11/MASP complex formation triggers activation of the lectin complement pathway–the fifth lectin pathway initiation complex. *J Innate Immun.* (2013) 5:242–50. doi: 10.1159/000345356

9. Garred P, Genster N, Pilely K, Bayarri-Olmos R, Rosbjerg A, Ma YJ, et al. A journey through the lectin pathway of complement-MBL and beyond. *Immunol Rev*. (2016) 274:74–97. doi: 10.1111/imr.12468
10. Alcorlo M, Tortajada A, Rodriguez De Cordoba S, Llorca O. Structural basis for the stabilization of the complement alternative pathway C3 convertase by properdin. *Proc Natl Acad Sci USA*. (2013) 110:13504–9. doi: 10.1073/pnas.1309618110
11. Fearon DT, Austen KF. Properdin: binding to C3b and stabilization of the C3b-dependent C3 convertase. *J Exp Med.* (1975) 142:856–63.
12. Ma YJ, Lee BL, Garred P. An overview of the synergy and crosstalk between pentraxins and collectins/ficolins: their functional relevance in complement activation. *Exp Mol Med.* (2017) 49:e320. doi: 10.1038/emm.2017.51
13. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
14. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol.* (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409–101305
15. Mantovani A, Garlanda C, Doni A, Bottazzi B. Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. *J Clin Immunol.* (2008) 28:1–13. doi: 10.1007/s10875-007-9126-7
16. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem.* (1997) 272:32 817–23.
17. Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, et al. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. *Eur J Immunol.* (2003) 33:465–73. doi: 10.1002/immu.200310022
18. Baruah P, Dumitriu IE, Peri G, Russo V, Mantovani A, Manfredi AA, et al. The tissue pentraxin PTX3 limits C1q-mediated complement activation and phagocytosis of apoptotic cells by dendritic cells. *J Leukoc Biol*. (2006) 80:87–95. doi: 10.1189/jlb.0805445
19. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. PTX3, a Humoral Pattern Recognition Molecule, in Innate Immunity, Tissue Repair, and Cancer. *Physiol Rev*. (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
20. Nauta AJ, Daha MR, Van Kooten C, and Roos A. Recognition and clearance of apoptotic cells: a role for complement and pentraxins. *Trends Immunol*. (2003) 24:148–54. doi: 10.1016/S1471-4906(03)00030-9
21. Gershov D, Kim S, Brot N, Elkon KB. C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. *J Exp Med*. (2000) 192:1353–64. doi: 10.1084/jem.192.9.1353
22. Ng PM, Le Saux A, Lee CM, Tan NS, Lu J, Thiel S, et al. C-reactive protein collaborates with plasma lectins to boost immune response against bacteria. *EMBO J*. (2007) 26:3431–40. doi: 10.1038/sj.emboj.7601762
23. Zhang J, Koh J, Lu J, Thiel S, Leong BS, Sethi S, et al. Local inflammation induces complement crosstalk which amplifies the antimicrobial response. *PLoS Pathog.* (2009) 5:e1000282. doi: 10.1371/journal.ppat.1000282
24. Ma YJ, Doni A, Hummelshoj T, Honore C, Bastone A, Mantovani A, et al. Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. *J Biol Chem.* (2009) 284:28263–75. doi: 10.1074/jbc.M109.009225
25. Deban L, Jaillon S, Garlanda C, Bottazzi B, Mantovani A. Pentraxins in innate immunity: lessons from PTX3. *Cell Tissue Res.* (2011) 343:237–49. doi: 10.1007/s00441–010-1018–0
26. Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, Nota R, et al. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature* (2002) 420:182–6. doi: 10.1038/nature01195
27. Ma YJ, Doni A, Skjoedt MO, Honore C, Arendrup M, Mantovani A, et al. Heterocomplexes of mannose-binding lectin and the pentraxins PTX3 or serum amyloid P component trigger cross-activation of the complement system. *J Biol Chem.* (2011) 286:3405–17. doi: 10.1074/jbc.M110.190637
28. Ma YJ, Hein E, Munthe-Fog L, Skjoedt MO, Bayarri-Olmos R, Romani L, et al. Soluble collectin-12 (CL-12) is a pattern recognition molecule initiating complement activation via the alternative pathway. *J Immunol.* (2015) 195:3365–73. doi: 10.4049/jimmunol.1500493
29. Hector RF, Yee E, Collins MS. Use of DBA/2N mice in models of systemic candidiasis and pulmonary and systemic aspergillosis. *Infect Immun.* (1990) 58:1476–8.
30. Svirshchevskaya EV, Shevchenko MA, Huet D, Femenia F, Latge JP, Boireau P, et al. Susceptibility of mice to invasive aspergillosis correlates with delayed cell influx into the lungs. *Int J Immunogenet.* (2009) 36:289–99. doi: 10.1111/j.1744–313X.2009.00869.x
31. Henwick S, Hetherington SV, Patrick CC. Complement binding to Aspergillus conidia correlates with pathogenicity. *J Lab Clin Med.* (1993) 122:27–35.
32. Bidula S, Kenawy H, Ali YM, Sexton D, Schwaeble WJ, Schelenz S. Role of ficolin-A and lectin complement pathway in the innate defense against pathogenic Aspergillus species. *Infect Immun.* (2013) 81:1730–40. doi: 10.1128/IAI.00032–13
33. Bidula S, Sexton DW, Schelenz S. Serum opsonin ficolin-A enhances host-fungal interactions and modulates cytokine expression from human monocyte-derived macrophages and neutrophils following Aspergillus fumigatus challenge. *Med Microbiol Immunol.* (2016) 205:133–42. doi: 10.1007/s00430–015-0435–9
34. Schelenz S, Kirchhof N, Bidula S, Wallis R, Sexton DW. Opsonizing properties of rat ficolin-A in the defence against Cryptococcus neoformans. *Immunobiology* (2013) 218:477–83. doi: 10.1016/j.imbio.2012.06.006
35. Bidula S, Sexton DW, Abdolrasouli A, Shah A, Reed A, Armstrong-James D, et al. The serum opsonin L-ficolin is detected in lungs of human transplant recipients following fungal infections and modulates inflammation and killing of Aspergillus fumigatus. *J Infect Dis*. (2015) 212:234–46. doi: 10.1093/infdis/jiv027
36. Martínez D, Vermeulen M, Trevani A, Ceballos A, Sabatté J, Gamberale R, et al. Extracellular acidosis induces neutrophil activation by a mechanism dependent on activation of phosphatidylinositol 3-kinase/Akt and ERK pathways. *J Immunol.* (2006) 176:1163–71. doi: 10.4049/jimmunol.176.2.1163
37. Kenawy HI, Boral I, Bevington A. Complement-coagulation cross-talk: a potential mediator of the physiological activation of complement by low pH. *Front Immunol.* (2015) 6:215. doi: 10.3389/fimmu.2015.00215
38. Genster N, Praestekjaer Cramer E, Rosbjerg A, Pilely K, Cowland JB, Garred P. Ficolins promote fungal clearance *in vivo* and modulate the inflammatory cytokine response in host defense against Aspergillus fumigatus. *J Innate Immun*. (2016) 8:579–88. doi: 10.1159/000447714
39. Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med.* (2014) 370:421–32. doi: 10.1056/NEJMoa1211161
40. Nakamura K, Funakoshi H, Miyamoto K, Tokunaga F, Nakamura T. Molecular cloning and functional characterization of a human scavenger receptor with C-type lectin (SRCL), a novel member of a scavenger receptor family. *Biochem Biophys Res C* (2001) 280:1028–35. doi: 10.1006/bbrc.2000.4210
41. Ohtani K, Suzuki Y, Eda S, Kawai T, Kase T, Keshi H, et al. The membrane-type collectin CL-P1 is a scavenger receptor on vascular endothelial cells. *J Biol Chem.* (2001) 276:44222–8. doi: 10.1074/jbc.M103942200M103942200[pii]
42. Roy N, Ohtani K, Hidaka Y, Amano Y, Matsuda Y, Mori K, et al. Three pentraxins C-reactive protein, serum amyloid p component and pentraxin 3 mediate complement activation using Collectin CL-P1. *Biochim Biophys Acta* (2017) 1861:1–14. doi: 10.1016/j.bbagen.2016.11.023
43. Braunschweig A, Jozsi M. Human pentraxin 3 binds to the complement regulator c4b-binding protein. *PLoS ONE* (2011) 6:e23991. doi: 10.1371/journal.pone.0023991
44. Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, et al. Binding of the long pentraxin PTX3 to factor H: interacting domains and function in the regulation of complement activation. *J Immunol.* (2008) 181:8433–40. doi: 10.4049/jimmunol.181.12.8433
45. Jarva H, Jokiranta TS, Hellwage J, Zipfel PF, Meri S. Regulation of complement activation by C-reactive protein: targeting the complement inhibitory activity of factor H by an interaction with short consensus repeat domains 7 and 8–11. *J Immunol.* (1999) 163:3957–62.

46. Okemefuna AI, Nan R, Miller A, Gor J, Perkins SJ. Complement factor H binds at two independent sites to C-reactive protein in acute phase concentrations. *J Biol Chem.* (2010) 285:1053–65. doi: 10.1074/jbc.M109.044529
47. Kelly U, Yu L, Kumar P, Ding JD, Jiang H, Hageman GS, et al. Heparan sulfate, including that in Bruch's membrane, inhibits the complement alternative pathway: implications for age-related macular degeneration. *J Immunol.* (2010) 185:5486–94. doi: 10.4049/jimmunol.0903596
48. Wang L, Cano M, Datta S, Wei H, Ebrahimi KB, Gorashi Y, et al. Pentraxin 3 recruits complement factor H to protect against oxidative stress-induced complement and inflammasome overactivation. *J Pathol.* (2016) 240:495–506. doi: 10.1002/path.4811
49. Reis ES, Mastellos DC, Ricklin D, Mantovani A, Lambris JD. Complement in cancer: untangling an intricate relationship. *Nat Rev Immunol.* (2018) 18:5–18. doi: 10.1038/nri.2017.97
50. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* (2008) 454:436–44. doi: 10.1038/nature07205
51. Markiewski MM, Deangelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, Gerard C, et al. Modulation of the antitumor immune response by complement. *Nat Immunol.* (2008) 9:1225–35. doi: 10.1038/ni.1655
52. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell* (2015) 160:700–14. doi: 10.1016/j.cell.2015.01.004
53. Chirco KR, Whitmore SS, Wang K, Potempa LA, Halder JA, Stone EM, et al. Monomeric C-reactive protein and inflammation in age-related macular degeneration. *J Pathol.* (2016) 240:173–83. doi: 10.1002/path.4766
54. Bhutto IA, Baba T, Merges C, Juriasinghani V, Mcleod DS, Lutty GA. C-reactive protein and complement factor H in aged human eyes and eyes with age-related macular degeneration. *Br J Ophthalmol.* (2011) 95:1323–30. doi: 10.1136/bjo.2010.199216
55. Wu Y, Potempa LA, El Kebir D, Filep JG. C-reactive protein and inflammation: conformational changes affect function. *Biol Chem.* (2015) 396:1181–97. doi: 10.1515/hsz-2015–0149
56. Johnson PT, Betts KE, Radeke MJ, Hageman GS, Anderson DH, Johnson LV. Individuals homozygous for the age-related macular degeneration risk-conferring variant of complement factor H have elevated levels of CRP in the choroid. *Proc Natl Acad Sci USA.* (2006) 103:17456–61. doi: 10.1073/pnas.0606234103
57. Sjoberg AP, Trouw LA, Clark SJ, Sjolander J, Heinegard D, Sim RB, et al. The factor H variant associated with age-related macular degeneration (His-384) and the non-disease-associated form bind differentially to C-reactive protein, fibromodulin, DNA, and necrotic cells. *J Biol Chem.* (2007) 282:10894–900. doi: 10.1074/jbc.M610256200
58. Laine M, Jarva H, Seitsonen S, Haapasalo K, Lehtinen MJ, Lindeman N, et al. Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein. *J Immunol.* (2007) 178:3831–6. doi: 10.4049/jimmunol.178.6.3831
59. Giannakis E, Jokiranta TS, Male DA, Ranganathan S, Ormsby RJ, Fischetti VA, et al. A common site within factor H SCR 7 responsible for binding heparin, C-reactive protein and streptococcal M protein. *Eur J Immunol.* (2003) 33:962–9. doi: 10.1002/eji.200323541
60. Mihlan M, Stippa S, Jozsi M, Zipfel PF. Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. *Cell Death Differ.* (2009) 16:1630–40. doi: 10.1038/cdd.2009.103
61. Hakobyan S, Harris CL, Van Den Berg CW, Fernandez-Alonso MC, De Jorge EG, De Cordoba SR, et al. Complement factor H binds to denatured rather than to native pentameric C-reactive protein. *J Biol Chem.* (2008) 283:30451–60. doi: 10.1074/jbc.M803648200
62. Pepys MB. The pentraxins 1975–2018: serendipity, diagnostics and drugs. *Front Immunol.* (2018) 9:2382. doi: 10.3389/fimmu.2018.02382


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 04 January 2019
doi: 10.3389/fimmu.2018.03048

# Source of Circulating Pentraxin 3 in Septic Shock Patients

*Chloé Albert Vega*[1,2], *Marine Mommert*[1,2], *Mathilde Boccard*[1,3], *Thomas Rimmelé*[4,5], *Fabienne Venet*[4,6], *Alexandre Pachot*[2], *Veronique Leray*[5], *Guillaume Monneret*[4,6], *Benjamin Delwarde*[5], *Karen Brengel-Pesce*[1,2], *François Mallet*[1,2,4] *and Sophie Trouillet-Assant*[1,7]*

[1] Joint Research Unit Hospices Civils de Lyon-bioMérieux, Hospices Civils de Lyon Centre Hospitalier Lyon Sud, Lyon, France, [2] Medical Diagnostic Discovery Department (MD3), bioMérieux S.A., Pierre Bénite, France, [3] Département des Maladies Infectieuses et tropicales, Hospices Civils de Lyon, Lyon, France, [4] EA 7426 Pathophysiology of Injury-Induced Immunosuppression, PI3, Claude Bernard Lyon 1 University-bioMérieux-Hospices Civils de Lyon, Edouard Herriot Hospital, Lyon, France, [5] Anesthesia and Critical Care Medicine Department, Hospices Civils de Lyon, Edouard Herriot Hospital, Lyon, France, [6] Hospices Civils de Lyon, Edouard Herriot Hospital, Immunology Laboratory, Lyon, France, [7] Faculté de Médecine Lyon Est, Virpath - Université Lyon, CIRI, INSERM U1111, CNRS 5308, ENS, UCBL, Lyon, France

OPEN ACCESS

**Edited by:**
*Cecilia Garlanda, Humanitas Università, Italy*

**Reviewed by:**
*Charles E. McCall, Wake Forest Baptist Medical Center, United States*
*Maria-Grazia De Simoni, Istituto Di Ricerche Farmacologiche Mario Negri, Italy*

***Correspondence:**
*Sophie Trouillet-Assant*
*sophie.assant@chu-lyon.fr*

**Specialty section:**
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

**Received:** *17 September 2018*
**Accepted:** *10 December 2018*
**Published:** *04 January 2019*

**Citation:**
*Albert Vega C, Mommert M, Boccard M, Rimmelé T, Venet F, Pachot A, Leray V, Monneret G, Delwarde B, Brengel-Pesce K, Mallet F and Trouillet-Assant S (2019) Source of Circulating Pentraxin 3 in Septic Shock Patients. Front. Immunol. 9:3048. doi: 10.3389/fimmu.2018.03048*

Sepsis, which is the leading cause of death in intensive care units (ICU), has been acknowledged as a global health priority by the WHO in 2017. Identification of biomarkers allowing early stratification and recognition of patients at higher risk of death is crucial. One promising biomarker candidate is pentraxin-3 (PTX3); initially elevated and persistently increased plasma concentration in septic patients has been associated with increased mortality. PTX3 is an acute phase protein mainly stored in neutrophil granules. These cells are responsible for rapid and prompt release of PTX3 in inflammatory context, but the cellular origin responsible for successive days' elevation in sepsis remains unknown. Upon inflammatory stimulation, PTX3 can also be produced by other cell types, including endothelial and immune cells. As in septic patients immune alterations have been described, we therefore sought to investigate whether such cells participated in the elevation of PTX3 over the first days after septic shock onset. To address this point, PTX3 was measured in plasma from septic shock patients at day 3 after ICU admission as well as in healthy volunteers (HV), and the capacity of whole blood cells to secrete PTX3 after inflammatory stimulation was evaluated *ex vivo*. A significantly mean higher (100-fold) concentration of plasma PTX3 was found in patients compared to HV, which was likely due to the inflammation-induced initial release of the pre-existing PTX3 reservoir contained in neutrophils. Strikingly, when whole blood was stimulated *ex vivo* with LPS no significant difference between patients and HV in PTX3 release was found. This was in contrast with TNFα which decreased production was illustrative of the endotoxin tolerance phenomenon occurring in septic patients. Then, the release of PTX3 protein from a HV neutrophil-free PBMC endotoxin tolerance model was investigated. At the transcriptional level, PTX3 seems to be a weakly tolerizable gene similar to TNFα. Conversely, increased protein levels observed in anergy condition reflects

a non-tolerizable phenotype, more likely to an anti-inflammatory marker. Hence, altered immune cells still have the ability to produce PTX3 in response to an inflammatory trigger, and therefore circulating white blood cell subset could be responsible of the sustained PTX3 plasma levels over the first days of sepsis setting.

**Keywords: pentraxin 3, sepsis, septic shock, immune dysfunction, endotoxin tolerance**

## INTRODUCTION

There are 31.5 million cases of sepsis per year that lead to 6 million deaths and 3 million people suffer from impairments leading to post-sepsis hospital re-admission (1). The official definition of sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (2) and, although infection is the initial trigger of sepsis response, the dysregulated immune response remains even after successful treatment of the infection (3). Due to the high heterogeneity among patients, early stratification and recognition of patients at higher risk of death is of importance as timely and appropriate decisions as to the best therapeutic approach is crucial to improve survival and decrease in-hospital mortality rates. In this context, several circulating biomarkers have been investigated in clinical studies and pentraxin 3 (PTX3) shows promising performance.

Pentraxin 3 is an acute phase protein which belongs to the long pentraxin subfamily, conserved in evolution, which acts as a key component of humoral innate immunity in microbial infections. Evidence suggests that PTX3 is a key homeostatic component at the crossroad of innate immunity, inflammation, tissue repair, and cancer (4). PTX3 binds conserved microbial structures and self-components under conditions of inflammation and activates effector functions (5). It has been found to be secreted by multiple cells including immune, vascular, lymphatic, endothelial, and epithelial cells (4). Neo-synthesis of PTX3 in these cells, except for neutrophils, is strongly induced by cytokines such as IL-1, TNF-α and by TLR agonists, but not by IL-6 or interferons (6). In neutrophils, PTX3 is synthetized in the bone marrow and stored in neutrophil granules, co-localized with lactoferrin (secondary granules) in physiological conditions, ready to be release upon inflammatory signals (7). Once released into the circulation, neutrophils lose the ability to produce PTX3 mRNA (8). The very high blood PTX3 levels observed as soon as onset of an injury is related to the release of preformed PTX3 contained in neutrophils. This was clearly described by Maugeri et al. who reported that neutrophils were responsible for plasma PTX3 concentration elevation within 6 h from onset of clinical symptoms of acute myocardial infarction, and that this returned to normal values 48 h (9).

PTX3 is barely detectable in the plasma of healthy individuals (<2 ng/mL), but its concentration can increase to up to 100 ng/ml during sepsis depending on the severity of disease (10). In septic shock patients, early high plasma PTX3 predicts subsequent new organ failure, and a smaller subsequent drop in circulating PTX3 over time predicts an increased risk of death (11, 12). Nevertheless, it is known that during septic shock immune cells are impaired (13); septic patients have markedly increased numbers of circulating neutrophils of various degrees of maturation with disrupted functions including impaired phagocytosis, reduced reactive oxygen species (ROS) production, and loss of chemotactic activity (14). Moreover, such patients have greater proportion of immature neutrophils with decreased levels of intracellular lactoferrin (15). Neutrophils may therefore not be the only source of PTX3 during sepsis. We then sought to investigate whether other immune cells participated in the elevation of PXT3 over the first days after septic shock onset. In the present study, using whole blood of sepsis patients and healthy volunteers (HV) as well as an *in vitro* model that mimics immune alterations found in patients, we examined the transcriptomic and proteomic changes of PTX3 upon *ex vivo* stimulation challenge.

## MATERIALS AND METHODS

### Study Population

This clinical study was conducted on septic shock patients admitted to the intensive care unit (ICU) of the Edouard Herriot Hospital (Hospices Civils de Lyon, Lyon, France) and is part of a wider study on ICU-induced immune dysfunctions. It was approved by the regional ethics committee (*Comité de Protection des Personnes Sud-Est II*, number 11236), which waived the need for written informed consent because the study was observational with a low risk for the patients and no specific procedure other than routine blood sampling was required. This study is also registered at the French ministry of research (*Ministère de l'Enseignement supérieur, de la Recherche et de l'Innovation*; DC-2008-509) and at the national data protection commission (*Commission Nationale de l'Informatique et des Libertés*). Oral information and non-opposition to inclusion in the study were mandatory and recorded in patients' clinical files.

Patients with septic shock were included prospectively. Septic shock was defined according to the Society of Critical Care Medicine and the European Society of Intensive Care Medicine (2): vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and serum lactate level >2 mmol/L (>18 mg/dL) in the absence of hypovolemia. The exclusion criteria were age <18 years, the presence of aplasia or immunosuppressive disease (e.g., HIV infection). At admission, data collected included demographic characteristics (age, gender), admission category (elective or emergency surgery, medicine) and site of primary infection; two clinical scores were recorded: the initial severity assessed by the Simplified Acute Physiology Score (SAPS II; range: 0–194) at admission and the Sequential Organ Failure Assessment (SOFA) score (range: 0–24), 24 h after ICU stay. Laboratory data during follow-up was also collected, as was death during the ICU stay. In addition,

study-specific experiments were performed (see details below) on routine blood samples (EDTA-coated tubes and heparin-coated tubes) taken at day 3–4 after septic shock onset.

Concomitantly, EDTA- and heparin-coated blood tubes from HV aged ≥50 years were obtained from the national blood service (*Etablissement Français du Sang*, EFS) and used immediately. According to EFS standardized procedures for blood donation and to provisions of the articles R.1243–49 and following ones of the French public health code, a written non-opposition to the use of donated blood for research purposes was obtained from HV. The blood donors' personal data were anonymised before transfer to our research laboratory. We obtained the favorable notice of the local ethical committee (Comité de Protection des Personnes Sud-Est II, Bâtiment Pinel, 59 Boulevard Pinel, 69,500 Bron) and the acceptance of the French ministry of research (*Ministère de l'Enseignement supérieur, de la Recherche et de l'Innovation*, DC-2008-64) for handling and conservation of these samples.

## Biological Samples and *in vitro* Experiment

### Plasma

Plasma samples were obtained after collection of whole blood in EDTA-coated tubes from 30 patients at day 3–4 after ICU admission and from 10 HV and were frozen until batch analysis.

### TruCulture® Stimulation

Heparinized-whole blood (1 mL), collected at the same time-point than plasma samples, from patients and HV was distributed into prewarmed TruCulture® tubes (Myriad Rbm, Austin, TX, USA) containing the medium alone (Null) or the medium with lipopolysaccharide (LPS; 100 ng/mL). These were then inserted into a dry block incubator and maintained at 37°C for 24 h. For the kinetic study, whole blood from 3 HV and 3 patients were incubated in TruCulture® tubes at 37°C, and 750 μL were collected for analysis at 1, 2, 4, and 24 h of incubation. Following incubation, the supernatant (media and plasma) and the cellular pellet were collected using a separation valve, according to manufacturer's instructions. Supernatants were aliquoted and immediately frozen at −20°C until batch quantification. Cell pellets were resuspended in 2 mL TRI Reagent® LS (Sigma-Aldrich, Deisenhofen, Germany), vortexed for 2 min, and rested for 10 min at room temperature before −80°C storage.

### Endotoxin Tolerance Model

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised venous blood of HV by Ficoll density gradient centrifugation (Eurobio Ingen, Courtaboeuf, France) and washed with PBS. Cells were cultured in 24-well plates at $2.10^6$ cells/mL in *X-Vivo* 20 Medium (Lonza, Verviers, Belgium). PBMCs were first cultured without or with 2 ng/mL LPS (mix of *Escherichia coli* O111:B4, O55:B5, and O127:B8; Sigma-Aldrich) to induce the LPS-primed state and incubated overnight at 37°C and 5% $CO_2$. In both conditions, LPS-primed (endotoxin tolerance condition) and unprimed (inflammation condition), PBMCs were incubated a second time for 4 h with 100 ng/mL LPS. A non-stimulated well was used as the control. For each condition, supernatants were collected and stored at −20°C. Cells were harvested, lysed in RLT buffer and stored at −80°C until further processing. The protocol was adapted from Allantaz-Frager et al. (16, 17).

### Monocyte Cell Line

THP1-Xblue™-MD2-CD14 cells (a human acute monocytic leukemia cell line stably expressing MD2/CD14 genes; Invivogen, San Diego, CA, USA) were cultured in 24-well plates at $1.10^6$ cells/mL in RPMI 1640 medium containing 2 mM L-glutamine, 25 mM HEPES (Thermofisher Scientific, Waltham, MA, USA), 10% heat-inactivated fetal bovine serum (FBS; Eurobio Ingen), 100 μg/mL Normocin (Invivogen) and 100 U/mL-100 μg-mL Pen-Strep (Thermofisher Scientific) (18). Endotoxin tolerance model set up on this cell line was above described.

## mRNA Decay and Intracellular Protein Stability Analysis

The endotoxin tolerance model, above described, performed on the THP1-Xblue™-MD2-CD14 cells was used to evaluate mRNA half-life and intracellular protein stability of PTX3 and TNFα. LPS-primed (endotoxin tolerance condition) and unprimed (inflammation condition) monocytes-like cells were incubated a second time, for 2 h, with LPS (100 ng/mL). Then, actinomycin D (5 μg/mL) or cycloheximide (10 μg/mL) were added to inhibit further transcription and translation, respectively, to both LPS-primed and LPS-unprimed conditions. The incubations were stopped at 0.5, 1, 2, 4, and 6 h for analysis. For untreated and actinomycing conditions, cells were harvested for molecular analysis. For untreated and cycloheximide condition, cells were harvested and solubilized in 200 μL lysis buffer (Human MxA protein ELISA, BioVendor, Brno, Czech Republic) dedicated to intracellular protein ELISA quantitation.

## Protein Analysis

Defective TNF-α production *ex-vivo* is a major trait of sepsis-induced immunosuppression (19), so this pro-inflammatory cytokine was evaluated as reference of PTX3 behavior. PTX3 and TNFα proteins from patients and HV in plasma and Truculture® supernatant were quantified using ELLA nanofluidic system (Biotechne, Minneapolis, MI, USA), according to the manufacturers' instructions. PTX3 and TNFα proteins after lysis of cellular pellets from untreated and cycloheximide conditions in the intracellular protein stability assay were also quantified using ELLA. Intracellular protein levels were expressed as a percentage of the maximal protein level.

These two proteins concentrations, from endotoxin tolerance model in PBMC and in THP1-Xblue™-MD2-CD14 cell culture supernatants, were detected using commercially-available ELISA kits Human TNF-alpha DuoSet ELISA and Human Pentraxin 3/TSG-14 DuoSet ELISA (R&D Systems, Biotechne), in accordance with the manufacturers' instructions. Results are expressed in pg/ml.

## Molecular Detection

For Truculture® cell pellet handling and RNA processing and detection, the protocol was followed according to Urrutia et al. study (20). Cell pellets from Truculture® stimulations kept in

TRI Reagen® LS (Sigma-Aldrich) were thawed under agitation. Before processing, thawed samples were centrifuged (3,000 g for 5 min at 4°C) to pellet cellular debris generated during the Trizol lysis. For extraction, a modified protocol of the NucleoSpin 96 RNA tissue kit (Macherey-Nagel Gmbh&Co. KG, Düren, Germany) was followed using a vacuum system. Briefly, 600 µL of clarified Trizol lysate was transferred to a tube preloaded with 900 µL 100% ethanol. The binding mixture was transferred to a silica column, then washed with buffers MW1 and MW2, and RNA was eluted using 30 µL RNase-free water. NanoString technology was used for mRNA detection, a hybridization-based multiplex assay characterized by its amplification-free step; 300 ng of RNA were hybridized to the probes (**Supplementary Table 1**) at 67°C for 18 h using a thermocycler (Biometra, Tprofesssional TRIO, Analytik Jena AG, Jena, Germany). After removal of excessive probes, samples were loaded into the nCounter Prep Station (NanoString Technologies, Seattle, WA, USA) for purification and immobilization onto the internal surface of a sample cartridge for 2–3 h. The sample cartridge was then transferred and imaged on the nCounter Digital Analyzer (NanoString Technologies) where color codes were counted and tabulated for PTX3 (NM_002852.3) and TNFα (NM_000594.2) genes. Counts number were normalized by the geometric mean of HPRT1 (NM_000194.1), DECR1 (NM_001359.1) and TBP (NM_001172085.1) housekeeping genes count number, as well as the negative and positive control values using nSolver analysis software (version 4.0, Nanostring technologies). Results are expressed in counts and fold change induction.

For endotoxin tolerance model, the protocol followed, with minor modifications, for PBMC and THP1-Xblue™-MD2-CD14 cell pellet handling, RNA processing and detection, was previously described (17). PTX3 and TNFα mRNA from endotoxin tolerance model in PBMC and in THP1-Xblue™-MD2-CD14 cells, but also from untreated and actimnomycin D conditions in the mRNA decay assay were quantified after RNA extraction from cellular pellet. RNeasy plus mini kit (Qiagen, Hilden, Germany) was used for total RNA extraction and RNA quantity was determined using Nanodrop (Thermofisher Scientific), according to the manufacturer's instructions. For mRNA detection, RNA was retro-transcribed using SuperScript VILO cDNA Synthesis kit (Thermofisher Scientific) followed by qPCR, performed using commercial Taqman probes for *TNF?* and *PTX3* (Invitrogen, Carlsbad, CA, USA) and normalized using the *PPIB* housekeeping gene. In the mRNA decay assay, mRNA levels were expressed as a percentage of the maximal mRNA level.

## mHLA-DR Measurement by Flow Cytometry

Circulating monocyte HLA-DR expression (mHLA-DR) from patients was assessed at day 3–4 on peripheral whole blood collected in EDTA anticoagulant tubes by flow cytometry (NAVIOS; Beckman-Coulter, Brea, CA, USA) as previously described (21). Results are expressed as the number of antibodies bound per cells (AB/C). Immunocompetence levels of mHLA-DR are defined as >15,000 AB/c and severe immunoparalysis as >5,000 AB/c (22, 23).

## Statistical Analysis

Results are expressed as median and interquartile ranges [IQR] for continuous variables. Non-parametric data were analyzed using the Mann–Whitney U test. Wilcoxon matched-pairs signed rank test was used for the analysis of THP1-MD2-CD14 experiments. Spearman test was used for correlation analysis. Statistical analyses were conducted using GraphPad Prism® software (version 5; GraphPad software, La Jolla, CA, USA). A *p*-value 0.05 was considered as statistically significant.

# RESULTS

## Clinical Characteristics of the Patients and Healthy Volunteers

From June 2017 to June 2018, 30 patients with septic shock were included (**Table 1**). The mean age was 65 (range: 19–86) years and 70% were male. The three major sites of infection were abdominal (30%), urinary tract infection (UTI) (13%) and skin and soft tissues (SST) (13%). The mean (range) SOFA score at day 1–2 was 9 (4–15) and SAPS II was 62 (26–93), indicating a high level of severity. 90% of patients had low mHLA-DR at 3–4 days after onset of shock, ranging from moderate to severe immunoparalysis. During ICU stay, mortality was 17% ($n = 5$) and the mean ICU stay among those who were discharged alive was 10 days. Concomitantly, a total of 10 HV were included, 50% were male and the mean age was 54.2 (range: 50–60) years.

## Plasma PTX3 Concentration

The median [IQR] PTX3 plasma levels at day 3–4 after ICU admission was significantly greater in septic shock patients (22031 [7518–52891] pg/mL) than in HV (438 [364–557] pg/mL, $p < 0.0001$; **Figure 1**). The 5 patients who died during ICU stay had significant higher PTX3 plasma concentration (204879 [47199–604280] pg/mL) compared to those who were discharged alive (14893 [6832–35336] pg/mL, $p < 0.05$; **Figure 1**). The correlation of plasma PTX3 levels with the degree of immunosuppression of the cells measured on the same day was explored, and no significant correlation was found (r spearman: −0.317, **Figure S1**).

## *Ex vivo* Whole-Blood LPS Stimulation

After 24 h incubation, we evaluated the *ex vivo* capacity of patient blood cells to express PTX3 and TNFα at the protein and molecular level upon LPS stimulation compared to an unstimulated condition. Due to the high level of PTX3 in septic patients at baseline (unstimulated condition, median [IQR]: 11128 [5052–21559] pg/mL), results were expressed as the difference in cytokine production between the LPS-stimulated condition and the unstimulated condition. Likewise, as PTX3 mRNA counts were higher in patients at baseline than in HV (unstimulated condition, median [IQR]: 233 [75–561] counts in patients and median [IQR]: 59 [38–98] counts in HV, $p < 0.05$),

**TABLE 1 |** Clinical and immunological data for patients with septic shock.

| | Patients at D3-D4 ($n = 30$) |
|---|---|
| Age, years (range) | 65 (19–86) |
| Sex, male, n (%) | 21 (70) |
| SOFA score (range) | 9 (4–15) |
| SAPS II (range) | 62 (26–93) |
| Missing data | 3 |
| mHLA-DR (AB/c) | 8240 (1644–32790) |
| **TYPE OF ADMISSION, *N* (%)** | |
| Medical | 13 (43) |
| Emergency surgery | 17 (57) |
| **SITE OF PRIMARY INFECTION, *N* (%)** | |
| Abdominal | 9 (30) |
| UTI | 4 (13) |
| SST | 4 (13) |
| Other | 13 (44) |
| LOS in the ICU, days (range) | 10 (2–34) |
| Death in the ICU, *n* (%) | 5 (17) |

*Numeric variables are presented as mean (range). Categorical variables are presented as number of cases, and percentages among the total of patients in parentheses. Simplified Acute Physiology Score (SAPS II) was calculated after admission and Sequential Organ Failure Assessment (SOFA) score was measured after 24 h of ICU stay. mHLA-DR expressed as numbers of anti-HLA-DR antibodies bound per monocyte (AB/C). LOS, length of stay; UTI, urinary tract infection; SST, skin and soft tissue.*

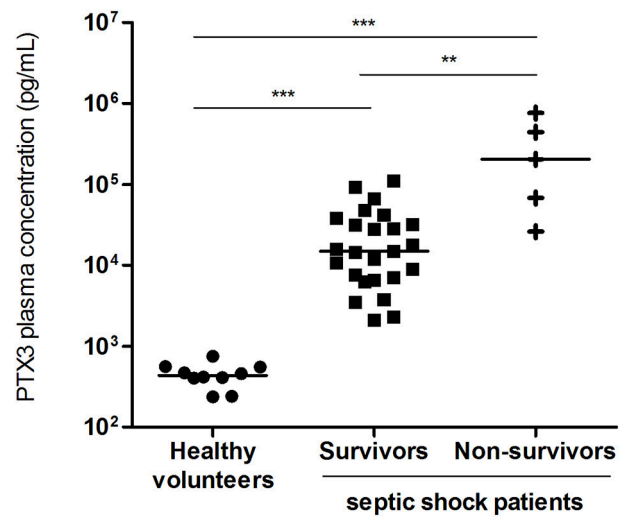


**FIGURE 1 |** Plasma PTX3 concentration. PTX3 plasma concentration in survivors ($n = 25$) and non-survivors ($n = 5$) septic shock patients, at day 3–4 after ICU admission and in healthy volunteers ($n = 10$). Bar represents the median. $^{**}p < 0.001$; $^{***}p < 0.001$.

results were expressed as the ratio in counts between the LPS-stimulated condition and the unstimulated condition. Septic shock patients had a significantly attenuated secretion of LPS-stimulated TNFα concentrations (median [IQR]: 701 [314–1301] pg/mL) compared to HV (median [IQR]: 5215 [3895–6014] pg/mL, $p < 0.0001$; **Figure 2A**). There was a corresponding 5-fold decrease in TNFα gene expression after LPS stimulation in septic shock patients compared to HV (fold change: 1.33 vs. 6.94 respectively, $p < 0.0001$; **Figure 2B**). Conversely, septic shock patients did not have altered capacity to secrete PTX3 after LPS stimulation. There was no significant difference (**Figure 2C**, $p = 0.08$) observed in PTX3 concentrations between patients (median [IQR]: 5,077 pg/mL [4128–8737] pg/mL) and HV (median [IQR]: 7393 [5464–9439] pg/mL). At the molecular level, PTX3 gene expression (**Figure 2D**) by blood cells after LPS stimulation did not seem to be affected in patients (fold change: 0.95). Furthermore, LPS did not induce a significant gene expression of PTX3 in HV (fold change: 1.66).

## mRNA and Protein Kinetic on Whole Blood After LPS-Stimulation

Kinetics studies were performed to decipher the apparently unusual dichotomy between PTX3 mRNA expression and protein secretion, in contrast to TNFα. *Ex vivo* whole blood from 3 healthy volunteers and 3 septic shock patients were stimulated with LPS and four time-points were analyzed post-stimulation: 1, 2, 4, and 24 h. In HV, we observed a massive early TNFα mRNA expression at 2 h post-stimulation (80-fold induction) which decreased by half 2 h later and reached low but significant levels of induction 24 h post-stimulation. Protein levels quickly raised 4 h post-stimulation to reach the highest 24 h post-stimulation (**Figure 3A**). For PTX3 mRNA, the peak expression was also observed 2 h post-stimulation (70-fold), and sharply decreased 2 h later until under significant levels 24 h post-stimulation. PTX3 protein required 24 h to reach high levels post-stimulation (**Figure 3B**), evidencing a time-lag between transcription and protein secretion. For septic shock patients, an overall decrease in induction efficiency was observed for both TNFα and PTX3 at the transcriptional level, the lowest level being reached after 24 h of stimulation, similarly to HV. Conversely, the highest level of TNFα protein was observed at the earliest time-points, to further decrease until very low levels(**Figure 3C**), while PTX3 protein levels were elevated during the first 4 h, to reach highest levels 24 h post-stimulation (**Figure 3D**), similar to 24-h HV levels.

## *In vitro* PBMC- and THP1-MD2-CD14 Cell Line-Based Endotoxin Tolerance Model

Given that mature neutrophils are not able to produce *de novo* PTX3 (8), we then hypothesized that the remaining circulating immune cells could be a possible source of PTX3 production during the first days of sepsis. To mimic the altered immune states observed in septic shock patients, healthy neutrophil-free PBMCs ($n = 12$) were stimulated with LPS to reproduce an inflammatory state and a monocyte-anergy state (endotoxin tolerance phenomenon) to study PTX3 behavior. As expected, median TNFα release was two-times lower in the anergy condition (median [IQR]: 1270 [642–1801] pg/mL) than in the inflammatory situation (median [IQR]: 2782 [2257–4124] pg/mL, $p < 0.001$; **Figure 4A**); a similar result was found at the molecular level (fold change 2 vs. 14 respectively, $p < 0.0001$; **Figure 4B**). For PTX3, strikingly, there was no significant difference between LPS-induced inflammation

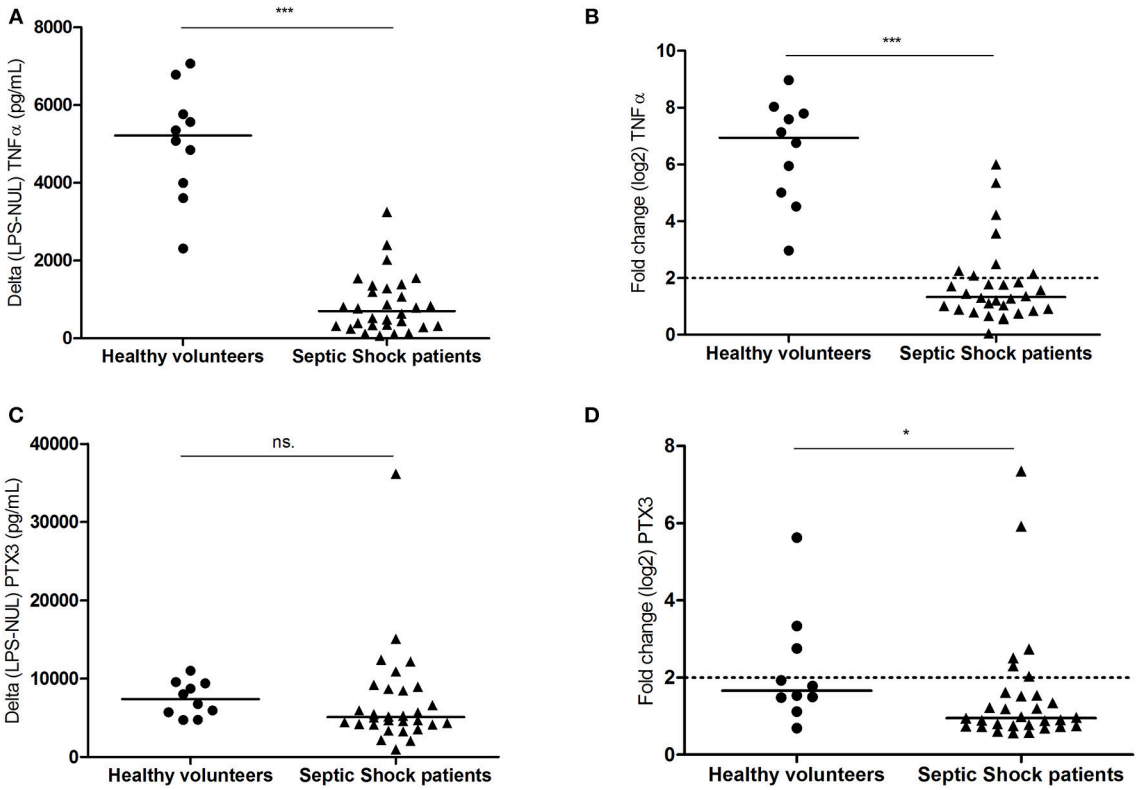


**FIGURE 2 |** *Ex vivo* whole-blood LPS-stimulation. Whole blood from 10 healthy volunteers and 30 septic shock patients stimulated *ex vivo* with LPS in Truculture® tubes for 24 h. Protein levels **(A–C)** and mRNA gene expression **(B–D)** are plotted for TNFα and PTX3. For cytokine quantification, results were expressed as the difference in cytokines production between LPS stimulated condition and unstimulated conditions (pg/mL). Counts number for mRNA gene expression, were normalized by the geometric mean of *hprt1*, *decr1,* and *tbp* housekeeping genes. The relative differential expression between LPS-stimulated and unstimulated condition was represented in the figure. Bar represents median. NS: not significant; $^{*}p < 0.05$; $^{***}p < 0.0001$.

(median [IQR]: 160.8 [91.8–238.5] pg/ml) and LPS-induced anergy conditions (median [IQR]: 325.6 [183.1–384.9] pg/mL), although there was a trend toward increased PTX3 secretion in the endotoxin tolerance condition ($p = 0.07$; **Figure 4C**). A conserved capacity to secrete PTX3 was observed in the *in vitro* model, independently of the inflammatory state. PTX3 gene expression was also not significantly different between both inflammatory *in vitro* conditions (fold change 8 vs. 5 respectively), although there was a trend toward a lower value in the anergy condition ($p = 0.24$, **Figure 4D**). The trends observed with PBMCs were statistically confirmed with the monocyte THP1-MD2-CD14 cell line (**Figure S2**).

## mRNA and Protein Decay Analysis

Because of the intriguing behavior of PTX3 mRNA and protein observed in whole blood, in PBMC and in THP1-MD2-CD14 cell line, we sought to investigate PTX3 mRNA half-life and its intracellular protein stability, as well as for TNFα, in inflammation and anergy conditions. Using actinomycin D to inhibit RNA synthesis, we reported that PTX3 mRNA half-life was approximately 4 h, compared to the shorter 0.5 h of TNFα mRNA half-life (**Figure S3A**). No difference was observed between inflammation and anergy conditions. By adding cycloheximide in cell culture 2 h after LPS stimulation, we observed the behavior of the intracellular content of PTX3 and TNFα in inflammatory and anergy conditions. We noticed that the intracellular content of PTX3 is maintained in the anergy condition compared to a slight decay in LPS-induced inflammation condition. Concerning TNFα, more than 50% of the intracellular content was diminished in both inflammation and anergy conditions only 1 h after the addition of cycloheximide (**Figure S3B**).

# DISCUSSION

Pentraxin-3, along with other biomarkers such as PCT and lactate, have been stated as clinically informative of disease severity and patient outcome in sepsis and septic shock (24). We confirmed that PTX3 is barely detectable in plasma from HV, around 2 ng/mL in the study reported by Yamasaki et al. (25) and <1 ng/mL herein, while it is much greater in sepsis patients, up to 100 ng/mL in the study reported by Daigo et al. (10) and up to 800-fold greater herein for highest values; this is in accordance with a prompt liberation of preformed PTX3 from granules after an inflammatory trigger (26). We also observed that patients with higher PTX3 plasma levels 3–4 days after ICU admission were

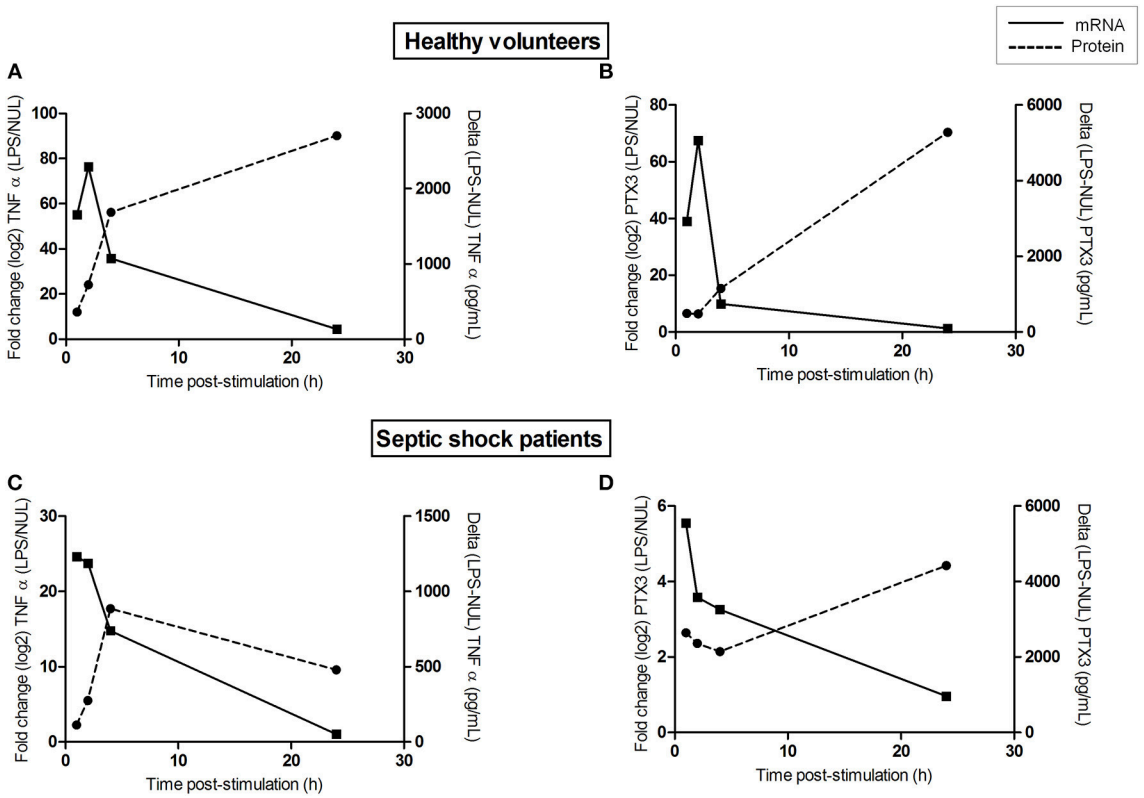


**FIGURE 3 |** mRNA and protein kinetic on whole blood after LPS-stimulation. Whole blood from 3 healthy volunteers **(A,B)** and 3 septic shock patients **(C,D)** stimulated *ex vivo* with LPS in Truculture® tubes for 1, 2, 4, and 24 h. Protein levels (dashed line) and mRNA gene expression (solid line) are plotted for TNFα **(A,C)** and PTX3 **(B,D)**. For cytokine quantification, results were expressed as the difference in cytokines production between LPS stimulated condition and unstimulated conditions (pg/mL, y right axis). Counts number for mRNA gene expression, were normalized by the geometric mean of *hprt1*, *decr1,* and *tbp* housekeeping genes. The relative differential expression between LPS-stimulated and unstimulated condition was represented in the figure (y left axis). Lines represents median of the three observations.

those who died during ICU stay. This is in line with the study reported by Huttunen et al. who found that PTX3 values were markedly higher in bacteraemic patients who died compared to survivors (27), also described in a septic shock cohort (11).

In the present study we aimed to assess whether circulating cells could be responsible for the maintenance of PTX3 concentration in the blood of patients over time during severe sepsis. LPS stimulation revealed a state of immunosuppression with a 5-fold lower capacity of patient blood cells to produce TNFα mRNA and release of this cytokine as compared to HV. Strikingly, patient blood cells still had the capacity to secrete PTX3 protein after *ex vivo* LPS stimulation, as did HV. In line with this, the kinetic analysis of PTX3 expression in whole blood stimulated by LPS, both in HV and patients, revealed that the mRNA content is globally decrease from 4 h on, reaching barely detectable levels 24 h post-stimulation whereas the continuous increase in PTX3 protein level reached its maximum at 24 h. The observed delay between the highest mRNA and protein amounts ranged roughly between 20 and 24 h in our conditions. Such observation has already been described in a human fibroblast cell line where such delay in PTX3 synthesis compared to mRNA production was shown (28). Altogether, these results indicate that sustained PTX3 plasma levels in the first week of sepsis could be driven by circulating blood cells, despite their altered immune functions.

Knowing that mature neutrophils are responsible for PTX3 secretion in the first hours of sepsis onset but are unable to produce *de novo* mRNA PTX3, we then aimed to decipher the cellular source of circulating PTX3 neo-synthesis in immunodysregulated septic shock patients. Onset of sepsis is characterized by dysfunctional host response of patients, where an acute hyper-inflammatory phase is established leading to organ damage and hence, early death. Simultaneously, a compensatory response is initiated and can consequently plunge the patient in a longer immunosuppressive phase. To reproduce these altered immune states, a neutrophil-free immune cell compartment was used to evaluate its capacity to produce and release PTX3 in different inflammatory conditions. The endotoxin tolerance model was set up on freshlyPBMC isolated from HV and on the monocyte THP1-MD2-CD14 cell line as well, to mimic an inflammatory- and a tolerant-immune state. At the transcriptional level, PTX3 seems to be a weakly tolerizable gene similar to TNFα, as previously reported in the literature (16, 17). Conversely, increased protein levels observed in anergy

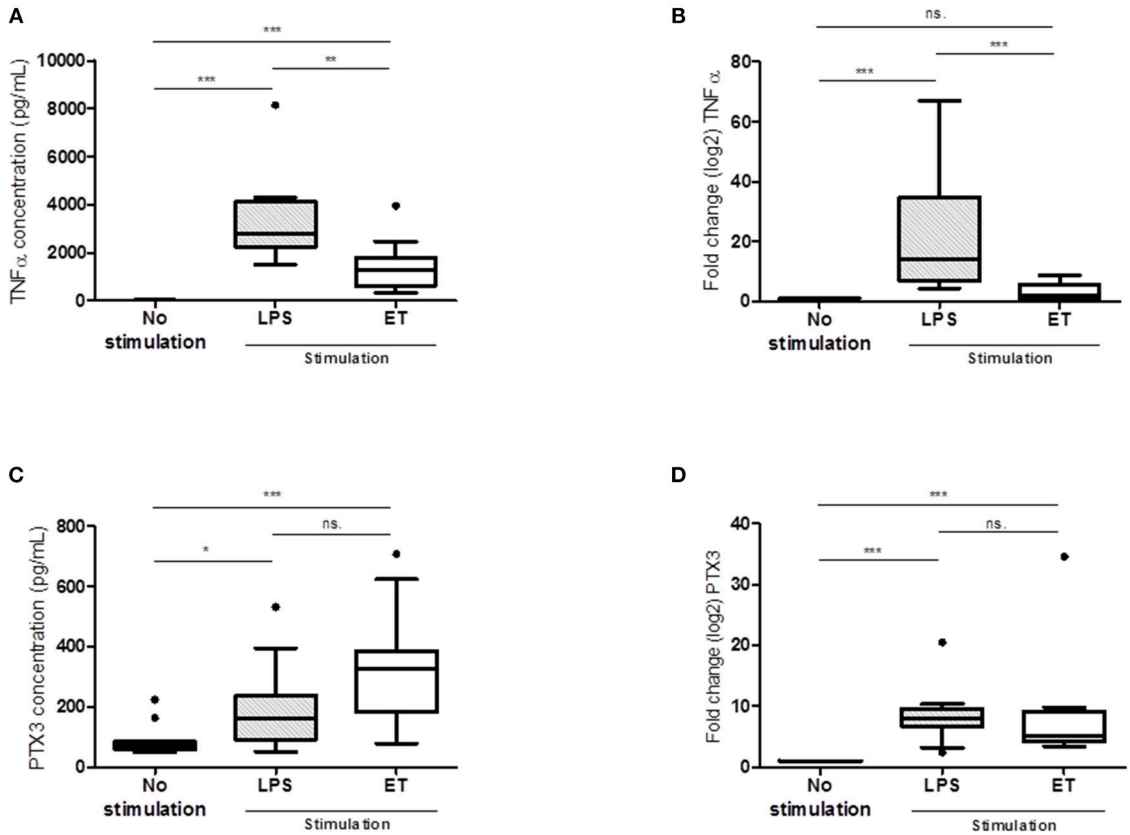


**FIGURE 4 |** *In vitro* PBMC-based endotoxin tolerance model. LPS condition: stimulation with 100 ng/mL LPS at day 1 and ET (endotoxin tolerance) condition: stimulation with 2 ng/mL LPS at day 0 and 100 ng/mL LPS at day 1. Protein levels **(A–C)** and mRNA gene expression **(B–D)** are plotted for TNFα and PTX3 ($n = 12$). For cytokine expression results are expressed in pg/mL. For mRNA expression results are expressed in fold change. NS: not significant; *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$.

condition reflects a non-tolerizable phenotype, different from TNFα but more likely to IL-10, as already described (29). Altogether, these observations, which demonstrate the capacity of PTX3 secretion by tolerizable circulating immune mononuclear cells, emphasize the anti-inflammatory role of PTX3 in sepsis. Of note, PTX3 has been described as associated with IL-10 in atherosclerosis experimental conditions (30) and could help to counteract the pro-inflammatory response observed in sepsis (14). The mechanisms supporting the discordant behavior of PTX3 at transcription and translation levels, different from TNFα, remained to be clearly determined. On one hand, the longer PTX3 mRNA half-life observed *in vitro*—4 vs. 0.5 h for TNFα–may support a sustained protein translation in the tolerant condition. Conversely, it was reported that miRNAs may interact with PTX3 mRNA, making it unstable and responsible for the delay with protein synthesis (28). In addition, we observed a very significantly higher stability of PTX3 protein compared to TNFα, potentially 4 times. Even if we were not able to determine PTX3 protein half-life in our experimental settings, we observed that PTX3 is more stable in the tolerant condition than in the LPS-induced inflammation condition, which would contribute to the higher protein levels in the tolerant condition. Of note, a 2 h PTX3 protein plasma half-life was measured using exogenously administered recombinant protein (31). Interestingly, the absence of difference in mRNA PTX3 half-life between inflammation and tolerant conditions and the apparently higher stability of PTX3 protein in anergy condition would suggest a complex phenomenon involving translation enhancement, although we cannot exclude an increased stability, which could be mediated by protein-protein interaction e.g., complement factors (32). Moreover, PTX3 is described as induced by TNFα (33, 34) but TNFα signaling pathway is known to be disrupted in sepsis (35) which suggests that PTX3 may be induced through other signaling pathways especially if PTX3 has a major anti-inflammatory effect in sepsis (36). This could explain why PTX3 was not altered during sepsis and can still be secreted over the course of the disease. Although this remains to be demonstrated, the PI3K/Akt pathway described in sepsis (37) represents a potential driver of PTX3 expression in sepsis, as recently demonstrated in cancer (38) and in inflammatory condition (39). This pathway, which seems to control the increased IL-1RA anti-inflammatory cytokine in sepsis patients and in LPS-adapted THP1 cells (40), acts indeed by enhancing translation efficacy without interfering with gene transcription.

Further experiments are required to evaluate this hypothesis to confirm if sustained circulating PTX3 levels in sepsis are achieved through this signaling pathway.

This study has some limitations to take into consideration regarding the mechanisms of production and action of PTX3 in sepsis pathophysiology. We studied the likely source of plasma PTX3 levels in septic shock patients by immune circulating cells, yet we cannot exclude a role of endothelial cells which was not investigated here (41). Indeed, although our results indicate that plasma PTX3 levels can be explained without endothelial cells contribution, their capacity to secrete PTX3 upon inflammatory signals and the observation that PTX3 protects against histone-mediated endothelial cell cytotoxicity in sepsis (42) and limits the vascular regenerative response (43) deserves further investigation. Moreover, *ex vivo* experiments as well as *in vit*ro models were performed on a limited number of healthy individuals and/or septic patients, and therefore the trends observed require confirmation in larger studies. Results obtained with the monocytic THP1-MD2-CD14 cell line, point out monocytes as (one of) the cell population(s) responsible for such plasma PTX3 source for the first days of sepsis. New pertinent cellular models are required to better understand the contribution of every (blood) cell type to PTX3 expression and the feedback loops with its environment during the host response in sepsis.

In conclusion, circulating PBMCs, despite their immune dysfunctions, could be responsible for the sustained PTX3 plasma levels over the first days of sepsis setting.

## AUTHOR CONTRIBUTIONS

All authors were involved in the analysis and interpretation of data as well as drafting the manuscript or revising it critically for important intellectual content. CAV and ST-A made substantial contributions to the conception and design of the study. CAV, ST-A and FM designed the experiments. CAV, MM, and MB performed the experiments. TR, FV, VL, GM, KB-P, and BD performed the data collection. CAV, ST-A, MM, FV, and FM performed the data analyses and data interpretation. CAV, FM, and ST-A wrote the paper. CAV, KB-P, TR, FV, MM, VL, GM, BD, AP, FM, and ST-A revised the manuscript content. All authors read and approved the final manuscript. ST-A takes responsibility for the integrity of the data analysis.

## FUNDING

This work was supported by Marie Sklodowska-Curie Innovative Training H2020-MSCA-ITN-2015.

## ACKNOWLEDGMENTS

We thank Dr. Jonathan Lopez (Plateforme de Recherche de transfert en Oncologie des Hospices Civils de Lyon) for his technical assistance on nanostring molecular biology. We thank Philip Robinson for language editing and critical reading the manuscript. We are grateful to the reviewers whose helpful comments contributed to improve this work, and to Olivier Gandrillon and Sylvie Pons for sharing their expertise.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2018.03048/full#supplementary-material

## REFERENCES

1. Fleischmann C, Thomas-Rueddel DO, Hartmann M, Hartog CS, Welte T, Heublein S, et al. Hospital incidence and mortality rates of sepsis. *Deutsch Arzteblatt int.* (2016) 113:159–66. doi: 10.3238/arztebl.2016.0159
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* (2016) 315:801–10. doi: 10.1001/jama.2016.0287
3. van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG. The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol.* (2017) 17:407–20. doi: 10.1038/nri.2017.36
4. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. PTX3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiol rev.* (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
5. Doni A, Garlanda C, Mantovani A. Innate immunity, hemostasis and matrix remodeling: PTX3 as a link. *Semin Immunol.* (2016) 28:570–7. doi: 10.1016/j.smim.2016.10.012
6. Alles VV, Bottazzi B, Peri G, Golay J, Introna M, Mantovani A. Inducible expression of PTX3, a new member of the pentraxin family, in human mononuclear phagocytes. *Blood* (1994) 84:3483–93.
7. Razvina O, Jiang S, Matsubara K, Ohashi R, Hasegawa G, Aoyama T, et al. Differential expression of pentraxin 3 in neutrophils. *Exp mol pathol.* (2015) 98:33–40. doi: 10.1016/j.yexmp.2014.11.009
8. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, et al. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med.* (2007) 204:793–804. doi: 10.1084/jem.20061301
9. Maugeri N, Rovere-Querini P, Slavich M, Coppi G, Doni A, Bottazzi B, et al. Early and transient release of leukocyte pentraxin 3 during acute myocardial infarction. *J Immunol.* (2011) 187:970–9. doi: 10.4049/jimmunol.1100261
10. Daigo K, Hamakubo T. Host-protective effect of circulating pentraxin 3 (PTX3) and complex formation with neutrophil extracellular traps. *Front immunol.* (2012) 3:378. doi: 10.3389/fimmu.2012.00378
11. Caironi P, Masson S, Mauri T, Bottazzi B, Leone R, Magnoli M, et al. Pentraxin 3 in patients with severe sepsis or shock: the ALBIOS trial. *Eur j clin invest.* (2017) 47:73–83. doi: 10.1111/eci.12704
12. Mauri T, Bellani G, Patroniti N, Coppadoro A, Peri G, Cuccovillo I, et al. Persisting high levels of plasma pentraxin 3 over the first days after severe sepsis and septic shock onset are associated with mortality. *Intens Care Med.* (2010) 36:621–9. doi: 10.1007/s00134-010-1752-5
13. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat rev Nephrol.* (2018) 14:121–37. doi: 10.1038/nrneph.2017.165
14. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol.* (2013) 13:862–74. doi: 10.1038/nri3552
15. Demaret J, Venet F, Friggeri A, Cazalis MA, Plassais J, Jallades L, et al. Marked alterations of neutrophil functions during sepsis-induced immunosuppression. *J Leukoc Biol.* (2015) 98:1081–90. doi: 10.1189/jlb.4A0415-168RR

16. Allantaz-Frager F, Turrel-Davin F, Venet F, Monnin C, De Saint Jean A, Barbalat V, et al. Identification of biomarkers of response to IFNg during endotoxin tolerance: application to septic shock. *PLoS ONE* (2013) 8:e68218. doi: 10.1371/journal.pone.0068218
17. Mommert M, Tabone O, Oriol G, Cerrato E, Guichard A, Naville M, et al. LTR-retrotransposon transcriptome modulation in response to endotoxin-induced stress in PBMCs. *BMC genom.* (2018) 19:522. doi: 10.1186/s12864-018-4901-9
18. Aneja R, Odoms K, Dunsmore K, Shanley TP, Wong HR. Extracellular heat shock protein-70 induces endotoxin tolerance in THP-1 cells. *J Immunol.* (2006) 177:7184–92. doi: 10.4049/jimmunol.177.10.7184
19. Antonakos N, Tsaganos T, Oberle V, Tsangaris I, Lada M, Pistiki A, et al. Decreased cytokine production by mononuclear cells after severe gram-negative infections: early clinical signs and association with final outcome. *Crit Care* (2017) 21:48. doi: 10.1186/s13054-017-1625-1
20. Urrutia A, Duffy D, Rouilly V, Posseme C, Djebali R, Illanes G, et al. Standardized whole-blood transcriptional profiling enables the deconvolution of complex induced immune responses. *Cell Rep*. (2016) 16:2777–91. doi: 10.1016/j.celrep.2016.08.011
21. Monneret G, Lepape A, Voirin N, Bohe J, Venet F, Debard AL, et al. Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. *Intens Care Med*. (2006) 32:1175–83. doi: 10.1007/s00134-006-0204-8
22. Docke WD, Hoflich C, Davis KA, Rottgers K, Meisel C, Kiefer P, et al. Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: a multicenter standardized study. *Clin chem.* (2005) 51:2341–7. doi: 10.1373/clinchem.2005.052639
23. Monneret G, Lepape A, Venet F. A dynamic view of mHLA-DR expression in management of severe septic patients. *Crit Care* (2011) 15:198. doi: 10.1186/cc10452
24. Hu C, Zhou Y, Liu C, Kang Y. Pentraxin-3, procalcitonin and lactate as prognostic markers in patients with sepsis and septic shock. *Oncotarget* (2018) 9:5125–36. doi: 10.18632/oncotarget.23701
25. Yamasaki K, Kurimura M, Kasai T, Sagara M, Kodama T, Inoue K. Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations. *Clin chem lab med.* (2009) 47:471–7. doi: 10.1515/CCLM.2009.110
26. Daigo K, Inforzato A, Barajon I, Garlanda C, Bottazzi B, Meri S, et al. Pentraxins in the activation and regulation of innate immunity. *Immunol Rev*. (2016) 274:202–17. doi: 10.1111/imr.12476
27. Huttunen R, Hurme M, Aittoniemi J, Huhtala H, Vuento R, Laine J, et al. High plasma level of long pentraxin 3 (PTX3) is associated with fatal disease in bacteremic patients: a prospective cohort study. *PLoS ONE* (2011) 6:e17653. doi: 10.1371/journal.pone.0017653
28. Rubino M. *Epigenetic Modifications Abolish the Expression of the Long Pentraxin PTX3 in Human Tumors.* Doctoral dissertation (2015). Available online at: https://air.unimi.it/handle/2434/254345?mode=full.73#s
29. Turrel-Davin F, Venet F, Monnin C, Barbalat V, Cerrato E, Pachot A, et al. mRNA-based approach to monitor recombinant gamma-interferon restoration of LPS-induced endotoxin tolerance. *Crit Care* (2011) 15:R252. doi: 10.1186/cc10513
30. Slusher AL, Mischo AB, Acevedo EO. Pentraxin 3 is an anti-inflammatory protein associated with lipid-induced interleukin 10 *in vitro*. *Cytokine* (2016) 86:36–40. doi: 10.1016/j.cyto.2016.07.012
31. Deban L, Russo RC, Sironi M, Moalli F, Scanziani M, Zambelli V, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nat Immunol*. (2010) 11:328–34. doi: 10.1038/ni.1854
32. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell* (2015) 160:700–14. doi: 10.1016/j.cell.2015.01.004
33. Zhang J, Koussih L, Shan L, Halayko AJ, Chen BK, Gounni AS. TNF up-regulates pentraxin3 expression in human airway smooth muscle cells via JNK and ERK1/2 MAPK pathways. *Allergy asthma clin immunol.* (2015) 11:37. doi: 10.1186/s13223-015-0104-y
34. Han B, Mura M, Andrade CF, Okutani D, Lodyga M, dos Santos CC, et al. TNFalpha-induced long pentraxin PTX3 expression in human lung epithelial cells via JNK. *J Immunol*. (2005) 175:8303–11. doi: 10.4049/jimmunol.175.12.8303
35. Hoogendijk AJ, Garcia-Laorden MI, van Vught LA, Wiewel MA, Belkasim-Bohoudi H, Duitman J, et al. Sepsis patients display a reduced capacity to activate nuclear factor-kappaB in multiple cell types. *Crit Care Med*. (2017) 45:e524–e31. doi: 10.1097/CCM.0000000000002294
36. Learn CA, Mizel SB, McCall CE. mRNA and protein stability regulate the differential expression of pro- and anti-inflammatory genes in endotoxin-tolerant THP-1 cells. *J Biol Chem*. (2000) 275:12185–93. doi: 10.1074/jbc.275.16.12185
37. Qiu P, Liu Y, Zhang J. Review: the role and mechanisms of macrophage autophagy in sepsis. *Inflammation* (2018). doi: 10.1007/s10753-018-0890-8. [Epub ahead of print].
38. Thomas C, Henry W, Cuiffo BG, Collmann AY, Marangoni E, Benhamo V, et al. Pentraxin-3 is a PI3K signaling target that promotes stem cell-like traits in basal-like breast cancers. *Sci signal.* (2017) 10:467. doi: 10.1126/scisignal.aah4674
39. Norata GD, Marchesi P, Pirillo A, Uboldi P, Chiesa G, Maina V, et al. Long pentraxin 3, a key component of innate immunity, is modulated by high-density lipoproteins in endothelial cells. *Arterioscler thromb vasc biol.* (2008) 28:925–31. doi: 10.1161/ATVBAHA.107.160606
40. Learn CA, Boger MS, Li L, McCall CE. The phosphatidylinositol 3-kinase pathway selectively controls sil-1ra not interleukin-1beta production in the septic leukocytes. *J Biol Chem*. (2001) 276:20234–9. doi: 10.1074/jbc.M100316200
41. Presta M, Camozzi M, Salvatori G, Rusnati M. Role of the soluble pattern recognition receptor PTX3 in vascular biology. *J cell mol med.* (2007) 11:723–38. doi: 10.1111/j.1582-4934.2007.00061.x
42. Daigo K, Nakakido M, Ohashi R, Fukuda R, Matsubara K, Minami T, et al. Protective effect of the long pentraxin PTX3 against histone-mediated endothelial cell cytotoxicity in sepsis. *Sci signal.* (2014) 7:ra88. doi: 10.1126/scisignal.2005522
43. O'Neill CL, Guduric-Fuchs J, Chambers SE, O'Doherty M, Bottazzi B, Stitt AW, et al. Endothelial cell-derived pentraxin 3 limits the vasoreparative therapeutic potential of circulating angiogenic cells. *Cardiovasc res.* (2016) 112:677–88. doi: 10.1093/cvr/cvw209

**Conflict of Interest Statement:** CAV, MM, AP, KB-P and FM are employed by an *in vitro* diagnostic company, bioMérieux.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 25 January 2019
doi: 10.3389/fimmu.2019.00029

# Circulating Pentraxin3-Specific B Cells Are Decreased in Lupus Nephritis

*Mariele Gatto[1,2], Annika Wiedemann[2], Nadja Nomovi[2], Karin Reiter[2], Eva Schrezenmeier[3], Thomas Rose[2], Franziska Szelinski[2], Andreia C. Lino[4], Sonia Valentino[5], Anna Ghirardello[1], Thomas Dörner[2†] and Andrea Doria[1*†]*

[1] Unit of Rheumatology, Department of Medicine, University of Padova, Padua, Italy, [2] Department Medicine, Rheumatology and Clinical Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany, [3] Department of Medicine, Nephrology and Medical Intensive Care, Charité – Universitätsmedizin Berlin, Berlin, Germany, [4] Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, Germany, [5] Humanitas Clinical and Research Center, Milan, Italy

**Background:** Pentraxin3 (PTX3) is overexpressed in kidneys of patients developing lupus nephritis (LN). Active LN is associated with reduced anti-PTX3 antibodies. However, abnormalities of B cell differentiation against PTX3 have not been characterized in systemic lupus erythematosus (SLE).

**Objective:** Characterization of PTX3-specific ($PTX3^+$) B cells in peripheral blood of SLE patients with or without LN and healthy donors (HD).

**Patients and Methods:** SLE patients without LN, biopsy-proven LN and matched HD were analyzed. Active LN was defined as proteinuria>0.5 g/day or CrCl<60 ml/min/1.73 $m^2$ with active urinary sediment. Peripheral B cells were analyzed for direct PTX3 binding by flow cytometry using PTX3 labeled with cyanine 5 (Cy5) and phycoerythrin (PE).

**Results:** Initially, a flow cytometry based assay to identify $PTX3^+$ B cells was developed by demonstrating simultaneous binding of PTX3-Cy5 and PTX3-PE. Specificity of B cells was validated by blocking experiments using unlabeled PTX3. We could identify circulating $PTX3^+$ B-cells in HD and patients. Notably, LN patients showed a significantly diminished number of $PTX3^+$ B cells (SLE vs. LN $p = 0.033$; HD vs. LN $p = 0.008$). This decrease was identified in naïve and memory B cell compartments (naïve: SLE vs. LN $p = 0.028$; HD vs. LN $p = 0.0001$; memory: SLE vs. LN $p = 0.038$, HD vs. LN $p = 0.011$).

**Conclusions:** Decreased $PTX3^+$ B cells in LN within the naïve and memory compartment suggest their negative selection at early stages of B cell development potentially related to a decreased regulatory function. $PTX3^+$ B cells could candidate for autoantigen-defined regulatory B cells as a striking abnormality of LN patients.

**Keywords: SLE, $PTX3^+$ B cells, lupus nephritis, biomarkers, flow-cytometry**

OPEN ACCESS

***Edited by:***
*Mauro Martins Teixeira, Universidade Federal de Minas Gerais, Brazil*

***Reviewed by:***
*Angelo A. Manfredi, Vita-Salute San Raffaele University, Italy*
*Luuk Hilbrands, Radboud University Nijmegen, Netherlands*

***Correspondence:**
*Andrea Doria*
*adoria@unipd.it*

*†These authors have contributed equally to this work*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *01 October 2018*
***Accepted:*** *08 January 2019*
***Published:*** *25 January 2019*

***Citation:***
*Gatto M, Wiedemann A, Nomovi N, Reiter K, Schrezenmeier E, Rose T, Szelinski F, Lino AC, Valentino S, Ghirardello A, Dörner T and Doria A (2019) Circulating Pentraxin3-Specific B Cells Are Decreased in Lupus Nephritis. Front. Immunol. 10:29. doi: 10.3389/fimmu.2019.00029*

## INTRODUCTION

Lupus nephritis (LN) is a severe manifestation of systemic lupus erythematosus (SLE), involving up to two thirds of patients at onset or during disease course (1–3). The inflammatory process in the kidney is driven by both cellular and humoral abnormalities, in particular formation of immune complexes (4–6).

Pentraxin3 (PTX3) belongs to the long pentraxin family, i.e., a superfamily of multimeric evolutionary conserved molecules which are released locally at sites of inflammation (7–12) and are involved in tissue homeostasis, with prominent antimicrobial functions and fine-tuning of inflammation (8–10). It has been proposed as a bridge between innate and adaptive immunity, being endowed with antibody-like properties i.e., the capability to provide opsonization of foreign or apoptotic bodies (9), to modulate antigen presentation and inflammatory responses. Indeed, soluble PTX3 exerts an anti-inflammatory function by sequestering C1q, while when bound to apoptotic debris PTX3 allows C1q fixation and subsequent enhanced activation of the classical complement pathway (8, 10, 13).

Lupus kidney is a source of autoantigens (14, 15), and current evidence supports PTX3 involvement in SLE-driven renal inflammation. Deposits of PTX3 have been characterized in renal samples of several immune-mediated kidney diseases including LN (16–18), where the extent of deposition correlated with proteinuria and renal fibrosis (11, 17, 18).

Moreover, clinical observations showed that SLE patients display high frequencies and titers of anti-PTX3 antibodies (18–20), which are inversely correlated with LN occurrence (18, 19, 21). Furthermore, PTX3 immunization of lupus-prone mice resulted in anti-PTX3 antibody occurrence and was associated with delayed and milder lupus-like nephritis and increased overall and disease-free survival (22), thus providing evidence for an immunomodulatory capacity of anti-PTX3 antibodies.

Despite the interaction of PTX3 with diverse cell types has been described (9, 13, 23), its relationship with B cells remains blurry. Recently, PTX3 released by specialized neutrophils was described to interact with marginal zone (MZ) B cell thereby promoting class-switch from IgM to IgG antibodies (24), however a direct interaction was not described.

In light of the aforementioned observations and open questions, and considering the established role of B cells in LN development (25), it is attractive to speculate that PTX3-specific ($PTX3^+$) B cells could bear a regulatory potential in lupus and particularly in LN. Herein, we sought to develop a new method that allows identification and characterization of peripheral $PTX3^+$ B cells. Using this methodology, we found $PTX3^+$ B cells in SLE patients and healthy donors (HD) which were virtually absent in LN patients. This data suggests that these autoantigen specific B cells may represent a layer of regulation that is lost in LN patients.

## PATIENTS AND METHODS

Thirty-eight consecutive SLE patients (American College of Rheumatology criteria), including 12 with biopsy-proven LN, and 22 HD were recruited.

Active LN was defined as proteinuria > 0.5 g/day or creatinine clearance < 60 ml/min/1.73 $m^2$, evaluated with Cockcroft and Gault formula, with active urinary sediment (3). Accordingly, LN was considered active in 7/12 patients. Demographics of all groups and clinical and therapeutic features of patients are given in **Table 1**.

**TABLE 1 |** Demographic and therapeutic features of healthy donors and patient groups.

| | Healthy donors | Patients | |
|---|---|---|---|
| | | Non-renal SLE | LN |
| No. | 22 | 26 | 12 |
| Mean age ± SD | 33.4 ± 8.6 | 34.6 ± 10.2 | 41.8 ± 10.37 |
| Female (%) | 77.2 | 84.6 | 75 |
| Active disease (cSLEDAI≥2) (%) | | 3/26 (11.5%) | 7/12 (58.3%) |
| cSLEDAI [mean ± SD] | | 1.14 ± 3.66 | 4.17 ± 3.86 |
| **LN class* (no.)** | | | |
| Proliferative (III or IV) | | N/A** | 7 |
| V or mixed | | | 5 |
| 24-h proteinuria (g) [mean ± SD] | | N/A | 4 ± 1.41 |
| Active urinary sediment§ (%) | | 0 | 41.7 |
| **CONCOMITANT TREATMENT** | | | |
| Oral prednisone (%) [mean daily dosage ± SD] | | 60.8 [4.04 ± 4.14] | 58.3 [3.98 ± 6.05] |
| HCQ (%) | | 69.4 | 58.3 |
| Immunosuppressants (%) | | 73.9 | 66.7 |
| MMF | | 34.7 | 41.7 |
| MTX | | 4.3 | 0 |
| AZA | | 30.4 | 25 |

**International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003. **These patients never underwent kidney biopsy due to lack of renal involvement. § > 5 white blood cells and/or >5 red blood cells/high power field and/or heme-granular/red cell casts. SLE, systemic lupus erythematosus; LN, lupus nephritis; SD, standard deviation; cSLEDAI, clinical SLE disease activity index; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil; MTX, methotrexate; AZA, azathioprine; N/A, not available.*

All subjects gave written informed consent, in accordance with the local ethics committee of the Charité Universitätsmedizin Berlin.

## Whole Blood Lysis

Blood was obtained before any induction therapy for LN in EDTA vacutainer tubes (BD Biosciences, San Jose, CA, USA) and lysed according to the manufacturer's instructions. Briefly, 1 ml of EDTA blood was incubated with 10 ml of lysing solution (Lysing Buffer BD Pharm Lyse™). The obtained cells were washed three times with phosphate-buffered saline/bovine serum albumin (PBS/BSA) (Miltenyi, Germany).

## Staining Procedure and Flow Cytometry

To identify PTX3$^+$ B cells, recombinant human purified PTX3 (10) was labeled with either cyanine 5 (Cy5) or phycoerythrin (PE). Tetanus (TT) staining was performed in parallel as control as previously described (24) in order to establish an optical reference range for PTX3$^+$ cells. Antigens were labeled at the German Rheumatism Research Centre (DRFZ), Berlin.

For flow cytometric analysis, the following fluorochrome-labeled antibodies were used: PTX3 staining: anti-CD19 Allophycocyanin (APC)-Cy7 (clone SJ25C1, BioLegend), anti-CD20 Brilliant Violet (BV)510 (clone 2H7, BioLegend), anti-CD27 Fluorescein isothiocynate (FITC) (clone M-T271, BD), IgG PECy7 (clone G18-145, BD), IgD Peridinin-Chlorophyll-protein (PerCp) Cy5.5 (clone IA6-2, BD), anti-CD3/anti-CD14 Pacific Blue (PacB) (clone UCHT1/M5E2, BD). TT staining: anti-CD19 PECy7 (clone SJ25C1, BD), anti-CD20 BV510 (clone 2H7, BioLegend), anti-CD27-FITC, IgD PerCp Cy5.5 (clone IA6-2, BD), anti-CD3/anti-CD14 PacB (clone UCHT1/M5E2, BD), anti-CD38 APC-Cy7 (clone HIT2, BioLegend).

PTX3$^+$B cells were identified as B cells binding both PTX3-PE and PTX3-Cy5 (**Figure 1**).

TT-specific B cells were identified by the binding of TT-Cy5. Specificity of this binding was checked in parallel stainings performed in our laboratory.

After Fc receptor blocking (Miltenyi Biotec, Germany), stainings were performed in the dark at 4°C for 15 min, followed by two washing steps with PBS/BSA and centrifugation for 5 min at 4°C and 330 × g. Stained cells were analyzed by flow cytometry using a FACS Canto II flow cytometer (BD, USA).

B cell subsets were defined as naïve (single CD3$^-$CD14$^-$Dapi$^-$CD19$^+$CD20$^+$CD27$^-$), memory (single CD3$^-$CD14$^-$Dapi$^-$CD19$^+$CD20$^+$CD27$^+$) and plasmablasts (single CD3$^-$CD14$^-$Dapi$^-$CD19$^+$CD27$^{hi}$CD20$^-$).

The gating strategy is exemplarily shown in **Supplementary Figure 1**. Absolute numbers of B subpopulations were calculated by using the absolute number of B cells/μl retrieved with Multitest 6-color TBNK analysis (BD, USA) according to the manufacturer's protocol.

## Data Analysis and Statistics

Samples included in analyses had at least $1 \times 10^6$ events with a minimum threshold for CD19$^+$ cells of 50,000 events.

Flow cytometric data was analyzed by FlowJo software 7.6.5 (TreeStar, Ashland, OR, USA). GraphPad Prism Version 5 (GraphPad software, San Diego, CA, USA) was used for statistical analysis. To test for significance, non-parametric tests were used.

# RESULTS

## Identification of PTX3$^+$ B Cells in Peripheral Blood of Patients and Controls

Initially, we developed a flow cytometric method to identify and quantify PTX3$^+$ B cells in human peripheral blood. B cells (CD3$^-$CD14$^-$CD19$^+$CD20$^+$) able to bind simultaneously PTX3-Cy5 and PTX3-PE were considered specific (**Figure 1A**) (gated as shown in **Supplementary Figure 1**). The specificity of PTX3 binding was further confirmed by blocking with unlabeled PTX3 prior to staining (**Figures 1A,B**). Blocking of single positive PE or Cy5-PTX3 B cells could not be efficiently performed (**Supplementary Figure 2**), thus these cells were not included in the present analysis.

## PTX3$^+$B Cells Are Decreased in LN Patients vs. SLE and HD

LN patients showed the lowest absolute numbers of PTX3$^+$ B cells among total peripheral B cells, which were also significantly decreased in comparison with HD and non-renal SLE [mean ± standard deviation (SD) cells/ml: LN 0.023 ± 0.027 vs. HD 33.09 ± 48.15, $p = 0.008$; LN 0.023 ± 0.027 vs. non-renal SLE 12.53 ± 20.24, $p = 0.033$] (**Figure 2A**, left).

Analyses of B cell subsets confirmed a substantial decrease in absolute numbers of both naïve (CD20$^+$CD27$^-$) and memory (CD20$^+$CD27$^+$) PTX3$^+$ B cells in LN compared to HD and non-renal SLE patients (mean ± SD naïve/ml: LN 0.18 ± 0.58 vs. HD 30.12 ± 42.96, $p = 0.0001$; LN SLE 0.18 ± 0.58 vs. non-renal 16.22 ± 24.88, $p = 0.028$; mean ± SD memory/ml: LN 0.97 ± 2.18 vs. HD 12.75 ± 24.88, $p = 0.011$; LN 0.97 ± 2.18 vs. non-renal SLE 4.07 ± 5.21, $p = 0.038$) (**Figure 2A**, middle and right).

Moreover, the frequencies of PTX3$^+$ B cells and B cell subsets were decreased in LN (**Figure 2B**), while there was no significant difference between HD and non-renal SLE.

Of note, no difference in PTX3$^+$ naïve and memory compartment was identified between active and inactive LN (data not shown), suggesting that the actual decrease in LN is not related to disease activity, rather mirroring a characteristic of LN.

We detected nearly no circulating PTX3$^+$ plasmablasts (CD27$^{hi}$CD20$^{-/low}$) in whole blood sampled for the majority of donors, where a minimum of $1 \times 10^6$ events was retrieved from each sample. These cells were absent even when a larger amount of cellular events ($27 \times 10^6$) from an SLE patient among a total of 7,648 plasmablasts was analyzed.

## Circulating PTX3$^+$ B Cells Reside Mainly Within Naïve (CD20$^+$CD27$^-$IgD$^+$) B Cells With a Similar Distribution Among HD and Non-renal SLE Patients

Using CD27 and IgD as surface markers, we further subdivided B cell subpopulations. We found that the majority of circulating PTX3$^+$B cells resided among the CD20$^+$CD27$^-$IgD$^+$ mature pre-switch naïve subset, followed by a lower number of

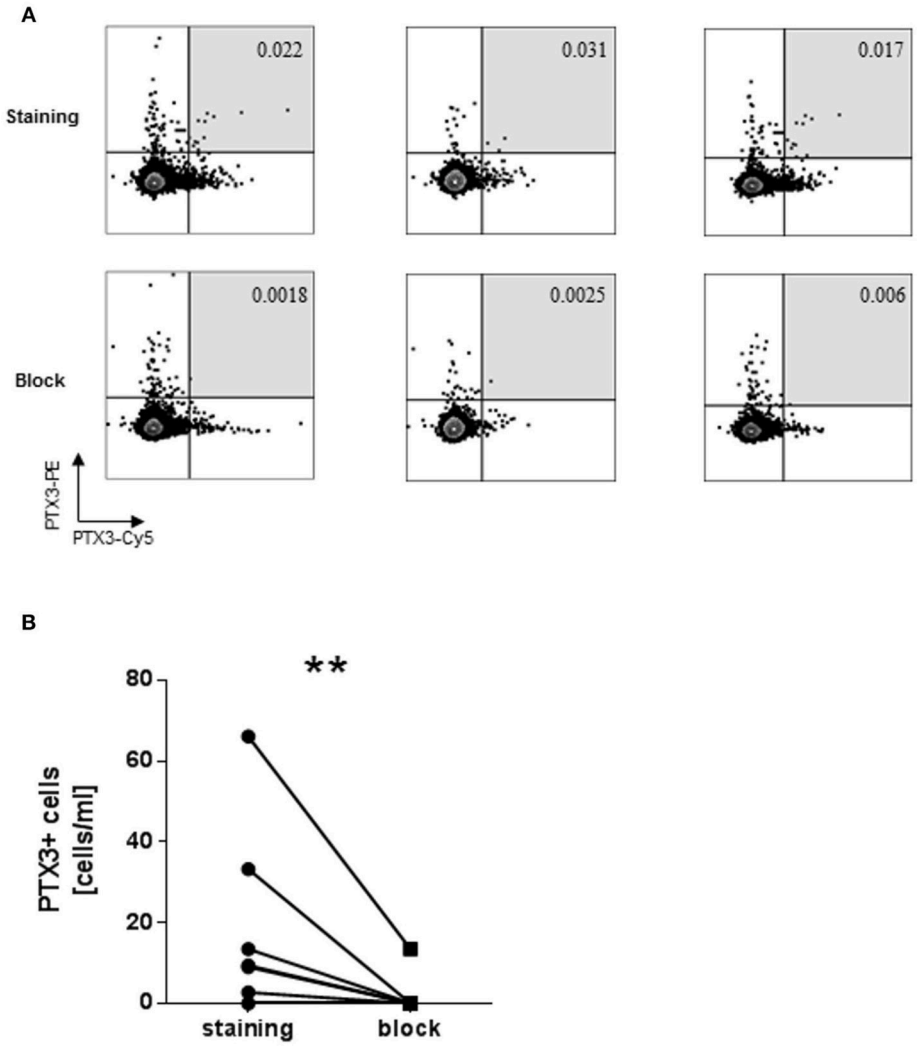


**FIGURE 1 |** Identification of PTX3+ B cells among CD19+CD20+ B cells. **(A)** Three representative dot plots of the PTX3-specific B cells before and after blocking with unlabeled PTX3. Only B cells staining positive for both PTX3-Cy5 and PTX3-PE were considered (light gray square). **(B)** Quantification of PTX3 binding among B cells before and after blocking of PTX3. Cy5, cyanin 5; PE, phycoerythrin. **<0.01.

$CD20^+CD27^+IgD^+$ B cells (**Figures 2C,D**), likely representing pre-switched memory B cells whose origin is still debated (26). This distribution remained consistent among HD and non-renal SLE patients (**Figure 2C**, left and middle), while LN patients did not show any difference among B cell subsets (**Figure 2C**, right). Proportions of $PTX3^+$ CD27IgD B cell subsets in relationship to the whole $PTX3^+$ B cell pool are shown in **Figure 2D**.

## DISCUSSION

In this study, we aimed at characterizing the distribution of an $PTX3^+$ B cells in peripheral blood of SLE patients, focusing on potential differences between LN and non-renal SLE. Most interestingly, our results show that LN patients bear strikingly reduced amounts of circulating $PTX3^+$ B cells both in the naïve and memory compartment vs. HD and non-renal SLE. This finding was observed regardless of LN activity, suggesting a characteristic divergence between patient subsets.

Moreover, the current results indicate that selection of PTX3 binding B cells appears to be defective in LN based on the analysis of sole $PTX3^+$ B cells and B cell subsets which were able to bind specifically PTX3 molecules using two different dyes blockable by unlabeled antigen. The observation that most circulating $PTX3^+$B cells belong to $CD27^-IgD^+$ and to $CD27^+IgD^+$ subsets is not surprising, since PTX3 is a self-antigen without enhanced antigenicity. In this context, lack of detectable $PTX3^+$ plasmablasts is consistent with the notion that the B and plasma cell compartments underlie independent regulations (27–30), thus at this point it is not possible to establish a direct link between these specific PTX3-B cell subsets and the production of anti-PTX3 antibodies.

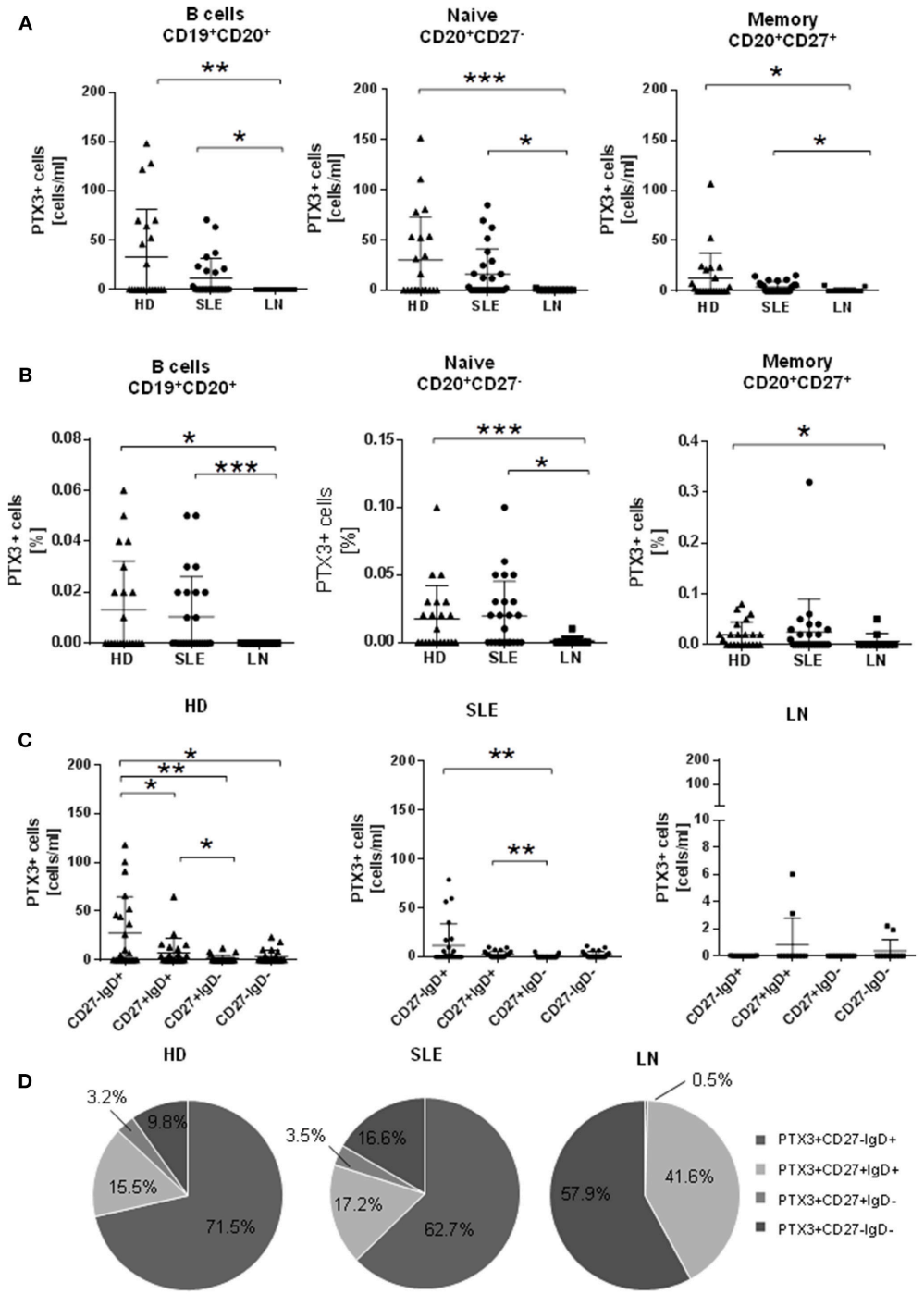


**FIGURE 2 |** PTX3$^{+}$ B cells are decreased in patients with lupus nephritis and are mainly confined to CD27$^{-}$IgD$^{+}$ B cells. **(A)** Absolute numbers of PTX3$^{+}$ B cells (cell/mL) within (left) total; (middle) naïve or (right) memory B cells in HD ($n = 22$) and SLE ($n = 26$) and LN ($n = 12$) patients. **(B)** Frequencies of PTX3$^{+}$ B cells (left), naïve (middle), and memory (right) are decreased in LN ($n = 12$) in comparison with HD ($n = 22$) and SLE ($n = 26$). **(C)** Distribution of CD27 and IgD expression by PTX3$^{+}$ B cell subsets are shown. Enrichment in the naïve pool with decreases in the other subsets was found in HD ($n = 22$) and SLE ($n = 26$), but not in LN ($n = 12$). **(D)** Pie charts of percentages of PTX3$^{+}$ CD27IgD subsets within the PTX3$^{+}$ B cell pool are consistent with distribution of absolute numbers. Mann-Whitney *U*-test (*< 0.05, **< 0.01, ***< 0.001). SLE, systemic lupus erythematosus; HD, healthy donors; LN, lupus nephritis; PTX3 pentraxin3.

Notably, by virtue of its octameric structure, PTX3 is able to bind diverse molecules (12, 31, 32) and either living or apoptotic cells (9, 13, 23). More recently, the binding of PTX3 to marginal zone (MZ) B cells was described which promoted production of IgM and IgG antibodies, thus suggesting that PTX3 is involved in the regulation of B cell differentiation or potentially in B helper

function (24). So far, however, the binding site of PTX3 other than Fcγ receptors or Toll-like receptors (23, 24) has not been fully delineated.

It is well-known that regulatory B cell function (both in numbers and functionality) is impaired in SLE (33, 34) and a stable decrease of naïve and memory PTX3$^+$B cells in LN patients may hint to an early negative selection or depletion of this specificity in LN. While previous pre-clinical and clinical data showed that anti-PTX3 autoantibodies carry a LN protective potential, it remains to be shown whether this also applies to a potential immunomodulatory function of PTX3$^+$ B cells. Alternatively, and not mutually exclusively, the abundance of naïve PTX3$^+$ B cells in non-renal SLE may represent polyreactive B cells, which is a common feature of SLE (35, 36), and may give rise to antibody secreting cells *via* an extrafollicular pathway (37). This would be consistent with the progressive decrease in PTX3$^+$B cell subsets from naïve, pre-switch to post-switch memory cells. The current finding of a similar level and distribution of PTX3$^+$ B cells in non-renal SLE and HD has some implications. First, their absence in LN suggests that their presence has relevance to protect the kidney. Second, selection processes leading to PTX3$^+$ B cell subsets might be intact in SLE as observed for HD. Alternatively and related to the proposed protective function, non-renal SLE patients are able to generate a sufficient amount of PTX3$^+$ B cells which could be involved in immune protection being more compromised in LN. As such, the absence of circulating PTX3$^+$ B cells in LN may hamper the kidney-protective effect provided by PTX3 specific autoantibodies.

Our study is the first addressing the distribution of autoantigen-specific B cells in lupus, focusing on a molecule of emerging importance, through an original method of selection and verification of autoantigen-specific B cells, which can be thereby identified from peripheral blood of patients or controls with a high level of certainty and in the absence of stimulation.

In summary, this study developed a method to reliably identify PTX3-specific B cells and their subsets. Application of this technology allowed the identification of an abnormal distribution of PTX3$^+$ B cells with their absence in LN and a very similar profile among controls and non-renal SLE. To which extent these findings relate to a potential immune regulatory role or protective function of this specificity as well as potential clinical applications in diagnostics or therapeutics remains to be delineated.

## AUTHOR CONTRIBUTIONS

TD, AD, and MG conceived the core idea of the study. KR, AW, AL, FS, and MG established the technology and experimental conditions. NN, AG, and SV provided antigen supply. ES, TR, KR, NN, and MG all contributed to patient recruitment. MG and KR performed laboratory and statistical analysis. MG wrote the manuscript. TD, AD, AL, AW, and AG provided revisions and all authors approved the final version.

## FUNDING

This work was supported by DFG grant Do 491/7-3 and 10-1.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00029/full#supplementary-material

**Supplementary Figure 1 |** Representative gating strategy of peripheral B cells according to FSC and SSC characteristics and further identification on their surface expression of CD19, CD20, and subsets according to CD27 surface expression. FSC, forward scatter; SSC, sideward scatter.

**Supplementary Figure 2 |** Quantification of PTX3 binding among B cells that only bind either PTX3-PE **(A)** or PTX3-Cy5 **(B)** before and after blocking of PTX3 is not significant (Wilcoxon rank test). PTX3, pentraxin 3; Cy5, cyanin 5; PE, phycoerythrin.

