



# On the functional overlap between complement and anti-microbial peptides

Jana Zimmer<sup>1</sup>, James Hobkirk<sup>2</sup>, Fatima Mohamed<sup>3</sup>, Michael J. Browning<sup>3,4</sup> and Cordula M. Stover<sup>3\*</sup>

<sup>1</sup> Department of Infectious Diseases – Medical Microbiology and Hygiene, Ruprecht-Karls-University of Heidelberg, Heidelberg, Germany

<sup>2</sup> Department of Academic Endocrinology, Diabetes and Metabolism, Hull York Medical School, University of Hull, Hull, UK

<sup>3</sup> Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK

<sup>4</sup> Department of Immunology, Leicester Royal Infirmary, Leicester, UK

## Edited by:

Uday Kishore, Brunel University, UK

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## \*Correspondence:

Cordula M. Stover, Department of Infection, Immunity and Inflammation, College of Medicine, Biological Sciences and Psychology, University of Leicester, University Road, Leicester LE1 9HN, UK  
e-mail: cms13@le.ac.uk

Intriguingly, activated complement and anti-microbial peptides share certain functionalities; lytic, phagocytic, and chemo-attractant activities and each may, in addition, exert cell instructive roles. Each has been shown to have distinct LPS detoxifying activity and may play a role in the development of endotoxin tolerance. In search of the origin of complement, a functional homolog of complement C3 involved in opsonization has been identified in horseshoe crabs. Horseshoe crabs possess anti-microbial peptides able to bind to acyl chains or phosphate groups/saccharides of endotoxin, LPS. Complement activity as a whole is detectable in marine invertebrates. These are also a source of anti-microbial peptides with potential pharmaceutical applicability. Investigating the locality for the production of complement pathway proteins and their role in modulating cellular immune responses are emerging fields. The significance of local synthesis of complement components is becoming clearer from *in vivo* studies of parenchymatous disease involving specifically generated, complement-deficient mouse lines. Complement C3 is a central component of complement activation. Its provision by cells of the myeloid lineage varies. Their effector functions in turn are increased in the presence of anti-microbial peptides. This may point to a potentiating range of activities, which should serve the maintenance of health but may also cause disease. Because of the therapeutic implications, this review will consider closely studies dealing with complement activation and anti-microbial peptide activity in acute inflammation (e.g., dialysis-related peritonitis, appendicitis, and ischemia).

**Keywords:** immune cells, innate immunity, histidine tag, deficiencies, acute inflammation

## BACKGROUND

The host defense against microorganisms relies on both innate and adaptive elements. Innate immunity is the first line of defense against a microbial pathogen, which exposes a pathogen-associated molecular pattern or more simply a prokaryotic surface membrane, differing from eukaryotic biphospholipid layers in the complete absence of cholesterol. For an efficient and directed response, complement uses both pattern recognition and missing self-recognition strategies [reviewed by Ref. (1)]. Besides, it involves a highly controlled, rapid cascade, and crosstalks with other biological systems, for example, with Toll-like receptors (2). Control of the complement system is maintained by a group of membrane-anchored proteins and soluble, circulating proteins referred to as complement regulatory proteins. Regulatory proteins can act at different points in the complement cascade and help control complement attack and adjust its severity, propagation, and endpoints to the cellular target (3). Cells expose membrane-anchored proteins like membrane cofactor protein (MCP or CD46), decay accelerating factor (DAF or CD55), complement receptor 1 (CR1 or CD35), and CD59 as complement regulatory proteins (4), while properdin and factor H may become membrane associated and

then are thought to fine tune locally the extent of complement activation (5).