## REFERENCES


1. Doria A, Iaccarino L, Ghirardello A, Zampieri S, Arienti S, Sarzi-Puttini P, et al. Long-term prognosis and causes of death in systemic lupus erythematosus. *Am J Med.* (2006) 119:700–6. doi: 10.1016/j.amjmed.2005.11.034
2. Hanly JG, Su L, Urowitz MB, Romero-Diaz J, Gordon C, Bae SC, et al. A longitudinal analysis of outcomes of lupus nephritis in an international inception cohort using a multistate model approach. *Arthritis Rheumatol.* (2016) 68:1932–44. doi: 10.1002/art.39674
3. Moroni G, Vercelloni PG, Quaglini S, Gatto M, Gianfreda D, Sacchi L, et al. Changing patterns in clinical-histological presentation and renal outcome over the last five decades in a cohort of 499 patients with lupus nephritis. *Ann Rheum Dis.* (2018) 77:1318–25. doi: 10.1136/annrheumdis-2017-212732
4. Gatto M, Iaccarino L, Ghirardello A, Punzi L, Doria A. Clinical and pathologic considerations of the qualitative and quantitative aspects of lupus nephritogenic autoantibodies: a comprehensive review. *J Autoimmun.* (2016) 69:1–11. doi: 10.1016/j.jaut.2016.02.003
5. Doria A, Gatto M. Nephritogenic-antinephritogenic antibody network in lupus glomerulonephritis. *Lupus* (2012) 21:1492–6. doi: 10.1177/0961203312462267
6. Schrezenmeier E, Jayne D, Dörner T. Targeting B cells and plasma cells in glomerular diseases: translational perspectives. *J Am Soc Nephrol.* (2018) 29:741–58. doi: 10.1681/ASN.2017040367
7. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive proteinand serum amyloid P component. *J Biol Chem.* (1997) 272:32817–23. doi: 10.1074/jbc.272.52.32817
8. Garlanda C, Jaillon S, Doni A, Bottazzi B, Mantovani A. PTX3, a humoral pattern recognition molecule at the interface between microbe and matrix recognition. *Curr Opin Immunol.* (2016) 38:39–44. doi: 10.1016/j.coi.2015.11.002
9. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol.* (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
10. Manfredi A, Rovere-Querini P, Bottazzi B, Garlanda C, Mantovani A. Pentraxins, humoral innate immunity and tissue injury. *Curr Opin Immunol.* (2008) 20:538–44. doi: 10.1016/j.coi.2008.05.004
11. Nauta AJ, de Haij S, Bottazzi B, Mantovani A, Borrias MC, Aten J, et al. Human renal epithelial cells produce the long pentraxin PTX3. *Kidney Int.* (2005) 67:543–53. doi: 10.1111/j.1523-1755.2005.67111.x

12. Jaillon S, Peri G, Delneste Y, Frémaux I, Doni A, Moalli F, et al. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med.* (2007) 204:793–804. doi: 10.1084/jem.20061301
13. Baruah P, Dumitriu IE, Peri G, Russo V, Mantovani A, Manfredi AA, et al. The tissue pentraxin PTX3 limits C1q-mediated complement activation and phagocytosis of apoptotic cells by dendritic cells. *J Leukoc Biol.* (2006) 80:87–95. doi: 10.1189/jlb.0805445
14. Mistry P, Kaplan MJ. Cell death in the pathogenesis of systemic lupus erythematosus and lupus nephritis. *Clin Immunol.* (2017)185:59–73. doi: 10.1016/j.clim.2016.08.010
15. Kang S, Rogers JL, Monteith AJ, Jiang C, Schmitz J, Clarke SH, et al. Apoptotic debris accumulates on hematopoietic cells and promotes disease in murine and human systemic lupus erythematosus. *J Immunol.* (2016)196:4030–9. doi: 10.4049/jimmunol.1500418
16. Bussolati B, Peri G, Salvidio G, Verzola D, Mantovani A, Camussi G. The long pentraxin PTX3 is synthesized in IgA glomerulonephritis and activates mesangial cells. *J Immunol.* (2003) 170:1466–72. doi: 10.4049/jimmunol.170.3.1466
17. Pang Y, Tan Y, Li Y, Zhang J, Guo Y, Guo Z, et al. Pentraxin 3 is closely associated with tubulointerstitial injury in lupus nephritis: a large multicenter cross-sectional study. *Medicine* (2016) 95:e2520. doi: 10.1097/MD.0000000000002520
18. Bassi N, Del Prete D, Ghirardello A, Gatto M, Ceol M, Zen M, et al. PTX3, Anti-PTX3, and anti-C1q autoantibodies in lupus glomerulonephritis. *Clin Rev Allergy Immunol.* (2015) 49:217–26. doi: 10.1007/s12016-015-8476-9
19. Bassi N, Ghirardello A, Blank M, Zampieri S, Sarzi-Puttini P, Mantovani A, et al. IgG anti-pentraxin 3 antibodies in systemic lupus erythematosus. *Ann Rheum Dis.* (2010) 69:1704–10. doi: 10.1136/ard.2009.117804
20. Augusto JF, Onno C, Blanchard S, Dubuquoi S, Mantovani A, Chevailler A, et al. Detection of anti-PTX3 autoantibodies in systemic lupus erythematosus. *Rheumatology* (2009) 48:442–4. doi: 10.1093/rheumatology/ken507
21. Yuan M, Tan Y, Pang Y, Li YZ, Song Y, Yu F, et al. Anti-pentraxin 3 autoantibodies might be protective in lupus nephritis: a large cohort study. *Ren Fail.* (2017) 39:465–73. doi: 10.1080/0886022X.2017.1308258
22. Gatto M, Ghirardello A, Luisetto R, Bassi N, Fedrigo M, Valente M, et al. Immunization with pentraxin 3 (PTX3) leads to anti-PTX3 antibody production and delayed lupus-like nephritis in NZB/NZW F1 mice. *J Autoimmun.* (2016) 74:208–16. doi: 10.1016/j.jaut.2016.07.002
23. Lu J, Marnell LL, Marjon KD, Mold C, Du Clos TW, Sun PD. Structural recognition and functional activation of FcgammaR by innate pentraxins. *Nature* (2008) 456:989–2. doi: 10.1038/nature07468
24. Chorny A, Casas-Recasens S, Sintes J, Shan M, Polentarutti N, García-Escudero R, et al. The soluble pattern recognition receptor PTX3 links humoral innate and adaptive immune responses by helping marginal zone B cells. *J Exp Med.* (2016) 213:2167–85. doi: 10.1084/jem.20150282
25. Ma K, Li J, Wang X, Lin X, Du W, Yang X, et al. TLR4(+)CXCR4(+) plasma cells drive nephritis development in systemic lupus erythematosus. *Ann Rheum Dis.* (2018) 77:1498–506. doi: 10.1136/annrheumdis-2018-213615
26. Berkowska MA, Driessen GJ, Bikos V, Grosserichter-Wagener C, Stamatopoulos K, Cerutti A, et al. Human memory B cells originate from three distinct germinal center-dependent and -independent maturation pathways. *Blood* (2011) 118:2150–8. doi: 10.1182/blood-2011-04-345579
27. Giesecke C, Frölich D, Reiter K, Mei HE, Wirries I, Kuhly R, et al. Tissue distribution and dependence of responsiveness of human antigen-specific memory B cells. *J Immunol.* (2014) 192:3091–100. doi: 10.4049/jimmunol.1302783
28. Leyendeckers H, Odendahl M, Löhndorf A, Irsch J, Spangfort M, Miltenyi S, et al. Correlation analysis between frequencies of circulating antigen-specific IgG-bearing memory B cells and serum titers of antigen-specific IgG. *Eur J Immunol.* (1999) 29:1406–17.
29. Mei HE, Frölich D, Giesecke C, Loddenkemper C, Reiter K, Schmidt S, et al. Steady-state generation of mucosal IgA+ plasmablasts is not abrogated by B-cell depletion therapy with rituximab. *Blood* (2010) 116:5181–90. doi: 10.1182/blood-2010-01-266536
30. Giesecke C, Meyer T, Durek P, Maul J, Preiß J, Jacobs JFM, et al. Simultaneous presence of non- and highly mutated Keyhole Limpet Hemocyanin (KLH)-specific plasmablasts early after primary KLH immunization suggests cross-reactive memory B cell activation. *J Immunol.* (2018) 200:3981–92. doi: 10.4049/jimmunol.1701728
31. Inforzato A, Baldock C, Jowitt TA, Holmes DF, Lindstedt R, Marcellini M, et al. The angiogenic inhibitor long pentraxin PTX3 forms an asymmetric octamer with two binding sites for FGF2. *J Biol Chem.* (2010) 285:17681–92. doi: 10.1074/jbc.M109.085639
32. Doni A, Garlanda C, Bottazzi B, Meri S, Garred P, Mantovani A. Interactions of the humoral pattern recognition molecule PTX3 with the complement system. *Immunobiology* (2012) 1122–8. doi: 10.1016/j.imbio.2012.07.004
33. Dörner T, Jacobi AM, Lee J, Lipsky PE. Abnormalities of B cell subsets in patients with systemic lupus erythematosus. *J Immunol Methods* (2011) 363:187–97. doi: 10.1016/j.jim.2010.06.009
34. Blair PA, Noreña LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* (2010) 32:129–40. doi: 10.1016/j.immuni.2009.11.009
35. Zhang J, Jacobi AM, Wang T, Berlin R, Volpe BT, Diamond B. Polyreactive autoantibodies in systemic lupus erythematosus have pathogenic potential. *J Autoimmun.* (2009) 33:270–4. doi: 10.1016/j.jaut.2009.03.011
36. Odendahl M, Jacobi A, Hansen A, Feist E, Hiepe F, Burmester GR, et al. Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J Immunol.* (2000)165:5970–9. doi: 10.4049/jimmunol.165.10.5970
37. Tipton CM, Fucile CF, Darce J, Chida A, Ichikawa T, Gregoretti I, et al. Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. *Nat Immunol.* (2015) 16:755–65. doi: 10.1038/ni.3175


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 31 January 2019
doi: 10.3389/fimmu.2019.00088

# PTX3 Polymorphisms Influence Cytomegalovirus Reactivation After Stem-Cell Transplantation

*Cláudia F. Campos[1,2], Luís Leite[3], Paulo Pereira[4], Carlos Pinho Vaz[3], Rosa Branca[3], Fernando Campilho[3], Fátima Freitas[5], Dário Ligeiro[6], António Marques[7], Egídio Torrado[1,2], Ricardo Silvestre[1,2], João F. Lacerda[4,8], António Campos Jr.[3], Cristina Cunha[1,2] and Agostinho Carvalho[1,2]**

[1] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, [2] ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal, [3] Serviço de Transplantação de Medula Óssea, Instituto Português de Oncologia do Porto, Porto, Portugal, [4] Faculdade de Medicina de Lisboa, Instituto de Medicina Molecular, Lisbon, Portugal, [5] Instituto Português do Sangue e Transplantação, IP, Porto, Portugal, [6] Instituto Português do Sangue e Transplantação, IP, Lisbon, Portugal, [7] Serviço de Imuno-Hemoterapia, Hospital de Braga, Braga, Portugal, [8] Serviço de Hematologia e Transplantação de Medula, Hospital de Santa Maria, Lisbon, Portugal

OPEN ACCESS

**Edited by:**
Barbara Bottazzi,
Milan University, Italy

**Reviewed by:**
Orla Morrissey,
The Alfred Hospital, Australia
Gunnar Houen,
State Serum Institute (SSI), Denmark

***Correspondence:**
Agostinho Carvalho
agostinhocarvalho@med.uminho.pt

**Specialty section:**
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

**Received:** 15 October 2018
**Accepted:** 14 January 2019
**Published:** 31 January 2019

**Citation:**
Campos CF, Leite L, Pereira P, Vaz CP, Branca R, Campilho F, Freitas F, Ligeiro D, Marques A, Torrado E, Silvestre R, Lacerda JF, Campos A Jr, Cunha C and Carvalho A (2019) PTX3 Polymorphisms Influence Cytomegalovirus Reactivation After Stem-Cell Transplantation. Front. Immunol. 10:88. doi: 10.3389/fimmu.2019.00088

**Background:** Reactivation of latent human cytomegalovirus (CMV) in patients undergoing allogeneic stem-cell transplantation (HSCT) predisposes to several clinical complications and is therefore a major cause of morbidity and mortality. Although pentraxin-3 (PTX3) has been previously described to bind both human and murine CMV and mediate several host antiviral mechanisms, whether genetic variation in the *PTX3* locus influences the risk of CMV infection is currently unknown.

**Methods:** To dissect the contribution of genetic variation within *PTX3* to the development of CMV infection, we analyzed described loss-of-function variants at the *PTX3* locus in 394 recipients of HSCT and their corresponding donors and assessed the associated risk of CMV reactivation.

**Results:** We report that the donor, but not recipient, h2/h2 haplotype in *PTX3* increased the risk of CMV reactivation after 24 months following transplantation, with a significant effect on survival. Among recipients with h2/h2 donors, CMV seropositive patients as well as those receiving grafts from unrelated donors, regardless of the CMV serostatus, were more prone to develop viral reactivation after transplantation. Most importantly, the h2/h2 haplotype was demonstrated to display an influence toward risk of CMV reactivation comparable to that conferred by the unrelated status of the donor alone.

**Conclusions:** Our findings demonstrate the important contribution of genetic variation in donor *PTX3* to the risk of CMV reactivation in patients undergoing HSCT, highlighting a promising prognostic value of donor *PTX3* to predict risk of CMV reactivation in this clinical setting.

**Keywords: cytomegalovirus, stem-cell transplantation, PTX3, single nucleotide polymorphism, precision medicine, genomics**

## INTRODUCTION

Human cytomegalovirus (CMV), a member of the *Herpesviridae* family, is a ubiquitous opportunistic pathogen that has intimately co-evolved with its human host and can establish latency after clearance of the primary infection (1). CMV asymptomatically infects the majority of the world's population (approximately 40–99%), with the highest seroprevalence in developing countries, and typically only leads to disease in the absence of an adequate cellular immunity (2). Asymptomatic long-term virus shedding in urine and saliva secretions usually marks the primary infection in healthy individuals (3). Throughout complex virus-host interactions, CMV evades a number of host pathways to enable its lifelong persistence, during which it may replicate chronically or reactivate from latency sporadically (4). In immunocompetent individuals, these reactivation events are tightly controlled by the immune system and rarely result in clinical presentation (5). However, it is becoming increasingly apparent that CMV may be associated with additional long-term health consequences due to its ability to establish lifelong persistence in critically ill patients (6, 7). Accordingly, reactivation of latent virus following allogeneic hematopoietic stem-cell transplantation (HSCT) has been increasingly associated with overt CMV disease, a major cause of morbidity and mortality in these patients. Despite important efforts centered in diagnostic and therapeutic advances, pre-emptive antiviral therapy is associated with significant myelotoxicity and impaired hematological reconstitution (8, 9), ultimately leading to other disease complications including superinfection by other viruses, bacteria and fungi, particularly *Aspergillus* species (10, 11).

The immune control of viral infections requires different components originating from both innate and adaptive arms of the immune system (12). Specifically, the innate immune system has evolved a multitude of unique antiviral humoral mechanisms through the participation of collectins, including surfactant protein (SP)-A and SP-D, and pentraxins (13–15). The long pentraxin-3 (PTX3) is a member of a superfamily of fluid-phase proteins, distinguished by their cyclic multimeric structure and the presence of a conserved amino acid signature in their C-terminal domain (16). In response to proinflammatory stimuli, PTX3 production is induced in a broad range of immune cells, including macrophages, dendritic cells and endothelial cells (17). Moreover, PTX3 is stored in the intracellular granules of neutrophils in a ready-made form and is rapidly released upon pathogen challenge or tissue damage, thereby covering a temporal window preceding PTX3 gene expression-dependent production. By acting as an ancestor of antibodies, PTX3 exerts a multifaceted nonredundant role in innate immunity against certain microbes by modulating complement activity and facilitating pathogen recognition by myeloid innate immune cells (18–20). As such, and although classic immunodeficiencies have not been linked to PTX3 deficiency (21), common polymorphisms have been disclosed as important risk factors across different infectious diseases, namely *Pseudomonas aeruginosa* colonization in cystic fibrosis patients (22), uropathogenic *Escherichia coli* infection (23), and invasive aspergillosis in recipients of HSCT (24, 25) and solid organ transplantation (26, 27), as well as patients with chronic obstructive pulmonary disease (28).

Despite a well-recognized role in innate host defense against selected bacteria and fungi, accumulating evidence also suggests the involvement of PTX3 in innate antiviral immunity (29). In fact, PTX3 has been described to act as a receptor decoy for the virus during CMV infection (30). Specifically, PTX3 was found to exert a protective role by binding both human and murine CMV, resulting in a reduced viral entry into permissive cells and resistance to *Aspergillus* superinfection, a mechanism entirely dependent on Toll-like receptors (TLRs) sensing pathways and activation of interferon (IFN) regulatory factor 3 (IRF3). Of note, the exogenous administration of PTX3 resulted in therapeutic efficacy against primary CMV infection and reactivation as well as *Aspergillus* superinfection in pre-clinical models of HSCT.

The compelling evidence that PTX3 is an effective mediator in preventing CMV infection and reactivation as well as subsequent superinfections pinpoints a potential role for PTX3 as a biomarker and therapeutic agent in viral infections and superinfections in the transplantation setting. However, the potential involvement of genetic variation in *PTX3* during CMV reactivation in at-high risk individuals has never been addressed. In this large genetic association study involving 394 eligible donor-recipient HSCT pairs, we provide crucial insights into the genetic contribution of PTX3 as a critical regulator of susceptibility to CMV infection. This information may ultimately lay the foundations toward risk stratification approaches aimed at a more effective and personalized management of CMV infection in this clinical setting.

## MATERIALS AND METHODS

### Patients

A total of 460 hematological patients of European descent undergoing allogeneic HSCT at Instituto Português de Oncologia, Porto, and at Hospital de Santa Maria, Lisbon, between 2009 and 2015, were enrolled. Both donor and recipient DNA samples as well as patient-level data were available for 394 of these. The demographic and clinical characteristics of the patients are summarized in **Table 1**. One hundred and ninety-six cases of CMV infection and 198 uninfected controls were identified through pp65/pUL83 antigenemia assay (>1/100 pp65/pUL83 antigen-positive cells) and blood quantitative PCR for detection of viral DNA (>400 copies/mL) according to the recent revised standard criteria (31). All patients were monitored weekly for viral infection (reactivation or primary infection) with CMV until day +90 post-HSCT and subsequently every second week. In the event of increasing viral loads, pre-emptive therapy with valganciclovir was initiated. Approval for the study was obtained from the Ethics Subcommittee for Life and Health Sciences of the University of Minho, Portugal (125/014), the Ethics Committee for Health of the Instituto Português de Oncologia, Porto, Portugal (26/015), the Ethics Committee of the Lisbon Academic Medical Center, Portugal (632/014), and the National Commission for the Protection of Data, Portugal

**TABLE 1 |** Demographic and transplant-related characteristics at baseline.

| Variable | Total ($n = 394$) | No CMV infection ($n = 198$) | CMV infection ($n = 196$) | *P*-value[†] |
|---|---|---|---|---|
| **AGE AT TRANSPLANTATION, NO (%)** | | | | |
| ≤20 years | 75 (19.1) | 42 (21.2) | 33 (16.8) | 0.37 |
| 21–40 years | 103 (26.1) | 54 (27.3) | 49 (25.0) | |
| >40 years | 216 (54.8) | 102 (51.5) | 114 (58.2) | |
| **GENDER, NO (%)** | | | | |
| Female | 169 (42.9) | 90 (45.5) | 79 (40.3) | 0.29 |
| Male | 225 (57.1) | 108 (54.5) | 117 (59.7) | |
| **UNDERLYING DISEASE, NO. (%)** | | | | |
| Acute leukemia | 213 (54.1) | 109 (55.1) | 104 (53.1) | 0.79 |
| Chronic lymphoproliferative diseases | 65 (16.5) | 28 (14.1) | 37 (18.9) | |
| Chronic myeloproliferative diseases | 25 (6.3) | 14 (7.1) | 11 (5.6) | |
| Myelodysplastic/myeloproliferative diseases | 57 (14.5) | 28 (14.1) | 29 (14.8) | |
| Aplastic anemia | 19 (4.8) | 10 (5.1) | 9 (4.6) | |
| Others or unknown | 15 (3.8) | 9 (4.5) | 6 (3.1) | |
| **TRANSPLANTATION TYPE, NO. (%)** | | | | |
| Matched, related | 180 (45.7) | 106 (53.6) | 74 (37.8) | 0.009 |
| Matched, unrelated | 106 (26.9) | 43 (21.7) | 63 (32.1) | |
| Mismatched, related | 6 (1.5) | 4 (2.0) | 2 (1.0) | |
| Mismatched, unrelated | 102 (25.9) | 45 (22.7) | 57 (29.1) | |
| **GRAFT SOURCE, NO. (%)** | | | | |
| Peripheral blood | 324 (82.2) | 166 (83.8) | 158 (80.6) | 0.10 |
| Bone-marrow | 62 (15.7) | 31 (15.7) | 31 (15.8) | |
| Cord blood | 8 (2.0) | 1 (0.5) | 7 (3.6) | |
| **DISEASE STAGE, NO. (%)** | | | | |
| First complete remission | 219 (55.6) | 120 (60.6) | 99 (50.5) | 0.13 |
| Second or subsequent remission, or relapse | 69 (17.5) | 30 (15.2) | 39 (19.9) | |
| Active disease | 106 (26.9) | 48 (24.2) | 58 (29.6) | |
| **CONDITIONING REGIMEN, NO (%)** | | | | |
| RIC | 274 (69.5) | 136 (68.7) | 138 (70.4) | 0.70 |
| Myeloablative | 120 (30.5) | 62 (31.3) | 58 (29.6) | |
| **CMV SEROSTATUS OF DONOR AND RECIPIENT, NO. (%)** | | | | |
| D+/R+ | 270 (68.5) | 133 (67.2) | 137 (69.9) | <0.0001 |
| D–/R+ | 81 (20.6) | 30 (15.2) | 51 (26.0) | |
| D+/R– | 23 (5.8) | 18 (9.1) | 5 (2.6) | |
| D–/R– | 20 (5.1) | 17 (8.6) | 3 (1.5) | |
| **DURATION OF NEUTROPENIA, MEAN DAYS (RANGE)[‡]** | | | | |
| | 14 (5–39) | 14 (6–39) | 13 (5–35) | 0.40 |
| **ACUTE GVHD, NO. (%)** | | | | |
| No GVHD or grades I–II | 329 (83.5) | 171 (86.4) | 158 (80.6) | 0.12 |
| Grades III–IV | 65 (16.5) | 27 (13.6) | 38 (19.4) | |

[†]*P-values were calculated by Fisher's exact probability t-test or Student's t-test for continuous variables, comparing the groups with and without CMV infection.* [‡]*Neutropenia was defined as ≤0.5 × $10^9$ cells/L. RIC, reduced intensity conditioning; CMV, cytomegalovirus; D, donor; R, recipient; GVHD, graft-vs.-host-disease.*

(1950/015). All participants provided written informed consent prior to transplantation in accordance with the Declaration of Helsinki.

## Single Nucleotide Polymorphism (SNP) Selection and Genotyping

Genetic variants in the *PTX3* gene analyzed in this study were selected based on their described functional consequences and previous association with infectious complications after HSCT (24). Genomic DNA was isolated from whole blood using the QIAmp DNA Blood Mini Kit according to the protocol supplied by the manufacturer (Qiagen, Hilden, Germany). Genotyping was performed using KASPar assays (LGC Genomics, Hertfordshire, United Kingdom) in an Applied Biosystem 7500 Fast Real-Time PCR system (Thermo Fisher Scientific, MA, United States), according to the manufacturer's instructions. Mean call rate for the SNP was >98%. Quality control for the genotyping results was achieved with negative controls and randomly selected samples with known genotypes.

## Statistical Analysis

The probability of CMV reactivation according to *PTX3* genotypes was determined using the cumulative incidence method and compared using the Gray's test (32). The cumulative incidence of CMV reactivation at 24 months after HSCT was computed with the *cmprsk* package for R version 2.10.1, with censoring of data at the date of last follow-up visit and relapse and death as competing events. All clinical and genetic variants achieving a $P \leq 0.15$ in the univariate analysis were entered one by one in a pairwise model together and kept in the final model if they remained significant ($P < 0.05$). Multivariate analysis was performed using the subdistribution regression model of Fine and Gray. Overall survival, defined as the time from transplantation to death from any cause, was estimated with the use of the Kaplan-Meier method and evaluated according to *PTX3* genotypes with the use of the log-rank test. Power calculations were performed using the *powerSurvEpi* package 0.0.9 for R. Our sample size provided 80% power and a type I error below 5% for genetic variants with allele frequencies between 0.15 and 0.20 conferring a relative risk of 2.0.

# RESULTS

## Genetic Variation in PTX3 Increases the Risk of CMV Reactivation After HSCT

The baseline demographic and transplantation characteristics of the enrolled HSCT patients are depicted in **Table 1**. No significant differences were observed among cases of CMV reactivation and control groups regarding the age at transplantation, gender, underlying hematological disease, graft source, disease stage at transplantation, conditioning regimen, development of acute graft-vs.-host disease (GVHD) and duration of neutropenia. However, an increased number of cases of CMV infection was detected among serologically positive recipients (R+), whereas recipients with negative CMV serostatus (R–) were instead more protected from viral reactivation ($P < 0.0001$). In addition, HSCT patients receiving grafts from unrelated donors were more prone to develop CMV infection, compared to those with related donors ($P = 0.009$).

To investigate the relationship between genetic variation in *PTX3* and the susceptibility to CMV reactivation, the cumulative incidence of infection among transplant recipients was assessed according to recipient or donor genotypes at 24 months after HSCT. We found that donor, but not recipient, SNPs influenced the risk of CMV reactivation (**Table 2**). The cumulative incidence of CMV infection for donor rs2305619 was 59% for GG, 38% for AG and 36% for AA ($P = 0.01$), whereas for rs3816527, cumulative incidence of CMV infection was 55% for AA, 32% for AC and 37% for CC ($P = 0.03$). Haplotype analysis comprising rs2305619 and rs3816527 revealed that cumulative incidence of infection among patients with G-A/G-A [referred to as h2/h2 (24)] donors was 63% ($P = 0.008$) compared to 44 and 38% incidence observed for A-C/G-A (h1/h2) ($P = 0.42$) and A-C/A-C (h1/h1) donors, respectively (**Figure 1A**). In accordance with the results obtained for the individual SNPs, no significant influence of recipient haplotypes was observed on viral reactivation, with the cumulative incidence being 55% for h1/h1 (reference), 41% for h1/h2 ($P = 0.07$) and 50% for h2/h2 ($P = 0.48$). Other haplotypes were rare and were not included in the analysis (data not shown). The key contribution of the h2/h2 haplotype to the risk of infection was further illustrated upon modeling a recessive mode of inheritance (cumulative incidence of CMV reactivation, 63% for h2/h2 vs. 42% for h1/h1 and h1/h2 haplotypes combined; $P = 0.004$) (**Figure 1B**).

In a multivariate model accounting for age, gender, donor relation and CMV serostatus, the donor h2/h2 haplotype was found to confer a 1.9-fold (95% confidence interval, 1.2–2.9; $P = 0.004$) increased risk of CMV reactivation after transplantation (**Table 3**). In addition, receiving grafts from either matched ($P = 0.005$) or mismatched ($P = 0.004$) unrelated donors also increased risk of infection, whereas CMV seronegative recipients displayed instead a decreased risk of infection ($P = 0.01$). Collectively, our results identify genetic variation in donor *PTX3* as a potentially critical risk factor influencing the susceptibility to CMV reactivation after HSCT.

## The H2/H2 Haplotype Predisposes to CMV Infection Regardless of Viral Serostatus and Type of Donor

Several studies have demonstrated that CMV seropositive patients retain a higher associated mortality in comparison with seronegative recipients who were transplanted from seronegative

**TABLE 2 |** Cumulative incidence of CMV reactivation according to recipient and donor *PTX3* genotypes, and association test results.

| RefSNP | Genotype(s) | Cumulative incidence of CMV reactivation at 24 Mo (%) | | | |
|---|---|---|---|---|---|
| | | Recipient | *P*-value | Donor | *P*-value |
| rs2305619 | AA | 52 | 0.12 | 36 | 0.01 |
| | AG | 38 | | 38 | |
| | GG | 49 | | 59 | |
| rs3816527 | AA | 51 | 0.20 | 55 | 0.03 |
| | AC | 35 | | 32 | |
| | CC | 52 | | 37 | |

*CMV, cytomegalovirus; SNP, single nucleotide polymorphism; Mo, months. The P-values are for Gray's test using cumulative incidence analysis.*

**TABLE 3 |** Multivariate analysis of the association of *PTX3* SNPs with the risk of CMV reactivation among transplant recipients.

| Genetic/clinical variables | Adjusted HR† (95% CI) | *P*-value |
|---|---|---|
| Donor h2/h2 haplotype in *PTX3* | 1.9 (1.2–2.9) | 0.004 |
| Matched unrelated donor | 1.9 (1.2–3.0) | 0.005 |
| Mismatched unrelated donor | 1.7 (1.2–2.5) | 0.004 |
| D+/R– | 0.26 (0.09–0.74) | 0.01 |

*HR, hazard ratio; CI, confidence interval. Multivariate analyses were based on the subdistribution regression model of Fine and Gray. †Hazard ratios were adjusted for patient age and gender, type of transplantation, graft source, disease stage at transplantation, CMV serostatus of donor and recipient, and duration of neutropenia. Only the variables remaining significant after adjustment are shown.*

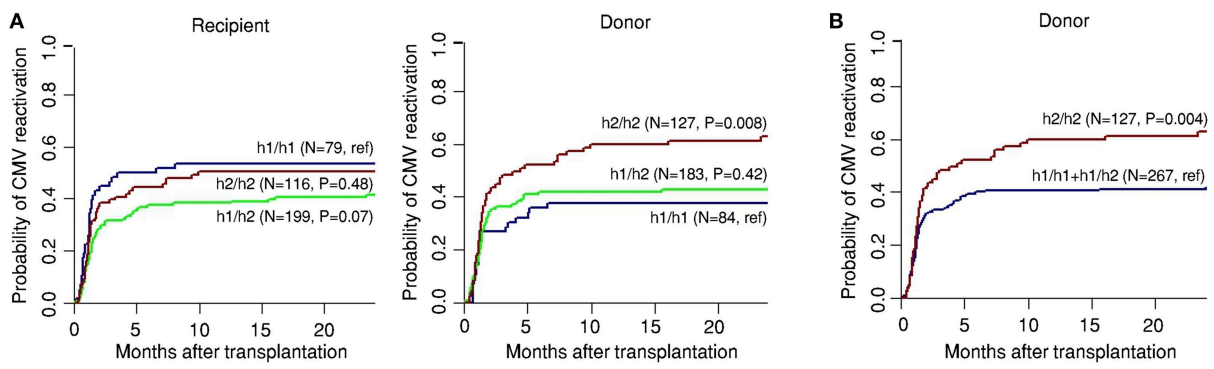


**FIGURE 1 |** Genetic variation in donor *PTX3* increases the risk of CMV reactivation after HSCT. Genetic association study comprising 394 eligible HSCT recipients with available clinical data and corresponding donors. **(A)** Cumulative incidence of CMV reactivation after HSCT according to recipient and donor haplotypes in *PTX3*. **(B)** Cumulative incidence of CMV reactivation after HSCT according to donor haplotypes in *PTX3* following a recessive genetic model. Data were censored at 24 months, and relapse and death were considered competing events. *P*-values were calculated using Gray's test.

donors (33, 34). In light of our genetic data disclosing the h2/h2 haplotype as a critical risk factor for CMV reactivation after HSCT, we further stratified patients according to the recipient CMV serostatus. We observed that CMV seropositive patients (R+) carrying the risk-conferring haplotype in *PTX3* displayed the highest risk of CMV reactivation after HSCT (**Figure 2A**). Specifically, the cumulative incidence of CMV reactivation was 68% for R+ and h2/h2 ($P < 0.001$), 44% for R+ and h1/h1+h1/h2 ($P = 0.03$), 30% for R– and h2/h2 ($P = 0.20$), and 14% for R– and h1/h1+h1/h2 (reference) (**Figure 2A**). These results indicate that the h2/h2 haplotype promoted a further increased risk of CMV reactivation among both CMV seropositive and seronegative recipients.

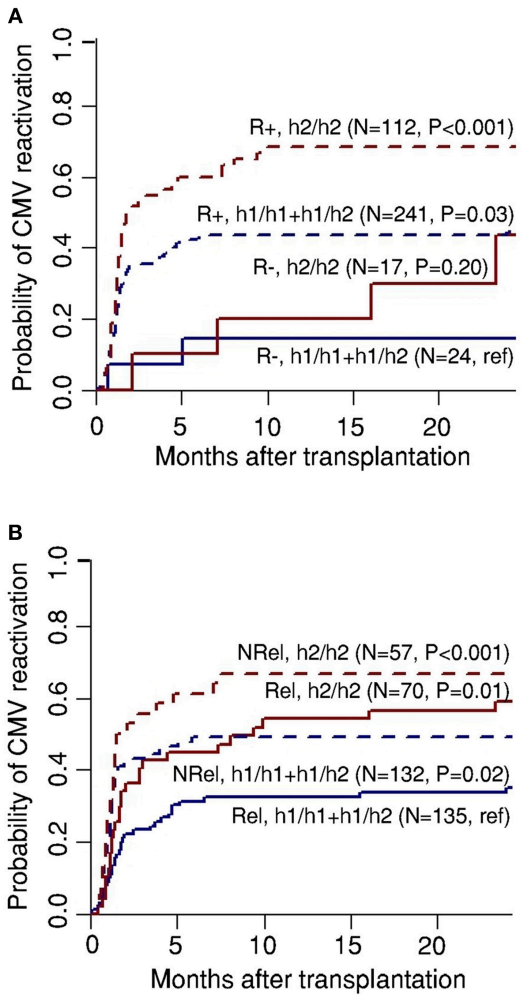


**FIGURE 2 |** Recipient serostatus and donor relation synergize with genetic variation in *PTX3* toward risk of CMV reactivation. Genetic association study comprising 394 eligible HSCT recipients with available clinical data and corresponding donors. Cumulative incidence of CMV reactivation after HSCT according to donor haplotypes in *PTX3* in combination with **(A)** recipient serostatus (negative, R–, or positive, R+) or **(B)** type of donor (related, Rel, or NRel, unrelated). Data were censored at 24 months, and relapse and death were considered competing events. *P*-values were calculated using Gray's test.

Given that the type of donor is also a well-described pre-transplantation predictive factor for CMV reactivation (35), we analyzed our genetic results according to the type of donor (related, Rel, and unrelated, NRel). We found that recipients harboring the h2/h2 haplotype were at higher risk of CMV infection following HSCT, regardless of the type of transplant (**Figure 2B**). The cumulative incidence of viral reactivation was 66.7% for NRel and h2/h2 ($P < 0.001$), 56.3% for Rel and h2/h2 ($P = 0.01$), 44.4% for NRel and h1/h1+h1/h2 ($P = 0.02$), and 33% for Rel and h1/h1+h1/h2 (reference). Of note, the similar incidence of CMV reactivation observed in recipients transplanted from Rel and h2/h2 donors and those transplanted from NRel and h1/h1+h1/h2 donors support a comparable effect of genetic variation in *PTX3* and the type of donor toward CMV reactivation. Collectively, these results suggest that PTX3 may constitute an ideal candidate for antiviral prophylactic measures aimed at counteracting the onset of CMV reactivation in HSCT patients.

## The H2/H2 Haplotype Influences Post-transplant Mortality

In view of our findings highlighting the genetic variation in *PTX3* as promising predictive clinical candidate for CMV reactivation in HSCT patients, we next sought to investigate whether the donor h2/h2 haplotype influenced post-transplant mortality. The probability of overall survival was evaluated at 36 months following transplantation and estimated according to donor *PTX3* haplotypes. We observed that, consistent with the increased risk of CMV reactivation, the donor h2/h2 haplotype influenced the post-transplant survival of HSCT patients (**Figure 3**). The probability of survival decreased from 54% among patients who received transplants from either h1/h1 or h1/h2 donors to 45% among patients who received transplants from donors carrying the risk-conferring h2/h2 haplotype ($P = 0.04$). Taken together, these results highlight the potential role of genetic variation in *PTX3* as an important predictor of the risk of infection, but also the outcome of the patients.

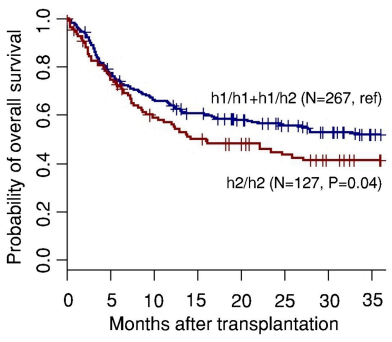


**FIGURE 3 |** The donor h2/h2 haplotype in *PTX3* influences post-transplant survival of HSCT recipients. Overall survival (OS) according to donor haplotypes in *PTX3*. Data were censored at 36 months. *P*-values were calculated using the log-rank test.

## DISCUSSION

Although remarkable advances in molecular virology and improvements in diagnostic methods and treatment regimen options have vastly enhanced our ability to manage CMV infection (36, 37), reactivation of latent virus remains one major cause of morbidity and mortality in patients undergoing HSCT (11). There is therefore a pressing demand for the development of novel prognostic markers for CMV reactivation aimed at supporting risk stratification measures and early diagnosis of infection. In this regard, the rs12979860 SNP upstream of the *IL28B* gene, known to be a critical factor associated with spontaneous clearance of hepatitis C (38), has been reported to influence the risk of CMV infection through the regulation of CMV-specific T-cell responses (39). Another study, despite failing to detect association, also revealed a contribution of the same *IL28B* variant to the levels of CMV DNAemia (40). In addition, SNPs in innate immunity genes, most notably TLR9 (41, 42), also appear to be important repositories of variability toward CMV infection across different studies. Collectively, these findings point to a strong genetic component in defining susceptibility to CMV reactivation after HSCT.

In this study, we have disclosed genetic variation in *PTX3* as an independent prognostic factor for CMV reactivation after transplantation, providing additional insights into human susceptibility to CMV infection. In addition, we determined a significant contribution of the donor h2/h2 haplotype to a poorer survival of HSCT recipients, similar to that previously reported among recipients of grafts from HLA-mismatched donors (24). This may reflect a key role of the h2/h2 haplotype in defining the outcome of HSCT patients by enhancing the risk of infectious complications. It remains to be assessed whether these genetic variants may also affect other non-infectious complications associated with HSCT. In this regard, it is worthwhile mentioning that plasma levels of PTX3 were increased at the onset of GVHD and were predictive of disease outcome (43), although the potential contribution of genetic variation in PTX3 to the risk and progression of GVHD remains to be assessed.

The integration of genetic markers into clinically valid processes to stratify the risk and progression of viral infection, and the efficacy of antiviral prophylaxis and therapy may represent a groundbreaking innovation for at-risk patients. In addition, the mechanistic involvement of PTX3 during CMV infection has been known for a long time (17, 44). By binding to CMV through sialic acids expressed on its glyosidic moiety, PTX3 was demonstrated to be effective in averting CMV infection and reactivation in selected *in vivo* and *in vitro* models of infection (30). Our genetic study associating a loss-of-function haplotype with the risk of CMV reactivation further supports the previously reported antiviral role of PTX3 and, considering its therapeutic potential in pre-clinical models (30), the administration of PTX3 could be envisaged as a promising immunotherapeutic approach to rescue the genetic deficiency in at-risk patients.

Although major advances have been accomplished regarding antiviral prophylactic strategies and preemptive therapy (9, 45), the donor and recipient CMV serological status still plays a major influence on the outcome of post-transplantation complications (46). Numerous investigations have demonstrated that CMV seronegative recipients transplanted from equally seronegative donors retain a reduced risk of transplant-related mortality, especially that caused by infections, in comparison with serologically positive recipients (47, 48). In line with the reported data, we found a significantly increased number of CMV reactivation cases among seropositive patients. Most importantly, our observation that R+ and h2/h2 recipients were at the highest risk of CMV reactivation highlights PTX3 as an ideal candidate for personalized medical interventions such as intensified diagnostics and targeted preemptive antiviral prophylaxis to prevent and counteract the onset of infection in specific subgroups of patients that are most at risk of viral reactivation.

Within the criteria for donor selection, the relation between donor and recipient constitutes one of the most relevant pre-transplantation predictors of CMV reactivation after transplantation (35, 49). In accordance, we found a significantly increased number of cases of CMV reactivation among patients with unrelated donors. Since the success of transplantation procedures hinges on the availability of suitable donors (50), our results suggest a pivotal role for PTX3 genetics as a pre-transplantation factor that could reshape current clinical approaches through the implementation of innovative risk stratification strategies that may involve the choice of alternative donors. Indeed, our results appear to indicate that selection of donors carrying the h2/h2 haplotype in *PTX3* may have a detrimental effect toward the reactivation of CMV after HSCT comparable to that conferred by the unrelated status of the donor alone.

Given its exploratory nature, our study presents certain limitations. The most important refer to the absence of definitive conclusions about the mechanism(s) through which genetic variation in donor *PTX3* influences the risk of developing CMV infection. Although it could presumably involve the transfer of CMV-specific lymphocytes, this hypothesis needs to be further explored. In addition, the low number of cases of overt CMV disease in our cohort precluded the appreciation of potential associations between severe viral disease and genetic variation in *PTX3*. Functional studies are ultimately required to understand how PTX3 is regulated in response and during CMV infection after HSCT, and what is the relative involvement of genetic variation in defining the levels of PTX3 and contributing to infection.

In conclusion, the evidence presented herein for a robust association between genetic variation in *PTX3* and CMV reactivation in patients undergoing HSCT highlights the significance of PTX3 as a promising marker for personalized medical intervention strategies, especially in the HSCT population, which may be particularly well-suited for genetically-targeted antiviral prophylaxis or enhanced diagnostic surveillance (51).

## AUTHOR CONTRIBUTIONS

CFC, CC, JL, AnC, and AgC designed the study. LL, PP, CV, RB, FC, JL, and AnC oversaw patient recruitment, and collection of patient-level data. FF and DL coordinated the collection and storage of DNA samples. CFC and CC performed genotyping and the statistical analyses. CFC, ET, RS, CC, and AgC interpreted the data. All authors critically revised and approved the manuscript and are accountable for the accuracy and integrity of the work.

## FUNDING

This work was supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER) (NORTE-01-0145-FEDER-000013), and the Fundação para a Ciência e Tecnologia (FCT) (SFRH/BPD/96176/2013 to CC, IF/01390/2014 to ET, IF/00021/2014 to RS, and IF/00735/2014 to AgC).

## REFERENCES

1. Gandhi MK, Khanna R. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. *Lancet Infect Dis.* (2004) 4:725–38. doi: 10.1016/S1473-3099(04)01202-2
2. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol.* (2010) 20:202–13. doi: 10.1002/rmv.655
3. Britt W. Manifestations of human cytomegalovirus infection: proposed mechanisms of acute and chronic disease. In: *Human Cytomegalovirus,* Shenk TE , Stinski MF editors. Berlin; Heidelberg: Springer Berlin Heidelberg (2008). p. 417–70. doi: 10.1007/978-3-540-77349-8_23
4. Goodrum F. Human cytomegalovirus latency: approaching the gordian knot. *Annu Rev Virol.* (2016) 3:333–57. doi: 10.1146/annurev-virology-110615-042422
5. Boeckh M, Geballe AP. Cytomegalovirus: pathogen, paradigm, and puzzle. *J Clin Invest.* (2011) 121:1673–80. doi: 10.1172/JCI45449
6. Razonable RR, Humar A, Practice ASTIDCO. Cytomegalovirus in solid organ transplantation. *Am J Transplant* (2013) 13(Suppl. 4):93–106. doi: 10.1111/ajt.12103
7. Locatelli F, Bertaina A, Bertaina V, Merli P. Cytomegalovirus in hematopoietic stem cell transplant recipients-management of infection. *Expert Rev Hematol.* (2016) 9:1093–105. doi: 10.1080/17474086.2016.1242406
8. Solano C, Giménez E, Piñana JL, Vinuesa V, Poujois S, Zaragoza S, et al. Preemptive antiviral therapy for CMV infection in allogeneic stem cell transplant recipients guided by the viral doubling time in the blood. *Bone Marrow Transplant.* (2015) 51:718. doi: 10.1038/bmt.2015.303
9. Kronfli N, Haider S. Management of cytomegalovirus in hematopoietic stem cell transplant recipients: a review of novel pharmacologic and cellular therapies. *Off J Assoc Med Microbiol Infect Dis Can.* (2017) 2:32–40. doi: 10.3138/jammi.2.1.005
10. Giménez E, Solano C, Nieto J, Remigia MJ, Clari MÁ, Costa E, et al. An investigation on the relationship between the occurrence of CMV DNAemia and the development of invasive aspergillosis in the allogeneic stem cell transplantation setting. *J Med Virol.* (2014) 86:568–75. doi: 10.1002/jmv.23735
11. De La Camara R. CMV in hematopoietic stem cell transplantation. *Mediterr J Hematol Infect Dis.* (2016) 8:e2016031. doi: 10.4084/mjhid.2016.031
12. Terrazzini N, Kern F. Cell-mediated immunity to human CMV infection: a brief overview. *F1000Prime Rep.* (2014) 6:28. doi: 10.12703/P6-28
13. Hawgood S, Brown C, Edmondson J, Stumbaugh A, Allen L, Goerke J, et al. Pulmonary collectins modulate strain-specific influenza a virus infection and host responses. *J Virol.* (2004) 78:8565–72. doi: 10.1128/JVI.78.16.8565-8572.2004
14. Tecle T, White MR, Crouch EC, Hartshorn KL. Inhibition of influenza viral neuraminidase activity by collectins. *Arch Virol.* (2007) 152:1731–42. doi: 10.1007/s00705-007-0983-4
15. Foo S-S, Reading PC, Jaillon S, Mantovani A, Mahalingam S. Pentraxins and collectins: friend or foe during pathogen invasion? *Trends Microbiol.* (2015) 23:799–811. doi: 10.1016/j.tim.2015.09.006
16. Erreni M, Manfredi AA, Garlanda C, Mantovani A, Rovere-Querini P. The long pentraxin PTX3: a prototypical sensor of tissue injury and a regulator of homeostasis. *Immunol Rev.* (2017) 280:112–25. doi: 10.1111/imr.12570
17. Magrini E, Mantovani A, Garlanda C. The dual complexity of PTX3 in health and disease: a balancing act? *Trends Mol Med.* (2016) 22:497–510. doi: 10.1016/j.molmed.2016.04.007
18. Moalli F, Doni A, Deban L, Zelante T, Zagarella S, Bottazzi B, et al. Role of complement and Fcγ receptors in the protective activity of the long pentraxin PTX3 against *Aspergillus fumigatus*. *Blood* (2010) 116:5170–80. doi: 10.1182/blood-2009-12-258376
19. Moalli F, Jaillon S, Inforzato A, Sironi M, Bottazzi B, Mantovani A, et al. Pathogen recognition by the long pentraxin PTX3. *J Biomed Biotechnol.* (2011) 2011:15. doi: 10.1155/2011/830421
20. Daigo K, Inforzato A, Barajon I, Garlanda C, Bottazzi B, Meri S, et al. Pentraxins in the activation and regulation of innate immunity. *Immunol Rev.* (2016) 274:202–17. doi: 10.1111/imr.12476
21. Campos CF, Van De Veerdonk FL, Goncalves SM, Cunha C, Netea MG, Carvalho A. Host genetic signatures of susceptibility to fungal disease. *Curr Top Microbiol Immunol.* (2018). doi: 10.1007/82_2018_113. [Epub ahead of print].
22. Chiarini M, Sabelli C, Melotti P, Garlanda C, Savoldi G, Mazza C, et al. PTX3 genetic variations affect the risk of Pseudomonas aeruginosa airway colonization in cystic fibrosis patients. *Genes Immun.* (2010) 11:665–70. doi: 10.1038/gene.2010.41
23. Jaillon S, Moalli F, Ragnarsdottir B, Bonavita E, Puthia M, Riva F, et al. The humoral pattern recognition molecule PTX3 is a key component of innate immunity against urinary tract infection. *Immunity* (2014) 40:621–32. doi: 10.1016/j.immuni.2014.02.015
24. Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med.* (2014) 370:421–32. doi: 10.1056/NEJMoa1211161
25. Fisher CE, Hohl TM, Fan W, Storer BE, Levine DM, Zhao LP, et al. Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. *Blood* (2017) 129:2693–701. doi: 10.1182/blood-2016-10-743294
26. Cunha C, Monteiro AA, Oliveira-Coelho A, Kuhne J, Rodrigues F, Sasaki SD, et al. PTX3-based genetic testing for risk of aspergillosis after lung transplant. *Clin Infect Dis.* (2015) 61:1893–4. doi: 10.1093/cid/civ679
27. Wojtowicz A, Lecompte TD, Bibert S, Manuel O, Rueger S, Berger C, et al. PTX3 polymorphisms and invasive mold infections after solid organ transplant. *Clin. Infect. Dis.* (2015) 61:619–22. doi: 10.1093/cid/civ386
28. He Q, Li H, Rui Y, Liu L, He B, Shi Y, et al. Pentraxin 3 gene polymorphisms and pulmonary aspergillosis in chronic obstructive pulmonary disease patients. *Clin Infect Dis.* (2018) 66:261–7. doi: 10.1093/cid/cix749
29. Reading PC, Bozza S, Gilbertson B, Tate M, Moretti S, Job ER, et al. Antiviral activity of the long chain pentraxin PTX3 against influenza viruses. *J Immunol.* (2008) 180:3391–8. doi: 10.4049/jimmunol.180.5.3391
30. Bozza S, Bistoni F, Gaziano R, Pitzurra L, Zelante T, Bonifazi P, et al. Pentraxin 3 protects from MCMV infection and reactivation through TLR sensing pathways leading to IRF3 activation. *Blood* (2006) 108:3387–96. doi: 10.1182/blood-2006-03-009266
31. Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. *Clin Infect Dis.* (2017) 64:87–91. doi: 10.1093/cid/ciw668
32. Gray RJ. A class of *K*-sample tests for comparing the cumulative incidence of a competing risk. *Ann Statist.* (1988) 16:1141–54. doi: 10.1214/aos/1176350951

33. Jaskula E, Bochenska J, Kocwin E, Tarnowska A, Lange A. CMV serostatus of donor-recipient pairs influences the risk of CMV Infection/Reactivation in HSCT Patients. *Bone Marrow Res.* (2012) 2012:8. doi: 10.1155/2012/375075
34. Kalra A, Williamson T, Daly A, Savoie ML, Stewart DA, Khan F, et al. Impact of donor and recipient cytomegalovirus serostatus on outcomes of antithymocyte globulin-conditioned hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* (2016) 22:1654–63. doi: 10.1016/j.bbmt.2016.05.020
35. Mack JP, Tan X, Vinh DC, Popradi G. Risk factors for progression of CMV viremia to CMV disease after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* (2015) 21:S296–7. doi: 10.1016/j.bbmt.2014.11.470
36. Kotton CN, Kumar D, Caliendo AM, Asberg A, Chou S, Danziger-Isakov L, et al. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation* (2013) 96:333–60. doi: 10.1097/TP.0b013e31829df29d
37. Boeckh M, Murphy WJ, Peggs KS. Recent advances in cytomegalovirus: an update on pharmacologic and cellular therapies. *Biol Blood Marrow Transplant.* (2015) 21:24–9. doi: 10.1016/j.bbmt.2014.11.002
38. Heim MH, Bochud PY, George J. Host-hepatitis C viral interactions: the role of genetics. *J Hepatol.* (2016) 65:S22–32. doi: 10.1016/j.jhep.2016.07.037
39. Corrales I, Solano C, Amat P, Gimenez E, De La Camara R, Nieto J, et al. IL28B genetic variation and cytomegalovirus-specific T-cell immunity in allogeneic stem cell transplant recipients. *J Med Virol.* (2017) 89:685–95. doi: 10.1002/jmv.24676
40. Bravo D, Solano C, Giménez E, Remigia MJ, Corrales I, Amat P, et al. Effect of the IL28B Rs12979860 C/T polymorphism on the incidence and features of active cytomegalovirus infection in allogeneic stem cell transplant patients. *J Med Virol.* (2014) 86:838–44. doi: 10.1002/jmv.23865
41. Carvalho A, Cunha C, Carotti A, Aloisi T, Guarrera O, Di Ianni M, et al. Polymorphisms in Toll-like receptor genes and susceptibility to infections in allogeneic stem cell transplantation. *Exp Hematol.* (2009) 37:1022–9. doi: 10.1016/j.exphem.2009.06.004
42. Xiao HW, Luo Y, Lai XY, Shi JM, Tan YM, He JS, et al. Donor TLR9 gene tagSNPs influence susceptibility to aGVHD and CMV reactivation in the allo-HSCT setting without polymorphisms in the TLR4 and NOD2 genes. *Bone Marrow Transplant.* (2014) 49:241–7. doi: 10.1038/bmt.2013.160
43. Dander E, De Lorenzo P, Bottazzi B, Quarello P, Vinci P, Balduzzi A, et al. Pentraxin 3 plasma levels at graft-versus-host disease onset predict disease severity and response to therapy in children given haematopoietic stem cell transplantation. *Oncotarget* (2016) 7:82123–38. doi: 10.18632/oncotarget.13488
44. Bottazzi B, Inforzato A, Messa M, Barbagallo M, Magrini E, Garlanda C, et al. The pentraxins PTX3 and SAP in innate immunity, regulation of inflammation and tissue remodelling. *J Hepatol.* (2016) 64:1416–27. doi: 10.1016/j.jhep.2016.02.029
45. Tan BH. Cytomegalovirus treatment. *Curr Treat Options Infect Dis* (2014) 6:256–70. doi: 10.1007/s40506-014-0021-5
46. Styczynski J. Who is the patient at risk of CMV recurrence: a review of the current scientific evidence with a focus on hematopoietic cell transplantation. *Infect Dis Ther.* (2018) 7:1–16. doi: 10.1007/s40121-017-0180-z
47. Schmidt-Hieber M, Labopin M, Beelen D, Volin L, Ehninger G, Finke J, et al. CMV serostatus still has an important prognostic impact in de novo acute leukemia patients after allogeneic stem cell transplantation: a report from the Acute Leukemia Working Party of EBMT. *Blood* (2013) 122:3359–64. doi: 10.1182/blood-2013-05-499830
48. Liu J, Kong J, Chang YJ, Chen H, Chen YH, Han W, et al. Patients with refractory cytomegalovirus (CMV) infection following allogeneic haematopoietic stem cell transplantation are at high risk for CMV disease and non-relapse mortality. *Clin Microbiol Infect.* (2015) 21:1121.e9–15. doi: 10.1016/j.cmi.2015.06.009
49. Sousa H, Boutolleau D, Ribeiro J, Teixeira AL, Pinho Vaz C, Campilho F, et al. Cytomegalovirus infection in patients who underwent allogeneic hematopoietic stem cell transplantation in Portugal: a five-year retrospective review. *Biol Blood Marrow Transplant.* (2014) 20:1958–67. doi: 10.1016/j.bbmt.2014.08.010
50. Kekre N, Antin JH. Hematopoietic stem cell transplantation donor sources in the 21st century: choosing the ideal donor when a perfect match does not exist. *Blood* (2014) 124:334–43. doi: 10.1182/blood-2014-02-514760
51. Wojtowicz A, Bochud PY. Risk stratification and immunogenetic risk for infections following stem cell transplantation. *Virulence* (2016) 7:917–29. doi: 10.1080/21505594.2016.1234566


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 25 February 2019
doi: 10.3389/fimmu.2019.00240

# Elevated Systemic Pentraxin-3 Is Associated With Complement Consumption in the Acute Phase of Thrombotic Microangiopathies

*Eszter Trojnar[1]*, Mihály Józsi[2], Zsóka Szabó[2], Marienn Réti[3], Péter Farkas[1], Kata Kelen[4], George S. Reusz[4], Attila J. Szabó[4,5], Nóra Garam[1], Bálint Mikes[1], György Sinkovits[1], Blanka Mező[1], Dorottya Csuka[1] and Zoltán Prohászka[1]*

[1] Research Laboratory, MTA-SE Research Group of Immunology and Hematology, 3rd Department of Internal Medicine, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary, [2] Complement Research Group, Department of Immunology, ELTE Eötvös Loránd University, Budapest, Hungary, [3] Department of Haematology and Stem Cell Transplantation, Central Hospital of Southern Pest, National Institute for Hematology and Infectious Diseases, Budapest, Hungary, [4] 1st Department of Pediatrics, Semmelweis University, Budapest, Hungary, [5] MTA-SE Pediatric and Nephrology Research Group, Budapest, Hungary

OPEN ACCESS

**Edited by:**
Barbara Bottazzi,
Humanitas Clinical and Research Center, Italy

**Reviewed by:**
Antonio Inforzato,
Istituto Clinico Humanitas, Italy
Giuseppe Remuzzi,
Istituto Di Ricerche Farmacologiche Mario Negri, Italy
Marie-Agnes Dragon-Durey,
Université Paris Descartes, France

***Correspondence:**
Eszter Trojnar
eszter.trojnar@gmail.com

**Specialty section:**
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology

**Received:** 30 September 2018
**Accepted:** 28 January 2019
**Published:** 25 February 2019

**Citation:**
Trojnar E, Józsi M, Szabó Z, Réti M, Farkas P, Kelen K, Reusz GS, Szabó AJ, Garam N, Mikes B, Sinkovits G, Mező B, Csuka D and Prohászka Z (2019) Elevated Systemic Pentraxin-3 Is Associated With Complement Consumption in the Acute Phase of Thrombotic Microangiopathies. Front. Immunol. 10:240. doi: 10.3389/fimmu.2019.00240

Pentraxin-3 (PTX3) and C-reactive protein (CRP) have been shown to regulate complement activation *in vitro*, but their role has not been investigated in complement consumption *in vivo*. Thrombotic microangiopathies (TMA) are often accompanied by complement overactivation and consumption, therefore we analyzed the relation of the systemic pentraxin levels to the complement profile, laboratory parameters and clinical outcome of TMA patients. We determined the PTX3 and CRP levels, complement factor and activation product concentrations in blood samples of 171 subjects with the diagnosis of typical hemolytic uremic syndrome (STEC-HUS) ($N = 34$), atypical HUS (aHUS) ($N = 44$), secondary TMA ($N = 63$), thrombotic thrombocytopenic purpura (TTP) ($N = 30$) and 69 age-matched healthy individuals. Clinical data, blood count and chemistry were collected from medical records. To determine the *in vitro* effect of PTX3 on alternative pathway (AP) activation, sheep red blood cell-based hemolytic assay and AP activity ELISA were used. We found that PTX3 levels were elevated in the acute phase of STEC-HUS, aHUS and secondary TMA, whereas PTX3 elevation was exceptional is TTP. Conversely, a significantly higher median CRP was present in all patient groups compared to controls. PTX3, but not CRP was associated with signs of complement consumption *in vivo*, and PTX3 significantly decreased the AP hemolytic activity *in vitro*. Our results provide a detailed description of acute phase-TMA patients' complement profile linked to changes in the systemic pentraxin levels that may support further molecular studies on the function of PTX3 in disease pathogenesis and add to the laboratory assessment of complement consumption in TMA.

**Keywords: pentraxin-3, C-reactive protein, thrombotic microangiopathies, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, alternative pathway, complement consumption**

## INTRODUCTION

Pentraxin-3 (PTX3) and C-reactive protein (CRP) are fluid phase pattern recognition molecules that have been shown to interact with the complement system on multiple levels. PTX3 consists of a unique N-terminal domain (1) and a highly conserved C-terminal pentraxin-like domain that is shared with CRP and allows octamer formation of the secreted PTX3 monomers through inter-chain disulfide bonds (2). Prompt release of PTX3 from neutrophil granulocytes is mediated at local sites of activation (3), whereas its enduring production is regulated via gene expression induction in innate immune cells and endothelial cells (3). Native CRP, a member of the short-pentraxin protein family, is stored as a pentamer in the endoplasmic reticulum of resting hepatocytes (4). Upon inflammatory stimuli CRP is secreted into the circulation and phosphocholine binding on target cell membranes induces the disassembly of the pentameric structure to CRP monomers in a calcium-dependent fashion (4).

PTX3 may facilitate phagocytosis of pathogens and clearance of cellular debris through the activation of the classical (CP) and lectin pathways of complement (2) upon binding to surface-associated mannan-binding lectin (5), ficolins, collectins (6) and C1q (7). Conversely, its interaction with C1q in the fluid phase restricts unwanted complement activation (3, 7). PTX3 also may recruit functionally active complement regulatory proteins, such as factor H (FH) (8) and C4b-binding protein (C4BP) (9) to the surface of apoptotic cells, which in turn facilitates C3b or C4b degradation and phagocytosis. Hence, by FH binding, PTX3 may prevent alternative pathway (AP) amplification and activation of the terminal pathway on non-activator surfaces *in vitro* (1). However, *in vivo* disease models of infection and tissue injury reported contradictory observations on the role of PTX3 during the inflammatory response. Both endogenous and exogenous PTX3 were shown to attenuate leukocyte recruitment and decrease apoptosis in experimental models of kidney and myocardial tissue injury (10, 11), whereas excess PTX3 was shown to intensify the inflammatory response in disease models of intestinal ischemia (12, 13) and certain respiratory pathologies (14).

CRP also has the ability to activate the CP of complement. Pentameric CRP however, may only bind solid phase C1q when complexed to phosphocholine (15), with concomitant restrain of the terminal pathway (16). By contrast, monomeric CRP may induce excess CP activation both *in vitro* and *in vivo* (15, 16), but at the same time it also allows for CRP to interact with the complement regulators C4BP, FH, but also with properdin (15, 17, 18), thus regulating both the CP and AP.

Thrombotic microangiopathies (TMA) are life threatening conditions that involve acute thrombocytopenia, hemolysis and organ impairment. Endothelial damage and subsequent microvascular thrombosis are key pathogenic factors in all forms of this disease (19, 20), despite differences in the clinical course and management of TMAs with distinct etiologies. Microvascular thrombosis has been linked to excessive complement activation in all forms of TMA (21–23) together with neutrophil activation and neutrophil extracellular trap (NET) release (24–28), which may provide excess PTX3 at the site of tissue injury (29) and thus influence the local complement activity.

Albeit numerous investigations have characterized the interaction of pentraxins with complement factors *in vitro*, no study has been designed so far to explore changes in the systemic level of pentraxins in complement mediated diseases, such as TMAs. Therefore, we performed a case-control study to determine the systemic levels of PTX3 and CRP in patients at the acute phase and remission of TMA. We explored the association between TMA-related complement consumption and circulatory pentraxin levels *in vivo* as well as the direct effect of PTX3 on AP activation *in vitro,* to reveal the potential role of pentraxins in complement mediated tissue injury. We further analyzed the relationship between the systemic level of pentraxins and TMA etiology, the clinical outcome of patients and classical laboratory markers of TMA.

## METHODS

### Patient Selection and Sample Collection

171 TMA patients with acute disease flare were enrolled in this study. Serum and plasma samples from all subjects were collected prior to the start of plasma exchange therapy; however, in 16 cases fresh frozen plasma had been administered to the patients prior to sampling. For appropriate comparison, 69 age-matched healthy individuals were selected, none of whom showed clinical or laboratory signs of TMA or an acute phase reaction that could have influenced the measured laboratory parameters. Diagnosis of TMA was established based on laboratory signs of thrombocytopenia (<150 G/L), and microangiopathic hemolytic anemia. Patients were included in the study only if all of the above criteria were met. For stratification of patients by disease etiology the following groups were formed: STEC-HUS ($N = 34$), aHUS ($N = 44$), secondary TMA ($N = 63$) and TTP ($N = 30$) (**Figure 1**), based on additional diagnostic criteria detailed in the **Supplementary Material**. Exclusion criteria were ongoing plasma exchange or complement inhibitory therapy at the time of sample collection (during the first acute flare), or the lack of available blood sample. For additional details on the study population please see the methods section of the **Supplementary Material**. This study was carried out in conformity with the Helsinki Declaration. Written informed consent was obtained from all participants, and the study was approved by the Scientific and Research Ethics Committee of the Medical Research Council (ETT TUKEB) in Budapest, Hungary (8361-1/2011-EKU).

### Determination of Laboratory Parameters

Complement activity-, component-, regulator-, and activation product determinations, CRP and PTX3 measurements were performed in this study. The AP activity was determined with the commercially available WIESLAB Alternative pathway ELISA kit (EuroDiagnostica, Malmö, Sweden), while total complement classical pathway activity was assessed using the sheep-erythrocyte hemolytic titration test. C3, C4 and hsCRP were measured by turbidimetry (Beckman Coulter, Brea, CA), complement factors B, and I were determined by radial

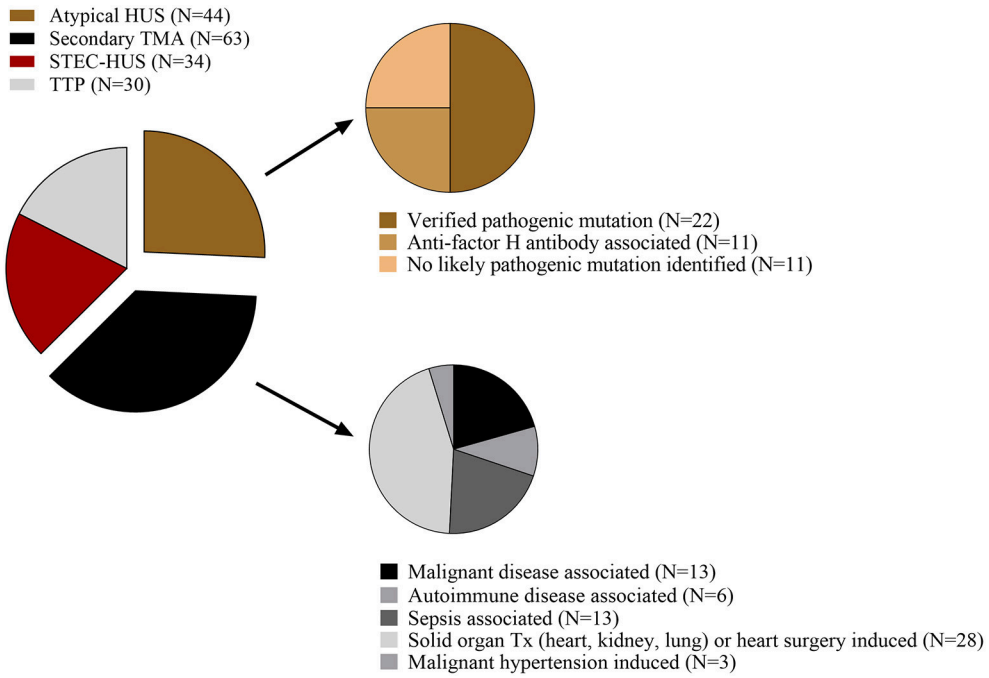


**FIGURE 1 |** Representation of TMA disease etiology in the studied population. The number of participants per group (N) is shown as proportion of a whole. HUS, hemolytic uremic syndrome; STEC-HUS, Shiga-like toxin associated HUS; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura; Tx, transplantation.

immunodiffusion assay. The level of the complement regulators C1q and FH and the titer of the anti-FH antibodies were measured using in-house ELISA techniques, described in detail elsewhere (22, 30, 31). A disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 (ADAMTS13) activity was evaluated by the application of the fluorogenic substrate FRETS-VWF73 (22). Commercially available kits were used to assess the levels of the complement activation products soluble C5b-9 (sC5b-9) and C3a (C3a des-arg) (Quidel, San Diego, CA) and for the measurement of PTX3 (R&D systems Minneapolis, MN). For the determination of CRP, PTX3, complement factor levels and pathway activities patient's sera were obtained. The complement activation products (sC5b-9 and C3a) were determined from EDTA anticoagulated plasma, whereas the ADAMTS13 activity was evaluated from sodium-citrate-anticoagulated plasma of the patients.

## *In vitro* Assessment of PTX3 Effect on AP Activation

We applied normal human serum (NHS) with additional recombinant human PTX3 in two established methods for the assessment of AP activity: the WIESLAB AP ELISA kit (EuroDiagnostica, Malmö, Sweden) and the C3 nephritic factor hemolytic assay (32), with modifications. The C3 nephritic factor assay was performed on washed sheep erythrocytes, where patient's samples were replaced by NHS spiked with recombinant human PTX3 (R&D systems Minneapolis, MN, USA) in gradually decreasing concentrations. Following a 20-min incubation of PTX3 with NHS, the solution was added to sheep erythrocytes. The formation of the C3 convertase was allowed within a 10-min incubation time at 30°C, and assembly of the terminal pathway membrane attack complex was achieved by the addition of undiluted rat serum to the cells, following multiple washes. After incubation at 37°C for 60 min, the extent of hemolysis was detected by reading the optical density (OD) at 412 nm. The effect of PTX3 on the assembly of C5b-9 on a plastic surface was assessed with the WIESLAB Alternative pathway ELISA kit (EuroDiagnostica, Malmö, Sweden). Similarly to the above, patient's sera were replaced by PTX3 spiked NHS, otherwise the assay was performed according to the manufacturer's instructions. To allow comparison of data, the hemolytic or AP activities in each experiment were expressed as ratio of the reference (mean OD of NHS with buffer control) in percentage.

## Statistical Analysis

Data analysis was performed using the GraphPad Prism version 6.00 (GraphPad Software, La Jolla, CA, www.graphpad.com). The statistical analysis applied for data comparison is indicated in each figure legend and detailed in the **Supplementary Material**.

# RESULTS

## Patient Characteristics

This study was performed to determine the systemic level of CRP and PTX3 in 171 TMA patients in acute disease flare and to investigate the role of PTX3 in complement dysregulation *in vivo* compared to 69 age and sex-matched healthy individuals. Basic

clinical and laboratory characteristics of the patients and controls are summarized in **Table 1**.

Our study group consisted of TMA patients with the following etiologies: STEC-HUS ($N = 34$), aHUS ($N = 44$), secondary TMA ($N = 63$), and TTP ($N = 30$) (**Figure 1**). Over 90% of the admitted patients presented with the first acute episode of the disease. Blood samples were obtained from all patients preceding the start of plasma exchange or complement inhibitory therapy, although 16 patients received fresh frozen plasma prior to sampling. **Figure 1** shows the distribution of patients with various etiologies in the aHUS and secondary TMA groups.

All acute phase-TMA patients presented with laboratory signs of hemolysis and thrombocytopenia (<150 G/L), with the lowest median platelet count (i.e., 16 G/L) in the TTP subgroup. ADAMTS13 activity was decreased in 79% of the patients and ADAMTS13 deficiency was present in all of the TTP patients. Organ involvement manifested in clinical and laboratory signs of kidney damage or neurological symptoms as a sign of central nervous system involvement in most of the TMA patients. Classical laboratory parameters indicative of ongoing TMA in each of the study groups are summarized in **Supplementary Table 1**.

## Pentraxin Levels in Acute Phase-TMA and Their Relation to the Laboratory Markers of Disease and Clinical Characteristics of Patients

We measured a significantly elevated median PTX3 level in acute phase-TMA compared to healthy controls (**Figure 2A**), with an elevated systemic PTX3 level in 64% of the acute phase TMA patients. CRP levels were also higher, exceeding

**TABLE 1 |** Characteristics of the TMA patients and healthy controls.

| Characteristics analyzed | TMA | Healthy controls | Result of statistical comparison |
|---|---|---|---|
| Number of individuals enrolled | 171 | 69 | NA |
| Age | 35.2 (7.7–56.9) | 33.0 (18.7–41.0) | $P = 0.608$ |
| Sex (male/female in %) | 43/57 | 48/52 | NA |
| First acute episode (%) | 93.6 | NA | NA |
| 31-days mortality (%) | 11.7 | 0 | NA |
| Complement C3 < 0.9 g/L (%) | 49.7 | 0 | NA |
| Complement FH < 250 mg/L (%) | 25.7 | 0 | NA |
| **LABORATORY PARAMETERS INDICATIVE OF ONGOING TMA** | | | |
| Red blood cell count ($10^9$/L) | 2.9 (2.6–3.4) | 4.9 (4.6–5.2) | $p < 0.001$ |
| Hemoglobin (g/L) | 85 (75–97) | 141 (134–152) | $p < 0.001$ |
| Platelet count ($10^9$/L) | 46 (22–75) | 262 (235–309) | $p < 0.001$ |
| Lactate dehydrogenase (U/L) | 1,819 (893–3,051) | Not done | NA |
| Creatinine (μmol/L) | 188 (86–320) | 71 (64–78) | $p < 0.001$ |
| Carbamide (mmol/L) | 16.9 (10.9–25.9) | 4.5 (3.8–5.6) | $p < 0.001$ |
| **PENTRAXIN LEVELS AND WHITE BLOOD CELL PROFILE** | | | |
| PTX3 level (μg/L) | 5.19 (2.08–13.17) | 1.08 (0.75–1.66) | $p < 0.001$ |
| CRP level (mg/L) | 16.9 (4.3–72.0) | 1.4 (0.8–2.0) | $p < 0.001$ |
| White blood cell count (G/L) | 10.4 (7.1–15.3) | 6.5 (5.4–7.9) | $p < 0.001$ |
| Absolute neutrophil count (G/L) | 7.1 (4.8–12.4) | 4.0 (3.0–4.7) | $p < 0.001$ |
| Absolute lymphocyte count (G/L) | 1.4 (0.8–2.6) | 2.0 (1.8–2.4) | $p < 0.001$ |
| **COMPLEMENT PARAMETERS** | | | |
| ADAMTS13 activity (%) (Reference range: 67–147%) | 38 (17–54) | Not done | NA |
| Classical pathway activity (CH50/ml) | 57 (45–71) | 70 (62–77) | $p < 0.001$ |
| Alternative pathway activity (%) | 86 (56–101) | 101 (78–117) | $p < 0.001$ |
| C3 level (g/L) | 0.90 (0.68–1.15) | 1.26 (1.18–1.47) | $p < 0.001$ |
| C4 level (g/L) | 0.23 (0.14–0.32) | 0.34 (0.27–0.40) | $p < 0.001$ |
| Factor H level (mg/L) | 390 (245–513) | 560 (462–692) | $p < 0.001$ |
| Factor I level (%) | 98 (81–123) | 102 (92–108) | $p = 0.192$ |
| Factor B level (%) | 98 (73–116) | 101 (91–113) | $p = 0.791$ |
| C1q level (mg/L) | 87 (56–112) | 100 (80–124) | $p = 0.020$ |
| sC5b-9 level (ng/mL) (Reference range: 110–252 ng/mL) | 352 (265–517) | Not done | NA |
| C3a level (ng/mL) (Reference range: 70–270 ng/mL) | 171 (120–259) | Not done | NA |

*Characteristics and laboratory data of the TMA patients and healthy controls. Data are shown as median with interquartile range, results of statistical comparison are indicated with the respective p-value of the Mann-Whitney test. ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CRP, C-reactive protein; FH, factor H; NA, not applicable; PTX3, pentraxin-3; sC5b-9, soluble C5b-9; TMA, thrombotic microangiopathy.*

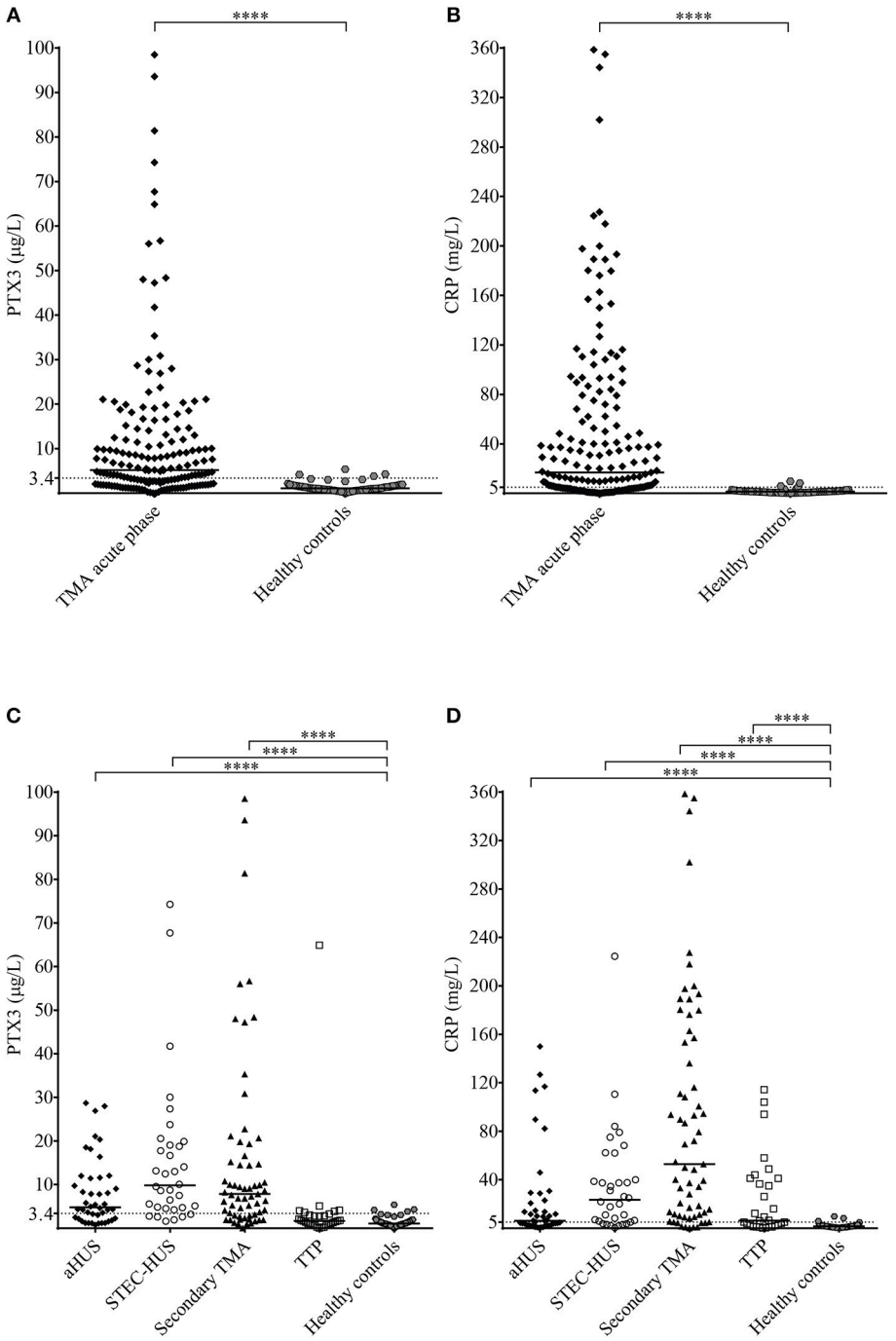


**FIGURE 2 |** PTX3 and CRP levels in acute TMA vs. healthy controls. **(A,B)** PTX3 and CRP levels of TMA patients at the acute disease onset compared to pentraxin levels of healthy individuals. **(C,D)** PTX3 and CRP levels of acute phase-TMA patients grouped by disease etiology. Data are expressed as mean of technical duplicates, the horizontal line indicates the median of each group, while an intermittent line shows the calculated cutoff of each pentraxin, respectively. Statistical analysis was performed with the Mann Whitney test **(A,B)** or the Kruskal-Wallis test corrected for multiple comparisons using the Dunn's *post hoc* test **(C,D)**, respectively. Statistical significance is indicated by asterisks (****$p < 0.0001$). aHUS, atypical hemolytic uremic syndrome; CRP, C-reactive protein; PTX3, pentraxin-3; STEC-HUS, Shiga-like toxin associated HUS; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura.

the upper limit of normal range (5 mg/L) in 70% of TMA patients (**Figure 2B**). The calculated cutoff of CRP levels (5.01 mg/L) was equivalent to the upper limit of normal range (5 mg/L) used in our laboratory in frames of diagnostics, whereas the cutoff of PTX3 levels was determined based on the levels measured in the healthy control group and set as

3.40 μg/L (mean + 2 times the standard deviation of healthy controls).

Elevated PTX3 and CRP levels could be detected in all etiology groups of TMA, although PTX3 elevation was exceptional in TTP, despite the elevated CRP level in 53% of the patients of this subgroup (**Figures 2C,D**). With further subdivision of the study groups, we found that the elevation of both pentraxins was independent of the molecular background in aHUS, since in each of the distinct aHUS subgroups PTX3 or CRP levels were significantly elevated compared to healthy controls (all $p < 0.05$, Mann-Whitney test) (**Figures 3A,B**), and we detected similar pentraxin levels in secondary TMAs with distinct etiologies, too (data not shown). PTX3 levels were associated with markers of disease activity and organ damage in TMA. We observed a positive correlation between lactate dehydrogenase and PTX3 levels, and a weaker yet significant correlation of the platelet count and laboratory signs of kidney damage to PTX3. By contrast, association between CRP and disease activity was not present, except a significant positive correlation to creatinine levels (**Table 2**). The parameters presented in **Table 2** were entered into two multiple regression models to explore relationship between them and PTX3 or CRP, respectively. LDH (standardized regression coefficient beta = 0.299) turned out to be significant predictor of PTX3 in the multivariable model, whereas platelet and kidney function measures did not. For CRP, significant predictors were hemoglobin (beta = 0.183), platelet number (beta = −0.179) and creatinine (beta = 0.338) levels. Since platelet count is a reliable marker of disease activity in TMA, we further explored its relationship to the systemic pentraxin levels by grouped analysis of patients according to platelet counts at the time of admission. We found that irrespective of the classification, median PTX3 and CRP levels of all subgroups remained significantly elevated compared to healthy controls (**Supplementary Figure 1**). Furthermore, PTX3 and CRP showed a strong positive correlation to each other and to markers of systemic inflammation such as the white blood cell count and absolute neutrophil count of the patients (**Supplementary Figures 2**, **3**). In the 16 FFP-treated patients enrolled in this study, administration of FFP did not results in an improvement of the clinical status or the classical laboratory signs of TMA until the time point of blood sample collection. We performed all our analysis with the exclusion of the FFP-treated patients as well, and it did not change any of our conclusions on the correlations observed at the acute phase of TMA.

## Elevated Pentraxin Levels Normalize in Disease Remission

We obtained follow-up samples from 31 aHUS patients and 19 of the TTP patients. In over 80% of aHUS both PTX3 and CRP levels decreased in remission compared to the paired acute phase samples, but the extent of decline did not reach statistical significance in patients with no clarified molecular background of the disease (**Figures 3A,B**). The median PTX3 level also remained significantly higher in aHUS remission compared to the control group, while the CRP levels in remission were similar to that of healthy controls (**Supplementary Figure 4**). The initially low PTX3 levels of TTP patients showed no remarkable difference in remission, and the CRP levels also normalized in over 80% of the cases (**Supplementary Figure 5**).

## Association of the Median PTX3 Level With the Acute Phase Mortality

The overall 11.7% acute phase mortality arose from the high mortality rate of the secondary TMA group, which exceeded 30% within a 31-days period. No deaths occurred in the STEC-HUS or aHUS study groups and one patient died in the TTP group. The median CRP levels did not differ significantly in secondary TMA patients who survived the acute phase compared to those who did not, but the median PTX3 level was significantly higher in the deceased individuals compared to those who survived the first month of the TMA episode (**Figures 4A,B**). The optimum PTX3 cut-point was 9 μg/mL to differentiate patients who died during follow up, from those who survived [odd's ratio 3.08 (95% CI 1.02–9.33)]. One-by-one adjustment for key activity indicators showed that high PTX3 levels are hemoglobin and creatinine independent predictors of mortality, whereas dependent on platelet and LDH levels.

## Signs of Complement Consumption in Acute Phase-TMA and Their Association With the Systemic Pentraxin Levels

Nearly 50% of the TMA patients presented with decreased C3 levels indicative of complement consumption (**Table 1**), while only 9% of the patients (15/171) showed no signs of complement alteration (with C3, C4, FH, C1q, factor I and factor B levels, CP and AP activities, and complement activation product levels within the laboratory normal range). To assess whether elevated pentraxin levels were associated with complement consumption in the acute phase of TMA, we grouped the patients based on PTX3 and CRP levels and observed a strong linkage between the gradual increase in PTX3 and signs of complement AP and CP consumption (**Figure 5** and **Supplementary Table 2**). As a result of complement overactivation and complement factor consumption, both C3 and C4 levels were significantly lower in patients with PTX3 above 20 μg/L compared to those below 5 μg/L. If the relationships between decreased C3 and C4 levels to the elevated PTX3 (**Figures 5A,C**) were further analyzed in subgroups of patients stratified according to the most important confounder, i.e., LDH level (**Supplementary Figure 6**), similar associations were observed. Although the gradual increase of PTX3 was not accompanied by a decrease in the FH levels, complement CP and AP activities were significantly lower in patients with PTX3 above 20 μg/L compared to those with PTX3 below 5 μg/L. Moreover, patients with a PTX3 level exceeding 20 μg/L had a median AP and CP activity below the normal range indicating explicit complement consumption. By contrast, CRP levels did not show an association with any of the measured complement activity parameters.

## Influence of PTX3 on AP Activation *in vitro*

*In vivo* complement consumption was accompanied by a gradual increase in the systemic PTX3 level in our acute phase-TMA

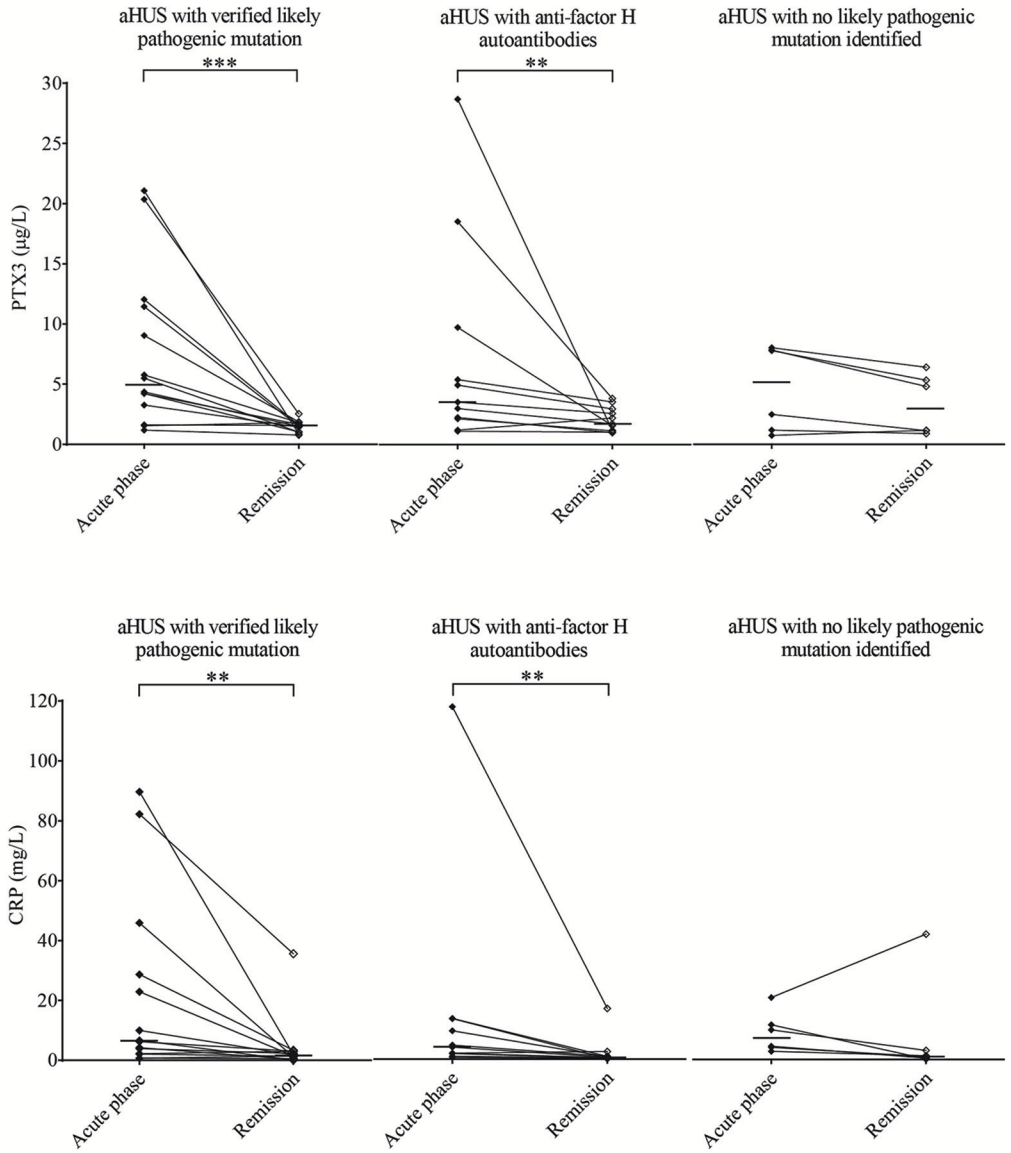


**FIGURE 3 |** PTX3 and CRP levels in aHUS acute phase and remission. PTX3 **(A)** and CRP **(B)** levels of aHUS patients are shown in the acute phase (black squares) and in remission (empty squares) with a continuous line connecting the respective sample pairs, while the medians of each group are indicated by a horizontal line. Half of the patients ($N = 22$, left) had a confirmed likely pathogenic mutation, 11 patients presented with anti-factor H antibodies (middle) and by the rest ($N = 11$, right) no likely pathogenic mutation has been identified in the complement genes (*CFH, CFHR5, CFI, CD46, C3, CFB*), *THBD* or *DGKE*. Data points represent mean of technical duplicates, statistical analysis was performed with the Wilcoxon-signed rank test, statistical significance is indicated by asterisks (**$p < 0.01$; ***$p < 0.001$). aHUS, atypical hemolytic uremic syndrome; CRP, C-reactive protein; PTX3, pentraxin-3.

patients. To explore the functional relevance of this phenomenon we tested whether PTX3 attenuates or stimulates the AP activity on the cellular surface. In a modified hemolytic assay (used to determine the C3 nephritic factor level) we built up the AP convertase on sheep erythrocytes and determined the hemolytic activity of NHS with the addition of recombinant human PTX3 or buffer control, respectively (**Figure 6A**). We found that addition of PTX3 significantly decreased the activity of the AP C3-convertase on sheep red blood cells. Conversely, addition of PTX3 to NHS did not influence AP activity on the surface of ELISA plates. Hence, no remarkable change was detected in C9 deposition through lipopolysaccharide (LPS)-induced activation of the AP (**Figure 6B**), whereas PTX3 alone did not bind to LPS in the ELISA wells (data not shown).

## DISCUSSION

Our study investigated the role of PTX3 and CRP in association with complement consumption in the acute phase of TMA. We provide a detailed description of acute phase-TMA patients'

**TABLE 2 |** Correlation of the systemic pentraxin levels to laboratory markers of TMA.

| Laboratory parameters analyzed | PTX3 | | | CRP | | |
|---|---|---|---|---|---|---|
| | **Spearman r** | ***p*-value** | ***N*** | **Spearman r** | ***p*-value** | ***N*** |
| Red blood cell count | 0.08785 | 0.3380 | 121 | 0.02669 | 0.7714 | 121 |
| Hemoglobin | 0.007054 | 0.9329 | 145 | 0.07903 | 0.3447 | 145 |
| Platelet count | **0.1975** | **0.0161** | **148** | −0.1107 | 0.1806 | 148 |
| Lactate dehydrogenase | **0.299** | **0.0004** | **134** | 0.003968 | 0.9637 | 134 |
| Creatinine | **0.2421** | **0.0023** | **156** | **0.2266** | **0.0045** | **156** |
| Carbamide | **0.2011** | **0.0257** | **123** | 0.1377 | 0.1289 | 123 |

*Spearman correlation analysis of PTX3 and CRP levels to laboratory markers of hemolysis, thrombocytopenia and kidney damage, characteristic to acute phase-TMA. Statistical analysis was performed by the Spearman correlation test, obtained r and p-values are indicated by each parameter, with the number of pairs analyzed. Significant correlations are highlighted in bold. Please note that blood count and chemistry data were not accessible by all the enrolled patients, therefore the correlation analyses might include less than the total number of patients (N = 171) in this study, CRP. C-reactive protein; PTX3, pentraxin-3; TMA, thrombotic microangiopathy.*

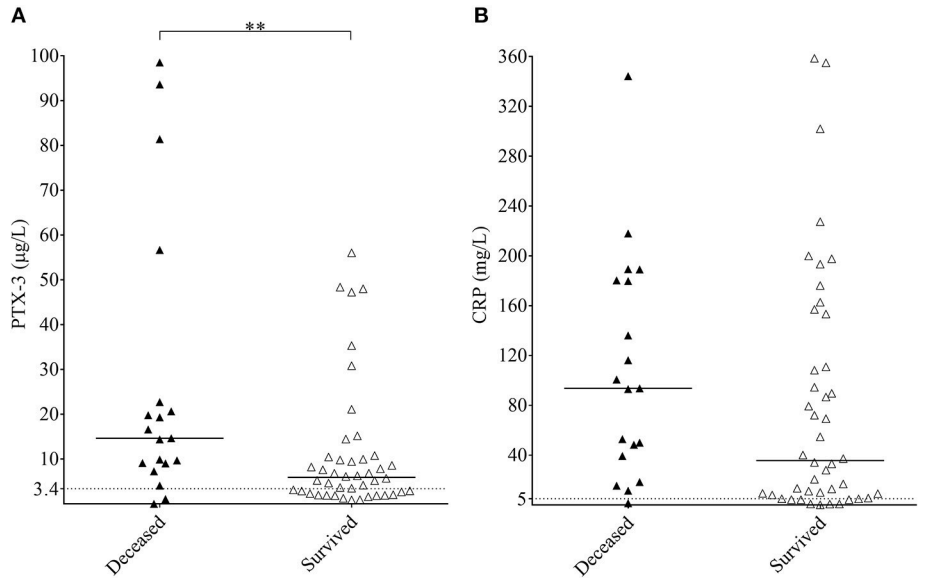


**FIGURE 4 |** Association of the systemic pentraxin levels to acute phase mortality in secondary TMA. PTX3 **(A)** and CRP **(B)** levels of acute phase secondary TMA patients are shown, grouped based on the 31-day survival of patients (deceased, black triangles; survived, white triangles). Data are expressed as mean of technical duplicates, the horizontal line indicates the median of each group, while an intermittent line shows the calculated cutoff of each pentraxin, respectively. Statistical analysis was performed with the Mann-Whitney test. Statistical significance is indicated by asterisks (**$p < 0.01$). CRP, C-reactive protein; PTX3, pentraxin-3; TMA, thrombotic microangiopathy.

complement profile linked to changes in the systemic pentraxin levels. We report that PTX3 elevation is present in the acute phase of STEC-HUS, aHUS and secondary TMA but is exceptional in TTP. Conversely, an elevation in the systemic CRP level is present regardless of disease etiology in the acute phase of TMA (**Figure 2**). Disease remission in aHUS was accompanied by a decline in the level of both pentraxins (**Figure 3**). However, while CRP decreased to values observed in healthy individuals, the median PTX3 level remained significantly higher in aHUS compared to controls (**Supplementary Figure 4**). In the remission of TTP no notable alteration of the PTX3 levels could be recorded (**Supplementary Figure 5**). We observed the highest acute phase mortality in secondary TMA patients, which was associated with high PTX3 but not CRP levels (**Figure 4**). TMA was accompanied by laboratory signs of complement activation and consumption in the majority of our patients. We show for the first time that AP and CP consumption is associated with elevated PTX3 in the acute phase of TMA (**Figure 5**). To explore a potential mechanism in the background of this observation, we confirmed *in vitro* that PTX3 limits AP activity on the surface of red blood cells, with no effect on terminal pathway assembly during LPS-induced AP activation on ELISA plates (**Figure 6**).

Microthrombus formation in TMA results in extensive inflammation that involves turnover of the complement and coagulation cascades together with the activation of innate immunity (21). The observed elevation of both pentraxins in acute phase-TMA and their strong positive correlation to the white blood cell and absolute neutrophil counts suggests that

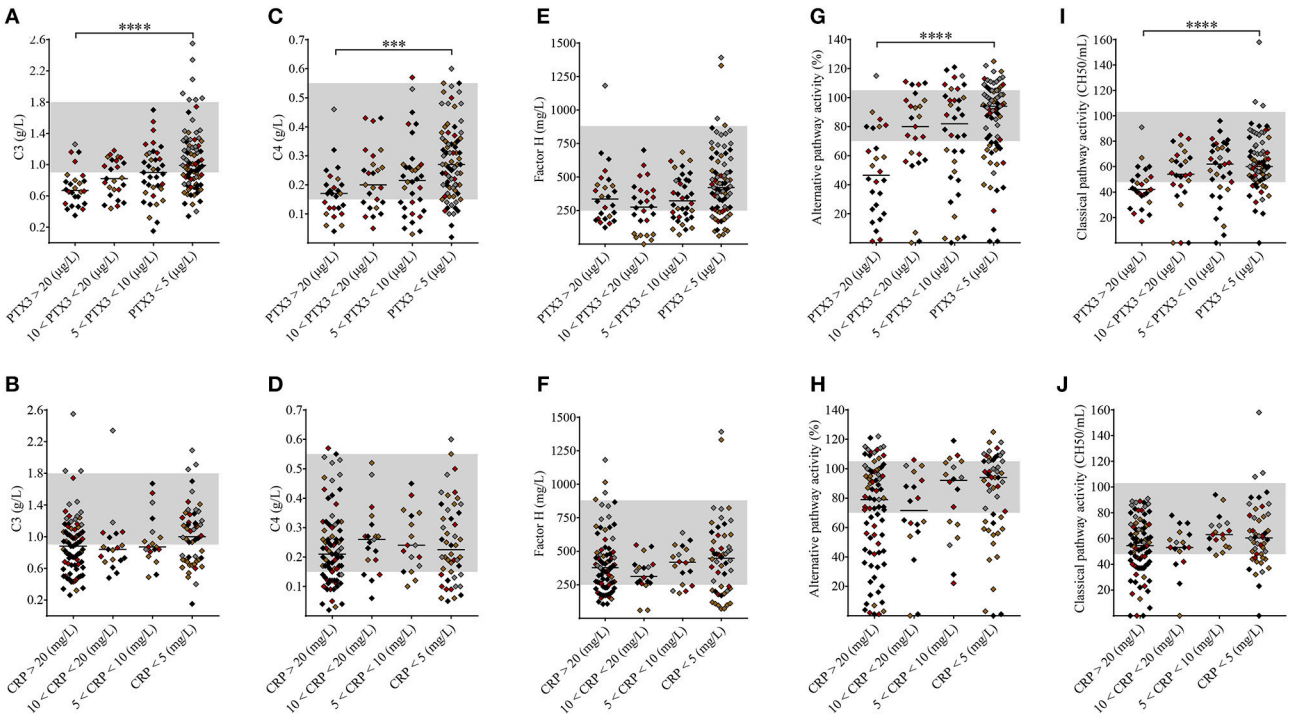


**FIGURE 5 |** Association of the systemic pentraxin levels with laboratory signs of complement consumption. The degree of complement activation and consumption was assessed from complement factor levels (**A–F**: C3, C4, FH) and complement activity parameters **(G–J)** in TMA patients subdivided based on the measured systemic PTX3 or CRP levels, respectively. Data are expressed as mean of technical duplicates, the horizontal lines show the median of each group and the laboratory normal range is indicated with gray shading. The color of each data point indicates the specific form of TMA corresponding to **Figure 1** (brown, aHUS; red, STEC-HUS; black, secondary TMA; gray, TTP). Statistical analysis was performed with the Kruskal-Wallis test corrected for multiple comparisons using the Dunn's *post hoc* test. ANOVA $\boldsymbol{p < 0.0001}$, $p = 0.1285$, $\boldsymbol{p = 0.0002}$, $p = 0.6713$, $\boldsymbol{p = 0.0358}$, $p = 0.2173$, $\boldsymbol{p < 0.0001}$, $p = 0.0717$, $\boldsymbol{p = 0.0001}$, $p = 0.053$ for **(A–J)**, statistical significance of the Dunn's tests are indicated by asterisks (***$p < 0.001$, ****$p < 0.0001$) on the respective figure panels **(A–J)**. aHUS, atypical hemolytic uremic syndrome; CRP, C-reactive protein; PTX3, pentraxin-3; STEC-HUS, Shiga-like toxin associated HUS; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura.