Defensins are able to kill or eliminate bacteria, fungi, protozoans, and viruses.  $\alpha$ - and  $\beta$ -defensins are synthesized as precursors that are proteolytically cleaved into their anti-microbially active forms (6). Human neutrophil peptides (HNP)1 to HNP3, for example, are found in high concentrations in granules of neutrophils (7) and released by degranulation in response to pro-inflammatory or bacterial stimuli (8). Human defensin (HD)5 and HD6 are present in Paneth cells in the crypts of the small intestine (9), whereas  $\beta$ -defensins are induced in epithelial cells by wounding, bacterial products, or pro-inflammatory cytokines (10–13). Based on the chemotactic effect exerted by anti-microbial peptides, much work was spent on identifying a receptor for their actions. It has now emerged that CCR2 and CCR6 are receptors for  $\beta$ -defensins (14), and that the interaction of, e.g., HD6 with glycosaminoglycans may modulate binding of one or the other to CCR2 (15). There are different ways of LL-37 uptake into a cell. The receptors FPRL-1 and P2X7 are important for LL-37 activity and lead to chemoattraction and IL-1 $\beta$  processing, respectively (16, 17). In contrast, cellular uptake of LL-37 into epithelial cells has been shown to be mediated by atypical endocytic processes (18).

## COMPLEMENT AND ANTI-MICROBIAL PEPTIDES SHAPE THE LOCAL ENVIRONMENT

Local production of complement components and their role in the inflammatory microenvironment is a currently emerging field. Most of the complement pathway proteins are synthesized in the liver (19); however, extrahepatic biosynthesis additionally occurs in a variety of other tissues and organs (20). Locally produced complement proteins, finely tuned according to the demands of the local environment, may allow differential regulation of inflammation and cellular activation within these tissues. Complement factor H, besides its hepatic expression, is further expressed at low levels in lung, heart, spleen, brain, eye, kidney, pancreas, placenta, as well as neurons and glial cells (21). Production of complement proteins and their regulators directly at sites of inflammation offers an underestimated variety of functions for complement proteins. So far, several cell types have been found to produce complement proteins including macrophages (22), fibroblasts (23), endothelial cells (24), as well as organ specific cells (25–28). Intriguingly, even cells such as peripheral monocytes that were thought to be incapable of synthesizing complement proteins unless activated have recently been shown to produce C1q (29). This suggests a new role for locally synthesized C1q in the immediate local response to pathogen- or danger-associated molecular patterns (PAMPs or DAMPs). Properdin, the positive regulator of the alternative pathway, is produced by a variety of cell types like neutrophils (30), peripheral blood monocytes (31), endothelial cells (32), and T cells (33). Properdin released by phagocytes was shown to bind to apoptotic and necrotic cells (33, 34), contributing to their direct removal or properdin-mediated complement activation. Likewise, local release of properdin may opsonize and kill microorganisms using the same mechanism, if indeed it can operate as a pattern-recognition molecule in its own right (35).

The role of complement in modulating inflammation and maintaining homeostasis is only recently becoming apparent. Local immune responses can be altered by C5a via modulating the local cytokine milieu, especially via the cytokines IL-17 and IL-23. While C5a has been shown to enhance IL-17F, it limits IL-17 and IL-23 production by macrophages or DCs (36). In agreement with these findings, another report determined that IL-17 levels in experimental asthma are reduced by signaling through C5aR (37). So far, little is known about local synthesis and specific function of complement proteins where produced away from the humoral environment that has led to the well-known diagrams of sequential assemblies and enzymatic cleavages. Due to functional studies, there is increasing evidence that locally produced complement proteins are biologically active and have a significant role in local environment. Local synthesis of complement proteins not only contributes to the systemic pool of complement (38) but also influences local tissue injury and provides a link with the antigen-specific immune response (39). The diverse range of extrahepatic sites for synthesis of complement proteins and their regulators suggests the importance and need for local availability of the proteins. It has been suggested recently that plasma-borne complement activation vs. cellular production of complement components sufficient to form convertases may pursue distinct, compartment-selective, biological functions (40). Understanding the relative importance of local and systemic

complement production could help to explain the differential involvement of complement in organ-specific pathology.

Locality of production plays an important role not only for complement proteins but also for anti-microbial peptides. Paneth cells in the small intestine have been shown to release granules into the lumen of the crypts thereby contributing to mucosal immunity (41). Those granules contain proteins that are associated with roles in host defense, including lysozyme (42), secretory phospholipase A2 (43), and  $\alpha$ -defensins termed cryptidins (44). Anti-microbial peptides secreted by Paneth cells are important for innate immunity as they protect mitotically active crypt cells from colonization by potential pathogens and confer protection from enteric infection (45). Moreover, secretion into the crypt lumen defines the apical environment of neighboring cells (46).