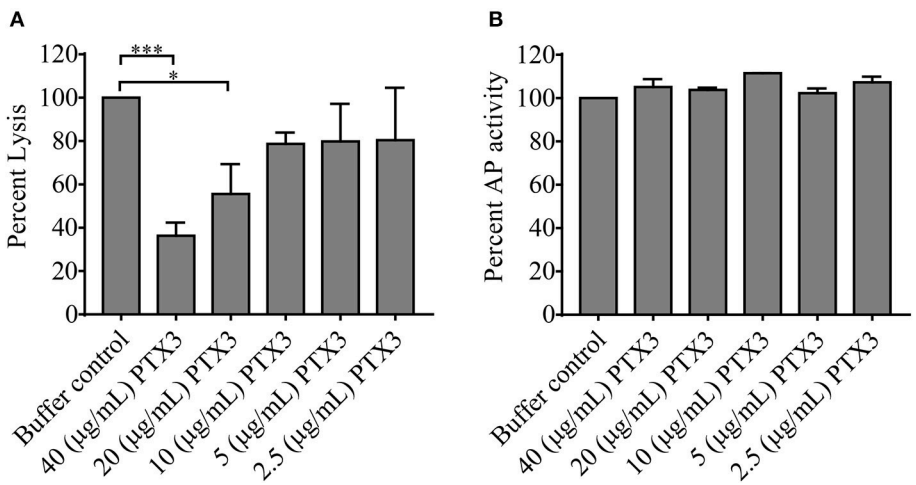


**FIGURE 6 |** Effect of PTX3 on AP activity *in vitro.* The effect of recombinant human PTX3 on AP hemolytic activity **(A)** and AP-mediated C9 deposition **(B)** is shown in percent compared to buffer control added to pooled serum of healthy individuals. Data represent mean of 3 times repeated experiments with technical duplicates, error bars indicate the standard error of mean. Statistical analysis was performed with the Kruskal-Wallis test corrected for multiple comparisons using the Dunn's *post hoc* test. Statistical significance is indicated by asterisks (*$p < 0.05$; ***$p < 0.001$). AP, alternative pathway; PTX3, pentraxin-3.

PTX3 and CRP production is induced in frames of the ongoing inflammatory response (**Supplementary Figures 2**, **3**). TMAs have recently been linked to neutrophil cell activation and NET formation (24–28), as a component of which PTX3 may be released on demand from leukocyte infiltrates that accumulate at the site of endothelial damage (3, 29). Furthermore, local levels of PTX3 may increase via its production by injured endothelial cells (3), hence providing a possible dual-source of PTX3 during the acute phase of TMA. Conversely, increased CRP production may be attributed to the induction of a systemic inflammatory response that induces the release of acute phase proteins.

In approximately 60% of aHUS cases mutations to the complement genes or antibodies directed against the complement regulator FH account for the pathophysiological process leading to AP dysregulation and consumption (33, 34), whereas in the remaining one-third of the cases the molecular background remains unrevealed. Our aHUS cohort had a somewhat higher representation of autoimmune aHUS (25 vs. 10%) and a relatively small proportion of unexplained cases (25 vs. 30–40%) compared to the previously reported prevalence (33, 34). We had a notable number of patients with low FH level in our patient cohort. This arose from FH mutations and antibodies in aHUS, albeit patients with a low FH level were also present in STEC-HUS, secondary TMA and TTP, indicating the presence of complement dysregulation in multiple forms of TMA. Nonetheless, elevated pentraxin levels were present in all aHUS subgroups independent of the molecular etiology. PTX3 and CRP elevation was also prominent in STEC-HUS and secondary TMA, regardless of the heterogenic etiological background of the patients. However, PTX3 elevation was exceptional in TTP, albeit neutrophil cell activation together with complement dysregulation have been described in TTP (22, 28). Laboratory signs of kidney damage were also absent in 70% of the TTP patients, while most patients with other forms of TMA presented with a varying degree of kidney injury. Both acute and chronic kidney damage have been linked to the elevation of PTX3 (35), the lack of which in TTP could provide a possible explanation for the absence of PTX3 elevation in TTP. However, it cannot be excluded that additional factors arising from the distinct pathogenesis of TTP (34) have also contributed to the observed difference.

Secondary TMA patients in our study cohort had an overall 30.2% in-hospital mortality, which is comparable to observations reported in literature (23, 36). Acute phase disease mortality was associated with a higher median PTX3 level in secondary TMA, and this relationship was independent of the hemoglobin and creatinine levels, but was non-independent of platelet and LDH. The difference between median CRP levels did not reach statistical significance in deceased patients compared to those who survived the first month of the TMA episode. This observation conforms published reports in regard to the association of PTX3 to acute disease mortality in multiple conditions including severe sepsis (37, 38), ventilation assisted pneumonia (39) and acute aortic type A dissection (40). Besides, PTX3 was reported to be a long-term prognostic marker of mortality in patients undergoing hemodialysis (41) and of cardiovascular death in patients with renal disease (42), whereas some studies even place PTX3 superior to CRP as a predictor of mortality (39), endothelial dysfunction (43) or indicator of local inflammatory response following vascular injury (44).

Even though both pentraxins have been described to interact with the complement system *in vitro* (1, 2), we only found an association between laboratory signs of complement consumption and elevated PTX3 in the acute phase of TMA. The net result of the PTX3-complement interaction is proposed to be restrain of complement-mediated damage on non-activator surfaces and stimulation of phagocytosis and clearance of cellular debris (1, 2). *In vivo* experimental models of tissue damage however, reported inconclusive data on the overall impact of PTX3 on tissue recovery. In murine models of ischemia-reperfusion injury both endogenous and exogenous PTX3 were described to alleviate leukocyte recruitment following renal ischemia (10), while the lack of PTX3 was associated with a higher degree of apoptosis and C3 deposition in damaged cardiac tissue (11). Nevertheless, others reported that the overexpression or external admission of PTX3 exacerbated the post-ischemic intestinal and remote pulmonary tissue damage (12, 13). In humans PTX3 has been shown to correlate with surrogate markers of disease severity in cardiovascular and renal diseases (35, 45, 46) and molecular characterization of this association suggests that PTX3 is involved in the fine tuning of inflammation with an overall tissue-protective effect (47, 48).

In endothelial damage associated with TMA, although NET formation may promote thrombosis and complement activation (49), as a NET component (29) PTX3 may recruit the complement regulator FH (8) and limit the expansion of tissue damage mediated by the AP. The potential regulatory role of PTX3 on AP activity is suggested by experimental evidence describing FH recruitment by PTX3 (8, 50) to the damaged cell surface, while the presence of anti-FH antibodies or mutations of the complement regulator have been linked to an impaired FH-PTX3 interaction that may aggravate the endothelial damage in aHUS (50). To better understand the potential role of PTX3 elevation in TMA, we measured the changes of AP activity in the presence or absence of external PTX3 using two distinct *in vitro* approaches. First, to determine the AP hemolytic activity, we built up the C3 convertase on sheep erythrocytes under conditions allowing for AP activation only. Second, we assessed C9 generation on the surface of ELISA plates via LPS-induced AP activation, with or without additional PTX3. Based on the gradual decline of the hemolytic activity parallel to the increment of PTX3 concentration in pooled human serum, we conclude that local release of PTX3 may indeed play an important role in the limitation of AP activity. However, based on previously published observations on the interaction of PTX3 with the regulators of complement (8, 9, 50), restrain of the AP activity by PTX3 is most probably due to an indirect effect (e.g., recruitment of complement regulators), which requires cellular attachment of the PRM, rather than direct inhibition of the activation pathway. This hypothesis is also supported by the observed lack of AP restrain, when the activation was induced on the surface of an ELISA plate, however detailed molecular investigation of this phenomenon would be necessary to identify each complement factor involved in the regulatory effect. Nonetheless, the observed

restrain of the AP indicates that local release of PTX3 could possibly attenuate complement activity and hence potentially limit the ongoing endothelial damage in TMA patients.

Finally, the lack of correlation between CRP levels and complement consumption could be attributed to the fact, that CRP production is induced in the liver in frames of a systemic inflammatory response that may not closely reflect the degree of local endothelial damage and subsequent complement consumption. However, *in vitro* evidence suggests that through the binding of complement regulators and the restrain of excess terminal pathway activity, CRP as well as PTX3 are able to regulate the AP and CP of complement (1, 15–18).

In conclusion, we report the association of PTX3 elevation with complement overactivation and consumption in TMA. The regulatory role of PTX3 on AP hemolytic activity *in vitro* suggests that PTX3 is an adjunct factor in the prevention of excess endothelial damage in TMA. Our observations are in line with previously published *in vitro* data describing the interaction of PTX3 and individual complement factors, and add to *in vivo* investigations emphasizing the potential tissue-protective role of PTX3. This is the first study where the association of PTX3 and CRP elevation has been investigated in a complement mediated disease *in vivo*, and thus our results provide a missing link between the numerous *in vitro* observations that described the interaction of PTX3 with the complement system under defined experimental conditions. On the other hand, our observations may indicate a potential practical use of PTX3 determination as a biomarker and determinant of complement consumption in the acute phase of TMA. However, apparent limitations of our study are the retrospective enrollment of patients and the rare nature of this disease that together may have caused some of our analyses to be underpowered. The limited number of study subjects and subsequently low case and event numbers in this study precluded multivariate analysis in different etiology based subgroups of TMAs, therefore some of our observation may represent overestimation of true effects due to the lack of adjustment for important clinical and/or laboratory covariates. Therefore, independent confirmation of our observations is necessary before firm conclusions can be reached on the contribution of PTX3 to the pathogenesis of TMA. Nonetheless, the reported association of elevated PTX3 levels and complement consumption may initiate further investigations to understand the exact role of PTX3 in TMA pathogenesis and may aid the better understanding of the heterogeneous clinical course of TMA.

## AUTHOR CONTRIBUTIONS

ZP and MJ: study concept and design. ET, ZS, NG, BáM, GS, BlM, and DC: experimental procedures. ET, BáM, MR, PF, KK, GR, AS, and ZP: acquisition of data. All authors: analysis and interpretation of data. ET and ZP: critical writing of the manuscript. All authors: critical revision of the manuscript for important intellectual content. ZP and MJ: study supervision. MJ, GR, DC, and ZP: acquisition of funding.

## FUNDING

The research was financed by the Higher Education Institutional Excellence Programme of the Ministry of Human Capacities in Hungary, within the framework of the molecular biology thematic programme of Semmelweis University. ET received financial support from the NTP-NFTÖ-18 project by the Human Capacities Grant Management Office and the Hungarian Ministry of Human Capacities and the EFOP-3.6.3-VEKOP-16-2017-00009 grant. This work was partly supported by the grant of the National Research Fund (National Research, Development and Innovation Office) of Hungary, PD116119 to DC, and K124549 to GR. MJ was supported by the Hungarian Academy of Sciences (MTA Lendület program, grant nr. LP-2012/43) and by the Institutional Excellence Program of the Ministry of Human Capacities of Hungary.

## ACKNOWLEDGMENTS

The authors would like to thank Sz. Walentin, I. Szigeti, Zs. Szendrei, M. Kókai, and E. Kertész for expert technical assistance, and E. Kenesei for help with sample collection from healthy individuals. Furthermore, we thank B. Uzonyi for valuable professional advice and methodological suggestions.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00240/full#supplementary-material

## REFERENCES

1. Inforzato A, Doni A, Barajon I, Leone R, Garlanda C, Bottazzi B, et al. PTX3 as a paradigm for the interaction of pentraxins with the complement system. *Semin Immunol.* (2013) 25:79–85. doi: 10.1016/j.smim.2013.05.002
2. Daigo K, Inforzato A, Barajon I, Garlanda C, Bottazzi B, Meri S, et al. Pentraxins in the activation and regulation of innate immunity. *Immunol Rev.* (2016) 274:202–17. doi: 10.1111/imr.12476
3. Kunes P, Holubcova Z, Kolackova M, Krejsek J. Pentraxin 3(PTX 3): an endogenous modulator of the inflammatory response. *Mediators Inflamm.* (2012) 2012:920517. doi: 10.1155/2012/920517
4. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Front Immunol.* (2018) 9:754. doi: 10.3389/fimmu.2018.00754
5. Ma YJ, Doni A, Skjoedt MO, Honore C, Arendrup M, Mantovani A, et al. Heterocomplexes of mannose-binding lectin and the pentraxins PTX3 or serum amyloid P component trigger cross-activation of the complement system. *J Biol Chem.* (2011) 286:3405–17. doi: 10.1074/jbc.M110.190637
6. Ma YJ, Doni A, Hummelshoj T, Honore C, Bastone A, Mantovani A, et al. Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. *J Biol Chem.* (2009) 284:28263–75. doi: 10.1074/jbc.M109.009225
7. Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, et al. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. *Eur J Immunol.* (2003) 33:465–73. doi: 10.1002/immu.200310022

8. Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, et al. Binding of the long pentraxin PTX3 to factor H: interacting domains and function in the regulation of complement activation. *J Immunol.* (2008) 181:8433–40. doi: 10.4049/jimmunol.181.12.8433
9. Braunschweig A, Jozsi M. Human pentraxin 3 binds to the complement regulator c4b-binding protein. *PLoS ONE* (2011) 6:e23991. doi: 10.1371/journal.pone.0023991
10. Lech M, Rommele C, Grobmayr R, Eka Susanti H, Kulkarni OP, Wang S, et al. Endogenous and exogenous pentraxin-3 limits postischemic acute and chronic kidney injury. *Kidney Int.* (2013) 83:647–61. doi: 10.1038/ki.2012.463
11. Salio M, Chimenti S, De Angelis N, Molla F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. *Circulation* (2008) 117:1055–64. doi: 10.1161/CIRCULATIONAHA.107.749234
12. Souza DG, Soares AC, Pinho V, Torloni H, Reis LF, Teixeira MM, et al. Increased mortality and inflammation in tumor necrosis factor-stimulated gene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol.* (2002) 160:1755–65. doi: 10.1016/S0002-9440(10)61122-4
13. Souza DG, Amaral FA, Fagundes CT, Coelho FM, Arantes RM, Sousa LP, et al. The long pentraxin PTX3 is crucial for tissue inflammation after intestinal ischemia and reperfusion in mice. *Am J Pathol.* (2009) 174:1309–18. doi: 10.2353/ajpath.2009.080240
14. He X, Han B, Liu M. Long pentraxin 3 in pulmonary infection and acute lung injury. *Am J Physiol Lung Cell Mol Physiol.* (2007) 292:L1039–49. doi: 10.1152/ajplung.00490.2006
15. Biro A, Rovo Z, Papp D, Cervenak L, Varga L, Fust G, et al. Studies on the interactions between C-reactive protein and complement proteins. *Immunology* (2007) 121:40–50. doi: 10.1111/j.1365-2567.2007.02535.x
16. Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, et al. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: *in vivo* proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *Circulation* (2014) 130:35–50. doi: 10.1161/CIRCULATIONAHA.113.007124
17. Sjoberg AP, Trouw LA, McGrath FD, Hack CE, Blom AM. Regulation of complement activation by C-reactive protein: targeting of the inhibitory activity of C4b-binding protein. *J Immunol.* (2006) 176:7612–20. doi: 10.4049/jimmunol.176.12.7612
18. O'Flynn J, van der Pol P, Dixon KO, Prohaszka Z, Daha MR, van Kooten C. Monomeric C-reactive protein inhibits renal cell-directed complement activation mediated by properdin. *Am J Physiol Renal Physiol.* (2016) 310:F1308–16. doi: 10.1152/ajprenal.00645.2014
19. Goldberg RJ, Nakagawa T, Johnson RJ, Thurman JM. The role of endothelial cell injury in thrombotic microangiopathy. *Am J Kidney Dis.* (2010) 56:1168–74. doi: 10.1053/j.ajkd.2010.06.006
20. Jokiranta TS. HUS and atypical HUS. *Blood* (2017) 129:2847–56. doi: 10.1182/blood-2016-11-709865
21. Noris M, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. *Nat Rev Nephrol.* (2012) 8:622–33. doi: 10.1038/nrneph.2012.195
22. Reti M, Farkas P, Csuka D, Razso K, Schlammadinger A, Udvardy ML, et al. Complement activation in thrombotic thrombocytopenic purpura. *J Thromb Haemost.* (2012) 10:791–8. doi: 10.1111/j.1538-7836.2012.04674.x
23. Farkas P, Csuka D, Mikes B, Sinkovits G, Reti M, Nemeth E, et al. Complement activation, inflammation and relative ADAMTS13 deficiency in secondary thrombotic microangiopathies. *Immunobiology* (2017) 222:119–27. doi: 10.1016/j.imbio.2016.10.014
24. Walters MD, Matthei IU, Kay R, Dillon MJ, Barratt TM. The polymorphonuclear leucocyte count in childhood haemolytic uraemic syndrome. *Pediatr Nephrol.* (1989) 3:130–4. doi: 10.1007/BF00852893
25. Ishikawa N, Kamitsuji H, Murakami T, Nakayama A, Umeki Y. Plasma levels of granulocyte elastase-alpha1-proteinase inhibitor complex in children with hemolytic uremic syndrome caused by verotoxin-producing *Escherichia coli*. *Pediatr Int.* (2000) 42:637–41. doi: 10.1046/j.1442-200x.2000.01309.x
26. Fuchs TA, Kremer Hovinga JA, Schatzberg D, Wagner DD, Lammle B. Circulating DNA and myeloperoxidase indicate disease activity in patients with thrombotic microangiopathies. *Blood* (2012) 120:1157–64. doi: 10.1182/blood-2012-02-412197
27. Fitzpatrick MM, Shah V, Filler G, Dillon MJ, Barratt TM. Neutrophil activation in the haemolytic uraemic syndrome: free and complexed elastase in plasma. *Pediatr Nephrol.* (1992) 6:50–3. doi: 10.1007/BF00856833
28. Mikes B, Sinkovits G, Farkas P, Csuka D, Schlammadinger A, Razso K, et al. Elevated plasma neutrophil elastase concentration is associated with disease activity in patients with thrombotic thrombocytopenic purpura. *Thromb Res.* (2014) 133:616–21. doi: 10.1016/j.thromres.2014.01.034
29. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, et al. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med.* (2007) 204:793–804. doi: 10.1084/jem.20061301
30. Trojnar E, Jozsi M, Uray K, Csuka D, Szilagyi A, Milosevic D, et al. Analysis of linear antibody epitopes on factor H and CFHR1 using sera of patients with autoimmune atypical hemolytic uremic syndrome. *Front Immunol.* (2017) 8:302. doi: 10.3389/fimmu.2017.00302
31. Delamarche C, Berger F, Pouplard A, Emile J. An ELISA technique for the measurement of C1q in cerebrospinal fluid. *J Immunol Methods* (1988) 114:101–6. doi: 10.1016/0022-1759(88)90160-3
32. Rother U. A new screening test for C3 nephritis factor based on a stable cell bound convertase on sheep erythrocytes. *J Immunol Methods* (1982) 51:101–7.
33. Kavanagh D, Goodship TH, Richards A. Atypical hemolytic uremic syndrome. *Semin Nephrol.* (2013) 33:508–30. doi: 10.1016/j.semnephrol.2013.08.003
34. Trojnár E, Szilágyi Á, Mikes B, Csuka D, Sinkovits G, Prohászka Z. Role of complement in the pathogenesis of thrombotic microangiopathies. *memo Magaz Eur Med Oncol.* (2018) 11:227–34. doi: 10.1007/s12254-017-0380-y
35. Speeckaert MM, Speeckaert R, Carrero JJ, Vanholder R, Delanghe JR. Biology of human pentraxin 3 (PTX3) in acute and chronic kidney disease. *J Clin Immunol.* (2013) 33:881–90. doi: 10.1007/s10875-013-9879-0
36. Sun F, Wang X, Wu W, Wang K, Chen Z, Li T, et al. TMA secondary to SLE: rituximab improves overall but not renal survival. *Clin Rheumatol.* (2018) 37:213–8. doi: 10.1007/s10067-017-3793-4
37. Hu C, Zhou Y, Liu C, Kang Y. Pentraxin-3, procalcitonin and lactate as prognostic markers in patients with sepsis and septic shock. *Oncotarget* (2018) 9:5125–36. doi: 10.18632/oncotarget.23701
38. Lee YT, Gong M, Chau A, Wong WT, Bazoukis G, Wong SH, et al. Pentraxin-3 as a marker of sepsis severity and predictor of mortality outcomes: a systematic review and meta-analysis. *J Infect.* (2018) 76:1–10. doi: 10.1016/j.jinf.2017.10.016
39. Lin Q, Fu F, Shen L, Zhu B. Pentraxin 3 in the assessment of ventilator-associated pneumonia: an early marker of severity. *Heart Lung* (2013) 42:139–45. doi: 10.1016/j.hrtlng.2012.11.005
40. Zhou Q, Chai XP, Fang ZF, Hu XQ, Tang L. Association of plasma pentraxin-3 levels on admission with in-hospital mortality in patients with acute type A aortic dissection. *Chin Med J.* (2016) 129:2589–95. doi: 10.4103/0366-6999.192785
41. Sjoberg B, Snaedal S, Stenvinkel P, Qureshi AR, Heimburger O, Barany P. Three-month variation of plasma pentraxin 3 compared with C-reactive protein, albumin and homocysteine levels in haemodialysis patients. *Clin Kidney J.* (2014) 7:373–9. doi: 10.1093/ckj/sfu071
42. Krzanowski M, Krzanowska K, Gajda M, Dumnicka P, Dziewierz A, Woziwodzka K, et al. Pentraxin 3 as a new indicator of cardiovascularrelated death in patients with advanced chronic kidney disease. *Pol Arch Intern Med.* (2017) 127:170–7. doi: 10.20452/pamw.3944
43. Kocyigit I, Eroglu E, Orscelik O, Unal A, Gungor O, Ozturk F, et al. Pentraxin 3 as a novel bio-marker of inflammation and endothelial dysfunction in autosomal dominant polycystic kidney disease. *J Nephrol.* (2014) 27:181–6. doi: 10.1007/s40620-014-0045-4
44. Hudzik B, Szkodzinski J, Pietka-Rzycka A, Danikiewicz A, Wojnar R, Lekston A, et al. Plasma pentraxin 3 may be a more sensitive marker of inflammatory response than high-sensitivity C-reactive protein after bare-metal stent compared to drug-eluting stent implantation. *J Interferon Cytokine Res.* (2013) 33:280–4. doi: 10.1089/jir.2012.0023
45. Lech M, Rommele C, Anders HJ. Pentraxins in nephrology: C-reactive protein, serum amyloid P and pentraxin-3. *Nephrol Dial Transplant.* (2013) 28:803–11. doi: 10.1093/ndt/gfs448

46. Maekawa Y, Nagai T, Anzai A. Pentraxins: CRP and PTX3 and cardiovascular disease. *Inflamm Allergy Drug Targets* (2011) 10:229–35. doi: 10.2174/187152811796117744
47. Maugeri N, Rovere-Querini P, Slavich M, Coppi G, Doni A, Bottazzi B, et al. Early and transient release of leukocyte pentraxin 3 during acute myocardial infarction. *J Immunol.* (2011) 187:970–9. doi: 10.4049/jimmunol.1100261
48. Bonacina F, Baragetti A, Catapano AL, Norata GD. Long pentraxin 3: experimental and clinical relevance in cardiovascular diseases. *Mediators Inflamm.* (2013) 2013:725102. doi: 10.1155/2013/725102
49. de Bont CM, Boelens WC, Pruijn GJM. NETosis, complement, and coagulation: a triangular relationship. *Cell Mol Immunol.* (2018) 16:19–27. doi: 10.1038/s41423-018-0024-0
50. Kopp A, Strobel S, Tortajada A, Rodriguez de Cordoba S, Sanchez-Corral P, Prohaszka Z, et al. Atypical hemolytic uremic syndrome-associated variants and autoantibodies impair binding of factor h and factor h-related protein 1 to pentraxin 3. *J Immunol.* (2012) 189:1858–67. doi: 10.4049/jimmunol.1200357

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 26 February 2019
doi: 10.3389/fimmu.2019.00166

# Structure-Function Relationships of C-Reactive Protein in Bacterial Infection

*Donald N. Ngwa and Alok Agrawal**

*Department of Biomedical Sciences, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, United States*

One host defense function of C-reactive protein (CRP) is to protect against *Streptococcus pneumoniae* infection as shown by experiments employing murine models of pneumococcal infection. The protective effect of CRP is due to reduction in bacteremia. There is a distinct relationship between the structure of CRP and its anti-pneumococcal function. CRP is functional in both native and non-native pentameric structural conformations. In the native conformation, CRP binds to pneumococci through the phosphocholine molecules present on the C-polysaccharide of the pneumococcus and the anti-pneumococcal function probably involves the known ability of ligand-complexed CRP to activate the complement system. In the native structure-function relationship, CRP is protective only when given to mice within a few hours of the administration of pneumococci. The non-native pentameric conformation of CRP is created when CRP is exposed to conditions mimicking inflammatory microenvironments, such as acidic pH and redox conditions. In the non-native conformation, CRP binds to immobilized complement inhibitor factor H in addition to being able to bind to phosphocholine. Recent data using CRP mutants suggest that the factor H-binding function of non-native CRP is beneficial: in the non-native structure-function relationship, CRP can be given to mice any time after the administration of pneumococci irrespective of whether the pneumococci became complement-resistant or not. In conclusion, while native CRP is protective only against early stage infection, non-native CRP is protective against both early stage and late stage infections. Because non-native CRP displays phosphocholine-independent anti-pneumococcal activity, it is quite possible that CRP functions as a general anti-bacterial molecule.

**Keywords: C-reactive protein, factor H, phosphocholine, pneumococcal C-polysaccharide, *Streptococcus pneumoniae***

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Milan University, Italy*

***Reviewed by:***
*Annette Karen Shrive, Keele University, United Kingdom*
*Tomohide Takaya, Shinshu University, Japan*

***Correspondence:**
*Alok Agrawal*
*agrawal@etsu.edu*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *28 November 2018*
***Accepted:*** *18 January 2019*
***Published:*** *26 February 2019*

***Citation:***
*Ngwa DN and Agrawal A (2019) Structure-Function Relationships of C-Reactive Protein in Bacterial Infection. Front. Immunol. 10:166. doi: 10.3389/fimmu.2019.00166*

## INTRODUCTION

C-reactive protein (CRP) is a multifunctional molecule of the innate immune system in humans (1–4). CRP is a cyclic pentameric protein comprised of five identical non-covalently attached subunits. Each subunit has an intra-disulfide bond and the molecular weight of each subunit is ∼23 kDa (5, 6). A phosphocholine (PCh)-binding site is located on the same face of each subunit in the homopentamer. The amino acids $Phe^{66}$, $Thr^{76}$, and $Glu^{81}$ in CRP are critical for the formation of

the PCh-binding site (7–9). Once CRP is complexed with a substance with exposed PCh group, the complex activates the complement system through the classical pathway (10–12).

*Streptococcus pneumoniae* are gram positive bacteria that asymptomatically colonize the upper respiratory tract (1, 13–15). It is the most common bacterium that causes community-acquired pneumonia and is also a significant cause of septicemia and meningitis (1, 13–15). Systemic pneumococcal infection raises the level of CRP in serum by up to several hundred-fold in humans as a part of the acute phase response (16–18). CRP binds to pneumococci through $Ca^{2+}$-dependent interaction with PCh residues present on the pneumococcal cell wall C-polysaccharide (PnC) (19, 20). In mice, however, CRP is only a minor acute phase protein; therefore, mice have been useful in investigating the functions of human CRP *in vivo* (21).

In murine models of pneumococcal infection, passively administered human CRP has been shown to be protective against lethal pneumococcal infection, that is, CRP decreases bacteremia and enhances survival of infected mice (1, 22–26). CRP-deficient mice are more susceptible to pneumococcal infection than are wild type mice, which indicates that the trace level of endogenous mouse CRP is capable of exerting anti-pneumococcal functions (27). Mice transgenic for human CRP are also protected against infection with *S. pneumoniae* (28). The mechanism of anti-pneumococcal action of CRP in mice, however, is unknown.

Current research on defining the mechanism of anti-pneumococcal actions of CRP benefited from a key finding made several decades ago using passive administration of purified human CRP into mice (29). CRP was protective when injected into mice 6 h before to 2 h after the administration of pneumococci. CRP was not protective when mice received CRP 24 h after infection, suggesting that CRP is protective during early stage infection but not in late stage infection. For early stage protection, it is believed that the mechanism of action of CRP involves the capability of CRP to bind to pneumococci through PCh groups present on their surfaces and subsequent activation of the classical complement pathway by pathogen-bound CRP. Obviously, this mechanism does not operate for late stage infection. A PCh-independent mechanism for anti-pneumococcal function of CRP has been proposed along with an explanation for the inability of CRP to be protective against late stage infection (1, 24–26). In this article, we review PCh-dependent, PCh-independent, and other proposed mechanisms for the anti-pneumococcal function of CRP during both early stage infection (when CRP and pneumococci are administered into mice 30 min apart) and late stage infection (when CRP and pneumococci are administered into mice 24 h apart).

## PCh-DEPENDENT ANTI-PNEUMOCOCCAL FUNCTION OF CRP

*In vivo* experiments employing a CRP mutant incapable of binding to PCh, PnC, and whole pneumococci provided results indicating that CRP-mediated protection of mice against infection is independent of binding of CRP to PCh; the CRP mutant was as effective as wild-type CRP in protecting mice against early stage infection (26). The PCh-binding mechanism, however, does contribute to the protection of mice during the early stage of infection (25, 26). The PCh-dependent mechanism contributes to the initial and immediate clearance of pneumococci as has been shown employing a variety of murine models of infection (26, 27). Overall, the combined data suggest that both PCh-dependent and PCh-independent mechanisms operate in the protection of mice against early stages of infection, although the PCh-dependent mechanism is not necessary (25, 26).

Indirect evidence has been presented to show the importance of the PCh-binding property of CRP and subsequent complement activation by CRP-complexes in protection from infection. It has been shown that CRP binds to gram negative bacterial lipopolysaccharide (LPS) if the LPS is modified by adding a few PCh residues to it. The binding of CRP to PCh-modified LPS increases based on the number of PCh residues added and subsequently affects the resistance of the organism to the killing effects of serum (30). Also, the pneumococcal surface protein PspA, which is a choline-binding protein, is known to bind to PCh. PspA thus competes and inhibits the binding of CRP to PCh on pneumococci and decreases complement activation (31). Similarly, pneumococci growing as a biofilm are avirulent due to a decrease in PnC production although with an increase in PCh expression, interference from pneumococcal surface protein PspC, reduced binding of C1q to CRP-PCh complexes, and subsequent failure to activate complement (32, 33). Biofilm formation in *S. pneumoniae* is an effective means of evading complement attack (33).

One study suggested that the property of CRP to activate the classical pathway of complement in human serum is irrelevant for the protective function of CRP in mice infected with *S. pneumoniae*, because human CRP does not activate murine complement via the classical pathway (23). Since complement-deficient mice do not show CRP-mediated protection to pneumococcal infection (34), it is possible that CRP-complexes are able to activate murine complement system via a pathway other than the classical pathway (1, 23). It has been proposed that human CRP-complexes are able to activate the lectin pathway in murine serum and are able to activate both the classical and lectin pathways in human serum (23). CRP has been shown to interact with both L-ficolin and M-ficolin and activate the lectin pathway of complement (35–39). The interaction between CRP and L-ficolin increases 100-fold under the conditions of slight acidosis and reduced calcium levels, and it has also been shown that the cross-talk between CRP and L-ficolin mediates killing of *Pseudomonas aeruginosa* in plasma (37). L-ficolin also recognizes PCh on pneumococcal strains and triggers activation of the lectin complement pathway (40). Lectin-like oxidized LDL receptor, LOX-1, can also recognize CRP and is involved in CRP-dependent complement activation (41, 42). CRP is a major hemolymph protein in the horseshoe crab *Carcinoscorpius rotundicauda*. When CRP is in the hemolymph, it binds to a range of bacteria through galactose-binding protein and ficolin. Accordingly, it has been proposed that CRP does not act

**Abbreviations:** CRP, C-reactive protein; FHR, factor H-related protein; LPS, lipopolysaccharide; mCRP, monomeric CRP; PCh, phosphocholine; PnC, pneumococcal C-polysaccharide.

alone but collaborates with other plasma lectins to form stable pathogen recognition complexes when targeting a wide range of bacteria for destruction (35).

## PCh-INDEPENDENT ANTI-PNEUMOCOCCAL FUNCTION OF CRP

Factor H, a regulator of complement activation, has been implicated in resistance of pneumococci to complement attack (43, 44). Factor H protects from complement attack by inhibiting the activation of the alternative pathway on host cells and on those pathogenic surfaces which are capable of recruiting factor H from the plasma. On the host cells, factor H binds to polyanionic structures and glycoproteins found on the cell surface (45). On *S. pneumoniae*, factor H binds to a surface protein called Hic (factor H-binding inhibitor of complement) which is a variant of PspC (46, 47). Thus, pneumococci use factor H to evade complement-mediated killing. The recruitment of factor H by pneumococci might be the reason why CRP does not protect mice from pneumococcal infections during late stage infection.

CRP does not bind to factor H under normal physiological conditions (48–52). Denaturation conditions for CRP enable CRP to bind to factor H (4, 48–51). For example, immobilization of CRP on to a surface enables CRP to bind to factor H (4, 53, 54). Monomeric CRP (mCRP) also binds to factor H, in a $Ca^{2+}$-independent manner (55). The Y384H polymorphism of factor H affects binding affinity for mCRP. CRP binds to factor H-$Tyr^{384}$ more strongly compared to factor H-$His^{384}$ which is the risk allele (56–60). PCh does not compete with factor H for binding to CRP (52). It has been suggested that when CRP immobilizes itself on *S. pneumoniae*, it limits excessive complement activation by recruiting factor H (61, 62). CRP has also been shown to modulate lectin pathway-dependent cytolysis by recruiting factor H (63, 64). When CRP binds to dead cells it does not recruit factor H (55). mCRP also binds to factor H-related proteins (FHR) FHR1 and FHR5 and to factor H like protein 1 (FHL-1) which inhibit subsequent recruitment of factor H (65–68). CRP has also been shown to recruit factor H on other cell types, for example, CRP recruits factor H after binding to collectin CL-P1 on the surface of placental cells (69, 70). Otherwise, the interaction of CRP with CL-P1 activates the classical complement pathway. The interaction of CL-P1 with factor H might be the key to prevent self-attack due to complement activation induced by the CL-P1 and CRP interaction (69, 70).

Based on results obtained from the experiments performed under defined conditions—native pentameric CRP does not bind to factor H while mCRP binds to factor H—it was hypothesized that a non-native pentameric CRP may also be able to bind to factor H (48). Indeed, the native pentameric structure of CRP could be modified *in vitro* to generate non-native pentameric CRP capable of binding to factor H (2, 48–50). Since non-native CRP and Hic can bind to factor H simultaneously, it is possible that non-native CRP can bind to factor H-coated pneumococci, cover the factor H-Hic complex formed on bacteria and therefore eliminate the repressive effect of factor H on complement activation (71–73). Recently, a CRP mutant capable of binding to immobilized factor H was evaluated for its ability to protect against late stage pneumococcal infection. The CRP mutant protected mice against infection regardless of the time of administration into mice (71–73). These data lead to the proposal that the PCh-independent mechanism first involves a structural change in CRP which is then followed by the interaction between structurally altered CRP and factor H-bound pneumococci. Once factor H on pneumococci is bound to structurally altered CRP, such pneumococci may not be resistant to complement attack any longer (1, 71–73).

Besides, factor H, *S. pneumoniae* have also been shown to recruit another complement inhibitor, C4b-binding protein (C4BP) via Hic that also recruits factor H (74, 75). Pneumococci also use another cell surface protein, enolase, to recruit C4BP (75). By recruiting C4BP, pneumococci are able to evade complement attack. We hypothesize that non-native CRP may also be protective against those pathogens which recruit C4BP for complement evasion: non-native CRP could bind to factor H/C4BP-coated pneumococci, and then the complex formed by CRP, factor H/C4BP, and Hic could activate the lectin pathway of complement and trigger killing of the pneumococci. The possibility cannot be ruled out that the PCh-independent mechanism may involve the binding of non-native CRP to pneumococcal surface proteins, as CRP has been shown to interact with several choline-binding proteins found on pneumococci in a $Ca^{2+}$-independent manner (76).

## CRP AS AN ANTI-BACTERIAL MOLECULE

CRP binds to several pathogenic serotypes of *S. pneumoniae* (77–79) and binds more avidly to those strains which contain PCh in both cell wall and capsular polysaccharides, such as type 27 (80). CRP, like lectins, also reacts with polysaccharides that do not contain PCh, such as depyruvylated type-IV capsular polysaccharide prepared from type 27, in the presence of calcium, and probably the reaction is due to N-acetylgalactosamine in the polysaccharide (81–84). CRP appears to have opsonin properties; it causes agglutination and lysis of gram positive bacteria *Staphylococcus aureus, Bacillus subtilis*, *Streptococcus pyogenes,* and *Streptococcus agalactiae* (77, 78).

The anti-bacterial action of CRP is not limited to gram positive bacteria only. CRP also protects mice from the early stages of infection with *Salmonella enterica* serovar Typhimurium, which is a gram negative bacterium and to which CRP does not bind *in vitro* (85). But CRP has been shown to bind to *S. enterica* in the presence of serum (35). CRP also binds to *Haemophilus influenzae* (86). *H. influenzae* undergoes phase variation in expression of the PCh on the cell surface-exposed outer core of the LPS. PCh-positive variants are more sensitive to the bactericidal activity of human serum which requires the binding of serum CRP to whole bacteria with subsequent activation of complement (86–88). The ability of *H. influenzae* to vary PCh expression to zero may relate to its ability to cause invasive infection by evading attack by CRP (86). Mouse

models of *H. influenzae* infection have not been established yet to determine whether CRP protects against infection with *H. influenzae* (27). CRP also binds to *Neisseriae spp*. in a $Ca^{2+}$-dependent manner (89–91). PCh is present on the LPS of several species of commensal *Neisseriae* and, like *H. influenzae*, *Neisseriae* also undergo phase variation in expression of the PCh on their LPS (91). Mouse protection experiments have not been performed for *Neisseriae* either, employing native or non-native pentameric CRP.

Some experiments suggest a role of CRP in protecting animals against lethal toxicity of LPS, although the subject has been controversial (92–96). In the hemolymph of horseshoe crab, *Carcinoscorpius rotundicauda*, CRP was identified as the major LPS-binding protein in infections with *Pseudomonas aeruginosa* (97). CRP bound to all bacteria tested in the horseshoe crab hemolymph (35). The binding of CRP to LPS is indirect; a third molecule called galactose-binding protein (GBP) participates in bridging CRP and LPS (98). Upon binding to LPS, GBP interacts with CRP to form a pathogen-recognition complex, which helps to eliminate invading microbes (35, 98). Combined data raise the possibility that CRP functions as a general anti-bacterial molecule; CRP may require a change in its pentameric conformation and also seek help from other serum proteins to form pathogen-recognition complexes.

## CRP AS AN ANTI-INFLAMMATORY MOLECULE

Native pentameric CRP can dissociate into mCRP via an intermediate non-native pentameric structure (50, 99–101). All three forms, native pentameric, non-native pentameric, and mCRP display different ligand recognition functions *in vitro* (2, 102–104). Under conditions of low pH, reduced calcium levels and oxidation-reduction, CRP is converted to a non-native conformation but remains pentameric (48–50, 105–107). When non-native CRP binds to a non-PCh ligand, it denatures further to mCRP. Similarly, when CRP binds to cell membranes, liposomes, and cell-derived microvesicles, it undergoes a structural change which involves spatial separation of the monomers from each other without disrupting the pentameric symmetry to form a transitional state CRP (108). The mechanism by which CRP recognizes membrane lipids and binds in a $Ca^{2+}$-independent manner depends on the combination of protein form, lipid composition, and membrane shape (109, 110). Surface-immobilization of CRP generates a preservable intermediate with dual antigenicity expression of both CRP and mCRP. The intermediate exhibits modified bioactivities, such as a high affinity with solution-phase proteins (107). It has been shown that mCRP but not CRP is the major isoform present in local inflammatory lesions (111). Since mCRP is insoluble, it is considered a tissue-bound form of CRP. Thus, an intermediate stage of CRP structure seems to be responsible for anti-inflammatory host defense functions of CRP *in vivo*. Structural changes *in vivo* may be converting CRP into an anti-inflammatory molecule assuming that the ultimate pro-inflammatory by-product, mCRP, is continuously being removed. An intrinsically disordered region of amino acid residues 35–47 in CRP is responsible for mediating the interactions of mCRP with diverse ligands (112), and possibly also responsible for mediating the interactions of non-native pentameric CRP with diverse ligands (48–50).

## CONCLUSIONS

While native CRP is protective only against early stage infection, non-native pentameric CRP is protective against both early stage and late stage infections in murine models of pneumococcal infection. Because non-native pentameric CRP displays PCh-independent anti-pneumococcal activity, it is quite possible that CRP functions as a general anti-bacterial molecule. Thus, pentameric CRP is an anti-inflammatory molecule.

A long-term goal could be to focus on the discovery and design of small-molecule compounds to target CRP, a compound that can change the structure of endogenous CRP so that the structurally altered CRP is capable of binding to factor H-bound pneumococci. A recent study showed that injections of sub-inhibitory concentrations of antibiotics enhanced the binding of CRP to three antibiotic-resistant *S. pneumoniae* strains in serum and enhanced antibody-dependent complement activation (113). Based on these findings, another goal could be to investigate the effects of combinations of non-native pentameric CRP with various antibiotics in pre-clinical studies.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

This work was supported by National Institutes of Health grant AI117730 to AA.

## ACKNOWLEDGMENTS

We are grateful to Irving Kushner, M.D., for reviewing the manuscript and for his helpful suggestions.

## REFERENCES

1. Agrawal A, Suresh MV, Singh SK, Ferguson DA Jr. The protective function of human C-reactive protein in mouse models of *Streptococcus pneumoniae* infection. *Endocr Metab Immune Disord Drug Targets* (2008) 8:231–7. doi: 10.2174/187153008786848321
2. Agrawal A, Gang TB, Rusiñol AE. Recognition functions of pentameric C-reactive protein in cardiovascular disease. *Mediators Inflamm.* (2014) 2014:1–6. doi: 10.1155/2014/319215

3. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
4. Vilahur G, Badimon L. Biological actions of pentraxins. *Vasc Pharmacol.* (2015) 73:38–44. doi: 10.1016/j.vph.2015.05.001
5. Shrive AK, Cheetham GM, Holden D, Myles DA, Turnell WG, Volanakis JE, et al. Three dimensional structure of human C-reactive protein. *Nat Struct Biol.* (1996) 3:346–54. doi: 10.1038/nsb0496-346
6. Lv JM, Lü SQ, Liu ZP, Zhang J, Gao BX, Yao ZY, et al. Conformational folding and disulfide bonding drive distinct stages of protein structure formation. *Sci Rep.* (2018) 8:1494. doi: 10.1038/s41598-018-20014-y
7. Agrawal A, Lee S, Carson M, Narayana SVL, Greenhough TJ, Volanakis JE. Site-directed mutagenesis of the phosphocholine-binding site of human C-reactive protein: Role of $Thr^{76}$ and $Trp^{67}$. *J Immunol.* (1997) 158:345–50.
8. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* (1999) 7:169–77. doi: 10.1016/S0969-2126(99)80023-9
9. Agrawal A, Simpson MJ, Black S, Carey MP, Samols D. A C-reactive protein mutant that does not bind to phosphocholine and pneumococcal C-polysaccharide. *J Immunol.* (2002) 169:3217–22. doi: 10.4049/jimmunol.169.6.3217
10. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin, and sphingomyelin. *J Immunol.* (1974) 112:2135–47.
11. Agrawal A, Volanakis JE. Probing the C1q-binding site on human C-reactive protein by site-directed mutagenesis. *J Immunol.* (1994) 152:5404–10.
12. Agrawal A, Shrive AK, Greenhough TJ, Volanakis JE. Topology and structure of the C1q-binding site on C-reactive protein. *J Immunol* (2001) 166:3998–400. doi: 10.4049/jimmunol.166.6.3998
13. Tuomanen EI, Austrian R, Masure HR. Pathogenesis of pneumococcal infection. *N Engl J Med.* (1995) 332:1280–4. doi: 10.1056/NEJM199505113321907
14. Van der Poll T, Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet* (2009) 374:1543–56. doi: 10.1016/S0140-6736(09)61114-4
15. Malley R, Anderson PW. Serotype-independent pneumococcal experimental vaccines that induce cellular as well as humoral immunity. *Proc Natl Acad Sci USA.* (2012) 109:3623–27. doi: 10.1073/pnas.1121383109
16. Tillett WS, Francis T Jr. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *J Exp Med.* (1930) 52:561–71. doi: 10.1084/jem.52.4.561
17. Kushner I. The phenomenon of the acute phase response. *Ann NY Acad Sci USA.* (1982) 389:39–48. doi: 10.1111/j.1749-6632.1982.tb2 2124.x
18. Song JY, Eun BW, Nahm MH. Diagnosis of pneumococcal pneumonia: current pitfalls and the way forward. *Infect Chemother.* (2013) 45:351–66. doi: 10.3947/ic.2013.45.4.351
19. Abernethy TJ, Avery OT. The occurrence during acute infections of a protein not normally present in the blood: I. Distribution of the reactive protein in patients' sera and the effect of calcium on the flocculation reaction with C polysaccharide of pneumococcus. *J Exp Med.* (1941) 73:173–82.
20. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med.* (1971) 136:612–4. doi: 10.3181/00379727-136-35323
21. Whitehead AS, Zahedi K, Rits M, Mortensen RF, Lelias JM. Mouse C-reactive protein: generation of cDNA clones, structural analysis, and induction of mRNA during inflammation. *Biochem J.* (1990) 266:283–90. doi: 10.1042/bj2660283
22. Yother J, Volanakis JE, Briles DE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in mice. *J Immunol.* (1982) 128:2374–6.
23. Suresh MV, Singh SK, Ferguson DA Jr, Agrawal A. Role of the property of C-reactive protein to activate the classical pathway of complement in protecting mice from pneumococcal infection. *J Immunol.* (2006) 176:4369–74. doi: 10.4049/jimmunol.176.7.4369
24. Suresh MV, Singh SK, Ferguson DA Jr, Agrawal A. Human C-reactive protein protects mice from *Streptococcus pneumoniae* infection without binding to pneumococcal C-polysaccharide. *J Immunol.* (2007) 178:1158–63. doi: 10.4049/jimmunol.178.2.1158
25. Gang TB, Hammond DJ Jr, Singh SK, Ferguson DA Jr, Mishra VK, Agrawal A. The phosphocholine-binding pocket on C-reactive protein is necessary for initial protection of mice against pneumococcal infection. *J Biol Chem.* (2012) 287:43116–25. doi: 10.1074/jbc.M112.427310
26. Gang TB, Hanley GA, Agrawal A. C-reactive protein protects mice against pneumococcal infection via both phosphocholine-dependent and phosphocholine-independent mechanisms. *Infect Immun.* (2015) 83:1845–52. doi: 10.1128/IAI.03058-14
27. Simons JP, Loeffler JM, Al-Shawi R, Ellmerich S, Hutchinson WL, Tennent GA, et al. C-reactive protein is essential for innate resistance to pneumococcal infection. *Immunology* (2014) 142:414–20. doi: 10.1111/imm.12266
28. Szalai AJ, Briles DE, Volanakis JE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in transgenic mice. *J Immunol.* (1995) 155:2557–63.
29. Nakayama S, Gewurz H, Holzer T, Du Clos TW, Mold C. The role of the spleen in the protective effect of C-reactive protein in *Streptococcus pneumoniae* infection. *Clin Exp Immunol.* (1983) 54:319–26.
30. Fox KL, Li J, Schweda EKH, Vitiazeva V, Makepeace K, Jennings MP, et al. Duplicate copies of lic1 direct the addition of multiple phosphocholine residues in the lipopolysaccharide of *Haemophilus influenzae*. *Infect Immun.* (2008) 76:588–600. doi: 10.1128/IAI.00748-07
31. Mukerji R, Mirza S, Roche AM, Widener RW, Croney CM, Rhee D-K, et al. Pneumococcal surface protein A inhibits complement deposition on the pneumococcal surface by competing with the binding of C-reactive protein to cell-surface phosphocholine. *J Immunol.* (2012) 189:5327–35. doi: 10.4049/jimmunol.1201967
32. Sanchez CJ, Kumar N, Lizcano A, Shivshankar P, Dunning Hotopp JC, Jorgensen JH, et al. *Streptococcus pneumoniae* in biofilms are unable to cause invasive disease due to altered virulence determinant production. *PLoS ONE* (2011) 6:e28738. doi: 10.1371/journal.pone.0028738
33. Domenech M, Ramos-Sevillano E, García E, Moscoso M, Yuste J. Biofilm formation avoids complement immunity and phagocytosis of *Streptococcus pneumoniae*. *Infect Immun.* (2013) 81:2606–15. doi: 10.1128/IAI.00491-13
34. Mold C, Rodic-Polic B, Du Clos TW. Protection from *Streptococcus pneumoniae* infection by C-reactive protein and natural antibody requires complement but not Fc gamma receptors. *J Immunol.* (2002) 168:6375–81. doi: 10.4049/jimmunol.168.12.6375
35. Ng PML, Le Saux A, Lee CM, Tan NS, Lu J, Thiel S, et al. C-reactive protein collaborates with plasma lectins to boost immune response against bacteria. *EMBO J.* (2007) 26:3431–40. doi: 10.1038/sj.emboj.7601762
36. Tanio M, Wakamatsu K, Kohno T. Binding site of C-reactive protein on M-ficolin. *Mol Immunol.* (2009) 47:215–21. doi: 10.1016/j.molimm.2009.09.032
37. Zhang J, Koh J, Lu J, Thiel S, Leong BSH, Sethi S, et al. Local inflammation induces complement crosstalk which amplifies the antimicrobial response. *PLoS Pathog.* (2009) 5:e1000282. doi: 10.1371/journal.ppat.1000282
38. Zhang J, Yang L, Ang Z, Yoong SL, Tran TTT, Anand GS, et al. Secreted M-ficolin anchors onto monocyte transmembrane G protein-coupled receptor 43 and cross talks with plasma C-reactive protein to mediate immune signaling and regulate host defense. *J Immunol.* (2010) 185:6899–910. doi: 10.4049/jimmunol.1001225
39. Ma YJ, Lee BL, Garred P. An overview of the synergy and crosstalk between pentraxins and collectins/ficolins: their functional relevance in complement activation. *Exp Mol Med.* (2017) 49:e320. doi: 10.1038/emm.2017.51
40. Vassal-Stermann E, Lacroix M, Gout E, Laffly E, Pedersen CM, Martin L, et al. Human L-ficolin recognizes phosphocholine moieties of pneumococcal teichoic acid. *J Immunol.* (2014) 193:5699–708. doi: 10.4049/jimmunol.1400127
41. Yoshimoto R, Fujita Y, Kakino A, Iwamoto S, Takaya T, Sawamura T. The discovery of LOX-1, its ligands and clinical significance. *Cardiovasc Drugs Ther.* (2011) 25:379–91. doi: 10.1007/s10557-011-6324-6
42. Fujita Y, Yamaguchi S, Kakino A, Iwamoto S, Yoshimoto R, Sawamura T. Lectin-like oxidized LDL receptor 1 is involved in CRP-mediated complement activation. *Clin Chem.* (2011) 57:1398–405. doi: 10.1373/clinchem.2011.168625
43. Zipfel PF, Hallström T, Hammerschmidt S, Skerka C. The complement fitness factor H: role in human diseases and for immune escape of pathogens, like pneumococci. *Vaccine* (2008) 26:I67–74. doi: 10.1016/j.vaccine.2008.11.015

44. Kopp A, Hebecker M, Svobodová E, Józsi M. Factor H: a complement regulator in health and disease, and a mediator of cellular interactions. *Biomolecules* (2012) 2:46–75. doi: 10.3390/biom2010046
45. Meri S, Pangburn MK. Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. *Proc Natl Acad Sci USA*. (1990) 87:3982–6. doi: 10.1073/pnas.87.10.3982
46. Janulczyk R, Iannelli F, Sjoholm AG, Pozzi G, Bjorck L. Hic, a novel surface protein of *Streptococcus pneumoniae* that interferes with complement function. *J Biol Chem.* (2000) 275:37257–63. doi: 10.1074/jbc.M004572200
47. Jarva H, Janulczyk R, Hellwage J, Zipfel PF, Björck L, Meri S. *Streptococcus pneumoniae* evades complement attack and opsonophagocytosis by expressing the *pspC* locus-encoded Hic protein that binds to short consensus repeats 8-11 of factor H. *J Immunol.* (2002) 168:1886–94. doi: 10.4049/jimmunol.168.4.1886
48. Hammond DJ Jr, Singh SK, Thompson JA, Beeler BW, Rusiñol AE, Pangburn MK, et al. Identification of acidic pH-dependent ligands of pentameric C-reactive protein. *J Biol Chem.* (2010) 285:36235–44. doi: 10.1074/jbc.M110.142026
49. Singh SK, Thirumalai A, Hammond DJ Jr, Pangburn MK, Mishra VK, Johnson DA, et al. Exposing a hidden functional site of C-reactive protein by site-directed mutagenesis. *J Biol Chem.* (2012) 287:3550–8. doi: 10.1074/jbc.M111.310011
50. Singh SK, Thirumalai A, Pathak A, Ngwa DN, Agrawal A. Functional transformation of C-reactive protein by hydrogen peroxide. *J Biol Chem.* (2017) 292:3129–36. doi: 10.1074/jbc.M116.773176
51. Hakobyan S, Harris CL, van den Berg CW, Fernandez-Alonso MC, de Jorge EG, de Cordoba SR, et al. Complement factor H binds to denatured rather than to native pentameric C-reactive protein. *J Biol Chem.* (2008) 283:30451–60. doi: 10.1074/jbc.M803648200
52. Okemefuna AI, Nan R, Miller A, Gor J, Perkins SJ. Complement factor H binds at two independent sites to C-reactive protein in acute phase concentrations. *J Biol Chem.* (2010) 285:1053–65. doi: 10.1074/jbc.M109.044529
53. Jarva H, Jokiranta TS, Hellwage J, Zipfel PF, Meri S. Regulation of complement activation by C-reactive protein: targeting the complement inhibitory activity of factor H by an interaction with short consensus repeat domains 7 and 8-11. *J Immunol* (1999) 163:3957–62.
54. Sjöberg AP, Trouw LA, Clark SJ, Sjölander J, Heinegård D, Sim RB, et al. The factor H variant associated with age-related macular degeneration (His-384) and the non-disease-associated form bind differentially to C-reactive protein, fibromodulin, DNA, and necrotic cells. *J Biol Chem.* (2007) 282:10894–900. doi: 10.1074/jbc.M610256200
55. Mihlan M, Stippa S, Józsi M, Zipfel PF. Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. *Cell Death Differ.* (2009) 16:1630–40. doi: 10.1038/cdd.2009.103
56. Clark SJ, Higman VA, Mulloy B, Perkins SJ, Lea SM, Sim RB, et al. His-384 allotypic variant of factor H associated with age-related macular degeneration has different heparin binding properties from the non-disease-associated form. *J Biol Chem.* (2006) 281:24713–20. doi: 10.1074/jbc.M605083200
57. Laine M, Jarva H, Seitsonen S, Haapasalo K, Lehtinen MJ, Lindeman N, et al. Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein. *J Immunol.* (2007) 178:3831–6. doi: 10.4049/jimmunol.178.6.3831
58. Herbert AP, Deakin JA, Schmidt CQ, Blaum BS, Egan C, Ferreira VP, et al. Structure shows that a glycosaminoglycan and protein recognition site in factor H is perturbed by age-related macular degeneration-linked single nucleotide polymorphism. *J Biol Chem.* (2007) 282:18960–8. doi: 10.1074/jbc.M609636200
59. Lauer N, Mihlan M, Hartmann A, Schlötzer-Schrehardt U, Keilhauer C, Scholl HPN, et al. Complement regulation at necrotic cell lesions is impaired by the age-related macular degeneration-associated factor-H $His^{402}$ risk variant. *J Immunol.* (2011) 187:4374–83. doi: 10.4049/jimmunol.1002488
60. Molins B, Fuentes-Prior P, Adán A, Antón R, Arostegui JI, Yagüe J, et al. Complement factor H binding of monomeric C-reactive protein downregulates proinflammatory activity and is impaired with at risk polymorphic CFH variants. *Sci Rep.* (2016) 6:22889. doi: 10.1038/srep22889
61. Mold C, Gewurz H. Inhibitory effect of C-reactive protein on alternative C pathway activation by liposomes and *Streptococcus pneumoniae*. *J Immunol.* (1981) 127:2089–92.
62. Mold C, Kingzette M, Gewurz H. C-reactive protein inhibits pneumococcal activation of the alternative pathway by increasing the interaction between factor H and C3b. *J Immunol.* (1984) 133:882–5.
63. Suankratay C, Mold C, Zhang Y, Potempa LA, Lint TF, Gewurz H. Complement regulation in innate immunity and the acute phase response: inhibition of mannan-binding lectin-initiated complement cytolysis by C-reactive protein (CRP). *Clin Exp Immunol.* (1998) 113:353–9. doi: 10.1046/j.1365-2249.1998.00663.x
64. Zhang Y, Suankratay C, Zhang XH, Lint TF, Gewurz H. Lysis via the lectin pathway of complement activation: minireview and lectin pathway enhancement of endotoxin-initiated hemolysis. *Immunopharmacology* (1999) 42:81–90. doi: 10.1016/S0162-3109(99)00029-6
65. Hebecker M, Okemefuna AI, Perkins SJ, Mihlan M, Huber-Lang M, Józsi M. Molecular basis of C-reactive protein binding and modulation of complement activation by factor H-related protein 4. *Mol Immunol.* (2010) 47:1347–55. doi: 10.1016/j.molimm.2009.12.005
66. Csincsi ÁI, Kopp A, Zöldi M, Bánlaki Z, Uzonyi B, Hebecker M, et al. Factor H–related protein 5 interacts with pentraxin 3 and the extracellular matrix and modulates complement activation. *J Immunol* (2015) 194:4963–73. doi: 10.4049/jimmunol.1403121
67. Csincsi ÁI, Szabó Z, Bánlaki Z, Uzonyi B, Cserhalmi M, Kárpáti É, et al. FHR-1 binds to C-reactive protein and enhances rather than inhibits complement activation. *J Immunol.* (2017) 199:292–303. doi: 10.4049/jimmunol.1600483
68. Swinkels M, Zhang JH, Tilakaratna V, Black G, Perveen R, McHarg S, et al. C-reactive protein and pentraxin-3 binding of factor H-like protein 1 differs from complement factor H: implications for retinal inflammation. *Sci Rep.* (2018) 8:1643. doi: 10.1038/s41598-017-18395-7
69. Roy N, Ohtani K, Matsuda Y, Mori K, Hwang I, Suzuki Y, et al. Collectin CL-P1 utilizes C-reactive protein for complement activation. *Biochim Biophys Acta* (2016) 1860:1118–28. doi: 10.1016/j.bbagen.2016.02.012
70. Roy N, Ohtani K, Hidaka Y, Amano Y, Matsuda Y, Mori K, et al. Three pentraxins C-reactive protein, serum amyloid P component and pentraxin 3 mediate complement activation using collectin CL-P1. *Biochim Biophys Acta* (2017) 1861:1–14. doi: 10.1016/j.bbagen.2016.11.023
71. Ngwa DN. *Comparison of Anti-pneumococcal Functions of Native and Modified Forms of C-Reactive Protein*. Dissertation/Master's thesis. Johnson City, TN: East Tennessee States University (2016).
72. Ngwa DN, Gang TB, Singh SK, Agrawal A. Engineered C-reactive protein with improved protective action against pneumococcal infection. *J Immunol.* (2016) 196:63.
73. Ngwa DN, Singh SK, Gang TB, Agrawal A. A C-reactive protein-based strategy to reduce antibiotic dosing. *J Immunol.* (2018) 200:170.
74. Sjöberg AP, Trouw LA, McGrath FDG, Hack CE, Blom AM. Regulation of complement activation by C-reactive protein: targeting of the inhibitory activity of C4b-binding protein. *J Immunol.* (2006) 176:7612–20. doi: 10.4049/jimmunol.176.12.7612
75. Agarwal V, Hammerschmidt S, Malm S, Bergmann S, Riesbeck K, Blom AM. Enolase of *Streptococcus pneumoniae* binds human complement inhibitor C4b-binding protein and contributes to complement evasion. *J Immunol.* (2012) 189:3575–84. doi: 10.4049/jimmunol.1102934
76. Frolet C, Beniazza M, Roux L, Gallet B, Noirclerc-Savoye M, Vernet T, et al. New adhesin functions of surface-exposed pneumococcal proteins. *BMC Microbiol.* (2010) 10:190. doi: 10.1186/1471-2180-10-190
77. Kindmark CO. *In vitro* binding of human C-reactive protein by some pathogenic bacteria and zymosan. *Clin Exp Immunol.* (1972) 11:283–9.
78. Mold C, Rodgers CP, Kaplan RL, Gewurz H. Binding of human C-reactive protein to bacteria. *Infect Immun.* (1982) 38:392–5.
79. De Beaufort AJ, Langermans JAM, Matze-van der Lans AM, Hiemstra PS, Vossen JM, van Furth R. Difference in binding of killed and live *Streptococcus pneumoniae* serotypes by C-reactive protein. *Scand J Immunol.* (1997) 46:597–600. doi: 10.1046/j.1365-3083.1997.d01-171.x

80. Edwards KM, Gewurz H, Lint TF Mold C. A role for C-reactive protein in the complement-mediated stimulation of human neutrophils by type 27 Streptococcus pneumoniae. *J Immunol.* (1982) 128:2493–6.
81. Higginbotham J, Heidelberger M, Gotschlich E. Degradation of a pneumococcal type-specific polysaccharide with exposure of group specificity. *Proc Natl Acad Sci USA*. (1970) 67:138–42. doi: 10.1073/pnas.67.1.138
82. Heidelberger M, Gotschlich EC, Higginbotham JD. Inhibition experiments with pneumococcal C and depyruvylated type-IV polysaccharides. *Carbohydr Res.* (1972) 22:1–4. doi: 10.1016/S0008-6215(00)85719-5
83. Köttgen E, Hell B, Kage A, Tauber R. Lectin specificity and binding characteristics of human C-reactive protein. *J Immunol.* (1992) 149:445–53.
84. Lee RT, Lee YC. Carbohydrate ligands of human C-reactive protein: binding of neoglycoproteins containing galactose-6-phosphate and galactose-terminated disaccharide. *Glycoconj J.* (2006) 23:317–27. doi: 10.1007/s10719-006-6173-x
85. Szalai AJ, VanCott JL, McGhee JR, Volanakis JE, Benjamin WH Jr. Human C-reactive protein is protective against fatal *Salmonella enterica* serovar typhimurium infection in transgenic mice. *Infect Immun.* (2000) 68:5652–6. doi: 10.1128/IAI.68.10.5652-5656.2000
86. Weiser JN, Pan N, McGowan KL, Musher D, Martin A, Richards J. Phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* contributes to persistence in the respiratory tract and sensitivity to serum killing mediated by C-reactive protein. *J Exp Med.* (1998) 187:631–40. doi: 10.1084/jem.187.4.631
87. Lysenko E, Richards JC, Cox AD, Stewart A, Martin A, Kapoor M, et al. The position of phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* affects binding and sensitivity to C-reactive protein-mediated killing. *Mol Microbiol.* (2000) 35:234–45. doi: 10.1046/j.1365-2958.2000.01707.x
88. Weiser JN, Pan N. Adaptation of *Haemophilus influenzae* to acquired and innate humoral immunity based on phase variation of lipopolysaccharide. *Mol Microbiol.* (1998) 30:767–75. doi: 10.1046/j.1365-2958.1998.01108.x
89. Serino L, Virji M. Genetic and functional analysis of the phosphorylcholine moiety of commensal *Neisseria* lipopolysaccharide. *Mol Microbiol.* (2002) 43:437–48. doi: 10.1046/j.1365-2958.2002.02755.x
90. Casey R, Newcombe J, McFadden J, Bodman-Smith KB. The acute-phase reactant C-reactive protein binds to phosphorylcholine-expressing *Neisseria meningitidis* and increases uptake by human phagocytes. *Infect Immun.* (2008) 76:1298–304. doi: 10.1128/IAI.00741-07
91. Serino L, Virji M. Phosphorylcholine decoration of lipopolysaccharide differentiates commensal *Neisseriae* from pathogenic strains: identification of licA-type genes in commensal *Neisseriae*. *Mol Microbiol.* (2000) 35:1550–9. doi: 10.1046/j.1365-2958.2000.01825.x
92. Xia D, Samols D. Transgenic mice expressing rabbit C-reactive protein are resistant to endotoxemia. *Proc Natl Acad Sci USA*. (1997) 94:2575–80. doi: 10.1073/pnas.94.6.2575
93. Noursadeghi M, Bickerstaff MCM, Gallimore JR, Herbert J, Cohen J, Pepys MB. Role of serum amyloid P component in bacterial infection: Protection of the host or protection of the pathogen. *Proc Natl Acad Sci USA*. (2000) 97:14584–9. doi: 10.1073/pnas.97.26.14584
94. Chae MR, Park BH, Kim JS, Rho HW, Park JW, Kim HR. Protective effect of C-reactive protein against the lethality induced by *Vibrio vulnificus* lipopolysaccharide. *Microbiol Immunol.* (2000) 44:335–40. doi: 10.1111/j.1348-0421.2000.tb02503.x
95. Mold C, Rodriguez W, Rodic-Polic B, Du Clos TW. C-reactive protein mediates protection from lipopolysaccharide through interactions with FcγR. *J Immunol.* (2002) 169:7019–25. doi: 10.4049/jimmunol.169.12.7019
96. Hirschfield GM, Herbert J, Kahan MC, Pepys MB. Human C-reactive protein does not protect against acute lipopolysaccharide challenge in mice. *J Immunol.* (2003) 171:6046–51. doi: 10.4049/jimmunol.171.11.6046
97. Ng PML, Jin Z, Tan SSH, Ho B, Ding JL. C-reactive protein: a predominant LPS-binding acute phase protein responsive to *Pseudomonas* infection. *J Endotoxin Res.* (2004) 10:163–74. doi: 10.1177/09680519040100030301
98. Low DHP, Frecer V, Le Saux A, Srinivasan GA, Ho B, Chen J, et al. Molecular interfaces of the galactose-binding protein tectonin domains in host-pathogen interaction. *J Biol Chem.* (2010) 285:9898–907. doi: 10.1074/jbc.M109.059774
99. Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, et al. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: *in vivo* proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *Circulation* (2014) 130:35–50. doi: 10.1161/CIRCULATIONAHA.113.007124
100. Braig D, Nero TL, Koch HG, Kaiser B, Wang X, Thiele JR, et al. Transitional changes in the CRP structure lead to the exposure of proinflammatory binding sites. *Nat Commun.* (2017) 8:14188. doi: 10.1038/ncomms14188
101. McFadyen JD, Kiefer J, Braig D, Loseff-Silver J, Potempa LA, Eisenhardt SU, et al. Dissociation of C-reactive protein localizes and amplifies inflammation: evidence for a direct biological role of C-reactive protein and its conformational changes. *Front Immunol.* (2018) 9:1351. doi: 10.3389/fimmu.2018.01351
102. Suresh MV, Singh SK, Agrawal A. Interaction of calcium-bound C-reactive protein with fibronectin is controlled by pH: *in vivo* implications. *J Biol Chem.* (2004) 279:52552–57. doi: 10.1074/jbc.M409054200
103. Salazar J, Martínez MS, Chávez-Castillo M, Núñez V, Añez R, Torres Y, et al. C-reactive protein: an in-depth look into structure, function, and regulation. *Int Sch Res Not.* (2014) 2014:653045. doi: 10.1155/2014/653045
104. Wu Y, Potempa LA, El Kebir D, Filep JG. C-reactive protein and inflammation: conformational changes affect function. *Biol Chem.* (2015) 396:1181–97. doi: 10.1515/hsz-2015-0149
105. Wang MY, Ji SR, Bai CJ, El Kebir D, Li HY, Shi JM, et al. A redox switch in C-reactive protein modulates activation of endothelial cells. *FASEB J.* (2011) 25:3186–96. doi: 10.1096/fj.11-182741
106. Kenawy HI, Boral I, Bevington A. Complement-coagulation cross-talk: a potential mediator of the physiological activation of complement by low pH. *Front Immunol.* (2015) 6:1–10. doi: 10.3389/fimmu.2015.00215
107. Lv JM, Wang MY. *In vitro* generation and bioactivity evaluation of C-reactive protein intermediate. *PLoS ONE* (2018) 13:e0198375. doi: 10.1371/journal.pone.0198375
108. Ji SR, Wu Y, Zhu L, Potempa LA, Sheng FL, Lu W, et al. Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: mCRP(m). *FASEB J.* (2007) 21:284–94. doi: 10.1096/fj.06-6722com
109. Alnaas AA, Moon CL, Alton M, Reed SM, Knowles MK. Conformational changes in C-reactive protein affect binding to curved membranes in a lipid bilayer model of the apoptotic cell surface. *J Phys Chem B.* (2017) 121:2631–9. doi: 10.1021/acs.jpcb.6b11505
110. Goda T, Miyahara Y. Specific binding of human C-reactive protein towards supported monolayers of binary and engineered phospholipids. *Colloids Surfaces B Biointerfaces* (2018) 161:662–9. doi: 10.1016/j.colsurfb.2017.11.036
111. Shi P, Li X-X, Zhu W, Yang H, Dong C, Li X-M. Immunohistochemical staining reveals C-reactive protein existing predominantly as altered conformation forms in inflammatory lesions. *Acta Biol Hung.* (2014) 65:265–73. doi: 10.1556/ABiol.65.2014.3.3
112. Li HY, Wang J, Meng F, Jia ZK, Su Y, Bai QF, et al. An intrinsically disordered motif mediates diverse actions of monomeric C-reactive protein. *J Biol Chem.* (2016) 291:8795–804. doi: 10.1074/jbc.M115.695023
113. Ramos-Sevillano E, Rodríguez-Sosa C, Cafini F, Giménez MJ, Navarro A, Sevillano D, et al. Cefditoren and ceftriaxone enhance complement-mediated immunity in the presence of specific antibodies against antibiotic-resistant pneumococcal strains. *PLoS ONE* (2012) 7:e44135. doi: 10.1371/journal.pone.0044135


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 14 March 2019
doi: 10.3389/fimmu.2019.00461

# Interaction of C1q With Pentraxin 3 and IgM Revisited: Mutational Studies With Recombinant C1q Variants

*Isabelle Bally*[1], *Antonio Inforzato*[2,3], *Fabien Dalonneau*[1], *Matteo Stravalaci*[2,3], *Barbara Bottazzi*[2], *Christine Gaboriaud*[1] *and Nicole M. Thielens*[1]*

[1] Université Grenoble Alpes, CEA, CNRS, IBS, Grenoble, France, [2] Humanitas Clinical and Research Center, Rozzano, Italy, [3] Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy

Pentraxins and complement defense collagens are soluble recognition proteins that sense pathogens and altered-self elements, and trigger immune responses including complement activation. PTX3 has been shown to interact with the globular recognition domains (gC1q) of the C1q protein of the classical complement pathway, thereby modulating complement activity. The C1q-PTX3 interaction has been characterized previously by site-specific mutagenesis using individual gC1q domains of each of the three C1q chains. The present study is aimed at revisiting this knowledge taking advantage of full-length recombinant C1q. Four mutations targeting exposed amino acid residues in the gC1q domain of each of the C1q chains ($Lys^{A200}$Asp-$Lys^{A201}$Asp, $Arg^{B108}$Asp-$Arg^{B109}$Glu, $Tyr^{B175}$Leu, and $Lys^{C170}$Glu) were introduced in recombinant C1q and the interaction properties of the mutants were analyzed using surface plasmon resonance. All C1q mutants retained binding to C1r and C1s proteases and mannose-binding lectin-associated serine proteases, indicating that the mutations did not affect the function of the collagen-like regions of C1q. The effect of these mutations on the interaction of C1q with PTX3 and IgM, and both the PTX3- and IgM-mediated activation of the classical complement pathway were investigated. The $Lys^{A200}$Asp-$Lys^{A201}$Asp and $Lys^{C170}$Glu mutants retained partial interaction with PTX3 and IgM, however they triggered efficient complement activation. In contrast, the $Arg^{B108}$Asp-$Arg^{B109}$Glu mutation abolished C1q binding to PTX3 and IgM, and significantly decreased complement activation. The $Tyr^{B175}$Leu mutant exhibited decreased PTX3- and IgM-dependent complement activation. Therefore, we provided evidence that, in the context of the full length C1q protein, a key contribution to the interaction with both PTX3 and IgM is given by the B chain Arg residues that line the side of the gC1q heterotrimer, with a minor participation of a Lys residue located at the apex of gC1q. Furthermore, we generated recombinant forms of the human PTX3 protein bearing either D or A at position 48, a polymorphic site of clinical relevance in a number of infections, and observed that both allelic variants equally recognized C1q.

**Keywords: complement C1q, PTX3, IgM, site-directed mutagenesis, molecular interactions, complement activation**

OPEN ACCESS

***Edited by:***
*Zvi Fishelson, Tel Aviv University, Israel*

***Reviewed by:***
*Péter Gál, Institute of Enzymology (MTA), Hungary*
*Uday Kishore, Brunel University London, United Kingdom*

***Correspondence:**
*Nicole M. Thielens*
*nicole.thielens@ibs.fr*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *10 December 2018*
***Accepted:*** *20 February 2019*
***Published:*** *14 March 2019*

***Citation:***
*Bally I, Inforzato A, Dalonneau F, Stravalaci M, Bottazzi B, Gaboriaud C and Thielens NM (2019) Interaction of C1q With Pentraxin 3 and IgM Revisited: Mutational Studies With Recombinant C1q Variants. Front. Immunol. 10:461. doi: 10.3389/fimmu.2019.00461*

## INTRODUCTION

Immune defense relies on the host capacity to identify pathogenic microorganisms and trigger an efficient anti-infectious response while protecting integrity of its own tissues. Pathogen sensing is mediated by constitutive innate immune molecules that are able to identify characteristic pathogen-associated molecular patterns at the surface of microbes, but also potentially noxious elements from self, such as dying cells. Recognition of these cell surface motifs elicits effector mechanisms aimed at containing early infection while instructing appropriate adaptive immune response, and supporting safe removal of apoptotic cell/debris by phagocytes (1, 2). Pentraxins and defense collagens are evolutionarily conserved multimeric pattern recognition proteins that are part of the humoral arm of innate immunity and play a vital role in the first line of anti-microbial defense and in the maintenance of tissue homeostasis (3).

The family of soluble complement defense collagens comprises C1q, collectins including mannose-binding lectin (MBL) and the newly described collectin-10 (CL-K1) and collectin-11 (CL-L1), and the lectin-like proteins ficolins. C1q is the most complex defense collagen since it is composed of 3 homologous yet distinct polypeptide chains A, B, and C that are encoded by three different genes. Each C1q chain comprises an N-terminal collagen-like sequence and a C-terminal globular gC1q module and 18 chains assemble into six heterotrimeric (A-B-C) subunits (4). This hexameric structure exhibits the characteristic shape of a bouquet of flowers, with six collagen-like triple helices (stems), each terminating in a C-terminal globular trimeric head (**Figure 1A**). Serum C1q circulates in association with a tetramer comprising two copies of each of the homologous C1r and C1s serine proteases. The resulting complex (C1) has the capacity to recognize targets through the globular regions of C1q, which triggers activation of the proteases associated to C1q collagen-like regions and subsequent cleavage of the complement components C4 and C2 (5). The activation fragments C4b and C2a assemble at the target surface to form the C3 convertase of the classical complement pathway that cleaves C3, the central component of the complement system. The classical C3 convertase can also be assembled through activation of the lectin pathway that is initiated by complexes of complement collectins or ficolins and MBL-associated serine proteases (MASPs), which are homologous to C1r and C1s and able to cleave C4 and C2. A third complement activation pathway involves assembly of an alternative C3 convertase containing the C3b fragment and serving to amplify C3 cleavage [reviewed in (6)].

Pentraxins are multimeric proteins with protomer subunits characterized by a conserved C-terminal domain (containing the canonical pentraxin signature HxCxS/TWxS) and assembled into distinctive quaternary structures. The short pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP) are pentameric (7), whereas pentraxin 3 (PTX3), the prototypic long pentraxin, contains an additional N terminal domain and is an octamer composed of two disulfide linked tetramers (8). Pentraxins are acute-phase proteins produced in response to inflammatory stimuli that provide protection against a wide variety of pathogens and participate in the clearance of apoptotic cells (9). PTX3 has regulatory roles in inflammation, where it has been shown to inhibit leukocyte extravasation, and plays additional functions in cancer and tissue repair (10). The three pentraxins have been shown to establish a complex crosstalk with diverse components of complement, thus affecting both the recognition and effector activities of this system. In particular, PTX3 modulates the three complement pathways through interactions with defense collagens (C1q, MBL, ficolin-1, and ficolin-2) and negative regulators of the alternative and classical/lectin C3 convertases, including factor H and C4bp [reviewed in (11)].

Several studies have analyzed single nucleotide polymorphisms (SNPs) in the PTX3 gene. Amongst the 22 SNPs spanning the PTX3 gene (∼25 kb) on chromosome 3, three are associated with susceptibility to a number of infections including those mediated by *Aspergillus fumigatus* (12–15), *Mycobacterium tuberculosis* (16) and *Pseudomonas aeruginosa* (17). Two of them are located in intronic regions of the gene (rs2305619 in intron 1, and rs1840680 in intron 2, respectively), and one (missense rs3816527 in exon 2) causes a single amino-acid substitution (p.D48A) at position 48 of the preprotein sequence (i.e., in the N-terminal domain). Epidemiological studies indicate that these three SNPs and the corresponding haplotypes are associated with different plasma levels of the protein, with the D48 exonic allele being enriched in individuals with lower systemic concentrations of PTX3 (18). This information notwithstanding, it is currently unknown whether this exonic polymorphism has qualitative (i.e., functional) in addition to quantitative effects on the crosstalk between PTX3 and the complement system, with major regard to the interaction of this long pentraxin with C1q.

In this regard, binding of C1q to immobilized PTX3 has been reported to trigger complement activation whereas fluid-phase PTX3 interferes with C1q binding to complement activators such as antigen-antibody complexes, in accordance with location of the PTX3 binding site of C1q in the gC1q regions (19). Previous mutagenesis studies on recombinant forms of the gC1qA, gC1qB. and gC1qC domains fused to maltose-binding protein provided initial information on the C1q amino acid residues at the interface of complexes formed with selected ligands, including immunoglobulins (IgG, IgM) and pentraxins (CRP, PTX3) (20–23). These data highlighted the key contribution of electrostatic forces to the interaction of C1q with most of its ligands, and the central role of two residues, $Tyr^{175}$ in gC1qB and $Lys^{170}$ in gC1qC, to recognition of PTX3 (20). We have recently produced the whole human C1q molecule in a recombinant form and demonstrated its structural similarity to serum-derived C1q, as judged from biochemical analysis and electron microscopy imaging. Recombinant C1q functionality was assessed by its capacity to associate with the C1s-C1r-C1r-C1s tetramer, to recognize physiological C1q ligands including IgG and PTX3, and to trigger complement activation (24). Using site-directed mutagenesis, we have also identified two homologous lysine

**Abbreviations:** gC1q, globular domain of C1q; MAA, *Maackia amurensis* agglutinin; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; SPR, surface plasmon resonance.

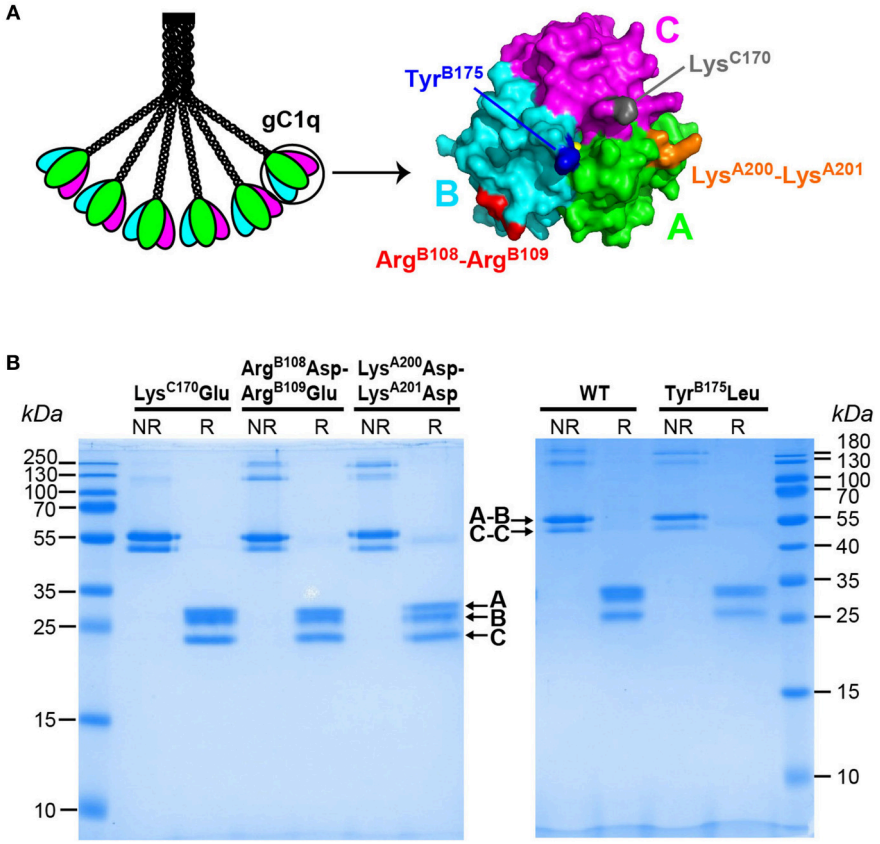


**FIGURE 1 |** Location of the mutated residues on gC1q structure and SDS-PAGE analysis of the C1q variants. **(A)** Schematic representation of the C1q molecule and 3-D structure of C1q globular domain (gC1q) with C1qA (pink), C1qB (cyan), and C1qC (green). The location of the mutated residues is indicated on the gC1q heterotrimer structure [figure generated using the Mac Pymol software; PDB ID 2wnv (35)]. $Tyr^{B175}$ and $Lys^{C170}$ are located at the apex of the gC1q heterotrimer and $Lys^{A200}$-$Lys^{A201}$ and $Arg^{B108}$-$Arg^{B109}$ are located on the side surfaces of the gC1qA and gC1qB modules, respectively. **(B)** SDS-PAGE analysis and Coomassie blue staining of the C1q variants, under non-reducing (NR) and reducing (R) conditions.

residues in the collagen-like sequences of the B ($Lys^{61}$) and C ($Lys^{58}$) chains of C1q that play a key role in the interaction with C1r and C1s and confirmed that C1q shares with MBL and ficolins a common mechanism of interaction with its associated proteases (24).

The availability of recombinant full-length C1q prompted us to revisit the C1q-PTX3 interaction using site-directed mutagenesis. To this end, we generated four C1q mutants targeting exposed amino acid residues in the gC1q domain of the different chains, including $Tyr^{B175}$ and $Lys^{C170}$ and investigated the impact of these mutations on the C1q-PTX3 interaction and the PTX3-mediated activation of the classical complement pathway. The effect on the interaction of C1q with its canonical ligand IgM was studied in parallel for comparison purposes. Furthermore, we addressed the functional impact of the p.D48A polymorphism on C1q recognition by PTX3.

## MATERIALS AND METHODS

### Proteins and Reagents

A recombinant form of the human PTX3 protein (with D at position 48) was made in a CHO cell line (25), and used in surface plasmon resonance (SPR) and complement activation experiments (see below). To assess the effect of the rs3816527 (p.D48A) polymorphism on the interaction with C1q in solid phase binding assays (see below), two PTX3 constructs were generated by overlapping PCR site-directed mutagenesis that contained triplets coding either for D or A at position 48. The corresponding recombinant proteins were expressed in and purified from a HEK293 cell line as previously reported (13). Molar concentration of the recombinant PTX3 from both cell lines was estimated using a Mr value of 340,000 (26). Human IgM, bovine serum albumin (BSA) and FLAG peptide were purchased from Sigma-Aldrich. Oligonucleotides were from Eurogentec and restriction and modification enzymes from New England Biolabs. Recombinant human MASP-2 was produced in S2 cells and quantified as described previously (27).

### Production of the Recombinant C1s-C1r-C1r-C1s Tetramer

The recombinant C1s-C1r-C1r-C1s tetramer was produced in the FreeStyle 293 Expression System (Thermo Fisher), using a pcDNA3.1/Neo(+) plasmid encoding human C1r with a

Ser637Ala mutation and a C-terminal Strep-tag (kindly provided by A. Amberger and R. Gröbner, Innsbruck Medical University, Austria) and a plasmid encoding human C1s with a C-terminal FLAG epitope. The latter was generated by fusing the FLAG tag (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) DNA sequence to C1s DNA (amplified using the VentR polymerase and the pFastBac-C1s plasmid (28) as a template) and cloning into a pcDNA3.1/Zeo(+) plasmid. 293-F cells grown in FreeStyle 293 medium were co-transfected with both plasmids using 293fectin and stable transfectants were selected with 400 μg/ml neomycin and 10 μg/ml zeocin (Thermo Fisher). Recombinant C1s-C1r-C1r-C1s was purified from the culture supernatant by chromatography on an anti-FLAG M2 affinity column (Sigma-Aldrich) as described by Bally et al. (24). The tetrameric assembly of the two proteins was assessed by size exclusion chromatography on a Superose 6 Increase 10/300 GL column (GE Healthcare). The molar concentration of the tetramer was estimated using a Mr value of 344,500, as determined by mass spectrometry analyses, and an absorption coefficient (A1%, 1 cm) at 280 nm of 13.45 (29).

## Production of C1q Variants

The $Lys^{A200}Asp\text{-}Lys^{A201}Asp$, $Arg^{B108}Asp\text{-}Arg^{B109}Glu$, $Tyr^{B175}Leu$, and $Lys^{C170}Glu$ mutations were introduced into the C1qA-, C1qB-, and C1qC-FLAG-containing pcDNA3.1/Neo(+), /Hygro(+), and /Zeo(+) plasmids, respectively, using the QuickChange XL site-directed mutagenesis kit (Agilent Technologies) (24). All constructs were checked by dsDNA sequencing (Eurofins Genomics).

Stable 293-F cell lines producing the individual B and C, A and C, or A and B chains of C1q (24), grown in FreeStyle 293 medium containing the appropriate selection antibiotics and 100 μg/ml ascorbic acid (Sigma-Aldrich), were transfected with the plasmids containing the C1qA $Lys^{200}Asp\text{-}Lys^{201}Asp$

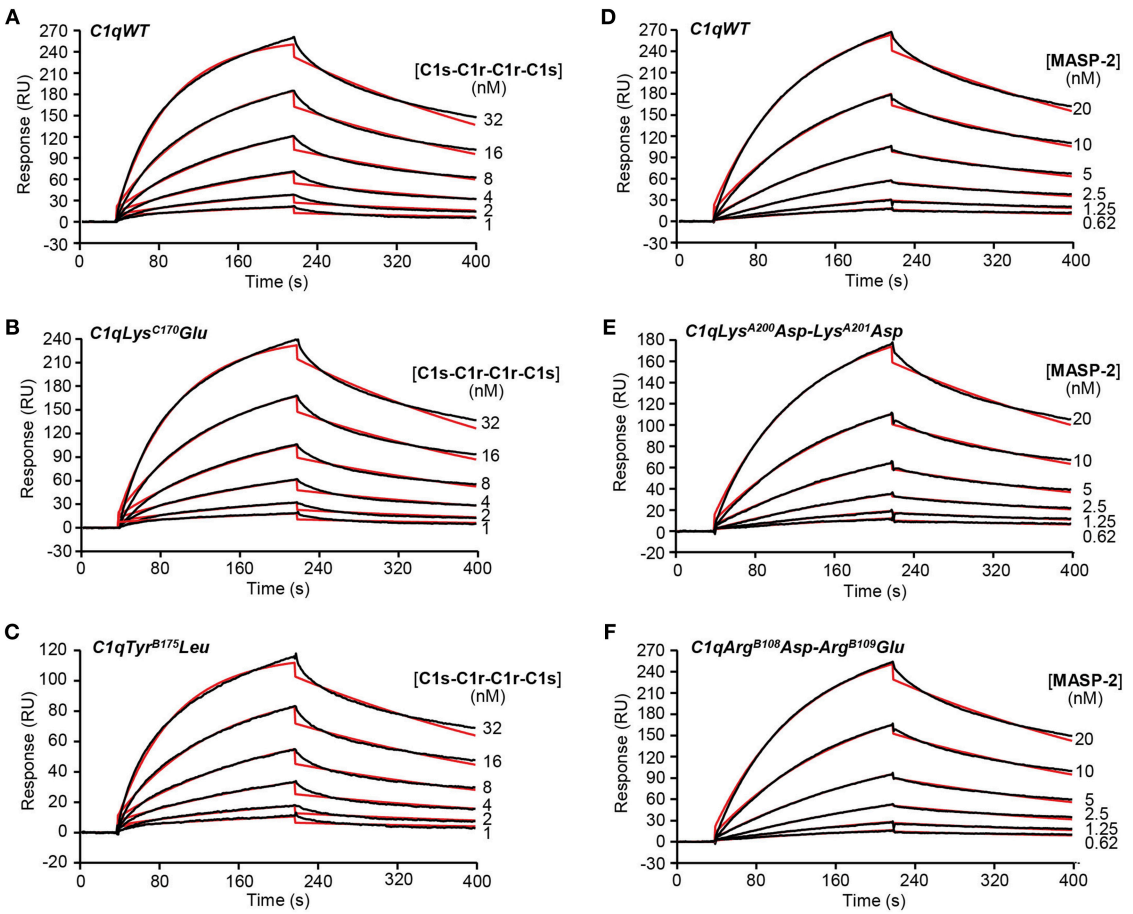


**FIGURE 2 |** Kinetic analyses of the interaction of the C1s-C1r-C1r-C1s tetramer and the MASP-2 dimer with immobilized C1q variants. Sixty microliter of the C1s-C1r-C1r-C1s tetramer at the indicated concentrations were injected over **(A)** C1qWT (16,300 RU), **(B)** $C1qLys^{C170}Glu$ (17,000 RU) and **(C)** $C1qTyr^{B175}Leu$ (12,200 RU) in 50 mM Tris-HCl, 150 mM NaCl, 2 mM $CaCl_2$, 0.005% surfactant P20, pH 7.4 at a flow rate of 20 μl/min. The MASP-2 dimer was injected over **(D)** C1qWT (13,600 RU), **(E)** $C1qLys^{A200}Asp\text{-}Lys^{A201}Asp$ (13,000 RU), and **(F)** $C1qArg^{B108}Asp\text{-}Arg^{B109}Glu$ (13,700 RU) under the same conditions as in **(A–C)**. The binding signals shown were obtained by subtracting the signal over the BSA reference surface and further subtraction of buffer blanks. Fits are shown as red lines and were obtained by global fitting of the data using a 1:1 Langmuir binding model. Chi2 values were between 0.9 and 5.9. Each kinetic analysis shown is representative of two independent experiments performed on separate sensor chips.

mutation, the C1qB $Arg^{108}$Asp-$Arg^{109}$Glu or $Tyr^{175}$Leu mutation, or the C1qC $Lys^{170}$Glu mutation, respectively, using 293fectin. Stable transfectants producing the three chains were generated following additional selection with 400 μg/ml neomycin (Fisher Scientific), 100 μg/ml hygromycin (Fisher Scientific), or 10 μg/ml zeocin (Sigma-Aldrich), respectively.

Recombinant wild-type (WT) and mutated C1q variants were purified from the stably transfected cell culture supernatants by adsorption on insoluble IgG-ovalbumin aggregates (30) and chromatography on an anti-FLAG M2 affinity column as described previously (24). The molar concentration of the C1q variants was estimated using a Mr of 460,000 and $A_{1\%,1cm}$ of 6.8.

## SPR Analyses

Analyses were performed at 25°C using a Biacore 3000 instrument (GE Healthcare). BSA and the C1q variants were diluted in 10 mM sodium acetate at the following concentration and pH: BSA, 25 μg/ml, pH 4.0; C1q variants, 50 μg/ml, pH 4.5 (wild-type) or 4.0 (C1q mutants), and immobilized on CM5 sensor chips (GE Healthcare) using the amine coupling chemistry in 10 mM Hepes, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4. Binding of C1q partners was measured at a flow rate of 20 μl/min in 50 mM Tris-HCl, 150 mM NaCl, 2 mM $CaCl_2$, 0.005% surfactant P20, pH 7.4. The specific binding signal was obtained by subtracting the signal over the BSA reference surface. Regeneration of the surfaces was achieved by 10 μl injections of 1 M NaCl, 10 mM EDTA, and, if needed, 10–20 mM NaOH. Kinetic data were analyzed by global fitting to a 1:1 Langmuir binding model for at least five concentrations simultaneously, using the BIAevaluation 3.2 software (GE Healthcare). Buffer blanks were subtracted from the data sets used for kinetic analyses. The apparent equilibrium dissociation constants ($K_D$) were calculated from the ratio of the dissociation and association rate constants ($k_d/k_a$). Chi2 values were below 6 in all cases.

## Complement Activation Assays

Microtiter plates (Maxisorp Nunc) were coated with PTX3 (10 μg/ml) or IgM (2 μg/ml) in 10 mM $NaHCO_3$, pH 9.6 overnight at 4°C. Wells were incubated for 1 h at 37°C with PBS containing 2% BSA (w/v) and washed with PBS containing 0.05% Tween 20 (PBS-T). C1q-depleted serum (CompTech), diluted 1:25 in 5 mM Na veronal, 145 mM NaCl, 5 mM $CaCl_2$, 1.5 mM $MgCl_2$, pH 7.5 and reconstituted with the recombinant C1q variants (4 μg/ml) was added to the wells and incubated for 1 h at 37°C. The wells were washed with 5 mM Na veronal, 145 mM NaCl, 5 mM EDTA, pH 7.5 and then a rabbit anti-C4 polyclonal antibody (1:1000 dilution) (Siemens Healthcare Diagnostics) was added to each well and incubated for 1 h at 37°C. After washing with PBS-T and incubation with a peroxidase-conjugated goat anti-rabbit polyclonal antibody (diluted 1:20,000 in PBS-T) (Jackson ImmunoResearch) for 1 h at 37°C, plates were washed with PBS-T and developed with 3,3',5,5'-tetramethylbenzidine (Tebu). The reaction was stopped with 1 N $H_2SO_4$ and absorbance was read at 450 nm. Each assay was performed in duplicate and absorbance values were determined after subtracting blank values obtained in the absence of added C1q. Normal human serum was obtained from the Etablissement Français du Sang Rhône-Alpes (agreement number 14-1940 regarding its use in research). Statistical analysis was performed using a paired two-tailed Student *t*-test (GraphPad software), with statistical significance defined as $P \leq 0.05$.

## Gel Electrophoresis and Lectin Blotting

Aliquots of purified recombinant PTX3 (either A48 and D48 from HEK293, or D48 from CHO) were run under denaturing conditions on Tris acetate 3–8% (w/v) gels (Thermo Fisher) and 8–18% (w/v) gel cards (GE Healthcare), in the absence and presence, respectively, of dithiothreitol, as reducing agent. Following separation, protein bands were stained either with silver nitrate (ProteoSilver™ Silver Stain Kit, Sigma-Aldrich) or Cy5, according to the electrophoretic apparatus used (XCell SureLock™ Mini-Cell Electrophoresis System, Thermo Fisher, or Amersham WB System, GE Healthcare, respectively).

The oligosaccharides linked to the A48 and D48 variants of PTX3 from HEK293 were probed for linkage and content of terminal residues of sialic acid by lectin staining using the DIG Glycan Differentiation Kit (Roche). Briefly, aliquots of both preparations were resolved by SDS-PAGE on Tris-glycine 10% (w/v) gels under reducing conditions and transferred onto Hybond-C Extra membranes. Following blocking, membranes were incubated with *Maackia amurensis* agglutinin (MAA, that recognizes α(2, 3)-linked sialic acid), and bound lectin revealed according to the manufacturer's instructions (26).

**TABLE 1 |** Kinetic and dissociation constants for binding of the $C1r_2$-$C1s_2$ tetramer and MASP-2 dimer to immobilized C1q variants.

| Soluble C1q ligand | Constants | Immobilized C1q variants | | | | |
|---|---|---|---|---|---|---|
| | | WT | $Lys^{C170}Glu$ | $Tyr^{B175}Leu$ | $Lys^{A200}Asp$-$Lys^{A201}Asp$ | $Arg^{B108}Asp$-$Arg^{B109}Glu$ |
| $C1r_2$-$C1s_2$ | $k_a$ ($M^{-1}$ $s^{-1}$) | $4.35 \pm 0.15 \times 10^5$ | $4.86 \pm 0.27 \times 10^5$ | $4.40 \pm 0.19 \times 10^5$ | | |
| | $k_d$ ($s^{-1}$) | $2.88 \pm 0.19 \times 10^{-3}$ | $2.56 \pm 0.05 \times 10^{-3}$ | $2.97 \pm 0.04 \times 10^{-3}$ | | |
| | $K_D$ (M) | $6.36 \pm 0.42 \times 10^{-9}$ | $5.29 \pm 0.40 \times 10^{-9}$ | $6.75 \pm 0.20 \times 10^{-9}$ | | |
| MASP-2 | $k_a$ ($M^{-1}$ $s^{-1}$) | $4.63 \pm 0.12 \times 10^5$ | | | $4.08 \pm 0.06 \times 10^5$ | $4.26 \pm 0.05 \times 10^5$ |
| | $k_d$ ($s^{-1}$) | $2.34 \pm 0.08 \times 10^{-3}$ | | | $2.64 \pm 0.11 \times 10^{-3}$ | $2.50 \pm 0.13 \times 10^{-3}$ |
| | $K_D$ (M) | $5.04 \pm 0.03 \times 10^{-9}$ | | | $6.03 \pm 0.18 \times 10^{-9}$ | $5.88 \pm 0.31 \times 10^{-9}$ |

*Values are the means ± SE of two to four separate experiments.*

## Solid Phase Binding Assays

Binding of the A48 and D48 variants of PTX3 from HEK293 to C1q was assessed using 96 well Maxisorp plates (Nunc) coated with C1q (purified from human serum; Merck Millipore). All dilutions, incubations, and washes were performed in 50 mM HEPES, 100 mM NaCl, 0.1% (v/v) Tween 20, pH 7.40 (HBS-T). Plates were coated overnight at room temperature with proteins in 20 mM $Na_2CO_3$, pH 9.6. Control wells were incubated with buffer alone and treated as for sample wells. Plates were blocked with 1% (w/v) BSA for 2 h at 37°C, and incubated with the PTX3 proteins for 1 h at 37°C. Bound proteins were detected using a rabbit anti-human PTX3 polyclonal antibody (200 ng/ml)

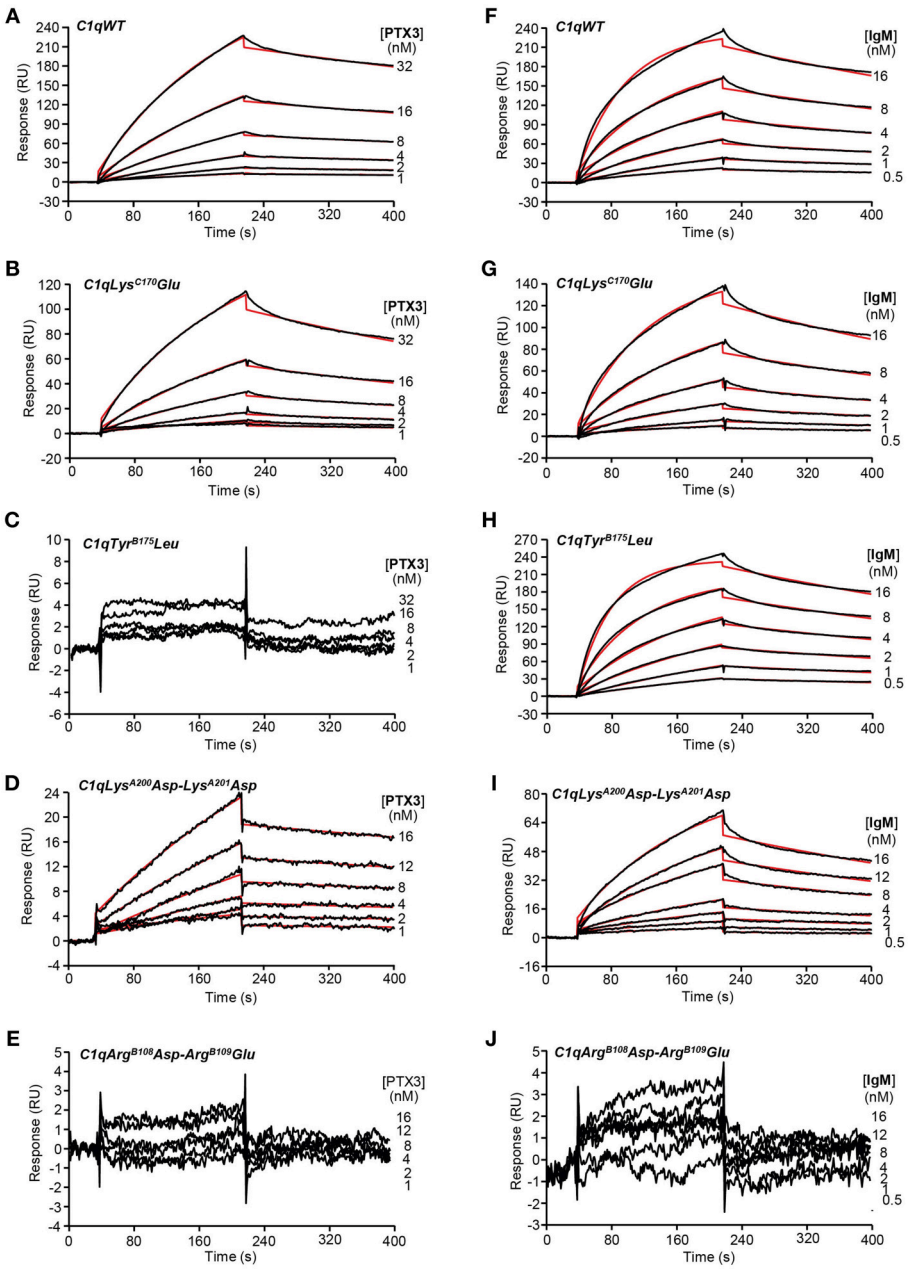


**FIGURE 3 |** Kinetic analyses of the interaction of PTX3 and IgM with immobilized C1q variants**.** Sixty microliter of PTX3 at the indicated concentrations were injected over **(A)** C1qWT (16,300 RU), **(B)** C1qLys$^{C170}$Glu (17,000 RU), **(C)** C1qTyr$^{B175}$Leu (12,200 RU), **(D)** C1qLys$^{A200}$Asp-Lys$^{A201}$Asp (18,400 RU), and **(E)** C1qArg$^{B108}$Asp-Arg$^{B109}$Glu (17,500 RU) in 50 mM Tris-HCl, 150 mM NaCl, 2 mM $CaCl_2$, 0.005% surfactant P20, pH 7.4 at a flow rate of 20 µl/min. **(F–J)** IgM was injected over the immobilized C1q variants under the same conditions as in **(A–E)**. The binding signals shown were obtained by subtracting the signal over the BSA reference surface and further subtraction of buffer blanks. Fits are shown as red lines and were obtained by global fitting of the data using a 1:1 Langmuir binding model. Chi2 values were between 0.25 and 5.8. Each kinetic analysis shown is representative of two to five independent experiments performed on separate sensor chips.

followed by a donkey anti-rabbit IgG HRP-conjugate whole antibody (GE Healthcare) and the 3,3',5,5'-tetramethylbenzidine substrate. Absorbance was read at 450 nm and background from uncoated wells subtracted.

# RESULTS AND DISCUSSION

## Generation and Quality Control of the C1q Mutants

Four C1q mutants have been produced, with two mutations targeting residues suggested to participate in the interaction with pentraxins ($Tyr^{B175}$Leu and $Lys^{C170}$Glu), and two mutations targeting exposed basic consecutive residues in the A and B chains ($Lys^{A200}$Asp-$Lys^{A201}$Asp, $Arg^{B108}$Asp-$Arg^{B109}$Glu), proposed to contribute to IgG and/or IgM binding (20, 21, 31). The single mutated residues are located at the apex of the gC1q heterotrimer whereas the tandem lysine and arginine residues are located on the side surface of gC1q (**Figure 1A**).

The C1q mutants were produced in stably transfected 293-F cells expressing the three C1q chains and the recombinant C1q variants purified from the cell culture supernatants as described for C1qWT. SDS-PAGE analysis of the four purified C1q mutants showed a band pattern similar to that obtained for C1qWT, with characteristic A-B and C-C dimers under non-reducing conditions (**Figure 1B**, NR lanes) and the three A, B, and C chains under reducing conditions (**Figure 1B**, R lanes). The minor extra bands above 100 kDa, observed only under non-reducing conditions, likely correspond to multimers of the C chain. This is corroborated by the lower intensity of the bands corresponding to C-C dimers compared to A-B dimers whereas the three chains are of equal intensity under reducing conditions. It should be mentioned that these extra bands are also observed with serum-derived C1q (**Figure S1**). Negative staining electron microscopy imaging revealed no difference between the wild-type protein and the four mutants, with individual molecules harboring a bouquet-like structure with six globular heads and a central stalk (data not shown), further indicating that the mutations had no impact on the assembly of mutated C1q.

The capacity of the C1q mutants to associate with the C1s-C1r-C1r-C1s tetramer or MASP-2 dimer, the homologous protease of the lectin complement pathway, was analyzed by SPR. The proteases bound to immobilized C1qWT, in accordance with our previous data (24) and to the four C1q mutants (**Figure 2**). The lower binding level of the C1s-C1r-C1r-C1s tetramer observed for the $Tyr^{B175}$Leu mutant (**Figure 2C**) can be related to the lower immobilization level of this mutant (12,200 RU) by comparison with C1qWT (16,300 RU, **Figure 2A**) and the $Lys^{C170}$Glu mutant (17,000 RU, **Figure 2B**). Kinetic analyses yielded similar binding parameters and dissociation constants for the interaction of the proteases with immobilized C1qWT and the four C1q mutants (**Table 1**). These data indicated that the mutations in the globular regions did not affect the capacity of the collagen-like regions of the C1q mutants to associate with the C1r/C1r or MASPs proteases.

## PTX3 and IgM Binding Properties of the C1q Variants

SPR was further used to investigate the functional impact of the mutations on the interaction of the C1q variants with PTX3 and with IgM, a major complement activating ligand of C1q. The amounts of immobilized C1q ranged from 16,200 to 18,400 RU, except for the $Tyr^{B175}$Leu mutant, for which the immobilization level could not exceed 12,200 RU despite repeated injections. No detectable PTX3 binding was observed for the two C1q variants with mutated B chain residues (**Figures 3C,E**) whereas the $Lys^{C170}$Glu mutant and the $Lys^{A200}$Asp-$Lys^{A201}$Asp mutant retained the ability of C1qWT to interact with PTX3 (**Figures 3B,D**), although lower binding levels were observed for the latter mutant (**Figures 3A,D**). However, kinetic analysis of the interactions yielded $K_D$ values of the same order, comprised between 5.65 and 10.9 nM (**Table 2**), even if small differences could be detected between the mutants and C1qWT. For example, the 1.5-fold higher $k_d$ value for the $Lys^{C170}$Glu mutant may reflect a slightly lower stability of the complex and the 1.5-fold lower $k_a$ value for the $Lys^{A200}$Asp-$Lys^{A201}$Asp mutant a slightly slower formation of the complex. Comparable effects were observed for binding of both C1q mutants to IgM, with $K_D$ values ranging from 1.92 to 3.31 nM (**Table 2**), reflecting a higher apparent affinity for IgM than for PTX3. Interestingly, the immobilized $Tyr^{B175}$Leu mutant retained the ability to interact with IgM (**Figure 3H**), with even a slightly better affinity (0.89 nM) than C1qWT, arising mainly from a 1.5-fold higher $k_a$ value. As observed for PTX3 binding, the $Arg^{B108}$Asp-$Arg^{B109}$Glu mutation abolished C1q capacity to

**TABLE 2 |** Kinetic and dissociation constants for binding of PTX3 and IgM to immobilized C1q variants.

| Soluble C1q ligand | Constants | Immobilized C1q variants | | | | |
|---|---|---|---|---|---|---|
| | | WT | $Lys^{C170}$Glu | $Tyr^{B175}$Leu | $Lys^{A200}$Asp-$Lys^{A201}$Asp | $Arg^{B108}$Asp-$Arg^{B109}$Glu |
| PTX3 | $k_a$ ($M^{-1}$ $s^{-1}$) | $1.55 \pm 0.21 \times 10^5$ | $1.40 \pm 0.01 \times 10^5$ | | $9.83 \pm 4.08 \times 10^4$ | |
| | $k_d$ ($s^{-1}$) | $8.59 \pm 0.10 \times 10^{-4}$ | $1.52 \pm 0.11 \times 10^{-3}$ | ND | $6.34 \pm 0.15 \times 10^{-4}$ | ND |
| | $K_D$ (M) | $5.65 \pm 0.82 \times 10^{-9}$ | $1.09 \pm 0.07 \times 10^{-8}$ | | $7.84 \pm 3.37 \times 10^{-9}$ | |
| IgM | $k_a$ ($M^{-1}$ $s^{-1}$) | $9.19 \pm 2.08 \times 10^5$ | $6.81 \pm 0.51 \times 10^5$ | $1.47 \pm 0.05 \times 10^6$ | $7.17 \pm 2.48 \times 10^5$ | |
| | $k_d$ ($s^{-1}$) | $1.54 \pm 0.19 \times 10^{-3}$ | $1.67 \pm 0.04 \times 10^{-3}$ | $1.31 \pm 0.01 \times 10^{-3}$ | $2.06 \pm 0.22 \times 10^{-3}$ | ND |
| | $K_D$ (M) | $1.92 \pm 0.68 \times 10^{-9}$ | $2.48 \pm 0.24 \times 10^{-9}$ | $8.92 \pm 0.41 \times 10^{-10}$ | $3.31 \pm 1.12 \times 10^{-9}$ | |

*Values are the means ± SE of two to five separate experiments. ND, not determined due to no detectable binding in the concentration range used.*

interact with IgM (**Figure 3J**) and the binding levels observed for the $Lys^{A200}Asp$-$Lys^{A201}Asp$ mutant were lower than those obtained with C1qWT (**Figure 3I**).

## PTX3- and IgM-Dependent Complement Activation by the C1q Variants

The capacity of the C1q variants to trigger complement activation when added to C1q-depleted serum in microwells coated with PTX3 or IgM was analyzed by ELISA. C4b deposition in the wells results from serum C4 cleavage by a functional C1 complex assembled from recombinant C1q and the serum C1r/C1s proteases. As expected, C1qWT yielded amounts of deposited C4b comparable to those obtained with complement-sufficient normal human serum (NHS) in both PTX3 and IgM coated plates. In accordance with the SPR data, no significant difference was observed between the $Lys^{C170}Glu$ mutant and C1qWT whereas the $Arg^{B108}Asp$-$Arg^{B109}Glu$ mutation strongly decreased both PTX3- and IgM-dependent complement activation (30 and 9% of the signal obtained with C1qWT, respectively) (**Figure 4**). The $Tyr^{B175}Leu$ mutant also exhibited significantly decreased PTX3- and IgM-mediated complement activating capacity (44.5 and 61.4% of the C1qWT value, respectively), in apparent discrepancy with the SPR data that detected no binding of PTX3 (**Figure 3C**) and a strong interaction with IgM (**Figure 3H**). As mentioned above, an immobilization level comparable to that of the other mutants (>16,000 RU) could not be reached for the $Tyr^{B175}Leu$ mutant (12,200 RU) and the lack of PTX3 binding might be explained by a possible threshold effect. The fact that the interaction with IgM was not affected under the same C1q immobilization conditions might result from a difference in the avidity component of the interactions between hexameric C1q and multivalent PTX3 or IgM. However, it cannot be excluded that the covalent immobilization of this mutant might have influenced its PTX3 binding capacity. In addition, the complement activating assay is performed using coated PTX3 or IgM and the SPR experiments in the reverse configuration (immobilized C1q variants), which might account for the observed discrepancy. Another interesting observation is the fact that the $Lys^{A200}Asp$-$Lys^{A201}Asp$ mutant exhibited significantly higher PTX3-dependent complement activating capacity (163% of C1qWT, **Figure 4A**), which might be linked to the slightly higher stability of the complex observed in SPR experiments.

## The D48 and A48 Allelic Variants of PTX3 and Their C1q Binding Properties

To assess if the exonic polymorphism p.D48A in the PTX3 gene affects the protein's binding to C1q, recombinant forms of the D48 and A48 allelic variants were made in a HEK293 cell line and purified by immunoaffinity chromatography as previously described (13). SDS-PAGE analysis, performed on Tris acetate 3–8% gels under non-reducing conditions (**Figure 5A**), revealed a major band with an apparent molecular mass of 340 kDa, consistent with PTX3 protomers being mainly assembled into octamers stabilized by disulfide bonds, in both preparations. Additional bands were detected at apparent molecular masses of 280, 210, 170, 80, and 65 kDa, and this pattern was consistent amongst the two allelic variants made in HEK293 and the CHO protein, here used as a reference (8). Upon reduction, a major band at 42 kDa (close to the expected molecular mass for PTX3 monomers, i.e., ∼42.5 kDa) and a minor one at 100 kDa (likely corresponding to dimers of the protein, originating from partial reduction) were observed following Cy5 staining in all PTX3 proteins (**Figure 5B**). Therefore, no significant difference in terms of quaternary structure and homogeneity was noticed amongst the D48 and A48 allelic variants, and the electrophoretic profiles of these proteins under both reducing and non-reducing conditions were comparable to that of the CHO-derived PTX3, taken as a reference. Given that protein glycosylation has been implicated in a number of PTX3 functions in innate immunity and inflammation (32), and, most importantly, sialic acid has been shown to modulate the interaction of PTX3 with

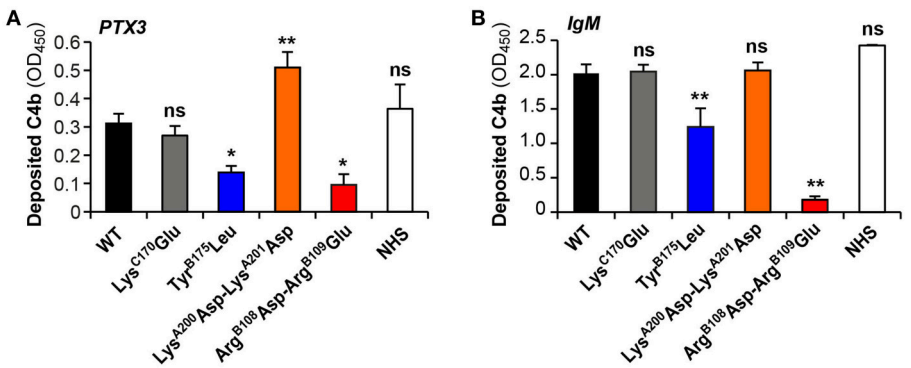


**FIGURE 4 |** PTX3- and IgM-dependent complement activation by the C1q variants. C1q-depleted serum (1:25 dilution) was reconstituted with the recombinant C1q variants (4 μg/ml) and added to microwells coated with 10 μg/ml PTX3 **(A)** or 2 μg/ml IgM **(B)**. Normal human serum (NHS, 1:25 dilution) was used as a control. The resulting C1-cleaving activity was measured by a C4b deposition assay as described under Material and Methods. Deposited C4b was detected with an anti-human polyclonal antibody, and results are expressed as absorbance at 450 nm ($OD_{450}$), following background subtraction [means ± SEM of three (IgM) and four (PTX3) independent experiments]. Comparisons between C1qWT and each of the mutants or C1q in normal human serum were made using a paired Student *t*-test. $^{*}P < 0.05$; $^{**}P < 0.005$; ns, not significant.

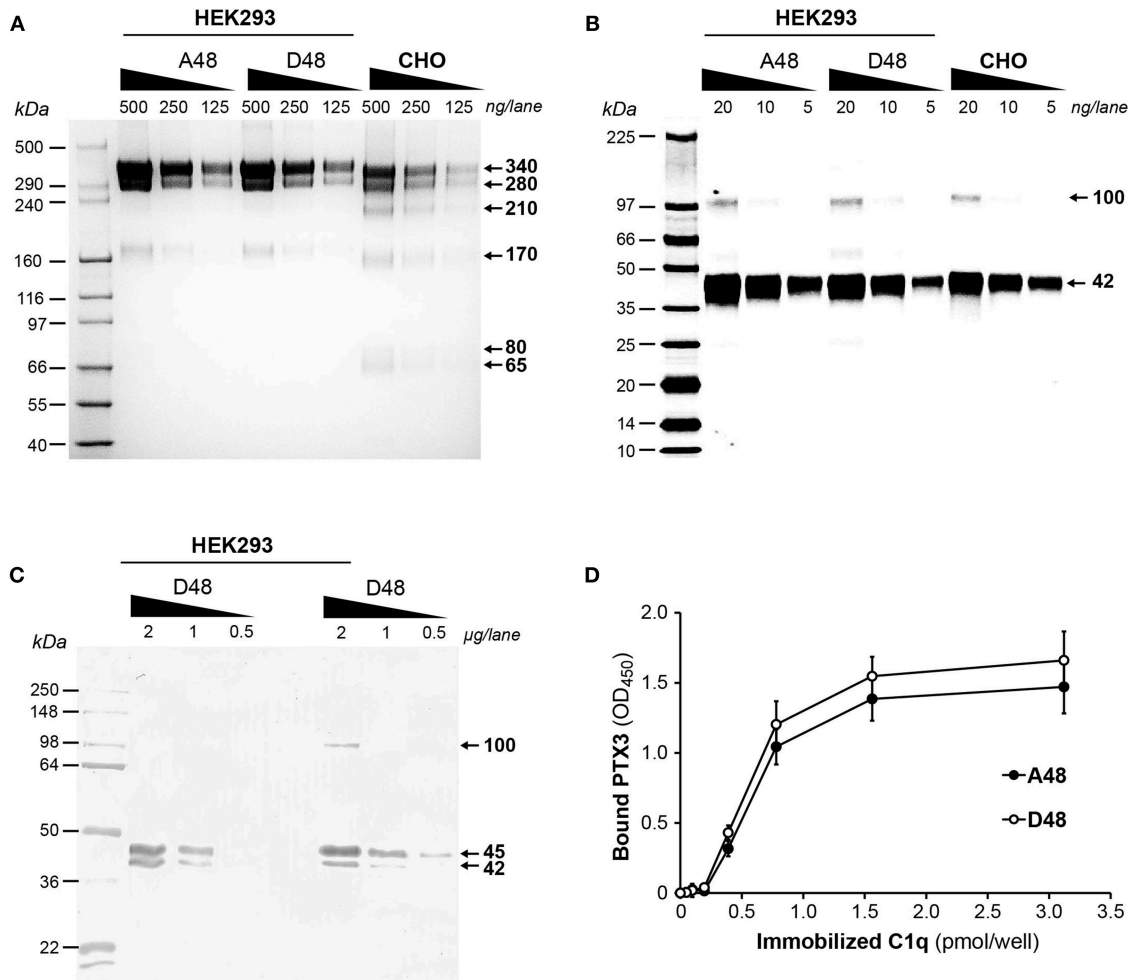


**FIGURE 5 |** Biochemical characterization of the D48 and A48 allelic variants of PTX3 and their binding to C1q. The indicated amounts of purified recombinant PTX3 (either A48 and D48 from HEK293, or D48 from CHO) were run under denaturing conditions on Tris acetate 3–8% (w/v) gels **(A)** and 8–18% (w/v) gel cards **(B)**, in the absence and presence, respectively, of dithiothreitol. Following separation, protein bands were stained either with silver nitrate **(A)** or Cy5 **(B)**. **(C)** Aliquots of both A48 and D48 preparations from HEK293 were resolved on Tris-glycine 10% (w/v) gels under reducing conditions, transferred onto membranes, and probed with MAA lectin. **(A–C)** representative gels from three independent experiments are shown, with molecular mass markers on the left, and apparent molecular mass values observed for the resolved bands on the right. **(D)** The effect of the p.D48A polymorphism on the interaction of PTX3 with C1q was assessed by solid phase binding assay using microwells coated with the indicated amounts of C1q that were incubated with the A48 and D48 variants (both at 3 nM). Bound PTX3 was revealed with an anti-human polyclonal antibody, and results are expressed as absorbance at 450 nm ($OD_{450}$), following background subtraction (three independent experiments performed in quadruplicate, $n = 12$, mean ± SD).

C1q (33), we analyzed the sialylation status of the D48 and A48 allelic variants by lectin blotting, using MAA to probe the terminal α(2, 3)-linked sialic acid residues. As shown in **Figure 5C**, both proteins gave two major MAA-reactive bands at 45 and 42 kDa, indicative of glycoform populations with distinct sialylation (possibly, bi- and tri-antennary complex oligosaccharides), and a minor signal at 100 kDa (likely corresponding to protein dimers, as described in **Figure 5B**). Therefore, the two recombinant variants of PTX3 were virtually identical in terms of quaternary structure and glycosylation, thus amenable to comparative functional studies. In this regard, when assayed in solid phase binding experiments, the D48 and A48 alleles had comparable binding to plastic-immobilized C1q at each applied concentration (**Figure 5D**), indicating that in the described experimental conditions the p.D48A polymorphism does not affect the interaction of PTX3 with C1q.

## CONCLUSION

The present study aimed at revisiting the PTX3-C1q interaction using mutagenesis of full-length recombinant C1q, as compared to the canonical C1q ligand IgM. We confirmed previous observations of an essential role of the B chain residues $Arg^{108}$, $Arg^{109}$, and $Tyr^{175}$ in the interaction with both PTX3 (20) and IgM (21, 34). Our results also suggest no significant contribution of the exposed C1qA $Lys^{200}$ and $Lys^{201}$ in IgM-mediated complement activation, in contrast to

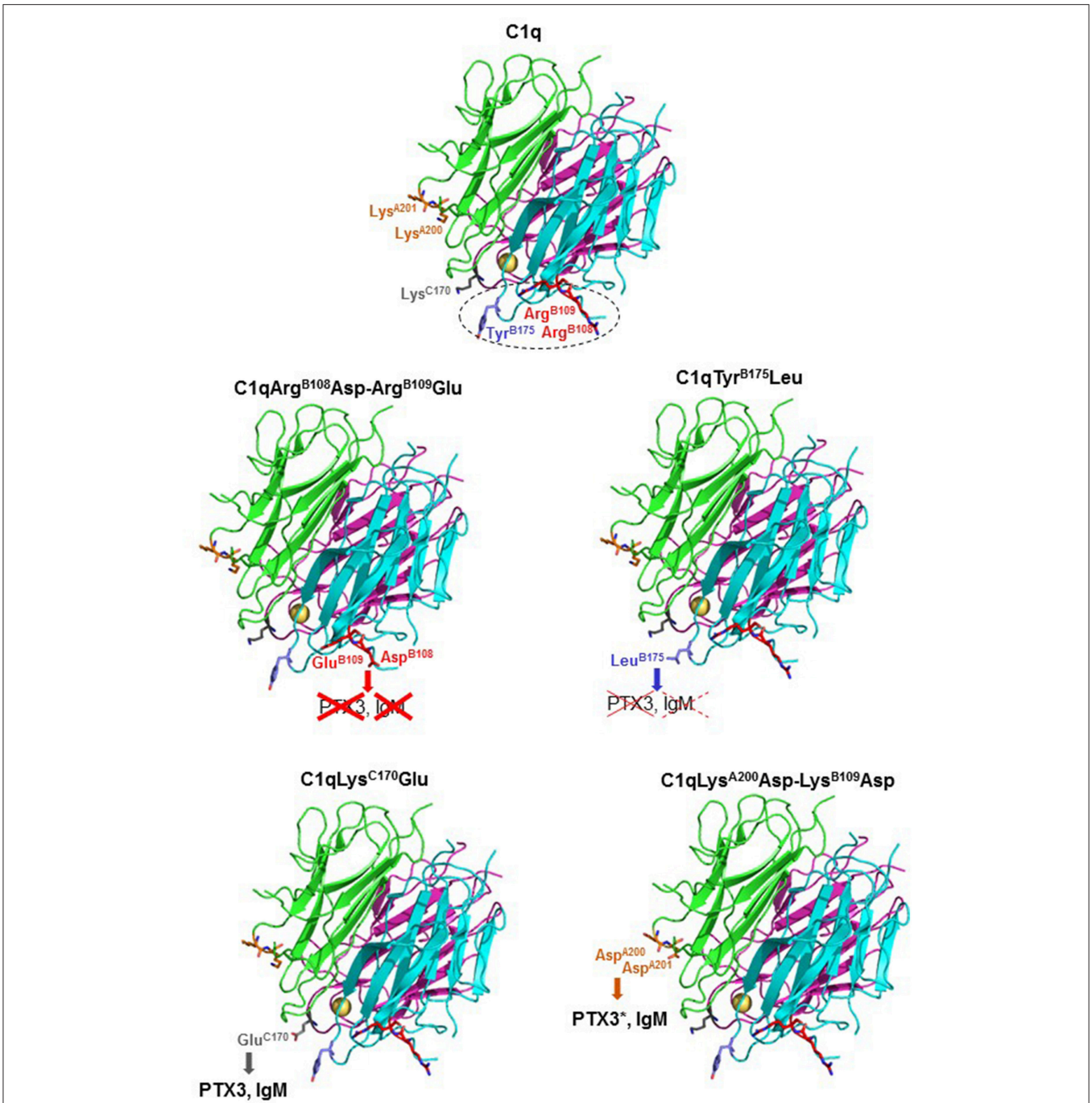


**FIGURE 6 |** Summary of the C1q mutagenesis results. Ribbon diagrams of the structure of the globular domains of wild-type C1q and of the four mutants are shown. The side chains of the mutated amino acid residues are represented as stick models. The color code for the three C1q chains and the mutated amino acids is the same as in **Figure 1**. The calcium ion is represented by a yellow sphere. The gC1q region found important for interaction with both PTX3 and IgM and complement activation is delineated by dots. The effects of the C1q mutations toward PTX3 and IgM binding and complement activation are represented as follows: red crosses (solid lines), inhibition of both activities; red crosses (dotted lines), inhibition of complement activation only; bold characters, no significant inhibition. PTX3* indicates that enhancement of PTX3-dependent complement activation was observed.

previous data obtained with the isolated recombinant gC1qA module showing that the Lys$^{A200}$Glu mutation resulted in a 27% reduction in the binding to solid-phase IgM (21). Intriguingly, the replacement of these two basic residues by acidic residues in the side part of gC1qA significantly enhanced the complement activating capacity of PTX3, which might be

related to the slightly better stability of the complex observed in SPR experiments. In a similar way, the C chain $Lys^{170}Glu$ mutation had no significant impact on the PTX3 or IgM binding and the complement activating properties of C1q, in contrast to previous observations showing a considerable reduction in gC1qC binding to solid-phase PTX3 (about 40%) (20) and IgM (>30%) (21). These data suggest a differential exposure of $Lys^{A200}$ and $Lys^{C170}$ in the isolated gC1qA/gC1qC modules and in the heterotrimeric globular heads of full-length C1q. Altogether, these mutagenesis results confirm a key electrostatic contribution in the interaction between C1q B chain and PTX3 or IgM (summarized in **Figure 6**), consistent with the hypothesis that binding of C1q to targets through this region triggers efficient activation of the C1 complex (35, 36).

Given that the exonic polymorphism p.D48A (or rs3816527) in the PTX3 gene forms with rs2305619 in intron 1 and rs1840680 in intron 2 an haplotypic block that has been linked to the susceptibility to selected infections as well as different circulating levels of the protein, it was important to assess whether this amino acid substitution had any effect on C1q recognition. To this end, we generated recombinant forms of the A48 and D48 alleles that were comparable in quaternary structure, homogeneity and sialylation status, thus being amenable to comparative studies. These preparations had similar binding to C1q, which indicates that the p.D48A polymorphism does not alter the C1q binding properties of PTX3, at least in the applied experimental conditions. In addition, we have previously shown that the C-terminal domain of PTX3 mostly mediates the interaction of the long pentraxin with C1q (25), and this is modulated by protein glycosylation (at $Asn^{220}$ in the C-terminal domain). Molecular dynamics simulations suggest that the PTX3 oligosaccharides (via their terminal residues of sialic acid) are in contact with polar amino acids on the solvent exposed surface of the C-terminal domain (33). Given the prominent electrostatic nature of the PTX3-C1q interaction, as supported by our study and previous evidence (20), it is tempting to speculate that these residues are involved in C1q recognition. Further studies are needed to challenge this hypothesis and identify the PTX3 residues that support C1q binding. Finally, the novel C1q mutants generated in this study should allow further exploration of the molecular bases of C1q binding versatility in different physiological contexts.

## DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

## AUTHOR CONTRIBUTIONS

NT and CG designed the study. IB, NT, MS, and AI performed the research. IB, CG, AI, BB, and NT analyzed the data. FD, AI, and BB contributed key reagents. NT wrote the manuscript draft. All authors revised and approved the final version of the manuscript.

## FUNDING

This work was supported by the French National Research Agency (grants ANR-16-CE11-0019 and ANR-16-CE91-0004-01). AI is recipient of a Young Investigator Grant from Ministero della Salute (GR-2011-02349539). The European Research Council (grant PHII-669415) is gratefully acknowledged.

## ACKNOWLEDGMENTS

We thank A. Amberger and R. Gröbner (Innsbruck Medical University, Austria) for kindly providing the pcDNA3.1/Zeo(+) plasmid encoding human C1r with a $Ser^{637}Ala$ mutation and a Strep-tag. This work used the platforms of the Grenoble Eric-Instruct center (ISBG; UMS 3518 CNRS-CEA-UJF-EMBL) with support from FRISBI (ANR-10-INSB-05-02) and GRAL (ANR-10-LABX-49-01) within the Grenoble Partnership for Structural Biology (PSB). We thank Anne Chouquet and Jean-Baptiste Reiser for assistance and access to the SPR facility.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00461/full#supplementary-material

## REFERENCES

1. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature.* (2007) 449:819–26. doi: 10.1038/nature06246
2. Paidassi H, Tacnet-Delorme P, Arlaud GJ, Frachet P. How phagocytes track down and respond to apoptotic cells. *Crit Rev Immunol.* (2009) 29:111–30. doi: 10.1615/CritRevImmunol.v29.i2.20
3. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol.* (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
4. Reid KBM. Complement component C1q: historical perspective of a functionally versatile, and structurally unusual, serum protein. *Front Immunol.* (2018) 9:764. doi: 10.3389/fimmu.2018.00764
5. Lu J, Kishore U. C1 Complex: an adaptable proteolytic module for complement and non-complement functions. *Front Immunol.* (2017) 8:592. doi: 10.3389/fimmu.2017.00592
6. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement system part I - molecular mechanisms of activation and regulation. *Front Immunol.* (2015) 6:262. doi: 10.3389/fimmu.2015.00262
7. Srinivasan N, White HE, Emsley J, Wood SP, Pepys MB, Blundell TL. Comparative analyses of pentraxins: implications for protomer assembly and ligand binding. *Structure.* (1994) 2:1017–27. doi: 10.1016/S0969-2126(94)00105-7
8. Inforzato A, Rivieccio V, Morreale AP, Bastone A, Salustri A, Scarchilli L, et al. Structural characterization of PTX3 disulfide bond network and its multimeric status in cumulus matrix organization. *J Biol Chem.* (2008) 283:10147–61. doi: 10.1074/jbc.M708535200

9. Du Clos TW. Pentraxins: structure, function, and role in inflammation. *ISRN Inflamm.* (2013) 2013:379040. doi: 10.1155/2013/379040
10. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. PTX3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiol Rev.* (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
11. Inforzato A, Doni A, Barajon I, Leone R, Garlanda C, Bottazzi B, et al. PTX3 as a paradigm for the interaction of pentraxins with the complement system. *Semin Immunol.* (2013) 25:79–85. doi: 10.1016/j.smim.2013.05.002
12. Brunel AS, Wojtowicz A, Lamoth F, Spertini O, Neofytos D, Calandra T, et al. Pentraxin-3 polymorphisms and invasive mold infections in acute leukemia patients receiving intensive chemotherapy. *Haematologica.* (2018) 103:e527–30. doi: 10.3324/haematol.2018.195453
13. Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med.* (2014) 370:421–32. doi: 10.1056/NEJMoa1211161
14. Fisher CE, Hohl TM, Fan W, Storer BE, Levine DM, Zhao LP, et al. Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. *Blood.* (2017) 129:2693–701. doi: 10.1182/blood-2016-10-743294
15. Wojtowicz A, Lecompte TD, Bibert S, Manuel O, Rueger S, Berger C, et al. PTX3 polymorphisms and invasive mold infections after solid organ transplant. *Clin Infect Dis.* (2015) 61:619–22. doi: 10.1093/cid/civ386
16. Olesen R, Wejse C, Velez DR, Bisseye C, Sodemann M, Aaby P, et al. DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. *Genes Immun.* (2007) 8:456–67. doi: 10.1038/sj.gene.6364410
17. Chiarini M, Sabelli C, Melotti P, Garlanda C, Savoldi G, Mazza C, et al. PTX3 genetic variations affect the risk of *Pseudomonas aeruginosa* airway colonization in cystic fibrosis patients. *Genes Immun.* (2010) 11:665–70. doi: 10.1038/gene.2010.41
18. Barbati E, Specchia C, Villella M, Rossi ML, Barlera S, Bottazzi B, et al. Influence of pentraxin 3 (PTX3) genetic variants on myocardial infarction risk and PTX3 plasma levels. *PLoS ONE.* (2012) 7:e53030. doi: 10.1371/journal.pone.0053030
19. Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, et al. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. *Eur J Immunol.* (2003) 33:465–73. doi: 10.1002/immu.200310022
20. Roumenina LT, Ruseva MM, Zlatarova A, Ghai R, Kolev M, Olova N, et al. Interaction of C1q with IgG1, C-reactive protein and pentraxin 3: mutational studies using recombinant globular head modules of human C1q A, B, and C chains. *Biochemistry.* (2006) 45:4093–104. doi: 10.1021/bi052646f
21. Gadjeva MG, Rouseva MM, Zlatarova AS, Reid KB, Kishore U, Kojouharova MS. Interaction of human C1q with IgG and IgM: revisited. *Biochemistry.* (2008) 47:13093–102. doi: 10.1021/bi801131h
22. Kishore U, Gupta SK, Perdikoulis MV, Kojouharova MS, Urban BC, Reid KB. Modular organization of the carboxyl-terminal, globular head region of human C1q A, B, and C chains. *J Immunol.* (2003) 171:812–20. doi: 10.4049/jimmunol.171.2.812
23. Kojouharova MS, Gadjeva MG, Tsacheva IG, Zlatarova A, Roumenina LT, Tchorbadjieva MI, et al. Mutational analyses of the recombinant globular regions of human C1q A, B, and C chains suggest an essential role for arginine and histidine residues in the C1q-IgG interaction. *J Immunol.* (2004) 172:4351–8. doi: 10.4049/jimmunol.172.7.4351
24. Bally I, Ancelet S, Moriscot C, Gonnet F, Mantovani A, Daniel R, et al. Expression of recombinant human complement C1q allows identification of the C1r/C1s-binding sites. *Proc Natl Acad Sci USA.* (2013) 110:8650–5. doi: 10.1073/pnas.1304894110
25. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem.* (1997) 272:32817–23. doi: 10.1074/jbc.272.52.32817
26. Inforzato A, Baldock C, Jowitt TA, Holmes DF, Lindstedt R, Marcellini M, et al. The angiogenic inhibitor long pentraxin PTX3 forms an asymmetric octamer with two binding sites for FGF2. *J Biol Chem.* (2010) 285:17681–92. doi: 10.1074/jbc.M109.085639
27. Lacroix M, Tessier A, Dumestre-Perard C, Vadon-Le Goff S, Gout E, Bruckner-Tuderman L, et al. Interaction of complement defence collagens C1q and mannose-binding lectin with BMP-1/tolloid-like proteinases. *Sci Rep.* (2017) 7:16958. doi: 10.1038/s41598-017-17318-w
28. Rossi V, Teillet F, Thielens NM, Bally I, Arlaud GJ. Functional characterization of complement proteases C1s/mannan-binding lectin-associated serine protease-2 (MASP-2) chimeras reveals the higher C4 recognition efficacy of the MASP-2 complement control protein modules. *J Biol Chem.* (2005) 280:41811–8. doi: 10.1074/jbc.M503813200
29. Bally I, Rossi V, Lunardi T, Thielens NM, Gaboriaud C, Arlaud GJ. Identification of the C1q-binding sites of human C1r and C1s: a refined three-dimensional model of the C1 complex of complement. *J Biol Chem.* (2009) 284:19340–8. doi: 10.1074/jbc.M109.004473
30. Arlaud GJ, Sim RB, Duplaa AM, Colomb MG. Differential elution of Clq, Clr and Cls from human Cl bound to immune aggregates. Use in the rapid purification of Cl subcomponents. *Mol Immunol.* (1979) 16:445–50. doi: 10.1016/0161-5890(79)90069-5
31. Gaboriaud C, Juanhuix J, Gruez A, Lacroix M, Darnault C, Pignol D, et al. The crystal structure of the globular head of complement protein C1q provides a basis for its versatile recognition properties. *J Biol Chem.* (2003) 278:46974–82. doi: 10.1074/jbc.M307764200
32. Inforzato A, Reading PC, Barbati E, Bottazzi B, Garlanda C, Mantovani A. The "sweet" side of a long pentraxin: how glycosylation affects PTX3 functions in innate immunity and inflammation. *Front Immunol.* (2012) 3:407. doi: 10.3389/fimmu.2012.00407
33. Inforzato A, Peri G, Doni A, Garlanda C, Mantovani A, Bastone A, et al. Structure and function of the long pentraxin PTX3 glycosidic moiety: fine-tuning of the interaction with C1q and complement activation. *Biochemistry.* (2006) 45:11540–51. doi: 10.1021/bi0607453
34. Zlatarova AS, Rouseva M, Roumenina LT, Gadjeva M, Kolev M, Dobrev I, et al. Existence of different but overlapping IgG- and IgM-binding sites on the globular domain of human C1q. *Biochemistry.* (2006) 45:9979–88. doi: 10.1021/bi060539v
35. Garlatti V, Chouquet A, Lunardi T, Vives R, Paidassi H, Lortat-Jacob H, et al. Cutting edge: C1q binds deoxyribose and heparan sulfate through neighboring sites of its recognition domain. *J Immunol.* (2010) 185:808–12. doi: 10.4049/jimmunol.1000184
36. Kojouharova M, Reid K, Gadjeva M. New insights into the molecular mechanisms of classical complement activation. *Mol Immunol.* (2010) 47:2154–60. doi: 10.1016/j.molimm.2010.05.011


**Conflict of Interest Statement:** Pending patent application by NT and IB: Method for preparing C1q recombinant protein (WO2014 057437).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 04 April 2019
doi: 10.3389/fimmu.2019.00712

# The Long Pentraxin PTX3 as a Link Between Innate Immunity, Tissue Remodeling, and Cancer

*Andrea Doni*[1], *Matteo Stravalaci*[2], *Antonio Inforzato*[1,2], *Elena Magrini*[1], *Alberto Mantovani*[1,2,3], *Cecilia Garlanda*[1,2]* *and Barbara Bottazzi*[1]*

[1] Humanitas Clinical and Research Institute—IRCCS, Milan, Italy, [2] Department of Biomedical Sciences, Humanitas University, Milan, Italy, [3] The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom

The innate immune system comprises a cellular and a humoral arm. Humoral pattern recognition molecules include complement components, collectins, ficolins, and pentraxins. These molecules are involved in innate immune responses by recognizing microbial moieties and damaged tissues, activating complement, exerting opsonic activity and facilitating phagocytosis, and regulating inflammation. The long pentraxin PTX3 is a prototypic humoral pattern recognition molecule that, in addition to providing defense against infectious agents, plays several functions in tissue repair and regulation of cancer-related inflammation. Characterization of the PTX3 molecular structure and biochemical properties, and insights into its interactome and multiple roles in tissue damage and remodeling support the view that microbial and matrix recognition are evolutionarily conserved functions of humoral innate immunity molecules.

**Keywords: pentraxins, PTX3, inflammation, tissue remodeling, wound healing**

OPEN ACCESS

***Edited by:***
*Massimo Gadina, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), United States*

***Reviewed by:***
*Darrell Pilling, Texas A&M University, United States*
*Marco Presta, University of Brescia, Italy*

***Correspondence:**
*Cecilia Garlanda*
*cecilia.garlanda@humanitasresearch.it*
*Barbara Bottazzi*
*barbara.bottazzi@humanitasresearch.it*

***Specialty section:***
*This article was submitted to Inflammation, a section of the journal Frontiers in Immunology*

***Received:*** *10 January 2019*
***Accepted:*** *15 March 2019*
***Published:*** *04 April 2019*

***Citation:***
*Doni A, Stravalaci M, Inforzato A, Magrini E, Mantovani A, Garlanda C and Bottazzi B (2019) The Long Pentraxin PTX3 as a Link Between Innate Immunity, Tissue Remodeling, and Cancer. Front. Immunol. 10:712. doi: 10.3389/fimmu.2019.00712*

## INTRODUCTION

Innate immune responses are the first strategies of host defense from invading pathogens and tissue damage. Their activation occurs when conserved structures on the surface of pathogens or associated with tissue damage, called pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), respectively, are recognized by cell-associated or soluble molecules known as pattern recognition molecules (PRMs). Among soluble PRMs, pentraxins are a superfamily of evolutionarily conserved molecules with multi-functional roles in innate immunity and inflammation, such as regulation of complement activation and opsonization of pathogens (1). C-reactive protein (CRP) and serum amyloid P component (SAP) are the short or "classical" pentraxins. CRP is mainly produced by hepatocytes as an acute phase protein in man as well as other mammalian species, but not in mouse, in response to interleukin (IL)-6, whereas SAP is the short pentraxin acting as an acute phase protein in mouse (2). Pentraxin 3 (PTX3) is the prototype of the long pentraxin subfamily, originally identified as an IL-1 or TNF-inducible gene. PTX3 is produced by different cell types in response to primary pro-inflammatory stimuli and microbial moieties, is an essential mediator of innate resistance to selected pathogens of fungal, bacterial and viral origin [as discussed elsewhere (1, 3)], and is involved in regulation of inflammation, tissue remodeling, and cancer.

Here we will review the main biological features of PTX3 focusing on its structure and involvement in sterile conditions of tissue damage and cancer, and providing evidence that microbial and matrix recognition are evolutionarily conserved properties shared by humoral innate immunity molecules.

## GENE REGULATION AND PROTEIN STRUCTURE

The human and the murine PTX3 gene map on chromosome 3 and are organized in three exons, the first two coding for the leader peptide and the N-terminal domain, and the third coding for the C-terminal-pentraxin domain (**Figure 1**).

PTX3 is mainly induced by pro-inflammatory cytokines, such as IL-1β and TNFα, and by TLR agonists, microbial components (e.g., LPS, lipoarabinomannan, and outer membrane proteins of selected Gram-negative bacteria), and intact microorganisms (**Figure 1**). PTX3 expression is inducible in a wide variety of cell types, including fibroblasts and endothelial cells, myeloid cells such monocytes, macrophages, and dendritic cells (DCs), synovial cells, chondrocytes, adipocytes, glial and mesangial cells, epithelial cells and retinal cells (1, 4, 5) (**Figure 1**). High density and oxidized low density lipoproteins (HDL and ox-LDL) induce PTX3 production in endothelial cells and primary vascular smooth muscle cells (SMC) (6, 7). Microbial ligands stimulate the release of PTX3 from neutrophils, where the protein, mostly produced by myeloid precursors, is constitutively stored in specific granules (8, 9). Among peripheral blood mononuclear cells, only monocytes exposed to inflammatory cytokines or LPS produce PTX3 mRNA (1). PTX3 expression is negatively regulated by IFN-γ, IL-4, dexamethasone, 1α,25-dihydroxivitamin D3, and prostaglandin E2 (5, 10, 11). PTX3 is also induced by ovulatory stimuli in granulosa cells, and when released it contributes to the structural architecture of cumulus oophorus extracellular matrix (12).

PTX3 expression and production is regulated by different signaling pathways, mainly depending on the cell type and/or stimuli. The NF-κB pathway controls PTX3 expression in conditions of IL-1 receptor- or TLR-dependent inflammation (13–15), while induction of the protein by TNFα in lung epithelial cells involves the c-Jun N-terminal kinase (JNK) pathway (16). HDL-induced PTX3 production in endothelial cells requires the activation of the PI3K/Akt pathway through G-coupled lysosphingolipid receptors (7).

The expression of the human PTX3 gene in physiological and inflammatory conditions is also regulated by epigenetic mechanisms. Hypermethylation of the promoter region and of an enhancer encompassing the second PTX3 exon (enhancer 2) (**Figure 2**) have been associated with PTX3 gene silencing in selected human tumors (e.g., colon rectal cancer and

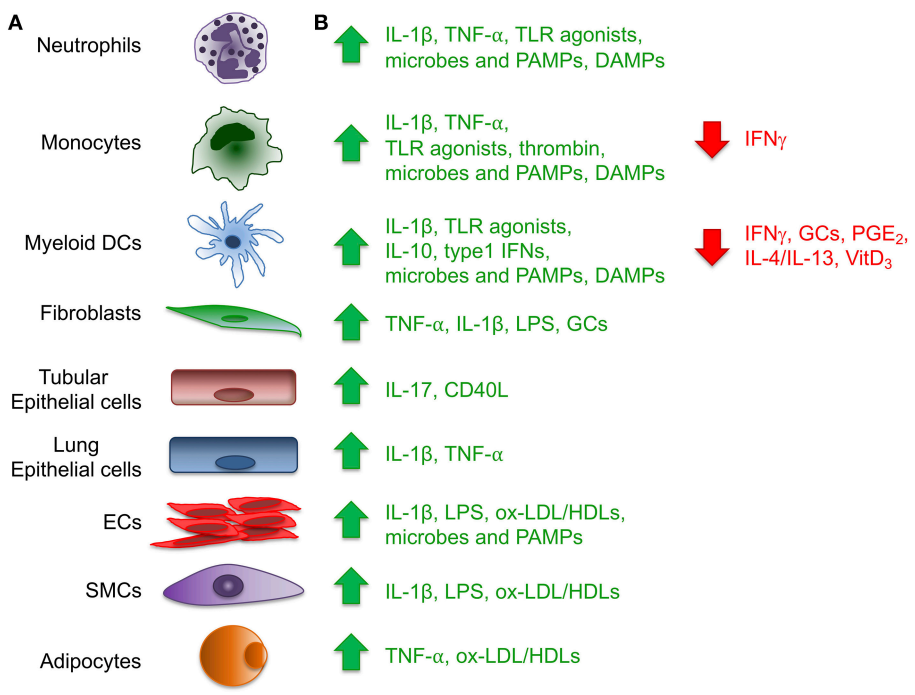


**FIGURE 1 |** Expression of the long pentraxin PTX3. Several inflammatory stimuli, including positive (green) and negative (red) regulators **(A)** can induce PTX3 expression in different cell types, including cells of the myeloid lineage, fibroblasts, epithelial cells derived from different tissues, vascular and lymphatic endothelial cells (ECs), smooth muscle cells (SMCs) and adipocytes **(B)**. (CD40L, CD40 ligand; DAMPs, damaged-associated molecular patterns; GCs, glucocorticoids; LPS, Lipopolysaccharide; ox-LDL/HDL, oxidized-low-density lipoprotein/high-density lipoprotein; PAMPs, pathogen-associated molecular patterns; $PGE_2$, prostaglandin E2; TLR, Toll like receptor; $VitD_3$, vitamin D3).

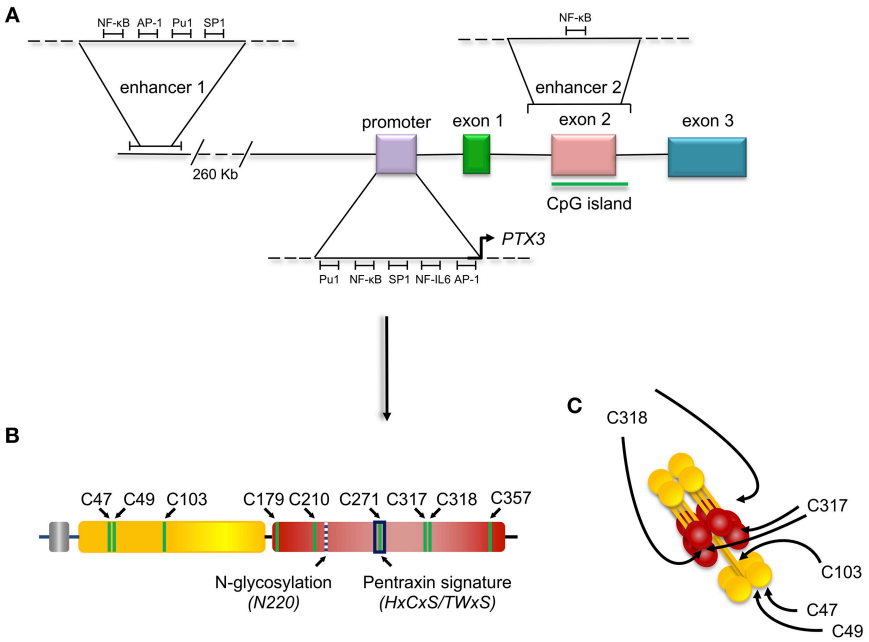


**FIGURE 2 |** Gene and protein structure of the long pentraxin PTX3. PTX3 gene is located in chromosome 3 and is organized in three exons: the first coding for the signal peptide, the second coding for the N-terminal domain, and the third coding for the C-terminal, pentraxin domain. The promoter of PTX3 contains several transcription factor binding sites, including Pu1, NF-κB, SP1, NF-IL6, and AP-1. Depicted are also the sites of PTX3 epigenetic regulation, mediated by two potentially active enhancers. The first enhancer—containing the transcription factor binding sites for NF-κB, AP-1, Pu1, and SP1 - is located 230 kb upstream of the promoter, while the second enhancer—containing the transcription factor binding site for NF-κB—encompasses the second PTX3 exon **(A)**. Schematic representation of the PTX3 protomer subunit with the leader peptide in gray, the N-terminal region in yellow, and the pentraxin C-terminal domain in red. Shown are the Cys residues involved in intra- (C179-C357, and C210-C271) and inter- (C47-C47, C49-C49, C103-C103, C317/318-C317/318) chain disulfide bonds, the N-glycosylation site at Asn220, and the pentraxin signature (a primary sequence motif highly conserved across pentraxins) **(B)**. 8 protomer subunits assemble into an octameric protein stabilized by inter-chain disulfides (as well as non-covalent interactions), which are pointed to by arrows in the picture **(C)**.

leiomyosarcoma) (14). Consistent with this, hypomethylation of these regulatory elements correlated with higher than normal protein levels in the plasma of coronary artery disease patients (17). Recent studies have characterized these epigenetic mechanisms in the context of different PTX3 expressing cells, including macrophages and fibroblasts, and have further addressed the epigenetic modifications occurring in the PTX3 gene in colorectal cancer (CRC) (18). These investigations identified a second enhancer located 230 kb upstream of the PTX3 gene promoter (enhancer 1, **Figure 2**). *In silico* and ChIP analysis revealed the binding of several transcription factors on this enhancer (18). Many of them, including the NF-κB subunit RelA, c-Jun, c-Fos, PU.1, and SP.1, are involved in the activation of inflammatory and immune responses, and are also known to control the activity of PTX3 promoter (**Figure 2**). The enhancer 2 was found only to bind NF-κB after TNF-α stimulation in macrophages, suggesting that this regulatory element could be important in the activation of tissue-specific transcription factors. However, the enhancer 2 could have a direct role in activating the expression of PTX3, since ChIP analysis showed its interaction with TAF1, a member of the transcription preinitiation complex (PIC) (18). Furthermore, STAT3-mediated hypermethylation of enhancer 1 has been associated with PTX3 gene silencing in colorectal cancers (18) (**Figure 2**). Interestingly, *in vitro* treatment of macrophages with glucocorticoid hormones, such as dexamethasone, results in M2 polarization, which is associated with immune suppression, and tumor progression (19). Noteworthy, one of the main markers of this phenotype is activation of STAT3, thus suggesting that STAT3-mediated PTX3 downregulation could be involved in carcinogenesis (see below).

Amongst PTX3 single nucleotide polymorphisms (SNPs), three (collectively forming an haplotypic block) have been found associated with susceptibility to infections including those caused by *Aspergillus fumigatus* (20–23), *Mycobacterium tuberculosis* (24), and *Pseudomonas aeruginosa* (25). Two of these SNPs are located in PTX3 intronic non-coding regions (rs2305619 in intron 1, and rs1840680 in intron 2), while the third is an exonic polymorphism that causes an amino acid substitution at position 48 (D48A, or rs3816527). Epidemiological studies have found a correlation between these three SNPs and PTX3 plasma levels, however the molecular mechanisms responsible for this association are still poorly understood. In this regard, individuals carrying the D48 allele have lower systemic concentrations of PTX3 (26). This might be due to faster rate of mRNA degradation, as proposed by Cunha et al. (20), or, alternatively, reduced activity of the second enhancer in the PTX3 gene (that encompasses the rs2305619, rs3816527, and rs1840680 SNPs)

(18). It is not currently possible to exclude a direct local effect of amino acid substitution on protein structure (therefore function).

PTX3 is a multimeric glycoprotein whose protomer subunits comprise 381 amino acids. The protein primary sequence is highly conserved in evolution (with 82% identity between human and murine PTX3), likely due to early selection and enduring maintenance in phylogenesis of fundamental structure/function relationships. Analogous to other members of the long pentraxin sub-family, which includes guinea pig apexin, rat, human, and murine neuronal pentraxins 1 (NP1, or NPTX1) and 2 (NP2, also known as Narp or NPTX2), the putative integral membrane pentraxin NRP, and PTX4 (27), the PTX3 protomer is organized into an N-terminal region and a 203 amino acids long C-terminal domain with homology to the short pentraxins CRP and SAP (28) (**Figure 2**).

The N-terminal domain has no obvious similarity to any protein of known structure. However, secondary structure predictions indicate that this domain mostly comprises α-helical elements, three of which are likely organized into coiled-coils (6). Furthermore, the N-terminal end of this domain (amino acids 18–54) is predicted to be intrinsically disordered, a property that might provide the PTX3 protein with structural and functional versatility (29), thus contributing to the remarkable complexity of its interaction network (3).

The C-terminal domain shares with the short pentraxins a considerable degree of homology (with up to 57% similarity), which has allowed generation of 3D models based on the crystal structures of CRP (PDBID:1b09) and SAP (PDBID:1sac) (30–32) indicating that it adopts a β-jelly roll topology, stabilized by two intra-chain disulfide bonds (33). Two additional cysteine residues (i.e., Cys317 and Cys318) are involved both in intra- and inter-chain disulfides that, in conjunction with inter-chain bonds made by cysteine residues of the N-terminal domain, support the quaternary structure of the mature PTX3 protein (34) (**Figure 2**).

The pentraxin domain of PTX3 bears a single N-glycosylation site at Asn220 that, in a recombinant form of the protein from CHO cells, is fully occupied by complex type oligosaccharides, mainly fucosylated and sialylated biantennary sugars with a minor fraction of tri-and tetraantennary glycans. N-linked complex type glycosylation occurs in the natural protein made by human cells too (32), and mediates some of the PTX3 biological functions, including inhibition of influenza A virus hemagglutination (35, 36) and recognition of P-selectin (37). Furthermore, protein glycosylation (with major regard to sialylation) modulates the interaction of PTX3 with C1q, and the regulatory effect of PTX3 on complement activation via the classical pathway (32). We speculate that the molecular crosstalk between PTX3 and a range of diverse ligands involves a common glycan code, whereby tissue- and microenvironment-specific changes in the protein glycosylation profile might regulate its biological properties [see (38) for a review].

The modular (i.e., N- and C-domains) and sub-modular (i.e., coiled-coils and intrinsically disordered regions of the N-domain) nature of the protomer likely endows PTX3 with the structural versatility that is required to support its diverse interactions, thereby its biological functions. In this regard, the N-terminal region of the protein contains binding sites for fibroblast growth factor 2 (FGF2), inter-α-inhibitor (IαI), TNF-α-induced protein 6 (TNFAIP6 or TSG-6), plasminogen (Plg), fibrin, and conidia of *A. fumigatus* (15, 39–42). C1q and P-selectin mostly interact with the pentraxin-like domain (28, 37), whereas both domains have been implicated in the recognition of complement factor H (43, 44), and Ficolin-1 (45).

In addition to the multidomain organization, PTX3 has a complex quaternary structure with high-order oligomers stabilized by disulfide bonds. Mass spectrometry and site-directed mutagenesis indicate that PTX3 is made of covalent octamers (i.e., with a molecular mass of 340 kDa), through inter-chain disulfides bridges (34) (**Figure 2**). A low-resolution model based on data from electron microscopy and small angle X-ray scattering shows that eight PTX3 protomers fold into an elongated molecule with two differently sized domains interconnected by a stalk region, and a pseudo 4 fold symmetry along the longitudinal axis (33). Such quaternary structure is unique among pentraxins, where CRP and SAP both share a prototypical pentameric planar symmetry (46, 47). The only other pentraxin that forms an octamer is SAP from *Limulus polyphemus*, which, however, folds into a doubly stacked octameric ring (48). In addition, the oligomeric organization has important implications in its ligand binding properties. For example, the PTX3 octamer contains two binding sites for FGF2, and tetrameric recombinant forms of the N-terminal domain recapitulate the inhibitory functions of the full length protein toward this factor both in angiogenesis (33, 39) and bone deposition (49). However, dimeric forms of the N-terminal domain retain binding to IαI and TSG-6, thereby the octameric PTX3 protein is likely endowed with multiple (at least four) binding sites for each of these ligands, and can act as a nodal molecule in cross-linking hyaluronic acid in the extracellular matrix (41, 50).

High resolution models are urgently needed to disentangle the structural complexity of this long pentraxin and shed light on its structure/function relationships, some of which are remarkably different to those classically described for the short pentraxins.

## ROLE OF PTX3 IN TISSUE REPAIR

Beyond its role as the first line of resistance against pathogens, innate immunity is involved in initiating the process of tissue repair (51–53). The cellular arm of the innate immune system senses specific DAMPs and regulates inflammatory responses at sites of damage (52, 54). The humoral arm of the innate immunity has different and complex roles ranging from the clearance of apoptotic cells and regulation of immune cell migration and activation, to regulation of remodeling cell activity (55, 56). For instance, SAP regulates fibrosis by inhibiting the alternative activation of macrophages via FcγRs (57) or by modulating immune cell activities via DC-SIGN (58). Pentraxins and components of the complement system also interact with elements present in the extracellular matrix (ECM), thus suggesting additional regulatory roles of the innate immune system in the tissue response to injury (53, 59). On the other hand, different ECM components, such as fibronectin,

**TABLE 1 |** Biological functions of PTX3 in tissue remodeling and cancer.

| | | Ligands | Functions |
|---|---|---|---|
| Tissue remodeling | Fertility | Hyaluronic acid/TSG-6/inter-α-trypsin inhibitor | Incorporation of PTX3 into the hyaluronic acid-rich ECM surrounding the pre-ovulatory oocyte (i.e., dependent on the presence of Iαl and TSG-6) is essential for cumulus matrix stability and female fertility (12) |
| | Synaptogenesis | ND | Pentraxin 3 regulates synaptic function by inducing AMPA receptor clustering via ECM remodeling and beta1-integrin (60) |
| | Bone turn-over | FGF2 | PTX3 is expressed by osteoblast progenitors, and is essential for matrix mineralization both in bone tissue homeostasis and fracture repair (49) |
| | Angiogenesis | FGFs | PTX3 recognizes selected FGFs via its N-terminal domain, and inhibits their binding to FGF receptors, thus preventing endothelial/smooth muscle cell proliferation *in vitro* and angiogenesis/neointima formation *in vivo* (61) |
| | Fibrinolysis | Fibrinogen/ fibrin/ plasminogen | PTX3 derived from macrophages and mesenchymal cells forms a tripartite PTX3/fibrin/plasminogen complex at acidic pH that promotes pericellular fibrinolysis (15)<br>In a mouse model of arterial thrombosis, PTX3 inhibits platelet adhesion and aggregation by targeting fibrinogen and collagen (62) |
| Cancer | Anti-tumoral | factor H | In murine models of chemically induced mesenchymal and epithelial carcinogenesis, PTX3 dampens cancer-related, complement-dependent inflammation (14) |
| | | FGFs | PTX3 inhibits the FGF-driven tumor cell proliferation *in vitro*, tumor growth, angiogenesis and metastatic potential *in vivo* in models of melanoma, prostate, breast and lung cancer (63) |
| | Pro-tumoral | Not defined | PTX3 promotes tumor cell migration, invasion and metastasis, and protein levels correlate with prognosis and/or tumor grade in different types of cancer (64, 65) |

mindin, osteopontin, and vitronectin, interact with microbes and have opsonic activity (53, 66), thus suggesting a close evolutionary link between recognition of microbial moieties and ECM components.

In different mouse models of non-infectious tissue damage, deficiency of the long pentraxin PTX3 was associated with altered thrombotic response to the lesion, increased deposition and persistence of fibrin, followed by increased collagen deposition (15, 62, 67–69) (**Figure 3**).

Following tissue damage, PTX3 was induced in the blood and locally in response to TLR activation and IL-1β amplification (15). Interestingly, PTX3 is reported to be among the genes induced by thrombin in monocytes (70). At sites of wound, PTX3, released by neutrophils (9), localized in the clot and in the pericellular matrix of macrophages and PDGFRα$^+$FAP$^+$ cells of mesenchymal origin that collectively invade the wound site (15, 68).

In skin wounding, PTX3-deficiency was associated with increased deposition of fibrin, followed by increased deposition of collagen, fibroplasia, epithelial hyperplasia, and delayed healing (15). A premature contraction of the wound was observed in PTX3-deficient mice, in agreement with an augmented content of platelet-derived factors (e.g., thrombin, serotonin, PDGF, TGFβ) known to be responsible of skin wound contraction by SMC located in the *panniculus carnosus* (71–73). Indeed, administration of pharmacological inhibitors of coagulation and platelet activation reverted these defects, including premature wound contraction and increased collagen deposition (15). Therefore, an altered haemostatic and fibrinolytic response triggered the alterations associated with PTX3-deficiency in skin wound healing.

In $CCl_4$-induced liver injury, PTX3 was localized in necroinflammatory areas and fibrotic portal tracts, and was associated with neutrophils, macrophages and mesenchymal stromal cells (MSCs) (15). In this setting, PTX3-deficiency was associated with increased centrilobular thrombosis and fibrin deposition in necroinflammatory areas, followed by severe impairment of repair and fibrosis, as assessed by increased α-SMA$^+$ fibroblastic cells and collagen deposition (15). Similar abnormalities were reported in different models of lung injury (15, 74). In addition, PTX3 played a protective role in a murine model of ischemic injury of the brain, where it was involved in the resolution of edema and glial scar formation (75). PTX3 administration reverted the IL-6/STAT3-dependent interstitial fibrosis in a mouse model of acute kidney injury (76).

Fibrin is deposited after tissue injury and its subsequent timely removal is essential for several aspects of tissue repair in major organs, as well as in a wide range of pathological conditions (77, 78). In these contexts, a defective fibrinolysis is described as an etiopathological factor leading to reduced remodeling and altered connective tissue formation (79–81). Macrophages and MSCs enter the wound site invading the inflammatory matrix through plasmin-mediated mechanisms, allowing fibrin removal and consequent deposition of granulation tissue rich in type

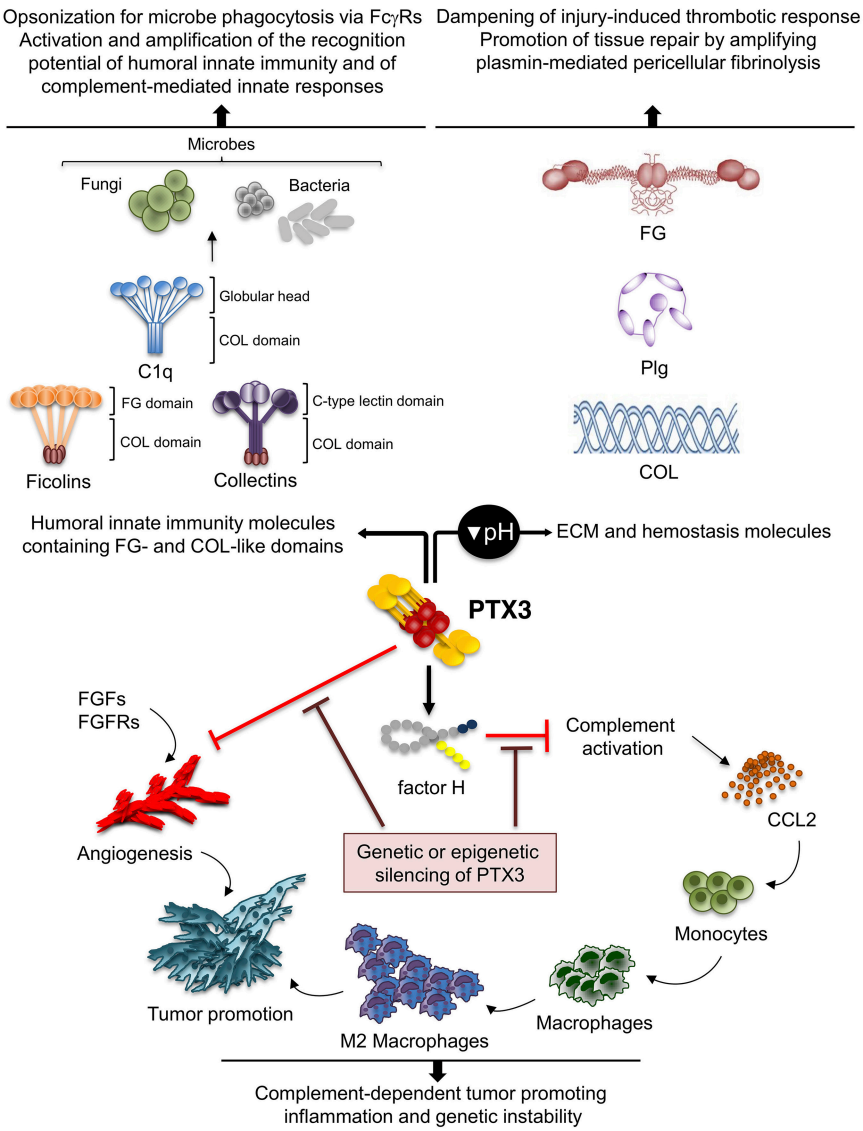


**FIGURE 3 |** Role of PTX3 at the crossroad between innate immunity, hemostasis and tissue repair, and cancer. By interacting with fibrinogen (FG)- and collagen (COL)-like domains of other fluid-phase PRMs, such as C1q, ficolins, and collectins, PTX3 amplifies the recognition potential of these members of the humoral innate immunity, favoring complement-mediated antimicrobial resistance, and effector functions (upper left panel). By interacting at acidic pH with fibrinogen/fibrin and collagen, as well as plasminogen (Plg), PTX3 tunes injury-induced thrombotic responses and favors pericellular fibrinolysis, contributing to tissue remodeling and repair (upper right panel). By interacting with factor H and FGFs, PTX3 controls complement-dependent tumor promoting inflammation, including CCL2-dependent macrophage recruitment and M2-like skewing, as well as FGF-dependent neo-angiogenesis. This anti-tumor potential of PTX3 is hampered by genetic or epigenetic silencing of PTX3 leading to enhanced tumor growth (lower panel).

I collagen, as well as other ECM proteins (71, 81, 82). The alterations in tissue repair observed in PTX3-deficient mice have been attributed to defective plasmin-mediated invasion and fibrinolysis by tissue remodeling cells, namely macrophages and MSCs (15, 68). Also *in vitro*, PTX3-deficient macrophages and fibroblasts showed defective fibrinolytic activity (15), thus suggesting that PTX3 contributes to the progression of a normal and efficient pericellular fibrinolysis which promotes repair.

PTX3 was shown to interact specifically with Plg and fibrin at acidic pH (optimal range from 6.5 to 5.5), but not at neutral pH (15) (**Figure 3**). Interestingly, the interaction of PTX3 with members of the ficolin and collectin family, occurring through their fibrinogen-like domain and collagen domains, is facilitated in an acidic microenvironment (45, 83, 84). Acidification of the wound site, which occurs as a result of cellular metabolic adaptation to trauma-induced tissue hypoperfusion, has functional relevance in the healing outcome and involves several processes including cell adhesion, migration, and proliferation (53, 85). The interaction of PTX3 with fibrin and Plg occurs through different sites in its N-terminal domain

and PTX3 does not interfere with the interaction between fibrin and Plg. In mapping experiments, PTX3 did not interact with the Plg Kringle 1 domain, indispensable for Plg initial recruitment on lysine-rich portions of fibrin and/or on cell surface, but specifically bound the Kringle 5 domain (15). This interaction could be crucial for triggering Plg conformational changes that allow a transition of the molecule from a closed-inactive to an open and functional form (86–88). This conformational transition is essential for Plg conversion into plasmin operated by Plg activators (PAs) and central in fibrin removal in the thrombus (78, 89). Indeed, in cell-free fibrinolysis assays, the interaction of PTX3 with Plg determined the enhancement of plasmin-mediated fibrin gel degradation triggered by urokinase PA (uPA) and tissue-type plasminogen activator (tPA) at acidic, but not neutral, pH. Plg activators are neutral proteases (90). The dependence on acidic pH of the interaction of PTX3 with fibrin and Plg ensures that it does not occur in the circulation but rather at sites of tissue repair and in thrombi, where it supports fibrinolysis in acidic environments. Thus, the acidic pH acts as a "switch on" signal for this function of PTX3 (**Figure 3**). The interaction with fibrin and Plg is restricted to PTX3, since no similar function has been reported for short pentraxins. Only one study reported the interaction of SAP with fibrin and consequent modulation of *in vitro* formation of clots (91), however the underlying molecular mechanism has never been characterized.

In a model of arterial thrombosis, PTX3 produced by the vessel wall had a critical protective role in the modulation of thrombus formation (62). Fibrinogen pre-incubation with PTX3 significantly reduced platelet aggregation in the presence of collagen. Likewise, pre-incubation of collagen with PTX3 attenuated platelet aggregation in the presence of fibrinogen. These effects were dependent, respectively, on the N-terminal or C-terminal domain of PTX3, and suggested that in arterial thrombosis PTX3 disfavored the pro-thrombotic activity of fibrinogen and collagen (62). PTX3 interacts with P-selectin and tunes P-selectin-dependent neutrophil extravasation (37). However, in arterial thrombosis PTX3 did not influence P-selectin-dependent platelet-leukocyte and platelet-endothelium aggregation (62, 92). Although initially PTX3 has been reported to induce tissue factor (TF) expression in endothelial cells and monocytes (93), subsequent *in vitro* and *in vivo* studies did not confirm this result (15, 62). Indeed, in the thrombosis model TF expression in the aorta of PTX3-deficient mice and controls was similar (62). These results are in line with the evidence that PTX3 plays protective functions in vascular pathologies. Indeed, PTX3 overexpression limited the neointimal thickening after rat carotid artery balloon injury (94) and PTX3-deficiency was associated with augmented infarct area following myocardial ischemia/reperfusion injury (13), increased atherosclerosis and augmented macrophage accumulation and inflammation in atherosclerotic plaques (95).

The administration of MSCs to acute or chronic wounds improves wound healing by increasing granulation tissue formation, accelerating re-epithelialization and stimulating angiogenesis through paracrine signaling (96), thus prompting new studies on the treatment of non-healing wounds resulting from burns (97) and Crohn's disease (98). In wounded skin, MSCs acted as a potent promoter of tissue repair and remodeling, whereas PTX3-deficient MSCs showed compromised recruitment and invasiveness at the site of damage, due to defective fibrinolysis, and therefore exerted a compromised therapeutic effect causing delayed healing (68).

Similar results were obtained in a mouse model of acid aspiration-dependent acute lung injury (69), mimicking acute respiratory distress syndrome (ARDS) caused by aspiration of gastric contents (99). In the mouse model, beneficial effects of treatment with MSCs on the early acute inflammatory reaction, pulmonary edema and long-term fibrotic evolution and pulmonary function have been observed. The administration of PTX3-deficient MSCs was less effective in limiting the pulmonary edema at 24 h after acid aspiration, and was associated with defective fibrinolytic activity, resulting at later time points in increased pulmonary fibrosis and therefore in a not significant increase of lung function. Levels of D-dimer significantly increased in mice after treatment with MSCs indicating their ability to modulate pulmonary fibrinolysis and thus affecting fibrotic scarring. The administration of PTX3-deficient MSCs resulted in decreased lung levels of D-dimer compared to PTX3-competent MSCs, thus attributing to a defective fibrinolysis the observed reduced therapeutic effects of PTX3-deficient MSCs (69).

Recently, PTX3 has been identified as an important molecule contributing to bone homeostasis and remodeling (49). Under homoeostatic conditions, histological analysis of distal femurs of PTX3-deficient mice did not show differences in the number of active trabecular and endosteal $TRAP^+$ osteoclasts. However, micro-computed tomography showed a lower bone volume attributable to suppression of the osteoblast function. In a fracture and regeneration model of the tibia diaphysis, PTX3-deficient mice showed a lower bone formation and repair rate than controls, in agreement with lower percentage of mineralized callous tissue and lower collagen I expression compared to controls. Under conditions of homeostasis and bone repair, the expression of PTX3 was associated with non-hematopoietic/non-endothelial periosteal cells, in particular, with $CD51^+$ and $\alpha$-$SMA^+$ osteoprogenitor subsets. FGF2 is expressed during the early stages of bone formation and is abundantly accumulated in the bone matrix, where it participates in osteoblastogenesis and skeletal remodeling (100). In agreement with the property of PTX3 to bind FGF2 and prevent FGF2-dependent activities, PTX3 reversed the negative effect of FGF2 on osteoblast differentiation from bone marrow stromal cells *in vitro*, and the PTX3 N-terminal domain alone recapitulated this activity. Therefore, PTX3 produced by osteoblast lineage cells, acts as a bone protective factor, important to unlock osteoblast maturation by antagonizing the FGF2 effect during bone formation (49). Bone formation during fracture repair initiates around extravascular deposits of fibrin-rich matrix and subsequent defects in fibrin clearance from the fracture site severely impair healing (101). Fibrinogen depletion in Plg-deficient animals restores a normal fracture repair (102), thus proving that inefficient fibrin turnover is essential for bone repair. Therefore, further studies are needed to address the relevance

of PTX3-dependent modulation of the fibrinolytic system in bone repair.

All together, these studies have provided several lines of evidence that the involvement of PTX3 in tissue remodeling and repair depends on the recognition of matrix molecules and highlight the connection and interplay between haemostasis and immunity (**Figure 3**).

## ROLE OF PTX3 IN CANCER

Inflammation is a component of the tumor microenvironment promoting tumor development and growth (103). Since PTX3 is expressed in inflammatory conditions and acts as a tuner of complement-activation and leukocyte recruitment, it was hypothesized that PTX3 was involved in cancer-related inflammation. Genetic studies in mice showed that PTX3-deficiency caused increased susceptibility to mesenchymal and epithelial carcinogenesis in the models of 3-methylcholanthrene (3-MCA)-induced sarcomagenesis, and 7,12-dimethylbenz [a] anthracene/terephthalic acid (DMBA/TPA)-induced skin carcinogenesis (14). In these tumors, infiltrating macrophages and endothelial cells were the major source of PTX3 in response to locally produced IL-1. PTX3-deficient tumors were characterized by increased macrophage infiltration, pro-inflammatory cytokine production, complement activation, and angiogenesis, as well as increased oxidative DNA damage and genetic instability, compared to wild type tumors (14). In this context, PTX3 regulated complement activation by interacting with factor H, a complement regulator, and as a consequence, macrophage recruitment and M2-like polarization (14) (**Figure 3**).

These data are in line with recent studies showing that the anaphylatoxins C3a and C5a may contribute to cancer-related inflammation, recruit myeloid suppressor cells, and promote IL-1β and IL-17 response in neutrophils thus promoting colon carcinogenesis (104–107).

In addition to regulate complement, PTX3 was shown to bind selected fibroblast growth factors (FGFs), including FGF2, and FGF8b through the N-terminal domain, and inhibit FGF-dependent angiogenic responses (6). This effect was shown to be responsible of the anti-tumor activity of PTX3 in FGF-dependent transplanted murine tumors, including prostate cancer and melanoma and fibrosarcoma (108–110) (**Figure 3**). The role of PTX3-mediated anti-angiogenic activity has not been addressed so far in primary carcinogenesis.

In line with these preclinical studies, the human PTX3 promoter and regulatory regions were shown to be epigenetically modified through hypermethylation in selected human mesenchymal and epithelial cancers, such as esophageal squamous cell carcinoma (111) and colorectal cancer (14, 18, 112), leading to silencing of PTX3 protein expression. Thus, genetic studies in mice and epigenetic studies in humans demonstrate that PTX3 behaves as an extrinsic oncosuppressor gene by acting at the level of complement-mediated, macrophage-sustained, tumor promoting inflammation.

In contrast to the genetic evidence outlined above, several studies performed with PTX3 overexpressing cells suggest that the protein may play a pro-tumorigenic role by promoting tumor cell migration and invasion (head and neck tumors, cervical cancer) or proliferation (glioma), epithelial-mesenchymal transition (hepatocellular carcinoma) and macrophage chemotaxis (64, 65, 113, 114). In basal-like breast cancers, PTX3 was found to be a critical target of oncogenic phosphoinositide 3-kinase signaling and NF-κB-dependent pathways, and to be associated with PI3K-induced stem cell-like traits (115). However, none of these pro-tumoral effects of PTX3 has been confirmed in gene targeted animals or in carcinogenesis models.

These contradictory results suggest that PTX3 may have a dual role in cancer, likely depending on the type of cancer, or on the cells producing it, in particular tumor cells or infiltrating macrophages, fibroblasts and endothelial cells. Further genetic studies in mice and humans will be necessary to clarify these context-dependent findings.

## PTX3 AS MARKER OF CANCER PROGRESSION

Several lines of evidence indicate that PTX3 could be a local or systemic marker of cancer-related inflammation. Upregulation of PTX3 gene expression was observed associated to a stromal signature in ovarian cancer (116), and has been described in aggressive breast cancer and distant bone metastases (117–119), anaplastic thyroid carcinoma (120), soft tissue liposarcoma (121), prostate cancer (122), and glioblastoma (123). Increased circulating levels of PTX3 were observed in myeloproliferative neoplasms (124), soft tissue sarcomas (125), lung cancers (126–128), pancreatic carcinomas (129), gliomas (130), and hepatocellular carcinomas (131). In pancreatic carcinoma, high PTX3 levels were associated with advanced clinical stage and poor overall survival. In the same cohort of patients with invasive ductal pancreatic carcinoma at stage III and IV, plasmatic CRP levels were similarly associated with a worst prognosis (129).

Different studies analyzed the role of PTX3 as biomarker in lung cancer. Through a proteomic effort, Planque et al. reported in 2009 that PTX3 is produced by lung cancer cells. This result was confirmed in patients with lung cancer, in which PTX3 plasma levels resulted significantly increased compared to healthy subjects (126). It was subsequently observed that PTX3 circulating levels were related to disease aggressiveness and progression, irrespective to the subtypes and histotypes of lung cancer (127). In addition, ROC analysis indicated that PTX3 could discriminate between cancer patients and heavy smokers at high risk for lung cancer (127). Similarly, high PTX3 levels were correlated with worse progression-free survival in patients with lung cancer and chronic obstructive pulmonary disease (132), and with overall survival and disease-free survival in small-cell lung carcinoma (SCLC) (133). A recent study on 1358 individuals at high risk for lung cancer demonstrated that PTX3 levels were not predictive of pathology occurrence (128). In the 110 patients of this cohort that developed resectable lung cancer, preoperative PTX3 plasma levels were higher compared with those of cancer-free heavy smokers, but were not predictor of outcomes (128).

In prostate cancer patients, circulating levels of PTX3 were higher compared to patients with prostatic inflammation,

while serum levels of prostate-specific antigen (PSA) and CRP were not different between the two groups (134). In CRC, PTX3 circulating levels were significantly increased compared to healthy individuals or to patients with colorectal polyps, representing an independent prognostic factor for CRC patients (135). PTX3 levels were reduced at discharge after surgery, and a subsequent increase during the follow-up was associated to recurrence. Preoperative PTX3 levels were significantly associated to clinical stage and to a better postoperative prognosis in a cohort of 263 primary CRC patients (136). In another small group of CRC patients, PTX3 serum levels combined with CXCL8 and VEGF levels were efficiently predicting relapsing cases (137). Since epigenetic studies showed PTX3 silencing in colorectal tumor cells (18), increased PTX3 plasma levels in these patients reasonably reflect cancer-related inflammation associated with tumor growth. Patients with hepatocellular carcinoma (HCC) showed higher PTX3 levels than individuals with fibrosis (131). Interestingly, in these patients the A/A genotype for rs1840680 and rs2305619, resulting in higher PTX3 plasma levels, was also significantly associated with the presence of HCC.

Beside an evaluation of PTX3 as soluble biomarker in cancer, some reports also investigated PTX3 expression in cancer tissues. In hepatocellular carcinoma, PTX3 expression was analyzed after liver resection in tumoral and adjacent normal tissue and a higher PTX3 expression was observed in the tumoral area. PTX3 expression was correlated with advanced stage, larger tumor size, presence of intra-hepatic metastases, portal vein tumor thrombosis and liver cirrhosis (65). Overall, high PTX3 expression in tumor tissue from HCC was associated with lower survival after surgery. Immunohistochemical analysis on tissue specimens from lung cancer revealed an interstitial expression of PTX3 in the neoplastic area associated with shorter survival, while no staining was observed in normal lung parenchyma (128). In tissue samples from prostate cancer patients, PTX3 is expressed at higher levels compared to patients with prostatic inflammation (134).

Overall the data reported above strongly suggest that PTX3 is overexpressed locally or systemically in different neoplastic conditions, and could likely represent a novel promising prognostic factor for cancer patients. In particular, as discussed above and by Giacomini et al. (63), PTX3 originated from endothelial cells, tumor-associated fibroblasts and infiltrating myeloid cells likely reflects microenvironment or systemic inflammation associated with tumor progression, and not its involvement in the pathogenesis. Indeed, the role of PTX3 in neoplastic transformation and growth has been shown to depend on the context and to be influenced by its property to interact with different molecules in the tumor environment.

## CONCLUDING REMARKS

Based on genetic studies in mice and human genetic associations, PTX3 is a well-recognized mediator of innate resistance to selected infections, acting by modulating complement activation, opsonizing microbes and facilitating their clearance through phagocytosis. Moreover, by interacting with the fibrinogen-like and collagen-like domains of ficolins and collectins, PTX3 amplifies the recognition potential of the humoral innate immunity (1) (**Figure 3**). These lines of evidence provide the rational for therapeutic and diagnostic translation of this molecule in infectious conditions.

Several studies presented in this review also indicate that PTX3 is involved in tissue remodeling and repair in sterile conditions through the recognition of matrix molecules, and regulates the thrombotic response and fibrin remodeling, thus playing a non-redundant role in the orchestration of the tissue repair process (**Figure 3**). Other humoral PRMs interact with ECM components (e.g., C1q, collectins, CRP, SAP), or contain collagen- and fibrinogen-like domains (e.g., ficolins, MBL, collectins), and several ECM molecules recognize microbial moieties and have opsonic activity (e.g., fibronectin, mindin, osteopontin, vitronectin). These lines of evidence support the view that inflammation, innate immunity, haemostasis, and tissue repair are functionally linked and that the recognition of microbial moieties and extracellular matrix molecules by the humoral arm of innate immunity is evolutionarily conserved.

Studies reported here finally show that PTX3 is involved in tuning carcinogenesis through the modulation of cancer-related inflammation or angiogenesis in specific cancer types (**Figure 3**). However, other studies propose that in specific models PTX3 has a pro-tumorigenic function, by promoting tumor cell migration and invasion and macrophage infiltration, suggesting that PTX3 may have different functions on carcinogenesis depending on the tissue and cancer type, and possibly cell- and stimulus-dependent PTX3 glycosylation (and sialylation) profiles, which needs further dissection.

Cancer is considered a "non-healing wound" (138), since wound-healing responses favoring tumor growth are activated in the tumor microenvironment. These include extravascular deposition of fibrin that acts as a provisional stroma for stromal and immune cells migration, angiogenesis and ECM deposition and remodeling (139). Fibrin degradation, vascular resorption and collagen synthesis result in formation of dense fibrous connective tissue ("scar" in wounds and "desmoplasia" in cancer). These responses are similar in tumors and wounds, but in tumors they are not self-limited. PTX3 by interacting with fibrin matrix (15), FGF2 (109), and complement components (14) regulates the main common processes in tissue repair (139–141) and in tumor-promoting angiogenesis and inflammation (**Table 1**) (61, 142, 143), thus suggesting that the roles of PTX3 in tissue repair and cancer are functionally associated.

## AUTHOR CONTRIBUTIONS

CG and BB revised the manuscript and redacted the final version. AD, MS, AI, EM, AM, CG, and BB contributed to the writing of the manuscript.

## ACKNOWLEDGMENTS

The financial support of Fondazione Cariplo [Contract no. 2015-0564 (to AM) and 2016-0568 (to EM)]; Ministero della Salute (RF-2013-02355470 to CG, GR-2011-02349539 to AI); Ministry of Education, University and Research (PRIN 2015YYKPNN); the European Research Council (ERC – N° 669415 to AM); Fondazione Beppe e Nuccy Angiolini; and the Italian Association for Cancer Research (AIRC IG 19014 to AM, AIRC IG 21714 to CG, AIRC 5x1000 9962 and 21147) is gratefully acknowledged.