## LYTIC ACTIVITIES OF COMPLEMENT AND ANTI-MICROBIAL PEPTIDES

Both complement proteins and anti-microbial peptides share lytic activities. Anti-microbial peptides attack bacteria, fungi, protozoa, and certain viruses by inserting into their membrane manifold causing pore formation and subsequent lysis (47, 48). Due to the cationic character of microbial peptides, electrostatic attraction to the negatively charged phospholipids of microbial membranes occurs resulting in integration into the microbial cell membrane and membrane disruption.

In the absence of regulators, complement proteins contribute to lysis of cells by forming a membrane attack complex (MAC). After cleavage of C5 into C5a and C5b by the highly specific C5 convertase, C5b initiates the terminal complement pathway involving a non-enzymatic assembly of C6, C7, C8, and C9 to form the MAC to cause lysis. Fusion of those proteins brings forth hydrophobic sites that can insert into the membrane to form a transmembrane channel (49). While only one mode of insertion to form a transmembrane channel for the MAC has been described (50), several models exist to explain the insertion of conformationally changed anti-microbial peptides into and across target membranes (51). Pathogens actively interfere with either of these lytic effector processes (52, 53).

Peptides synthesized from the C-terminal portion of complement C3a have inhibitory effect on the growth of *P. aeruginosa*, *E. coli*, *B. subtilis*, and *C. albicans*, which does not exceed the activity of equal molar amounts of LL-37 (54). Native human C3a, however, showed inhibitory effect on *C. albicans* growth, which exceeded that of LL-37 at equimolar amounts [50  $\mu$ M; (55)]. While 6  $\mu$ M C-terminal C3a peptide was needed to observe membrane disruption of *P. aeruginosa* (54), 1  $\mu$ M native C3a produced leakage of liposomes (55).

## PHAGOCYtic AND CHEMO-ATTRACTANT ACTIVITIES OF COMPLEMENT AND ANTI-MICROBIAL PEPTIDES

The peritoneal cavity is a site in which complement and anti-microbial peptides are key components of the innate immune response and have been investigated with regard to peritoneal dialysis (56, 57). Both mesothelium and leukocytes are the source for this production (58). While the opsonophagocytic activity of complement is well known (via C3b/iC3b), recent findings show that LL-37 can modulate the expression of receptors, which determine

the extent of the phagocytic response of human macrophages *in vitro* (59). Both components of the innate immune response are thereby able to influence the adaptive immune response by altering the phenotype of phagocytic cells to become more mature, i.e., acquire characteristics, which will make them more potent to present antigen in a suitable, germinal center environment. Chemotactic activity of complement *per se* (via generation of C3a, C5a, and engagement with their receptors, C3aR, C5aR, C5L2) has been described (60). In addition, however, bradykinin, which may be released after activation of kininogen by the lectin pathway of complement activation (61), has chemotactic activity (62). Contact and complement system cooperate in a pro-inflammatory way. Interestingly,  $\beta$ -defensins can bind to chemokine receptors, in particular, CCR6 present on dendritic cells and T cells (14) and CCR2 (see above). Complement C3a and CXCL12 cooperate in the chemotaxis of CD34<sup>+</sup> progenitor cells in bone marrow, but the receptor has not yet been described, though C3aR has been excluded (60).

### CELL INSTRUCTIVE ROLES OF COMPLEMENT AND ANTI-MICROBIAL PEPTIDES

Anti-microbial peptides and complement are constitutively expressed and are upregulated during inflammation. While anti-microbial peptides are commonly known to be synthesized by epithelial cells to partake in the innate host defense (63), the contribution of complement expression in non-lymphoid cells is not well appreciated yet, although the pattern of expression in crypts follows that of anti-microbial peptides (26). Beyond their chemo-attractant ability, complement and anti-microbial peptides may assume immunoadjuvant, i.e., adaptive immunity supportive, properties (63, 64). The type of cellular response is co-determined by the integration of signaling events triggered by mediators. So complement activation products and anti-microbial peptides, which can alter their expression manifold acutely and remain altered chronically, are relevant determinants of this cell activity (65, 66).