## REFERENCES

1. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol.* (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
2. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest.* (2003) 111:1805–12. doi: 10.1172/JCI200318921
3. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. Ptx3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiol Rev.* (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
4. Doni A, Peri G, Chieppa M, Allavena P, Pasqualini F, Vago L, et al. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. *Eur J Immunol.* (2003) 33:2886–93. doi: 10.1002/eji.200324390
5. Woo JM, Kwon MY, Shin DY, Kang YH, Hwang N, Chung SW. Human retinal pigment epithelial cells express the long pentraxin PTX3. *Mol Vis.* (2013) 19:303–10.
6. Presta M, Camozzi M, Salvatori G, Rusnati M. Role of the soluble pattern recognition receptor PTX3 in vascular biology. *J Cell Mol Med.* (2007) 11:723–38. doi: 10.1111/j.1582-4934.2007.00061.x
7. Norata GD, Marchesi P, Pirillo A, Uboldi P, Chiesa G, Maina V, et al. Long pentraxin 3, a key component of innate immunity, is modulated by high-density lipoproteins in endothelial cells. *Arterioscler Thromb Vasc Biol.* (2008) 28:925–31. doi: 10.1161/ATVBAHA.107.160606
8. Imamura M, Kawasaki T, Savchenko AS, Ohashi R, Jiang S, Miyamoto K, et al. Lipopolysaccharide induced expression of pentraxin 3 in human neutrophils and monocyte-derived macrophages. *Cell Immunol.* (2007) 248:86–94. doi: 10.1016/j.cellimm.2007.09.003
9. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, et al. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med.* (2007) 204:793–804. doi: 10.1084/jem.20061301
10. Doni A, Michela M, Bottazzi B, Peri G, Valentino S, Polentarutti N, et al. Regulation of PTX3, a key component of humoral innate immunity in human dendritic cells: stimulation by IL-10 and inhibition by IFN-gamma. *J Leukoc Biol.* (2006) 79:797–802. doi: 10.1189/jlb.0905493
11. Doni A, Mantovani G, Porta C, Tuckermann J, Reichardt HM, Kleiman A, et al. Cell-specific regulation of PTX3 by glucocorticoid hormones in hematopoietic and non-hematopoietic cells. *J Biol Chem.* (2008) 283:29983–92. doi: 10.1074/jbc.M805631200
12. Camaioni A, Klinger FG, Campagnolo L, Salustri A. The influence of Pentraxin 3 on the ovarian function and its impact on fertility. *Front Immunol.* (2018) 9:2808. doi: 10.3389/fimmu.2018.02808
13. Salio M, Chimenti S, De Angelis N, Molla F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. *Circulation.* (2008) 117:1055–64. doi: 10.1161/CIRCULATIONAHA.107.749234
14. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell.* (2015) 160:700–14. doi: 10.1016/j.cell.2015.01.004
15. Doni A, Musso T, Morone D, Bastone A, Zambelli V, Sironi M, et al. An acidic microenvironment sets the humoral pattern recognition molecule PTX3 in a tissue repair mode. *J Exp Med.* (2015) 212:905–25. doi: 10.1084/jem.20141268
16. Han B, Mura M, Andrade CF, Okutani D, Lodyga M, dos Santos CC, et al. TNFalpha-induced long pentraxin PTX3 expression in human lung epithelial cells via JNK. *J Immunol.* (2005) 175:8303–11. doi: 10.4049/jimmunol.175.12.8303
17. Guo TM, Huang LL, Liu K, Ke L, Luo ZJ, Li YQ, et al. Pentraxin 3 (PTX3) promoter methylation associated with PTX3 plasma levels and neutrophil to lymphocyte ratio in coronary artery disease. *J Geriatr Cardiol.* (2016) 13:712–7. doi: 10.11909/j.issn.1671-5411.2016.08.010
18. Rubino M, Kunderfranco P, Basso G, Greco CM, Pasqualini F, Serio S, et al. Epigenetic regulation of the extrinsic oncosuppressor PTX3 gene in inflammation and cancer. *Oncoimmunology.* (2017) 6:e1333215. doi: 10.1080/2162402X.2017.1333215
19. Sica A, Mantovani A. Macrophage plasticity and polarization: *in vivo* veritas. *J Clin Invest.* (2012) 122:787–95. doi: 10.1172/JCI59643
20. Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med.* (2014) 370:421–32. doi: 10.1056/NEJMoa1211161
21. Wojtowicz A, Lecompte TD, Bibert S, Manuel O, Rueger S, Berger C, et al. PTX3 Polymorphisms and invasive mold infections after solid organ transplant. *Clin Infect Dis.* (2015) 61:619–22. doi: 10.1093/cid/civ386
22. Fisher CE, Hohl TM, Fan W, Storer BE, Levine DM, Zhao LP, et al. Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. *Blood.* (2017) 129:2693–701. doi: 10.1182/blood-2016-10-743294
23. Brunel AS, Wojtowicz A, Lamoth F, Spertini O, Neofytos D, Calandra T, et al. Pentraxin-3 polymorphisms and invasive mold infections in acute leukemia patients with intensive chemotherapy. *Haematologica.* (2018) 103:e527–30. doi: 10.3324/haematol.2018.195453
24. Olesen R, Wejse C, Velez DR, Bisseye C, Sodemann M, Aaby P, et al. DC-SIGN (CD209), Pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West-Africans. *Genes Immun.* (2007) 8:456–67. doi: 10.1038/sj.gene.6364410
25. Chiarini M, Sabelli C, Melotti P, Garlanda C, Savoldi G, Mazza C, et al. PTX3 genetic variations affect the risk of *Pseudomonas aeruginosa* airway colonization in cystic fibrosis patients. *Genes Immun.* (2010) 11:665–70. doi: 10.1038/gene.2010.41
26. Barbati E, Specchia C, Villella M, Rossi ML, Barlera S, Bottazzi B, et al. Influence of pentraxin 3 (PTX3) genetic variants on myocardial infarction risk and PTX3 plasma levels. *PLoS ONE.* (2012) 7:e53030. doi: 10.1371/journal.pone.0053030
27. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
28. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem.* (1997) 272:32817–23. doi: 10.1074/jbc.272.52.32817
29. Dyson HJ. Making sense of intrinsically disordered proteins. *Biophys J.* (2016) 110:1013–6. doi: 10.1016/j.bpj.2016.01.030
30. Goodman AR, Cardozo T, Abagyan R, Altmeyer A, Wisniewski HG, Vilcek J. Long pentraxins: an emerging group of proteins with diverse functions. *Cytokine Growth Factor Rev.* (1996) 7:191–202. doi: 10.1016/1359-6101(96)00019-6
31. Introna M, Alles VV, Castellano M, Picardi G, De Gioia L, Bottazzai B, et al. Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites. *Blood.* (1996) 87:1862–72.
32. Inforzato A, Peri G, Doni A, Garlanda C, Mantovani A, Bastone A, et al. Structure and function of the long pentraxin PTX3 glycosidic moiety: fine-tuning of the interaction with C1q and complement activation. *Biochemistry.* (2006) 45:11540–51. doi: 10.1021/bi0607453

33. Inforzato A, Baldock C, Jowitt TA, Holmes DF, Lindstedt R, Marcellini M, et al. The angiogenic inhibitor long pentraxin PTX3 forms an asymmetric octamer with two binding sites for FGF2. *J Biol Chem.* (2010) 285:17681–92. doi: 10.1074/jbc.M109.085639
34. Inforzato A, Rivieccio V, Morreale AP, Bastone A, Salustri A, Scarchilli L, et al. Structural characterization of PTX3 disulfide bond network and its multimeric status in cumulus matrix organization. *J Biol Chem.* (2008) 283:10147–61. doi: 10.1074/jbc.M708535200
35. Reading PC, Bozza S, Gilbertson B, Tate M, Moretti S, Job ER, et al. Antiviral activity of the long chain Pentraxin PTX3 against influenza viruses. *J Immunol.* (2008) 180:3391–8. doi: 10.4049/jimmunol.180.5.3391
36. Job ER, Bottazzi B, Short KR, Deng YM, Mantovani A, Brooks AG, et al. A single amino acid substitution in the hemagglutinin of H3N2 subtype influenza A viruses is associated with resistance to the long pentraxin PTX3 and enhanced virulence in mice. *J Immunol.* (2014) 192:271–81. doi: 10.4049/jimmunol.1301814
37. Deban L, Castro Russo R, Sironi M, Moalli F, Scanziani M, Zambelli V, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nat Immunol.* (2010) 11:328–34. doi: 10.1038/ni.1854
38. Inforzato A, Doni A, Barajon I, Leone R, Garlanda C, Bottazzi B, et al. PTX3 as a paradigm for the interaction of pentraxins with the complement system. *Semin Immunol.* (2013) 25:79–85. doi: 10.1016/j.smim.2013.05.002
39. Camozzi M, Rusnati M, Bugatti A, Bottazzi B, Mantovani A, Bastone A, et al. Identification of an antiangiogenic FGF2-binding site in the N terminus of the soluble pattern recognition receptor PTX3. *J Biol Chem.* (2006) 281:22605–13. doi: 10.1074/jbc.M601023200
40. Moalli F, Doni A, Deban L, Zelante T, Zagarella S, Bottazzi B, et al. Role of complement and Fc{gamma} receptors in the protective activity of the long pentraxin PTX3 against *Aspergillus fumigatus*. *Blood.* (2010) 116:5170–80. doi: 10.1182/blood-2009-12-258376
41. Ievoli E, Lindstedt R, Inforzato A, Camaioni A, Palone F, Day AJ, et al. Implication of the oligomeric state of the N-terminal PTX3 domain in cumulus matrix assembly. *Matrix Biol.* (2011) 30:330–7. doi: 10.1016/j.matbio.2011.05.002
42. Leali D, Inforzato A, Ronca R, Bianchi R, Belleri M, Coltrini D, et al. Long pentraxin 3/tumor necrosis factor-stimulated gene-6 interaction: a biological rheostat for fibroblast growth factor 2-mediated angiogenesis. *Arterioscler Thromb Vasc Biol.* (2012) 32:696–703. doi: 10.1161/ATVBAHA.111.243998
43. Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, et al. Binding of the long pentraxin PTX3 to Factor H: Interacting domains and function in the regulation of complement activation. *J Immunol.* (2008) 181:8433–40. doi: 10.4049/jimmunol.181.12.8433
44. Parente R, Clark SJ, Inforzato A, Day AJ. Complement factor H in host defense and immune evasion. *Cell Mol Life Sci.* (2017) 74:1605–24. doi: 10.1007/s00018-016-2418-4
45. Ma YJ, Doni A, Skjoedt MO, Honore C, Arendrup M, Mantovani A, et al. Heterocomplexes of mannose-binding lectin and the pentraxins PTX3 or serum amyloid P component trigger cross-activation of the complement system. *J Biol Chem.* (2011) 286:3405–17. doi: 10.1074/jbc.M110.190637
46. Emsley J, White HE, O'Hara BP, Oliva G, Srinivasan N, Tickle IJ, et al. Structure of pentameric human serum amyloid P component. *Nature.* (1994) 367:338–45. doi: 10.1038/367338a0
47. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure.* (1999) 7:169–77. doi: 10.1016/S0969-2126(99)80023-9
48. Shrive AK, Metcalfe AM, Cartwright JR, Greenhough TJ. C-reactive protein and SAP-like pentraxin are both present in Limulus polyphemus haemolymph: crystal structure of Limulus SAP. *J Mol Biol.* (1999) 290:997–1008. doi: 10.1006/jmbi.1999.2956
49. Grcevic D, Sironi M, Valentino S, Deban L, Cvija H, Inforzato A, et al. The long Pentraxin 3 plays a role in bone turnover and repair. *Front Immunol.* (2018) 9:417. doi: 10.3389/fimmu.2018.00417
50. Baranova NS, Inforzato A, Briggs DC, Tilakaratna V, Enghild JJ, Thakar D, et al. Incorporation of pentraxin 3 into hyaluronan matrices is tightly regulated and promotes matrix cross-linking. *J Biol Chem.* (2014) 289:30481–98. doi: 10.1074/jbc.M114.568154
51. Medzhitov R. Origin and physiological roles of inflammation. *Nature.* (2008) 454:428–35. doi: 10.1038/nature07201
52. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. *Nat Rev Cancer.* (2009) 9:57–63. doi: 10.1038/nrc2541
53. Doni A, D'Amico G, Morone D, Mantovani A, Garlanda C. Humoral innate immunity at the crossroad between microbe and matrix recognition: The role of PTX3 in tissue damage. *Semin Cell Dev Biol.* (2016) 61:31–40. doi: 10.1016/j.semcdb.2016.07.026
54. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol.* (2003) 21:335–76. doi: 10.1146/annurev.immunol.21.120601.141126
55. Holmskov U, Thiel S, Jensenius JC. Collections and ficolins: humoral lectins of the innate immune defense. *Annu Rev Immunol.* (2003) 21:547–78. doi: 10.1146/annurev.immunol.21.120601.140954
56. Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD. Interactions between coagulation and complement–their role in inflammation. *Semin Immunopathol.* (2012) 34:151–65. doi: 10.1007/s00281-011-0280-x
57. Pilling D, Gomer RH. Persistent lung inflammation and fibrosis in serum amyloid P component (APCs-/-) knockout mice. *PLoS ONE.* (2014) 9:e93730. doi: 10.1371/journal.pone.0093730
58. Cox N, Pilling D, Gomer RH. DC-SIGN activation mediates the differential effects of SAP and CRP on the innate immune system and inhibits fibrosis in mice. *Proc Natl Acad Sci USA.* (2015) 112:8385–90. doi: 10.1073/pnas.1500956112
59. Bottazzi B, Inforzato A, Messa M, Barbagallo M, Magrini E, Garlanda C, et al. The pentraxins PTX3 and SAP in innate immunity, regulation of inflammation and tissue remodelling. *J Hepatol.* (2016) 64:1416–27. doi: 10.1016/j.jhep.2016.02.029
60. Fossati G, Pozzi D, Canzi A, Mirabella F, Valentino S, Morini R, et al. Pentraxin 3 regulates synaptic function by inducing AMPA receptor clustering via ECM remodeling and beta1-integrin. *EMBO J.* (2019) 38:e99529. doi: 10.15252/embj.201899529
61. Presta M, Foglio E, Churruca Schuind A, Ronca R. Long Pentraxin-3 modulates the angiogenic activity of fibroblast growth factor-2. *Front Immunol.* (2018) 9:2327. doi: 10.3389/fimmu.2018.02327
62. Bonacina F, Barbieri SS, Cutuli L, Amadio P, Doni A, Sironi M, et al. Vascular pentraxin 3 controls arterial thrombosis by targeting collagen and fibrinogen induced platelets aggregation. *Biochim Biophys Acta.* (2016) 1862:1182–90. doi: 10.1016/j.bbadis.2016.03.007
63. Giacomini A, Ghedini GC, Presta M, Ronca R. Long pentraxin 3: a novel multifaceted player in cancer. *Biochim Biophys Acta Rev Cancer.* (2018) 1869:53–63. doi: 10.1016/j.bbcan.2017.11.004
64. Chang WC, Wu SL, Huang WC, Hsu JY, Chan SH, Wang JM, et al. PTX3 gene activation in EGF-induced head and neck cancer cell metastasis. *Oncotarget.* (2015) 6:7741–57. doi: 10.18632/oncotarget.3482
65. Song T, Wang C, Guo C, Liu Q, Zheng X. Pentraxin 3 overexpression accelerated tumor metastasis and indicated poor prognosis in hepatocellular carcinoma via driving epithelial-mesenchymal transition. *J Cancer.* (2018) 9:2650–8. doi: 10.7150/jca.25188
66. Doni A, Garlanda C, Mantovani A. Innate immunity, hemostasis and matrix remodeling: PTX3 as a link. *Semin Immunol.* (2016b) 28:570–7. doi: 10.1016/j.smim.2016.10.012
67. Han B, Haitsma JJ, Zhang Y, Bai X, Rubacha M, Keshavjee S, et al. Long pentraxin PTX3 deficiency worsens LPS-induced acute lung injury. *Intensive Care Med.* (2011) 37:334–42. doi: 10.1007/s00134-010-2067-2
68. Cappuzzello C, Doni A, Dander E, Pasqualini F, Nebuloni M, Bottazzi B, et al. Mesenchymal stromal cell-derived PTX3 promotes wound healing via fibrin remodeling. *J Invest Dermatol.* (2016) 136:293–300. doi: 10.1038/JID.2015.346
69. Mauri T, Zambelli V, Cappuzzello C, Bellani G, Dander E, Sironi M, et al. Intraperitoneal adoptive transfer of mesenchymal stem cells enhances recovery from acid aspiration acute lung injury in mice. *Intensive Care Med Exp.* (2017) 5:13. doi: 10.1186/s40635-017-0126-5
70. Lopez ML, Bruges G, Crespo G, Salazar V, Deglesne PA, Schneider H, et al. Thrombin selectively induces transcription of genes in human monocytes involved in inflammation and wound healing. *Thromb Haemost.* (2014) 112:992–1001. doi: 10.1160/th14-01-0034
71. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med.* (1999) 341:738–46. doi: 10.1056/NEJM199909023411006

72. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol.* (2002) 3:349–63. doi: 10.1038/nrm809
73. Wong VW, Sorkin M, Glotzbach JP, Longaker MT, Gurtner GC. Surgical approaches to create murine models of human wound healing. *J Biomed Biotechnol.* (2011) 2011:969618. doi: 10.1155/2011/969618
74. Han B, Ma X, Zhang J, Zhang Y, Bai X, Hwang DM, et al. Protective effects of long pentraxin PTX3 on lung injury in a severe acute respiratory syndrome model in mice. *Lab Invest.* (2012) 92:1285–96. doi: 10.1038/labinvest.2012.92
75. Rodriguez-Grande B, Swana M, Nguyen L, Englezou P, Maysami S, Allan SM, et al. The acute-phase protein PTX3 is an essential mediator of glial scar formation and resolution of brain edema after ischemic injury. *J Cereb Blood Flow Metab.* (2014) 34:480–8. doi: 10.1038/jcbfm.2013.224
76. Xiao Y, Yang N, Zhang Q, Wang Y, Yang S, Liu Z. Pentraxin 3 inhibits acute renal injury-induced interstitial fibrosis through suppression of IL-6/Stat3 pathway. *Inflammation.* (2014) 37:1895–901. doi: 10.1007/s10753-014-9921-2
77. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degen J, Bronson R, et al. Physiological consequences of loss of plasminogen activator gene function in mice. *Nature.* (1994) 368:419–24. doi: 10.1038/368419a0
78. Bugge TH, Kombrinck KW, Flick MJ, Daugherty CC, Danton MJ, Degen JL. Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell.* (1996) 87:709–19. doi: 10.1016/S0092-8674(00)81390-2
79. Tuan TL, Zhu JY, Sun B, Nichter LS, Nimni ME, Laug WE. Elevated levels of plasminogen activator inhibitor-1 may account for the altered fibrinolysis by keloid fibroblasts. *J Invest Dermatol.* (1996) 106:1007–11. doi: 10.1111/1523-1747.ep12338552
80. Idell S. Endothelium and disordered fibrin turnover in the injured lung: newly recognized pathways. *Crit Care Med.* (2002) 30:S274–280. doi: 10.1097/00003246-200205001-00017
81. de Giorgio-Miller A, Bottoms S, Laurent G, Carmeliet P, Herrick S. Fibrin-induced skin fibrosis in mice deficient in tissue plasminogen activator. *Am J Pathol.* (2005) 167:721–32. doi: 10.1016/S0002-9440(10)62046-9
82. Romer J, Bugge TH, Pyke C, Lund LR, Flick MJ, Degen JL, et al. Impaired wound healing in mice with a disrupted plasminogen gene. *Nat Med.* (1996) 2:287–92. doi: 10.1038/nm0396-287
83. Ma YJ, Doni A, Hummelshoj T, Honore C, Bastone A, Mantovani A, et al. Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. *J Biol Chem.* (2009) 284:28263–75. doi: 10.1074/jbc.M109.009225
84. Ma YJ, Doni A, Romani L, Jurgensen HJ, Behrendt N, Mantovani A, et al. Ficolin-1-PTX3 complex formation promotes clearance of altered self-cells and modulates IL-8 production. *J Immunol.* (2013) 191:1324–33. doi: 10.4049/jimmunol.1300382
85. Schneider LA, Korber A, Grabbe S, Dissemond J. Influence of pH on wound-healing: a new perspective for wound-therapy? *Arch Dermatol Res.* (2007) 298:413–20. doi: 10.1007/s00403-006-0713-x
86. Mangel WF, Lin BH, Ramakrishnan V. Characterization of an extremely large, ligand-induced conformational change in plasminogen. *Science.* (1990) 248:69–73. doi: 10.1126/science.2108500
87. Castellino FJ, Ploplis VA. Structure and function of the plasminogen/plasmin system. *Thromb Haemost.* (2005) 93:647–54. doi: 10.1160/TH04-12-0842
88. Law RH, Caradoc-Davies T, Cowieson N, Horvath AJ, Quek AJ, Encarnacao JA, et al. The X-ray crystal structure of full-length human plasminogen. *Cell Rep.* (2012) 1:185–90. doi: 10.1016/j.celrep.2012.02.012
89. Ploplis VA, Carmeliet P, Vazirzadeh S, Van Vlaenderen I, Moons L, Plow EF, et al. Effects of disruption of the plasminogen gene on thrombosis, growth, and health in mice. *Circulation.* (1995) 92:2585–93. doi: 10.1161/01.CIR.92.9.2585
90. Hamilton JA. Stimulation of macrophage prostaglandin and neutral protease production by phorbol esters as a model for the induction of vascular changes associated with tumor promotion. *Cancer Res.* (1980) 40:2273–80.
91. Ku CS, Fiedel BA. Modulation of fibrin clot formation by human serum amyloid P component (SAP) and heparin. *J Exp Med.* (1983) 158:767–80. doi: 10.1084/jem.158.3.767
92. Vandendries ER, Furie BC, Furie B. Role of P-selectin and PSGL-1 in coagulation and thrombosis. *Thromb Haemost.* (2004) 92:459–66. doi: 10.1160/TH04-05-0306
93. Napoleone E, di Santo A, Peri G, Mantovani A, de Gaetano G, Donati MB, et al. The long pentraxin PTX3 up-regulates tissue factor in activated monocytes: another link between inflammation and clotting activation. *J Leukoc Biol.* (2004) 76:203–9. doi: 10.1189/jlb.1003528
94. Camozzi M, Zacchigna S, Rusnati M, Coltrini D, Ramirez-Correa G, Bottazzi B, et al. Pentraxin 3 inhibits fibroblast growth factor 2-dependent activation of smooth muscle cells *in vitro* and neointima formation *in vivo*. *Arterioscler Thromb Vasc Biol.* (2005) 25:1837–42. doi: 10.1161/01.ATV.0000177807.54959.7d
95. Norata GD, Marchesi P, Pulakazhi Venu VK, Pasqualini F, Anselmo A, Moalli F, et al. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. *Circulation.* (2009) 120:699–708. doi: 10.1161/CIRCULATIONAHA.108.806547
96. Sellheyer K, Krahl D. Skin mesenchymal stem cells: prospects for clinical dermatology. *J Am Acad Dermatol.* (2010) 63:859–65. doi: 10.1016/j.jaad.2009.09.022
97. Bey E, Prat M, Duhamel P, Benderitter M, Brachet M, Trompier F, et al. Emerging therapy for improving wound repair of severe radiation burns using local bone marrow-derived stem cell administrations. *Wound Repair Regen.* (2010) 18:50–8. doi: 10.1111/j.1524-475X.2009.00562.x
98. Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut.* (2011) 60:788–98. doi: 10.1136/gut.2010.214841
99. Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. *J Clin Invest.* (2012) 122:2731–40. doi: 10.1172/JCI60331
100. Long F. Building strong bones: molecular regulation of the osteoblast lineage. *Nat Rev Mol Cell Biol.* (2011) 13:27–38. doi: 10.1038/nrm3254
101. O'Keefe RJ. Fibrinolysis as a target to enhance fracture healing. *N Engl J Med.* (2015) 373:1776–8. doi: 10.1056/NEJMcibr1510090
102. Yuasa M, Mignemi NA, Nyman JS, Duvall CL, Schwartz HS, Okawa A, et al. Fibrinolysis is essential for fracture repair and prevention of heterotopic ossification. *J Clin Invest.* (2015) 125:3117–31. doi: 10.1172/JCI80313
103. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* (2008) 454:436–44. doi: 10.1038/nature07205
104. Markiewski MM, DeAngelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, Gerard C, et al. Modulation of the antitumor immune response by complement. *Nat Immunol.* (2008) 9:1225–35. doi: 10.1038/ni.1655
105. Ning C, Li YY, Wang Y, Han GC, Wang RX, Xiao H, et al. Complement activation promotes colitis-associated carcinogenesis through activating intestinal IL-1beta/IL-17A axis. *Mucosal Immunol.* (2015) 8:1275–84. doi: 10.1038/mi.2015.18
106. Guglietta S, Rescigno M. Hypercoagulation and complement: connected players in tumor development and metastases. *Semin Immunol.* (2016) 28:578–86. doi: 10.1016/j.smim.2016.10.011
107. Medler TR, Murugan D, Horton W, Kumar S, Cotechini T, Forsyth AM, et al. Complement C5a fosters squamous carcinogenesis and limits T cell response to chemotherapy. *Cancer Cell.* (2018) 34:561–578.e566. doi: 10.1016/j.ccell.2018.09.003
108. Ronca R, Alessi P, Coltrini D, Salle ED, Giacomini A, Leali D, et al. Long Pentraxin-3 As An epithelial-stromal fibroblast growth factor-targeting inhibitor in prostate cancer. *J Pathol.* (2013) 230:228–38. doi: 10.1002/path.4181
109. Ronca R, Giacomini A, Di Salle E, Coltrini D, Pagano K, Ragona L, et al. Long-Pentraxin 3 derivative as a small-molecule FGF trap for cancer therapy. *Cancer Cell.* (2015) 28:225–39. doi: 10.1016/j.ccell.2015.07.002
110. Rodrigues PF, Matarazzo S, Maccarinelli F, Foglio E, Giacomini A, Silva Nunes JP, et al. Long Pentraxin 3-mediated fibroblast growth factor trapping impairs fibrosarcoma growth. *Front Oncol.* (2018) 8:472. doi: 10.3389/fonc.2018.00472
111. Wang JX, He YL, Zhu ST, Yang S, Zhang ST. Aberrant methylation of the 3q25 tumor suppressor gene PTX3 in human esophageal squamous cell carcinoma. *World J Gastroenterol.* (2011) 17:4225–30. doi: 10.3748/wjg.v17.i37.4225

112. Tsuji S, Midorikawa Y, Seki M, Takayama T, Sugiyama Y, Aburatani H. Network-based analysis for identification of candidate genes for colorectal cancer progression. *Biochem Biophys Res Commun.* (2016) 476:534–40. doi: 10.1016/j.bbrc.2016.05.158
113. Choi B, Lee EJ, Park YS, Kim SM, Kim EY, Song Y, et al. Pentraxin-3 silencing suppresses gastric cancer-related inflammation by inhibiting chemotactic migration of macrophages. *Anticancer Res.* (2015) 35:2663–8.
114. Ying TH, Lee CH, Chiou HL, Yang SF, Lin CL, Hung CH, et al. Knockdown of Pentraxin 3 suppresses tumorigenicity and metastasis of human cervical cancer cells. *Sci Rep.* (2016) 6:29385. doi: 10.1038/srep29385
115. Thomas C, Henry W, Cuiffo BG, Collmann AY, Marangoni E, Benhamo V, et al. Pentraxin-3 is a PI3K signaling target that promotes stem cell-like traits in basal-like breast cancers. *Sci Signal.* (2017) 10:eaah4674. doi: 10.1126/scisignal.aah4674
116. Tothill RW, Tinker AV, George J, Brown R, Fox SB, Lade S, et al. Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. *Clin Cancer Res.* (2008) 14:5198–208. doi: 10.1158/1078-0432.CCR-08-0196
117. Choi B, Lee EJ, Song DH, Yoon SC, Chung YH, Jang Y, et al. Elevated Pentraxin 3 in bone metastatic breast cancer is correlated with osteolytic function. *Oncotarget.* (2014) 5:481–92. doi: 10.18632/oncotarget.1664
118. Chen WY, Wu F, You ZY, Zhang ZM, Guo YL, Zhong LX. Analyzing the differentially expressed genes and pathway cross-talk in aggressive breast cancer. *J Obstet Gynaecol Res.* (2015) 41:132–40. doi: 10.1111/jog.12495
119. Player A, Abraham N, Burrell K, Bengone IO, Harris A, Nunez L, et al. Identification of candidate genes associated with triple negative breast cancer. *Genes Cancer.* (2017) 8:659–72. doi: 10.18632/genesandcancer.147
120. Espinal-Enriquez J, Munoz-Montero S, Imaz-Rosshandler I, Huerta-Verde A, Mejia C, Hernandez-Lemus E. Genome-wide expression analysis suggests a crucial role of dysregulation of matrix metalloproteinases pathway in undifferentiated thyroid carcinoma. *BMC Genomics.* (2015) 16:207. doi: 10.1186/s12864-015-1372-0
121. Willeke F, Assad A, Findeisen P, Schromm E, Grobholz R, von Gerstenbergk B, et al. Overexpression of a member of the pentraxin family (PTX3) in human soft tissue liposarcoma. *Eur J Cancer.* (2006) 42:2639–46. doi: 10.1016/j.ejca.2006.05.035
122. Ravenna L, Sale P, Di Vito M, Russo A, Salvatori L, Tafani M, et al. Up-regulation of the inflammatory-reparative phenotype in human prostate carcinoma. *Prostate.* (2009) 69:1245–55. doi: 10.1002/pros.20966
123. Tafani M, Di Vito M, Frati A, Pellegrini L, De Santis E, Sette G, et al. Pro-inflammatory gene expression in solid glioblastoma microenvironment and in hypoxic stem cells from human glioblastoma. *J Neuroinflamm.* (2011) 8:32. doi: 10.1186/1742-2094-8-32
124. Barbui T, Carobbio A, Finazzi G, Vannucchi AM, Barosi G, Antonioli E, et al. Inflammation and thrombosis in essential thrombocythemia and polycythemia vera: different role of C-reactive protein and pentraxin 3. *Haematologica.* (2011) 96:315–8. doi: 10.3324/haematol.2010.031070
125. Germano G, Frapolli R, Simone M, Tavecchio M, Erba E, Pesce S, et al. Antitumor and anti-inflammatory effects of trabectedin on human myxoid liposarcoma cells. *Cancer Res.* (2010) 70:2235–44. doi: 10.1158/0008-5472.CAN-09-2335
126. Planque C, Kulasingam V, Smith CR, Reckamp K, Goodglick L, Diamandis EP. Identification of five candidate lung cancer biomarkers by proteomics analysis of conditioned media of four lung cancer cell lines. *Mol Cell Proteomics.* (2009) 8:2746–58. doi: 10.1074/mcp.M900134-MCP200
127. Diamandis EP, Goodglick L, Planque C, Thornquist MD. Pentraxin-3 is a novel biomarker of lung carcinoma. *Clin Cancer Res.* (2011) 17:2395–9. doi: 10.1158/1078-0432.CCR-10-3024
128. Infante M, Allavena P, Garlanda C, Nebuloni M, Morenghi E, Rahal D, et al. Prognostic and diagnostic potential of local and circulating levels of pentraxin 3 in lung cancer patients. *Int J Cancer.* (2016) 138:983–91. doi: 10.1002/ijc.29822
129. Kondo S, Ueno H, Hosoi H, Hashimoto J, Morizane C, Koizumi F, et al. Clinical impact of pentraxin family expression on prognosis of pancreatic carcinoma. *Br J Cancer.* (2013) 109:739–46. doi: 10.1038/bjc.2013.348
130. Locatelli M, Ferrero S, Martinelli Boneschi F, Boiocchi L, Zavanone M, Maria Gaini S, et al. The long pentraxin PTX3 as a correlate of cancer-related inflammation and prognosis of malignancy in gliomas. *J Neuroimmunol.* (2013) 260:99–106. doi: 10.1016/j.jneuroim.2013.04.009
131. Carmo RF, Aroucha D, Vasconcelos LR, Pereira LM, Moura P, Cavalcanti MS. Genetic variation in PTX3 and plasma levels associated with hepatocellular carcinoma in patients with HCV. *J Viral Hepat.* (2016) 23:116–22. doi: 10.1111/jvh.12472
132. Berg J, Halvorsen AR, Bengtson MB, Tasken KA, Maelandsmo GM, Yndestad A, et al. Levels and prognostic impact of circulating markers of inflammation, endothelial activation and extracellular matrix remodelling in patients with lung cancer and chronic obstructive pulmonary disease. *BMC Cancer.* (2018) 18:739. doi: 10.1186/s12885-018-4659-0
133. Liu C, Yao Y, Wang W. Pentraxin-3 as a prognostic marker in patients with small-cell lung cancer. *Med Oncol.* (2014) 31:207. doi: 10.1007/s12032-014-0207-1
134. Stallone G, Cormio L, Netti GS, Infante B, Selvaggio O, Fino GD, et al. Pentraxin 3: a novel biomarker for predicting progression from prostatic inflammation to prostate cancer. *Cancer Res.* (2014) 74:4230–8. doi: 10.1158/0008-5472.CAN-14-0369
135. Zhang J, Wang TY, Niu XC. Increased plasma levels of Pentraxin 3 are associated with poor prognosis of colorectal carcinoma patients. *Tohoku J Exp Med.* (2016) 240:39–46. doi: 10.1620/tjem.240.39
136. Liu B, Zhao Y, Guo L. Increased serum pentraxin-3 level predicts poor prognosis in patients with colorectal cancer after curative surgery, a cohort study. *Medicine.* (2018) 97:e11780. doi: 10.1097/MD.0000000000011780
137. Di Caro G, Carvello M, Pesce S, Erreni M, Marchesi F, Todoric J, et al. Circulating inflammatory mediators as potential prognostic markers of human colorectal cancer. *PLoS ONE.* (2016) 11:e0148186. doi: 10.1371/journal.pone.0148186
138. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med.* (1986) 315:1650–9. doi: 10.1056/NEJM198612253152606
139. Clark RA. Fibrin and wound healing. *Ann N Y Acad Sci.* (2001) 936:355–67. doi: 10.1111/j.1749-6632.2001.tb03522.x
140. Ortega S, Ittmann M, Tsang SH, Ehrlich M, Basilico C. Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. *Proc Nat Acad Sci USA.* (1998) 95:5672–7. doi: 10.1073/pnas.95.10.5672
141. Rafail S, Kourtzelis I, Foukas PG, Markiewski MM, DeAngelis RA, Guariento M, et al. Complement deficiency promotes cutaneous wound healing in mice. *J Immunol.* (2015) 194:1285–91. doi: 10.4049/jimmunol.1402354
142. Dvorak HF. Tumors: wounds that do not heal-redux. *Cancer Immunol Res.* (2015) 3:1–11. doi: 10.1158/2326-6066.CIR-14-0209
143. Reis ES, Mastellos DC, Ricklin D, Mantovani A, Lambris JD. Complement in cancer: untangling an intricate relationship. *Nat Rev Immunol.* (2018) 18:5–18. doi: 10.1038/nri.2017.97


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 17 April 2019
doi: 10.3389/fimmu.2019.00823

# Pentraxin 3 in Cardiovascular Disease

*Giuseppe Ristagno[1]*, Francesca Fumagalli[1], Barbara Bottazzi[2], Alberto Mantovani[2,3,4], Davide Olivari[1], Deborah Novelli[1] and Roberto Latini[1]*

[1] Department of Cardiovascular Research, Mario Negri Institute for Pharmacological Research IRCCS, Milan, Italy, [2] Humanitas Clinical and Research Center-IRCCS, Milan, Italy, [3] Humanitas University, Milan, Italy, [4] The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom

The long pentraxin PTX3 is a member of the pentraxin family produced locally by stromal and myeloid cells in response to proinflammatory signals and microbial moieties. The prototype of the pentraxin family is C reactive protein (CRP), a widely-used biomarker in human pathologies with an inflammatory or infectious origin. Data so far describe PTX3 as a multifunctional protein acting as a functional ancestor of antibodies and playing a regulatory role in inflammation. Cardiovascular disease (CVD) is a leading cause of mortality worldwide, and inflammation is crucial in promoting it. Data from animal models indicate that PTX3 can have cardioprotective and atheroprotective roles regulating inflammation. PTX3 has been investigated in several clinical settings as possible biomarker of CVD. Data collected so far indicate that PTX3 plasma levels rise rapidly in acute myocardial infarction, heart failure and cardiac arrest, reflecting the extent of tissue damage and predicting the risk of mortality.

**Keywords: PTX 3, pentraxin, cardiovascular disease, cardiac arrest (CA), heart failure, biomarker**

OPEN ACCESS

***Edited by:***
*Cees Van Kooten,*
*Leiden University, Netherlands*

***Reviewed by:***
*Lubka T. Roumenina,*
*INSERM U1138 Centre de Recherche des Cordeliers, France*
*Livija Deban,*
*Prokarium, United Kingdom*

****Correspondence:***
*Giuseppe Ristagno*
*giuseppe.ristagno@marionegri.it*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *21 December 2018*
***Accepted:*** *28 March 2019*
***Published:*** *17 April 2019*

***Citation:***
*Ristagno G, Fumagalli F, Bottazzi B, Mantovani A, Olivari D, Novelli D and Latini R (2019) Pentraxin 3 in Cardiovascular Disease. Front. Immunol. 10:823. doi: 10.3389/fimmu.2019.00823*

## INTRODUCTION

According to the World Health Organization (WHO), an estimated 17 million people globally die of cardiovascular diseases (CVD) every year, with important implications in terms of quality of life and social costs[1] Experimental and clinical evidence points to inflammation as a major cause of atherosclerosis, the underlying mechanism of CVD (1, 2). Accordingly, therapies targeting inflammation show promising results, as demonstrated by the successes of statins therapy, due not only to their effects on cholesterol, but also on the control of inflammation (3); or anti-interleukin-1β (IL-1β) appears to lower cardiovascular event rates (4).

The inflammatory response is mediated by a set of cells and soluble proteins belonging to the innate immune system. The humoral arm of the innate immune response includes components of the complement cascade and soluble pattern recognition molecules (PRM), particularly collectins (surfactant protein-A, [SP-A], and SP-D), ficolins, (ficolin-1;−2;−3) and members of the pentraxin family (C-reactive protein [CRP], serum amyloid P component [SAP], and long pentraxin 3 [PTX3]) (5–7). Therefore, soluble PRM are a heterogeneous group of proteins acting as functional ancestors of antibodies and key roles as regulators of inflammation playing as effectors and modulators of the innate immune response in animals and man.

[1]Source: http://www.euro.who.int/en/health-topics/noncommunicable-diseases/cardiovascular-diseases/cardiovascular-diseases2.

CRP, one of the prototypic molecules of the pentraxin family, is a systemic biomarker of inflammation widely used in the clinic to monitor infections and inflammatory conditions (7). Epidemiological studies have consistently associated raised CRP serum levels with an increased risk of acute myocardial infarction (MI), stroke, and peripheral artery disease (8). In studies to date, CRP has emerged not only as a biomarker of CVD, but also as an independent predictor of adverse cardiovascular events.

PTX3, identified as a cognate molecule of CRP, is a multifunctional protein with complex regulatory roles in inflammation and extracellular matrix organization and remodeling (9). In men and mice PTX3 blood levels rise rapidly and dramatically in different pathological conditions with an inflammatory and/or infectious origin and have been investigated in several studies (**Figure 1**). The main characteristic of PTX3 is that it rises faster than CRP (peak at 6–8 h for PTX3; 24–48 h for CRP), very likely because of local vs. systemic production of the two proteins (9). The question is "How can a member of the humoral innate immunity be involved in cardiovascular health and disease?" Here we review the key properties of PTX3 as prototypic member of the pentraxin superfamily in relation to cardiovascular pathology.

The properties of PTX3 have been widely studied in humans and mice, using genetic approaches made possible by the high level of conservation of this molecule among species (5). The review will specifically deal with (1) vascular disorders, in which PTX3 has been found to play a role, but also (2) cardiac diseases such as myocardial infarction, heart failure (HF) and cardiac arrest (CA). While it has long been known that atherosclerosis is an inflammatory disease (10) and consequently innate and adaptive immune responses are expected to play a role, the involvement of PTX3 in cardiac diseases is somewhat less evident.

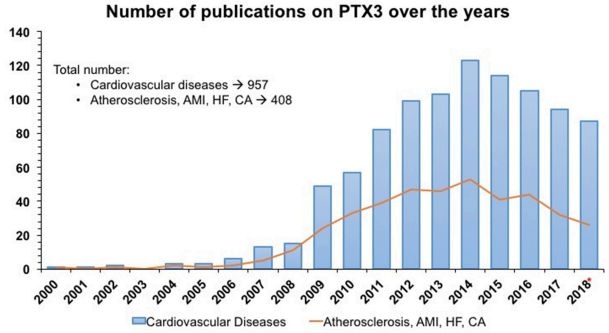


**FIGURE 1 |** Number of publications/year from PubMed and Embase on PTX3 as an early indicator of acute myocardial infarction and cardiovascular diseases in humans. SEARCH QUERY: (ptx3 OR "pentraxin 3"/exp/ descriptor) AND "cardiovascular disease"/exp/descriptor AND 2000–2018*. Same search strategy was applied for PTX3 AND Atherosclerosis, Acute Myocardial Infarction (AMI), Heart Failure (HF) and cardiac arrest (CA). * 2018 included only 10 months.

## THE PENTRAXIN SUPERFAMILY: CRP AND PTX3

The Pentraxin superfamily comprises long and short pentraxins (9). CRP and SAP were identified as the prototypes of the short pentraxin family; PTX3 was cloned in the late 1980's and is considered the prototype of the long pentraxin arm, its gene and protein sequences being almost twice the sequences of CRP and SAP. PTX3 is a key molecule playing complex regulatory roles at the crossroads of innate immunity, inflammation, tissue repair and cancer (9). A strong association has been reported between PTX3 genetic variants, affecting circulating levels of the protein, and susceptibility to fungal infections, suggests therapeutic use of the protein (11–15).

The main biochemical and biological characteristics of CRP and PTX3 have been amply described in several reviews, some of which published very recently (7, 9). Here we will only underline the main differences between the two proteins and some aspects possibly helpful to define their role in CVD.

Although both CRP and PTX3 are considered acute phase proteins, they differ in their producing cells and inducing stimuli. The short pentraxins CRP and SAP are produced primarily in the liver in response to IL-6, reflecting a systemic response, while PTX3 is produced locally by a wide range of stromal and myeloid cells, including monocytes, endothelial cells (EC), and fibroblasts, but not hepatocytes (**Figure 2**). Primary pro-inflammatory signals, interleukin- (IL-) 1β (IL-1β) and TNFα, or bacterial moieties engaging Toll-like receptors (TLR), such as bacterial lipopolysaccharides (LPS), flagellin and outer membrane proteins, are major inducers of PTX3, while IL-6 is ineffective. Polymorphonuclear leucocytes (PMN) have a store of mature PTX3 produced during the differentiation from bone marrow precursors and accumulated in their granules, ready to be released in response to microbial recognition or tissue damage (16).

Vascular EC are a major source of PTX3 in response to inflammatory signals. Anti-inflammatory and atheroprotective signals, such as high density lipoproteins (HDL) and IL-10 induce PTX3 expression. This suggests a potential regulatory role of PTX3 in the innate and adaptive immune responses as well as being an anti-atherogenic molecule (17–19).

The NF-κB pathway is involved in the cascade of molecular events leading to PTX3 expression, as initially demonstrated in a model of acute myocardial ischemia (AMI) and reperfusion in mice (20), and subsequently confirmed by other studies (21, 22). In the model of myocardial infarction in mice, induction of ischemia resulted in upregulation of PTX3 production, an effect almost completely absent in *il-1r1*- or *myd88*-deficient mice.

Recent data have indicated a role of PTX3 in tissue remodeling and repair. In different models of tissue damage, PTX3 was localized in the pericellular provisional fibrin matrix, where it promoted migration and invasive phenotype of remodeling cells (23). Excessive fibrin accumulation was observed in skin, liver and lung injury models in *ptx3*-deficient mice, while in a murine model of arterial thrombosis PTX3 released by EC inhibited platelet aggregation, dampening thrombogenesis (23, 24). In addition, PTX3 is involved in edema resolution and scar formation in a model of brain ischemic injury in mice (25). Finally, we recently reported a non-redundant role

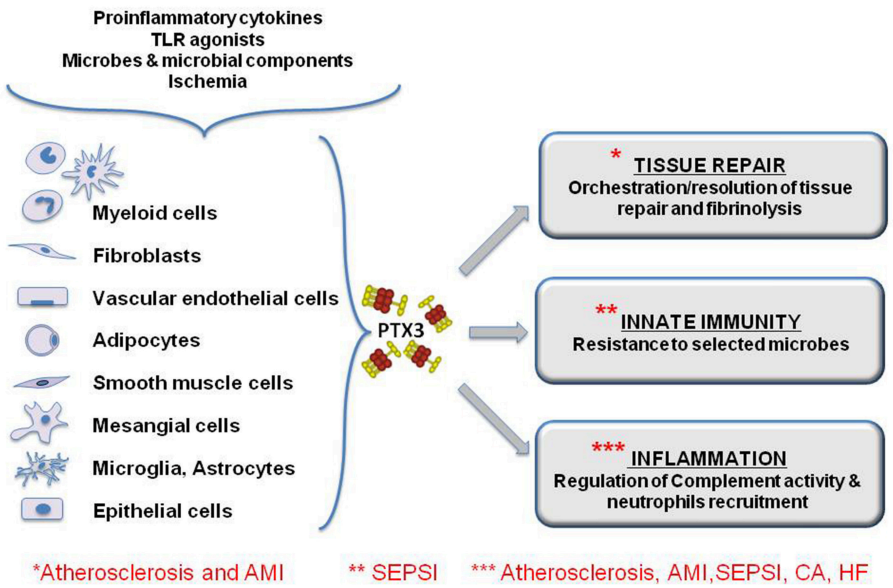


**FIGURE 2 |** Cellular sources and main functions of the long pentraxin PTX3. The protein is induced by primary proinflammatory stimuli, TLR engagement, microbe recognition and ischemia by myeloid and stromal cells. PTX3 is a multifunctional protein playing a role in the orchestration of tissue repair through the regulation of fibrin deposition, and it regulates inflammation, modulating complement activity and limiting neutrophils infiltration in inflamed tissues. Finally, PTX3 is a molecule of the innate immunity and exerts protective roles against selected pathogens acting as and opsonin. The functional role of PTX3 in the main discussed pathology is highlighted by asterisks. However, for some of them, PTX3 has been explored only as a biomarker and there is little evidence of its functional involvement.

of PTX3 in physiological skeletal remodeling and in proper matrix mineralization during bone fracture repair (26). These observations illustrated how PTX3 can play essential roles in tissue remodeling and repair.

The data summarized here indicate the complex regulation of PTX3 production from different cell types in response to different stimuli, and very likely reflect the different roles of this multifunctional protein in the innate immune response and as a constituent of the extracellular matrix. In addition, the induction of PTX3 by anti-inflammatory and atheroprotective signals such as HDL and IL-10 may reflect a possible protective function of PTX3 on EC and vascular integrity.

PTX3 is also expressed during sterile inflammation. For instance, in the model of experimental myocardial infarction (MI) mentioned, *ptx3*-deficient mice had greater myocardial lesions, more leukocyte infiltration, more cell death and higher complement C3 deposition in the infarcted area (20). This suggests that PTX3 might have a non-redundant cardioprotective role in mice, acting on the inflammation and tissue damage associated with reperfusion possibly by affecting the classical and the alternative pathways of complement activation (20). In addition, PTX3 can regulate leukocyte extravasation through an interaction with P-selectin (27), reducing neutrophil recruitment in inflamed sites. PTX3 can also interact with platelets via P-selectin exposed on their surface, and dampens the proinflammatory and prothrombotic effects of activated platelets, further contributing to a cardioprotective role (28).

In contrast, in a mouse model of transverse aortic constriction (TAC), PTX3 modulated the hypertrophic response and ventricular dysfunction following an increased afterload. Specifically, echocardiography indicated that PTX3 overexpression promoted tissue remodeling, left ventricular dysfunction, and increased myocardial fibrosis, while these responses were suppressed in *ptx3*-deficient mice (29).

## PTX3 IN CVD

The findings summarized above underline the dual role of PTX3 in sterile and non-sterile inflammation. Here we will examine the role of PTX3 in four cardiovascular disorders:

1. Atherosclerosis,
2. Acute MI (AMI)
3. Heart failure (HF)
4. Cardiac arrest (CA).

We briefly discuss the evidences of the possible roles played by PTX3 and its potential as a circulating biomarker of diagnosis and/or prognosis in each disease.

### Atherosclerosis

PTX3 is produced by different cell types potentially involved in atherosclerosis, in particular EC, smooth muscle cells and macrophages (**Figure 2**). Staining of advanced atherosclerotic lesions in humans showed strong expression of PTX3, mainly by macrophages and EC, but also by smooth

muscle cells (30). Different pro-inflammatory molecules are produced in an atherosclerotic lesion, particularly cytokines such as TNFα and IL-1, and oxidized low-density lipoproteins (oxLDL) These soluble factors may well be responsible for the production of PTX3 by target cells (31).

Investigations were made in PTX3/apolipoprotein E double knockout mice (*ptx3/apoE-/-*) The lack of PTX3 in animals with a genetic background making them susceptible to atherosclerosis resulted in larger areas of atherosclerotic lesions, greater accumulation of macrophages, higher expression of adhesion molecules, cytokines and chemokines in the vascular wall (19). Vascular inflammation was more marked, suggesting that PTX3 could exert an atheroprotective effect in mice.

Smooth muscle cells are important players in atherosclerosis and are activated after arterial injury. The soluble mediators produced by injured arteries include fibroblast growth factor 2 (FGF2), one of the well-characterized ligands recognized by PTX3 (32, 33). FGF2 plays important roles *in vivo* by promoting angiogenesis and revascularization during wound healing, inflammation, atherosclerosis, and tumor growth. PTX3 has been reported to act as a competitor of FGF2, blocking its interaction to its receptor and thus influencing neo-angiogenesis. In addition, it has been recently reported that PTX3 interaction with FGF2 might contribute to the maintenance of bone mass in homeostatic and pathological conditions, affecting the cross-talk between inflammatory cells and endothelium (26, 34). The specific interaction between PTX3 and FGF2 also results in the inhibition of FGF-dependent proliferation *in vitro* (35). In addition, FGF2 exerts a potent inhibitory effect on the activation of smooth muscle cells, suggesting that PTX3 might affect the activation of SMC after arterial injury (35).

In summary, there are various evidences that PTX3 may play a role in atherosclerosis:

1. PTX3 is expressed more in leukocytes and in adipose tissue from patients with high levels of low-density lipoprotein (LDL) compared to those with low levels (36).
2. PTX3 expression in visceral fat of obese individuals is determined by both LDL/ high density lipoprotein (HDL) ratio and fibrinogen (37).
3. Treatment of EC with lysophosphatidic acid led to a marked upregulation of PTX3 both in terms of mRNA and protein level (38).
4. Immunohistochemistry (ICH) on human atherosclerotic lesions showed that macrophages and PMN cells infiltrating the atherosclerotic plaques were positive for PTX3 (30, 39).
5. PTX3 expression in human EC was upregulated by HDL, whereas there were no effects on CRP and SAP expression (19).
6. In *apolipoprotein E*-deficient mice, the inflammatory reaction of the vascular wall and macrophage accumulation in the plaque were markedly increased by the lack of PTX3 (19, 31).
7. PTX3 plays a protective role in arterial thrombosis by dampening the pro-thrombotic effects of fibrinogen and collagen (23, 24)

## Acute Myocardial Infarction (AMI)

One of the first *in vivo* findings on PTX3 was its high expression in murine hearts after injection of LPS (40). Specific immunostaining for PTX3 was also observed in heart tissues of patients who died of MI (41). In early ischemic lesions PTX3 expression was high primarily in PMN cells, while in more advanced lesions PTX3 positivty of granulocyte was lost and was mainly acquired by macrophages, EC and sometimes myocardiocytes (**Figure 2**).

The high conservation of PTX3 in evolution allows us to translate to humans the observations in mice, whereas CRP and SAP expression is regulated differently in mice and man. Based on this consideration, a model of experimental MI based on coronary artery ligation and reperfusion was applied to *ptx3*-deficient mice (20). In this model, PTX3 mRNA expression was upregulated in the left ventricle (LV) of wild- type animals and circulating levels of the protein were increased, with a peak at 24 h. IHC and confocal microscopy confirmed that major sources of PTX3 in the infarcted heart are first granulocytes and EC (24 h after reperfusion), followed by macrophages, that became positive 3 days after reperfusion. Similarly, PTX3 is released from neutrophils in the early phases of AMI in humans (28), contributing to the rapid increases in different studies (see below). Infarct sizes were measured in wild type and *ptx3*-deficient mice, and the larger damaged area was in the absence of the protein (20). Thus, the presence of PTX3 observed by IHC in tissue samples from mice after ischemic injury and confirmed in the heart of patients who died from MI, supports a pathophysiologic role of the protein in myocardial damage and repair.

Regulation of complement activation by PTX3 has been considered a possible mechanism involved in tissue damage after ischemia and reperfusion. The interaction of PTX3 with Factor H (FH), the most important regulator of the alternative complement pathway, was important to limit FH deposition on PTX3-coated surfaces and to protect against oxidative stress-induced complement and inflammasome activation (42, 43). In addition, deposition of FH and higher complement activation was lower in tumors growing in *ptx3*-deficient mice (44). These observations strongly sustain the hypothesis that the PTX3-FH interaction may constitute a mechanism to prevent excessive complement activation. In the infarcted heart, C3 deposition was higher in *ptx3*-deficient mice and complement depletion canceled the difference between wild- type and $ptx3^{-/-}$ mice (20).

Whether PTX3 plays a role in the progression of post-infarction left ventricular dysfunction and failure has been the subject of research in the mouse after coronary ligation. The results have been mostly inconclusive but nonetheless it can be concluded with some confidence that the role of PTX3 in left ventricular remodeling after MI is practically irrelevant.

The production of PTX3 by vascular cells in response to inflammatory signals and ox-LDL (17, 31) and its occurrence in atherosclerotic lesions (30, 45), prompted investigations of PTX3 levels in AMI (46–52). A high- sensitive (lower detection limit 0.1 ng/mL), specific (no cross-reaction with human CRP

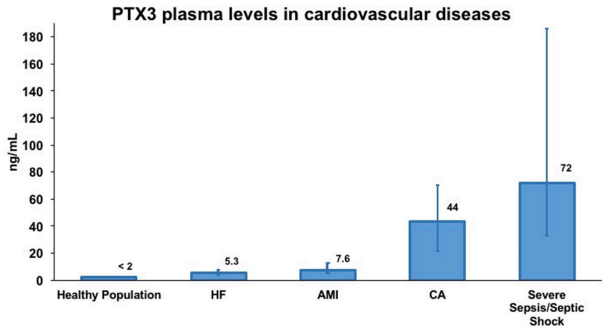


**FIGURE 3 |** Representative concentrations of PTX3 in healthy volunteers and in different cardiovascular diseases. Data were extrapolated from four different publications evaluating PTX3 levels in plasma samples by sandwich ELISA. Specifically, PTX3 levels in healthy volunteers and in acute myocardial infarction (AMI) patients are from Peri et al. (53) in heart failure (HF) patients are from Latini et al. (56) in cardiac arrest (CA) patients are from Ristagno et al. (57) and in severe sepsis/septic shock patients are from Caironi et al. (58). PTX3 levels are presented as median (interquartile range). Numbers over bars represents the median value of plasma concentration of PTX3 expressed in ng/mL.

and SAP) ELISA based on original reagents was developed and used to measure circulating levels of PTX3 in patients and healthy volunteers. Plasma PTX3 in healthy subjects was $\leq$ 2 ng/mL, with higher levels in females than males and levels increased with aging (53–55). Patients with AMI showed an early peak of PTX3 plasma levels observed within 6–8 h from symptom onset (**Figure 3**), and baseline levels were reached within 3 days (53, 59). In a cohort of 748 patients with MI and ST elevation enrolled in the Lipid Assessment Trial Italian Network (LATIN), PTX3, CRP, pro b-type natriuretic peptide (NT-proBNP) and troponin-T were measured within the first day from the onset of symptoms. Among all these markers, PTX3 levels >10.73 ng/mL within the first day after MI were the only independent predictor of three-month mortality (56). High PTX3 also predicted long-term mortality in several subsequent prospective observational studies (50–52).

Besides being a biomarker of MI reflecting the degree of tissue damage, PTX3 was proposed as a prognostic tool in two large studies aiming to identify predictive factors of CVD: the Cardiovascular Health Study (1,583 patients analyzed) and the Multi-Ethnic Study of Atherosclerosis (2,880 patients). These two studies illustrated a significant relation between PTX3 levels and cardiovascular mortality and all-cause death (60, 61). In addition, it was recently shown that higher PTX3 levels predicted occurrence of MI in a cohort of young or middle-aged individuals followed for a first-time MI (62).

It is not clear yet whether the impressive relation with fatal outcome seen in most of the clinical studies actually reflects a role of PTX3 on the pathogenesis of damage, for instance through amplification of the complement and coagulation cascades (63, 64), or a marked protective response to severe cardiac injury. This question has not yet been addressed, nor has that more related to the potential role of PTX3 as an early prognostic biomarker in MI.

## Heart Failure (HF)

The role of inflammation in the progression and outcome of heart failure (HF) is still under discussion. High levels of circulating inflammatory molecules, in particular cytokines and CRP, are related to more severe HF and worse outcomes. However, whether inflammation is a cause or just a consequence of the disease is a matter of controversy. In addition, none of the inflammatory cytokines measurable in the plasma of HF patients can be used singly as a basis for prognosis (65), or even when a multi-marker approach including a range of soluble inflammatory mediators and PTX3, was considered (66).

The prognostic role of PTX3 in chronic HF with reduced (67, 68) or preserved (69, 70) ejection fraction has been reported in several small studies (with $\leq$ 200 patients each). PTX3 levels correlated weakly with those of brain natriuretic peptide (BNP), and ROC analysis suggested that PTX3 was superior to BNP in the prediction of adverse outcomes (68). Other studies (with 37–164 patients) showed that the best risk prediction was achieved by combining three biomarkers: BNP, H-FABP and PTX3 (67, 71). Importantly, high levels of PTX3 correlated significantly with the presence of HF among patients with normal LVEF and LV diastolic dysfunction (69). PTX3 was assayed at randomization and after 3 months in 1,233 patients from the GISSI-Heart Failure trial (GISSI-HF) and 1,457 patients from the Controlled Rosuvastatin Multinational Trial in HF (CORONA) (72). PTX3 was independently and significantly related to the severity of HF. In addition, PTX3 levels were higher in older individuals with ventricular dysfunction, worse symptoms and co-morbidity, i.e., atrial fibrillation or diabetes. Most important, baseline concentrations of PTX3 and three-month changes were significantly related to fatal outcome (72). Similarly, a long-term prospective study on patients with HF and normal ejection fraction indicated that high baseline circulating levels of PTX3 were predictive of all-cause mortality, cardiovascular mortality or hospitalization for worsening HF (70).

The effects of rosuvastatin in the GISSI-HF and CORONA studies deserve a comment. Rosuvastatin (10 mg/day for 3 months) consistently reduced circulating levels of high-sensitive CRP (hsCRP) in both the CORONA trial and GISSI-HF, in line with its anti-inflammatory properties. In contrast, the CORONA trial found an unexpected, opposite effect on PTX3, which increased significantly more in rosuvastatin- treated patients than with placebo (72). This controversial observation might be explained by the hypothesis that statins alter the innate immunity behavior of stimulated phagocytic cells and enhance the production of macrophage and neutrophil extracellular traps that contain antimicrobial proteins and PTX3 (16, 73). This hypothesis, based on clinical and epidemiological observations of lower susceptibility to severe bacterial infection in patients receiving statin therapy, remains highly speculative but calls for further investigation. According to these findings CRP and PTX3 seem to exert different but, to a certain extent, overlapping roles in systemic and local inflammation.

## Cardiac Arrest (CA)

Despite the return of spontaneous circulation (ROSC), mortality after resuscitation from cardiac arrest remains extremely high

(74). The well-known "post-cardiac arrest syndrome" (PCAS) is characterized by myocardial dysfunction with circulatory shock, systemic inflammation, and evolving brain injury (75). Thus, clear similarities between sepsis, septic shock and PCAS have been acknowledged (76). Indeed, upon reperfusion after ROSC, a systemic inflammatory response occurs and ultimately contributes to worsening of circulatory shock and neurological damage.

Recently PTX3 has been investigated in comparison with the classic hsCRP for the prediction of early multiple organ dysfunction syndrome (MODS), early death, and long-term outcome after CA. More specifically, PTX3 and hsCRP were assayed at admission to intensive care unit (ICU) and 2 days later in 278 out-of-hospital CA patients enrolled in the prospective observational cohort study FINNRESUSCI, conducted in 21 hospitals in Finland (57). In this population, at ICU admission hsCRP was normal, i.e., 2.8 [1.2–9.8] mg/L, while PTX3 already showed large increases, i.e., 19.1 [9.2–41.8] ng/mL and levels were higher in older patients and in patients resuscitated after longer CA. Higher plasma levels of PTX3 were significantly associated with MODS [AUCs 0.78 ($p < 0.0001$)], and values above 24 ng/mL showed 0.8 sensitivity and 0.7 specificity for predicting MODS. HsCRP, instead, presented a lower accuracy (AUC of 0.6, $p = 0.033$) compared to PTX3 ($p < 0.003$) in predicting MODS occurrence. PTX3 plasma levels were already significantly higher at ICU admission in patients who developed MODS and died in the ICU compared to those who did not experience MODS and survived to ICU discharge. HsCRP levels discriminated for MODS and ICU death only 48 h after admission (57).

After ROSC, the levels of soluble intercellular adhesion molecule-1, soluble vascular-cell adhesion molecule-1, and P- and E-selectins showed early increases indicating leucocyte and endothelial activation. This condition is ultimately associated with a rapid PTX3 increase, as illustrated by the 10-fold higher plasma levels already observed at ICU admission in the cohort study. Thus, early PTX3 levels, i.e., at ICU admission after ROSC, are independent predictors of MODS and early death, while CRP is not. Moreover, since PTX3 levels continue to rise during ICU recovery, the post-CA pro-inflammatory response is prolonged and could be therefore a potential target for intervention (57).

## PTX3 as Circulating Biomarker in CVD

Preclinical data in the mouse and the homology with CRP, a molecule used to monitor inflammatory diseases and infection in clinical practice, prompted investigation of PTX3 as possible marker of human pathology. As seen above, PTX3 has been investigated as a possible circulating biomarker in MI, HF and CA, but the numbers of pathological conditions potentially involving PTX3 as biomarker are larger. The rapid rises in PTX3 plasma levels are compatible with an acute phase response. PTX3 blood levels can reach 800 ng/ml in patients with endotoxin shock, sepsis and infections of viral, bacterial or fungal origin (58, 77–83). In general, PTX3 circulating levels were significantly correlated with the severity of disease and mortality, and served to monitor the response to therapy. In addition, PTX3 levels rarely correlate with CRP, indicating that the two proteins might have different roles. This lack of correlation was useful to distinguish the presence and absence of shock in a small cohort of patients with meningococcal disease (80). In addition, myeloproliferative disorder patients with major thrombosis had higher levels of hsCRP and lower levels of PTX3, thus confirming that the two molecules modulate cardiovascular risk factors in opposite ways (84).

The diagnostic and prognostic value of circulating PTX3 was tested in recent studies on patients with severe sepsis and septic shock. The Albumin Italian Outcome Sepsis (ALBIOS) trial reported that high levels of PTX3 measured in a cohort of 958 patients on day 1 after admission to the ICU were able to predict new organ failures, while a smaller drop in circulating PTX3 over time predicted an increased risk of death (58). Similar increases in plasmatic levels of PTX3 and associations with mortality in patients with sepsis or septic shock were reported in other studies (85–88).

PTX3 plasma levels were significantly elevated in patients with arterial inflammation who underwent percutaneous coronary intervention (PCI) (54, 89, 90). Systemic PTX3 levels before PCI were associated with larger plaque area and volume, a higher risk of plaque rupture at the culprit site and impaired post-PCI myocardial perfusion (49). A study on 594 patients with stable coronary artery disease (CAD) reported that PTX3 plasma levels were higher 24 h after PCI than before (91). During the follow-up for major adverse cardiovascular events, patients with higher post-PCI levels of PTX3 had a higher incidence of events. Similarly, in patients with angina who underwent PCI, PTX3 levels resulted to be an independent risk factor associated to troponin increase after PCI (92). These data suggest that PTX3 could provide a reliable marker for risk stratification in patients undergoing PCI. Patients with unstable angina pectoris have higher PTX3 levels than healthy controls, suggesting that this long pentraxin might be a candidate marker to unstable angina (54).

PTX3 has also been proposed as a novel marker for stent-induced inflammation in patients with CAD after PCI (93). PTX3 was increased in peripheral blood and in the coronary sinus of 20 patients undergoing coronary stenting. Expression of CD11b/CD18 on neutrophils correlated with PTX3 levels. The relative PTX3 increase observed at 24 h was the most powerful predictor of late lumen loss. In the same setting, CRP could not discriminate between patients with and without re-stenosis. These data suggested that, after vascular injury PTX3 may be used as a marker of the inflammatory response and neointimal thickening. Patients with re-stenosis after PCI presented a positive transcardiac gradient, indicative of PTX3 production by the coronary vasculature (93). The role of PTX3 in patients with CAD was confirmed in a subsequent prospective observational study on 75 ST elevation MI patients. High levels of PTX3 before PCI were associated with higher frequencies of plaque rupture (49).

PTX3 levels were high in patients with small vessel vasculitis and rheumatoid arthritis, but not in those with systemic lupus erythematosus (94, 95). In small vessel vasculitis, a group of autoimmune disorders characterized by inflammation of the blood vessels, PTX3 plasma levels were higher in patients with

active disease than in those with quiescent disease (95). EC are responsible for PTX3 production, as shown by IHC performed on skin sections at sites of vasculitis (96). Moreover, PTX3 is more abundant at sites of leukocytoclastic infiltration (97). PTX3, in contrast to the short pentraxin SAP, inhibits the uptake of apoptotic PMNs by macrophages (97), suggesting that the long pentraxin is a key factor in the incomplete clearance of apoptotic and secondary necrotic PMNs observed in small-vessels vasculitis (96). High circulating PTX3 levels were associated with vascular injury in systemic lupus erythematosus patients, thus increasing the dysfunction on the vascular endothelium (98).

PTX3 plasma levels also increase in patients with chronic kidney disease (CKD) (99), and correlate with the severity of the disease (100, 101). Hemodialysis (HD) patients have higher circulating levels of PTX3 compared to peritoneal hemodialysis patients (102). During the HD session, PTX3 plasma levels are increased, suggesting that the protein could be a biomarker of the HD-induced inflammation (103). In addition, in the presence of peripheral or coronary artery disease, PTX3 levels are significantly increased (102). Finally, high PTX3 levels predict all-cause mortality and cardiovascular mortality in patients with CKD (99), a finding reminiscent of MI data (56). PTX3 was associated with proteinuria and endothelial dysfunction in patients with advanced CKD or type 2 diabetes (104), suggesting that PTX3 is more than just an additional marker of inflammation in chronic HF (105).

Preeclampsia, a pathological condition causing an exaggerated inflammatory response resulting in endothelial dysfunction (106–108), is a major complication of pregnancy. Circulating levels of PTX3 are high in preeclampsia, underlining the strong inflammatory response (108, 109). In addition, PTX3 levels in the first trimester were altered in women who subsequently developed preeclampsia, this confirming that an excessive inflammatory response is one of the causal factors causing preeclampsia in pregnant women (110).

## CONCLUDING REMARKS

PTX3, the prototype member of the long pentraxin family, is a soluble pattern recognition molecule with multifunctional properties. Genetic approaches indicate that PTX3 is an essential component of innate immunity and a modulator of the inflammatory response. Not surprisingly in an intricate field such as the immune/inflammatory response, PTX3 has a dual character, one is good-protective against excessive inflammatory response, the other is harmful- antiangiogenic in cardiovascular diseases or inhibitor of phagocytosis in nasopharyngeal carcinoma (111). In addition, the recent observations on the involvement of PTX3 in tissue remodeling and repair (23–26) may cast further light on the role of this molecule in the cardiovascular pathology.

Current data are consistent with a role of PTX3 as a novel marker of CVD (**Figure 3**). In general, PTX3 levels rise rapidly, reflecting the inflammatory response affecting vascular involvement. Thus, PTX3 has a different kinetics of production and different patterns of recognized ligands from CRP, a much more widely used biomarker of inflammation and infection. Data available so far propose that CRP and PTX3 could serve as complementary biomarkers of pathological conditions, with CRP reflecting a systemic response while PTX3 is mainly produced locally. However, one must acknowledge that, while the functional role of PTX3 in some vascular disorders, i.e., atherosclerosis and MI, has been well-described, in HF and CA, PTX3 has been explored only as a biomarker and there is little evidence of its functional involvement. In fact, the crucial question on each candidate biomarker is "How long can a biomarker be called "emerging"? Are 10 years enough? Or better 20? How long should we keep searching for evidence? It is impossible to answer this rationallly. Although almost 50 years passed since the discovery of natriuretic peptides and their functions, the evidence of benefits obtained from their clinical monitoring is still incomplete.

## AUTHOR CONTRIBUTIONS

GR, BB, and RL equally contributed to work conception and draft manuscript. FF, DO, and DN contributed to literature review and draft parts of the manuscript. AM contributed to manuscript revision.

## FUNDING

The contribution of the European Commission (HOMAGE FP7 305507, ESCAPE-NET H2020 agreement no. 733381), Cluster Alisei (MEDINTECH CTN01_00177_962865) and European Research Council (ERC project PHII—agreement no. 669415) are gratefully acknowledged.

## ACKNOWLEDGMENTS

We are grateful to Vanna Pistotti for the literature search used for **Figure 1**.

## REFERENCES

1. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. (2010) 464:1357–61. doi: 10.1038/nature08938
2. Geovanini GR, Libby P. Atherosclerosis and inflammation: overview and updates. *Clin Sci*. (2018) 132:1243–52. doi: 10.1042/cs20180306
3. Ridker PM, MacFadyen J, Libby P, Glynn RJ. Relation of baseline high-sensitivity C-reactive protein level to cardiovascular outcomes with rosuvastatin in the Justification for Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER). *Am J Cardiol*. (2010) 106:204–9. doi: 10.1016/j.amjcard.2010.03.018
4. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic

disease. *N Engl J Med*. (2017) 377:1119–31. doi: 10.1056/NEJMoa1707914
5. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol*. (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
6. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol*. (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
7. Pepys MB. The Pentraxins 1975–2018: serendipity, diagnostics and drugs. *Front Immunol*. 9:2382. doi: 10.3389/fimmu.2018.02382
8. Avan A, Tavakoly Sany SB, Ghayour-Mobarhan M, Rahimi HR, Tajfard M, Ferns G. Serum C-reactive protein in the prediction of cardiovascular diseases: overview of the latest clinical studies and public health practice. *J Cell Physiol*. (2018) 233:8508–25. doi: 10.1002/jcp.26791
9. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. Ptx3, a Humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiol Rev*. (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
10. Ross R. Atherosclerosis — An Inflammatory Disease. *N Engl J Med*. (1999) 340:115–26. doi: 10.1056/nejm199901143400207
11. Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med*. (2014) 370:421–32. doi: 10.1056/NEJMoa1211161
12. Wojtowicz A, Lecompte TD, Bibert S, Manuel O, Rueger S, Berger C, et al. PTX3 polymorphisms and invasive mold infections after solid organ transplant. *Clin Infect Dis*. (2015) 61:619–22. doi: 10.1093/cid/civ386
13. Fisher CE, Hohl TM, Fan W, Storer BE, Levine DM, Zhao LP, et al. Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. *Blood*. (2017) 129:2693–701. doi: 10.1182/blood-2016-10-743294
14. Kabbani D, Bhaskaran A, Singer LG, Bhimji A, Rotstein C, Keshavjee S, et al. Pentraxin 3 levels in bronchoalveolar lavage fluid of lung transplant recipients with invasive aspergillosis. *J Heart Lung Transplant*. (2017) 36:973–9. doi: 10.1016/j.healun.2017.04.007
15. Herrero-Sanchez MC, Angomas EB, de Ramon C, Telleria JJ, Corchete LA, Alonso S, et al. Polymorphisms in receptors involved in opsonic and non-opsonic phagocytosis and the risk of infection in oncohematological patients. *Infect Immun*. (2018) 86:e00709-18. doi: 10.1128/iai.00709-18.
16. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, et al. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med*. (2007) 204:793–804. doi: 10.1084/jem.20061301
17. Breviario F, d'Aniello EM, Golay J, Peri G, Bottazzi B, Bairoch A, et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem*. (1992) 267:22190–7.
18. Norata GD, Marchesi P, Pirillo A, Uboldi P, Chiesa G, Maina V, et al. Long pentraxin 3, a key component of innate immunity, is modulated by high-density lipoproteins in endothelial cells. *Arterioscler Thromb Vasc Biol*. (2008) 28:925–31. doi: 10.1161/ATVBAHA.107.160606
19. Norata GD, Marchesi P, Pulakazhi Venu VK, Pasqualini F, Anselmo A, Moalli F, et al. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. *Circulation*. (2009) 120:699–708. doi: 10.1161/CIRCULATIONAHA.108.806547
20. Salio M, Chimenti S, De Angelis N, Molla F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. *Circulation*. (2008) 117:1055–64. doi: 10.1161/CIRCULATIONAHA.107.749234
21. Halle M, Gabrielsen A, Paulsson-Berne G, Gahm C, Agardh HE, Farnebo F, et al. Sustained inflammation due to nuclear factor-kappa B activation in irradiated human arteries. *J Am Coll Cardiol*. (2010) 55:1227–36. doi: 10.1016/j.jacc.2009.10.047
22. Zhao Y, Feng G, Wang Y, Yue Y, Zhao W. A key mediator, PTX3, of IKK/IkappaB/NF-kappaB exacerbates human umbilical vein endothelial cell injury and dysfunction. *Int J Clin Exp Pathol*. (2014) 7:7699–707.
23. Doni A, Musso T, Morone D, Bastone A, Zambelli V, Sironi M, et al. An acidic microenvironment sets the humoral pattern recognition molecule PTX3 in a tissue repair mode. *J Exp Med*. (2015) 212:905–25. doi: 10.1084/jem.20141268
24. Bonacina F, Barbieri SS, Cutuli L, Amadio P, Doni A, Sironi M, et al. Vascular pentraxin 3 controls arterial thrombosis by targeting collagen and fibrinogen induced platelets aggregation. *Biochim Biophys Acta*. (2016) 1862:1182–90. doi: 10.1016/j.bbadis.2016.03.007
25. Rodriguez-Grande B, Swana M, Nguyen L, Englezou P, Maysami S, Allan SM, et al. The acute-phase protein PTX3 is an essential mediator of glial scar formation and resolution of brain edema after ischemic injury. *J Cereb Blood Flow Metab*. (2014) 34:480–8. doi: 10.1038/jcbfm.2013.224
26. Grcevic D, Sironi M, Valentino S, Deban L, Cvija H, Inforzato A, et al. The long pentraxin 3 plays a role in bone turnover and repair. *Front Immunol*. (2018) 9:417. doi: 10.3389/fimmu.2018.00417
27. Deban L, Castro Russo R, Sironi M, Moalli F, Scanziani M, Zambelli V, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nat Immunol*. (2010) 11:328–34. doi: 10.1038/ni.1854
28. Maugeri N, Rovere-Querini P, Slavich M, Coppi G, Doni A, Bottazzi B, et al. Early and transient release of leukocyte pentraxin 3 during acute myocardial infarction. *J Immunol*. (2011) 187:970–9. doi: 10.4049/jimmunol.1100261
29. Suzuki S, Shishido T, Funayama A, Netsu S, Ishino M, Kitahara T, et al. Long pentraxin PTX3 exacerbates pressure overload-induced left ventricular dysfunction. *PLoS ONE*. (2013) 8:e53133. doi: 10.1371/journal.pone.0053133
30. Rolph MS, Zimmer S, Bottazzi B, Garlanda C, Mantovani A, Hansson GK. Production of the long pentraxin PTX3 in advanced atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. (2002) 22:e10–14. doi: 10.1161/01.ATV.0000015595.95497.2F
31. Klouche M, Peri G, Knabbe C, Eckstein HH, Schmid FX, Schmitz G, et al. Modified atherogenic lipoproteins induce expression of pentraxin-3 by human vascular smooth muscle cells. *Atherosclerosis*. (2004) 175:221–8. doi: 10.1016/j.atherosclerosis.2004.03.020
32. Rusnati M, Camozzi M, Moroni E, Bottazzi B, Peri G, Indraccolo S, et al. Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood*. (2004) 104:92–9. doi: 10.1182/blood-2003-10-3433
33. Presta M, Foglio E, Churruca Schuind A, Ronca R. Long Pentraxin-3 modulates the angiogenic activity of fibroblast growth factor-2. *Front Immunol*. (2018) 9:2327. doi: 10.3389/fimmu.2018.02327
34. Presta M, Camozzi M, Salvatori G, Rusnati M. Role of the soluble pattern recognition receptor PTX3 in vascular biology. *J Cell Mol Med*. (2007) 11:723–38. doi: 10.1111/j.1582-4934.2007.00061.x
35. Camozzi M, Zacchigna S, Rusnati M, Coltrini D, Ramirez-Correa G, Bottazzi B, et al. Pentraxin 3 inhibits fibroblast growth factor 2-dependent activation of smooth muscle cells *in vitro* and neointima formation *in vivo*. *Arterioscler Thromb Vasc Biol*. (2005) 25:1837–42. doi: 10.1161/01.ATV.0000177807.54959.7d
36. Bosutti A, Grassi G, Zanetti M, Aleksova A, Zecchin M, Sinagra G, et al. Relation between the plasma levels of LDL-cholesterol and the expression of the early marker of inflammation long pentraxin PTX3 and the stress response gene p66ShcA in pacemaker-implanted patients. *Clin Exp Med*. (2007) 7:16–23. doi: 10.1007/s10238-007-0118-y
37. Alberti L, Gilardini L, Zulian A, Micheletto G, Peri G, Doni A, et al. Expression of long pentraxin PTX3 in human adipose tissue and its relation with cardiovascular risk factors. *Atherosclerosis*. (2008) 202:455–60. doi: 10.1016/j.atherosclerosis.2008.05.015
38. Gustin C, Delaive E, Dieu M, Calay D, Raes M. Upregulation of pentraxin-3 in human endothelial cells after lysophosphatidic acid exposure. *Arterioscler Thromb Vasc Biol*. (2008) 28:491–7. doi: 10.1161/ATVBAHA.107.158642
39. Savchenko A, Imamura M, Ohashi R, Jiang S, Kawasaki T, Hasegawa G, et al. Expression of pentraxin 3 (PTX3) in human atherosclerotic lesions. *J Pathol*. (2008) 215:48–55. doi: 10.1002/path.2314
40. Introna M, Alles VV, Castellano M, Picardi G, De Gioia L, Bottazzai B, et al. Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites. *Blood*. (1996) 87:1862–72.
41. Nebuloni M, Pasqualini F, Zerbi P, Lauri E, Mantovani A, Vago L, et al. PTX3 expression in the heart tissues of patients with myocardial infarction and infectious myocarditis. *Cardiovasc Pathol*. (2011) 20:e27–35. doi: 10.1016/j.carpath.2010.02.005

42. Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, et al. Binding of the long pentraxin PTX3 to Factor H: interacting domains and function in the regulation of complement activation. *J Immunol*. (2008) 181:8433–40. doi: 10.4049/jimmunol.181.12.8433
43. Wang L, Cano M, Datta S, Wei H, Ebrahimi KB, Gorashi Y, et al. Pentraxin 3 recruits complement factor H to protect against oxidative stress-induced complement and inflammasome overactivation. *J Pathol*. (2016) 240:495–506. doi: 10.1002/path.4811
44. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell*. (2015) 160:700–14. doi: 10.1016/j.cell.2015.01.004
45. Savchenko AS, Inoue A, Ohashi R, Jiang S, Hasegawa G, Tanaka T, et al. Long pentraxin 3 (PTX3) expression and release by neutrophils *in vitro* and in ulcerative colitis. *Pathol Int*. (2011) 61:290–7. doi: 10.1111/j.1440-1827.2011.02651.x
46. Matsui S, Ishii J, Kitagawa F, Kuno A, Hattori K, Ishikawa M, et al. Pentraxin 3 in unstable angina and non-ST-segment elevation myocardial infarction. *Atherosclerosis*. (2010) 210:220–5. doi: 10.1016/j.atherosclerosis.2009.10.033
47. Eggers KM, Armstrong PW, Califf RM, Johnston N, Simoons ML, Venge P, et al. Clinical and prognostic implications of circulating pentraxin 3 levels in non ST-elevation acute coronary syndrome. *Clin Biochem*. (2013) 46:1655–9. doi: 10.1016/j.clinbiochem.2013.08.014
48. Guo R, Li Y, Wen J, Li W, Xu Y. Elevated plasma level of pentraxin-3 predicts in-hospital and 30-day clinical outcomes in patients with non-ST-segment elevation myocardial infarction who have undergone percutaneous coronary intervention. *Cardiology*. (2014) 129:178–88. doi: 10.1159/000364996
49. Kimura S, Inagaki H, Haraguchi G, Sugiyama T, Miyazaki T, Hatano Y, et al. Relationships of elevated systemic pentraxin-3 levels with high-risk coronary plaque components and impaired myocardial perfusion after percutaneous coronary intervention in patients with ST-elevation acute myocardial infarction. *Circ J*. (2014) 78:159–69. doi: 10.1253/circj.CJ-13-0329
50. Akgul O, Baycan OF, Bulut U, Somuncu MU, Pusuroglu H, Ozyilmaz S, et al. Long-term prognostic value of elevated pentraxin 3 in patients undergoing primary angioplasty for ST-elevation myocardial infarction. *Coron Artery Dis*. (2015) 26:592–7. doi: 10.1097/mca.0000000000000280
51. Mjelva OR, Ponitz V, Brugger-Andersen T, Grundt H, Staines H, Nilsen DW. Long-term prognostic utility of pentraxin 3 and D-dimer as compared to high-sensitivity C-reactive protein and B-type natriuretic peptide in suspected acute coronary syndrome. *Eur J Prev Cardiol*. (2016) 23:1130–40. doi: 10.1177/2047487315619733
52. Altay S, Cakmak HA, Kemaloglu Oz T, Ozpamuk Karadeniz F, Turer A, Erer HB, et al. Long-term prognostic significance of pentraxin-3 in patients with acute myocardial infarction: 5-year prospective cohort study. *Anatol J Cardiol*. (2017) 17:202–9. doi: 10.14744/AnatolJCardiol.2016.7307
53. Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, et al. PTX3, A prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. *Circulation*. (2000) 102:636–41. doi: 10.1161/01.CIR.102.6.636
54. Inoue K, Sugiyama A, Reid PC, Ito Y, Miyauchi K, Mukai S, et al. Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris. *Arterioscler Thromb Vasc Biol*. (2007) 27:161–7. doi: 10.1161/01.ATV.0000252126.48375.d5
55. Yamasaki K, Kurimura M, Kasai T, Sagara M, Kodama T, Inoue K. Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations. *Clin Chem Lab Med*. (2009) 47:471–7. doi: 10.1515/CCLM.2009.110
56. Latini R, Maggioni AP, Peri G, Gonzini L, Lucci D, Mocarelli P, et al. Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. *Circulation*. (2004) 110:2349–54. doi: 10.1161/01.CIR.0000145167.30987.2E
57. Ristagno G, Varpula T, Masson S, Greco M, Bottazzi B, Milani V, et al. Elevations of inflammatory markers PTX3 and sST2 after resuscitation from cardiac arrest are associated with multiple organ dysfunction syndrome and early death. *Clin Chem Lab Med*. (2015) 53:1847–57. doi: 10.1515/cclm-2014-1271
58. Caironi P, Masson S, Mauri T, Bottazzi B, Leone R, Magnoli M, et al. Pentraxin 3 in patients with severe sepsis or shock: the ALBIOS trial. *Eur J Clin Invest*. (2017) 47:73–83. doi: 10.1111/eci.12704
59. Helseth R, Solheim S, Opstad T, Hoffmann P, Arnesen H, Seljeflot I. The time profile of Pentraxin 3 in patients with acute ST-elevation myocardial infarction and stable angina pectoris undergoing percutaneous coronary intervention. *Mediators Inflamm*. (2014) 2014:608414. doi: 10.1155/2014/608414
60. Jenny NS, Arnold AM, Kuller LH, Tracy RP, Psaty BM. Associations of pentraxin 3 with cardiovascular disease and all-cause death: the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol*. (2009) 29:594–9. doi: 10.1161/ATVBAHA.108.178947
61. Jenny NS, Blumenthal RS, Kronmal RA, Rotter JI, Siscovick DS, Psaty BM. Associations of pentraxin 3 with cardiovascular disease: the multi-ethnic study of atherosclerosis. *J Thromb Haemost*. (2014) 12:999–1005. doi: 10.1111/jth.12557
62. Vengen IT, Enger TB, Videm V, Garred P. Pentraxin 3, ficolin-2 and lectin pathway associated serine protease MASP-3 as early predictors of myocardial infarction - the HUNT2 study. *Sci Rep*. (2017) 7:43045. doi: 10.1038/srep43045
63. Napoleone E, Di Santo A, Bastone A, Peri G, Mantovani A, de Gaetano G, et al. Long pentraxin PTX3 upregulates tissue factor expression in human endothelial cells: a novel link between vascular inflammation and clotting activation. *Arterioscler Thromb Vasc Biol*. (2002) 22:782–7. doi: 10.1161/01.ATV.0000012282.39306.64
64. Napoleone E, di Santo A, Peri G, Mantovani A, de Gaetano G, Donati MB, et al. The long pentraxin PTX3 up-regulates tissue factor in activated monocytes: another link between inflammation and clotting activation. *J Leukoc Biol*. (2004) 76:203–9. doi: 10.1189/jlb.1003528
65. Ueland T, Gullestad L, Nymo SH, Yndestad A, Aukrust P, Askevold ET. Inflammatory cytokines as biomarkers in heart failure. *Clin Chim Acta*. (2015) 443:71–7. doi: 10.1016/j.cca.2014.09.001
66. Nymo SH, Aukrust P, Kjekshus J, McMurray JJ, Cleland JG, Wikstrand J, et al. Limited added value of circulating inflammatory biomarkers in chronic heart failure. *JACC Heart Fail*. (2017) 5:256–64. doi: 10.1016/j.jchf.2017.01.008
67. Kotooka N, Inoue T, Aoki S, Anan M, Komoda H, Node K. Prognostic value of pentraxin 3 in patients with chronic heart failure. *Int J Cardiol*. (2008) 130:19–22. doi: 10.1016/j.ijcard.2007.07.168
68. Suzuki S, Takeishi Y, Niizeki T, Koyama Y, Kitahara T, Sasaki T, et al. Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure. *Am Heart J*. (2008) 155:75–81. doi: 10.1016/j.ahj.2007.08.013
69. Matsubara J, Sugiyama S, Nozaki T, Sugamura K, Konishi M, Ohba K, et al. Pentraxin 3 is a new inflammatory marker correlated with left ventricular diastolic dysfunction and heart failure with normal ejection fraction. *J Am Coll Cardiol*. (2011) 57:861–9. doi: 10.1016/j.jacc.2010.10.018
70. Matsubara J, Sugiyama S, Nozaki T, Akiyama E, Matsuzawa Y, Kurokawa H, et al. Incremental prognostic significance of the elevated levels of pentraxin 3 in patients with heart failure with normal left ventricular ejection fraction. *J Am Heart Assoc*. (2014) 3:928 doi: 10.1161/jaha.114.000928
71. Ishino M, Takeishi Y, Niizeki T, Watanabe T, Nitobe J, Miyamoto T, et al. Risk stratification of chronic heart failure patients by multiple biomarkers: implications of BNP, H-FABP, and PTX3. *Circ J*. (2008) 72:1800–5. doi: 10.1253/circj.CJ-08-0157
72. Latini R, Gullestad L, Masson S, Nymo SH, Ueland T, Cuccovillo I, et al. Pentraxin-3 in chronic heart failure: the CORONA and GISSI-HF trials. *Eur J Heart Fail*. (2012) 14:992–9. doi: 10.1093/eurjhf/hfs092
73. Chow OA, von Kockritz-Blickwede M, Bright AT, Hensler ME, Zinkernagel AS, Cogen AL, et al. Statins enhance formation of phagocyte extracellular traps. *Cell Host Microbe*. (2010) 8:445–54. doi: 10.1016/j.chom.2010.10.005
74. Sasson C, Rogers MA, Dahl J, Kellermann AL. Predictors of survival from out-of-hospital cardiac arrest: a systematic review and meta-analysis. *Circ Cardiovasc Qual Outcomes*. (2010) 3:63–81. doi: 10.1161/circoutcomes.109.889576
75. Nolan JP, Soar J, Cariou A, Cronberg T, Moulaert VR, Deakin CD, et al. European resuscitation council and european society of intensive care medicine guidelines for post-resuscitation care 2015: section 5 of

the european resuscitation council guidelines for resuscitation 2015. *Resuscitation*. (2015) 95:202–22. doi: 10.1016/j.resuscitation.2015.07.018
76. Adrie C, Adib-Conquy M, Laurent I, Monchi M, Vinsonneau C, Fitting C, et al. Successful cardiopulmonary resuscitation after cardiac arrest as a "sepsis-like" syndrome. *Circulation*. (2002) 106:562–8. doi: 10.1161/01.CIR.0000023891.80661.AD
77. Muller B, Peri G, Doni A, Torri V, Landmann R, Bottazzi B, et al. Circulating levels of the long pentraxin PTX3 correlate with severity of infection in critically ill patients. *Crit Care Med*. (2001) 29:1404–7. doi: 10.1097/00003246-200107000-00017
78. Azzurri A, Sow OY, Amedei A, Bah B, Diallo S, Peri G, et al. IFN-gamma-inducible protein 10 and pentraxin 3 plasma levels are tools for monitoring inflammation and disease activity in Mycobacterium tuberculosis infection. *Microbes Infect*. (2005) 7:1–8. doi: 10.1016/j.micinf.2004.09.004
79. Mairuhu AT, Peri G, Setiati TE, Hack CE, Koraka P, Soemantri A, et al. Elevated plasma levels of the long pentraxin, pentraxin 3, in severe dengue virus infections. *J Med Virol*. (2005) 76:547–52. doi: 10.1002/jmv.20397
80. Sprong T, Peri G, Neeleman C, Mantovani A, Signorini S, van der Meer JW, et al. Ptx3 and C-reactive protein in severe meningococcal disease. *Shock*. (2009) 31:28–32. doi: 10.1097/SHK.0b013e31817fd543
81. Wagenaar JF, Goris MG, Gasem MH, Isbandrio B, Moalli F, Mantovani A, et al. Long pentraxin PTX3 is associated with mortality and disease severity in severe Leptospirosis. *J Infect*. (2009) 58:425–32. doi: 10.1016/j.jinf.2009.04.004
82. Foo SS, Chen W, Taylor A, Sheng KC, Yu X, Teng TS, et al. Role of pentraxin 3 in shaping arthritogenic alphaviral disease: from enhanced viral replication to immunomodulation. *PLoS Pathog*. (2015) 11:e1004649. doi: 10.1371/journal.ppat.1004649
83. Ciancarella V, Lembo-Fazio L, Paciello I, Bruno AK, Jaillon S, Berardi S, et al. Role of a fluid-phase PRR in fighting an intracellular pathogen: PTX3 in Shigella infection. *PLoS Pathog*. (2018) 14:e1007469. doi: 10.1371/journal.ppat.1007469
84. Barbui T, Carobbio A, Finazzi G, Vannucchi AM, Barosi G, Antonioli E, et al. Inflammation and thrombosis in essential thrombocythemia and polycythemia vera: different role of C-reactive protein and pentraxin 3. *Haematologica*. (2011) 96:315–8. doi: 10.3324/haematol.2010.031070
85. Hamed S, Behnes M, Pauly D, Lepiorz D, Barre M, Becher T, et al. Diagnostic value of Pentraxin-3 in patients with sepsis and septic shock in accordance with latest sepsis-3 definitions. *BMC Infect Dis*. (2017) 17:554. doi: 10.1186/s12879-017-2606-3
86. Jie H, Li Y, Pu X, Ye J. Pentraxin 3, a predicator for 28-day mortality in patients with septic shock. *Am J Med Sci*. (2017) 353:242–6. doi: 10.1016/j.amjms.2017.01.003
87. Kim SB, Lee KH, Lee JU, Ann HW, Ahn JY, Jeon YD, et al. Long pentraxin 3 as a predictive marker of mortality in severe septic patients who received successful early goal-directed therapy. *Yonsei Med J*. (2017) 58:370–9. doi: 10.3349/ymj.2017.58.2.370
88. Hu C, Zhou Y, Liu C, Kang Y. Pentraxin-3, procalcitonin and lactate as prognostic markers in patients with sepsis and septic shock. *Oncotarget*. (2018) 9:5125–36. doi: 10.18632/oncotarget.23701
89. Munk PS, Breland UM, Aukrust P, Skadberg O, Ueland T, Larsen AI. Inflammatory response to percutaneous coronary intervention in stable coronary artery disease. *J Thromb Thrombolysis*. (2011) 31:92–8. doi: 10.1007/s11239-010-0471-7
90. Alipour-Parsa S, Haybar H, Namazi MH, Safi M, Khaheshi I, Memaryan M, et al. Evaluation of pentraxin-3 level and its related factors in patients undergoing primary percutaneous coronary intervention. *ARYA Atheroscler*. (2017) 13:73–8.
91. Haibo L, Xiaofang G, Chunming W, Jie Y, Guozhong C, Limei Z, et al. Prognostic value of plasma pentraxin-3 levels in patients with stable coronary artery disease after drug-eluting stent implantation. *Mediators Inflamm*. (2014) 2014:963096. doi: 10.1155/2014/963096
92. Wang Z, Sato A, Akiyama D, Kimura T, Tajiri K, Hoshi T, et al. Clinical value of plasma pentraxin 3 levels for predicting cardiac troponin elevation after percutaneous coronary intervention. *Life Sci*. (2014) 95:40–4. doi: 10.1016/j.lfs.2013.11.021
93. Kotooka N, Inoue T, Fujimatsu D, Morooka T, Hashimoto S, Hikichi Y, et al. Pentraxin3 is a novel marker for stent-induced inflammation and neointimal thickening. *Atherosclerosis*. (2008) 197:368–74. doi: 10.1016/j.atherosclerosis.2007.05.031
94. Luchetti MM, Piccinini G, Mantovani A, Peri G, Matteucci C, Pomponio G, et al. Expression and production of the long pentraxin PTX3 in rheumatoid arthritis (RA). *Clin Exp Immunol*. (2000) 119:196–202. doi: 10.1046/j.1365-2249.2000.01110.x
95. Fazzini F, Peri G, Doni A, Dell'Antonio G, Dal Cin E, Bozzolo E, et al. PTX3 in small-vessel vasculitides: an independent indicator of disease activity produced at sites of inflammation. *Arthritis Rheum*. (2001) 44:2841–50. doi: 10.1002/1529-0131(200112)44:12<2841::AID-ART472>3.0.CO;2-6
96. van Rossum AP, Pas HH, Fazzini F, Huitema MG, Limburg PC, Jonkman MF, et al. Abundance of the long pentraxin PTX3 at sites of leukocytoclastic lesions in patients with small-vessel vasculitis. *Arthritis Rheum*. (2006) 54:986–91. doi: 10.1002/art.21669
97. van Rossum AP, Fazzini F, Limburg PC, Manfredi AA, Rovere-Querini P, Mantovani A, et al. The prototypic tissue pentraxin PTX3, in contrast to the short pentraxin serum amyloid P, inhibits phagocytosis of late apoptotic neutrophils by macrophages. *Arthritis Rheum*. (2004) 50:2667–74. doi: 10.1002/art.20370
98. Cieslik P, Hrycek A. Pentraxin 3 as a biomarker of local inflammatory response to vascular injury in systemic lupus erythematosus. *Autoimmunity*. (2015) 48:242–50. doi: 10.3109/08916934.2014.983264
99. Tong MCJ, Qureshi AR, Anderstam B, Heimburger O, Barany P, et al. Plasma Pentraxin 3 in chronic kidney disease patients: association with renal function, protein-energy wasting, cardiovascular disease and mortality. *Clin J Am Soc Nephrol*. (2007) 2:889–97. doi: 10.2215/CJN.00870207
100. Yilmaz MI, Sonmez A, Ortiz A, Saglam M, Kilic S, Eyileten T, et al. Soluble TWEAK and PTX3 in Nondialysis CKD patients: impact on endothelial dysfunction and cardiovascular outcomes. *Clin J Am Soc Nephrol*. (2011) 6:785–92. doi: 10.2215/cjn.09231010
101. Witasp A, Ryden M, Carrero JJ, Qureshi AR, Nordfors L, Naslund E, et al. Elevated circulating levels and tissue expression of pentraxin 3 in uremia: a reflection of endothelial dysfunction. *PLoS ONE*. (2013) 8:e63493. doi: 10.1371/journal.pone.0063493
102. Boehme M, Kaehne F, Kuehne A, Bernhardt W, Schroder M, Pommer W, et al. Pentraxin 3 is elevated in haemodialysis patients and is associated with cardiovascular disease. *Nephrol Dial Transplant*. (2007). 22:2224–9. doi: 10.1093/ndt/gfl747
103. Oldani S, Finazzi S, Bottazzi B, Garlanda C, Baldassarre E, Valaperta S, et al. Plasma pentraxin-3 as a marker of bioincompatibility in hemodialysis patients. *J Nephrol*. (2012) 25:120–6. doi: 10.5301/JN.2011.8432
104. Suliman ME, Yilmaz MI, Carrero JJ, Qureshi AR, Saglam M, Ipcioglu OM, et al. Novel links between the long pentraxin 3, endothelial dysfunction, and albuminuria in early and advanced chronic kidney disease. *Clin J Am Soc Nephrol*. (2008) 3:976–85. doi: 10.2215/CJN.03960907
105. Malaponte G, Libra M, Bevelacqua Y, Merito P, Fatuzzo P, Rapisarda F, et al. Inflammatory status in patients with chronic renal failure: the role of PTX3 and pro-inflammatory cytokines. *Int J Mol Med*. (2007) 20:471–81. doi: 10.3892/ijmm.20.4.471
106. Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin JNJr, et al. Expression of the placental cytokines tumor necrosis factor alpha, interleukin 1beta, and interleukin 10 is increased in preeclampsia. *Am J Obstet Gynecol*. (1999) 181:915–20. doi: 10.1016/S0002-9378(99)70325-X
107. Benyo DF, Smarason A, Redman CW, Sims C, Conrad KP. Expression of inflammatory cytokines in placentas from women with preeclampsia. *J Clin Endocrinol Metab*. (2001) 86:2505–12. doi: 10.1210/jc.86.6.2505
108. Rovere-Querini P, Antonacci S, Dell'antonio G, Angeli A, Almirante G, Cin ED, et al. Plasma and tissue expression of the long

pentraxin 3 during normal pregnancy and preeclampsia. *Obstet Gynecol*. (2006) 108:148–55. doi: 10.1097/01.AOG.0000224607.46622.bc

109. Cetin I, Cozzi V, Pasqualini F, Nebuloni M, Garlanda C, Vago L, et al. Elevated maternal levels of the long pentraxin 3 (PTX3) in preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol*. (2006) 194:1347–53. doi: 10.1016/j.ajog.2005.11.018
110. Cetin I, Cozzi V, Papageorghiou AT, Maina V, Montanelli A, Garlanda C, et al. First trimester PTX3 levels in women who subsequently develop preeclampsia and fetal growth restriction. *Acta Obstet Gynecol Scand*. (2009) 88:846–9. doi: 10.1080/00016340902971441
111. Hsiao YW, Li CF, Chi JY, Tseng JT, Chang Y, Hsu LJ, et al. CCAAT/enhancer binding protein delta in macrophages contributes to immunosuppression and inhibits phagocytosis in nasopharyngeal carcinoma. *Sci Signal*. (2013) 6:ra59. doi: 10.1126/scisignal.2003648

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer LD declared a past co-authorship with several of the authors to the handling editor.

frontiers in Immunology

REVIEW
published: 30 April 2019
doi: 10.3389/fimmu.2019.00943

# Evolution of C-Reactive Protein

*Asmita Pathak and Alok Agrawal**

*Department of Biomedical Sciences, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, United States*

C-reactive protein (CRP) is an evolutionarily conserved protein. From arthropods to humans, CRP has been found in every organism where the presence of CRP has been sought. Human CRP is a pentamer made up of five identical subunits which binds to phosphocholine (PCh) in a $Ca^{2+}$-dependent manner. In various species, we define a protein as CRP if it has any two of the following three characteristics: First, it is a cyclic oligomer of almost identical subunits of molecular weight 20–30 kDa. Second, it binds to PCh in a $Ca^{2+}$-dependent manner. Third, it exhibits immunological cross-reactivity with human CRP. In the arthropod horseshoe crab, CRP is a constitutively expressed protein, while in humans, CRP is an acute phase plasma protein and a component of the acute phase response. As the nature of CRP gene expression evolved from a constitutively expressed protein in arthropods to an acute phase protein in humans, the definition of CRP became distinctive. In humans, CRP can be distinguished from other homologous proteins such as serum amyloid P, but this is not the case for most other vertebrates and invertebrates. Literature indicates that the binding ability of CRP to PCh is less relevant than its binding to other ligands. Human CRP displays structure-based ligand-binding specificities, but it is not known if that is true for invertebrate CRP. During evolution, changes in the intrachain disulfide and interchain disulfide bonds and changes in the glycosylation status of CRP may be responsible for different structure-function relationships of CRP in various species. More studies of invertebrate CRP are needed to understand the reasons behind such evolution of CRP. Also, CRP evolved as a component of and along with the development of the immune system. It is important to understand the biology of ancient CRP molecules because the knowledge could be useful for immunodeficient individuals.

**Keywords: C-reactive protein, pentraxin, serum amyloid P, phosphocholine, PTX3**

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Humanitas Clinical and Research Center, Milan University, Italy*

***Reviewed by:***
*Alexander J. Szalai, University of Alabama at Birmingham, United States*
*Lawrence Albert Potempa, Roosevelt University, United States*

****Correspondence:***
*Alok Agrawal*
*agrawal@etsu.edu*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *04 February 2019*
***Accepted:*** *12 April 2019*
***Published:*** *30 April 2019*

***Citation:***
*Pathak A and Agrawal A (2019) Evolution of C-Reactive Protein. Front. Immunol. 10:943. doi: 10.3389/fimmu.2019.00943*

## INTRODUCTION

Human C-reactive protein (CRP) was identified as a plasma protein which, in the presence of $Ca^{2+}$, precipitated C-polysaccharide (PnC) isolated from the cell wall of *Streptococcus pneumoniae* (1). The precipitation was due to the binding of CRP to phosphocholine (PCh) moiety present in PnC (2). In animals, we define a protein as CRP if it has at least two of the following three characteristics: First, it is a cyclic oligomer of almost identical subunits of molecular weight 20–30 kDa. Second, it binds to PCh in a $Ca^{2+}$-dependent manner. Third, it exhibits immunological cross-reactivity with human CRP.

CRP is an evolutionarily conserved protein. From arthropods to humans, CRP has been found in every organism where the presence of CRP has been sought (3–8). In the arthropod horseshoe crab, CRP is a constitutively expressed protein found in the hemolymph (8). After ∼500 million

years of evolution, in humans and some other species, CRP became a protein which is expressed as a component of the acute phase response (9). The aim of this paper was to review the changes observed in the structure and ligand-binding specificities of CRP during evolution. We reviewed the literature on the structure and ligand-binding specificities of CRP from the following animals from arthropods to humans: American horseshoe crab, giant African snail, 17 different species of fish, chicken, frog, cow, dog, guinea pig, horse, hamster, mouse, goat, rat, rabbit, monkey, pig, mink, elephant, squirrel, seal, phascogale, and man. We compared the primary structure of CRP and searched for the conservation of functionally critical amino acid residues that are known for human CRP (**Figure 1**). We also compared the overall quaternary structure (**Table 1**), ligand-binding specificities, and immunological cross-reactivity of CRP (**Table 2**), if known. Two other proteins, serum amyloid P component (SAP), also known as pentraxin-2, and long pentraxin (PTX3), which share several structural and functional properties with CRP, are not reviewed here (3, 56).

## CRP IN AN ARTHROPOD

A protein that fits the definition of CRP is present in the hemolymph of the arthropod American horseshoe crab, *Limulus polyphemus* (5, 6, 10–13, 57–59). *Limulus* CRP binds to PCh in a $Ca^{2+}$-dependent manner and precipitates PnC. *Limulus* CRP exhibits immunological cross-reactivity against snail CRP; the cross-reactivity against human CRP and rabbit CRP is weak. *Limulus* CRP is made of three types of subunits, each subunit having 218 amino acid residues, encoded by three homologous genes. The three subunits, which share an identical N-terminal sequence of 44 amino acid residues and a C-terminal sequence from amino acid residues 206–218, exist approximately in equimolar amount and are tightly associated. Hexagonal *Limulus* CRP, as revealed by electron microscopy, consists of two copies of each type of subunit. The positions of six half-cystines that form three intrachain disulfide bonds and the site of glycosylation are constant in all subunits. The molecular weight of *Limulus* CRP is ∼300 kDa. The molecular weight of the subunits is ∼25 kDa. Thus, there are 12 subunits in one *Limulus* CRP molecule, that is, two hexamers stacked together. The concentration of CRP in *Limulus* hemolymph is ∼2.0 mg/ml. It remains to be determined whether *Limulus* CRP is an acute phase protein. *Limulus* CRP has also been shown to chelate the heavy metals mercury and cadmium, and hence playing a role in detoxification of heavy metals. Such a detoxification function of *Limulus* CRP is not known for human CRP. Uniquely, *Limulus* CRP has also been shown to exhibit $Ca^{2+}$-independent binding to membranes mimicking the outer membrane of Gram-negative bacteria and then create pores in the lipid bilayer (5, 6, 10–13, 57–59).

**Abbreviations:** CRP, C-reactive protein; FP, female protein; LDL, low density lipoprotein; PCh, phosphocholine; PnC, pneumococcal C-polysaccharide; SAP, serum amyloid P component.

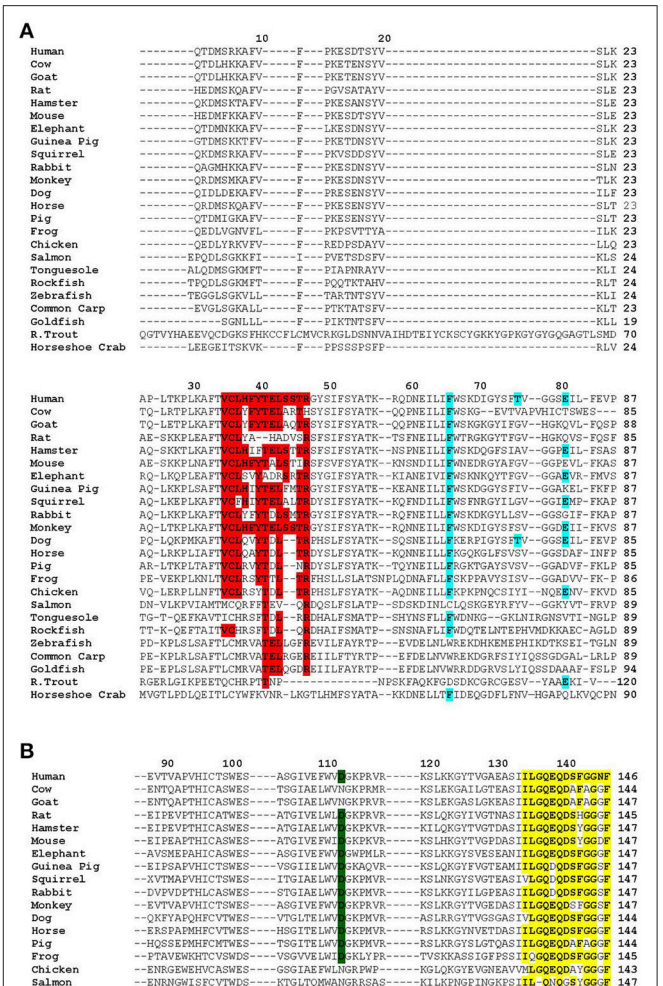


**FIGURE 1 |** Alignment of the primary structure of CRP from various species using Clustal Omega (1.2.4) EMBL-EBI multiple sequence alignment software. Sequences were obtained from NCBI in FASTA format and copied into the EMBL-EBI alignment software where the output result was obtained in the format of ClustalW with character counts. For horse and horseshoe crab, there were several sequences due to the presence of isoforms. Since the microheterogeneity between these isoforms was <10%, the first isoform sequence was selected. Accession numbers of the sequences are: Horseshoe crab, AAA28270; Rainbow trout, NP001118197.1; Goldfish, AK022072.1;

*(Continued)*

**FIGURE 1 |** Common carp, AEU04519; Zebrafish, AGB69036; Rockfish, AKR17056; Tonguefish, NP001281151; Salmon, NP001134140; Chicken, ABD16281; Frog, NP001165686; Pig, NP999009; Horse, XP001504452; Dog, CDF47287; Monkey, XP001117250; Rabbit, NP001075734; Squirrel, XP026263752.1; Guinea pig, AAC60662; Elephant, XP006895510.1; Mouse, AFA37877; Hamster, XP005078251; Rat, AFA37869; Goat, XP017901842; Cow, NP001137569; and Human, AAL48218. The sequence of the signal peptide is not shown. The column on the right shows the number of amino acid residues in each CRP. **(A)** Sequence of amino acid residues 1–87 of human CRP aligned with the sequence of CRP from other animals. Conserved amino acid residues in the following functional sites are highlighted: The intrinsically disordered region in CRP (amino acid residues 35–46 in human CRP) is highlighted in red. The PCh-binding site (amino acid residues 66, 76, and 81 in human CRP) is highlighted in blue. **(B)** Sequence of amino acid residues 88–206 of human CRP aligned with the sequence of CRP from other animals. Conserved amino acid residues in the following functional sites are highlighted: The C1q-binding site (amino acid residues 112 and 175 in human CRP) is highlighted in green. The $Ca^{2+}$-binding site (amino acid residues 134–148 in human CRP) is highlighted in yellow.

## CRP IN A MOLLUSC

Based on the reactivity with anti-*Limulus* CRP antibodies, CRP was detected in the hemolymph of the mollusc, giant African snail, *Achatina fulica* (14, 15, 60–62). *Achatina* CRP binds to PCh in a $Ca^{2+}$-dependent manner. The molecular weight of *Achatina* CRP is ∼400 kDa. *Achatina* CRP is glycosylated and has two types of subunits, of molecular weight 20 and 24 kDa. Although anti-*Limulus* CRP antibodies react with *Achatina* CRP, anti-*Achatina* CRP antibodies do not react with *Limulus* CRP. *Achatina* CRP is a constitutively expressed protein and one of the major components of the haemolymph with a normal level of ∼2.0 mg/ml. Like *Limulus* CRP, *Achatina* CRP has also been shown to bind to heavy metals and it has been proposed that *Achatina* CRP may be utilized as a viable exogenous agent of cytoprotection against heavy metal-related toxicity. In addition, *Achatina* CRP has been found to be bacteriostatic against gram negative bacteria and bactericidal against gram positive bacteria (14, 15, 60–62).

## CRP IN FISH

CRP was first found in the serum of a marine teleost fish, plaice, *Pleuronectes platessa* (16–19) and has been isolated and characterized mostly from teleost fish. Plaice CRP binds to PCh in a $Ca^{2+}$-dependent manner and looks pentameric in its electron microscopic appearance. The molecular weight of plaice CRP is ∼187 kDa, consisting of 10 non-covalently associated subunits arranged in two pentameric discs. There are two distinct subunits in plaice CRP; the difference in size between the subunits is due to carbohydrates since the heavier subunit is glycosylated while the lighter one is not. Plaice CRP is present at a concentration of 55 μg/ml and is not an acute phase protein because its serum concentration does not increase in response to turpentine. However, adrenal hormones and endotoxins do cause an increase in circulating CRP in plaice (16–19). A CRP-like protein has also been purified from the eggs of another marine teleost, lumpsucker, *Cyclopterus lumpus* (20, 21). The molecular weight of lumpsucker CRP is in the range of 125 and 150 kDa. Lumpsucker CRP consists of identical, non-covalently bound subunits of molecular weight 20–21.5 kDa.

CRP is also present in the sera of another teleost, the rainbow trout species, *Salmo gairdneri* and *Oncorhynchus mykiss* (22, 63–67). Trout CRP binds to PCh in a $Ca^{2+}$-dependent manner, precipitates PnC, is a glycoprotein, and has 179 amino acid residues. According to one study, the molecular weight of rainbow trout CRP is 110 kDa, while that of the subunits is ∼20 kDa. According to another study, the molecular weight of rainbow trout CRP is 81.4 kDa, it is a trimer, and composed of one monomer subunit (26.6 kDa) and one disulfide-linked dimer (43.7 kDa). However, there exists the possibility that a hexamer or a double-stacked hexamer was separated into two or four trimers. The CRP level in normal trout sera is in the range of 30–88 μg/ml. Trout CRP is an acute phase protein because its concentration increases in response to toxic chemicals and bacterial pathogens (22, 63–67). Cod, *Gadus morhua*, CRP that exhibits $Ca^{2+}$-dependent binding to PCh, is glycosylated, and is a pentamer in its electron microscopic appearance. The size of the subunits in cod CRP varies in the range of 22–29 kDa (23, 24).