Innate lymphoid cells located in the mucosa contribute to the barrier by releasing IL-22, which stimulates the production of anti-microbial peptides (67). IL-22 is also expressed, in the context of TGF- $\beta$ , by IL-17A and IL-17F expressing CD4<sup>+</sup>Th17 cells. Synergistically, IL-22 and IL-17A lead to significant induction of mRNA expression for hBD2, S100A7–9 by keratinocytes (68). Because, on its own, IL-17A is a potent stimulator of anti-microbial peptide production (68), those studies reporting a deviation in complement activity, which impact on the Th17 cell population (69, 70), have to be viewed with care. It is likely that a greater component within the immune response is significantly determined by the relative amounts of anti-microbial peptides, which escape attention in the complement field. In this sense, it is a matter of discussion whether the phenotype observed in the properdin-deficient mice when infected with *Listeria monocytogenes* could be significantly influenced by a lack of anti-microbial peptides, which would be due to significantly lower IL-17 levels, which, importantly, do not adequately upregulate during infection (71). C5a and an N-terminal peptide of human lactoferrin with anti-microbial activity, by stimulating macrophages or dendritic cells, respectively, are able to enhance production

of Th17 cells (72, 73), which act in a pro-inflammatory, Treg opposing, way.

### ROLE OF COMPLEMENT AND ANTI-MICROBIAL PEPTIDES IN ENDOTOXIN CLEARANCE

Intact complement activation in the humoral system (blood) is needed for efficient endotoxin clearance (74), while it exerts at the same time a modulatory effect on cellular, pro-inflammatory activity (75). Anti-microbial peptides may have LPS-neutralizing effect, which is important for the beneficial outcome from sepsis (76). Avoiding exhaustion of these systems would obviate the detrimental development of endotoxin tolerance in sepsis. In severe sepsis, significantly lower levels of plasma C3 have been reported (77) and a failure of PBLs to induce defensins *ex vivo* in response to endotoxins (78). Low Vitamin D3 levels have been linked to mortality in sepsis (79). Interestingly, Vitamin D3 promotes production of LL-37 and  $\beta$ -Defensin (80) as well as C2 and C3 (81, 82) *in vitro*. The complement receptor C5aR is upregulated in lung, liver, kidney, and heart during the early phases of sepsis. Blocking of C5aR has been correlated to improved survival in murine models of sepsis (83).

### MONOCYTES AND MACROPHAGES ARE DISTINCT PRODUCERS FOR C3 AND ANTI-MICROBIAL PEPTIDES

Monocytes appear to need LPS stimulation to produce C3 (84), whereas macrophages were shown to produce basal levels of C3 even without stimulation (85, 86). As a recurring point, most of the papers suggest that macrophage differentiation has to have taken place before considerable C3 production occurs (85–92). This observation is also supported by Affymetrix array data (<http://www.ncbi.nlm.nih.gov/geo/profile/60640353>), showing more C3 mRNA in macrophages compared to monocytes.

Both monocytes and macrophages are also affected by anti-microbial peptides. The honeybee anti-microbial peptide apidaecin, for example, has been shown to bind both to human macrophages and monocytes (93) without inducing cytotoxic effects. However, apidaecin shows a different subcellular localization in the cytoplasm or in endosomal compartments for macrophages or monocytes, respectively. Besides, the effect upon LPS stimulation differs. Antagonizing LPS-stimulatory effects on both macrophages and monocytes at low concentrations, a high concentration of apidaecin stimulated pro-inflammatory and pro-immune functions of macrophages. Not only for complement production but also for anti-microbial peptides, monocyte to macrophage differentiation plays an important role. The peptide hLF1–11 applied on monocytes during GM-CSF-driven differentiation has been shown to modulate differentiation toward a macrophage subset characterized by both pro- and anti-inflammatory cytokine production and increased responsiveness to microbial structures (94, 95).