Among other teleosts, CRP has been isolated from the sera of eels *Anguilla anguilla* and *Anguilla japonica* (25, 26). Eel CRP binds to PCh in a $Ca^{2+}$-dependent manner and agglutinates *S. pneumoniae*. The molecular weight of eel CRP is 120 kDa and the subunits are non-glycosylated, with a molecular weight of 24 kDa. The serum levels of eel CRP is ∼1 μg/ml. CRP is present in channel catfish *Ictalurus punctatus* (27). Catfish CRP binds to PCh and precipitates PnC in a $Ca^{2+}$-dependent manner. Catfish CRP is a non-glycosylated protein with a molecular weight of ∼100 kDa. Electron microscopy has shown that catfish CRP has a planar pentagonal symmetry. The serum titer of catfish CRP follows an acute phase pattern in catfish injected with turpentine. Striped catfish, *Pangasianodon hypophthalmus,* CRP binds to PCh in a $Ca^{2+}$-dependent manner, is a trimer of 28 kDa subunits, can exist as tetramers of trimers, is devoid of interchain disulfide bonds, is glycosylated, and agglutinates a few species of pathogenic bacteria (28). A CRP gene in ayu, *Plecoglossus altivelis*, has been identified (29). The expression of ayu CRP is upregulated following bacterial infection. Ayu CRP agglutinates both gram negative and positive bacteria in a $Ca^{2+}$-dependent manner. Ayu CRP is not glycosylated, has a molecular weight of 25.2 kDa, and has 225 amino acid residues.

In cyprinids, the carp family fish rohu, *Labeo rohita*, CRP has been purified by their $Ca^{2+}$-dependent binding to PCh (30, 68, 69). There are three types of glycosylated subunits in rohu CRP, and all three types of subunits move to identical position after desialylation and deglycosylation. Rohu CRP appears pentameric under electron microscope and is composed of identical subunits of molecular weight 33 kDa. Rohu CRP is an acute phase protein because its concentration in serum increases in response to heavy metal poisoning of water. In common carp *Cyprinus carpio*, CRP CRP displays $Ca^{2+}$-dependent binding to phosphate monoesters (31, 37, 70–72). Common carp CRP is glycosylated, has 208 amino acid residues, and the molecular weight of the subunits is 27 kDa. A potential commercial use of CRP, which is constitutively expressed in common carp, is as a biomarker of

**TABLE 1 |** Properties of CRP isolated from various animals. See the text for scientific names of the animals.

| Animal | Approximate molecular weight (kDa) | Symmetry (quaternary structure) | Approximate molecular of subunits (kDa) | Glycosylation | Acute phase protein | References |
|---|---|---|---|---|---|---|
| Horseshoe crab | 300 | Hexamer (stack of two hexamers) | 25 | Yes | No | (10–13) |
| Giant African Snail | 400 | | 20 and 24 | Yes | No | (14, 15) |
| Plaice | 187 | Pentamer (10 subunits) | 18.7 | Yes | No | (16–19) |
| Lumpsucker | 125–150 | Pentamer | 20–21.5 | | | (20, 21) |
| Rainbow trout | 81.4 | Trimer | 26.6 | Yes | Yes | (22) |
| Cod | | Pentamer | 22–29 | Yes | | (23, 24) |
| Eel | 120 | Pentamer | 24 | No | | (25, 26) |
| Channel catfish | 100 | Pentamer | | No | Yes | (27) |
| Striped catfish | | Trimer | 28 | Yes | | (28) |
| Ayu | | | 25.2 | No | Yes | (29) |
| Rohu | | Pentamer | 33 | Yes | Yes | (30) |
| Common carp | | | 27 | Yes | Yes | (31) |
| Major carp | | | 22 and 29 | Yes | Yes | (32, 33) |
| Goldfish | | | 25.6 | | Yes | (34) |
| Dogfish | 250 | Pentamer of dimers | 25 | | | (35, 36) |
| Zebrafish | | Trimer | | | Yes | (37, 38) |
| Tonguefish | | | 26 | | Yes | (39) |
| Rockfish | 160 and 152 | | 30 and 26 | Yes | Yes | (40) |
| Frog | | | 24 | | No | (41) |
| Cow | 100 | Pentamer | 23 | No | No | (42, 43) |
| Dog | 115 | Pentamer | 21 | Yes | Yes | (44, 45) |
| Harbor seal | | | 25 | | Yes | (46) |
| Goat | 120 | Pentamer | 24 | Yes | No | (47) |
| Horse | 118 | Pentamer | 24 | No | Yes | (48) |
| Hamster | 128–150 | Pentamer | 27–30 | Yes | Yes | (49–51) |
| Rabbit | 115–140 | Pentamer | 23.5 | No | Yes | (52, 53) |
| Rat | 129 | Pentamer | 23 | Yes | Yes | (54) |
| Human | 115 | Pentamer | 23 | No | Yes | (55) |

health status in cultured carp. Serum level of CRP in common carp infected with some but not all pathogens increases several-fold, suggesting that common carp CRP is a minor acute phase protein. CRP has also been purified from the sera of major carp *Catla catla* (32, 33). Kinetic studies of metal intoxication in major carp indicated that a unique molecular variant of CRP is present in the serum at the peak level of acute phase induction, and this variant coexists with normal CRP. Major carp CRP is a glycoprotein, contains two non-identical subunits of molecular weight 22 and 29 kDa, and binds to PCh in a $Ca^{2+}$-dependent manner. The electrophoretic mobility of the subunits is identical after desialylation and deglycosylation implying that the molecular variants vary in the glycan parts. The expression of CRP during the course of parasitic infection in the goldfish, *Carassius auratus*, was also determined (34); goldfish CRP, which has 214 amino acid residues and has subunits of 25.6 kDa, enhances complement-mediated killing of trypanosomes *in vitro*, and lysis increases after addition of immune serum.

Dogfish, *Mustelus canis,* CRP also binds to PCh in a $Ca^{2+}$-dependent manner and precipitates PnC (35, 36, 73). Dogfish CRP has a molecular weight of ∼250 kDa with subunits of molecular weight of ∼25 kDa. Dogfish CRP probably exists as pentamers of two disulfide-linked dimers; however, the crystals of the protein were found to contain two hexamers in the asymmetric unit. Dogfish CRP is present at a concentration of 400 μg/ml. Dogfish CRP exhibits immunological cross-reactivity with rabbit CRP. The CRP gene from zebrafish, *Danio rerio*, a bony fish, has been cloned, expressed, protein purified, and crystallized (37, 38, 74, 75). There are seven CRP-like genes in zebrafish which are differentially expressed both normally and in acute phase and have anti-viral activities. Zebrafish CRP is trimeric, and each subunit has 208 amino acid residues. In tonguefish, a flat fish, CRP is composed of 210 amino acid residues with a subunit molecular weight of ∼26 kDa (39). Expression of tonguefish CRP is upregulated by pathogen infection. Tonguefish CRP has been found to interact with both gram positive and negative bacteria.

In rockfish, *Sebastes taczanowskii*, CRP is a sex-limited protein (40). CRP is induced in the serum of males by estrogen administration. Serum levels in females during vitellogenic and

**TABLE 2 |** Immunological cross-reactivity among CRP from various animals.

| Reactivity with serum or CRP | Anti-CRP antibodies or antiserum | | | | | | |
|---|---|---|---|---|---|---|---|
| | Human | Sheep | Rabbit | Dog | Cow | Goat | Horseshoe crab |
| Human | ✓ | | | | ✓ | ✓ | ✓ |
| Sheep | | ✓ | | | | ✓ | |
| Rabbit | ✓ | | ✓ | | | | ✓ |
| Dog | ✓ | | | ✓ | | | |
| Cow | ✓ | | | | ✓ | ✓ | |
| Goat | ✓ | | | | ✓ | ✓ | |
| Rat | | | | | | | |
| Mouse | | ✓ | | | | | |
| Elephant | | | | ✓ | | | |
| Horse | ✓ | | | | | | |
| Monkey | ✓ | ✓ | | | | | |
| Cat | ✓ | | | | | | |
| Sheep | ✓ | | | | ✓ | | |
| Dogfish | | | ✓ | | | | |
| Snail | | | | | | | ✓ |
| Horseshoe crab | ✓ | | ✓ | | | | ✓ |

*Only those animals are shown for which at least one immunological cross-reactivity is known. See the text for references.*

gestation periods are about 1,000 times higher than those in the normal males. In the presence of $Ca^{2+}$, rockfish CRP binds to PCh and agglutinates *S. pneumoniae*. In rockfish, two types of CRP are found, with molecular weights 160 and 152 kDa, with subunits of 30 and 26 kDa, respectively. Both subunits are glycosylated. In another species of rockfish, *Sebastes schlegelii*, CRP contains 208 amino acid residues with a molecular weight of 25 kDa (76). In Atlantic salmon *Salmo salar*, five CRP-like molecules are present (77). Salmon CRP has 208 amino acid residues, is not an acute phase protein, and only one of five CRP species is upregulated by cytokines.

## CRP IN BIRDS, REPTILES, AND AMPHIBIANS

So far, the presence of CRP has been investigated only in the fowl *Gallus gallus*. In chickens, CRP has 206 amino acid residues. The mRNA for CRP was found in many tissues from the fowl by using a probe derived from human CRP cDNA (78, 79). Although genes are present in lizards, the CRP protein has not been isolated and characterized from any reptile (77). Among amphibians, CRP has been isolated from the frog *Xenopus laevis* (41). Frog CRP has 210 amino acid residues. Xenopus CRP is present at an intermediate low level of ∼1 μg/ml in the normal serum. Frog CRP level in the serum is not induced by turpentine. It is suggested that frog CRP represents a transitional period in the evolution of CRP, when host defenses switched from primitive innate immunity to the immune system. The constitutive functions of CRP gradually became less essential as a result of the development of a complex immune system (41).

## CRP IN MAMMALS

CRP from cow, *Bos taurus*, has been purified from the serum (42, 43, 80). Unlike human CRP, bovine CRP does not precipitate PnC. However, bovine CRP exhibits immunological cross-reactivity with human, goat and sheep CRP. Bovine CRP is a pentameric molecule with a molecular weight of ∼100 kDa and is composed of five identical non-glycosylated subunits of molecular weight of ∼23 kDa. Each subunit has one intrachain disulfide bond. The pentameric structure of bovine CRP was seen by electron microscopy. The concentration of bovine CRP is in the range of 5–40 μg/ml and it is not an acute phase protein (42, 43, 80).

CRP from dogs, *Canis lupus*, exhibits $Ca^{2+}$-dependent binding to PCh (44, 45, 81–88). Dog CRP has the typical cyclic pentameric disc-like structure in its electron microscopic appearance, although the pentamer stacks. Dog CRP has 204 amino acid residues and a molecular weight of ∼100 kDa. Dog CRP is composed of five subunits of ∼20 kDa with an intrachain disulfide bond in each subunit. In another study, the molecular weight of dog CRP was estimated to be ∼156 kDa. In one study, two isotypes of CRP with different molecular weights, 22 and 25 kDa were found, with the 25 kDa subunit glycosylated. Two of the five subunits in the pentamer were glycosylated. Antibodies to human CRP react with dog CRP, but antibodies to dog CRP do not react with human CRP. However, antibodies to dog CRP was used to detect CRP in elephants. CRP Normal healthy dogs contain ∼5–60 μg/ml of CRP but, following a stimulus, CRP behaves as an acute phase protein (44, 45, 81–88).

Guinea pig CRP has 206 amino acid residues and has not been characterized fully, but the gene has organization typical of the CRP genes of other mammals. Guinea pig CRP is not an acute phase protein (89). Harbor seal, *Phoca vitulina,* CRP

binds to PCh in a $Ca^{2+}$-dependent manner and has a molecular weight of ∼25 kDa (46). A CRP-like protein has also been isolated from goat serum (47). Direct binding of goat CRP to PCh has not been shown; however, fluid phase PCh inhibits the $Ca^{2+}$-dependent binding of goat CRP to its ligand agarose. Goat CRP is composed of five identical, glycosylated, non-covalently associated subunits, each of molecular weight ∼24 kDa. Goat CRP possesses immunological cross-reactivity with human, cow and sheep CRP. Like in cows and guinea pigs, CRP in goats is not an acute phase protein (47). CRP in horses has pentameric structure as revealed by electron microscopy and binds to PCh in a $Ca^{2+}$-dependent manner (48, 90). Horse CRP has 204 amino acid residues and a molecular weight of ∼118 kDa. Horse CRP is composed of five identical, non-glycosylated and non-covalently associated subunits with molecular weight of ∼23 kDa. Equine CRP displays immunochemical cross-reactivity with human CRP. In horses, CRP is an acute phase protein (48, 90). Monkey CRP has 206 amino acid residues and precipitates PnC; however, monkey CRP was first isolated by $Ca^{2+}$-dependent binding to organic monophosphates. Monkey CRP reacts with anti-sheep CRP antibodies but not with anti-dog CRP antibodies (91). CRP from pigs has 203 amino acid residues but not been characterized fully yet. Porcine CRP is an acute phase protein (92, 93).

A CRP-like protein named female protein (FP) was found in Syrian and Armenian hamsters (49–51, 94–96). FP is a prominent serum constituent of normal female hamsters but is under hormonal control in males. However, in normal adult male hamsters, FP in serum increases only about 5-fold during an acute phase response, in contrast to human CRP which may increase 1,000-fold or more. FP has a pentameric structure as indicated by electron microscopy and binds to PCh in a $Ca^{2+}$-dependent manner. FP has 206 amino acid residues with a molecular weight in the range of 128–150 kDa. The molecular weight of each of the five non-covalantly assembled glycosylated subunits is in the range of 26–30 kDa; each subunit contains a single intrachain disulphide bond. In the presence of $Ca^{2+}$, FP aggregates, probably to form decamers (49–51, 94–96). In mice (97–100), CRP is not an acute phase protein. Murine CRP agglutinates several strains of gram-positive bacteria *in vitro*. Murine CRP has 206 amino acid residues. Protein modeling has demonstrated that adaptively selected amino acid residues in murine CRP lie in the ligand-binding region and contact region between subunits (97–100).

Rabbit CRP reacts with PCh and precipitates PnC in a $Ca^{2+}$-dependent manner (52, 53, 91, 101–120). CRP was found localized at sites of inflammation in rabbits and was not observed at the inflammatory site before appearance in the blood. The concentration of rabbit CRP in the serum is ∼1.5 μg/ml. Investigation of rabbit CRP provided evidence that CRP molecules expressing a structure and antigenicity that are distinct from native CRP occurs *in vivo*, and that such molecules accumulate at sites of inflammation. Rabbit CRP has 205 amino acid residues and is pentameric. The molecular weight of rabbit CRP lies between 115–140 kDa. The subunit size is 23.5 kDa. For precipitation of the PCh-ligands, only the binding of the phosphoryl group of PCh to rabbit CRP is required, unlike for human CRP where binding to both the phosphoryl and cationic groups of PCh are needed for precipitation. Transgenic mice expressing rabbit CRP are partially protected from a lethal challenge of endotoxin or platelet activating factor. Rabbit CRP is capable of activating complement when bound to a ligand; however, complement activation is not required to mediate protection against either endotoxins or platelet activating factor. Immobilized rabbit CRP binds to low-density lipoprotein (LDL) also. In rabbits, CRP has been found associated with the progression of atherosclerosis (52, 53, 101–120).

Rat CRP is unique when compared to CRP from other mammalian species (54, 111, 112, 121–133). Rat CRP has five subunits arranged as a cyclic pentamer and is the only mammalian CRP which is glycosylated and contains a covalently linked dimer in its pentameric structure. Rat CRP binds to PCh in a $Ca^{2+}$-dependent manner but does not precipitate PnC. The non-glycosylated rat CRP is also able to bind to PCh. Rat CRP is made up of 206 amino acid residues. One pair of the subunits per molecule is linked by two interchain disulphide bonds, that is, the five subunits are not non-covalently associated. The other three subunits have intrachain disulfide bonds. Rat CRP is present at a concentration of 0.3–0.5 mg/ml and is not an acute phase protein. Immobilized rat CRP is capable of binding to LDL also in a $Ca^{2+}$-dependent and PCh-inhibitable manner; however, the binding ability of rat CRP to PCh is not a sufficient requirement for the interaction between rat CRP and LDL. A sialic acid moiety must also be present on rat CRP for binding to LDL. LDL is modified once it is complexed with rat CRP. Like Limulus CRP, rat CRP has also been shown to play a role in detoxification of heavy metals such as mercury (54, 111, 112, 121–133).

Mink presents an unusual case (134): it is called mink SAP. However, mink SAP is not glycosylated unlike SAP in other mammals. Mink SAP binds to PCh also, unlike SAP in other mammals. The molecular weight of mink SAP subunits is ∼26 kDa. The presence of mRNA for CRP has also been found by using a probe derived from human CRP cDNA in many tissues from Asian elephant *Elephas maximus* (135, 136) and ground squirrel *Spermophilus richarsonii* (137). CRP is also present in elephant seal *Mirounga angustirostris* (138). A gene sequence for CRP is also found in marsupial genome, red-tailed phascogale *Phascogale calura* (139).

## HUMAN CRP

In humans, CRP is a major acute phase protein whose concentration may increase more than 1,000-fold in severe inflammatory states (9). Human CRP is a pentameric protein composed of five identical non-covalently bound subunits of 206 amino acid residues with a molecular weight of ∼23 kDa. Human CRP binds to PCh in a $Ca^{2+}$-dependent manner. There are five PCh-binding sites, one located on each subunit. Each subunit binds two $Ca^{2+}$ ions (55, 140). In human CRP, $Glu^{81}$ in the PCh-binding site interacts with the nitrogen atom of choline in PCh, $Phe^{66}$ interacts with three methyl groups of choline, and $Thr^{76}$ is critical for creating the appropriately sized pocket to accommodate PCh. The phosphate group of PCh directly coordinates with the two $Ca^{2+}$ bound to CRP. Using synthetic peptides derived from CRP, direct binding of $Ca^{2+}$ to a peptide of residues 134–148 has been shown. Crystallography of CRP

has demonstrated that two $Ca^{2+}$ ions are co-ordinated by $Asp^{60}$, $Asn^{61}$, and by residues $Glu^{138}$, $Gln^{139}$, $Asp^{140}$, $Glu^{147}$, and $Gln^{150}$ in a loop (55, 141–144). Once CRP is bound to a PCh-containing ligand, it activates the classical complement pathway. Residues $Asp^{112}$ and $Tyr^{175}$ play critical roles in the formation of the C1q-binding site in CRP (145–147).

Acidic pH transforms native pentameric CRP into another pentameric configuration, called as non-native CRP, which exposes a hidden ligand-binding site for non-PCh ligands, and which enables CRP to bind to immobilized, denatured and aggregated proteins. For example, CRP does not bind to oxidized LDL (ox-LDL) at physiological pH but gains the ability to bind to ox-LDL at acidic pH (148). $H_2O_2$-treated CRP also gains a ligand recognition property not exhibited by native CRP, indicating that $H_2O_2$, like acidic pH, is another modifier of the structure and ligand recognition function of CRP (149). Immobilization of CRP or mutagenesis of $Glu^{42}$ in the inter-subunit contact region in pentameric CRP also convert CRP into molecules that bind to a variety of immobilized, denatured and aggregated proteins (148–151). A possible binding site in non-native CRP for immobilized, denatured and aggregated proteins could be formed involving the single intrinsically disordered region present in CRP (152). It has been shown that when CRP dissociates into its monomers, monomeric CRP recognizes such protein ligands through the intrinsically disordered region (152).

CRP is a multifunctional component of the human innate host defense machinery. In mouse models of pneumococcal infection, transgenic or passively administered human CRP has been shown to be protective against lethal infection with *S. pneumoniae* (153–160). Similarly, CRP may be an atheroprotective molecule, as shown by using transgenic CRP in animal models of human like atherosclerosis (161–166). CRP has been found deposited at sites of inflammation, indicating the presence of non-native CRP *in vivo*. The functions of CRP at sites of inflammation have not been defined yet; however, it has been suggested that a structural change in CRP and the resulting shift from the ligand recognition function of CRP in its native conformation to another ligand recognition function in its non-native conformation occurs at sites of inflammation (151, 152, 167).

Interestingly, human CRP also possesses sites for glycosylation, although the sites are hidden in native CRP (168–170). When CRP was isolated from patients with six different pathological conditions, CRP was found to be differentially glycosylated. A few amino acids at the N-terminus and a few amino acids near the C-terminus are missing in glycosylated human CRP. The cleavage of these peptides from CRP exposes two potential sites of glycosylation and these sites are located on CRP on the face opposite to the PCh-binding face of CRP. It has been proposed that glycosylated CRP has a protective role toward the clearance of damaged erythrocytes in diseases.

## EVOLUTION OF CRP

As the nature of CRP gene expression evolved from a constitutively expressed protein in arthropods to an acute phase protein in humans, the definition of CRP also became distinctive. In humans, CRP can easily be distinguished from other homologous proteins such as SAP, but this is not the case in invertebrates. For invertebrates, it has always been difficult to define a protein as either CRP or SAP because of the similarities in their structures and functions. Also, whereas a single CRP gene is present in the human, multiple genes are present in some species, such as *Limulus*.

There is sequence similarity and homology among the known functional sites of CRP from all species (**Figure 1**). The PCh-binding property of CRP has been conserved. However, employing animal models of pneumococcal infection it has been shown that the PCh-binding property of human CRP is not the only relevant ligand recognition function of human CRP. Apparently, another ligand-binding property of CRP, such as recognition of complement regulator protein factor H by CRP in its non-native conformation, is responsible for its host defense functions (160). Because some ancient CRP molecules including bovine CRP do not bind PCh, it has been proposed that the recognition of PCh by CRP is less relevant to the role of the protein than its interaction with other ligands (42, 151, 160).

Human CRP in its non-native structural conformation expresses the capability to bind to deposited and conformationally altered proteins and which can be achieved by several means including treatment of CRP with acidic pH (151). The ligand-binding property of human CRP in its non-native structure has implications for toxic and inflammatory conditions and favors the conservation of CRP throughout evolution. It seems that the host-defense functions of CRP evolved to expose a ligand-binding site only when needed, that is, an inflammatory microenvironment would have to be sensed by CRP first and that CRP would change its structure to execute a function. It is not known, however, whether CRP from invertebrates also exhibits structure-based ligand-binding properties. A recent study has shown that *Limulus* CRP is capable of binding to immobilized ox-LDL without being pre-treated with acidic pH (171). During evolution, changes in the intrachain disulfide and interchain disulfide bonds and the changes in the glycosylation status of CRP may also be responsible for the structure-function relationships of CRP in various species.

## CONCLUSIONS

CRP evolved as a component of and along with the development of the entire immune system. Both structure and function of CRP have evolved; however, more studies on CRP from all invertebrates and vertebrates are needed to understand fully the reasons behind the evolution of CRP. Structure-function relationships of CRP from most animals are unknown. We know that the ligand-binding properties of *Limulus* CRP are not identical to that of native human CRP but overlap the ligand-binding properties of non-native pentameric human CRP that can be generated at inflammatory microenvironments (143, 158). Since the ligand recognition functions of CRP lead to effector functions, it is important to understand the biology of ancient CRP molecules because the knowledge could be useful for immunodeficient individuals: all humans have CRP, but it is not known whether human CRP is functional in all humans.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## ACKNOWLEDGMENTS

We are grateful to Irving Kushner, M.D., for reviewing the manuscript and for his helpful suggestions.

## REFERENCES


1. Tillett WS, Francis T Jr. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *J Exp Med*. (1930) 52:561–71.
2. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med*. (1971) 136:612–4.
3. Pepys MB, Dash AC, Fletcher TC, Richardson N, Munn EA, Feinstein A. Analogues in other mammals and in fish of human plasma proteins, C-reactive protein and amyloid P component. *Nature*. (1978) 273:168–70.
4. Baltz ML, De Beer FC, Feinstein A, Munn EA, Milstein CP, Fletcher TC, et al. Phylogenetic aspects of C-reactive protein and related proteins. *Ann NY Acad Sci USA*. (1982) 389:49–75.
5. Maudsley S, Pepys MB. Immunochemical cross-reactions between pentraxins of different species. *Immunology*. (1987) 62:17–22.
6. Ying S-C, Marchalonis JJ, Gewurz AT, Siegel JN, Jiang H, Gewurz BE, et al. Reactivity of anti-human C-reactive protein (CRP) and serum amyloid P component (SAP) monoclonal antibodies with limulin and pentraxins of other species. *Immunology*. (1992) 76:324–30.
7. Nunomura W. C-reactive protein (CRP) in animals: its chemical properties and biological functions. *Zool Sci*. (1992) 9:499–513.
8. Armstrong PB. Comparative biology of the pentraxin protein family: evolutionarily conserved component of innate immune system. *Int Rev Cell Mol Biol*. (2015) 316:1–47. doi: 10.1016/bs.ircmb.2015.01.002
9. Kushner I. The phenomenon of the acute phase response. *Ann NY Acad Sci USA*. (1982) 389:39–48.
10. Robey FA, Liu T-Y. Limulin: a C-reactive protein from *Limulus polyphemus*. *J Biol Chem*. (1981) 256:969–75.
11. Nguyen NY, Suzuki A, Cheng S-M, Zon G, Liu T-Y. Isolation and characterization of *Limulus* C-reactive protein genes. *J Biol Chem*. (1986) 261:10450–5.
12. Nguyen NY, Suzuki A, Boykins RA, Liu TY. The amino acid sequence of *Limulus* C-reactive protein: evidence of polymorphism. *J Biol Chem*. (1986) 261:10456–65.
13. Tennent GA, Bulter PJG, Hutton T, Woolfitt AR, Harvey DJ, Rademacher TW, et al. Molecular characterization of *Limulus polyphemus* C-reactive protein. I. Subunit composition. *Eur J Biochem*. (1993) 214:91–7.
14. Mandal C, Biswas M, Nagpurkar A, Mookerjea S. Isolation of a phosphoryl choline-binding protein from the hemolymph of the snail, *Achatina fulica*. *Dev Comp Immunol*. (1991) 15:227–39.
15. Mukherjee S, Chatterjee S, Sarkar S, Agarwal S, Kundu R, Maitra S, et al. Mollusc C-reactive protein crosses species barrier and reverses hepatotoxicity of lead in rodent models. *Indian J Exp Biol*. (2013) 51:623–34.
16. Pepys MB, De Beer FC, Milstein CP, March JF, Feinstein A, Butress N, et al. C-reactive protein and serum amyloid P component in the plaice (*Pleuronectes platessa* L.), a marine teleost, are homologous with their human counterparts. *Biochim Biophys Acta*. (1982) 704:123–33.
17. White A, Fletcher TC, Pepys MB, Baldo BA. The effect of inflammatory agents on C-reactive protein and serum amyloid P-component levels in plaice (*Pleuronectes platessa* L.) serum. *Comp Biochem Physiol*. (1981) 69:325–9.
18. White A, Fletcher TC. The effects of adrenal hormones, endotoxin and turpentine on serum components of the plaice (*Pleuronectes platessa* L.). *Comp Biochem Physiol*. (1982) 73:195–200.
19. White A, Fletcher TC. The influence of hormones and inflammatory agents on C-reactive protein, cortisol and alanine aminotransferase in the plaice (*Pleuronectes platessa* L). *Comp Biochem Physiol*. (1985) 80C:99–104.
20. White A, Fletcher TC, Towler CM, Baldo BA. Isolation of a C-reactive protein-like precipitin from the eggs of the lumpsucker (*Cyclopterus lumpus* L.). *Comp Biochem Physiol*. (1978) 61C:331–6.
21. Fletcher TC, White A, Youngson A, Pusztai A, Baldo BA. Characterization of C-reactive protein from the eggs of the marine teleost *Cyclopterus lumpus* L. *Biochim Biophys Acta*. (1981) 671:44–9.
22. Murai T, Kodama H, Naiki M, Mikami T, Izawa H. Isolation and characterization of rainbow trout C-reactive protein. *Dev Comp Immunol*. (1990) 14:49–58.
23. Lund V, Olafsen JA. A comparative study of pentraxin-like proteins in different fish species. *Dev Comp Immunol*. (1998) 22:185–94.
24. Gisladottir B, Gudmundsdottir S, Brown L, Jonsson ZO, Magnadottir B. Isolation of two C-reactive protein homologues from cod (*Gadus morhua* L.) serum. Fish Shellfish Immunol. (2009) 26:210–9. doi: 10.1016/j.fsi.2008.03.015
25. Uhlenbruck G, Janssen E, Javeri S. Two different anti-galactan lectins in eel serum. *Immunobiology*. (1982) 163:36–47.
26. Nunomura W. C-reactive protein in eel: purification and agglutinating activity. *Biochim Biophys Acta*. (1991) 1076:191–6.
27. Szalai AJ, Norcum MT, Bly JE, Clem LW. Isolation of an acute-phase phosphorylcholine-reactive pentraxin from channel catfish (*Ictalurus punctatus*). *Comp Biochem Physiol*. (1992) 102B:535–43.
28. Huong Giang DT, Driessche EV, Vandenberghe I, Devreese B, Beeckmans S. Isolation and characterization of SAP and CRP, two pentraxins from *Pangasianodon* (*Pangasius*) *hypophthalmus*. *Fish Shellfish Immunol*. (2010) 28:743–53. doi: 10.1016/j.fsi.2010.01.007
29. Shi Y-H, Chen K, Ma WJ, Chen J. Ayu C-reactive protein/serum amyloid P agglutinates bacteria and inhibits complement-mediated opsonophagocytosis by monocytes/macrophages. *Fish Shellfish Immunol*. (2018) 76:58–67. doi: 10.1016/j.fsi.2018.02.038
30. Sinha S, Mandal C. Microheterogeneity of C-reactive protein in the sera of fish *Labeo rohita* induced by metal pollutants. *Biochem Biophys Res Comm*. (1996) 226:681–7.
31. Cartwright JR, Tharia HA, Burns I, Shrive AK, Hoole D, Greenhough TJ. Isolation and characterisation of pentraxin-like serum proteins from the common carp *Cyprinus carpio*. *Dev Comp Immunol*. (2004) 28:113–25. doi: 10.1016/S0145-305X(03)00123-X ·
32. Paul I, Mandal C, Mandal C. Effect of environmental pollutants on the C-reactive protein of a freshwater major carp, *Catla catla*. *Dev Comp Immunol*. (1998) 22:519–32.
33. Paul I, Mandal C, Allen AK, Mandal C. Glycosylated molecular variants of C-reactive proteins from the major carp *Catla catla* in fresh and polluted aquatic environments. *Glycoconj J*. (2001) 18:547–56. doi: 10.1023/A:1019696430477
34. Kovacevic N, Hagen MO, Xie J, Belosevic M. The analysis of the acute phase response during the course of *Trypanosoma carassii* infection in the goldfish (*Carassius auratus* L.). *Dev Comp Immunol*. (2015) 53:112–22. doi: 10.1016/j.dci.2015.06.009
35. Robey FA, Tanaka T, Liu TY. Isolation and characterization of two major serum proteins from the dogfish, *Mustelus canis*, C-reactive protein and amyloid P component. *J Biol Chem*. (1983) 258:3889–94.
36. Robey FA, Liu TY. Synthesis and use of new spin labeled derivatives of phosphorylcholine in a comparative study of human, dogfish, and *Limulus* C-reactive proteins. *J Biol Chem*. (1983) 258:3895–900.
37. Falco A, Cartwright JR, Wiegertjes GF, Hoole D. Molecular characterization and expression analysis of two new C-reactive protein genes from common carp (*Cyprinus carpio*). Dev Comp Immunol. (2012) 37:127–38. doi: 10.1016/j.dci.2011.10.005
38. Chen R, Qi J, Yao S, Pan X, Gao F, Xia C. Expression, crystallization and preliminary crystallographic analysis of C-reactive protein from zebrafish. *Acta Crystallogr Sect F Struct Biol Cryst Commun*. (2011) 67:1633–6. doi: 10.1107/S1744309111037390

39. Li MF, Chen C, Li J, Sun L. The C-reactive protein of tongue sole *Cynoglossus semilaevis* is an acute phase protein that interacts with bacterial pathogens and stimulates the antibacterial activity of peripheral blood leukocytes. *Fish Shellfish Immunol*. (2013) 34:623–31. doi: 10.1016/j.fsi.2012.12.001
40. Takemura A, Nunomura W, Takano K, Hirai H. Novel female protein with biological activities of C-reactive protein and lectin in a viviparous rockfish, *Sebastes taczanowskii*. *J Exp Zool*. (1993) 266:188–94.
41. Lin L, Liu TY. Isolation and characterization of C-reactive protein (CRP) cDNA and genomic DNA from *Xenopus laevis*: a species representing an intermediate stage in CRP evolution. *J Biol Chem*. (1993) 268:6809–15.
42. Maudsley S, Rowe IF, De Beer FC, Munn EA, Herbert J, Feinstein A, et al. Identification and isolation of two pentraxins from bovine serum. *Clin Exp Immunol*. (1987) 67:662–73.
43. Morimatsu M, Sakai H, Yoshimatsu K, Minowa O, Yamamoto S, Yatomi K, et al. Isolation and characterization of C-reactive protein and serum amyloid P component from bovine serum. *Nippon Juigaku Zasshi*. (1989) 51:723–32.
44. Caspi D, Baltz ML, Snel F, Gruys E, Niv D, Batt RM, et al. Isolation and characterization of C-reactive protein from the dog. *Immunology*. (1984) 53:307–13.
45. Yamamoto S, Tagata K, Nagahata H, Ishikawa Y, Morimatsu M, Naiki M. Isolation of canine C-reactive protein and characterization of its properties. *Vet Immunol Immunopathol*. (1992) 30:329–39.
46. Funke C, King DP, Brotheridge RM, Adelung D, Stott JL. Harbor seal (*Phoca vitulina*) C-reactive protein (CRP): purification, characterization of specific monoclonal antibodies and development of an immunoassay to measure serum CRP concentrations. *Vet Immunol Immunopathol*. (1997) 59:151–62.
47. Maudsley S, Baltz ML, Munn EA, Buttress N, Herbert J, Feinstein A, et al. Isolation and characterisation of goat C-reactive protein. *Biochim Biophys Acta*. (1987) 924:75–80.
48. Takiguchi M, Fujinaga T, Naiki M, Mizuno S, Otomo K. Isolation, characterization, and quantitative analysis of C-reactive protein from horses. *Am J Vet Res*. (1990) 51:1215–20.
49. Coe JE, Margossian SS, Slayter HS, Sogn JA. Hamster female protein: a new pentraxin structurally and functionally similar to C-reactive protein and amyloid P component. *J Exp Med*. (1981) 153:977–91.
50. Coe JE, Ross MJ. Hamster female protein: a divergent acute phase protein in male and female Syrian hamsters. *J Exp Med*. (1983) 157:1421–33.
51. Tennent GA, Baltz ML, Osborn GD, Butler PJG, Noble GE, Hawkins PN, et al. Studies of the structure and binding properties of hamster female protein. *Immunology*. (1993) 80:645–51.
52. Kushner I, Somerville JA. Estimation of the molecular size of C-reactive protein and Cx-reactive protein in serum. *Biochim Biophys Acta*. (1970) 207:105–14.
53. Bach BA, Gewurz H, Osmand AP. C-reactive protein in the rabbit: isolation, characterization and binding affinity to phosphocholine. *Immunochemistry*. (1977) 14:215–9.
54. De Beer FC, Baltz ML, Munn EA, Feinstein A, Taylor J, Bruton C, et al. Isolation and characterization of C-reactive protein and serum amyloid P component in the rat. *Immunology*. (1982) 45:55–70.
55. Shrive AK, Cheetham GMT, Holden D, Myles DAA, Turnell WG, Volanakis JE, et al. Three-dimensional structure of human C-reactive protein. *Nature Struct Biol*. (1996) 3:346–54.
56. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol*. (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
57. Liu TY, Syin C, Nguyen NY, Suzuki A, Boykins RA, Lei KJ, et al. Comparison of protein structure and genomic structure of human, rabbit, and *Limulus* C-reactive proteins: possible implications for function and evolution. *J Prot Chem*. (1987) 6:263–71.
58. Agrawal A, Bhattacharya S. Binding property of rat and *Limulus* C-reactive proteins (CRP) to mercury. *Experientia*. (1989) 45:567–70.
59. Harrington JM, Chou HT, Gutsmann T, Gelhaus C, Stahlberg H, Leippe M, et al. Membrane pore formation by pentraxin proteins from *Limulus*, the American horseshoe crab. *Biochem J*. (2008) 413:305–13. doi: 10.1042/BJ20071357
60. Agrawal A, Mitra S, Ghosh N, Bhattacharya S. C-reactive protein (CRP) in hemolymph of a mollusc, *Achatina fulica* Bowdich. *Indian J Exp Biol*. (1990) 28:788–9.
61. Bose R, Bhattacharya S. C-reactive protein in the hemolymph of *Achatina fulica*: interrelationship with sex steroids and metallothionein. *Comp Biochem Physiol*. (2000) 125:485–95. doi: 10.1016/S1095-6433(00)00176-8
62. Mukherjee S, Barman S, Sarkar S, Mandal NC, Bhattacharya S. Anti-bacterial activity of *Achatina* CRP and its mechanism of action. *Indian J Exp Biol*. (2014) 52:692–704.
63. Winkelhake JL, Chang RJ. Acute phase (C-reactive) protein-like macromolecules from rainbow trout (*Salmo gairdneri*). *Dev Comp Immunol*. (1982) 6:481–9.
64. Winkelhake JL, Vodicnik MJ, Taylor JL. Induction in rainbow trout of an acute phase (C-reactive) protein by chemicals of environmental concern. *Comp Biochem Physiol*. (1983) 74:55–8.
65. Nakanishi Y, Kodama H, Murai T, Mikami T, Izawa H. Activation of rainbow trout complement by C-reactive protein. *Am J Vet Res*. (1991) 52:397–401.
66. Liu Y, Iwasaki T, Watarai S, Kodama H. Effect of turpentine oil on C-reactive protein (CRP) production in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol*. (2004) 17:203–10. doi: 10.1016/j.fsi.2004.03.003
67. Kodama H, Matsuoka Y, Tanaka Y, Liu Y, Iwasaki T, Watarai S. Changes of C-reactive protein levels in rainbow trout (*Oncorhynchus mykiss*) sera after exposure to anti-ectoparasitic chemicals used in aquaculture. *Fish Shellfish Immunol*. (2004) 16:589–97. doi: 10.1016/j.fsi.2003.09.007
68. Mandal C, Sinha S, Mandal C. Lectin like properties and differential sugar binding characteristics of C-reactive proteins purified from sera of normal and pollutant induced *Labeo rohita*. *Glycoconj J*. (1999) 16:741–50.
69. Sinha S, Mandal C, Allen AK, Mandal C. Acute phase response of C-reactive protein of *Labeo rohita* to aquatic pollutants is accompanied by the appearance of distinct molecular forms. *Arch Biochem Biophys*. (2001) 396:139–50. doi: 10.1006/abbi.2001.2592
70. MacCarthy EM, Burns I, Irnazarow I, Polwart A, Greenhough TJ, Shrive AK, et al. Serum CRP-like protein profile in common carp *Cyprinus carpio* challenged with *Aeromonas hydrophila* and *Escherichia coli* lipopolysaccharide. *Dev Comp Immunol*. (2008) 32:1281–9. doi: 10.1016/j.dci.2008.04.004
71. Pionnier N, Falco A, Miest J, Frost P, Irnazarow I, Shrive A, et al. Dietary β-glucan stimulate complement and C-reactive protein acute phase responses in common carp (*Cyprinus carpio*) during an *Aeromonas salmonicida* infection. *Fish Shellfish Immunol*. (2013) 34:819–31. doi: 10.1016/j.fsi.2012.12.017
72. Pionnier N, Adamek M, Miest JJ, Harris SJ, Matras M, Rakus KL, et al. C-reactive protein and complement as acute phase reactants in common carp *Cyprinus carpio* during CyHV-3 infection. *Dis Aquat Organ*. (2014) 109:187–99. doi: 10.3354/dao02727
73. Samudzi CT, Nguyen NY, Rubin JR. Crystallization and preliminary x-ray diffraction studies of dogfish C-reactive protein. *Proteins Struct Funct Genet*. (1993) 15:100–2.
74. Bello-Perez M, Falco A, Medina R, Encinar JA, Novoa B, Perez L, et al. Structure and functionalities of the human C-reactive protein compared to the zebrafish multigene family of C-reactive-like proteins. *Dev Comp Immunol*. (2016) 69:33–40. doi: 10.1016/j.dci.2016.12.001
75. Bello-Perez M, Falco A, Medina-Gali R, Pereiro P, Encinar JA, Novoa B, et al. Neutralization of viral infectivity by zebrafish C-reactive protein isoforms. *Mol Immunol*. (2017) 91:145–55. doi: 10.1016/j.molimm.2017.09.005
76. Elvitigala DAS, Wan Q, Kim HC, Lee J. Identification of a C-reactive protein like homologue from black rockfish (*Sebastes schlegelii*) evidencing its potent anti-microbial properties at molecular level. *Dev Comp Immunol*. (2015) 53:169–78. doi: 10.1016/j.dci.2015.07.007
77. Lee PT, Bird S, Zou J, Martin SAM. Phylogeny and expression analysis of C-reactive protein (CRP) and serum amyloid-P (SAP) like genes reveal two distinct groups in fish. *Fish Shellfish Immunol*. (2017) 65:42–51. doi: 10.1016/j.fsi.2017.03.037
78. Patterson LT, Mora EC. Occurrence of a substance analogous to C-reactive protein in the blood of the domestic fowl. *Tex Rep Biol Med*. (1964) 22:716–21.
79. Marques AT, Nordio L, Lecchi C, Grilli G, Giudice C, Ceciliani F. Widespread extrahepatic expression of acute-phase proteins in healthy chicken (*Gallus gallus*) tissues. *Vet Immunol Immunopathol*. (2017) 190:10–7. doi: 10.1016/j.vetimm.2017.06.006

80. Sarikaputi M, Morimatsu M, Syuto B, Saito M, Naiki M. A new purification procedure for bovine C-reactive protein and serum amyloid P component. *Int J Biochem*. (1991) 23:1137–42.
81. Riley RF, Zontine W. Further observations on the properties of dog C-reactive protein and the C-reactive protein response in the dog. *J Lab Clin Med*. (1972) 80:698–703.
82. Ganrot K. Plasma protein response in experimental inflammation in the dog. *Res Exp Med*. (1973) 161:251–61.
83. Caspi D, Snel FW, Batt RM, Bennett D, Rutteman GR, Hartman EG, et al. C-reactive protein in dogs. *Am J Vet Res*. (1987) 48:919–21.
84. Fujise H, Takanami H, Yamamoto M, Ohta I, Yamamoto S, Fukase T, et al. Simple isolation of canine C-reactive protein (CRP) by phosphorylcholine (PC) affinity chromatography. *J Vet Med Sci*. (1992) 54:165–67.
85. Yamamoto S, Abe N, Santsuka H, Shida T, Kishida K, Kuwajima S, et al. Efficient preparation of monospecific anti-canine C-reactive protein serum and purification of canine C-reactive protein by affinity chromatography. *Vet Immunol Immunopathol*. (1993) 36:293–301.
86. Yamamoto S, Miyaji S, Abe N, Otabe K, Furukawa E, Naiki M. Canine C-reactive protein (CRP) does not share common antigenicity with human CRP. *Vet Res Commun*. (1993) 17:259–66.
87. Jasensky AK, Bondzio A, Murugaiyan J, Siebert U, Roesler U, Kohn B, et al. Characterization of the native C-reactive protein (cCRP) and the corresponding liver mRNA in dogs. *Biochem Biophys Res Commun*. (2014) 452:462–7. doi: 10.1016/j.bbrc.2014.08.090
88. Vasiu I, Dabrowski R, Martinez-Subiela S, Ceron JJ, Wdowiak A, Pop RA, et al. Milk C-reactive protein in canine mastitis. *Vet Immunol Immunopathol*. (2017) 186:41–4. doi: 10.1016/j.vetimm.2017.02.005
89. Rubio N, Sharp PM, Rits M, Zahedi K, Whitehead AS. Structure, expression, and evolution of guinea pig serum amyloid P component and C-reactive protein. *J Biochem*. (1993) 113:277–84.
90. Yamashita K, Fujinaga T, Okumura M, Takiguchi M, Tsunoda N, Mizuno S. Serum C-reactive protein (CRP) in horses: the effect of aging, sex, delivery and inflammations on its concentration. *J Vet Med Sci*. (1991) 53:1019–24.
91. Riley RF, Coleman MK. Isolation of C-reactive proteins of man, monkey, rabbit and dog by affinity chromatography on phosphorylated cellulose. *Clin Chim Acta*. (1970) 30:483–96.
92. Sorensen NS, Tegtmeier C, Andresen LO, Piñeiro M, Toussaint MJM, Campbell FM, et al. The porcine acute phase protein response to acute clinical and subclinical experimental infection with *Streptococcus suis*. *Vet Immunol Immunopathol*. (2006) 113:157–68. doi: 10.1016/j.vetimm.2006.04.008
93. Birck MM, Pesonen E, Odermarsky M, Hansen AK, Persson K, Frikke-Schmidt H, et al. Infection-induced coronary dysfunction and systemic inflammation in piglets are dampened in hypercholesterolemic milieu. *Am J Physiol Heart Circ Physiol*. (2011) 300:H1595–601. doi: 10.1152/ajpheart.01253.2010
94. Dowton SB, Woods DE, Mantzouranis EC, Colten HR. Syrian hamster female protein: analysis of female protein primary structure and gene expression. *Science*. (1985) 228:1206–8.
95. Dowton SB, Holden SN. C-reactive protein (CRP) of the Syrian hamster. *Biochemistry*. (1991) 30:9531–8.
96. Dowton SB, Waggoner DJ. Armenian hamster female protein (serum amyloid P component): comparison with the sex-regulated homolog in Syrian hamster. *J Immunol*. (1989) 143:3776–80.
97. Whitehead AS, Zahedi K, Rits M, Mortensen RF, Lelias JM. Mouse C-reactive protein: generation of cDNA clones, structural analysis, and induction of mRNA during inflammation. *Biochem J*. (1990) 266:283–90.
98. Bodmer B, Siboo R. Isolation of mouse C-reactive protein from liver and serum. *J Immunol*. (1977) 118:1086–9.
99. Li Y, Robins JH, Ye J, Huang Z, Wen Q, Zhang G. Adaptive diversity of innate immune receptor family short pentraxins in Murinae. FEBS Lett. (2012) 586:798–803. doi: 10.1016/j.febslet.2012.01.048
100. Patterson LT, Higginbotham RD. Mouse C-reactive protein and endotoxin-induced resistance. *J Bacteriol*. (1965) 90:1520–4.
101. Anderson HC, McCarty M. The occurrence in the rabbit of an acute phase protein analogous to human C reactive protein. *J Exp Med*. (1951) 93:25–36.
102. Kushner I, Kaplan MH. Studies of acute phase protein. I. An immunohistochemical method for the localization of Cx-reactive protein in rabbits: association with necrosis in local inflammatory lesions. *J Exp Med*. (1961) 114:961–73.
103. Riley RF, Coleman MK, Hokama Y. Effects of drugs on Cx-protein responses in the rabbit. *Arch Int Pharmacodyn Ther*. (1964) 148:61–81.
104. Riley RF, Coleman MK, Snow HD. Site of formation of Cx-reactive protein. *Experientia*. (1969) 25:277–8.
105. Osmand AP, Gewurz H, Friedenson B. Partial amino-acid sequences of human and rabbit C-reactive proteins: homology with immunoglobulins and histocompatibility antigens. *Proc Natl Acad Sci USA*. (1977) 74:1214–8.
106. Oliveira EB, Gotschlich EC, Liu T-Y. Comparative studies on the binding properties of human and rabbit C-reactive proteins. *J Immunol*. (1980) 124:1396–402.
107. Wang C-M, Nguyen NY, Yonaha K, Robey F, Liu TY. Primary structure of rabbit C-reactive protein. *J Biol Chem*. (1982) 257:13610–5.
108. Cabana VG, Gewurz H, Siegel JN. Interaction of very low-density lipoproteins. (VLDL) with rabbit C-reactive protein. *J Immunol*. (1982) 128:2342–8.
109. Rowe IF, Soutar AK, Trayner IM, Baltz ML, de Beer FC, Walker L, et al. Rabbit and rat C-reactive proteins bind apolipoprotein B-containing lipoproteins. *J Exp Med*. (1984) 159:604–16.
110. Saxena U, Nagpurkar A, Mookerjea S. Contrasting effect of phosphorylcholine-binding protein from rat and rabbit on heparin-lipoprotein interaction: a role of sialic acid. *Can J Biochem Cell Biol*. (1985) 63:1014–21.
111. Syin C, Gotschlich EC, Liu TY. Rabbit C-reactive protein: biosynthesis and characterization of cDNA clones. *J Biol Chem*. (1986) 261:5473–9.
112. Hu SI, Miller SM, Samols D. Cloning and characterization of the gene for rabbit C-reactive protein. *Biochemistry*. (1986) 25:7834–9.
113. Rees RF, Gewurz H, Siegel JN, Coon J, Potempa LA. Expression of a C-reactive protein neoantigen (neo-CRP) in inflamed rabbit liver and muscle. *Clin Immunol Immunopathol*. (1988) 48:95–107.
114. Rénia L, Xia D, Samols D, Nussenzweig V. Transgenic mice expressing C-reactive protein are susceptible to infection with *Plasmodium yoelii* sporozoites. *Infect Immun*. (1993) 61:348–9.
115. Lin CS, Xia D, Yun JS, Wagner T, Magnuson T, Mold C, et al. Expression of rabbit C-reactive protein in transgenic mice. *Immunol Cell Biol*. (1995) 73:521–31.
116. Xia D, Samols D. Transgenic mice expressing rabbit C-reactive protein are resistant to endotoxemia. *Proc Natl Acad Sci USA*. (1997) 94:2575–80.
117. Black S, Wilson A, Samols D. An intact phosphocholine binding site is necessary for transgenic rabbit C-reactive protein to protect mice against challenge with platelet-activating factor. *J Immunol*. (2005) 175:1192–6. doi: 10.4049/jimmunol.175.2.1192
118. Jiang S, Xia D, Samols D. Expression of rabbit C-reactive protein in transgenic mice inhibits development of antigen-induced arthritis. *Scand J Rheumatol*. (2006) 35:351–5. doi: 10.1080/03009740600757963
119. Yu Q, Li Y, Wan Y, Zhao S, Yang P, Chen Y, et al. C-reactive protein levels are associated with the progression of atherosclerotic lesions in rabbits. Histol Histopathol. (2012) 27:529–35. doi: 10.14670/HH-27.529
120. Mattecka S, Bock C, Vogt B, Yapici G, Schrödl W, Janko C, et al. CRP and SAP from different species have different membrane ligand specificities. Autoimmunity. (2013) 46:347–50. doi: 10.3109/08916934.2013.780601
121. Schade R, Gotz F, Porstmann B, Friedrich A, Nugel E. The acute phase proteins of the rat, their regulation by hormones and biological significance. *Agents Actions Suppl*. (1982) 10:213–31.
122. Taylor JA, Bruton CJ, Anderson JK, Mole JE, De Beer FC, Baltz ML, et al. Amino acid sequence homology between rat and human C-reactive protein. *Biochem J*. (1984) 221:903–6.
123. Bout D, Joseph M, Pontet M, Vorng H, Deslee D, Capron A. Rat resistance to schistosomiasis: platelet-mediated cytotoxicity induced by C-reactive protein. *Science*. (1986) 231:153–6.
124. Hirai H, Nunomura W, Hatakeyama M. Monoclonal antibodies to human C-reactive protein (CRP) and some characterization of a rat serum protein related to CRP. *Protides Biol fluid*. (1986) 283–6.
125. Agrawal A, Bhattacharya S. Appearance of C-reactive protein (CRP) in serum and liver cytosol of cadmium-treated rats. *Indian J Exp Biol*. (1989) 27:1024–7.

126. Randell E, Mookerjea S, Nagpurkar A. Interaction between rat serum phosphorylcholine binding protein and platelet activating factor. *Biochem Biophys Res Comm*. (1990) 167:444–9.
127. Rassouli M, Sambasivam H, Azadi P, Dell A, Morris HR, Nagpurkar A, et al. Derivation of the amino acid sequence of rat C-reactive protein from cDNA cloning with additional studies on the nature of its dimeric component. *J Biol Chem*. (1992) 267:2947–54.
128. Schwalbe RA, Dahlbäck B, Coe JE, Nelsestuen GL. Pentraxin family of proteins interacts specifically with phosphorylcholine and/or phosphorylethanolamine. *Biochemistry*. (1992) 31:4907–15.
129. Sambasivam H, Rassouli M, Murray RK, Nagpurkar A, Mookeerjea S, Azadi P, et al. Studies on the carbohydrate moiety and on the biosynthesis of rat C-reactive protein. *J Biol Chem*. (1993) 268:10007–16.
130. Mookerjea S, Francis J, Hunt D, Yang CY, Nagpurkar A. Rat C-reactive protein causes a charge modification of LDL and stimulates its degradation by macrophages. *Arterioscler Thromb*. (1994) 14:282–7.
131. Mookerjea S, Hunt D. A novel phosphatidylcholine hydrolysing action of C-reactive protein. *Biochem Biophys Res Comm*. (1995) 208:1046–52.
132. Schwalbe RA, Coe JE, Nelsestuen GL. Association of rat C-reactive protein and other pentraxins with rat lipoproteins containing apolipoproteins E and A1. *Biochemistry*. (1995) 34:10432–9.
133. Agrawal A, Bhattacharya S. Possible role of C-reactive protein in detoxication of mercury. *Indian J Exp Biol*. (1990) 28:638–41.
134. Omtvedt LA, Wien TN, Myran T, Sletten K, Husby G. Serum amyloid P component in mink, a non-glycosylated protein with affinity for phosphorylethanolamine and phosphorylcholine. *Amyloid J Prot Fold Disorders*. (2004) 11:101–8. doi: 10.1080/13506120410001728063
135. Bertelsen MF, Kjelgaard-Hansen M, Grøndahl C, Heegaard PM, Jacobsen S. Identification of acute phase proteins and assays applicable in nondomesticated mammals. *J Zoo Wildl Med*. (2009) 40:199–203. doi: 10.1638/2007-0125.1
136. Isaza R, Wiedner E, Hiser S, Cray C. Reference intervals for acute phase protein and serum protein electrophoresis values in captive Asian elephants (*Elephas maximus*). *J Vet Diagn Invest*. (2014) 26:616–21. doi: 10.1177/1040638714543923
137. Srere HK, Belke D, Wang LC, Martin SL. Alpha 2-macroglobulin gene expression during hibernation in ground squirrels is independent of acute phase response. *Am J Physiol*. (1995) 268:R1507–12.
138. Sheldon JD, Johnson SP, Hernandez JA, Cray C, Stacy NI. Acute-phase responses in healthy, malnourished, and otostrongylus-infected juvenile northern elephant seals (*Mirounga angustirostris*). *J Zoo Wildl Med*. (2017) 48:767–75. doi: 10.1638/2016-0267.1
139. Ong OTW, Green-Barber JM, Kanuri A, Young LJ, Old JM. Antimicrobial activity of red-tailed phascogale (*Phascogale calura*) serum. *Comp Immunol Microbiol Infect Dis*. (2017) 51:41–8. doi: 10.1016/j.cimid.2017.03.001
140. Woo P, Korenberg JR, Whitehead AS. Characterization of genomic and complementary DNA sequence of human C-reactive protein, and comparison with the complementary DNA sequence of serum amyloid P component. *J Biol Chem*. (1985) 260:13384–8.
141. Agrawal A, Lee S, Carson M, Narayana SVL, Greenhough TJ, Volanakis JE. Site-directed mutagenesis of the phosphocholine-binding site of human C-reactive protein: role of $Thr^{76}$ and $Trp^{67}$. *J Immunol*. (1997) 158, 345–50.
142. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure*. (1999) 7:169–77.
143. Agrawal A, Simpson MJ, Black S, Carey MP, Samols D. A C-reactive protein mutant that does not bind to phosphocholine and pneumococcal C-polysaccharide. *J Immunol*. (2002) 169:3217–22. doi: 10.4049/jimmunol.169.6.3217
144. Mikolajek H, Kolstoe SE, Pye VE, Mangione P, Pepys MB, Wood SP. Structural basis of ligand specificity in the human pentraxins, C-reactive protein and serum amyloid P component. *J Mol Recognit*. (2011) 24:371–7. doi: 10.1002/jmr.1090.
145. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. *J Immunol*. (1974) 112:2135–47.
146. Agrawal A, Volanakis JE. Probing the C1q-binding site on human C-reactive protein by site-directed mutagenesis. *J Immunol*. (1994) 152:5404–10.
147. Agrawal A, Shrive AK, Greenhough TJ, Volanakis JE. Topology and structure of the C1q-binding site on C-reactive protein. *J Immunol*. (2001) 166:3998–4004. doi: 10.4049/jimmunol.166.6.3998
148. Hammond DJ Jr., Singh SK, Thompson JA, Beeler BW, Rusinol AE, Pangburn MK, et al. Identification of acidic pH-dependent ligands of pentameric C-reactive protein. *J Biol Chem*. (2010) 285:36235–44. doi: 10.1074/jbc.M110.142026.
149. Singh SK, Thirumalai A, Pathak A, Ngwa DN, Agrawal A. Functional transformation of C-reactive protein by hydrogen peroxide. *J Biol Chem*. (2017) 292:3129–36. doi: 10.1074/jbc.M116.773176
150. Singh SK, Thirumalai A, Hammond DJ Jr., Pangburn MK, Mishra VK, Johnson DA, et al. Exposing a hidden functional site of C-reactive protein by site-directed mutagenesis. *J Biol Chem*. (2012) 287:3550–8. doi: 10.1074/jbc.M111.310011
151. Agrawal A, Gang TB, Rusiñol AE. Recognition functions of pentameric C-reactive protein in cardiovascular disease. *Mediators Inflamm*. (2014) 2014:1–6. doi: 10.1155/2014/319215
152. Li H-Y, Wang J, Meng F, Jia ZK, Su Y, Bai Q-F, et al. An intrinsically disordered motif mediates diverse actions of monomeric C-reactive protein. *J Biol Chem*. (2016) 291:8795–804. doi: 10.1074/jbc.M115.695023
153. Yother J, Volanakis JE, Briles DE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in mice. *J Immunol*. (1982) 128:2374–6.
154. Szalai AJ, Briles DE, Volanakis JE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in transgenic mice. *J Immunol*. (1995) 155:2557–63.
155. Suresh MV, Singh SK, Ferguson DA Jr., Agrawal A. Role of the property of C-reactive protein to activate the classical pathway of complement in protecting mice from *Streptococcus pneumoniae* infection. *J Immunol*. (2006) 176:4369–74. doi: 10.4049/jimmunol.176.7.4369
156. Suresh MV, Singh SK, Ferguson DA Jr., Agrawal A. Human C-reactive protein protects mice from *Streptococcus pneumoniae* infection without binding to pneumococcal C-polysaccharide. *J Immunol*. (2007) 178:1158–63. doi: 10.4049/jimmunol.178.2.1158
157. Agrawal A, Suresh MV, Singh SK, Ferguson DA Jr. The protective function of human C-reactive protein in mouse models of *Streptococcus pneumoniae* infection. *Endocr Metab Immune Disord Drug Targets*. (2008) 8:231–7. doi: 10.2174/187153008786848321
158. Gang TB, Hammond DJ Jr., Singh SK, Ferguson DA Jr., Mishra VK, Agrawal A. The phosphocholine-binding pocket on C-reactive protein is necessary for initial protection of mice against pneumococcal infection. *J Biol Chem*. (2012) 287:43116–25. doi: 10.1074/jbc.M112.427310
159. Simons JP, Loeffler JM, Al-Shawi R, Ellmerich S, Hutchinson WL, Tennent GA, et al. C-reactive protein is essential for innate resistance to pneumococcal infection. *Immunology*. (2014) 142:414–20. doi: 10.1111/imm.12266.
160. Gang TB, Hanley GA, Agrawal A. C-reactive protein protects mice against *Streptococcus pneumoniae* infection via both phosphocholine-dependent and phosphocholine-independent mechanisms. *Infect Immun*. (2015) 83:1845–52. doi: 10.1128/IAI.03058-14
161. Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis. Binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler Thromb Vasc Biol*. (1999) 19:2348–54.
162. Singh SK, Suresh MV, Prayther DC, Moorman JP, Rusinol AE, Agrawal A. C-reactive protein-bound enzymatically modified low-density lipoprotein does not transform macrophages into foam cells. *J Immunol*. (2008) 180:4316–22. doi: 10.1016/j.jvs.2009.07.006
163. Agrawal A, Hammond DJ Jr., and Singh SK. Atherosclerosis-related functions of C-reactive protein. *Cardiovasc Haematol Disorders Drug Targets*. (2010) 10:235–40. doi: 10.1161/01.ATV.0000079512.66448.1D
164. Singh SK, Suresh MV, Voleti B, Agrawal A. The connection between C-reactive protein and atherosclerosis. *Ann Med*. (2008) 40:110–20. doi: 10.1080/07853890701749225

165. Teupser D, Weber O, Rao TN, Sass K, Thiery J, Fehling HJ. No reduction of atherosclerosis in C-reactive protein (CRP)-deficient mice. *J Biol Chem*. (2011) 286:6272–9. doi: 10.1074/jbc.M110.161414
166. Kovacs A, Tornvall P, Nilsson R, Tegnér J, Hamsten A, Björkegren J. Human C-reactive protein slows atherosclerosis development in a mouse model with human-like hypercholesterolemia. *Proc Natl Acad Sci USA*. (2007) 104:13768–73. doi: 10.1073/pnas.0706027104
167. Braig D, Nero TL, Koch HG, Kaiser B, Wang X, Thiele JR, et al. Transitional changes in the CRP structure lead to the exposure of proinflammatory binding sites. *Nature Commun*. (2017) 8:14188. doi: 10.1038/ncomms14188
168. Ansar W, Mukhopadhyay S, Habib SK, Basu S, Saha B, Sen AK, et al. Disease-associated glycosylated molecular variants of human C-reactive protein activate complement-mediated hemolysis of erythrocytes in tuberculosis and Indian visceral leishmaniasis. *Glycoconj J*. (2009) 26:1151–69. doi: 10.1007/s10719-009-9236-y
169. Das T, Sen A, Kempf T, Pramanik SR, Mandal C, Mandal C. Induction of glycosylation in human C-reactive protein under different pathological conditions. *Biochem J*. (2003) 373:345–55. doi: 10.1042/BJ20021701
170. Das T, Mandal C, Mandal C. Variations in binding characteristics of glycosylated human C-reactive proteins in different pathological conditions. *Glycoconj J*. (2004) 20:537–43. doi: 10.1023/B:GLYC.0000043290.90182.e6
171. Pathak A, Singh SK, Thirumalai A, Armstrong PB, Agrawal A. Evolution of a host-defense function of C-reactive protein from horseshoe crab to humans. *J Immunol*. (2016) 196(Suppl. 1):132.5.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 29 May 2019
doi: 10.3389/fimmu.2019.01135

# PTX3 Intercepts Vascular Inflammation in Systemic Immune-Mediated Diseases

*Giuseppe A. Ramirez[1,2,3], Patrizia Rovere-Querini[1,3], Miriam Blasi[1,3], Silvia Sartorelli[1], Maria Chiara Di Chio[1], Mattia Baldini[1,3], Rebecca De Lorenzo[1,3], Enrica P. Bozzolo[2], Roberto Leone[4], Alberto Mantovani[4,5,6], Angelo A. Manfredi[1,2,3]* and Enrico Tombetti[1,2,3]*

[1] Università Vita-Salute San Raffaele, Milan, Italy, [2] Unit of Immunology, Rheumatology, Allergy and Rare Diseases, IRCCS Ospedale San Raffaele, Milan, Italy, [3] Division of Immunology, Transplantation and Infectious Immunity, IRCCS Ospedale San Raffaele, Milan, Italy, [4] Humanitas Research Center - IRCCS, Rozzano, Italy, [5] Humanitas University, Rozzano, Italy, [6] The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom

PTX3 is a prototypic soluble pattern recognition receptor, expressed at sites of inflammation and involved in regulation of the tissue homeostasis. PTX3 systemic levels increase in many (but not all) immune-mediated inflammatory conditions. Research on PTX3 as a biomarker has so far focused on single diseases. Here, we performed a multi-group comparative study with the aim of identifying clinical and pathophysiological phenotypes associated with PTX3 release. PTX3 concentration was measured by ELISA in the plasma of 366 subjects, including 96 patients with giant cell arteritis (GCA), 42 with Takayasu's arteritis (TA), 10 with polymyalgia rheumatica (PMR), 63 with ANCA-associated systemic small vessel vasculitides (AAV), 55 with systemic lupus erythematosus (SLE), 21 with rheumatoid arthritis (RA) and 79 healthy controls (HC). Patients with SLE, AAV, TA and GCA, but not patients with RA and PMR, had higher PTX3 levels than HC. PTX3 concentration correlated with disease activity, acute phase reactants and prednisone dose. It was higher in females, in patients with recent-onset disease and in those with previous or current active vasculitis at univariate analysis. Active small- or large- vessel vasculitis were the main independent variables influencing PTX3 levels at multivariate analysis. High levels of PTX3 in the blood can contribute to identify an increased risk of vascular involvement in patients with systemic immune-mediated diseases.

**Keywords: PTX3, autoimmunity, lupus, rheumatoid arthritis, Takayasu arteritis, giant cell arteritis, ANCA associated small vessel vasculitis, intravascular immunity**

OPEN ACCESS

***Edited by:***
*Mauro Martins Teixeira, Federal University of Minas Gerais, Brazil*

***Reviewed by:***
*Luis Enrique Munoz, University of Erlangen Nuremberg, Germany*
*Simon John Clark, University of Manchester, United Kingdom*

***Correspondence:**
*Angelo A. Manfredi*
*manfredi.angelo@hsr.it*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *04 February 2019*
***Accepted:*** *07 May 2019*
***Published:*** *29 May 2019*

***Citation:***
*Ramirez GA, Rovere-Querini P, Blasi M, Sartorelli S, Di Chio MC, Baldini M, De Lorenzo R, Bozzolo EP, Leone R, Mantovani A, Manfredi AA and Tombetti E (2019) PTX3 Intercepts Vascular Inflammation in Systemic Immune-Mediated Diseases. Front. Immunol. 10:1135. doi: 10.3389/fimmu.2019.01135*

## INTRODUCTION

Vascular inflammation reflects the dynamic interaction of circulating cells, blood molecules and vascular structures which plays a role in vascular homeostasis and in systemic or tissue/organ-limited autoimmunity (1–5). The vasculature recruits cellular and immune effectors to facilitate the intercellular signaling required to deploy an inflammatory and immune response (5, 6). Vessels are frequently targeted in immune mediated-diseases, although in a subset of patients vascular inflammation is prominent, resulting in overt vasculitis.

Pentraxins are part of an ancestral humoral innate network, evolutionarily rooted before the divergence of the immune and the haemolymphatic system. Pentraxin-3 (PTX3), a member of the long-pentraxin family, has changed little during the evolution, likely due to its role in multiple biological events (7, 8). In contrast to other pentraxins such as C-reactive protein (CRP), PTX3 is mostly generated at sites of inflammation rather than as a consequence of centralized hepatic synthesis. Neutrophils massively release PTX3 upon activation, while endothelial cells or macrophages synthetize the molecule, sustaining PTX3 production for longer times (9–11). In the extracellular space, PTX3 opsonizes self and foreign antigens and contributes to the structural and functional fitness of the extracellular matrix. Evidence has been acquired for a potential pathogenic role of PTX3 in a broad range of events from host defense to fertility, cancer biology, autoimmunity, regulation of angiogenesis and tissue repair (7, 12–19).

Enhanced expression of PTX3 has been reported in multiple systemic autoimmune diseases (20–27). Given that most cellular sources of PTX3 can be involved in vascular inflammation, and that PTX3 has been shown to be specifically involved in the regulation of the cross talk between the main players of intravascular immunity, including neutrophils, apoptotic cells, platelets, endothelial and antigen presenting cells (22, 28, 29), we undertook an observational study assessing systemic expression of PTX3 in healthy subjects and multiple inflammatory diseases with variable vascular involvement.

## PATIENTS AND METHODS

Upon written informed consent, 366 subjects followed up at San Raffaele University Hospital, Milan, Italy were recruited including: 96 patients with GCA, 42 with TA, 10 with PMR, 38 with granulomatosis with polyangiitis (GPA), 15 with eosinophilic granulomatosis with polyangiitis (EGPA), 10 with microscopic polyangiitis (MPA), 55 with systemic lupus erythematosus (SLE), and 21 with rheumatoid arthritis (RA). Seventy-nine healthy volunteers served as controls. All patients gave their written informed consent for participation in this study (Autoimmuno-Mol protocol, approved by the Ethics Committee of the San Raffaele Institute, Milan, Italy; reference number 2/2013/INT). Patients were classified according to the 1990 American College of Rheumatology (ACR) classification criteria for GCA and PMR (30), the 1996 Sharma's diagnostic criteria for TA (31), the European Medicine Agency algorithm for classification of GPA, EGPA and MPA (32), the revised 1997 ACR or the 2012 SLE International Collaborating Clinics (SLICC) classification criteria for SLE (33, 34) and the 2010 ACR/European League Against Rheumatism(EULAR) classification criteria for RA (35).

Basic demographics (including gender, age at sampling and disease duration), disease activity and accrued irreversible damage, dose of prednisone or equivalents, erythrocyte sedimentation rate (ESR) and CRP values at time of sampling were recorded. In patients with SLE, complement levels and anti-DNA titres were collected. Disease activity for group comparison was quantitated by employing a 28-joint disease activity score (DAS-28) for RA, the SLE disease activity index 2000 version (SLEDAI-2K) for SLE (36), the Birmingham Vasculitis Activity Score version 3 (BVAS v3) for anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitides (AAV) (37) and the Indian Takayasu Activity Score for TA (ITAS2010) (38). In patients with SLE, disease activity was also estimated by employing the British Isles Lupus Assessment Group (BILAG) 2004 version index (39) and a 0.0-3.0 physician global assessment scale (PGA). A 0-10 visual analog scale (VAS) also measured SLE patients' impression about their global health status. Organ damage was determined by the SLICC/ACR Damage Index (SDI) for SLE (40), the Vasculitis Damage Index (VDI) for AAV and GCA (41) and the TA Damage Score (TADS) for TA (42). Disease activity and damage scores were made homogeneous by calculating Z-scores (i.e., $\frac{x-mean}{standard\ deviation}$) for activity and damage (Z-activity and Z-damage). In parallel to quantitative assessment, a binary evaluation of disease activity and damage was performed. The former was based on the Physician Global Assessment of disease avidity (Inactive vs. Active/smoldering), the latter by the presence vs. absence of items related to vasculitic manifestations in the abovementioned scores. In patients with SLE, lupus chilblains, skin/digital vasculitis/ischemia, urticarial vasculitis, gastrointestinal vasculitis, choroidopathy or retinal vasculitis, cerebral vasculitis and alveolar hemorrhage were considered as relevant vasculitic manifestation, whereas Raynaud's phenomenon was not. In patients with AAV, "pure" vasculitic manifestations included absence of ear-nose-throat (ENT) or orbital involvement and presence of purpura, scleritis, episcleritis, optic neuritis, renal involvement, peripheral neuropathy, hemorrhagic alveolitis, or diagnosis of MPA.

PTX3 plasma levels were measured by a sandwich ELISA based on original reagents developed in house. 96 well plates (Nunc MaxiSorp cat. 446612) were coated with 100 µl anti-hPTX3 monoclonal antibody (MNB4 (43) 1 µg/ml−100 ng/well) in coating buffer (15 mM carbonate buffer pH 9.6) and incubated overnight at 4°C. Plates were washed after each step with 300 µl/well of washing buffer (PBS 1X with $Ca^{++}$ $Mg^{++}$ + 0.05% Tween 20, pH 7.00). After coating, non-specific binding to the plates was blocked with 5% dry milk in washing buffer (2 h at room temperature), then 50 µl in duplicate of recombinant human PTX3 standard (from 75 pg/ml to 2.4 ng/ml) and human plasma (diluted in PBS 1X w/o $Ca^{++}$ $Mg^{++}$ + 2% BSA, pH 7.00), were plated. 1 µl of 2.5% Polybrene was added to 50 µl of plasma and incubated at room temperature for 10 min before dilution. After 2 h at

**Abbreviations:** AAV, ANCA-associated vasculitis; ACR, American College of Rheumatology; ANCA, anti-neutrophil cytoplasmic antibodies; BILAG, British Isles Lupus Assessment Group; BVAS, Birmingham Vasculitis Activity Score; CRP, C-reactive protein; DAS-28, 28-joint disease activity score EGPA, eosinophilic granulomatosis with polyangiitis; ESR, erythrocyte sedimentation rate; GPA, granulomatosis with polyangiitis; HC, healthy controls; ITAS, Indian Takayasu Activity Score; MPA, microscopic polyangiitis; PDN, prednisone; PGA, Physician's global assessment scale; PMR, polymyalgia rheumatica; PTX3, pentraxin-3; RA, rheumatoid arthritis; SDI, SLICC/ACR damage index; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; SLICC, SLE international collaborating clinics; TA, Takayasu's arteritis; TADS, TA Damage Score; VAS, visual analog scale; VDI, vasculitis damage index.

**TABLE 1 |** General features of patients included in the study.

| | RA ($n = 21$) | SLE ($n = 55$) | PMR ($n = 10$) | AAV ($n = 63$) | GCA ($n = 96$) | TA ($n = 42$) |
|---|---|---|---|---|---|---|
| Age at diagnosis (year) | ND | 26.2 (18.6- 34.2) | 72.0 (68.5- 73) | 48.5 (36.5 - 61.5) | 74.0 (67.0 - 77.0) | 30.0 (24.0 - 40.5) |
| Age at sampling (years) | 63.0 (43.0- 67.0) | 38.2 (31.2- 48.9) | 73.0 (69.3- 77) | 59.5 (47.8 - 67.9) | 75.2 (69.4 - 79.5) | 46.0 (35 - 53) |
| ESR (mm/h) | 16.5 (12.8 - 23.5) | 20.0 (7.0 - 38.5) | 30.0 (20.0 - 50.0) | 13.0 (6.5- 31.5) | 31.0 (18.0 - 57.8) | 15 (7.8- 30.3) |
| CRP (mg/l) | 3.5 (2 - 6.7) | 2.1 (0.3 - 6.1) | 10.4 (8.7 - 22.3) | 0.8 (0.23 - 6.2) | 11.0 (2.26 - 31.4) | 2.25 (1.03 - 7.3) |
| PDN dose at sampling (mg/day) | 5.0 (2.5 - 5) | 3.8 (0.0 - 5.0) | 1.9 (0.0 - 17.2) | 5.0 (5.0 - 6.5) | 5.0 (0.0 - 12.5) | 5.0 (0.0 - 5.0) |
| Z-activity | −0.42 (−0.47 - −0.28) | −0.33 (−0.56 - 0.35) | NA | −0.4 (−0.4- −0.07) | −0.23 (−0.23 - −0.07) | −0.42 (−0.47 - −0.28) |
| Z-damage | ND | −0.2 (−0.2 - −0.07) | NA | −0.06 (−0.06 - 0.01) | −0.14 (−0.14 - −0.06) | −0.12 (−0.57 - 0.19) |

37°C, plates were incubated with 100 µl /well of purified and biotinylated rabbit IgG anti hPTX3 (5 ng/well) in washing buffer (1 h at room temperature), followed by incubation with 100 µl/well-streptavidin conjugated to horseradish peroxidase (cat. SB01-61, Biospa, Milan, Italy) diluted 1:2,000 in washing buffer (1 h at room temperature). Finally, 100 µl of 1-Step™ Ultra TMB-ELISA Substrate Solution (cat. 34029, Thermo Scientific, Rockford, IL, USA) were added and the reaction was blocked after 10 min with 50 µl of 2M Sulphuric Acid ($H_2SO_4$) before reading the plates at 450 nm in an automatic ELISA reader. All the procedure was performed by personnel blind to patients' characteristics. For each biological sample, 2 dilutions in duplicate wells were evaluated and mean PTX3 content was calculated converting Abs450 values to protein concentration by means of the standard curve with recombinant purified hPTX3. Analysis was performed with SoftMax Pro software v5.3 (MDS Analytical Technologies, USA) and linear regression was used to interpolate unknown samples. Lower limit of detection of the assay was 75 pg/ml, interassay variability was from 8 to 10%; no cross reaction was observed with short pentraxins CRP and SAP.

PTX3 was measured in four different batches. Inter-batch variability was corrected by normalization based on HC samples. The relative frequencies of laboratory and clinical categorical variables were compared by using chi-square test with Fisher's exact correction as appropriate. Quantitative variables were compared by using Spearman's correlation tests. Differences in quantitative variables among groups were assessed by employing Mann-Whitney U-test or Kruskal-Wallis' test for multiple comparisons. We also employed generalized linear models with gamma distribution of the dependent variables and log function as a link function to assess the effect of each quantitative or qualitative variable on PTX3 levels. Data were processed and analyzed by employing Microsoft Excel® 2013 and IBM SPSS® version 15-23. Data are expressed as median (interquartile range, IQR) unless otherwise specified.

# RESULTS

## Expression of PTX3 in Systemic Autoimmune Diseases

We enrolled 287 patients diagnosed with systemic autoimmune diseases. Seventy-nine volunteers served as controls. Twenty-one patients had RA, a systemic inflammatory disease targeting the synovial membrane, 55 had SLE, the prototypic systemic autoimmune disease, including 19 patients with vasculitic features, ten had PMR, a fruste form of GCA with prominent osteoarticular inflammation sparing the vasculature. Moreover, we enrolled patients with primary systemic vasculitis of small vessels ($n = 63$, including 38 with GPA, 15 with EGPA) and large vessels ($n = 138$, including 96 with GCA and 42 with TA). **Table 1** summarizes patients' main characteristics.

PTX3 levels were significantly higher in patients with systemic inflammatory immune-mediated and autoimmune diseases (2.33 ng/ml, IQR: 1.26–4.89, $n = 287$) than in HCs (1.22 ng/ml, IQR:0.80–1.98, $n = 79$; $p < 0.001$ ). However, despite often comparable levels of systemic inflammation, PTX3 expression was heterogeneous in patients with different diseases. RA and PMR had plasma PTX3 levels comparable to those of HC. In contrast, PTX3 plasma levels of patients with SLE, AAV, GCA, and TA were significantly higher. Moreover, patients with SLE had higher levels of PTX3 compared to patients with AAV, GCA and TA ($p<0.001$ for AAV and TA, $p = 0.001$ for GCA; **Figure 1**).

## PTX3 Correlates With Disease Activity but Not With Accrued Damage

On univariate analysis in the general group of subjects who had been studied, PTX3 levels were higher in females than in males (**Figure 2A**) and did not correlate with disease duration (**Table 2**). Patients with recent onset disease (i.e., up to 6 months) had higher PTX3 levels compared to patients with longer disease duration (**Figure 2B**). PTX3 levels positively correlated with Z-activity (Rho = 0.181, $p = 0.016$, $n = 176$ **Figure 3A**), with acute phase reactants (ESR and CRP, Rho = 0.229 and Rho = 0.128, $p$

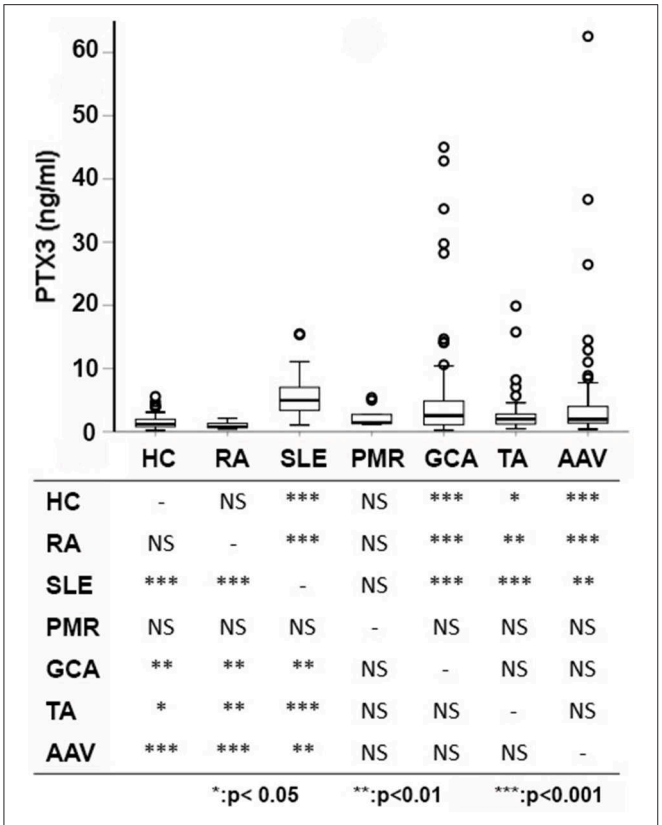


**FIGURE 1 |** PTX3 levels among different diseases. PTX3 plasma levels were compared among patients with multiple distinct autoimmune diseases and HC. Pairwise comparisons among groups are reported below. Patients with PMR and RA did not differ from HC, whereas patients with other immune-mediated diseases showed significantly higher levels of PTX3 compared to patients with RA and HC. Patients with SLE had higher levels of PTX3 compared to patients with large- (TA, GCA) and small-vessel vasculitides (AAV). AAV, ANCA-associated vasculitides; GCA, giant cell arteritis; HC, healthy controls; PMR, polymyalgia rheumatica; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TA, Takayasu's arteritis; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

$< 0.001$ and $p = 0.035$, $n = 261$ and $n = 269$, respectively) and with steroid dose (Rho = 0.198, $p = 0.001$, $n = 259$; **Table 1** and **Figure 3B**). On the contrary, PTX3 levels did not correlate with Z-damage (Rho = −0.006, $p = 0.930$). PTX3 levels were higher in patients with active disease as compared to those with quiescent disease (3.28, IQR 1.27–7.21, $n = 123$ vs. 1.74, IQR: 1.07–3.16, $n = 222$), while similar levels were observed in patients with or without organ damage. Accordingly, PTX3 levels were higher in patients with active disease and on corticosteroid treatment, but did not differ between patients with or without chronic damage (**Figures 4A–C**).

## PTX3 Reflects Vascular Inflammation

Subjects with previous vasculitic manifestations had higher PTX3 levels than those without (2.3 ng/ml, IQR = 1.26–4.83, $n = 220$ vs. 1.43 ng/ml, IQR = 0.88–2.81, $n = 146$; $p < 0.001$) and patients with active vasculitis had higher levels of PTX3 compared to patients without evidence of vasculitis at time of sampling (3.15 ng/ml, IQR = 1.31–7.75, $n = 85$ vs. 1.72 ng/ml, IQR = 1.08–3.37, $n = 278$; $p < 0.001$ **Figure 4D**). PTX3 plasma levels were higher either in patients with a history of small-vessel inflammation (3.21 ng/ml, IQR = 1.57–6.58, $n = 70$) or large vessel inflammation (2.27 ng/ml, IQR 1.12–4.29, $n = 138$) as compared to those without history of vasculitic features (1.43 ng/ml, IQR = 0.88–2.81, $n = 157$; $p < 0.001$ and $p = 0.003$, respectively, **Figure 4E**). Moreover, active small-vessel inflammation (4.10 ng/ml, IQR = 2.16-8.09, $n = 21$) or active large-vessel inflammation (2.49 ng/ml, IQR = 1.10–7.95, $n = 64$) identified two subsets of patients with higher PTX3 plasma levels than patients without active vascular inflammation (1.72 ng/ml, IQR = 1.09–3.39, $n = 281$; $p < 0.001$ for both tests, **Figure 4F**).

## PTX3 and Disease-Specific Clinical Features

Both in SLE and AAV, pure vasculitic manifestations are not detectable in all cases. Analysis of the patients with AAV revealed a positive correlation of PTX3 and CRP levels (rho = 0.362; $p = 0.005$, $n = 58$), ESR (rho = 0.272; $p = 0.037$, $n = 59$) and ANCA titres at blood sampling (rho = 0.266; p = 0.049, $n = 55$). PTX3 also correlated with disease activity as measured by BVAS in patients with GPA and MPA (rho = 0.362; $p = 0.014$, $n = 46$). PTX3 levels were higher in patients with exclusive vasculitic manifestations (3.51 ng/ml, IQR = 2.00–7.92, $n = 16$ vs. 1.83 ng/ml, IQR = 1.28–2.97 in patients with concomitant granulomatous lesions, $n = 47$; $p = 0.011$) and lower in patients with ear-nose-throat involvement (1.84 ng/ml, IQR = 1.32–2.97, $n = 43$ vs. 3.37 ng/ml, IQR = 1.74–7.92, $n = 20$; $p = 0.039$).

PTX3 levels of patients with SLE correlated with disease activity as assessed by SLEDAI-2K in the whole group of patients (rho = 0.361; $p = 0.007$, $n = 55$) and in those who were off corticosteroids ($p < 0.001$, $n = 19$; **Table 3**), but not in patients receiving prednisone. In the latter patients, a positive correlation was observed between PTX3 levels and prednisone dose (rho = 0.198; $p = 0.001$, $n = 36$). Patients with >1 moderately-to-highly active (A, B) BILAG domain had significantly higher PTX3 levels than those with more limited disease activity extent (7.23 ng/ml, IQR = 5.51–9.58, $n = 9$ vs. 4.29 ng/ml. IQR = 3.09–6.34, $n = 46$; $p = 0.041$). PTX3 also directly correlated with PGA (rho = 0.383; $p = 0.004$, $n = 55$) and inversely with patient-reported VAS (rho = −0.331; $p = 0.013$, $n = 55$) and C4 levels (rho = −0.458; $p = 0.001$, $n = 51$). There was no significant correlation with age, disease duration or with CRP concentration or anti-DNA antibodies titres (**Table 3**). CRP was higher in patients with >1 A/B BILAG domain ($p = 0.004$), but its concentration did not correlate with SLEDAI-2K or prednisone dose. SLE patients with active disease tended to have higher levels of PTX3 compared to patients with inactive disease. This trend was more evident in patients with past or current evidence of vascular inflammation (**Supplementary Figure 1**).

## PTX3 Levels Reflect Small- and Large-Vessel Inflammation at Multivariate Analysis

We performed two multivariate linear regressions of PTX3 plasma levels with a stepwise backward approach. The first

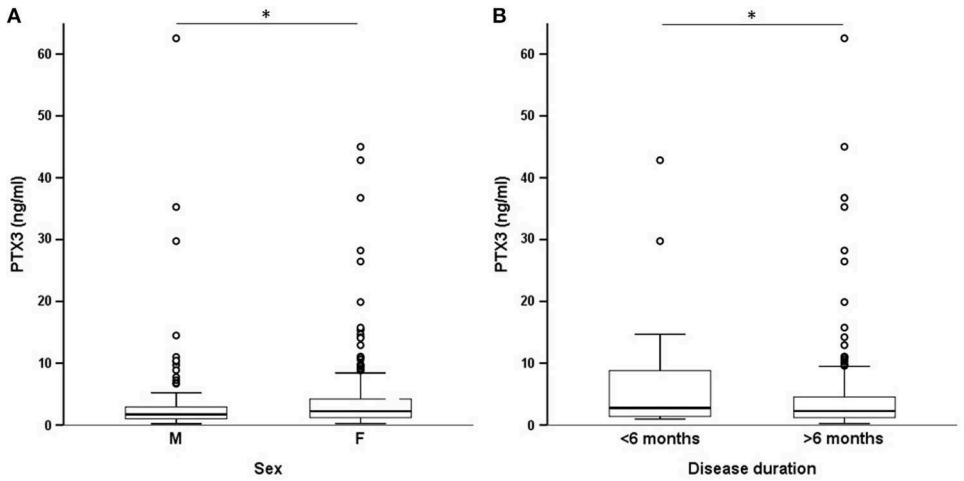


**FIGURE 2 |** PTX3 levels and demographics. These panels show the existing differences in systemic expression of PTX3 by selected demographics. PTX3 levels were higher in females **(A)** and in patients with early-onset disease **(B)**. *$p < 0.05$.

**TABLE 2 |** Correlations among PTX3 plasma levels and clinical features at univariate analysis.

| Variable | Spearman's Rho | *p* |
|---|---|---|
| Age (years) | NS | NS |
| Age at disease onset (years) | NS | NS |
| Z-score (activity) | 0.181 | 0.016 |
| Z-score (damage) | NS | NS |
| ESR (mm/h) | 0.229 | 0.0002 |
| CRP (mg/l) | 0.128 | 0.035 |
| Prednisone dose (mg) | 0.198 | 0.001 |

regression included sex, diagnosis, disease activity, history and activity of small-vessel inflammation, history, and activity of large-vessel inflammation, and steroid therapy. The stepwise algorithm resulted in a model including disease activity (B = 3.162, Std.Err = 0.738, p<0.001) and activity of small vessel inflammation (B = 4.200, Std.Err = 1.579, $p = 0.008$). In a second iteration, disease activity was excluded due to high colinearity with the other variables. The final regression model (**Table 4**) included activity of small vessel inflammation (B = 6.706, Std.Err = 1.473, $p < 0.001$), activity of large vessel inflammation (B = 4.269, Std.Err = 0.844, $p = 0.008$), and diagnosis (B = 0.243, Std.Err = 0.121, $p = 0.046$).

## DISCUSSION

In this brief report, we present a multi-disease comparison of PTX3 plasma profile in patients with various systemic autoimmune and inflammatory conditions. In line with previous evidence from other groups and us (20, 21, 23, 24, 26, 44–46), we observed that PTX3 levels rise in acutely inflamed patients. Accordingly, PTX3 levels are higher in patients with recent onset disease [see also (23, 47)] and correlated with disease activity and with conventional inflammatory markers such as ESR and CRP. However, PTX3 blood levels do not merely reflect systemic inflammation, and indeed they failed to increase in conditions such as RA and PMR. The relative lack of PTX3 increase well-agrees with the role of PTX3 as a tissue-generated signal: the inflamed *synoviae* possibly represent the preferential site of PTX3 generation and it has been reported that PTX3 assessment in the synovial fluid might indeed be more informative (27, 48).

Clinically overt vascular inflammation involving small or large vessels was associated with elevated PTX3 levels. Identification of reliable biomarkers for vascular inflammation assessment constitutes a significant unmet need in current Rheumatology practice (49–55). The present study supports the contention that vascular inflammation is a major driver of PTX3 elevation (20, 23, 56).

Patients with SLE had the highest PTX3 levels. PTX3 correlated with active SLE as estimated by the number of high-score BILAG domains. Aberrant presentation of autoantigens due to non-physiological release of PTX3 (13, 28, 57) could be involved, as indicated by the protective role of anti-PTX3 antibodies in SLE (58, 59). Corticosteroids are major inducers of PTX3 at a systemic level (60) and can constitute an additional modulatory variable in this setting. In particular, systemic administration of corticosteroid drugs or exposure to higher endogenous glucocorticoid levels cause an overall rise in blood PTX3 levels. Nonetheless, glucocorticoids have divergent effects on different cell types as they dampen PTX3 expression in monocyte-derived dendritic cells, but significantly induce PTX3 in endothelial cells and fibroblasts (60). Consistently, corticosteroids also exert distinct biological effects over different pathogenic backgrounds (61). Alternatively, smoldering vascular inflammation might be advocated as a potential explanation for plasma PTX3 elevation in patients with SLE and for conflicting results in the literature regarding associations with clinically overt vasculitis (26, 62–65).

Single tissue/organ-limited inflammatory events not involving the vascular bed might not represent effective stimuli for PTX3 elevation in the circulating blood. In line with this view,

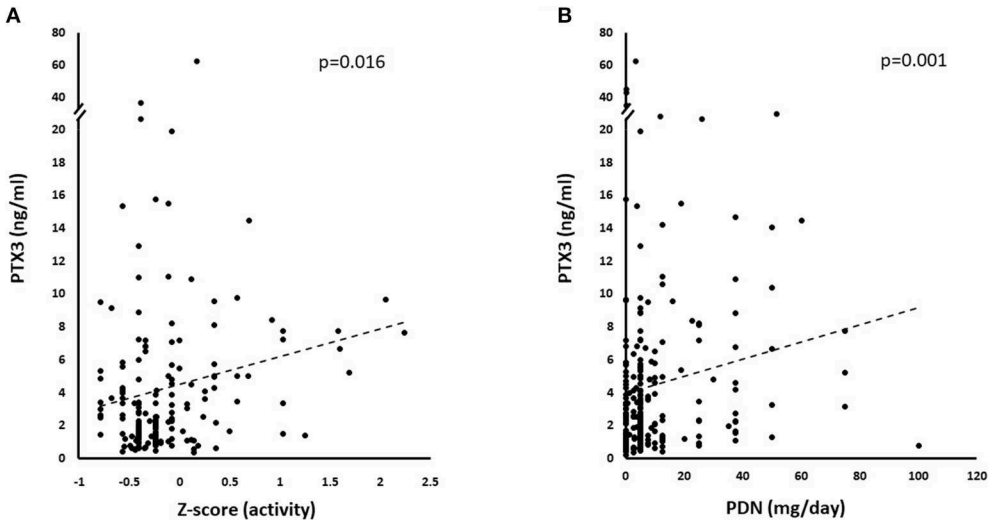


**FIGURE 3 |** Correlations with PTX3 levels. PTX3 correlated with multiple disease and treatment-related variables at univariate analysis. **(A)** Depicts the linkage between increasing normalized activity score (Z-activity) and PTX3 plasma levels. **(B)** Shows the potential influence of corticosteroid treatment on PTX3 circulating levels.

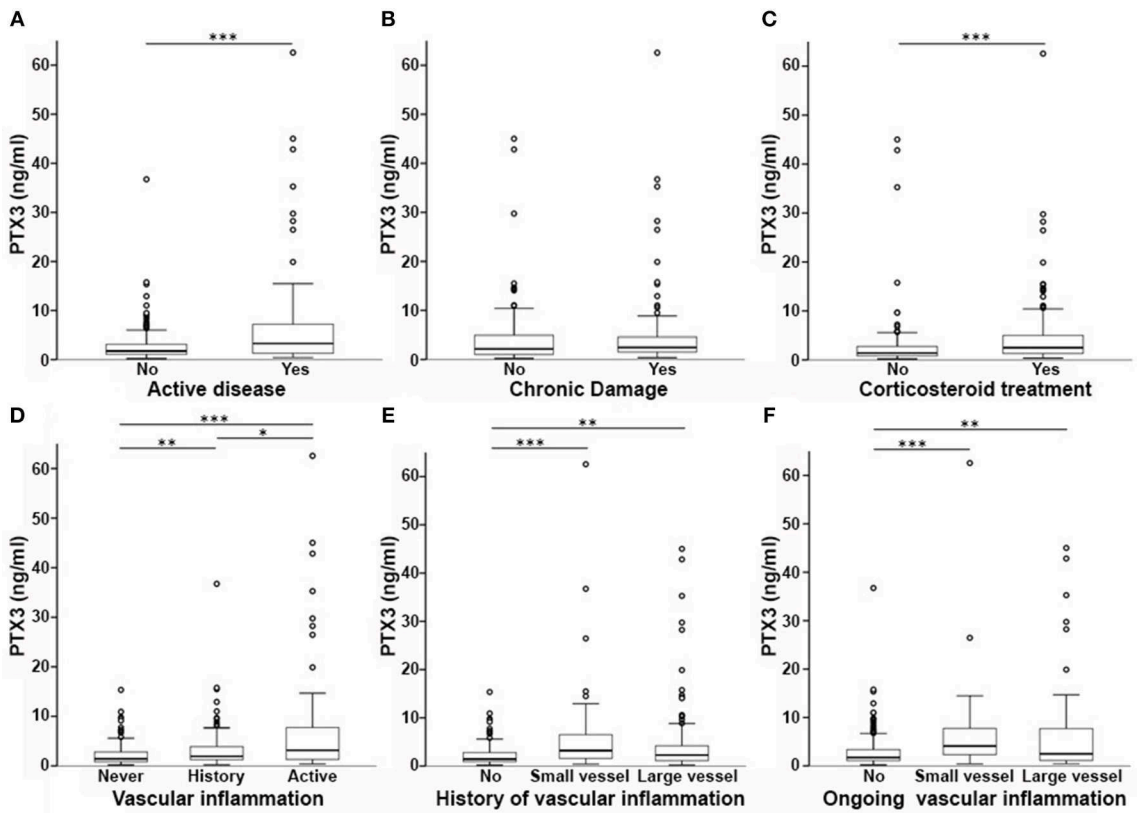


**FIGURE 4 |** PTX3 levels and disease phenotypes. In this multi-panel graph, differences in PTX3 plasma levels among phenotype groups are highlighted. PTX3 levels were higher in patients with active disease **(A)**, but not with accrued irreversible damage **(B)**. PTX3 was also higher in patients on corticosteroids **(C)**. **(D)** Depicts the existing differences between patients with vs. without a history of vasculitis and between active or quiescent vasculitis at time of sampling. In **(E,F)** patients are stratified according to a history **(E)** or ongoing activity **(F)** of small- or large-vessel vasculitis associated with higher levels of PTX3 when compared to no vasculitis. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

**TABLE 3 |** Correlations among PTX3 levels and clinical variables in patients with SLE.

| | Correlation with PTX3 | |
|---|---|---|
| | **All patients** | **Patients on PDN** |
| Age | No | No |
| Disease duration | No | No |
| SLEDAI-2K | Yes | No |
| PGA | Yes | No |
| Patient's VAS | Yes (inverse) | No |
| Erythrocyte sedimentation rate | No | No |
| CRP levels | No | No |
| C3 levels | No | No |
| C4 levels | Yes (inverse) | No |
| Anti-DNA titres | No | No |
| PDN dose | No | Yes |

**TABLE 4 |** Multivariate linear model of PTX3 levels.

| | B | Std. error | *p*-value |
|---|---|---|---|
| Active small-vessel inflammation | 6.706 | 1.473 | <0.001 |
| Active large-vessel inflammation | 4.269 | 0.844 | 0.008 |
| Diagnosis | 0.243 | 0.121 | 0.046 |
| History of small-vessel inflammation | 1.148 | 1.122 | 0.307 |
| History of large-vessel inflammation | 0.607 | 0.862 | 0.482 |
| Sex | −0.354 | 0.750 | 0.637 |
| Steroid therapy | −0.260 | 0.828 | 0.754 |

*$R^2$ of the model including the significant variables: 0.122.*

relatively low PTX3 concentrations were found in plasma from patients with RA, PMR and AAV without vasculitic features in this and other studies (20, 44). Neutrophil, endothelial cells and vessel-residing mononuclear cells can all concur to PTX3 release in the circulating blood during acute and chronic vascular injury (8, 11). PTX3 might be part of a protective response to the extension and exacerbation of organ damage due to post-ischemic inflammation (21, 29). However, PTX3 can also promote vascular injury under septic conditions (66). Furthermore, as a constituent of the antimicrobial array embedded in neutrophil extracellular traps (NETs) (10), PTX3 can concur to NETs-related immunothrombosis (58, 67) and similarly to neutrophil myeloperoxidase and proteinase-3 (which are also enclosed in NETs), promote the generation of pathogenic antibodies (68). Anti-PTX3 antibodies have been proposed represent atypical ANCA and, in contrast to SLE, might correlate with disease activity in patients with AAV (69).

This study has limitations. Systemic diseases without primary vessel inflammation are relatively underrepresented, which warrants caution in the interpretation of PTX3 dynamics in these settings. In addition, this study only explored the clinical relevance of PTX3 as a biomarker of vascular inflammation, without any deeper insight into the pathogenic drivers of this phenotype. Further mechanistic studies are thus needed to address this issue and possibly refine our knowledge on potential applications of PTX3 in diagnostics and therapy. To this purpose, dissecting the role of glucocorticoids as confounding factors for PTX3 expression would be of particular relevance, due to the widespread use of corticosteroid drugs in immune-mediated diseases.