Macrophages are considered classically activated (M1) when stimulated by IFN $\gamma$  or LPS and alternatively activated (M2) when stimulated by IL-4 or IL-13 (96). The arising question is therefore, which subpopulation of macrophages produces C3 predominantly. There were some hints pointing toward M1 macrophages like fact that synthesis of C3 in various organs can be directly upregulated by IFN $\gamma$  during an inflammatory response (97). In

addition, IFN $\gamma$  can induce C3 synthesis directly (98) as well as stabilize C3 mRNA (99). Recent studies using guinea pigs deficient for complement C3 showed an impaired antibody response to T-dependent antigens (100), a response dependent on M1 macrophages as well. Those data reveal that C3 production is a highly regulated process and can be modulated by a variety of cytokines, determining whether a macrophage will differentiate into an M1 or M2 macrophage and therefore produce more or less C3, respectively.

Anti-microbial peptides were shown to modulate inflammatory responses as well. LL-37, for example, dramatically reduced levels of pro-inflammatory cytokines such as TNF- $\alpha$  and NO in M1 and M2 bone marrow-derived macrophages, whereas anti-inflammatory functions remained unaltered (101). The same effect could also be observed for human THP-1 cells (102). Another example is the Vitamin D inducible LL-37 anti-microbial peptide, which is expressed mainly by M1 macrophages (103). A recent review sheds light on the feature of monocytes and macrophages to respond differently: they are of heterogeneous origin and do not necessarily follow the differentiation pathway of monocyte-macrophage (104).