Taken together, these data suggest that PTX3 could be implicated in multiple distinct pathophysiological events causing and maintaining inflammation in immune-mediated diseases. From a diagnostic point of view, PTX3 elevation in the circulating blood marks the occurrence of inflammatory events in blood vessels and might find a specific niche in clinical practice as a tool to identify vasculitic subsets among patients with autoimmune diseases.

## ETHICS STATEMENT

The study was performed under the Autoimmuno-Mol protocol, approved by the Ethics Committee of the San Raffaele Institute, Milan, Italy; reference number 2/2013/INT.

## AUTHOR CONTRIBUTIONS

AAM, PR-Q, GR, and ET designed the study. MaB, GR, ET, MiB, SS, MD, and RD collected clinical data. RL performed PTX3 evaluation. AM supervised the laboratory analysis and provided critical intellectual contribution to the study design and implementation. GR and ET analyzed clinical and laboratory data. GR, ET, and AAM drafted and revised the manuscript. The final version of the manuscript was approved by all authors.

## FUNDING

This work was supported by grants from the MIUR (2015MGBEM2_002), and the Ministero della Salute (RF_2013_02358715) to AAM as well as the PR-Q and European Research Council (ERC – No 69415), Fondazione Cariplo (Contract no. 2015-0564) and Cluster Alisei (MEDINTECH CTN01_00177_962865) to AM.

## ACKNOWLEDGMENTS

We are grateful to the B.I.R.D. Foundation Europe, Costozza di Longare (VI), Italy for its support to our School of Allergy and Immunology.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01135/full#supplementary-material

**Supplementary Figure 1 |** PTX3 levels in SLE. Boxplots depicting the existing differences in PTX3 plasma levels among patients with SLE stratified by disease activity, history of vascular inflammation and vasculitic activity. Patients with active disease not comprising any vascular involvement (V.I.) and patients with active vascular involvement in SLE showed a trend toward higher PTX3 levels compared to patients with clinically quiescent disease.

## REFERENCES


1. Hickey MJ, Kubes P. Intravascular immunity: the host-pathogen encounter in blood vessels. *Nat Rev Immunol.* (2009) 9:364–75. doi: 10.1038/nri2532
2. Stark K, Eckart A, Haidari S, Tirniceriu A, Lorenz M, von Bruhl ML, et al. Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and 'instruct' them with pattern-recognition and motility programs. *Nat Immunol.* (2013) 14:41–51. doi: 10.1038/ni.2477
3. Ramirez GA, Rovere-Querini P, Sabbadini MG, Manfredi AA. Parietal and intravascular innate mechanisms of vascular inflammation. *Arth Res Therap.* (2015) 17:16. doi: 10.1186/s13075-015-0528-2
4. Gaertner F, Massberg S. Blood coagulation in immunothrombosis-At the frontline of intravascular immunity. *Semin Immunol.* (2016) 28:561–9. doi: 10.1016/j.smim.2016.10.010
5. Maugeri N, Rovere-Querini P, Manfredi AA. Disruption of a regulatory network consisting of neutrophils and platelets fosters persisting inflammation in rheumatic diseases. *Front Immunol.* (2016) 7:182. doi: 10.3389/fimmu.2016.00182
6. Semple JW, Italiano JEJr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol.* (2011) 11:264–74. doi: 10.1038/nri2956
7. Erreni M, Manfredi AA, Garlanda C, Mantovani A, Rovere-Querini P. The long pentraxin PTX3: A prototypical sensor of tissue injury and a regulator of homeostasis. *Immunol Rev.* (2017) 280:112–25. doi: 10.1111/imr.12570
8. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. PTX3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiol Rev.* (2018) 98:623–39. doi: 10.1152/physrev.00016.2017
9. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
10. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, et al. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med.* (2007) 204:793–804. doi: 10.1084/jem.20061301
11. Manfredi AA, Rovere-Querini P, Bottazzi B, Garlanda C, Mantovani A. Pentraxins, humoral innate immunity and tissue injury. *Curr Opin Immunol.* (2008) 20:538–44. doi: 10.1016/j.coi.2008.05.004
12. Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, et al. PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in *in vivo* fertilization. *Development.* (2004) 131:1577–86. doi: 10.1242/dev.01056
13. Baruah P, Propato A, Dumitriu IE, Rovere-Querini P, Russo V, Fontana R, et al. The pattern recognition receptor PTX3 is recruited at the synapse between dying and dendritic cells, and edits the cross-presentation of self, viral, and tumor antigens. *Blood.* (2006) 107:151–8. doi: 10.1182/blood-2005-03-1112.
14. Canti V, Castiglioni MT, Rosa S, Franchini S, Sabbadini MG, Manfredi AA, et al. Pregnancy outcomes in patients with systemic autoimmunity. *Autoimmunity.* (2012) 45:169–75. doi: 10.3109/08916934.2011.593600
15. Assandri R, Monari M, Colombo A, Dossi A, Montanelli A. Pentraxin 3 plasma levels and disease activity in systemic lupus erythematosus. *Autoimmune Dis.* (2015) 2015:354014. doi: 10.1155/2015/354014
16. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell.* (2015) 160:700–14. doi: 10.1016/j.cell.2015.01.004
17. Grcevic D, Sironi M, Valentino S, Deban L, Cvija H, Inforzato A, et al. The long pentraxin 3 plays a role in bone turnover and repair. *Front Immunol.* (2018) 9:417. doi: 10.3389/fimmu.2018.00417
18. Presta M, Foglio E, Churruca Schuind A, Ronca R. Long Pentraxin-3 modulates the angiogenic activity of fibroblast growth factor-2. *Front Immunol.* (2018) 9:2327. doi: 10.3389/fimmu.2018.02327
19. Rodrigues PF, Matarazzo S, Maccarinelli F, Foglio E, Giacomini A, Silva Nunes JP, et al. Long pentraxin 3-mediated fibroblast growth factor trapping impairs fibrosarcoma growth. *Front Oncol.* (2018) 8:472. doi: 10.3389/fonc.2018.00472
20. Fazzini F, Peri G, Doni A, Dell'Antonio G, Dal Cin E, Bozzolo E, et al. PTX3 in small-vessel vasculitides: an independent indicator of disease activity produced at sites of inflammation. *Arthritis Rheum.* (2001) 44:2841–2850.doi: 10.1002/1529-0131(200112)44:12<2841::AID-ART472>3.0.CO;2-6
21. Garlanda C, Bottazzi B, Moalli F, Deban L, Molla F, Latini R, et al. Pentraxins and atherosclerosis: the role of PTX3. *Curr Pharm Des.* (2011) 17:38–46. doi: 10.2174/138161211795049750
22. Maugeri N, Rovere-Querini P, Slavich M, Coppi G, Doni A, Bottazzi B, et al. Early and transient release of leukocyte pentraxin 3 during acute myocardial infarction. *J Immunol.* (2011) 187:970–9. doi: 10.4049/jimmunol.1100261
23. Baldini M, Maugeri N, Ramirez GA, Giacomassi C, Castiglioni A, Prieto-Gonzalez S, et al. Selective up-regulation of the soluble pattern-recognition receptor pentraxin 3 and of vascular endothelial growth factor in giant cell arteritis: relevance for recent optic nerve ischemia. *Arthritis Rheum.* (2012) 64:854–65. doi: 10.1002/art.33411
24. Tombetti E, Di Chio MC, Sartorelli S, Papa M, Salerno A, Bottazzi B, et al. Systemic pentraxin-3 levels reflect vascular enhancement and progression in Takayasu arteritis. *Arthritis Res Ther.* (2014) 16:479. doi: 10.1186/s13075-014-0479-z
25. Shirai Y, Okazaki Y, Inoue Y, Tamura Y, Yasuoka H, Takeuchi T, et al. Elevated levels of pentraxin 3 in systemic sclerosis: associations with vascular manifestations and defective vasculogenesis. *Arthritis Rheumatol.* (2015) 67:498–507. doi: 10.1002/art.38953
26. Sahin S, Adrovic A, Barut K, Durmus S, Gelisgen R, Uzun H, et al. Pentraxin-3 levels are associated with vasculitis and disease activity in childhood-onset systemic lupus erythematosus. *Lupus.* (2017) 26:1089–94. doi: 10.1177/0961203317699286
27. Weitoft T, Larsson A, Saxne T, Manivel VA, Lysholm J, Knight A, et al. Pentraxin 3 in serum and synovial fluid of patients with rheumatoid arthritis with and without autoantibodies. *Scand J Rheumatol.* (2017) 46:346–52. doi: 10.1080/03009742.2016.1244288
28. Rovere P, Peri G, Fazzini F, Bottazzi B, Doni A, Bondanza A, et al. The long pentraxin PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells. *Blood.* (2000) 96:4300–6.
29. Deban L, Russo RC, Sironi M, Moalli F, Scanziani M, Zambelli V, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nat Immunol.* (2010) 11:328–34. doi: 10.1038/ni.1854
30. Leavitt RY, Fauci AS, Bloch DA, Michel BA, Hunder GG, Arend WP, et al. The American college of rheumatology 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum.* (1990) 33:1101–7. doi: 10.1002/art.1780330807
31. Sharma BK, Jain S, Suri S, Numano F. Diagnostic criteria for Takayasu arteritis. *Int J Cardiol.* (1996) 54 (Suppl.):S141–7. doi: 10.1016/0167-527302647-2
32. Watts R, Lane S, Hanslik T, Hauser T, Hellmich B, Koldingsnes W, et al. Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. *Ann Rheum Dis.* (2007) 66:222–7. doi: 10.1136/ard.2006.054593
33. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* (1997) 40:1725. doi: 10.1002/art.1780400928
34. Pineton de Chambrun M, Wechsler B, Geri G, Cacoub P, Saadoun D. New insights into the pathogenesis of Behcet's disease. *Autoimmun Rev.* (2012) 11:687–98. doi: 10.1016/j.autrev.2011.11.026
35. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham COIII, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* (2010) 62:2569–81. doi: 10.1002/art.27584
36. Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol.* (2002) 29:288–91.
37. Mukhtyar C, Lee R, Brown D, Carruthers D, Dasgupta B, Dubey S, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis.* (2009) 68:1827–32. doi: 10.1136/ard.2008.101279
38. Misra R, Danda D, Rajappa SM, Ghosh A, Gupta R, Mahendranath KM, et al. Development and initial validation of the Indian Takayasu Clinical Activity Score (ITAS2010). *Rheumatology.* (2013) 52:1795–801. doi: 10.1093/rheumatology/ket128
39. Isenberg DA, Rahman A, Allen E, Farewell V, Akil M, Bruce IN, et al. BILAG 2004. Development and initial validation of an updated version

of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. *Rheumatology.* (2005) 44:902–6. doi: 10.1093/rheumatology/keh624
40. Gladman DD, Goldsmith CH, Urowitz MB, Bacon P, Fortin P, Ginzler E, et al. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for Systemic Lupus Erythematosus International Comparison. *J Rheumatol.* (2000) 27:373–6.
41. Mohammad AJ, Bakoush O, Sturfelt G, Segelmark M. The extent and pattern of organ damage in small vessel vasculitis measured by the Vasculitis Damage Index (VDI). *Scand J Rheumatol.* (2009) 38:268–75. doi: 10.1080/03009740802668554
42. Sivakumar M. Outcome of vascular interventions in takayasu arteritis using the takayasu arteritis damage score. *Arthritis Rheumatism.* (2011) 63.
43. Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, et al. PTX3, A prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. *Circulation*. (2000) 102:636–641. doi: 10.1161/01.CIR.102.6.636
44. Pulsatelli L, Peri G, Macchioni P, Boiardi L, Salvarani C, Cantini F, et al. Serum (10): S588. levels of long pentraxin PTX3 in patients with polymyalgia rheumatica. *Clin Exp Rheumatol.* (2010) 28:756–8.
45. Dagna L, Salvo F, Tiraboschi M, Bozzolo EP, Franchini S, Doglioni C, et al. Pentraxin-3 as a marker of disease activity in Takayasu arteritis. *Ann Intern Med.* (2011) 155:425–33. doi: 10.7326/0003-4819-155-7-201110040-00005
46. Zhang J, Shan L, Koussih L, Redhu NS, Halayko AJ, Chakir J, et al. Pentraxin 3 (PTX3) expression in allergic asthmatic airways: role in airway smooth muscle migration and chemokine production. *PLoS ONE.* (2012) 7:e34965. doi: 10.1371/journal.pone.0034965
47. Klimek E, Skalska A, Kwasny-Krochin B, Surdacki A, Sulicka J, Korkosz M, et al. Differential associations of inflammatory and endothelial biomarkers with disease activity in rheumatoid arthritis of short duration. *Mediators Inflamm.* (2014) 2014:681635. doi: 10.1155/2014/681635
48. Luchetti MM, Piccinini G, Mantovani A, Peri G, Matteucci C, Pomponio G, et al. Expression and production of the long pentraxin PTX3 in rheumatoid arthritis (RA). *Clin Exp Immunol.* (2000) 119:196–202. doi: 10.1046/j.1365-2249.2000.01110.x
49. Monach PA. Biomarkers in vasculitis. *Curr Opin Rheumatol.* (2014) 26:24–30. doi: 10.1097/BOR.0000000000000009
50. Weyand CM, Goronzy JJ. Clinical practice. Giant-cell arteritis and polymyalgia rheumatica. *N Engl J Med.* (2014) 371:50–7. doi: 10.1056/NEJMcp1214825
51. Carmona FD, Martín J, González-Gay MA. New insights into the pathogenesis of giant cell arteritis and hopes for the clinic. *Expert Rev Clin Immunol.* (2016) 12:57–66. doi: 10.1586/1744666X.2016.1089173
52. Tombetti E, Colombo B, Di Chio MC, Sartorelli S, Papa M, Salerno A, et al. Chromogranin-A production and fragmentation in patients with Takayasu arteritis. *Arthritis Res Ther.* (2016) 18:187. doi: 10.1186/s13075-016-1082-2
53. Tombetti E, Maugeri N, Rovere-Querini P, Manfredi AA. Biomarkers of vascular inflammation. Cell stress offers new clues. *Int J Cardiol.* (2017) 246:18–9. doi: 10.1016/j.ijcard.2017.05.115
54. Tombetti E, Godi C, Ambrosi A, Doyle F, Jacobs A, Kiprianos AP, et al. Novel angiographic scores for evaluation of large vessel vasculitis. *Sci Rep.* (2018) 8:15979. doi: 10.1038/s41598-018-34395-7
55. Tombetti E, Mason JC. Takayasu arteritis: recent advances and hopes for the future. *Rheumatology.* (2019) [epub ahead of print].
56. Baragetti A, Knoflach M, Cuccovillo I, Grigore L, Casula M, Garlaschelli K, et al. Pentraxin 3 (PTX3) plasma levels and carotid intima media thickness progression in the general population. *Nutr Metab Cardiovasc Dis.* (2014) 24:518–23. doi: 10.1016/j.numecd.2013.10.030
57. Jaillon S, Jeannin P, Hamon Y, Fremaux I, Doni A, Bottazzi B, et al. Endogenous PTX3 translocates at the membrane of late apoptotic human neutrophils and is involved in their engulfment by macrophages. *Cell Death Differ.* (2009) 16:465–74. doi: 10.1038/cdd.2008.173
58. Bassi N, Ghirardello A, Blank M, Zampieri S, Sarzi-Puttini P, Mantovani A, et al. IgG anti-pentraxin 3 antibodies in systemic lupus erythematosus. *Ann Rheum Dis.* (2010) 69:1704–10. doi: 10.1136/ard.2009.117804
59. Gatto M, Ghirardello A, Luisetto R, Bassi N, Fedrigo M, Valente M, et al. Immunization with pentraxin 3 (PTX3) leads to anti-PTX3 antibody production and delayed lupus-like nephritis in NZB/NZW F1 mice. *J Autoimmun.* (2016) 74:208–16. doi: 10.1016/j.jaut.2016.07.002
60. Doni A, Mantovani G, Porta C, Tuckermann J, Reichardt HM, Kleiman A, et al. Cell-specific regulation of PTX3 by glucocorticoid hormones in hematopoietic and nonhematopoietic cells. *J Biol Chem.* (2008) 283:29983–92. doi: 10.1074/jbc.M805631200
61. Wu GC, Li J, Leng RX, Li XP, Li XM, Wang DG, et al. Identification of long non-coding RNAs GAS5, linc0597 and lnc-DC in plasma as novel biomarkers for systemic lupus erythematosus. *Oncotarget.* (2017) 8:23650–63. doi: 10.18632/oncotarget.15569
62. Lech M, Rommele C, Kulkarni OP, Susanti HE, Migliorini A, Garlanda C, et al. Lack of the long pentraxin PTX3 promotes autoimmune lung disease but not glomerulonephritis in murine systemic lupus erythematosus. *PLoS ONE.* (2011) 6:e20118. doi: 10.1371/journal.pone.0020118
63. Shimada Y, Asanuma YF, Yokota K, Yoshida Y, Kajiyama H, Sato K, et al. Pentraxin 3 is associated with disease activity but not atherosclerosis in patients with systemic lupus erythematosus. *Mod Rheumatol.* (2014) 24:78–85. doi: 10.3109/14397595.2013.852837
64. Skare TL, Nisihara R, Ramos GP, Utiyama SR, Messias-Reason I. Pentraxin-3 levels in systemic lupus erythematosus: association with cumulative damage but not with disease activity. *Joint Bone Spine.* (2015) 82:466–7. doi: 10.1016/j.jbspin.2014.09.003
65. Wirestam L, Enocsson H, Skogh T, Eloranta ML, Ronnblom L, Sjowall C, et al. Interferon-alpha coincides with suppressed levels of pentraxin-3 (PTX3) in systemic lupus erythematosus and regulates leucocyte PTX3 *in vitro*. *Clin Exp Immunol.* (2017) 189:83–91. doi: 10.1111/cei.12957
66. Souza DG, Amaral FA, Fagundes CT, Coelho FM, Arantes RM, Sousa LP, et al. The long pentraxin PTX3 is crucial for tissue inflammation after intestinal ischemia and reperfusion in mice. *Am J Pathol.* (2009) 174:1309–18. doi: 10.2353/ajpath.2009.080240
67. Ramirez GA, Manfredi AA, Rovere-Querini P, Maugeri N. Bet on NETs! Or on how to translate basic science into clinical practice. *Front Immunol.* (2016) 7:417. doi: 10.3389/fimmu.2016.00417
68. Sangaletti S, Tripodo C, Chiodoni C, Guarnotta C, Cappetti B, Casalini P, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood.* (2012) 120:3007–18. doi: 10.1182/blood-2012-03-416156
69. Simon A, Subra JF, Guilpain P, Jeannin P, Pignon P, Blanchard S, et al. Detection of anti-pentraxin-3 autoantibodies in ANCA-associated vasculitis. *PLoS ONE.* (2016) 11:e0147091. doi: 10.1371/journal.pone.0147091

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

CORRECTION
published: 24 July 2019
doi: 10.3389/fimmu.2019.01755

# Corrigendum: PTX3 Intercepts Vascular Inflammation in Systemic Immune-Mediated Diseases

*Giuseppe A. Ramirez[1,2,3], Patrizia Rovere-Querini[1,3], Miriam Blasi[1,3], Silvia Sartorelli[1], Maria Chiara Di Chio[1], Mattia Baldini[1,3], Rebecca De Lorenzo[1,3], Enrica P. Bozzolo[2], Roberto Leone[4], Alberto Mantovani[4,5,6], Angelo A. Manfredi[1,2,3]* and Enrico Tombetti[1,2,3]*

[1] Università Vita-Salute San Raffaele, Milan, Italy, [2] Unit of Immunology, Rheumatology, Allergy and Rare Diseases, IRCCS Ospedale San Raffaele, Milan, Italy, [3] Division of Immunology, Transplantation and Infectious Immunity, IRCCS Ospedale San Raffaele, Milan, Italy, [4] Humanitas Research Center - IRCCS, Rozzano, Italy, [5] Humanitas University, Rozzano, Italy, [6] The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom

OPEN ACCESS

**Approved by:**
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

***Correspondence:**
Angelo A. Manfredi
manfredi.angelo@hsr.it

**Specialty section:**
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

**Received:** 11 July 2019
**Accepted:** 11 July 2019
**Published:** 24 July 2019

**Citation:**
Ramirez GA, Rovere-Querini P, Blasi M, Sartorelli S, Di Chio MC, Baldini M, De Lorenzo R, Bozzolo EP, Leone R, Mantovani A, Manfredi AA and Tombetti E (2019) Corrigendum: PTX3 Intercepts Vascular Inflammation in Systemic Immune-Mediated Diseases. Front. Immunol. 10:1755. doi: 10.3389/fimmu.2019.01755

**Keywords: PTX3, autoimmunity, lupus, rheumatoid arthritis, Takayasu arteritis, giant cell arteritis, ANCA associated small vessel vasculitis, intravascular immunity**

## A Corrigendum on

**PTX3 Intercepts Vascular Inflammation in Systemic Immune-Mediated Diseases**
*by Ramirez, G. A., Rovere-Querini, P., Blasi, M., Sartorelli, S., Di Chio, M. C., Baldini, M., et al. (2019). Front. Immunol. 10:1135. doi: 10.3389/fimmu.2019.01135*

In the original article, we neglected to include the funder "Cluster Alisei (MEDINTECH CTN01_00177_962865)" to Alberto Mantovani.

A correction has therefore been made to the **Funding** statement:

"This work was supported by grants from the MIUR (2015MGBEM2_002), and the Ministero della Salute (RF_2013_02358715) to AAM as well as the PR-Q and European Research Council (ERC – No 69415), Fondazione Cariplo (Contract no. 2015-0564) and Cluster Alisei (MEDINTECH CTN01_00177_962865) to AM."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

frontiers in Immunology

REVIEW
published: 10 July 2019
doi: 10.3389/fimmu.2019.01461

# Tissue Dependent Role of PTX3 During Ischemia-Reperfusion Injury

*Thiago Henrique Caldeira de Oliveira*[1], *Danielle G. Souza*[2], *Mauro Martins Teixeira*[1] *and Flávio Almeida Amaral*[1]*

[1] *Immunopharmacology Laboratory, Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil,* [2] *Host-Microorganism Interaction Laboratory, Department of Microbiology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Reperfusion of an ischemic tissue is the treatment of choice for several diseases, including myocardial infarction and stroke. However, reperfusion of an ischemic tissue causes injury, known as Ischemia and Reperfusion Injury (IRI), that limits the benefit of blood flow restoration. IRI also occurs during solid organ transplantation. During IRI, there is activation of the innate immune system, especially neutrophils, which contributes to the degree of injury. It has been shown that PTX3 can regulate multiple aspects of innate immunity and tissue inflammation during sterile injury, as observed during IRI. In humans, levels of PTX3 increase in blood and elevated levels associate with extent of IRI. In mice, there is also enhanced expression of PTX3 in tissues and plasma after IRI. In general, absence of PTX3, as seen in PTX3-deficient mice, results in worse outcome after IRI. On the contrary, increased expression of PTX3, as seen in PTX3 transgenic mice and after PTX3 administration, is associated with better outcome after IRI. The exception is the gut where PTX3 seems to have a clear deleterious role. Here, we discuss mechanisms by which PTX3 contributes to IRI and the potential of taming this system for the treatment of injuries associated with reperfusion of solid organs.

**Keywords: PTX3, ischemia and reperfusion injury, sterile inflammation, hypoxia, adhesion moleculaes, neutrophil**

OPEN ACCESS

***Edited by:***
*Patrizia Rovere Querini, Vita-Salute San Raffaele University, Italy*

***Reviewed by:***
*Lara Campana, University of Edinburgh, United Kingdom*
*Mariele Gatto, University of Padova, Italy*

***Correspondence:**
*Flávio Almeida Amaral*
*famaral@icb.ufmg.br*

***Specialty section:***
*This article was submitted to Inflammation, a section of the journal Frontiers in Immunology*

***Received:*** *30 October 2018*
***Accepted:*** *10 June 2019*
***Published:*** *10 July 2019*

***Citation:***
*de Oliveira THC, Souza DG, Teixeira MM and Amaral FA (2019) Tissue Dependent Role of PTX3 During Ischemia-Reperfusion Injury. Front. Immunol. 10:1461. doi: 10.3389/fimmu.2019.01461*

## INTRODUCTION

Impaired blood flow to tissues caused by reduced or obstructed arterial inflow (ischemia) and consequent decreasing of oxygen and nutrient supply is an intrinsic condition during clinical procedures, including coronary angioplasty, vascular reconstruction, organ transplantation, and vascular diseases, such as stroke, myocardial, renal, and intestinal infarction (1–3). Although reperfusion brings blood flow and oxygen back, which are essential to prevent irreversible tissue injury, it may paradoxically worsen ischemic tissue damage. During reperfusion, there is excessive production of pro-inflammatory molecules by the ischemic tissue and systemic distribution of these molecules. This phenomenon is known as ischemia-reperfusion injury (IRI) and is a major issue during organ transplantation, as it directly correlates to graft rejection (4–6). IRI is responsible for up to 10% of early transplant failures and is also associated with high rates of acute and chronic rejection (7–9).

During ischemia, adenosine triphosphate (ATP) production is impaired due to decreased oxygen supply (10). In addition, ischemic tissue produces high levels of pro-inflammatory cytokines, vasoactive agents, adhesion molecules, and reactive oxygen species (ROS) (4). Particularly, ROS generation modifies intracellular pH that is associated with organelle damage and cell death (11). In this sterile inflammatory context, the innate immune response is activated

when dead cells release their contents into the extracellular environment, which are recognized by pattern recognition receptors (PRRs) expressed on resident immune cells. Furthermore, soluble pattern recognition molecules work as fluid-phases receptors, distributed mainly in distinct liquid compartments. This humoral arm of the innate immune system consists of three clearly defined subgroups of molecules, represented by collectins, ficolins, and pentraxins (12).

Pentraxins belong to a family of phylogenetically conserved proteins and are divided into two groups according to the length of their primary structure: the short and long chain pentraxins (13). The classical short pentraxins, represented by C-reactive protein (CRP) and serum amyloid P component (SAP) are produced in the liver under pro-inflammatory stimuli, most prominently by IL-6. Both CRP and SAP bind to different ligands of microbes and host components in a calcium-dependent manner, a mechanism associated to innate immunity against pathogens and also for scavenging of cellular debris (14). Long pentraxins are characterized by an unrelated N-terminal domain coupled to a pentraxin-like C-terminal domain (15). The prototypic long pentraxin 3 (PTX3), also formerly referred to as TSG-14 (TNF-stimulated gene 14) was identified in the early 1990s in human endothelial cells and fibroblasts as a TNF or IL-1β-inducible mRNA and protein, respectively (16, 17). Here, we provide an overview of currently available data about the role of PTX3 in the complex mechanisms involved in the immune response during IRI in different organs. Then, we discuss possible options for IRI therapy based on the knowledge of PTX3 biology.

## MECHANISMS OF TISSUE DAMAGE DURING ISCHEMIA AND REPERFUSION INJURY (IRI)

Several pathological processes contribute to IRI, including impaired endothelial cell barrier function (18, 19), activation of cell death programs (20) and activation of innate and adaptive immune responses (21). IRI occurs as the result of a biphasic condition. During ischemia, when the oxygen levels decrease, there is a dysfunction of the electron transport chain in mitochondria and a shift from aerobic to anaerobic metabolism, which impairs ATP production. Moreover, there is accumulation of lactic acid and ketone bodies, leading to decrease of pH in tissues and cells, known as metabolic acidosis. The lack of energetic substrate also interferes with transmembrane transports, causing dysfunction of sodium-potassium and calcium pumps on the cell surface, which results in cell hyperosmolarity and flow of water into the cytoplasm and cell swelling (22). In ischemic tissues, a large number of ROS are produced by mitochondria. ROS production can cause damage to membrane lipids, proteins, and DNA, leading to endothelial cell dysfunction and consequently cell death (23). In addition, the deleterious effects of low oxygen levels spread along different cell types in the affected tissue (24–27). There is a variation of the resistance to ischemia among cell populations of a given tissue. For example, cardiac cells are more resistant to periods of ischemia as compared to hepatocytes and Kupffer cells (28).

The magnitude and duration of ischemia will determine the degree of cell dysfunction and death. Cells that died during the ischemic phase release a range of intracellular molecules called danger associated molecular patterns (DAMPs), also known as alarmins. Under homeostatic conditions, these molecules are hidden into intracellular compartments. However, under conditions of cellular stress, DAMPs are released to the extracellular environment or kept on cell membrane (29). Different molecules have been described as DAMPs, including ROS, ATP, high mobility group box 1 (HMGB1), DNA, mitochondrial formyl peptides, IL-1, urate, and S100 proteins. These molecules bind to a variety of PRR and trigger inflammatory responses through the activation of various signaling pathways (30). Innate immune, parenchymal and endothelial cells express PRRs on their surface and in their cytoplasm, which recognize DAMPs. PRRs include Toll-like receptors (TLRs), Retinoic Acid-Inducible Gene I-like receptors, nucleotide-binding oligomerization domain-like receptors (NLRs), including the inflammasomes, and C-type lectin receptors. Thus, it seems clear that DAMPs released during ischemia contribute to the intense inflammatory response seen in IRI (31).

The reperfusion phase occurs when the blood flow is restored to the ischemic tissue. During the first minutes, the blood flow to ischemic tissue may not happen immediately, a phenomenon known as no-reflow. It is believed that this intravascular obstruction may be caused by leukocytes and platelets (32). Although reperfusion is required to restore oxygen to the tissue, the metabolic distress caused during ischemia creates a condition that triggers a set of excessive innate immune response, which exacerbates the injury to vascular and parenchymal cells during reperfusion. The reperfusion can be separated in two phases. Initially, there is intense oxidant stress, leading to impaired production of antioxidative molecules that increases ROS generation further. ROS initiate a series of cellular events that cause inflammation, promoting cellular injury through endothelial dysfunction, DNA damage, necrosis and/or apoptosis (11). An important pathway of ROS production involves reduction of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (32). Indeed, abrogation of NADPH component in mice reduces the deleterious effects of IRI (33). In addition, the reperfusion phase sets deposition of complement, upregulation of adhesion molecules, inflammatory cell infiltration, mainly neutrophils, and further pro-inflammatory mediators production (33, 34). The local presence of DAMPS and molecules produced in response to DAMPs will feed into this increasing inflammatory reaction seen during IRI.

Although the degree of injury may vary in different tissues, a common feature in all organs is microvascular dysfunction. The vascular injury induced by IR is a consequence of local and systemic inflammatory response and includes vascular permeability, endothelial cell activation, platelet–leukocyte interaction, complement activation, and imbalance between vasodilating and vasoconstricting factors (3). Tissue hypoxia during ischemia directly influences the increase of vascular permeability, as demonstrated by studies with

endothelial cells exposed to an environment with low oxygen concentration, a phenomenon that alters endothelial cell barrier function in a mechanism dependent on reduced adenylate cyclase activity and intracellular cAMP levels (19). Moreover, studies *in vivo* demonstrated that animals exposed to a hypoxic environment showed vascular leakage in multiple organs and increased hypoxia-associated pulmonary edema (35, 36).

The migration of neutrophils from blood into tissue during vascular inflammation occurs by a multistep cascade. There is initial tethering and rolling on vessel wall via selectins interactions followed by firm adhesion and emigration out of the vasculature to the parenchyma. These events are well-established in different microvasculatures including the peritoneum, mesentery, skeletal muscle, and skin (37). In this regard, tissue and resident cells produce chemoattractant factors, such as chemokines, that guide neutrophil infiltration into the site of inflammation. To induce neutrophil migration, chemokines are maintained in high concentration on the endothelium cell surface by binding to glycosaminoglycans (38). Moreover, other factors contribute to neutrophil migration and microvascular dysfunction after reperfusion, including complement components and leukocyte interactions with platelets (39). Upon leaving the vessels and entering the tissues, activated leukocytes release ROS and proteases, causing increased microvascular permeability, edema, thrombosis, and parenchymal cell death (39). Indeed, previous reports have shown that Reparixin, a non-competitive allosteric antagonist of chemokine receptor CXCR2 was able to prevent neutrophil migration and reduce liver and intestinal damage, suggesting that excessive neutrophil migration is detrimental to tissues following reperfusion (40, 41).

## ROLE OF PTX3 DURING STERILE INFLAMMATION

There are several actions of PTX3 that are relevant to the sterile inflammation that occurs during IRI (**Figure 1**). PTX3 can be considered an acute-phase protein. In normal conditions, its serum levels is low (around 25 ng/ml in the mouse, <2 ng/ml in humans), but quickly increases during inflammation (200–800 ng/ml in humans and mice) (42). Innate immune factors stimulate the production of PTX3 locally, including pattern molecules (DAMPs) and cytokines (43). Particularly in the field of sterile inflammation, IL-1 is a potent inducer of PTX3 production during tissue damage, as occurs in mouse models of acute myocardial infarction (AMI) (44).

In terms of kinetics, local production of PTX3 begins before the synthesis of classical pentraxins by hepatic parenchyma cells. PTX3 can be produced in several organs, especially in the heart and striated muscle, but also in the lungs, ovarium, thymus, and the skin (45). Resident leukocytes and parenchymal cells are important sources of PTX3 (46, 47). PTX3 is also stored in neutrophil granules, which are released under inflammatory stimuli. It is estimated that neutrophils release about 25% of their PTX3 to the extracellular compartment, much of them associated with neutrophil extracellular traps (NETs) (48). Thus, while CRP and SAP are produced in the liver and carried to the inflammatory foci by blood flow, PTX3 is formed locally at sites of ongoing inflammatory reaction (45, 49).

The multifunctional properties of PTX3 include interactions with different ligands, such as complement C1q component, the extracellular matrix component TSG6, apoptotic cells, endothelial cells, and leukocytes (50). Complement factors and PTX3 have been considered important regulators in the clearance of dying cells. In this regard, an investigation in mammalian cells showed that soluble PTX3 binds to immobilized C1q and, reciprocally, C1q bound to immobilized PTX3 (51). In addition, C1q and PTX3 present different functions during the phagocytosis of apoptotic cells. Previous reports have demonstrated that complement C1q is necessary for effective phagocytosis of apoptotic cells by macrophages, whereas PTX3 inhibits this process (52, 53). This mechanism was elucidated by Baruah and coworkers' study, who showed that C1q and PTX3 have different affinity for apoptotic cell domains. However, the presence of PTX3 in the solution removed bound C1q from apoptotic cells, leading to inhibition of complement activation by C1q on apoptotic cells and their phagocytosis by dendritic cells. Moreover, it has been shown that although PTX3 decreased the internalization of dying cells by human dendritic cells, it did not affect the capture of soluble or inert particulate substrates, such as fluorescent ovalbumin and latex beads. Furthermore, apoptotic cells preincubated first with PTX3 did not modify binding of C1q to these dying cells. Thus, these findings suggest that PTX3 and C1q interaction may occur in soluble phase, reducing the availability of C1q-mediated phagocytosis (54). These results suggest that although PTX3 prevents cell phagocytosis by dendritic cells, it favors the sequestration of cell debris by antigen-presenting cells, which could contribute to reduce self-antigen presentation and a possible development of autoimmune disorders (55). Furthermore, deficiencies of C1q is associated with development of systemic erythematosus lupus (SLE) and accumulation of apoptotic cells in renal glomeruli, which emphasizes the importance of C1q in the clearance of cellular debris (56). It has been demonstrated for a long time that patients with SLE have a well-characterized defect in the production of pentraxins during active phases of the disease (57). Thus, these data suggest that the interaction of C1q and PTX3 may have important implications in the healthy removal of cellular debris under inflammatory conditions and protection against autoimmunity.

PTX3 also interacts with endothelial cell adhesion molecules. Deban and coworkers reported that PTX3 released by hematopoietic cells prevent excessive neutrophil recruitment under P-selectin interaction. This observation was also demonstrated using exogenous PTX3.This finding suggests a natural anti-inflammatory effect of PTX3 in P-selectin-dependent models of leukocyte recruitment and inflammation (58). Moreover, models of sterile inflammation, such as AMI induced by coronary artery ligation and reperfusion or cerebral IRI, showed that absence of PTX3 was associated with increased neutrophil migration and tissue damage (44, 59). These results suggest that PTX3 provides a feedback loop by preventing neutrophil recruitment and tissue damage in

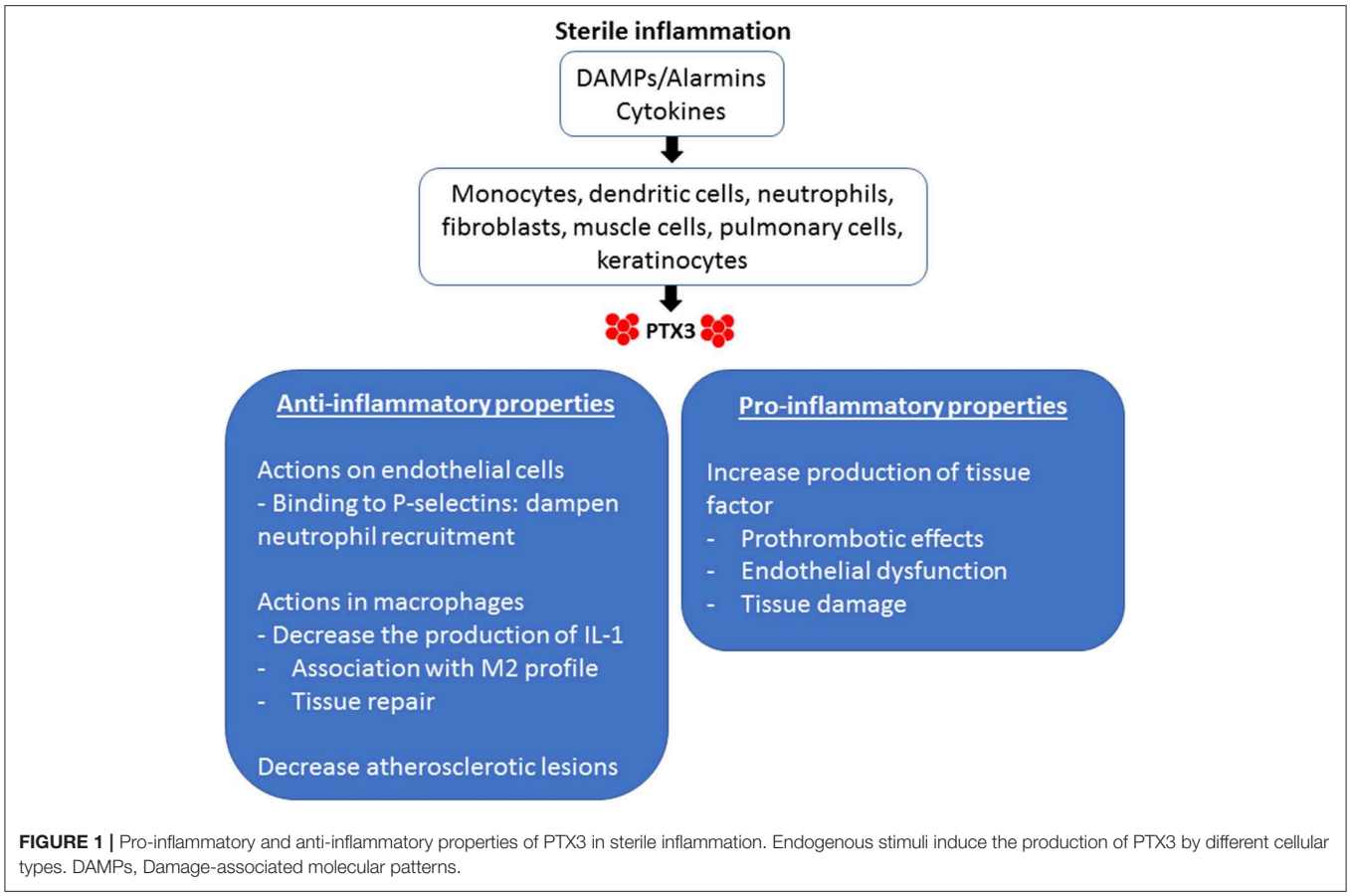


**FIGURE 1 |** Pro-inflammatory and anti-inflammatory properties of PTX3 in sterile inflammation. Endogenous stimuli induce the production of PTX3 by different cellular types. DAMPs, Damage-associated molecular patterns.

models of sterile inflammation. In addition, PTX3 influences macrophages function. PTX3 impairs the production IL-1β, TNF, and CCL2 levels, whilst stimulates TGF-β production by THP-1 macrophages. These results were associated with Akt phosphorylation and reduced NF-κB activation in the presence of PTX3. Silencing PTX3 increased IL-1β production by macrophages (60). Moreover, it has been reported that mice lacking PTX3 subjected to wire-mediated endovascular injury exhibited higher deteriorated neointimal hyperplasia after vascular injury via the effects of macrophage accumulation (61). Thus, considering the function of PTX3 in control pro-inflammatory molecules production by macrophages, half of macrophages positive for PTX3 in coronary atherosclerosis presented M2-like phenotype (62). Therefore, all these findings suggest a role for PTX3 in resolving inflammation by suppressing the activity of macrophages at inflamed sites and inducing healing process.

Recent studies using genetic-modified mice demonstrated that PTX3 has an important action in regulating vascular sterile inflammation. Norata and coworkers have shown increased expression of PTX3 in the vasculature during atherogenesis. Mice deficient for PTX3 fed with an atherogenic diet showed larger atherosclerotic lesions compared with WT mice. These mice also showed increased expression of adhesion molecules, cytokines, and chemokines in the vascular wall, associated with intense accumulation of macrophages within atherosclerotic plaque (63). On the other hand, although these results suggest atheroprotective and cardiovascular protective effects of PTX3 by modulating the vascular-associated inflammatory response, this molecule induces tissue factor in endothelial cells, presenting potential proinflammatory and prothrombotic properties (64, 65). Thus, PTX3 may orchestrate different roles depending on the scenario of vascular pathology. In this regard, increased levels of PTX3 is observed in vascular disorders, such as myocardial infarction and small vessel vasculitis that correlate with worsen outcome or disease activity. In fact, during inflammation, blood vessels produce large amounts of PTX3 (66). PTX3 has been linked to vascular endothelial dysfunction in several diseases, including chronic kidney disease and preeclampsia, a condition associated with hypertension (67, 68). Carrizzo and coworkers have shown that PTX3 promotes endothelial dysfunction and morphological changes by a mechanism dependent on P-selectin and matrix metalloproteinase-1 (MMP1) pathway. *In vivo* administration of PTX3 induced endothelial dysfunction and increased blood pressure. Moreover, inhibition of MMP1 protected mesenteric arteries against the endothelial dysfunction promoted by PTX3, an effect absent in P-selectin-deficient mice (69). In addition, overexpression of PTX3 attenuates the production of nitric oxide by a mechanism dependent

on the upregulation of MMP1 and P-selectin (69). Therefore, these studies suggest that a high plasma concentration of PTX3 could be a biomarker of altered endothelial function in different diseases.

## ROLE OF PTX3 IN ORGAN SPECIFIC IRI

In the last decades, the mechanisms associated to IRI pathogenesis has been extensively investigated, although they have not yet been completely elucidated. As discussed above, IRI is characterized by intense tissue inflammation due to high production of local pro-inflammatory cytokines and with massive accumulation of neutrophils. As described below, the role of PTX3 during IRI seems to be organ specific, depends on the amount and source of this protein, and the related disease (70–73) (**Figure 2**).

### Renal IRI

Renal IRI syndrome develops after a sudden transient decrease in total or regional blood flow to the kidney (74). The sterile inflammatory disease observed in this condition occurs due to endothelial cell activation caused by endothelial cell-leucocyte interaction and by reduced vascular blood flow. In addition to endothelial cell damage, IRI is associated with endothelial-leukocyte interactions through the up-regulation of adhesion molecules.

A previous study has shown that injured renal cells released endogenous HMGB1 after IRI. HMGB1 binds to endothelial Toll-like receptor 4 (TLR4), promoting an increase of adhesion molecules expression in vasculature (75). Interestingly, PTX3 is regulated by TLR4 activation, since TLR4-deficient mice subject to renal IRI showed reduced PTX3 production and lower renal damage when compared with WT animals. This modification in PTX3 expression occurred together with other markers of endothelial activation and was associated to reduced kidney injury and lower expression of endothelial adhesion molecules and chemokines when compared to wild type mice (76).

Although the study above suggested a deleterious role of PTX3 in the context of IRI, others provide more direct evidence for a beneficial role of this protein. Renal injury was aggravated in PTX3-deficient mice subject to IRI by a mechanism dependent on the control of neutrophil and macrophage recruitment into the postischemic kidney (77). Mechanistically, absence of PTX3 could affect PTX3-P-selectin interaction (58). The neutralization of P-selectin by specific antibody completely abrogated IRI-induced tissue damage. Interestingly, administration of recombinant PTX3 injection in the reperfusion phase effectively prevented renal inflammation, as observed by reduction of leukocyte accumulation by suppressing leukocyte-selectin interaction and consequent leukocyte rolling on endothelial cells (77). In this regard, lack of PTX3 increase the expression of P-selectin, favoring the interaction of circulating leukocytes with activated endothelial cells (78). Thus, in the kidney undergoing IRI injury, local PTX3 production tends to avoid excessive organ inflammation and dysfunction. In addition, PTX3 injection recovered kidney function as observed by the reduction of IRI-induced interstitial fibrosis by a mechanism associated with the reduction of IL-6 and p-STAT3 (79).

### Cardiac IRI

Cardiovascular diseases (CVD) are responsible for high number of deaths in the developed world and numerous studies have indicated that PTX3 has a potential contribution to prevent the progression of CVD (80, 81). The reperfusion of affected

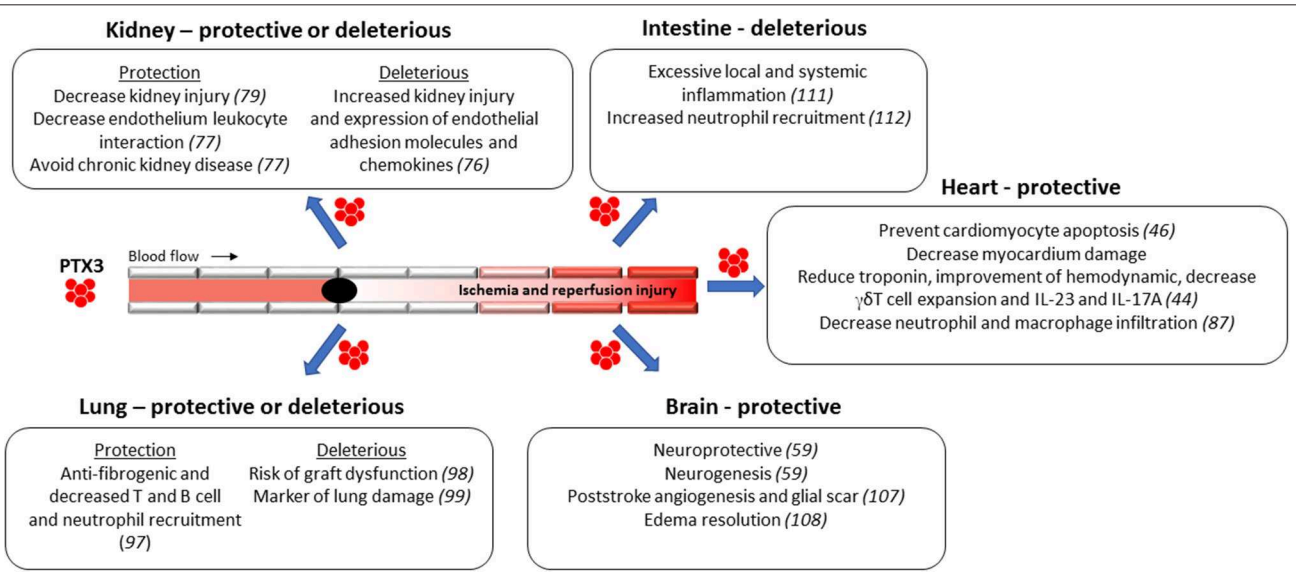


**FIGURE 2 |** Protective and deleterious functions of PTX3 during Ischemia and Reperfusion Injury (IRI) in different organs. The role of PTX3 during IRI seems to be organ specific, depends on the amount and source of this protein, and the related disease.

coronary arteries is a crucial step for an effective therapy after a myocardial infarction. However, as it occurs in other organs, the restoration of blood flow is associated to myocardium damage that limits the benefit of blood flow restoration, known as myocardial IRI. A similar phenomenon is also seen during cardiac transplantation, which is associated with organ dysfunction that impairs cardiac recovery (82). Local inflammation is the major problem that contributes to cardiac IRI, associated with intense and rapid production of cytokines and accumulation of leukocytes in the affected area. Neutrophils migrate rapidly to the infarct zone guided by chemoattractants during the first 24 h of myocardial IR and release degradative enzymes that contribute to irreversible myocardial damage (83). PTX3 can be released by neutrophils early and by macrophages and endothelial cells in the late phase of myocardial infarcted patients (84) and there is evidence to suggest that the heart is a major site for PTX3 expression (85), which could contribute to its involvement in multiple cardiovascular disorders.

A transcriptomic analysis of the whole blood obtained after cardiac surgery identified PTX3 as a potential indicator for infarction and irreversible injury of the myocyte in ischemic cardiomyopathy (86). Using an experimental model of myocardial infarction and samples of myocardial infarction of patients, Maugeri and coworkers demonstrated that neutrophils were the main source of increased PTX3 in blood of patients with AMI in the early phase of the symptoms (within 6 h). Moreover, activated platelets were responsible to trigger neutrophil PTX3 release. Indeed, a substantial fraction of PTX3 was observed on cell membranes of circulating platelets in patients with AMI. In the presence of PTX3, the formation of platelet-neutrophil aggregation was inhibited, which was associated to less effectiveness of platelets at upregulating CD11b/CD18 integrin expression, a critical step for leukocytes to adhere to and transmigrate within inflamed tissues (87). These results suggested that PTX3 decreases the inflammatory response triggered by activated platelets, limiting noxious effects of neutrophils in the heart.

Other studies have also suggested that PTX3 has important functions for the protection of AMI. For instance, exogenous PTX3 played a protective role in myocardial IRI by preventing cardiomyocyte apoptosis and reducing troponin production in mice, which was associated to an improvement of hemodynamic performance (46). That study also demonstrated an important effect of PTX3 on cell function, restricting γδT cell expansion and activation, decreasing local expression of the proinflammatory cytokines IL-23 and IL-17A and neutrophil and macrophage infiltration in the tissue. Furthermore, using an experimental model of acute cardiac ischemia and reperfusion in mice, researchers identified a kinetics of PTX3 mRNA in the circulation which peaked after 24 h and returned to basal levels after 3 days (44). In the same study, mice deficient for PTX3 presented increased myocardial damage after cardiac IRI, with extended area without reflow, intense accumulation of leukocytes into affected area, and elevated number of apoptotic cardiomyocytes. Interestingly, the infusion of exogenous PTX3 in these mice reversed that phenotype (44). Thus, PTX3 seems to have a protective role to reduce myocardium damage by reducing heart inflammation. In addition to its potential therapeutic role, it is suggested that PTX3 could be used as an early indicator of CVD and an important inflammatory component of ischemic heart disease in humans. Peri and coworkers demonstrated that plasma levels of PTX3 were elevated after myocardial infarction faster than C-reactive protein, suggesting that PTX3 could be used as an earlier indication of cardiac IRI (88). PTX3 is present in normal cardiomyocytes (88). The increased PTX3 in blood may be a consequence of its release from dying or necrotic cells due to increased permeability of necrotic cardiomyocyte (89).

## Pulmonary IRI

Pulmonary IRI frequently occurs during lung transplantation, especially in the earlier stages of transplantation, as a form of acute lung injury (ALI) (90). Importantly, the development of ALI in the first 3 days after lung transplantation is associated to the development of chronic lung allograft dysfunction (CLAD), a condition that reduces up to 50% survival in the first 5 years after surgery (91–93). In ALI and acute respiratory distress syndrome patients, plasma PTX3 is elevated and is positively correlated with lung injury parameters (94). In transplantation models, IRI has been directly related to the activation of the innate immune system, which involves recognition TLR signaling pathways, complement activation and natural killer cell migration in transplantation models, and leads to decreased allograft tolerance in many organs (95, 96). In the lung, IRI leads to five main processes that result in regional injury, including sterile immunity, activation of coagulation, activation of cell death pathways and endothelial dysfunction (97).

A few studies indicate that PTX3 has protective effects in lung IRI. For instance, PTX3-deficient mice subjected to orthotopic lung transplantation showed increased lung parenchymal fibrosis 28 days after lung transplantation. These mice had significantly larger numbers of T cells and B cells, which is associated with CLAD (98). This is in line with others models of ALI, where PTX3 dampened neutrophil extravasation to lung parenchyma, while PTX3-deficient mice had worsen lung injury (58). Thus, these results indicate acute beneficial effects of PTX3 in lung transplant recipients and protection against the development of chronic rejection. On the other hand, a study by Diamond and colleagues reported that patients with idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease showed higher levels of PTX3 6 h and 24 h after reperfusion when compared with controls. Moreover, there was a positive correlation between elevated PTX3 levels and the elevated risk of graft dysfunction in lung transplant recipients with idiopathic pulmonary fibrosis (99). In this sense, PTX3 could be used as a marker of lung damage and severity of disease since is quickly detected in ALI patients (94). It is important to mention the differences among those studies. The protective role of PTX3 was performed mice. In humans, there was only a positive correlation between PTX3 levels and lung injury. Indeed, there are no data to explain whether increased levels of PTX3 are protective or harmful, as demonstrated above in cardiac IRI, where elevated PTX3 levels promote negative feedback on the inflammatory response to the heart (46). Thus, it is not possible to define a causal relationship between PTX3 release leading to lung injury in humans.

## Brain IRI

Different organs exhibit different levels of susceptibility to IRI with the brain being perhaps the most IRI sensitive organ, as irreversible brain damage can become evident within 20 min of ischemia (100). Cerebral ischemia is associated with high mortality and disability rates worldwide, as evidenced in stroke, intracerebral or subarachnoid hemorrhage, traumatic brain injury or perinatal hypoxia, and the intense production of pro-inflammatory mediators in acute cerebral ischemia is directly associated with brain damage (101). Increased number of circulating leukocytes and intense recruitment of neutrophils to the brain can be observed up to 24 h after the first symptoms of stroke (102). In addition, the inflammatory state at the affected site is associated to high levels of cytokines, including IL-1β, IL-6, TNFα, IL-10, TGF-β, and chemokines, such as CCL2, CCL3, CXCL1, and CX3CL1 (101, 103). Among them, IL-1β has been considered critical for the brain inflammation after stroke. Its expression is rapidly produced and contributes to brain neurotoxicity. In addition, the blockade of IL-1 receptor prevents ischemic and excitotoxic neuronal damage in rat (104).

As observed in other tissues, PTX3 has been considered a new mediator of inflammation in cerebrovascular disorders and also be considered a potential prognostic marker in ischemic stroke (105). Early after ischemic stroke, peri-infarct astrocytes are important source of PTX3 (106, 107). The production of PTX3 in brain is dependent on IL-1β release after cerebral ischemia and it mediates the formation of the glial scar and resolution of brain edema. Interestingly, mice deficient for PTX3 had marked increase in tissue damage and unresolved cerebral edema after 6 days of cerebral ischemia (108). In accordance, PTX3 deletion impaired blood brain barrier integrity, increased brain inflammation and decreased the resolution of tissue damage (108). Another report showed that PTX3-deficient mice subjected to experimental cerebral ischemia showed reduced neurogenesis in the dentate gyrus of the hippocampus. Furthermore, absence of PTX3 was associated to marked reduction in poststroke angiogenesis when compared to wild type mice 2 weeks after cerebral ischemia. In addition, recombinant PTX3 demonstrated important neurogenic role *in vitro* (59). These data indicate that PTX3 contributes to recovery after stroke through regulation of neurogenesis and angiogenesis and glial scar formation.

## Intestinal IRI

Intestinal ischemia occurs following mesenteric artery blockade with consequent reduction of blood flow to the area. Gust ischemia is very lethal and reperfusion is the only therapy of choice in these cases and may culminate in intense intestinal tissue inflammation and damage. Different conditions and procedures may cause intestinal ischemia, including necrotizing enterocolitis, allograft rejection in small bowel transplantation, complications of abdominal aortic aneurysm surgery, cardiopulmonary bypass, and inflammatory bowel disease (3, 109). Another critical point during intestinal IRI is the risk of loss of the intestinal barrier, facilitating bacterial translocation into the circulation, that could be associated with the development of sepsis (110).

To date, two studies have addressed the role of PTX3 in the context of intestinal IRI. The first one showed that transgenic mice overexpressing up to 4 extra copies of PTX3 had reduced survival rate after intestinal IRI when compared to wild type mice. This phenotype was associated with increased production of proinflammatory cytokines locally, systemically, and in the lungs (remote organ). This was accompanied by intense tissue damage and hemorrhage in both intestine and remote tissue, as observed in lungs (111). In addition, PTX3-deficient mice were protected from intestinal IRI. In PTX3-deficient mice, there was decreased NF-kB translocation and TNF and CXCL1 production when compared to wild type mice. The reduced inflammation was associated with decreased neutrophil influx, preservation of intestinal architecture and significant prevention of lethality. To assert the deleterious effect of PTX3 during intestinal IRI, intravenously infusion of PTX3 reversed the protected phenotype in PTX3-deficient mice (112). Thus, those results show that endogenous PTX3 is essential for the cascade of events leading to tissue inflammation and injury after IR. Moreover, they suggest that PTX3 blockade may be useful as therapy for intestinal IRI.

# CONCLUDING REMARKS

PTX3 has clear role in the induction of sterile inflammation, as observed during IRI (**Figure 2**). In humans, levels of PTX3 increase in blood and elevated levels associate with extent of IRI. In general, absence of PTX3, as seen in PTX3-deficient mice, results in worse outcome after IRI. On the contrary, increased expression of PTX3, as seen in PTX3 transgenic mice and after PTX3 administration, is associated with better outcome after IRI. The overall protective effects of PTX3 are associated with decreased local edema formation and decreased neutrophil-endothelial cell interactions. As neutrophils contribute significantly to IRI, these effects of PTX3 may underlie its beneficial effects in these models. In this regard, it the administration of PTX3 may be beneficial in patients undergoing IRI.

The situation is dramatically different in a model of intestinal IR injury. In the latter model, systemic levels and local expression of PTX3 also increases after reperfusion. However, and in contrast to findings in other systems, decreased PTX3 expression is associated with decreased damage and enhanced expression is associated with more significant and lethal damage in a model of intestinal IR injury (111, 112). It is difficult to reconcile these findings with the overall contrasting effects of PTX3 in models of IR injury in other sites. Intestinal IRI is in general much more severe than IRI to other organs and accompanied by very significant lethality rates within the first few hours after reperfusion. In addition, there is significantly more systemic inflammation and remote damage than in the other models of IRI. There are no studies directly comparing whether local and systemic severity accounts for the differences observed. Regardless of the explanation, it is

clear that one should take great caution when considering the administration of PTX3 in instances of severe IRI, as seen in the gut.

## AUTHOR CONTRIBUTIONS

TdO, DS, MT, and FA designed the article and wrote the manuscript.

## FUNDING

This work was supported by the National Institute of Science and Technology (INCT) in dengue and host-parasite interactions (465425/2014-3), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo á Pesquisa de Minas Gerais (FAPEMIG) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

## REFERENCES

1. Bellanti F. Ischemia-reperfusion injury: evidences for translational research. *Ann Transl Med*. (2016) 4(Suppl 1):S55. doi: 10.21037/atm.2016.10.52
2. Bonventre JV, Zuk A. Ischemic acute renal failure: an inflammatory disease? *Kidney Int*. (2004) 66:480–5. doi: 10.1111/j.1523-1755.2004.761_2.x
3. Eltzschig HK, Eckle T. Ischemia and reperfusion–from mechanism to translation. *Nat Med*. (2011) 17:1391–401. doi: 10.1038/nm.2507
4. Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado JA, Pina E, Geller DA. Factors in the pathophysiology of the liver ischemia-reperfusion injury. *J Surg Res*. (2008) 147:153–9. doi: 10.1016/j.jss.2007.06.015
5. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. *Am J Surg*. (2001) 181:160–6. doi: 10.1016/S0002-9610(00)00573-0
6. Romanque UP, Uribe MM, Videla LA. Molecular mechanisms in liver ischemic-reperfusion injury and ischemic preconditioning. *Rev Med Chil*. (2005) 133:469–76. doi: 10.1016/S0034-98872005000400012
7. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. *Liver Transpl*. (2003) 9:651–63. doi: 10.1053/jlts.2003.50105
8. Mendes-Braz M, Elias-Miro M, Jimenez-Castro MB, Casillas-Ramirez A, Ramalho FS, Peralta C. The current state of knowledge of hepatic ischemia-reperfusion injury based on its study in experimental models. *J Biomed Biotechnol*. (2012) 2012:298657. doi: 10.1155/2012/298657
9. Papadopoulos D, Siempis T, Theodorakou E, Tsoulfas G. Hepatic ischemia and reperfusion injury and trauma: current concepts. *Arch Trauma Res*. (2013) 2:63–70. doi: 10.5812/atr.12501
10. Selzner M, Selzner N, Jochum W, Graf R, Clavien PA. Increased ischemic injury in old mouse liver: an ATP-dependent mechanism. *Liver Transpl*. (2007) 13:382–90. doi: 10.1002/lt.21100
11. Guan LY, Fu PY, Li PD, Li ZN, Liu HY, Xin MG, et al. Mechanisms of hepatic ischemia-reperfusion injury and protective effects of nitric oxide. *World J Gastrointest Surg*. (2014) 6:122–8. doi: 10.4240/wjgs.v6.i7.122
12. Lu J, Marnell LL, Marjon KD, Mold C, Du Clos TW, Sun PD. Structural recognition and functional activation of FcgammaR by innate pentraxins. *Nature*. (2008) 456:989–92. doi: 10.1038/nature07468
13. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol*. (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756
14. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Investig*. (2003) 111:1805–12. doi: 10.1172/JCI18921
15. Mantovani A, Garlanda C, Bottazzi B. Pentraxin 3, a non-redundant soluble pattern recognition receptor involved in innate immunity. *Vaccine*. (2003) 21 (Suppl 2):S43–7. doi: 10.1016/S0264-410X(03)00199-3
16. Breviario F, d'Aniello EM, Golay J, Peri G, Bottazzi B, Bairoch A, et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem*. (1992) 267:22190–7.
17. Lee GW, Lee TH, Vilcek J. TSG-14, a tumor necrosis factor- and IL-1-inducible protein, is a novel member of the pentaxin family of acute phase proteins. *J Immunol*. (1993) 150:1804–12.
18. Ogawa S, Gerlach H, Esposito C, Pasagian-Macaulay A, Brett J, Stern D. Hypoxia modulates the barrier and coagulant function of cultured bovine endothelium. Increased monolayer permeability and induction of procoagulant properties. *J Clin Investig*. (1990) 85:1090–8. doi: 10.1172/JCI114540
19. Ogawa S, Koga S, Kuwabara K, Brett J, Morrow B, Morris SA, et al. Hypoxia-induced increased permeability of endothelial monolayers occurs through lowering of cellular cAMP levels. *Am J Physiol*. (1992) 262(3 Pt 1):C546–54. doi: 10.1152/ajpcell.1992.262.3.C546
20. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE. Cell death. *N Engl J Med*. (2009) 361:1570–83. doi: 10.1056/NEJMra0901217
21. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol*. (2010) 10:826–37. doi: 10.1038/nri2873
22. Akhtar MZ, Henderson T, Sutherland A, Vogel T, Friend PJ. Novel approaches to preventing ischemia-reperfusion injury during liver transplantation. *Transpl Proc*. (2013) 45:2083–92. doi: 10.1016/j.transproceed.2013.04.004
23. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, et al. Current mechanistic concepts in ischemia and reperfusion injury. *Cell Phys Biochem*. (2018) 46:1650–67. doi: 10.1159/000489241
24. Solaini G, Baracca A, Lenaz G, Sgarbi G. Hypoxia and mitochondrial oxidative metabolism. *Biochim Biophys Acta*. (2010) 1797:1171–7. doi: 10.1016/j.bbabio.2010.02.011
25. Brahimi-Horn MC, Pouyssegur J. Oxygen, a source of life and stress. *FEBS Lett*. (2007) 581:3582–91. doi: 10.1016/j.febslet.2007.06.018
26. Wheaton WW, Chandel NS. Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am J Physiol Cell Physiol*. (2011) 300:C385–93. doi: 10.1152/ajpcell.00485.2010
27. Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL. Mitochondrial dysfunction in cardiac disease: ischemia–reperfusion, aging, and heart failure. *J Mol Cell Cardiol*. (2001) 33:1065–89. doi: 10.1006/jmcc.2001.1378
28. Pompilio G, Polvani GL, Rossoni G, Porqueddu M, Berti F, Barajon I, et al. Effects of warm ischemia on valve endothelium. *Ann Thorac Surg*. (1997) 63:656–62. doi: 10.1016/S0003-4975(96)01035-1
29. Kunes P, Holubcova Z, Kolackova M, Krejsek J. Pentraxin 3(PTX 3): an endogenous modulator of the inflammatory response. *Mediators Inflamm*. (2012) 2012:920517. doi: 10.1155/2012/920517
30. Land WG. The role of damage-associated molecular patterns in human diseases: part I - promoting inflammation and immunity. *Sultan Qaboos Univ Med J*. (2015) 15:e9-21.
31. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. (2010) 140:805–20. doi: 10.1016/j.cell.2010.01.022
32. Rezkalla SH, Kloner RA. No-reflow phenomenon. *Circulation*. (2002) 105:656–62. doi: 10.1161/hc0502.102867
33. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. (2010) 11:785–97. doi: 10.1038/ni.1923
34. Slegtenhorst BR, Dor FJ, Rodriguez H, Voskuil FJ, Tullius SG. Ischemia/reperfusion Injury and its consequences on Immunity and Inflammation. *Curr Transpl Rep*. (2014) 1:147–54. doi: 10.1007/s40472-014-0017-6
35. Morote-Garcia JC, Rosenberger P, Kuhlicke J, Eltzschig HK. HIF-1-dependent repression of adenosine kinase attenuates hypoxia-induced vascular leak. *Blood*. (2008) 111:5571–80. doi: 10.1182/blood-2007-11-126763
36. Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK. A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood*. (2008) 111:2024–35. doi: 10.1182/blood-2007-10-117044
37. Takayanagi K, Dawson S, Reynolds SE, Mayer RJ. Specific developmental changes in the regulatory subunits of the 26 S proteasome in intersegmental

muscles preceding eclosion in Manduca sexta. *Biochem Biophys Res Commun*. (1996) 228:517–23. doi: 10.1006/bbrc.1996.1692
38. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annu Rev Immunol*. (2004) 22:891–928. doi: 10.1146/annurev.immunol.22.012703.104543
39. Eltzschig HK, Collard CD. Vascular ischaemia and reperfusion injury. *Br Med Bull*. (2004) 70:71–86. doi: 10.1093/bmb/ldh025
40. de Oliveira THC, Marques PE, Poosti F, Ruytinx P, Amaral FA, Brandolini L, et al. Intravital microscopic evaluation of the effects of a CXCR2 antagonist in a model of liver ischemia reperfusion injury in mice. *Front Immunol*. (2017) 8:1917. doi: 10.3389/fimmu.2017.01917
41. Souza DG, Bertini R, Vieira AT, Cunha FQ, Poole S, Allegretti M, et al. Repertaxin, a novel inhibitor of rat CXCR2 function, inhibits inflammatory responses that follow intestinal ischaemia and reperfusion injury. *Br J Pharmacol*. (2004) 143:132–42. doi: 10.1038/sj.bjp.0705862
42. Bottazzi B, Garlanda C, Cotena A, Moalli F, Jaillon S, Deban L, et al. The long pentraxin PTX3 as a prototypic humoral pattern recognition receptor: interplay with cellular innate immunity. *Immunol Rev*. (2009) 227:9–18. doi: 10.1111/j.1600-065X.2008.00719.x
43. Inforzato A, Jaillon S, Moalli F, Barbati E, Bonavita E, Bottazzi B, et al. The long pentraxin PTX3 at the crossroads between innate immunity and tissue remodelling. *Tissue Antigens*. (2011) 77:271–82. doi: 10.1111/j.1399-0039.2011.01645.x
44. Salio M, Chimenti S, De Angelis N, Molla F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. *Circulation*. (2008) 117:1055–64. doi: 10.1161/CIRCULATIONAHA.107.749234
45. Kunes P. The role of pentraxin 3 in the inflammatory and immune response. *Cas Lek Cesk*. (2005) 144:377–82; discussion 82–4.
46. Zhu H, Cui D, Liu K, Wang L, Huang L, Li J. Long pentraxin PTX3 attenuates ischemia reperfusion injury in a cardiac transplantation model. *Transpl Int*. (2014) 27:87–95. doi: 10.1111/tri.12197
47. Doni A, Peri G, Chieppa M, Allavena P, Pasqualini F, Vago L, et al. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. *Eur J Immunol*. (2003) 33:2886–93. doi: 10.1002/eji.200324390
48. Daigo K, Yamaguchi N, Kawamura T, Matsubara K, Jiang S, Ohashi R, et al. The proteomic profile of circulating pentraxin 3 (PTX3) complex in sepsis demonstrates the interaction with azurocidin 1 and other components of neutrophil extracellular traps. *Mol Cell Proteomics*. (2012) 11:M111.015073. doi: 10.1074/mcp.M111.015073
49. Polentarutti N, Bottazzi B, Di Santo E, Blasi E, Agnello D, Ghezzi P, et al. Inducible expression of the long pentraxin PTX3 in the central nervous system. *J Neuroimmunol*. (2000) 106:87–94. doi: 10.1016/S0165-5728(00)00214-9
50. Agrawal A, Singh PP, Bottazzi B, Garlanda C, Mantovani A. Pattern recognition by pentraxins. *Adv Exp Med Biol*. (2009) 653:98–116. doi: 10.1007/978-1-4419-0901-5_7
51. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem*. (1997) 272:32817–23. doi: 10.1074/jbc.272.52.32817
52. Castellano G, Woltman AM, Nauta AJ, Roos A, Trouw LA, Seelen MA, et al. Maturation of dendritic cells abrogates C1q production *in vivo* and *in vitro*. *Blood*. (2004) 103:3813–20. doi: 10.1182/blood-2003-09-3046
53. van Rossum AP, Fazzini F, Limburg PC, Manfredi AA, Rovere-Querini P, Mantovani A, et al. The prototypic tissue pentraxin PTX3, in contrast to the short pentraxin serum amyloid P, inhibits phagocytosis of late apoptotic neutrophils by macrophages. *Arthritis Rheum*. (2004) 50:2667–74. doi: 10.1002/art.20370
54. Baruah P, Dumitriu IE, Peri G, Russo V, Mantovani A, Manfredi AA, et al. The tissue pentraxin PTX3 limits C1q-mediated complement activation and phagocytosis of apoptotic cells by dendritic cells. *J Leukoc Biol*. (2006) 80:87–95. doi: 10.1189/jlb.0805445
55. Rovere P, Peri G, Fazzini F, Bottazzi B, Doni A, Bondanza A, et al. The long pentraxin PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells. *Blood*. (2000) 96:4300–6.
56. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, et al. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet*. (1998) 19:56–9. doi: 10.1038/ng0598-56
57. Hind CR, Ng SC, Feng PH, Pepys MB. Serum C-reactive protein measurement in the detection of intercurrent infection in Oriental patients with systemic lupus erythematosus. *Ann Rheum Dis*. (1985) 44:260–1. doi: 10.1136/ard.44.4.260
58. Deban L, Russo RC, Sironi M, Moalli F, Scanziani M, Zambelli V, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nat Immunol*. (2010) 11:328–34. doi: 10.1038/ni.1854
59. Rodriguez-Grande B, Varghese L, Molina-Holgado F, Rajkovic O, Garlanda C, Denes A, et al. Pentraxin 3 mediates neurogenesis and angiogenesis after cerebral ischaemia. *J Neuroinflamm*. (2015) 12:15. doi: 10.1186/s12974-014-0227-y
60. Shiraki A, Kotooka N, Komoda H, Hirase T, Oyama JI, Node K. Pentraxin-3 regulates the inflammatory activity of macrophages. *Biochem Biophys Rep*. (2016) 5:290–5. doi: 10.1016/j.bbrep.2016.01.009
61. Ishino M, Shishido T, Suzuki S, Katoh S, Sasaki T, Funayama A, et al. Deficiency of long pentraxin PTX3 promoted neointimal hyperplasia after vascular injury. *J Atheroscler Thromb*. (2015) 22:372–8. doi: 10.5551/jat.26740
62. Pucci S, Fisco T, Zonetti MJ, Bonanno E, Mazzarelli P, Mauriello A. PTX3: a modulator of human coronary plaque vulnerability acting by macrophages type 2. *Int J Cardiol*. (2014) 176:710–7. doi: 10.1016/j.ijcard.2014.07.109
63. Norata GD, Marchesi P, Pulakazhi Venu VK, Pasqualini F, Anselmo A, Moalli F, et al. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. *Circulation*. (2009) 120:699–708. doi: 10.1161/CIRCULATIONAHA.108.806547
64. Napoleone E, Di Santo A, Bastone A, Peri G, Mantovani A, de Gaetano G, et al. Long pentraxin PTX3 upregulates tissue factor expression in human endothelial cells: a novel link between vascular inflammation and clotting activation. *Arterioscler Thromb Vasc Biol*. (2002) 22:782–7. doi: 10.1161/01.ATV.0000012282.39306.64
65. Napoleone E, di Santo A, Peri G, Mantovani A, de Gaetano G, Donati MB, et al. The long pentraxin PTX3 up-regulates tissue factor in activated monocytes: another link between inflammation and clotting activation. *J Leukoc Biol*. (2004) 76:203–9. doi: 10.1189/jlb.1003528
66. Parlak A, Aydogan U, Iyisoy A, Dikililer MA, Kut A, Cakir E, et al. Elevated pentraxin-3 levels are related to blood pressure levels in hypertensive patients: an observational study. *Anadolu Kardiyol Derg*. (2012) 12:298–304. doi: 10.5152/akd.2012.092
67. Witasp A, Ryden M, Carrero JJ, Qureshi AR, Nordfors L, Naslund E, et al. Elevated circulating levels and tissue expression of pentraxin 3 in uremia: a reflection of endothelial dysfunction. *PLoS ONE*. (2013) 8:e63493. doi: 10.1371/journal.pone.0063493
68. Cozzi V, Garlanda C, Nebuloni M, Maina V, Martinelli A, Calabrese S, et al. PTX3 as a potential endothelial dysfunction biomarker for severity of preeclampsia and IUGR. *Placenta*. (2012) 33:1039–44. doi: 10.1016/j.placenta.2012.09.009
69. Carrizzo A, Lenzi P, Procaccini C, Damato A, Biagioni F, Ambrosio M, et al. Pentraxin 3 induces vascular endothelial dysfunction through a P-selectin/Matrix metalloproteinase-1 pathway. *Circulation*. (2015) 131:1495–505; discussion 505. doi: 10.1161/CIRCULATIONAHA.114.014822
70. Garlanda C, Jaillon S, Doni A, Bottazzi B, Mantovani A. PTX3, a humoral pattern recognition molecule at the interface between microbe and matrix recognition. *Curr Opin Immunol*. (2016) 38:39–44. doi: 10.1016/j.coi.2015.11.002
71. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell*. (2015) 160:700–14. doi: 10.1016/j.cell.2015.01.004
72. Soares AC, Souza DG, Pinho V, Vieira AT, Nicoli JR, Cunha FQ, et al. Dual function of the long pentraxin PTX3 in resistance against pulmonary infection with Klebsiella pneumoniae in transgenic mice. *Microb Infect*. (2006) 8:1321–9. doi: 10.1016/j.micinf.2005.12.017

73. Chi JY, Hsiao YW, Li CF, Lo YC, Lin ZY, Hong JY, et al. Targeting chemotherapy-induced PTX3 in tumor stroma to prevent the progression of drug-resistant cancers. *Oncotarget*. (2015) 6:23987–4001. doi: 10.18632/oncotarget.4364
74. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol*. (2003) 14:2199–210. doi: 10.1097/01.ASN.0000079785.13922.F6
75. Chen J, John R, Richardson JA, Shelton JM, Zhou XJ, Wang Y, et al. Toll-like receptor 4 regulates early endothelial activation during ischemic acute kidney injury. *Kidney Int*. (2011) 79:288–99. doi: 10.1038/ki.2010.381
76. Chen J, Matzuk MM, Zhou XJ, Lu CY. Endothelial pentraxin 3 contributes to murine ischemic acute kidney injury. *Kidney Int*. (2012) 82:1195–207. doi: 10.1038/ki.2012.268
77. Lech M, Rommele C, Grobmayr R, Eka Susanti H, Kulkarni OP, Wang S, et al. Endogenous and exogenous pentraxin-3 limits postischemic acute and chronic kidney injury. *Kidney Int*. (2013) 83:647–61. doi: 10.1038/ki.2012.463
78. Williams MR, Azcutia V, Newton G, Alcaide P, Luscinskas FW. Emerging mechanisms of neutrophil recruitment across endothelium. *Trends Immunol*. (2011) 32:461–9. doi: 10.1016/j.it.2011.06.009
79. Xiao Y, Yang N, Zhang Q, Wang Y, Yang S, Liu Z. Pentraxin 3 inhibits acute renal injury-induced interstitial fibrosis through suppression of IL-6/Stat3 pathway. *Inflammation*. (2014) 37:1895–901. doi: 10.1007/s10753-014-9921-2
80. Fuster V, Mearns BM. The CVD paradox: mortality vs prevalence. *Nat Rev Cardiol*. (2009) 6:669. doi: 10.1038/nrcardio.2009.187
81. Latini R, Maggioni AP, Peri G, Gonzini L, Lucci D, Mocarelli P, et al. Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. *Circulation*. (2004) 110:2349–54. doi: 10.1161/01.CIR.0000145167.30987.2E
82. Wiedemann D, Schneeberger S, Friedl P, Zacharowski K, Wick N, Boesch F, et al. The fibrin-derived peptide Bbeta(15-42) significantly attenuates ischemia-reperfusion injury in a cardiac transplant model. *Transplantation*. (2010) 89:824–9. doi: 10.1097/TP.0b013e3181ccd822
83. Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. *Cardiovasc Res*. (2004) 61:481–97. doi: 10.1016/j.cardiores.2003.10.011
84. Nebuloni M, Pasqualini F, Zerbi P, Lauri E, Mantovani A, Vago L, et al. PTX3 expression in the heart tissues of patients with myocardial infarction and infectious myocarditis. *Cardiovasc Pathol*. (2011) 20:e27–35. doi: 10.1016/j.carpath.2010.02.005
85. Introna M, Alles VV, Castellano M, Picardi G, De Gioia L, Bottazzai B, et al. Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites. *Blood*. (1996) 87:1862–72.
86. Liangos O, Domhan S, Schwager C, Zeier M, Huber PE, Addabbo F, et al. Whole blood transcriptomics in cardiac surgery identifies a gene regulatory network connecting ischemia reperfusion with systemic inflammation. *PLoS ONE*. (2010) 5:e13658. doi: 10.1371/journal.pone.0013658
87. Maugeri N, Rovere-Querini P, Slavich M, Coppi G, Doni A, Bottazzi B, et al. Early and transient release of leukocyte pentraxin 3 during acute myocardial infarction. *J Immunol*. (2011) 187:970–9. doi: 10.4049/jimmunol.1100261
88. Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, et al. PTX3, A prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. *Circulation*. (2000) 102:636–41. doi: 10.1161/01.CIR.102.6.636
89. Lagrand WK, Niessen HW, Wolbink GJ, Jaspars LH, Visser CA, Verheugt FW, et al. C-reactive protein colocalizes with complement in human hearts during acute myocardial infarction. *Circulation*. (1997) 95:97–103. doi: 10.1161/01.CIR.95.1.97
90. Zhang Y, McDyer JF. Pentraxin 3 in primary graft dysfunction: the long and short of it. *Am J Resp Crit Care Med*. (2012) 186:475–7. doi: 10.1164/rccm.201207-1158ED
91. den Hengst WA, Gielis JF, Lin JY, Van Schil PE, De Windt LJ, Moens AL. Lung ischemia-reperfusion injury: a molecular and clinical view on a complex pathophysiological process. *Am J Physiol Heart Circul Physiol*. (2010) 299:H1283–99. doi: 10.1152/ajpheart.00251.2010
92. Lund LH, Edwards LB, Kucheryavaya AY, Dipchand AI, Benden C, Christie JD, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report—2013; focus theme: age. *J Heart Lung Transpl*. (2013) 32:951–64. doi: 10.1016/j.healun.2013.08.006
93. Ozegovic L. T. mentagrophytes and T. quinckeanum in relation to the soil. *Med Arh*. (1970) 24:55–61.
94. He X, Han B, Liu M. Long pentraxin 3 in pulmonary infection and acute lung injury. *Am J Physiol Lung Cell Mol Physiol*. (2007) 292:L1039–49. doi: 10.1152/ajplung.00490.2006
95. Millington TM, Madsen JC. Innate immunity and cardiac allograft rejection. *Kidney Int Suppl*. (2010) 119: S18–21. doi: 10.1038/ki.2010.417
96. Kosieradzki M, Rowinski W. Ischemia/reperfusion injury in kidney transplantation: mechanisms and prevention. *Transpl Proc*. (2008) 40:3279–88. doi: 10.1016/j.transproceed.2008.10.004
97. Weyker PD, Webb CA, Kiamanesh D, Flynn BC. Lung ischemia reperfusion injury: a bench-to-bedside review. *Semin Cardiothorac Vasc Anesth*. (2013) 17:28–43. doi: 10.1177/1089253212458329
98. Yoshida M, Oishi H, Martinu T, Hwang DM, Takizawa H, Sugihara J, et al. Pentraxin 3 deficiency enhances features of chronic rejection in a mouse orthotopic lung transplantation model. *Oncotarget*. (2018) 9:8489–501. doi: 10.18632/oncotarget.23902
99. Diamond JM, Lederer DJ, Kawut SM, Lee J, Ahya VN, Bellamy S, et al. Elevated plasma long pentraxin-3 levels and primary graft dysfunction after lung transplantation for idiopathic pulmonary fibrosis. *Am J Transpl*. (2011) 11:2517–22. doi: 10.1111/j.1600-6143.2011.03702.x
100. Ordy JM, Wengenack TM, Bialobok P, Coleman PD, Rodier P, Baggs RB, et al. Selective vulnerability and early progression of hippocampal CA1 pyramidal cell degeneration and GFAP-positive astrocyte reactivity in the rat four-vessel occlusion model of transient global ischemia. *Exp Neurol*. (1993) 119:128–39. doi: 10.1006/exnr.1993.1014
101. Denes A, Thornton P, Rothwell NJ, Allan SM. Inflammation and brain injury: acute cerebral ischaemia, peripheral and central inflammation. *Brain Behav Immun*. (2010) 24:708–23. doi: 10.1016/j.bbi.2009.09.010
102. Emsley HC, Smith CJ, Gavin CM, Georgiou RF, Vail A, Barberan EM, et al. An early and sustained peripheral inflammatory response in acute ischaemic stroke: relationships with infection and atherosclerosis. *J Neuroimmunol*. (2003) 139:93–101. doi: 10.1016/S0165-5728(03)00134-6
103. Di Napoli M, Schwaninger M, Cappelli R, Ceccarelli E, Di Gianfilippo G, Donati C, et al. Evaluation of C-reactive protein measurement for assessing the risk and prognosis in ischemic stroke: a statement for health care professionals from the CRP Pooling Project members. *Stroke*. (2005) 36:1316–29. doi: 10.1161/01.STR.0000165929.78756.ed
104. Relton JK, Rothwell NJ. Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. *Brain Res Bull*. (1992) 29:243–6. doi: 10.1016/0361-9230(92)90033-T
105. Ryu WS, Kim CK, Kim BJ, Kim C, Lee SH, Yoon BW. Pentraxin 3: a novel and independent prognostic marker in ischemic stroke. *Atherosclerosis*. (2012) 220:581–6. doi: 10.1016/j.atherosclerosis.2011.11.036
106. Jeon H, Lee S, Lee WH, Suk K. Analysis of glial secretome: the long pentraxin PTX3 modulates phagocytic activity of microglia. *J Neuroimmunol*. (2010) 229:63–72. doi: 10.1016/j.jneuroim.2010.07.001
107. Shindo A, Maki T, Mandeville ET, Liang AC, Egawa N, Itoh K, et al. Astrocyte-derived pentraxin 3 supports blood-brain barrier integrity under acute phase of stroke. *Stroke*. (2016) 47:1094–100. doi: 10.1161/STROKEAHA.115.012133
108. Rodriguez-Grande B, Swana M, Nguyen L, Englezou P, Maysami S, Allan SM, et al. The acute-phase protein PTX3 is an essential mediator of glial scar formation and resolution of brain edema after ischemic injury. *J Cereb Blood Flow Metab*. (2014) 34:480–8. doi: 10.1038/jcbfm.2013.224
109. Lenaerts K, Ceulemans LJ, Hundscheid IH, Grootjans J, Dejong CH, Olde Damink SW. New insights in intestinal ischemia-reperfusion injury: implications for intestinal transplantation. *Curr Opin Organ Transpl*. (2013) 18:298–303. doi: 10.1097/MOT.0b013e32835ef1eb
110. Fink MP, Delude RL. Epithelial barrier dysfunction: a unifying theme to explain the pathogenesis of multiple organ dysfunction at the

cellular level. *Crit Care Clin*. (2005) 21:177–96. doi: 10.1016/j.ccc.2005.01.005

111. Souza DG, Soares AC, Pinho V, Torloni H, Reis LF, Teixeira MM, et al. Increased mortality and inflammation in tumor necrosis factor-stimulated gene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol*. (2002) 160:1755–65. doi: 10.1016/S0002-9440(10)61122-4
112. Souza DG, Amaral FA, Fagundes CT, Coelho FM, Arantes RM, Sousa LP, et al. The long pentraxin PTX3 is crucial for tissue inflammation after intestinal ischemia and reperfusion in mice. *Am J Pathol*. (2009) 174:1309–18. doi: 10.2353/ajpath.2009.080240

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 16 July 2019
doi: 10.3389/fimmu.2019.01655

# Functionality of C-Reactive Protein for Atheroprotection

*Sanjay K. Singh and Alok Agrawal**

*Department of Biomedical Sciences, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, United States*

C-reactive protein (CRP) is a pentameric molecule made up of identical monomers. CRP can be seen in three different forms: native pentameric CRP (native CRP), non-native pentameric CRP (non-native CRP), and monomeric CRP (mCRP). Both native and non-native CRP execute ligand-recognition functions for host defense. The fate of any pentameric CRP after binding to a ligand is dissociation into ligand-bound mCRP. If ligand-bound mCRP is proinflammatory, like free mCRP has been shown to be *in vitro*, then mCRP along with the bound ligand must be cleared from the site of inflammation. Once pentameric CRP is bound to atherogenic low-density lipoprotein (LDL), it reduces both formation of foam cells and proinflammatory effects of atherogenic LDL. A CRP mutant, that is non-native CRP, which readily binds to atherogenic LDL, has been found to be atheroprotective in a murine model of atherosclerosis. Thus, unlike statins, a drug that can lower only cholesterol levels but not CRP levels should be developed. Since non-native CRP has been shown to bind to all kinds of malformed proteins in general, it is possible that non-native CRP would be protective against all inflammatory states in which host proteins become pathogenic. If it is proven through experimentation employing transgenic mice that non-native CRP is beneficial for the host, then using a small-molecule compound to target CRP with the goal of changing the conformation of endogenous native CRP would be preferred over using recombinant non-native CRP as a biologic to treat diseases caused by pathogenic proteins such as oxidized LDL.

**Keywords: C-reactive protein, low-density lipoprotein, cholesterol, atherosclerosis, amyloid**

OPEN ACCESS

***Edited by:***
*Philippe Saas, INSERM U1098 Interactions Hôte-Greffon-Tumeur & Ingénierie Cellulaire et Génique, France*

***Reviewed by:***
*Shang-Rong Ji, Lanzhou University, China*
*Johannes Zeller, Freiburg University Medical Center, Germany*

***Correspondence:**
*Alok Agrawal*
*Agrawal@etsu.edu*

***Specialty section:***
*This article was submitted to Inflammation, a section of the journal Frontiers in Immunology*

***Received:*** *08 May 2019*
***Accepted:*** *03 July 2019*
***Published:*** *16 July 2019*

***Citation:***
*Singh SK and Agrawal A (2019) Functionality of C-Reactive Protein for Atheroprotection. Front. Immunol. 10:1655. doi: 10.3389/fimmu.2019.01655*

## INTRODUCTION

C-reactive protein (CRP) is a pentamer of identical subunits which functions in two different structural states, as native pentameric CRP (native CRP) in normal physiological environment and as non-native pentameric CRP (non-native CRP) in localized pathological and inflammatory environments (1–7). During making of CRP in the liver, first, the five subunits fold to almost a native core and the single C-terminal helix is correctly positioned. Then, the intrachain disulfide bond between $Cys^{36}$ and $Cys^{97}$ is formed. Further folding of the subunit is driven by the newly formed disulfide bond and $Ca^{2+}$-binding. Finally, CRP is assembled as pentamers and secreted into the circulation (8). It has been shown that recombinant CRP is not assembled and not secreted from the transfected cells if there is a mutation in the region coding for its $Ca^{2+}$-binding site (9). When CRP enters an inflammatory microenvironment and is exposed to pathological conditions, the data obtained from *in vitro* experiments suggest that the pentameric structure of CRP is converted from its native conformation to a non-native conformation (2, 10, 11). Whether it is native CRP or

non-native CRP, binding of CRP to a ligand causes dissociation of pentameric CRP and generation of monomeric CRP (mCRP) on the surface of the ligand (10).

Atherosclerosis is an inflammatory disease caused by the deposition and subsequent modification of low-density lipoprotein (LDL) in artery walls (12–14). Modified LDL is atherogenic: it is recognized and engulfed by macrophages to form LDL-loaded foam cells that contribute to the development of atherosclerotic lesions (14–16). It has been suggested that in areas in which inflammation takes place, including in atherosclerosis, the pH may be acidic due to hypoxia, lactate generation, activated macrophages and proton generation (17–21). Since CRP has been found to localize with LDL and macrophages in atherosclerotic lesions in both humans and experimental animals, CRP has been implicated in modulating the pathogenesis of atherosclerosis (22–26). Here, we review the literature on the structure-function relationships of CRP *in vitro* and *in vivo* as applied to atherosclerosis and conclude that CRP plays a defensive role in the pathogenesis of atherosclerosis (27, 28).

## FUNCTIONS OF CRP (NATIVE CRP) IN ATHEROSCLEROSIS

CRP, in its native pentameric conformation and in the presence of $Ca^{2+}$, binds to cells and molecules with uncovered phosphocholine (PCh) groups, such as the membrane of damaged cells and platelet-activating factor (29–31). Each subunit in the pentamer has a PCh-binding site. The three-dimensional structure and mutagenesis of the PCh-binding site revealed that $Glu^{81}$, $Phe^{66}$ and $Thr^{76}$ are critical for creating the pocket on CRP to bind and accommodate PCh (32–35). Once CRP is bound to a PCh-containing ligand, it activates the classical complement pathway (36).

Many kinds of modifications can occur to deposited LDL in arteries; however, two types of modified LDL prepared *in vitro*, oxidized LDL (ox-LDL) and enzymatically-modified LDL (E-LDL), are mostly used in experiments to define the role of CRP in atherosclerosis (37–39). Since the PCh groups present in LDL are exposed in E-LDL, CRP is able to bind to E-LDL in a $Ca^{2+}$-dependent manner (40, 41). CRP does not bind to ox-LDL; however, CRP can bind to ox-LDL if LDL is oxidized enough to expose its PCh moiety (42–45). If CRP binds to ox-LDL independent of the exposure of PCh on ox-LDL, it would be possible only in a pathological milieu that can affect CRP structurally (10, 11). CRP has also been shown to bind to complexes consisting of ox-LDL and β2-glycoprotein I (46, 47). CRP also binds to cholesterol crystals and it has been shown that CRP is located mainly in the cholesterol-rich necrotic core in atherosclerotic lesions (48). It has been shown that CRP also binds to LOX-1 which is a receptor for ox-LDL (49, 50).

CRP, ox-LDL and E-LDL all are known to be involved in interrelated pathophysiological pathways including in the formation of LDL-loaded macrophage foam cells (16, 51). However, the literature on the effects of CRP on the formation of foam cells has been controversial. Since CRP was found to be located intracellularly in foam cells, it was hypothesized that CRP complexes with LDL, enhances the binding of LDL to macrophages, and thus facilitates the cellular uptake of LDL along with CRP (52–57). When pure complexes of CRP and E-LDL were used for treatment of macrophages, it was found that CRP-bound E-LDL was unable to form foam cells, clearly suggesting for the first time that CRP possesses the ability to prevent the formation of foam cells (58). Indeed, in another study, the complexes of CRP and LDL were found to be unable to enter macrophages (59). In addition, when endothelial cells and a third type of modified LDL, acetylated LDL, were used in foam cell experiments, mCRP was found to decrease the uptake of acetylated LDL by endothelial cells (60). In another study employing endothelial cells as a model for foam cell formation, CRP was found to increase LDL transcytosis across endothelial cells (61). mCRP has also been shown to decrease uptake of ox-LDL by macrophages and it has been proposed that the interaction of mCRP with ox-LDL may contribute to retardation of the foam cell formation by reducing the aggressive macrophage response to ox-LDL (43, 62). Additionally, it has been proposed that mCRP may exert a protective role by facilitating the clearance of retained native LDL from extracellular space, and thus lower the risk of LDL modifications (43). But, since foam cell formation is inhibited whenever CRP is complexed with modified LDL such as CRP-E-LDL and mCRP-acetylated LDL, it has been proposed that if each LDL molecule retained in the arterial wall becomes CRP-bound, the development of atherosclerosis should be retarded (58).

Besides the effects of CRP on the formation of foam cells, other consequences of the interactions between CRP and modified LDL have been reported, although it is unclear whether it was ensured that CRP was free of spontaneously generated mCRP. CRP, after binding to LDL, causes charge modification of LDL (59). The production of proinflammatory cytokines by macrophages decreases when the cells are treated with a combination of CRP and ox-LDL (62). CRP inhibits the susceptibility of copper-induced oxidation of LDL, that is, once CRP is bound to ox-LDL, further oxidation is prevented, and CRP does so by prolonging the time it takes for copper ions to oxidize LDL (63, 64). By sequestering minimally modified LDL (mmLDL), CRP can prevent binding of mmLDL to monocytes and attenuate mmLDL-induced monocyte adhesion and activation (65). CRP was also found to suppress the proatherogenic effects of macrophages when bound to lysophosphatidylcholine present in ox-LDL and inhibit the association of ox-LDL to macrophages; this effect may in part retard the progression of atherosclerosis (66). These findings suggest that not only does CRP prevent foam cell formation but also reduce the proinflammatory effects of modified LDL and foam cells.

**Abbreviations:** CRP, C-reactive protein; CRP or native CRP, native pentameric CRP; non-native CRP, non-native pentameric CRP; mCRP, monomeric CRP; LDL, low-density lipoprotein; ox-LDL, oxidized LDL; E-LDL, enzymatically-modified LDL; PCh, phosphocholine.

Human CRP, mouse CRP and rabbit CRP have all been used to determine the effects of CRP on the development of atherosclerosis. For human CRP, three different murine models of atherosclerosis, *ApoE*$^{-/-}$ mice, *LDLr*$^{-/-}$ mice and *ApoB*$^{100/100}$*LDLr*$^{-/-}$ mice, and a rabbit model of atherosclerosis have been employed. CRP was either transgenic or passively administered. In most studies employing *ApoE*$^{-/-}$ mice, CRP was found to be neither proatherogenic nor atheroprotective: both passively administered human CRP and transgenically expressed human CRP had no effect on the development, progression, or severity of atherosclerosis (67–71). In two studies employing *ApoE*$^{-/-}$ mice, CRP slightly worsened the disease (72, 73). In another study employing *ApoE*$^{-/-}$ mice, CRP promoted early changes of atherosclerosis by directly increasing the transcytosis of LDL across endothelial cells and increasing LDL retention in vascular walls (61). In *LDLr*$^{-/-}$ mice also, there was no effect of CRP on the development of atherosclerosis (74). When *ApoB*$^{100/100}$*LDLr*$^{-/-}$ mice were employed, which are rich in LDL and develop human-like hypercholesterolemia, CRP slowed the development of atherosclerosis, suggesting an atheroprotective role of CRP (75). In the rabbit model of atherosclerosis also, there was no effect of transgenic human CRP on either aortic or coronary atherosclerotic lesion formation (76). CRP-deficient mice were employed to observe any possible role of endogenous murine CRP in atherosclerosis (77). In both *ApoE*$^{-/-}$*CRP*$^{-/-}$ and *LDLr*$^{-/-}$*CRP*$^{-/-}$ mice, the size of atherosclerotic lesions was either equivalent or increased when compared to that of *ApoE*$^{-/-}$ and *LDLr*$^{-/-}$ mice, suggesting that murine CRP had the ability to mediate atheroprotective effects (77). Besides human and murine CRP, the effect of rabbit CRP on the development of atherosclerosis in rabbits has also been investigated by using CRP antisense oligonucleotides (78). CRP antisense oligonucleotide treatment led to a significant reduction of CRP levels in rabbits; however, both aortic and coronary atherosclerotic lesions were not significantly changed, suggesting that inhibition of plasma CRP does not affect the development of atherosclerosis in rabbits (78). The combined data suggest that native CRP was either incapable or only partly capable for protecting against atherosclerosis in animal models.

## FUNCTIONS OF NON-NATIVE PENTAMERIC CRP (NON-NATIVE CRP) IN ATHEROSCLEROSIS

In the presence of a biological protein modifier, the structure of CRP is altered leading to the production of non-native CRP which ultimately generates mCRP (1–5, 79). Dissociation of CRP to mCRP thus involves an intermediate stage of non-native CRP, and it has been shown that antibodies specific for mCRP react with non-native CRP also (1). There are several modifiers of CRP structure. CRP is modified in the presence of abundant damaged cell membranes (1). The binding of CRP to activated platelets and apoptotic cells has also been shown to change the structure of CRP to generate mCRP (80, 81). CRP, by binding to cell-derived microvesicles, undergoes a structural change without disrupting the pentameric symmetry and constitutes the major CRP species deposited in inflamed tissue (4). mCRP has also been seen deposited at burn wounds having necrotic and inflamed tissue (82). Acidic pH condition modifies CRP (10, 83). CRP is also modified by hydrogen peroxide and hypochlorous acid (11, 84). Hypochlorous acid modifies CRP by oxidation and chlorination of amino acids, leading to protein unfolding, greater surface hydrophobicity and the formation of aggregates (84). These findings suggest that when CRP enters an inflammatory microenvironment and is exposed to pathological conditions, the structure of CRP is changed first to a non-native pentameric conformation leading to complete dissociation of CRP and generation of mCRP.