## DEFICIENCIES OF COMPLEMENT AND ANTI-MICROBIAL PEPTIDES

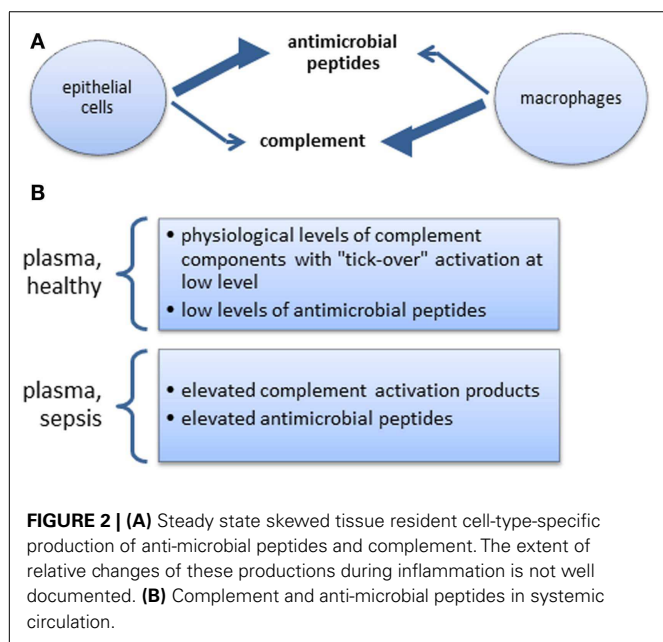
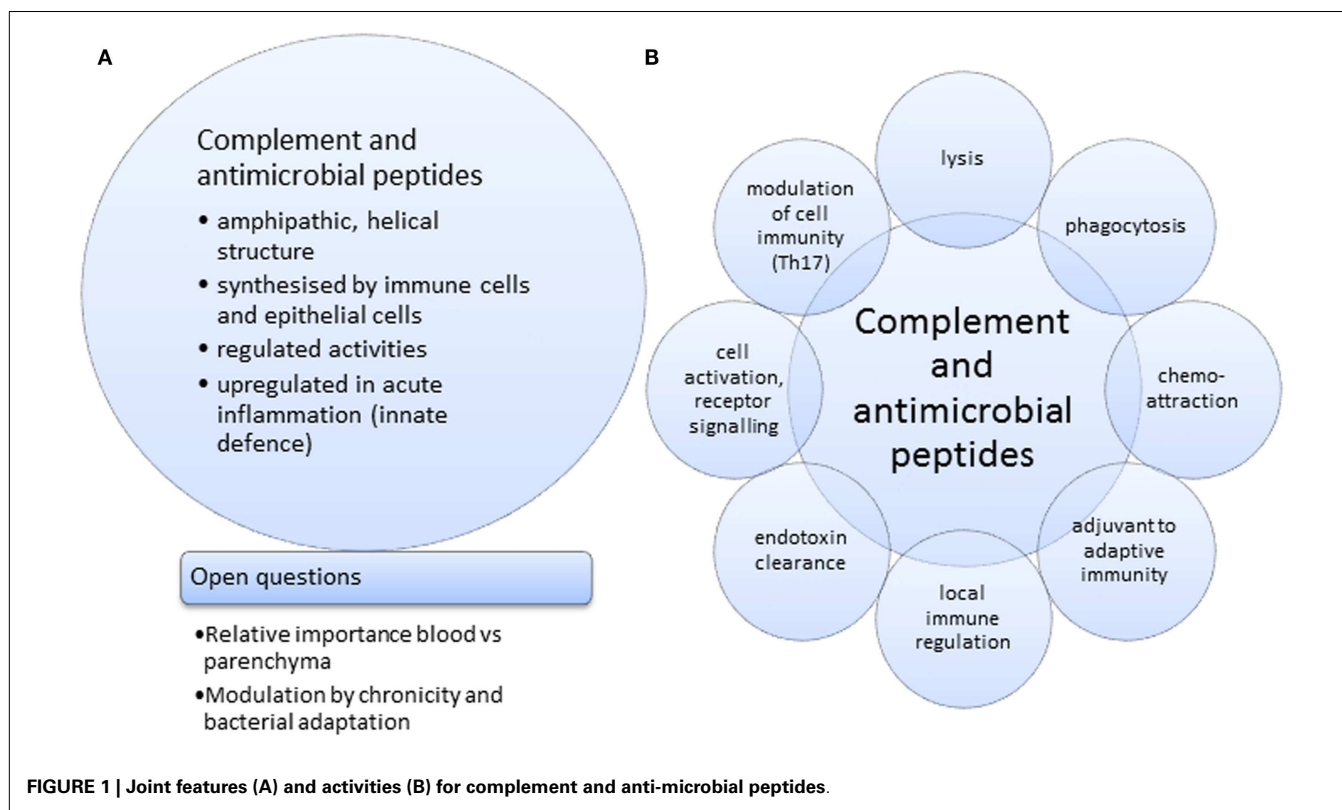
In humans, genetic deficiencies of the great majority of complement components have been described, giving insights into their functions in both infectious and non-infectious diseases. It is beyond the scope of this article to give a detailed review of genetically determined deficiencies of the complement system. [For a more comprehensive review, see in Ref. (105) or (106).] Deficiencies of most complement components give rise to increased susceptibility to specific pathogens or groups of pathogens. In broad terms, deficiencies of components of the classical pathway (C1q,r,s, C4, and C2) are associated with infections with encapsulated bacteria, such as *S. pneumoniae* and *N. meningitidis*. Deficiencies of lectin pathway components (MBL, MASP-2, and ficolin) have been associated with increased frequencies of (usually less severe) respiratory infections. However, asymptomatic lectin pathway-deficient individuals have also been described. C3-deficient patients suffer from a broader range of pyogenic infections, including more severe respiratory infections and meningitis (e.g., *S. pneumoniae*, *N. meningitidis*, *S. pyogenes*, *H. influenzae*, *S. aureus*). Deficiencies of the regulatory proteins properdin and Factor D, as well as of the terminal components of complement activation (C5–C9), are associated with an increase in susceptibility to *Neisseria* infections, reflecting the important role of cytolytic complement activity in the innate immune response against *Neisseria*. Deficiencies of Factors H and I are associated with increased pyogenic infections (*N. meningitidis*, *H. influenzae*, and *S. pneumoniae*). For some complement deficiencies, the lack of complement function in antibacterial immunity may be compensated for by the production of high levels of pathogen-specific IgG antibodies (107). Consequently, the infections may be more prevalent in childhood. Interestingly, deficiencies of some complement components are also associated with non-infectious conditions. For example, deficiencies of C1q,r,s, C4, and C2 are associated with systemic lupus erythematosus (SLE)-like disease, reflecting the