Except for binding to PCh, the recognition functions of non-native CRP are different from those of CRP (2, 7). One function of CRP in its non-native pentameric conformation is to bind to modified LDL irrespective of the presence of PCh and $Ca^{2+}$. Unlike CRP, non-native CRP readily binds to ox-LDL regardless of the extent and nature of the oxidation status (10, 11). To E-LDL, non-native CRP binds more avidly than CRP does (83). It has also been shown that, in the absence of $Ca^{2+}$, a new lysophosphatidylcholine-binding site located on the opposite side of the known PCh-binding site becomes functional (85, 86). The binding to and actions of CRP on endothelial cells also requires a conformational rearrangement in CRP (87). Taken together, the deposition of CRP and its co-localization with LDL in atherosclerotic lesions indicate the presence of non-native CRP at the lesions. Besides PCh, the other moieties on LDL molecules that interact with CRP include apolipoprotein B and cholesterol. However, the moiety on modified LDL with which non-native CRP interacts is unknown (88–90). The binding site on non-native CRP for modified LDL has not been elucidated as yet either. It has been proposed though that the binding site may involve amino acid residues participating in the formation of intersubunit contact region since this region is buried in CRP and accessible in non-native CRP (2, 10). In addition, a single sequence motif called the cholesterol binding sequence, from amino acid residue 35 to 47, has been found to be responsible for mediating the interactions of mCRP with diverse ligands. The versatility of the cholesterol binding sequence appears to originate from its intrinsically disordered conformation (91).

Although the investigations to determine the effects of CRP on the development of atherosclerosis in animals provide conflicting results, a study employing mCRP in *ApoE*$^{-/-}$ mice indicated that mCRP was atheroprotective (73). Additionally, the data obtained from *in vitro* experiments raised hopes that non-native CRP may be more atheroprotective than CRP, considering the difference between the LDL-binding recognition functions of CRP and non-native CRP. Employing site-directed mutagenesis, it was possible to create CRP mutants capable of binding to ox-LDL without the requirement of any further structural change, and one such mutant has been reported earlier (92). Recently, the impact of such a CRP mutant on the development of atherosclerosis was evaluated employing the *LDLr*$^{-/-}$ mouse model of atherosclerosis (93). The development

of atherosclerotic lesions in the whole aorta was reduced in mice receiving mutant CRP that had a non-native pentameric structure. Considering the findings made on all forms of CRP structure, it seems clear that CRP is an atheroprotective molecule (93).

## PROINFLAMMATORY FUNCTIONS OF LIGAND-BOUND mCRP

Once CRP, either native or non-native, is bound to certain types of ligands, mCRP is generated on the surface of the ligand, due to complete dissociation of the five subunits of CRP. It has been shown that the binding of non-native CRP to immobilized protein ligands results in expression of mCRP epitopes and that mCRP cannot be detached from the ligand (10). Thus, mCRP is not a free molecule; instead, mCRP is always ligand-bound and found in CRP-derived debris. The presence of mCRP can be detected at the sites where CRP-ligands are present. The detection of autoantibodies against mCRP provided further evidence for the *in vivo* existence of non-native CRP and mCRP, probably ligand-bound (94–96). The mCRP form is the predominant form of CRP existing in atherosclerotic lesions (80, 97–100). It has also been shown that the expression of proinflammatory properties of CRP requires sequential conformational changes beginning with the loss of pentameric symmetry, followed by reduction of the intrasubunit disulfide bond, generating mCRP (87, 101). Since free mCRP is proinflammatory in *in vitro* experiments, it can only be assumed that ligand-bound mCRP may also be proinflammatory. Ligand-associated mCRP must be removed along with the ligand.

## CRP, STATINS, AND ATHEROSCLEROSIS

Statins, the inhibitors of a key enzyme in the cholesterol biosynthesis pathway, are used in humans as cholesterol-lowering drugs (102). However, statins also lower CRP levels in humans and human CRP-transgenic mice (103–108). Statins lower CRP levels independently of their cholesterol-lowering activity (103, 104). Statins lower CRP by inhibiting the biosynthesis of CRP by hepatocytes (109, 110). Nitric oxide also inhibits the biosynthesis of CRP (109). It is possible that nitric oxide acts as the mediator of the CRP-lowering effect of statins, since statins are known to generate nitric oxide production (109–112). Because CRP is beneficial, to get rid of CRP from the circulation is not a good idea; a drug that can lower cholesterol levels, but not the CRP levels, should be of choice over statins which lower both (113, 114).

## CONCLUSIONS

CRP appears in the body in response to inflammation and CRP requires exposure to an inflammatory milieu to change its structure and execute functions (2, 115). We have hypothesized earlier that one of the functions of CRP at sites of inflammation is to sense the inflammatory microenvironment, change its own structure in response but remain pentameric, and then bind to pathogenic proteins deposited at those sites (11). CRP does not show an effect on the development of atherosclerosis likely because the inflammatory microenvironment in the arterial wall in animal models of atherosclerosis may not be appropriate in terms of pH and redox conditions and, therefore, the structure of CRP remains unchanged. Consistent with this hypothesis, a CRP molecule which was modified *in vitro* and was capable of binding to atherogenic LDL, did reduce the development of atherosclerosis in mice (93). Thus, CRP has atheroprotective functions displayed by its non-native pentameric form. It has also been proposed that CRP-mediated lipoprotein removal likely underlies the regression of early lesions and perhaps CRP should be considered as an antiatherogenic agent (39).

Non-native CRP binds not only to atherogenic LDL but to all immobilized proteins, including proteins that might be deposited in the host body or recruited on pathogenic surfaces (10, 116). We have suggested previously that deposited, aggregated and conformationally denatured proteins expose a CRP-ligand, regardless of the protein's identity (10). Accordingly, non-native CRP has also been found to be protective against pneumococcal infection (117–119). Although it is not clear what structure on immobilized proteins is recognized by non-native CRP, it has been proposed that an amyloid-like structure is formed on all such proteins and that is what is being recognized by non-native CRP, consistent with the hypothesis that CRP is a pattern recognition molecule of the innate immune system (10). Indeed, an amyloid-like structure appears on LDL by oxidation (120, 121). Non-native CRP may serve as a tool to investigate the functions of CRP in every inflammatory disease involving deposition and aggregation of proteins, such as amyloidosis and autoimmune diseases (122). CRP may have been conserved throughout evolution for protection against disease and toxicity caused by protein misfolding and conformationally altered pathogenic proteins (123, 124).

Considering all the properties of all forms of CRP, it can be said that CRP possesses the functionality of a host defense molecule against not only atherosclerosis but against all diseases caused by proteins when proteins behave like a pathogen or a toxic molecule, in a life cycle that begins as free CRP in circulation and ends in ligand-bound mCRP at sites of inflammation via an intermediate stage of non-native pentamers. If it is validated through further experimentation employing mice transgenic for non-native CRP that non-native CRP is beneficial, the focus should be on the designing and synthesis of a small-molecule compound to target CRP with the goal of changing the conformation of endogenous CRP, which would be preferred over using recombinant non-native CRP as a biologic to treat diseases caused by pathogenic proteins such as ox-LDL.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

This work was supported by National Institutes of Health grant AR068787 to AA.

## ACKNOWLEDGMENTS

We are grateful to Irving Kushner, M.D., for reviewing the manuscript and for his helpful suggestions.

## REFERENCES

1. Ji S-R, Wu Y, Zhu L, Potempa LA, Sheng F-L, Lu W, et al. Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: $mCRP_m$. *FASEB J.* (2007) 21:284–94. doi: 10.1096/fj.06-6722com
2. Agrawal A, Gang TB, Rusiñol AE. Recognition functions of pentameric C-reactive protein in cardiovascular disease. *Mediators Inflamm*. (2014) 2014:319215. doi: 10.1155/2014/319215
3. Wu Y, Potempa LA, El Kebir D, Filep JG. C-reactive protein and inflammation: conformational changes affect function. *Biol Chem*. (2015) 396:1181–97. doi: 10.1515/hsz-2015-0149
4. Braig D, Nero TL, Koch H-G, Kaiser B, Wang X, Thiele JR, et al. Transitional changes in the CRP structure lead to the exposure of proinflammatory binding sites. *Nat Commun*. (2017) 8:14188. doi: 10.1038/ncomms14188
5. McFadyen JD, Kiefer J, Braig D, Loseff-Silver J, Potempa LA, Eisenhardt SU. Dissociation of C-reactive protein localizes and amplifies inflammation: evidence for a direct biological role of C-reactive protein and its conformational changes. *Front Immunol*. (2018) 9:1351. doi: 10.3389/fimmu.2018.01351
6. Salazar J, Martínez MS, Chávez-Castillo M, Núñez V, Añez R, Torres Y, et al. C-reactive protein: an in-depth look into structure, function, and regulation. *Int Sch Res Notices.* (2014) 2014:653045. doi: 10.1155/2014/653045
7. Lv J-M, Wang M-Y. *In vitro* generation and bioactivity evaluation of C-reactive protein intermediate. *PLoS ONE*. (2018) 13:e0198375. doi: 10.1371/journal.pone.0198375
8. Lv J-M, Lü S-Q, Liu Z-P, Zhang J, Gao B-X, Yao Z-Y, et al. Conformational folding and disulfide bonding drive distinct stages of protein structure formation. *Sci Rep*. (2018) 8:1494. doi: 10.1038/s41598-018-20014-y
9. Suresh MV, Singh SK, Agrawal A. Interaction of calcium-bound C-reactive protein with fibronectin is controlled by pH: *in vivo* implications. *J Biol Chem*. (2004) 279:52552–7. doi: 10.1074/jbc.M409054200
10. Hammond DJ Jr, Singh SK, Thompson JA, Beeler BW, Rusiñol AE, Pangburn MK, et al. Identification of acidic pH-dependent ligands of pentameric C-reactive protein. *J Biol Chem.* (2010) 285:36235–44. doi: 10.1074/jbc.M110.142026
11. Singh SK, Thirumalai A, Pathak A, Ngwa DN, Agrawal A. Functional transformation of C-reactive protein by hydrogen peroxide. *J Biol Chem*. (2017) 292:3129–36. doi: 10.1074/jbc.M116.773176
12. Libby P. Inflammation in atherosclerosis. *Nature*. (2002) 420:868–74. doi: 10.1038/nature01323
13. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol.* (2009) 27:165–97. doi: 10.1146/annurev.immunol.021908.132620
14. Lu H, Daugherty A. Atherosclerosis: cell biology and lipoproteins. *Curr Opin Lipidol.* (2013) 24:455–6. doi: 10.1097/MOL.0b013e3283654ef9
15. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. (2011) 145:341–55. doi: 10.1016/j.cell.2011.04.005
16. Orsó E, Grandl M, Schmitz G. Oxidized LDL-induced endolysosomal phospholipidosis and enzymatically modified LDL-induced foam cell formation determine specific lipid species modulation in human macrophages. *Chem Phys Lipids*. (2011) 164:479–87. doi: 10.1016/j.chemphyslip.2011.06.001
17. Leake DS. Does an acidic pH explain why low density lipoprotein is oxidised in atherosclerotic lesions? *Atherosclerosis*. (1997) 129:149–57. doi: 10.1016/S0021-9150(96)06035-2
18. Björnheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall *in vivo*. *Arterioscler Thromb Vasc Biol*. (1999) 19:870–6. doi: 10.1161/01.ATV.19.4.870
19. Naghavi M, John R, Naguib S, Siadaty MS, Grasu R, Kurian KC, et al. pH heterogeneity of human and rabbit atherosclerotic plaques: a new insight into detection of vulnerable plaque. *Atherosclerosis.* (2002) 164:27–35. doi: 10.1016/S0021-9150(02)00018-7
20. Sneck M, Kovanen PT, Öörni K. Decrease in pH strongly enhances binding of native, proteolyzed, lipolyzed, and oxidized low density lipoprotein particles to human aortic proteoglycans. *J Biol Chem.* (2005) 280:37449–54. doi: 10.1074/jbc.M508565200
21. Haka AS, Grosheva I, Chiang E, Buxbaum AR, Baird BA, Pierini LM, et al. Macrophages create an acidic extracellular hydrolytic compartment to digest aggregated lipoproteins. *Mol Biol Cell*. (2009) 20:4932–40. doi: 10.1091/mbc.e09-07-0559
22. Torzewski J, Torzewski M, Bowyer DE, Fröhlich M, Koenig W, Waltenberger J, et al. C-reactive protein frequently colocalizes with the terminal complement complex in the intima of early atherosclerotic lesions of human coronary arteries. *Arterioscler Thromb Vasc Biol*. (1998) 18:1386–92. doi: 10.1161/01.ATV.18.9.1386
23. Zhang YX, Cliff WJ, Schoefl GI, Higgins G. Coronary C-reactive protein distribution: its relation to development of atherosclerosis. *Atherosclerosis*. (1999) 145:375–9. doi: 10.1016/S0021-9150(99)00105-7
24. Sun H, Koike T, Ichikawa T, Hatakeyama K, Shiomi M, Zhang B, et al. C-reactive protein in atherosclerotic lesions: its origin and pathophysiological significance. *Am J Pathol.* (2005) 167:1139–48. doi: 10.1016/S0002-9440(10)61202-3
25. Norja S, Nuutila L, Karhunen PJ, Goebeler S. C-reactive protein in vulnerable coronary plaques. *J Clin Pathol.* (2007) 60:545–8. doi: 10.1136/jcp.2006.038729
26. Yu Q, Li Y, Wang Y, Zhao S, Yang P, Chen Y, et al. C-reactive protein levels are associated with the progression of atherosclerotic lesions in rabbits. *Histol Histopathol.* (2012) 27:529–35. doi: 10.1155/2012/506159
27. Singh SK, Suresh MV, Voleti B, Agrawal A. The connection between C-reactive protein and atherosclerosis. *Ann Med*. (2008) 40:110–20. doi: 10.1080/07853890701749225
28. Agrawal A, Hammond DJ Jr, Singh SK. Atherosclerosis-related functions of C-reactive protein. *Cardiovasc Haematol Disord Drug Targets.* (2010) 10:235–40. doi: 10.2174/187152910793743841
29. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med*. (1971) 136:612–4. doi: 10.3181/00379727-136-35323
30. Volanakis JE, Wirtz KWA. Interaction of C-reactive protein with artificial phosphatidylcholine bilayers. *Nature*. (1979) 281:155–7. doi: 10.1038/281155a0
31. Black S, Wilson A, Samols D. An intact phosphocholine binding site is necessary for transgenic rabbit C-reactive protein to protect mice against challenge with platelet-activating factor. *J Immunol*. (2005) 175:1192–6. doi: 10.4049/jimmunol.175.2.1192
32. Shrive AK, Cheetham GMT, Holden D, Myles DAA, Turnell WG, Volanakis JE, et al. Three-dimensional structure of human C-reactive protein. *Nat Struct Biol*. (1996) 3:346–54. doi: 10.1038/nsb0496-346
33. Thompson D, Pepys MB, Wood S. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure*. (1999) 7:169–77. doi: 10.1016/S0969-2126(99)80023-9
34. Agrawal A, Lee S, Carson M, Narayana SVL, Greenhough TJ, Volanakis JE. Site-directed mutagenesis of the phosphocholine-binding site of human C-reactive protein: Role of $Thr^{76}$ and $Trp^{67}$. *J Immunol.* (1997) 158:345–50.
35. Agrawal A, Simpson MJ, Black S, Carey MP, Samols D. A C-reactive protein mutant that does not bind to phosphocholine and pneumococcal C-polysaccharide. *J Immunol.* (2002) 169:3217–22. doi: 10.4049/jimmunol.169.6.3217

36. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. *J. Immunol.* (1974) 112:2135–47.
37. Levitan I, Volkov S, Subbaiah PV. Oxidized LDL: Diversity, patterns of recognition, and pathophysiology. *Antioxid Redox Signal*. (2010) 13:39–75. doi: 10.1089/ars.2009.2733
38. Tsimikas S, Miller YI. Oxidative modification of lipoproteins: Mechanisms, role in inflammation and potential clinical applications in cardiovascular disease. *Curr Pharm Des*. (2011) 17:27–37. doi: 10.2174/138161211795049831
39. Torzewski M. Enzymatically modified LDL, atherosclerosis and beyond: paving the way to acceptance. *Front Biosci*. (2018) 23:1257–71. doi: 10.2741/4642
40. Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler Thromb Vasc Biol*. (1999) 19:2348–54. doi: 10.1161/01.ATV.19.10.2348
41. He W, Ren Y, Wang X, Chen Q, Ding S. C-reactive protein and enzymatically modified LDL cooperatively promote dendritic cell-mediated T cell activation. *Cardiovasc Pathol*. (2017) 29:1–6. doi: 10.1016/j.carpath.2017.03.009
42. De Beer FC, Soutar AK, Baltz ML, Trayner IM, Feinstein A, Pepys MB. Low density lipoprotein and very low density lipoprotein are selectively bound by aggregated C-reactive protein. *J Exp Med*. (1982) 156:230–42. doi: 10.1084/jem.156.1.230
43. Ji S-R, Wu Y, Potempa LA, Qiu Q, Zhao J. Interactions of C-reactive protein with low-density lipoproteins: Implications for an active role of modified C-reactive protein in atherosclerosis. *Int J Biochem Cell Biol*. (2006) 38:648–61. doi: 10.1016/j.biocel.2005.11.004
44. Chang M-K, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized phospholipids. *Proc Natl Acad Sci USA*. (2002) 99:13043–8. doi: 10.1073/pnas.192399699
45. Biró A, Thielens NM, Cervenák L, Prohászka Z, Füst G, Arlaud GJ. Modified low density lipoproteins differentially bind and activate the C1 complex of complement. *Mol Immunol*. (2007) 44:1169–77. doi: 10.1016/j.molimm.2006.06.013
46. Zhang R, Zhou S-J, Li C-J, Wang X-N, Tang Y-Z, Chen R, et al. C-reactive protein/oxidised low-density lipoprotein/β2-glycoprotein I complex promotes atherosclerosis in diabetic BALB/c mice via p38mitogen-activated protein kinase signal pathway. *Lipids Health Dis*. (2013) 12:42. doi: 10.1186/1476-511X-12-42
47. Wang J, Feng M-J, Zhang R, Yu D-M, Zhou S-J, Chen R, et al. C-reactive protein/oxidized low density lipoprotein/$\beta_2$-glycoprotein I complexes induce lipid accumulation and inflammatory reaction in macrophages via p38/mitogen-activated protein kinase and nuclear factor-κB signaling pathways. *Mol Med Rep*. (2016) 14:3490–8. doi: 10.3892/mmr.2016.5622
48. Pilely K, Fumagalli S, Rosbjerg A, Genster N, Skjoedt M-O, Perego C, et al. C-reactive protein binds to cholesterol crystals and co-localizes with the terminal complement complex in human atherosclerotic plaques. *Front Immunol*. (2017) 8:1040. doi: 10.3389/fimmu.2017.01040
49. Fujita Y, Kakino A, Nishimichi N, Yamaguchi S, Sato Y, Machida S, et al. Oxidized LDL receptor LOX-1 binds to C-reactive protein and mediates its vascular effects. *Clin Chem*. (2009) 55:285–94. doi: 10.1373/clinchem.2008.119750
50. Shih HH, Zhang S, Cao W, Hahn A, Wang J, Paulsen JE, et al. CRP is a novel ligand for the oxidized LDL receptor LOX-1. *Am J Physiol Heart Circ Physiol*. (2009) 296:H1643–50. doi: 10.1152/ajpheart.00938.2008
51. Obradovic MM, Trpkovic A, Bajic V, Soskic S, Jovanovic A, Stanimirovic J, et al. Interrelatedness between C-reactive protein and oxidized low-density lipoprotein. *Clin Chem Lab Med*. (2015) 53:29–34. doi: 10.1515/cclm-2014-0590
52. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. *Circulation*. (2001) 103:1194–7. doi: 10.1161/01.CIR.103.9.1194
53. Fu T, Borensztajn J. Macrophage uptake of low-density lipoprotein bound to aggregated C-reactive protein: possible mechanism of foam-cell formation in atherosclerotic lesions. *Biochem J*. (2002) 366:195–201. doi: 10.1042/bj20020045
54. Verma S, Li S-H, Badiwala MV, Weisel RD, Fedak PWM, Li R-K, et al. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation*. (2002) 105:1890–6. doi: 10.1161/01.CIR.0000015126.83143.B4
55. Van Tits L, de Graaf J, Toenhake H, van Heerde W, Stalenhoef A. C-reactive protein and annexin A5 bind to distinct sites of negatively charged phospholipids present in oxidized low-density lipoprotein. *Arterioscler Thromb Vasc Biol*. (2005) 25:717–22. doi: 10.1161/01.ATV.0000157979.51673.2c
56. Singh U, Dasu MR, Yancey PG, Afify A, Devaraj S, Jialal I. Human C-reactive protein promotes oxidized low density lipoprotein uptake and matrix metalloproteinase-9 release in Wistar rats. *J Lipid Res*. (2008) 49:1015–23. doi: 10.1194/jlr.M700535-JLR200
57. Torzewski M, Rist C, Mortensen RF, Zwaka TP, Bienek M, Waltenberger J, et al. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb Vasc Biol*. (2000) 20:2094–9. doi: 10.1161/01.ATV.20.9.2094
58. Singh SK, Suresh MV, Prayther DC, Moorman JP, Rusiñol AE, Agrawal A. C-reactive protein-bound enzymatically modified low-density lipoprotein does not transform macrophages into foam cells. *J Immunol*. (2008) 180:4316–22. doi: 10.4049/jimmunol.180.6.4316
59. Mookerjea S, Francis J, Hunt D, Yong CY, Nagpurkar A. Rat C-reactive protein causes a charge modification of LDL and stimulates its degradation by macrophages. *Arterioscler Thromb*. (1994) 14:282–7. doi: 10.1161/01.ATV.14.2.282
60. Schwedler SB, Hansen-Hagge T, Reichert M, Schmiedeke D, Schneider R, Galle J, et al. Monomeric C-reactive protein decreases acetylated LDL uptake in human endothelial cells. *Clin Chem*. (2009) 55:1728–31. doi: 10.1373/clinchem.2009.125732
61. Bian F, Yang X, Zhou F, Wu P-H, Xing S, Xu G, et al. C-reactive protein promotes atherosclerosis by increasing LDL transcytosis across endothelial cells. *Br J Pharmacol*. (2014) 171:2671–84. doi: 10.1111/bph.12616
62. Krayem I, Bazzi S, Karam M. The combination of CRP isoforms with oxLDL decreases TNF-α and IL-6 release by U937-derived macrophages. *Biomed Rep*. (2017) 7:272–6. doi: 10.3892/br.2017.949
63. Rufail ML, Ramage SC, van Antwerpen R. C-reactive protein inhibits *in vitro* oxidation of low-density lipoprotein. *FEBS Lett*. (2006) 580:5155–60. doi: 10.1016/j.febslet.2006.08.045
64. Nayeri H, Naderi GA, Moghadam MS, Mohamadzadeh S, Boshtam M, Dinani NJ, et al. Effect of CRP on some of the *in vitro* physicochemical properties of LDL. *ARYA Atheroscler*. (2010) 6:85–9.
65. Eisenhardt SU, Starke J, Thiele JR, Murphy A, Stark GB, Bassler N, et al. Pentameric CRP attenuates inflammatory effects of mmLDL by inhibiting mmLDL-monocyte interactions. *Atherosclerosis*. (2012) 224:384–93. doi: 10.1016/j.atherosclerosis.2012.07.039
66. Chang M-K, Hartvigsen K, Ryu J, Kim Y, Han KH. The pro-atherogenic effects of macrophages are reduced upon formation of a complex between C-reactive protein and lysophosphatidylcholine. *J Inflamm*. (2012) 9:42. doi: 10.1186/1476-9255-9-42
67. Hirschfield GM, Gallimore JR, Kahan MC, Hutchinson WL, Sabin CA, Benson GM, et al. Transgenic human C-reactive protein is not proatherogenic in apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA*. (2005) 102:8309–14. doi: 10.1073/pnas.0503202102
68. Reifenberg K, Lehr H-A, Baskal D, Wiese E, Schaefer SC, Black S, et al. Role of C-reactive protein in atherogenesis: can the apolipoprotein E knockout mouse provide the answer? *Arterioscler Thromb Vasc Biol*. (2005) 25:1641–6. doi: 10.1161/01.ATV.0000171983.95612.90
69. Trion A, de Matt MPM, Jukema JW, van der Laarse A, Mass MC, Offerman EH, et al. No effect of C-reactive protein on early atherosclerosis development in apolipoprotein E*3-leiden/human C-reactive protein transgenic mice. *Arterioscler Thromb Vasc Biol*. (2005) 25:1635–40. doi: 10.1161/01.ATV.0000171992.36710.1e

70. Tennent GA, Hutchinson WL, Kahan MC, Hirschfield GM, Gallimore JR, Lewin J, et al. Transgenic human CRP is not pro-atherogenic, pro-atherothrombotic or pro-inflammatory in apoE$^{-/-}$ mice. *Atherosclerosis*. (2008) 196:248–55. doi: 10.1016/j.atherosclerosis.2007.05.010
71. Ortiz MA, Campana GL, Woods JR, Boguslawski G, Sosa MJ, Walker CL, et al. Continuously-infused human C-reactive protein is neither proatherosclerotic nor proinflammatory in apolipoprotein E-deficient mice. *Exp Biol Med*. (2009) 234:624–31. doi: 10.3181/0812-RM-347
72. Paul A, Ko KWS, Li L, Yechoor V, McCrory MA, Szalai AJ, et al. C-reactive protein accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. (2004) 109:647–55. doi: 10.1161/01.CIR.0000114526.50618.24
73. Schwedler SB, Amann K, Wernicke K, Krebs A, Nauck M, Wanner C, et al. Native C-reactive protein increases whereas modified C-reactive protein reduces atherosclerosis in apolipoprotein E-knockout mice. *Circulation*. (2005) 112:1016–23. doi: 10.1161/CIRCULATIONAHA.105.556530
74. Torzewski M, Reifenberg K, Cheng F, Wiese E, Küpper I, Crain J, et al. No effect of C-reactive protein on early atherosclerosis in LDLR$^{-/-}$/human C-reactive protein transgenic mice. *Thromb Haemost*. (2008) 99:196–201. doi: 10.1160/TH07-10-0595
75. Kovacs A, Tornvall P, Nilsson R, Tegnér J, Hamsten A, Björkegren J. Human C-reactive protein slows atherosclerosis development in a mouse model with human-like hypercholesterolemia. *Proc Natl Acad Sci USA*. (2007) 104:13768–73. doi: 10.1073/pnas.0706027104
76. Koike T, Kitajima S, Yu Y, Nishijima K, Zhang J, Ozaki Y, et al. Human C-reactive protein does not promote atherosclerosis in transgenic rabbits. *Circulation*. (2009) 120:2088–94. doi: 10.1161/CIRCULATIONAHA.109.872796
77. Teupser D, Weber O, Rao TN, Sass K, Thiery J, Fehling HJ. No reduction of atherosclerosis in C-reactive protein (CRP)-deficient mice. *J Biol Chem*. (2011) 286:6272–9. doi: 10.1074/jbc.M110.161414
78. Yu Q, Liu Z, Waqar AB, Ning B, Yang X, Shiomi M, et al. Effects of antisense oligonucleotides against C-reactive protein on the development of atherosclerosis in WHHL rabbits. *Mediators Inflamm*. (2014) 2014:979132. doi: 10.1155/2014/979132
79. Caprio V, Badimon L, Di Napoli M, Fang W-H, Ferris GR, Guo B, et al. pCRP-mCRP dissociation mechanisms as potential targets for the development of small-molecule anti-inflammatory chemotherapeutics. *Front Immunol*. (2018) 9:1089. doi: 10.3389/fimmu.2018.01089
80. Eisenhardt SU, Habersberger J, Murphy A, Chen Y-C, Woollard KJ, Bassler N, et al. Dissociation of pentameric to monomeric C-reactive protein on activated platelets localizes inflammation to atherosclerotic plaques. *Circ Res*. (2009) 105:128–37. doi: 10.1161/CIRCRESAHA.108.190611
81. Molins B, Peña E, de la Torre R, Badimon L. Monomeric C-reactive protein is prothrombotic and dissociates from circulating pentameric C-reactive protein on adhered activated platelets under flow. *Cardiovasc Res*. (2011) 92:328–37. doi: 10.1093/cvr/cvr226
82. Braig D, Kaiser B, Thiele JR, Bannasch H, Peter K, Stark GB, et al. A conformational change of C-reactive protein in burn wounds unmasks its proinflammatory properties. *Int Immunol*. (2014) 26:467–78. doi: 10.1093/intimm/dxu056
83. Singh SK, Hammond DJ Jr, Beeler BW, Agrawal A. The binding of C-reactive protein, in the presence of phosphoethanolamine, to low-density lipoproteins is due to phosphoethanolamine-generated acidic pH. *Clin Chim Acta*. (2009) 409:143–4. doi: 10.1016/j.cca.2009.08.013
84. Boncler M, Kehrel B, Szewczyk R, Stec-Martyna E, Bednarek R, Brodde M, et al. Oxidation of C-reactive protein by hypochlorous acid leads to the formation of potent platelet activator. *Int J Biol Macromol*. (2018) 107:2701–14. doi: 10.1016/j.ijbiomac.2017.10.159
85. Goda T, Miyahara Y. Calcium-independent binding of human C-reactive protein to lysophosphatidylcholine in supported planar phospholipid monolayers. *Acta Biomater*. (2017) 48:206–14. doi: 10.1016/j.actbio.2016.10.043
86. Goda T, Miyahara Y. Specific binding of human C-reactive protein towards supported monolayers of binary and engineered phospholipids. *Colloids Surf B Biointerfaces*. (2018) 161:662–9. doi: 10.1016/j.colsurfb.2017.11.036
87. Khreiss T, József L, Potempa LA, Filep JG. Conformational rearrangement in C-reactive protein is required for proinflammatory actions on human endothelial cells. *Circulation*. (2004) 109:2016–22. doi: 10.1161/01.CIR.0000125527.41598.68
88. Nunomura W, Hatakeyama M. Binding of low density lipoprotein (LDL) to C-reactive protein (CRP): A possible binding through apolipoprotein B in LDL at phosphorylcholine-binding site of CRP. *Hokkaido Igaku Zasshi*. (1990) 65:474–80.
89. Saxena U, Nagpurkar A, Dolphin PJ, Mookerjea S. A study on the selective binding of apoprotein B- and E-containing human plasma lipoproteins to immobilized rat serum phosphorylcholine-binding protein. *J Biol Chem*. (1987) 262:3011–16.
90. Taskinen S, Kovanen PT, Jarva H, Meri S, Pentikäinen MO. Binding of C-reactive protein to modified low-density-lipoprotein particles: Identification of cholesterol as a novel ligand for C-reactive protein. *Biochem J*. (2002) 367:403–12. doi: 10.1042/bj20020492
91. Li H-Y, Wang J, Meng F, Jia Z-K, Su Y, Bai Q-F, et al. An intrinsically disordered motif mediates diverse actions of monomeric C-reactive protein. *J Biol Chem*. (2016) 291:8795–804. doi: 10.1074/jbc.M115.695023
92. Singh SK, Thirumalai A, Hammond DJ Jr, Pangburn MK, Mishra VK, Johnson DA, et al. Exposing a hidden functional site of C-reactive protein by site-directed mutagenesis. *J Biol Chem*. (2012) 287:3550–8. doi: 10.1074/jbc.M111.310011
93. Pathak A, Singh SK, Agrawal A. C-reactive protein is an atheroprotective molecule. *J Immunol*. (2018) 200:170.16.
94. Sjöwall C, Bengtsson AA, Sturfelt G, Skogh T. Serum levels of autoantibodies against monomeric C-reactive protein are correlated with disease activity in systemic lupus erythematosus. *Arthrit Res Ther*. (2004) 6:R87–94. doi: 10.1186/ar1032
95. Wetterö J, Nilsson L, Jonasson L, Sjöwall C. Reduced serum levels of autoantibodies against monomeric C-reactive protein (CRP) in patients with acute coronary syndrome. *Clin Chim Acta*. (2009) 400:128–31. doi: 10.1016/j.cca.2008.10.002
96. Tan Y, Yu F, Yang H, Chen M, Fang Q, Zhao M-H. Autoantibodies against monomeric C-reactive protein in sera from patients with lupus nephritis are associated with disease activity and renal tubulointerstitial lesions. *Hum Immunol*. (2008) 69:840–4. doi: 10.1016/j.humimm.2008.09.006
97. Shi P, Li X-X, Zhu W, Yang H, Dong C, Li X-M. Immunohistochemical staining reveals C-reactive protein existing predominantly as altered conformation forms in inflammatory lesions. *Acta Biol Hung*. (2014) 65:265–73. doi: 10.1556/ABiol.65.2014.3.3
98. Thiele JR, Zeller J, Bannasch H, Stark GB, Peter K, Eisenhardt SU. Targeting C-reactive protein in inflammatory disease by preventing conformational changes. *Mediators Inflamm*. (2015) 2015:372432. doi: 10.1155/2015/372432
99. Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, et al. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: *in vivo* proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *Circulation*. (2014) 130:35–50. doi: 10.1161/CIRCULATIONAHA.113.007124
100. Ji S-R, Wu Y, Potempa LA, Liang Y-H, Zhao J. Effect of modified C-reactive protein on complement activation: a possible complement regulatory role of modified or monomeric C-reactive protein in atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. (2006) 26:935–41. doi: 10.1161/01.ATV.0000206211.21895.73
101. Wang M-Y, Ji S-R, Bai C-J, El Kebir D, Li H-Y, Shi J-M, et al. A redox switch in C-reactive protein modulates activation of endothelial cells. *FASEB J*. (2011) 25:3186–96. doi: 10.1096/fj.11-182741
102. Endres M. Statins: potential new indications in inflammatory conditions. *Atheroscler Suppl*. (2006) 7:31–5. doi: 10.1016/j.atherosclerosissup.2006.01.005
103. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med*. (2005) 352:20–8. doi: 10.1056/NEJMoa042378
104. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med*. (2005) 352:29–38. doi: 10.1056/NEJMoa042000
105. Strandberg TE, Vanhanen H, Tikkanen MJ. Effect of statins on C-reactive protein in patients with coronary artery disease. *Lancet*. (1999) 353:118–9. doi: 10.1016/S0140-6736(05)76154-7

106. Kleemann R, Verschuren L, de Rooij BJ, Lindeman J, de Maat MM, Szalai AJ, et al. Evidence for anti-inflammatory activity of statins and PPARα activators in human C-reactive protein transgenic mice *in vivo* and in cultured human hepatocytes *in vitro*. *Blood*. (2004) 103:4188–94. doi: 10.1182/blood-2003-11-3791
107. Verschuren L, Kleemann R, Offerman EH, Szalai AJ, Emeis SJ, Princen HMG, et al. Effect of low dose atorvastatin versus diet-induced cholesterol lowering on atherosclerotic lesion progression and inflammation in apolipoprotein E* 3-leiden transgenic mice. *Arterioscler Thromb Vasc Biol.* (2005) 25:161–7. doi: 10.1161/01.ATV.0000148866.29829.19
108. Trion A, de Maat M, Jukema W, Maas A, Offerman E, Havekes L, et al. Anti-atherosclerotic effect of amlodipine, alone and in combination with atorvastatin, in APOE* 3-leiden /hCRP transgenic mice. *J Cardiovasc Pharmacol.* (2006) 47:89–95. doi: 10.1097/01.fjc.0000195603.65858.27
109. Voleti B, Agrawal A. Statins and nitric oxide reduce C-reactive protein production while inflammatory conditions persist. *Mol Immunol*. (2006) 43:891–6. doi: 10.1016/j.molimm.2005.06.045
110. Arnaud C, Burger F, Steffens S, Veillard NR, Nguyen TH, Trono D, et al. Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: new evidence for direct antiinflammatory effects of statins. *Arterioscler Thromb Vasc Biol*. (2005) 25:1231–6. doi: 10.1161/01.ATV.0000163840.63685.0c
111. Kaesemeyer WH, Caldwell RB, Huang J, Caldwell RW. Pravastatin sodium activates endothelial nitric oxide synthase independent of its cholesterol-lowering actions. *J Am Coll Cardiol*. (1999) 33:234–41. doi: 10.1016/S0735-1097(98)00514-2
112. Harris MB, Blackstone MA, Sood SG, Li C, Goolsby JM, Venema VJ, et al. Acute activation and phosphorylation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors. *Am J Physiol Heart Circ Physiol*. (2004) 287:H560–6. doi: 10.1152/ajpheart.00214.2004
113. Agrawal A. CRP after 2004. *Mol Immunol*. (2005) 42:927–30. doi: 10.1016/j.molimm.2004.09.028
114. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med*. (2001) 344:1959–65. doi: 10.1056/NEJM200106283442601
115. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. (1999) 340:448–54. doi: 10.1056/NEJM199902113400607
116. Agrawal A. Not only immunoglobulins, C-reactive protein too. *Mol Immunol*. (2013) 56:561–2. doi: 10.1016/j.molimm.2013.05.234
117. Ngwa DN, Gang TB, Singh SK, Agrawal A. Engineered C-reactive protein with improved protective action against pneumococcal infection. *J Immunol*. (2016) 196:63.4.
118. Ngwa DN, Singh SK, Gang TB, Agrawal A. A C-reactive protein-based strategy to reduce antibiotic dosing. *J Immunol*. (2018) 200:170.14.
119. Ngwa DN, Agrawal A. Structure-function relationships of C-reactive protein in bacterial infection. *Front Immunol*. (2019) 10:166. doi: 10.3389/fimmu.2019.00166
120. Miller YI, Choi S-H, Wiesner P, Fang L, Harkewicz R, Hartvigsen K, et al. Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circ Res*. (2011) 108:235–48. doi: 10.1161/CIRCRESAHA.110.223875
121. Stewart CR, Tseng AA, Mok Y-F, Staples MK, Schiesser CH, Lawrence LJ, et al. Oxidation of low-density lipoproteins induces amyloid-like structures that are recognized by macrophages. *Biochemistry*. (2005) 44:9108–16. doi: 10.1021/bi050497v
122. Jones NR, Pegues MA, McCrory MA, Kerr SW, Jiang H, Sellati R, et al. Collagen-induced arthritis is exacerbated in C-reactive protein-deficient mice. *Arthrit Rheum*. (2011) 63:2641–50. doi: 10.1002/art.30444
123. Pathak A, Singh SK, Thirumalai A, Armstrong PB, Agrawal A. Evolution of a host-defense function of C-reactive protein from horseshoe crab to humans. *J Immunol*. (2016) 196:132.5.
124. Pathak A, Agrawal A. Evolution of C-reactive protein. *Front Immunol*. (2019) 10:943. doi: 10.3389/fimmu.2019.00943


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 02 August 2019
doi: 10.3389/fimmu.2019.01750

# Regulation of the Complement System by Pentraxins

*Karita Haapasalo*[1] *and Seppo Meri*[1,2,3]*

[1] Department of Bacteriology and Immunology and Translational Immunology Research Program, University of Helsinki, Helsinki, Finland, [2] HUSLAB, Helsinki University Hospital, Helsinki, Finland, [3] Department of Biomedical Sciences, Humanitas University, Milan, Italy

The functions of pentraxins, like C-reactive protein (CRP), serum amyloid protein P (SAP) and pentraxin-3 (PTX3), are to coordinate spatially and temporally targeted clearance of injured tissue components, to protect against infections and to regulate related inflammation together with the complement system. For this, pentraxins have a dual relationship with the complement system. Initially, after a focused binding to their targets, e.g., exposed phospholipids or cholesterol in the injured tissue area, or microbial components, the pentraxins activate complement by binding its first component C1q. However, the emerging inflammation needs to be limited to the target area. Therefore, pentraxins inhibit complement at the C3b stage to prevent excessive damage. The complement inhibitory functions of pentraxins are based on their ability to interact with complement inhibitors C4bp or factor H (FH). C4bp binds to SAP, while FH binds to both CRP and PTX3. FH promotes opsonophagocytosis through inactivation of C3b to iC3b, and inhibits AP activity thus preventing formation of the C5a anaphylatoxin and the complement membrane attack complex (MAC). Monitoring CRP levels gives important clinical information about the extent of tissue damage and severity of infections. CRP is a valuable marker for distinguishing bacterial infections from viral infections. Disturbances in the functions and interactions of pentraxins and complement are also involved in a number of human diseases. This review will summarize what is currently known about the FH family proteins and pentraxins that interact with FH. Furthermore, we will discuss diseases, where interactions between these molecules may play a role.

**Keywords: CRP–C-reactive protein, complement factor H, PTX3, innate, age-related macular degeneration (AMD), factor H-related protein, complement C1q, cholesterol**

OPEN ACCESS

***Edited by:***
*Barbara Bottazzi, Milan University, Italy*

***Reviewed by:***
*Livija Deban, Prokarium, United Kingdom*
*Kenji Daigo, Nippon Medical School, Japan*
*Takao Hamakubo, Nippon Medical School, Japan*

***Correspondence:**
*Seppo Meri*
*seppo.meri@helsinki.fi*

***Specialty section:***
*This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology*

***Received:*** *07 June 2019*
***Accepted:*** *10 July 2019*
***Published:*** *02 August 2019*

***Citation:***
*Haapasalo K and Meri S (2019) Regulation of the Complement System by Pentraxins. Front. Immunol. 10:1750. doi: 10.3389/fimmu.2019.01750*

## INTRODUCTION

As a part of the host defense, the immune system enables us to cope with unwanted materials threatening our body. Innate immunity acts stereotypically and rapidly (in minutes to hours) to recognize and clear away unwanted materials, while the adaptive immunity generates antigen-specific responses during a longer time course (days to weeks). The central players in the humoral arm of innate immunity include complement (C) system components and soluble pattern recognition molecules, such as pentraxins and collectins. The interplay between these components has a crucial role in the recognition and clearance of both foreign and endogenous unwanted particles from the human body. Any disturbances in these interactions may have a significant impact on the immune response and health.

The complement system was first described in 1888–1889, when both George Nuttall and Hans Buchner independently demonstrated that blood serum was able to kill bacteria. Buchner called this activity "alexin." However, due to the "complementing" function, 10 years later the system was named "complement" by Paul Ehrlich. Jules Bordet observed that for bacterial killing serum contains a heat-stable component, i.e., antibodies, and a heat-labile component, complement. Within the next 50 years it was generally believed that complement requires antibodies for activation. In 1954, however, Louis Pillemer demonstrated that the complement system can be activated independently from antibodies by the so called "properdin" system, thereby playing a central role in innate immunity (1). Because this pathway does not require antibodies nor humoral lectins for activation, like the classical (CP) and lectin pathways (LP), it was later named as the alternative pathway (AP). The AP can act as a separate pathway and as an amplification system of activation triggered by the other pathways. We now know that the first identified heat-labile components, C1 subcomponents C1r and C1s, belong to an activation cascade containing over 40 different molecules. The heat-labile components include also other serine proteases of the C system, like C2, factor B, and the lectin-associated serine proteases (MASPs). Many of the complement factors also interact e.g., with the coagulation, fibrinolytic, and kinin system components. Complement also closely links the innate and adaptive immune systems together e.g., in antigen recognition and delivery to the adaptive immune system players: dendritic cells, follicular dendritic cells, macrophages, B cells and T cells (2). Importantly, the immune system also maintains tolerance and controls excessive inflammatory reactions.

A unique and separate system of targeted complement activation involves a group of evolutionarily relatively old molecules, the pentraxins. C-reactive protein (CRP), serum amyloid P component (SAP), and pentraxin-3 (PTX3) belong to the pentraxin family of pattern recognition molecules. The listed three members have been shown to interact with distinct C components. The first interaction between the C component C1q and CRP was described by Volanakis and Narkates (3). Thereafter, an interaction between SAP and C1q was soon reported (4). Years later, PTX3 was found to bind C1q, as well (5). These data and further studies have shown that pentraxins play a crucial role in inflammation in directing C activation toward, for example, foreign microbes, apoptotic cells and injured tissue. They interact with C components at different stages of the activation cascade. It has been generally accepted that, together with the C system, they contribute to host defense, tissue clearance and regulation of inflammation.

In addition, but very importantly, after activating the complement classical pathway the pentraxins regulate further activation to prevent excessive tissue damage and to coordinate targeted clearance of the injured tissue components. The complement inhibitory function of pentraxins is partially based on their ability to interact with factor H (FH), a complement regulator that interferes with AP activity at the C3b stage and thus prevents formation of the complement membrane attack complex C5b-9 (MAC). Pentraxins and C components such as C3b, C5b-9, and FH are often found in pathological deposits. Changes in their temporal behavior correlate and associate with the same diseases (6–8). Mutations or polymorphisms in these molecules can influence the interactions and have an impact on the progression of the diseases (6–8). The roles of FH, pentraxins and the interactions between these molecules during the course of inflammation have been the subject of many investigations. Pentraxins have been considered either as inflammatory or as anti-inflammatory factors. Thus, their potential causal or protective roles in various diseases still remain to be sorted out. This review summarizes studies on the interactions between pentraxins and the complement system, We will highlight current observations and discuss aspects, where more research is needed.

## THE COMPLEMENT SYSTEM

The complement cascade can be activated through three pathways, the classical, lectin and alternative pathways (**Figure 1**). C3 is the key component of all three pathways, since all pathways converge on it, and major effector functions of complement are mediated through activation of this molecule.

### Alternative Pathway

Distinct from the CP and LP, the AP is activated spontaneously, because C3 is continuously hydrolyzed at a low rate in human plasma to form a metastable C3($H_20$) without cleavage of C3 to C3a and C3b (**Figure 1**). C3($H_20$) is able to bind factor B in a $Mg^{2+}$-dependent manner exposing it to cleavage by factor D thus forming the C3($H_20$)Bb complex, the initial C3 convertase, in the fluid phase. This enzyme cleaves fluid phase C3 to C3a and C3b, and the freshly formed C3b can then target any nearby surface that has available hydroxyl or amino groups for covalent attachment. Soluble or fluid phase associated C3bBb enzyme has a strong catalytic activity for cleaving new C3 molecules to C3a and C3b and thus to amplify AP activation. The smaller cleavage fragment, C3a, is released into solution and acts as an anaphylatoxin and as a chemotactic and activating factor for leukocytes (2).

A key to the properly directed and efficient complement attack by AP is the ability to discriminate the target cells from host cells. In general, on the host cell surface the C3b molecules are rapidly inactivated, while on foreign cells and particles the deposited C3b molecules remain active and can lead to rapid amplification of AP activation. The C3 convertases (C3bBb) also activate the terminal complement cascade by cleaving fluid phase C5. Additional nearby C3b molecules may be needed for the attraction and proper orientation of C5 molecules. C5 activation leads to the release of the strongly proinflammatory chemotactic and anaphylatoxic protein fragment C5a and assembly of the potentially lytic C5b-9 membrane attack complex (MAC) onto the target membrane. Therefore, the fate of C3b deposits on a cell membrane dictates whether complement activation eliminates the target or not. Because of the strong biological activities of the C system, its activation needs carefully directed and efficient regulation at different times, occasions and locations. For this, additional molecules like the pentraxins are needed.

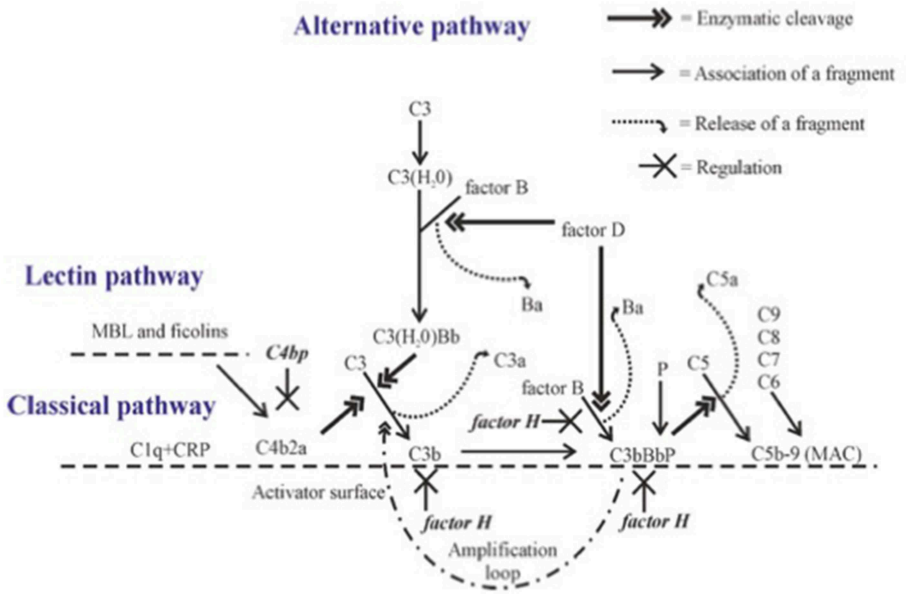


**FIGURE 1 |** Complement activation with emphasis on alternative pathway amplification. Complement proteins interact with each other in sequence leading to cleavage of C3 to C3b. Activation on a suitable target leads to opsonization (coating with C1q, C4b, C3b, or iC3b), release of chemotactic and anaphylatoxic fragments (C5a, C3a) and formation of the membrane attack complex (MAC). C4bp inhibits the CP C3 convertase C4b2a. The alternative pathway gets amplified, when C3 convertase (C3bBb) activates additional C3 molecules by cleavage to C3b to generate new C3 convertase enzymes. This amplification step efficiently opsonizes the target with C3b molecules and its inactivation fragment iC3b. Factor H is the main inhibitor of the amplification loop. Its function is to promote C3b inactivation, inhibit binding of factor B to C3b and accelerate the dissociation of the AP C3 convertases.

## FACTOR H AND FACTOR H-RELATED PROTEINS

The molecular mechanism, how our own cells are protected from the AP attack, is based on the recognition of C3b on host cells by factor H (FH), the main AP regulator in plasma and other body fluids. FH is an elongated molecule composed of 20 short-consensus repeat (SCR) or complement control protein (CCP) domains. The N-terminal domains 1-4 are responsible for the regulatory activity, while the C-terminal domains 19-20 are responsible for simultaneous recognition of C3b (9) and either sialic acids or glycosaminoglycans present on self surfaces (10). In addition, domains 6-7 can bind to surface polyanions (11, 12), As a result of these interactions, FH blocks AP activation and amplification on host structures. FH does this by acting (i) as a cofactor for factor I in the proteolytic cleavage of C3b to iC3b, (ii) by inhibiting the formation or (iii) by promoting the decay of the surface-bound C3bBb convertases (**Figure 1**) (13–15).

The essential role of FH in keeping spontaneous complement activation in check is obvious. It is based on the clinical consequences of *CFH* gene mutations or anti-FH autoantibodies that prevent full function of FH (16–18). Although the initiation of AP activation in the fluid phase relies on a spontaneous low-grade process without a need for a trigger, the activation will be enhanced under suitable conditions. Disease-related FH abnormalities usually lead to an imbalance between AP activation and regulation in the fluid phase or to a mistargeted attack against endothelial and blood cell surfaces (19). On surfaces recognized as activators AP amplification readily takes place, because the generated C3b molecules can bind covalently to the surface in the immediate neighborhood of the activating C3 convertase.

In addition to FH, the factor H family includes an alternatively spliced variant of FH, called factor H-like protein (FHL-1), and five factor H-related proteins (FHR-1 to 5) (**Figure 2**). While FHL-1 contains the first seven domains of FH (plus an extra 4 unique amino acids) and possesses AP regulatory activity, FHRs in general lack these regulatory domains. Therefore, FHRs have no strong direct regulatory activity, although they all interact with C3b (20). Instead, they can compete with the binding of the C-terminus of factor H and thereby regulate its activity with a net result to promote complement activation (21). The most homologous regions between FH and the FHRs are the 2 most C-terminal regions (19-20 in FH), which bind to the C3d region of C3b (22).

The gene cluster coding for FH and FHR-proteins is located on chromosome 1q32. The full-length FH is encoded by 22 exons, while the sequence for FHL-1 stops after alternative splicing at exon 10. The *CFHR* genes are located downstream from the *CFH* gene (23).

There are several known genetic variations and mutations within the *FH* gene cluster. Of these, some have no observable effect on the phenotype, while others are associated with diseases or other harmful effects on the carrier. Most of the disease-related mutations in FH are located within the

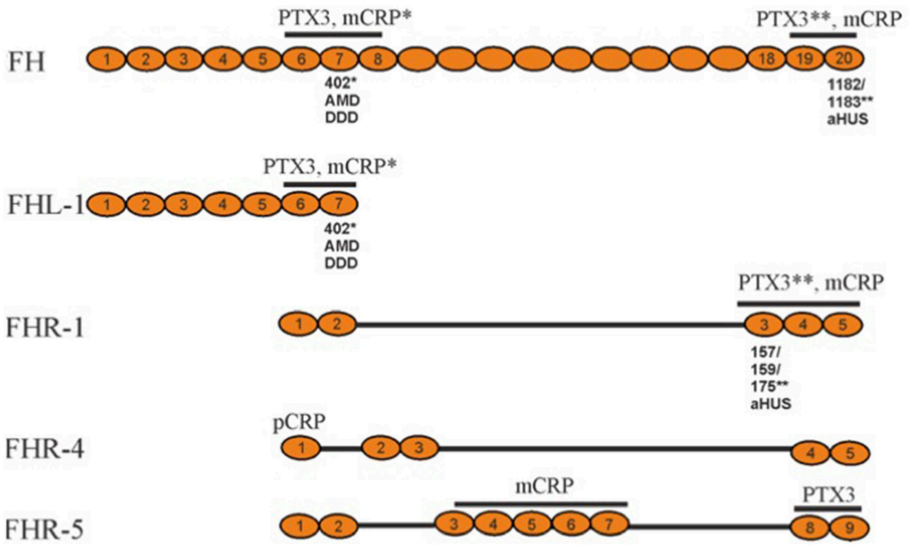


**FIGURE 2 |** Schematic structures of representative factor H family proteins, their interactions with pentraxins CRP and PTX3 and disease associations. The pentraxin interacting domains in FH family proteins (marked above) display disease-associated polymorphisms that alter the protein/pentraxin interactions. The substituted amino acid are marked below. The asterisk indicates the pentraxin, whose binding to the protein is affected by the polymorphism. FHR-2 and FHR-3 are not shown, because their plasma concentrations are low. AMD, Age-related Macular Degeneration; DDD, Dense Deposit Disease; aHUS, atypical Hemolytic Uremic Syndrome; PTX, pentraxin; CRP, C-reactive protein; mCRP, monomeric CRP; pCRP, pentameric CRP.

carboxyl-terminal domains 19-20. They are associated with the atypical hemolytic-uremic syndrome (aHUS) (24). Mutations in the amino-terminus are associated with dense deposit disease (DDD), earlier called membranoproliferative glomerulonephritis type II (MPGN) and rarely also with partial lipodystrophy (PLD). Some polymorphisms have been found to be associated with age-related macular degeneration (AMD), which is the most common cause of visual loss in the elderly people in the industrialized countries. The strongest genetic risk factor for AMD is the Y402H (Tyr402His) polymorphism, which is located in the domain seven (CCP7) of FH (25–27). In addition to polymorphisms and mutations, also autoantibodies against FH can predispose to diseases similar to aHUS or DDD (28, 29). Individuals with factor H deficiency have an over 1,000-fold increased risk to develop meningococcal meningitis, which is due to a secondary C3 and C5 deficiency following overactivation of the alternative pathway in the fluid phase.

## FH INTERACTIONS WITH PENTRAXINS

### Pentraxins

Pentraxins (PTX) are innate pattern recognition molecules, some of which are produced as a response to infection and tissue damage. The name pentraxin comes from the ability of at least some of these molecules to form multimers with five nearly identical subunits. Pentraxins have multiple functions. The best characterized function is activation of the classical pathway of complement on certain microbes and necrotic cells, and thereby contribution to removal of cellular debris. Further observations also imply antibody-like functions, which in evolution would predate the emergence of adaptive immunity (30). The pentraxins are divided into two groups, the short pentraxins: C-reactive protein (CRP) and serum amyloid P component (SAP) and long pentraxins: neuronal PTX1 (NPTX1), neuronal PTX2 (NPTX2), PTX3 and PTX4. All PTXs contain an approximately 200 amino acid-long PTX domain, while the long PTXs have an additional N-terminal domain. The neuronal pentraxins, NPTX1 and NPTX2, are expressed particularly, but not exclusively, in neurons, They have been suggested to be involved in the clearance of synaptic debris during neuronal synapse remodeling (31). However, no role in complement activation by these molecules has been reported. In contrast, CRP, SAP, and PTX3 are all known to activate complement, interact with multiple complement components and thereby contribute to innate immunity. Sometimes, they have been referred to as ancestors of antibodies (**Figure 3**).

SAP shares approximately 51% sequence identity with CRP, which supports the hypothesis that SAP and CRP are products of an earlier gene duplication event. SAP is the glycoprotein precursor of the amyloid P protein. SAP occurs in association with amyloid deposits, including those associated with Alzheimer's disease (34). SAP binds C1q to activate the CP similarly as CRP and PTX3. However, according to the current knowledge, SAP does not interact with any of the FH family proteins. Instead, SAP binds the fluid phase regulator of the CP, C4b-binding protein (C4bp), and plays a potential role in the regulation of CP (35).

CRP was originally named by its ability to bind to the phosphocholine (PC) part of the C-type polysaccharide of pneumococcus in a calcium-dependent manner. It also binds on carbohydrate structures of many other microorganisms such as fungi, yeasts, bacteria and parasites. Moreover, it recognizes

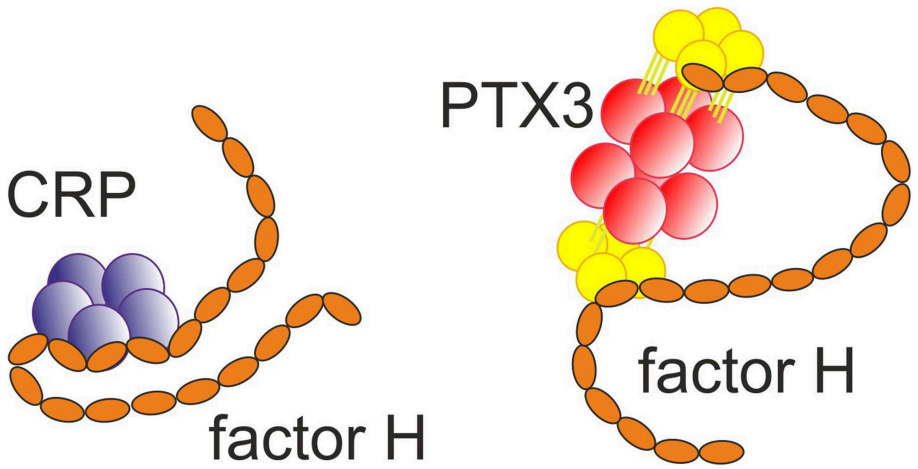


**FIGURE 3 |** Binding of the short pentraxin CRP (32) and the long pentraxin PTX3 (33) to factor H. Multiple interactions between the molecules exist.

modified low-density lipoproteins (LDL) and necrotic and apoptotic cells, and thereby participates in the phagocytosis and clearance mechanisms of the innate immune response (36). One of the specific targets for CRP in LDL particles is cholesterol itself, to which CRP binding was found to be dependent on the 3beta-OH group (37). CRP is produced by hepatocytes as a response to infection or tissue damage, mainly in response to the proinflammatory cytokine IL-6. CRP is therefore commonly used as a non-specific laboratory indicator for infection, systemic inflammation, and tissue damage (34). Highly elevated levels are usually seen in serious bacterial infections, but not so commonly in viral infections. Binding of CRP to apoptotic and necrotic cells enhances their opsonization and phagocytosis by macrophages.

Importantly, CRP has been observed to bind the alternative complement pathway inhibitor factor H (FH) to potentially recruit it to areas of tissue damage (32). This would limit AP activation and excessive inflammation in these areas and promote a non-inflammatory clearance of dying cells (38) (**Figure 4**). With the help of complement, CRP thus demarcates the area destined to clearance.

In addition to FH, also FHR-1, FHR-4, and FHR-5 (**Figure 2**), have been shown to bind CRP on necrotic cells (39–41). When compared to full length FH and FHL-1, the FHRs, however (with the possible exception of FHR-5), possess no direct complement regulatory activity. It has been suggested that FH, FHL-1, and different FHRs possess different binding properties to CRP than FH. FHL-1 domains 6-7, FHR-1 domains 3-5, and FHR-5 domains 3-7 preferentially interact with the monomeric (mCRP), while FHR-4 domain 1 mainly binds the pentameric form of CRP (pCRP) (39, 41–43). Both CRP forms are known to exist in humans. They have been shown to possess similar functions in modulating CP activation on necrotic cells, but they differ in their relative abundance in different tissues. The pCRP is present in plasma, while the mCRP is detected mainly on the surfaces of damaged cells and platelets (44). While the molecular function of the FH-CRP interaction is known, it is still unclear whether binding of FHRs to CRP will enhance C activation and/or promote CRP-mediated opsonization. The FHRs, however, appear to play a particular role in C activation, as exemplified by the association of several reported genetic variations, e.g., FHR deletions and hybrid molecules, with various diseases (45).

Unlike CRP and SAP, PTX3 has been described as an octamer composed of eight identical subunits. It is produced locally in a number of tissues and expressed by several cell types, including fibroblasts, monocytes, macrophages, myeloid dendritic cells and neutrophils (3). It can opsonize target surfaces, such as fungal (*Aspergillus*) and bacterial pathogens and apoptotic cells to initiate complement activation. PTX3 binds C1q, mannan-binding lectin, M-ficolin (ficolin-1) and L-ficolin (ficolin-2), and thereby activates both the CP and LP (36). Binding of PTX3 to C1q is calcium-independent, as opposed to CRP and SAP that both require this divalent cation for their interaction with C1q (34). In addition, PTX3 binds FH, and FHL-1 to inhibit excessive complement activation (27). Also, FHR-1 and FHR-5 have been observed to bind PTX3. By competing out factor H FHRs may actually promote complement activation.

## ALTERATIONS IN FH-PENTRAXIN INTERACTIONS AND THEIR POSSIBLE DISEASE ASSOCIATIONS

Recently, it has become clear that AP dysregulation is a central event in the development of several complement related-diseases involving factor H mutations or polymorphisms in domains FH1-5, FH7, and FH19-20 (**Table 1**). While mutations in FH19-20, or autoantibodies against this region, are associated with atypical hemolytic uremic syndrome (aHUS), the Y402H polymorphism in domain 7 is associated with age-related macular degeneration (AMD) (54, 55) and dense deposit disease (DDD) or C3-glomerulonephritis (C3GN) (26). DDD and C3GN are collectively referred to as C3 glomerulopathy (C3G), which is linked to mutations in the N-terminus of

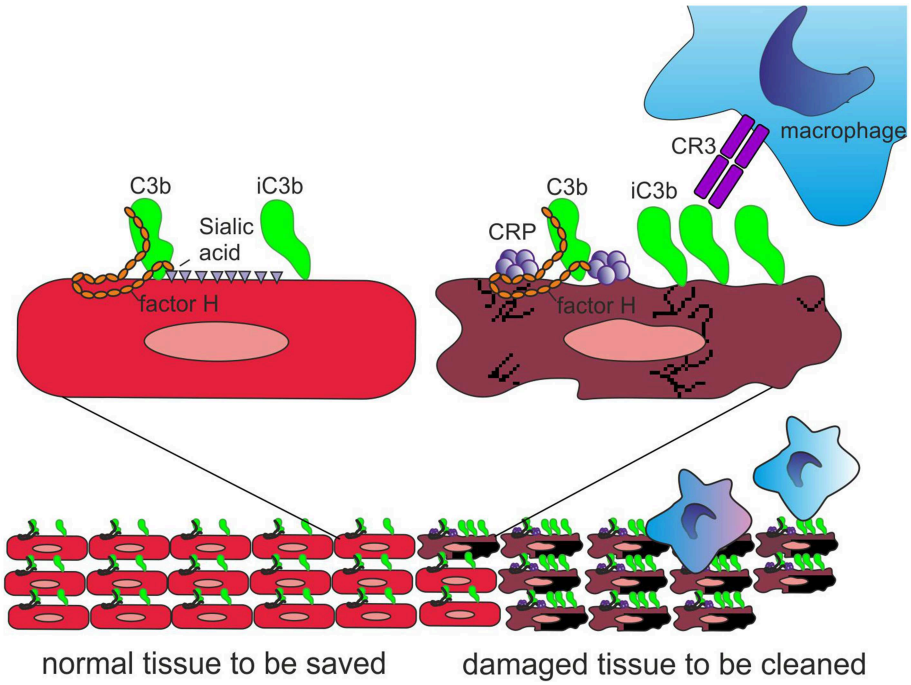


**FIGURE 4 |** Role of CRP and FH in promoting clearance of dying cells. On viable cells **(Left)** accidentally deposited C3b is rapidly inactivated to iC3b and no C activation takes place. On surfaces, from where protective polyanions, like sialic acids, are lost as a consequence of cell damage, factor H binding is decreased **(Right)**. This together with CRP-mediated classical pathway activation leads to complement activation. During a prolonged time course, the large number of deposited C3b molecules, will, however, be inactivated to iC3b with the help of factor H recruitment by CRP. Deposited iC3b molecules promote phagocytosis of the debris without leading to further activation of the terminal pathway.

**TABLE 1 |** Diseases related to factor H mutations or variants that have potential effects on interactions with CRP, PTX3, C3b or polyanions.

| Disease | Factor H or FHR polymorphisms/mutations | Interactions affected | Functional effect of disease-related variant | References |
|---|---|---|---|---|
| AMD | FH Y402H (domain 7)<br>FHL-1 | CRP, polyanions | Insufficient clearance of drusen, inflammation | (46–50) |
| aHUS | FH mutations in domains 19-20 | PTX3, C3b/d, sialic acid | C attack against vascular and blood cells, C-mediated inflammation | (24, 27, 51) |
| Atherosclerosis | FH I62V (associated with high MMP-8 levels) | C3b | Increased release of MMP-8 from neutrophils | (52) |
| DDD | FH domains 1-5 (e.g., R83S) | C3b | AP overactivation in the fluid phase, C3b deposition on basement membranes | (51, 53) |
| | FH Y402H (domain 7) | CRP, polyanions | Inflammation | (26) |
| C3GN | FHR abnormalities (e.g., hybrids), FHR5 | CRP, C3b | Competition with factor H, AP dysregulation | (21) |
| | FH Y402H (domain 7) | CRP | Inflammation | (26) |

*AMD, age-related macular degeneration; aHUS, atypical hemolytic-uremic syndrome; DDD, dense deposit disease; C3GN, C3 glomerulonephritis; FHR, factor H-related protein; AP, alternative pathway.*

FH or to FHR abnormalities. Interestingly, the AMD/DDD-associated domain 7 of FH mediates binding to CRP as well as to glycosaminoglycans (56). In addition to this short pentraxin, the long pentraxin 3 (PTX3) interacts with FH (33). However, unlike CRP binding to FH, the PTX3 binding to FH is not affected by the AMD-associated polymorphism. This implies different molecular functions for these two pentraxins within the complement regulatory system. Because CRP and PTX3 are both acute phase proteins, while FH is the main regulator of the AP, these interactions most likely are relevant during episodes of inflammation and/or tissue injury.

## Age-Related Macular Degeneration (AMD)

AMD is a progressive blinding disease that makes the individual unable to perform basic activities requiring vision, such as reading, recognizing faces, and driving. Globally, AMD affects

170 million people. Therefore, it is the leading cause of visual disability in the industrialized countries. While age is the strongest risk factor for AMD, several genetic risk factors have also been reported. Of these, theY402H polymorphism in FH is the strongest (6).

FH binds CRP at three sites, one located at domain 7, the second within domains 8 to 11 (32) and the third in domains 19-20 (57). CRP is thought to play an important role in helping to direct CP activation and suppressing AP activation at the site of tissue damage and during local inflammation. While CRP induces CP activation and C3b formation on apoptotic and damaged cells by recruiting C1q, the binding of FH to CRP blocks further AP activation and inflammation caused by accelerated C attack (**Figure 5**). Therefore, blocking of AP at this stage is crucial to prevent excessive damage of autologous cells and tissues at the site of inflammation.

An aberrant complement regulation may contribute to the etiology of inflammatory diseases, as exemplified by the strong association of the FH Y402H polymorphism with AMD (46–48). As a result of a single nucleotide polymorphism that leads to the substitution of tyrosine in position 402 in domain 7 of FH by a histidine, the binding of FH to CRP is reduced (49). It has also been observed that the 402 polymorphism may affect FH binding to certain local polyanions in the retinal tissue (58). The reduced binding of FH to CRP and/or to polyanions could partially explain why individuals homozygous for 402H have an up to 10-fold increased risk for developing AMD than individuals homozygous for 402Y. This is supported by the finding that drusen, lesions developed in early AMD between the basal surface of the retinal pigmented epithelium (RPE) and Bruch's membrane, contain numerous proteins associated with the complement system, including the membrane attack complex (MAC) (59). This same study found that drusen contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis and DDD. Thus, suggests partially shared pathogenetic mechanisms for these diseases. However, the results of studies analyzing associations of FH and CRP with these diseases are still controversial.

The FH Y402H polymorphism is strongly associated with AMD. However, it is still unclear how diminished CRP interaction with FH contributes to the disease development. Ultimately, the binding of FH to both CRP and PTX3 prevents further complement activation. In the case of AMD, the described effect on the molecular interaction between CRP and FH is logical and supported by the synergistic effects between 402H homozygosity, CRP expression and AMD (60, 61). No genetic association to AMD has been observed with FH family proteins FHR-4 and FHR-5, although they are known to interact with mCRP. In contrast, individuals with an FHR-3–FHR-1 deletion have a smaller risk for AMD (40, 62). Because the binding sites in the C-terminal domains of FH and FHR-1 are nearly identical, it is possible that the protective effect of FHR-3–FHR-1 deletion could be primarily caused by the FHR-1 deficiency. Because FHR-1 competes out FH it could actually promote, rather than inhibit, AP activation on CRP-coated necrotic cells, although contradictory results have also been reported (41, 63). In addition to Y402H in FH, the same polymorphism is found in FHL-1. It has been suggested that FHL-1 is a major regulator of complement in the retinal Bruch's membrane, as it can passively diffuse through the membrane, whereas the full-sized FH cannot (50). In addition, FHL-1 was reported to have slightly different binding properties to CRP and PTX3 than FH (64).

## Atypical Hemolytic Uremic Syndrome (aHUS)

Hemolytic uremic syndrome (HUS) is a disease characterized by thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure. The more frequent, "typical" form of HUS is associated with infections caused by Shiga-like toxin-(verotoxin) producing bacteria, such as enterohemorrhagic *E. coli* (EHEC), while aHUS is usually linked to mutations in complement proteins (FH, factor I, membrane-cofactor protein/MCP, factor B, C3), thrombomodulin or to antibodies against FH. aHUS is characterized by severe endothelial and blood cell damage, which is caused by a dysregulated and misdirected complement attack. Endothelial injury can be simulated *ex vivo* by the patient serum also in cases, where no mutations or autoantibodies have been found. These observations indicate that dysregulation of the AP on the cell surfaces is the central event in aHUS pathology (65). An abnormal recognition of cell or platelet surface sialic acids or C3b by mutated FH is the key mechanism behind the FH-mutation associated aHUS (66).

Binding of C1q to PTX3 has previously been shown to have a dual role, enhancing or inhibitory, upon C function. This depends on whether PTX3 recruits C1q to fluid phase molecules or to cellular surfaces, such as bacteria or apoptotic cells (67). Binding of the C-terminal domains of FH or of FHR-1 to PTX3 has been shown to be affected by aHUS-associated mutations within domains 19-20 of FH and by autoantibodies against FH and FHR-1. These findings suggest that a reduced binding of FH/FHR-1 to PTX3 could also have a role in the enhanced local C-mediated inflammation and endothelial damage in aHUS (27). Genomic rearrangements resulting in the generation of hybrid genes between FH and FHR-1 or FHR-3 or deletions are not unusual. From these, some have been reported to associate with aHUS or C3G but their interactions with PTX3 have not yet been studied.

## Atherosclerosis

Atherosclerosis is a disease, where arterial walls lose their dynamic properties because of lipid accumulation. The arteries may become narrow and in later stages obstructed because of plaque formation. Total obstruction, because of e.g., of plaque rupture, may lead to a local infarction e.g., in the myocardial coronary arteries. Atherosclerosis is considered to be a multifactorial disease driven by inflammation. Somewhat elevated levels of CRP are related to the long-term risk of death from cardiac causes (68). CRP is known to bind to phosphocholine (PC) and cholesterol in modified LDL particles and colocalize with LDL in human atherosclerotic lesions (37, 69). It has been suggested that FH has a protective role in the development of atherosclerosis, as it binds to apolipoprotein E and thereby increases cholesterol efflux by macrophages (70, 71). A marker of atherosclerosis, elevated level of matrix

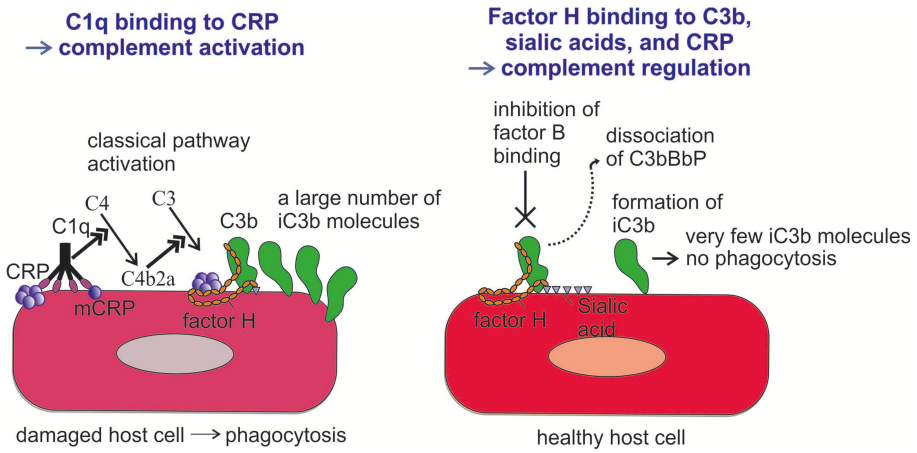


**FIGURE 5 |** Role of factor H-CRP interaction in regulating complement activation on host cells during the course of inflammation. CRP deposition initially activates the classical pathway of complement on injured cells, which leads to deposition of large amounts of C3b. Binding of factor H to CRP blocks further AP activation and inflammation on the damaged cells by promoting inactivation of the C3b molecules to iC3b. In contrast, only a few iC3b molecules are formed on healthy cells due to the rapid inactivation accidentally bound C3b-molecules by sialic acid-bound factor H. On injured cells the density of iC3b molecules is high enough to lead to phagocytosis.

metalloproteinase 8 (MMP-8), was also strongly linked to FH gene polymorphisms in a large unbiased population study (52).

Accumulation of lipids in the lesions caused by inefficient removal of modified LDL by macrophages has been recognized in both atherosclerosis and AMD. Interestingly, AMD and atherosclerosis partially share similar pathological and histological features (72). Complement dysregulation may play a role in the development and progression of both diseases. However, the results of studies investigating the link between CRP, FH Y402H polymorphism and atherosclerosis have yielded controversial results (60, 73). Studies showing that mCRP dissociated from pCRP mediates local proinflammatory effects suggest that mCRP is a proatherogenic factor. mCRP might thus contribute to the formation of atherosclerotic plaques and induce plaque rupture or destabilization (74). To what extent polymorphisms or binding properties of FH or FHL-1 could alter mCRP effector functions has not yet been elucidated.

## C3 Glomerulopathy (C3G)

Dense deposit disease (DDD, membranoproliferative glomerulonephritis type II) and C3 glomerulonephritis (C3GN) constitute a group of rare kidney diseases (C3G). The kidney histology in DDD is characterized by the presence of dense deposits in the glomerular basement membranes in electron microscopy. The deposits stain for complement C3/C3b, while immunoglobulins are absent. The fundamental cause of DDD is relatively well-understood. The disease is due to hypercatabolism of the alternative complement pathway in the fluid phase and C3b deposition to targets (basement membranes) that lack membrane regulators of complement, like CD46 or CD55. C3 glomerulonephritis, however, is less well-understood. It is characterized by C3 deposits in the absence of glomerular dense deposits and immunoglobulins, although they may be present in small amounts. In a proportion of cases C3G is associated with monoclonal gammopathy (17, 75, 76).

Mutations, allelic variants, sequence duplications and deletions within the *FH/FHR* gene cluster are known to associate with C3GN and DDD (26, 53, 77). They include the Y402H polymorphism in the CRP interacting domain 7 on FH. One significant SNP in FHR-5 associates strongly with a particular type of C3GN. This SNP is located in the FHR-5 domain 1 that is homologous to the short consensus repeat 6 of FH, which interacts with CRP. This is particularly interesting as this could affect the FHR-5-CRP interaction, and thereby influence complement activation and control in C3GN (26).

## Other Diseases

According to what has repeatedly been shown, interactions between pentraxins, and the C system play a crucial role in the development and regulation of inflammation. These interactions play an important role in handling tissue damage and priming it for clearance. Thus, they are involved also in conditions such as cancer and infectious diseases, where tissue damage and necrosis often occur. It has been suggested that FH expression levels could be increased in certain tumors, such as urinary bladder and skin tumors (78, 79). In humans, PTX3 expression is increased in different cancers, while in mice FH recruitment by PTX3 to C3b deposited on tumor cells has been shown to restrict the development of local inflammation. This indicates that PTX3-FH interaction could play a role in tumor-associated inflammation (80). In a few studies, genetic polymorphisms in FH/FHRs have been associated with microbial infections (81–84), but further studies will be necessary to define their real significance. Probably indicating its importance, CRP only shows polymorphism in the non-coding regions that could

influence its expression levels. Reduced expression of CRP has been observed e.g., systemic lupus. No deficiencies in CRP have been observed.

## CONCLUDING REMARKS

After the first discovery of the interaction between FH and pentraxins (32), it is now widely accepted that these molecules together regulate the balance between C activation and inhibition. Biochemical, histological and genetic data clearly link these factors to various inflammatory diseases indicating that they participate in the development and progression of these diseases. There are several polymorphisms and mutations in the pentraxin interacting domains of the FH family proteins. Some of them alter pentraxin-FH interactions suggesting a role for these molecules in disease development. However, further work is needed to characterize the exact molecular mechanisms and roles of pentraxin-FH interactions in the initiation and progression of inflammation in these diseases.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

Research funding was provided by The Helsinki University Hospital Funds (TYH2019311), Laboratoriolääketieteen Edistämissäätiö, Finska Vetenskaps-Societeten, The Sigrid Jusélius Foundation (years 2017–2019), and the Jane and Aatos Erkko foundation (190002).

## REFERENCES

1. Pillemer L, Blum L, Lepow IH, Ross OA, Todd EW, Wardlaw AC. The properdin system and immunity. I Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science.* (1954) 120:279–85. doi: 10.1126/science.120.3112.279
2. Walport MJ. Complement. First of two parts. *N Engl J Med.* (2001) 344:1058–66. doi: 10.1056/NEJM200104053441406
3. Volanakis JE, Narkates AJ. Interaction of C-reactive protein with artificial phosphatidylcholine bilayers and complement. *J Immunol.* (1981) 126:1820–5.
4. Bristow CL, Boackle RJ. Evidence for the binding of human serum amyloid P component to Clq and Fab gamma. *Mol Immunol.* (1986) 23:1045–52. doi: 10.1016/0161-5890(86)90003-9
5. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem.* (1997) 272:32817–23. doi: 10.1074/jbc.272.52.32817
6. Pennington KL, DeAngelis MM. Epidemiology of age-related macular degeneration (AMD): associations with cardiovascular disease phenotypes and lipid factors. *Eye Vis.* (2016) 3:34. doi: 10.1186/s40662-016-0063-5
7. Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler Thromb Vasc Biol.* (1999) 19:2348–54. doi: 10.1161/01.ATV.19.10.2348
8. Bhutto IA, Baba T, Merges C, Juriasinghani V, McLeod DS, Lutty GA. C-reactive protein and complement factor H in aged human eyes and eyes with age-related macular degeneration. *Br J Ophthalmol.* (2011) 95:1323–30. doi: 10.1136/bjo.2010.199216
9. Pangburn MK. Cutting edge: localization of the host recognition functions of complement factor H at the carboxyl-terminal: implications for hemolytic uremic syndrome. *J Immunol.* (2002) 169:4702–6. doi: 10.4049/jimmunol.169.9.4702
10. Kajander T, Lehtinen MJ, Hyvarinen S, Bhattacharjee A, Leung E, Isenman DE, et al. Dual interaction of factor H with C3d and glycosaminoglycans in host-nonhost discrimination by complement. *Proc Natl Acad Sci USA.* (2011) 108:2897–902. doi: 10.1073/pnas.1017087108
11. Blackmore TK, Sadlon TA, Ward HM, Lublin DM, Gordon DL. Identification of a heparin binding domain in the seventh short consensus repeat of complement factor H. *J Immunol.* (1996) 157:5422–7.
12. Giannakis E, Jokiranta TS, Male DA, Ranganathan S, Ormsby RJ, Fischetti VA, et al. A common site within factor H SCR 7 responsible for binding heparin, C-reactive protein and streptococcal M protein. *Eur J Immunol.* (2003) 33:962–9. doi: 10.1002/eji.200323541
13. Pangburn MK, Schreiber RD, Muller-Eberhard HJ. Human complement C3b inactivator: isolation, characterization, and demonstration of an absolute requirement for the serum protein beta1H for cleavage of C3b and C4b in solution. *J Exp Med.* (1977) 146:257–70. doi: 10.1084/jem.146.1.257
14. Pangburn MK, Müller-Eberhard HJ. Complement C3 convertase: cell surface restriction of beta1H control and generation of restriction on neuraminidase-treated cells. *Proc Natl Acad Sci USA.* (1978) 75:2416–20. doi: 10.1073/pnas.75.5.2416
15. Weiler JM, Daha MR, Austen KF, Fearon DT. Control of the amplification convertase of complement by the plasma protein beta1H. *Proc Natl Acad Sci USA.* (1976) 73:3268–72. doi: 10.1073/pnas.73.9.3268
16. Pangburn MK, Schreiber RD, Müller-Eberhard HJ. Formation of the initial C3 convertase of the alternative complement pathway. Acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. *J Exp Med.* (1981) 154:856–67. doi: 10.1084/jem.154.3.856
17. Meri S, Koistinen V, Miettinen A, Tornroth T, Seppala IJ. Activation of the alternative pathway of complement by monoclonal lambda light chains in membranoproliferative glomerulonephritis. *J Exp Med.* (1992) 175:939–50. doi: 10.1084/jem.175.4.939
18. Jokiranta TS, Solomon A, Pangburn MK, Zipfel PF, Meri S. Nephritogenic lambda light chain dimer: a unique human miniautoantibody against complement factor H. *J Immunol.* (1999) 163:4590–6.
19. Meri S. Self-nonself discrimination by the complement system. *FEBS Lett.* (2016) 590:2418–34. doi: 10.1002/1873-3468.12284
20. Jozsi M, Meri S. Factor H-related proteins. *Methods Mol Biol.* (2014) 1100:225–36. doi: 10.1007/978-1-62703-724-2_18
21. Goicoechea de Jorge E, Caesar JJ, Malik TH, Patel M, Colledge M, Johnson S, et al. Dimerization of complement factor H-related proteins modulates complement activation *in vivo*. *Proc Natl Acad Sci USA.* (2013) 110:4685–90. doi: 10.1073/pnas.1219260110
22. Prodinger WM, Hellwage J, Spruth M, Dierich MP, Zipfel PF. The C-terminus of factor H: monoclonal antibodies inhibit heparin binding and identify epitopes common to factor H and factor H-related proteins. *Biochem J.* (1998) 331:41–7. doi: 10.1042/bj3310041
23. Zipfel PF, Edey M, Heinen S, Jozsi M, Richter H, Misselwitz J, et al. Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. *PLoS Genet.* (2007) 3:e41. doi: 10.1371/journal.pgen.0030041
24. Neumann HP, Salzmann M, Bohnert-Iwan B, Mannuelian T, Skerka C, Lenk D, et al. Haemolytic uraemic syndrome and mutations of the factor H gene: a registry-based study of German speaking countries. *J Med Genet.* (2003) 40:676–81. doi: 10.1136/jmg.40.9.676
25. Schramm EC, Clark SJ, Triebwasser MP, Raychaudhuri S, Seddon J, Atkinson JP. Genetic variants in the complement system predisposing to age-related macular degeneration: a review. *Mol Immunol.* (2014) 61:118–25. doi: 10.1016/j.molimm.2014.06.032
26. Abrera-Abeleda MA, Nishimura C, Smith JL, Sethi S, McRae JL, Murphy BF, et al. Variations in the complement regulatory genes factor H (CFH)

and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease). *J Med Genet.* (2006) 43:582–9. doi: 10.1136/jmg.2005.038315
27. Kopp A, Strobel S, Tortajada A, Rodriguez de Cordoba S, Sanchez-Corral P, Prohaszka Z, et al. Atypical hemolytic uremic syndrome-associated variants and autoantibodies impair binding of factor h and factor h-related protein 1 to pentraxin 3. *J Immunol.* (2012) 189:1858–67. doi: 10.4049/jimmunol.1200357
28. Warwicker P, Goodship TH, Donne RL, Pirson Y, Nicholls A, Ward RM, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int.* (1998) 53:836–44. doi: 10.1111/j.1523-1755.1998.00824.x
29. Dragon-Durey MA, Loirat C, Cloarec S, Macher MA, Blouin J, Nivet H, et al. Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol.* (2005) 16:555–63. doi: 10.1681/ASN.2004050380
30. Lu J, Marnell LL, Marjon KD, Mold C, Du Clos TW, Sun PD. Structural recognition and functional activation of FcgammaR by innate pentraxins. *Nature.* (2008) 456:989–92. doi: 10.1038/nature07468
31. Osera C, Pascale A, Amadio M, Venturini L, Govoni S, Ricevuti G. Pentraxins and Alzheimer's disease: at the interface between biomarkers and pharmacological targets. *Ageing Res Rev.* (2012) 11:189–98. doi: 10.1016/j.arr.2011.12.004
32. Jarva H, Jokiranta TS, Hellwage J, Zipfel PF, Meri S. Regulation of complement activation by C-reactive protein: targeting the complement inhibitory activity of factor H by an interaction with short consensus repeat domains 7 and 8-11. *J Immunol.* (1999) 163:3957–62.
33. Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, et al. Binding of the long pentraxin PTX3 to factor H: interacting domains and function in the regulation of complement activation. *J Immunol.* (2008) 181:8433–40. doi: 10.4049/jimmunol.181.12.8433
34. Goodman AR, Cardozo T, Abagyan R, Altmeyer A, Wisniewski HG, Vilcek J. Long pentraxins: an emerging group of proteins with diverse functions. *Cytokine Growth Factor Rev.* (1996) 7:191–202. doi: 10.1016/1359-6101(96)00019-6
35. Garcia de Frutos P, Dahlback B. Interaction between serum amyloid P component and C4b-binding protein associated with inhibition of factor I-mediated C4b degradation. *J Immunol.* (1994) 152:2430–7.
36. Bottazzi B, Doni A, Garlanda C, Mantovani A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol.* (2010) 28:157–83. doi: 10.1146/annurev-immunol-030409-101305
37. Taskinen S, Hyvonen M, Kovanen PT, Meri S, Pentikainen MO. C-reactive protein binds to the 3beta-OH group of cholesterol in LDL particles. *Biochem Biophys Res Commun.* (2005) 329:1208–16. doi: 10.1016/j.bbrc.2005.02.091
38. Gershov D, Kim S, Brot N, Elkon KB. C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. *J Exp Med.* (2000) 192:1353–64. doi: 10.1084/jem.192.9.1353
39. Mihlan M, Hebecker M, Dahse HM, Halbich S, Huber-Lang M, Dahse R, et al. Human complement factor H-related protein 4 binds and recruits native pentameric C-reactive protein to necrotic cells. *Mol Immunol.* (2009) 46:335–44. doi: 10.1016/j.molimm.2008.10.029
40. McRae JL, Duthy TG, Griggs KM, Ormsby RJ, Cowan PJ, Cromer BA, et al. Human factor H-related protein 5 has cofactor activity, inhibits C3 convertase activity, binds heparin and C-reactive protein, and associates with lipoprotein. *J Immunol.* (2005) 174:6250–6. doi: 10.4049/jimmunol.174.10.6250
41. Csincsi AI, Szabo Z, Banlaki Z, Uzonyi B, Cserhalmi M, Karpati E, et al. FHR-1 Binds to C-reactive protein and enhances rather than inhibits complement activation. *J Immunol.* (2017) 199:292–303. doi: 10.4049/jimmunol.1600483
42. Hakobyan S, Harris CL, van den Berg CW, Fernandez-Alonso MC, de Jorge EG, de Cordoba SR, et al. Complement factor H binds to denatured rather than to native pentameric C-reactive protein. *J Biol Chem.* (2008) 283:30451–60. doi: 10.1074/jbc.M803648200
43. Csincsi AI, Kopp A, Zoldi M, Banlaki Z, Uzonyi B, Hebecker M, et al. Factor H-related protein 5 interacts with pentraxin 3 and the extracellular matrix and modulates complement activation. *J Immunol.* (2015) 194:4963–73. doi: 10.4049/jimmunol.1403121
44. Mihlan M, Blom AM, Kupreishvili K, Lauer N, Stelzner K, Bergstrom F, et al. Monomeric C-reactive protein modulates classic complement activation on necrotic cells. *FASEB J.* (2011) 25:4198–210. doi: 10.1096/fj.11-186460
45. Jozsi M, Tortajada A, Uzonyi B, Goicoechea de. Jorge E, Rodriguez de Cordoba S. Factor H-related proteins determine complement-activating surfaces. *Trends Immunol.* (2015) 36:374–84. doi: 10.1016/j.it.2015.04.008
46. Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science.* (2005) 308:421–4. doi: 10.1126/science.1110189
47. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science.* (2005) 308:419–21. doi: 10.1126/science.1110359
48. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* (2005) 308:385–9. doi: 10.1126/science.1109557
49. Laine M, Jarva H, Seitsonen S, Haapasalo K, Lehtinen MJ, Lindeman N, et al. Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein. *J Immunol.* (2007) 178:3831–6. doi: 10.4049/jimmunol.178.6.3831
50. Clark SJ, Schmidt CQ, White AM, Hakobyan S, Morgan BP, Bishop PN. Identification of factor H-like protein 1 as the predominant complement regulator in Bruch's membrane: implications for age-related macular degeneration. *J Immunol.* (2014) 193:4962–70. doi: 10.4049/jimmunol.1401613
51. Osborne AJ, Breno M, Borsa NG, Bu F, Frémeaux-Bacchi V, Gale DP, et al. Statistical validation of rare complement variants provides insights into the molecular basis of atypical Hemolytic Uremic Syndrome and C3 Glomerulopathy. *J Immunol.* (2018) 200:2464–78 doi: 10.4049/jimmunol.1701695
52. Salminen A, Vlachopoulou E, Havulinna AS, Tervahartiala T, Sattler W, Lokki ML, et al. Genetic variants contributing to circulating matrix metalloproteinase 8 levels and their association with cardiovascular diseases: a genome-wide analysis. *Circ Cardiovasc Genet.* (2017) 10:e001731. doi: 10.1161/CIRCGENETICS.117.001731
53. Wong EK, Anderson HE, Herbert AP, Challis RC, Brown P, Reis GS, et al. Characterization of a factor H mutation that perturbs the alternative pathway of complement in a family with membranoproliferative GN. *J Am Soc Nephrol.* (2014) 25:2425–33. doi: 10.1681/ASN.2013070732
54. Richards A, Buddles MR, Donne RL, Kaplan BS, Kirk E, Venning MC, et al. Factor H mutations in hemolytic uremic syndrome cluster in exons 18-20, a domain important for host cell recognition. *Am J Hum Genet.* (2001) 68:485–90. doi: 10.1086/318203
55. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci USA.* (2005) 102:7227–32. doi: 10.1073/pnas.0501536102
56. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv Immunol.* (1983) 34:141–212. doi: 10.1016/S0065-2776(08)60379-X
57. Mihlan M, Stippa S, Jozsi M, Zipfel PF. Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. *Cell Death Differ.* (2009) 16:1630–40. doi: 10.1038/cdd.2009.103
58. Clark SJ, Perveen R, Hakobyan S, Morgan BP, Sim RB, Bishop PN, et al. Impaired binding of the age-related macular degeneration-associated complement factor H 402H allotype to Bruch's membrane in human retina. *J Biol Chem.* (2010) 285:30192–202. doi: 10.1074/jbc.M110.103986
59. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J.* (2000) 14:835–46. doi: 10.1096/fasebj.14.7.835
60. Robman L, Baird PN, Dimitrov PN, Richardson AJ, Guymer RH. C-reactive protein levels and complement factor H polymorphism interaction in age-related macular degeneration and its progression. *Ophthalmology.* (2010) 117:1982–8. doi: 10.1016/j.ophtha.2010.02.003
61. Johnson PT, Betts KE, Radeke MJ, Hageman GS, Anderson DH, Johnson LV. Individuals homozygous for the age-related macular degeneration risk-conferring variant of complement factor H have elevated levels of CRP in the choroid. *Proc Natl Acad Sci USA.* (2006) 103:17456–61. doi: 10.1073/pnas.0606234103

62. Hughes AE, Orr N, Esfandiary H, Diaz-Torres M, Goodship T, Chakravarthy U. A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration. *Nat Genet*. (2006) 38:1173–7. doi: 10.1038/ng1890
63. Skerka C, Lauer N, Weinberger AA, Keilhauer CN, Suhnel J, Smith R, et al. Defective complement control of factor H (Y402H) and FHL-1 in age-related macular degeneration. *Mol Immunol*. (2007) 44:3398–406. doi: 10.1016/j.molimm.2007.02.012
64. Swinkels M, Zhang JH, Tilakaratna V, Black G, Perveen R, McHarg S, et al. C-reactive protein and pentraxin-3 binding of factor H-like protein 1 differs from complement factor H: implications for retinal inflammation. *Sci Rep*. (2018) 8:1643. doi: 10.1038/s41598-017-18395-7
65. Jokiranta TS. HUS and atypical HUS. *Blood*. (2017) 129:2847–56. doi: 10.1182/blood-2016-11-709865
66. Hyvarinen S, Meri S, Jokiranta TS. Disturbed sialic acid recognition on endothelial cells and platelets in complement attack causes atypical hemolytic uremic syndrome. *Blood*. (2016) 127:2701–10. doi: 10.1182/blood-2015-11-680009
67. Nauta AJ, Daha MR, van Kooten C, Roos A. Recognition and clearance of apoptotic cells: a role for complement and pentraxins. *Trends Immunol*. (2003) 24:148–54. doi: 10.1016/S1471-4906(03)00030-9
68. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group Fragmin during Instability in Coronary Artery Disease. *N Engl J Med*. (2000) 343:1139–47. doi: 10.1056/NEJM200010193431602
69. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: phosphorylcholine of oxidized phospholipids. *Proc Natl Acad Sci USA*. (2002) 99:13043–8. doi: 10.1073/pnas.192399699
70. Haapasalo K, van Kessel K, Nissila E, Metso J, Johansson T, Miettinen S, et al. Complement factor H binds to human serum apolipoprotein e and mediates complement regulation on high density lipoprotein particles. *J Biol Chem*. (2015) 290:28977–87. doi: 10.1074/jbc.M115.669226
71. Nissila E, Hakala P, Leskinen K, Roig A, Syed S, Van Kessel KPM, et al. Complement factor H and apolipoprotein E participate in regulation of inflammation in THP-1 macrophages. *Front Immunol*. (2018) 9:2701. doi: 10.3389/fimmu.2018.02701
72. Machalinska A, Kawa MP, Marlicz W, Machalinski B. Complement system activation and endothelial dysfunction in patients with age-related macular degeneration (AMD): possible relationship between AMD and atherosclerosis. *Acta Ophthalmol*. (2012) 90:695–703. doi: 10.1111/j.1755-3768.2011.02295.x
73. Sofat R, Casas JP, Kumari M, Talmud PJ, Ireland H, Kivimaki M, et al. Genetic variation in complement factor H and risk of coronary heart disease: eight new studies and a meta-analysis of around 48,000 individuals. *Atherosclerosis*. (2010) 213:184–90. doi: 10.1016/j.atherosclerosis.2010.07.021
74. Eisenhardt SU, Habersberger J, Peter K. Monomeric C-reactive protein generation on activatedplatelets: the missing link between inflammation and atherothrombotic risk. *Trends Cardiovasc Med*. (2009) 19:232–7. doi: 10.1016/j.tcm.2010.02.002
75. Sethi S, Zand L, Leung N, Smith RJ, Jevremonic D, Herrmann SS, et al. Membranoproliferative glomerulonephritis secondary to monoclonal gammopathy. *Clin J Am Soc Nephrol*. (2010) 5:770–82. doi: 10.2215/CJN.06760909
76. Zand L, Kattah A, Fervenza FC, Smith RJ, Nasr SH, Zhang Y, et al. C3 glomerulonephritis associated with monoclonal gammopathy: a case series. *Am J Kidney Dis*. (2013) 62:506–14. doi: 10.1053/j.ajkd.2013.02.370
77. Chen Q, Wiesener M, Eberhardt HU, Hartmann A, Uzonyi B, Kirschfink M, et al. Complement factor H-related hybrid protein deregulates complement in dense deposit disease. *J Clin Invest*. (2014) 124:145–55. doi: 10.1172/JCI71866
78. Cheng ZZ, Corey MJ, Parepalo M, Majno S, Hellwage J, Zipfel PF, et al. Complement factor H as a marker for detection of bladder cancer. *Clin Chem*. (2005) 51:856–63. doi: 10.1373/clinchem.2004.042192
79. Riihila PM, Nissinen LM, Ala-aho R, Kallajoki M, Grenman R, Meri S, et al. Complement factor H: a biomarker for progression of cutaneous squamous cell carcinoma. *J Invest Dermatol*. (2014) 134:498–506. doi: 10.1038/jid.2013.346
80. Daigo K, Inforzato A, Barajon I, Garlanda C, Bottazzi B, Meri S, et al. Pentraxins in the activation and regulation of innate immunity. *Immunol Rev*. (2016) 274:202–17. doi: 10.1111/imr.12476
81. Emonts M, Uitterlinden AG, Nouwen JL, Kardys I, Maat MP, Melles DC, et al. Host polymorphisms in interleukin 4, complement factor H, and C-reactive protein associated with nasal carriage of Staphylococcus aureus and occurrence of boils. *J Infect Dis*. (2008) 197:1244–53. doi: 10.1086/533501
82. Haapasalo K, Vuopio J, Syrjanen J, Suvilehto J, Massinen S, Karppelin M, et al. Acquisition of complement factor H is important for pathogenesis of Streptococcus pyogenes infections: evidence from bacterial in vitro survival and human genetic association. *J Immunol*. (2012) 188:426–35. doi: 10.4049/jimmunol.1102545
83. Bradley DT, Bourke TW, Fairley DJ, Borrow R, Shields MD, Zipfel PF, et al. Susceptibility to invasive meningococcal disease: polymorphism of complement system genes and Neisseria meningitidis factor H binding protein. *PLoS ONE*. (2015) 10:e0120757. doi: 10.1371/journal.pone.0120757
84. Davila S, Wright VJ, Khor CC, Sim KS, Binder A, Breunis WB, et al. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat Genet*. (2010) 42:772–6. doi: 10.1038/ng.640


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# Advantages of publishing in Frontiers

**OPEN ACCESS**
Articles are free to read for greatest visibility and readership

**FAST PUBLICATION**
Around 90 days from submission to decision

**HIGH QUALITY PEER-REVIEW**
Rigorous, collaborative, and constructive peer-review

**TRANSPARENT PEER-REVIEW**
Editors and reviewers acknowledged by name on published articles

**Frontiers**
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

**Visit us:** www.frontiersin.org
**Contact us:** info@frontiersin.org | +41 21 510 17 00

**REPRODUCIBILITY OF RESEARCH**
Support open data and methods to enhance research reproducibility

**DIGITAL PUBLISHING**
Articles designed for optimal readership across devices

**FOLLOW US**
@frontiersin

**IMPACT METRICS**
Advanced article metrics track visibility across digital media

**EXTENSIVE PROMOTION**
Marketing and promotion of impactful research

**LOOP RESEARCH NETWORK**
Our network increases your article's readership