important role of the classical complement pathway in clearance of immune complexes from the body. In these complement deficiencies, the autoimmune manifestations may be of greater clinical significance than the increased susceptibility to infections. Similarly, deficiencies of factors H or I most commonly present with atypical hemolytic uremic syndrome. The most obvious example of a non-infectious condition associated with a complement component deficiency is the association between C1 inhibitor deficiency and hereditary angioedema, in which patients suffer from (potentially life threatening) episodic attacks of tissue edema, due to loss of the inhibitory role of C1 inhibitor in cleavage of high molecular weight kininogen to produce bradykinin.

Deficiencies of anti-microbial peptides are less well defined. Anti-microbial peptides play an important role in immune defense in *Drosophila* (108). LL-37-knockout mice have been generated, and are described as having an increased susceptibility to a number of Gram-negative bacterial infections (109–113), suggesting a broad role for anti-microbial peptides in the immune response to infections in mammals. To date, genetic deficiencies of anti-microbial peptides have not been defined in humans. However, reduced expression of anti-microbial peptides in patients has been associated with increased susceptibility to infections of skin and periodontal gingiva (114–116). As we move toward an era in which exome sequencing becomes a feasible approach for defining genetic defects predisposing to immune deficiencies in patients, the significance of deficiencies of anti-microbial peptides in defense against infections may become apparent.

## ROLE OF COMPLEMENT AND ANTI-MICROBIAL PEPTIDES IN ACUTE INFLAMMATION

Activation of complement reveals beneficial functions such as pathogen sensing and defense and clearing injured cells on the one hand; however, complement has been shown to play a major role in pathogenesis of various inflammatory processes on the other hand. In response to pathogens or tissue damage, complement is highly capable of inducing all classical signs of inflammation such as redness, pain, hyperthermia, and swelling. Complement products lead to a release of pro-inflammatory mediators, upregulation of adhesion molecules, and increased vascular permeability of endothelial cells (117). Besides the beneficial effect of clearing an infection locally, complement activation may also contribute to a life-threatening systemic inflammatory response (118). Both the classical and alternative complement pathways appear to be activated during sepsis (119) resulting in elevated levels of the complement activation products C3a, C4a, and C5a (120). Among those, C5a appears to be the most harmful molecule (121). Complement activation seems to play a role in acute inflammation in lung and liver, where it has been correlated to acute respiratory distress syndrome and to acute humoral rejection, respectively (122, 123). Part of its detrimental complement activation derives from the crosstalk to other activation systems, such as the kininogen pathway and coagulation cascade (124). Besides, systemic complement activation has been confirmed in stroke patients (125). The anaphylatoxins C3a and C5a exert both protective and harmful functions in the central nervous system (126, 127). Direct contact between blood and cerebrospinal fluid in blood-brain barrier dysfunction leads to production of C1q and generation of C3a,



and C5a, which in turn contributes to intracranial inflammation by induction of blood–brain barrier damage and increase in vascular permeability (128, 129). Another example for complement activation is ischemia–reperfusion injury. In ischemia and during reperfusion, complement is activated via the classical, the alternative, and the MBL pathway (130–132). Inhibition of the

complement cascade greatly reduced myocardial damage after myocardial infarction (133–135). The role of complement in atherosclerosis remains controversial. Several studies revealed a protective role of complement activation in cardiovascular diseases such as atherosclerosis or vasculitis. The protective effect of complement in the pathogenesis of atherosclerosis has been shown by C3<sup>-/-</sup> mice exhibiting accelerated development of atherosclerosis (136). We have previously reported on the complexity in design and analysis of complement-targeted mouse models (137). However, a recent population based cohort study showed that unlike C3a, C3, and C5a are not associated with atherosclerosis (138). This suggests that C3a and C3 have distinct roles in pathways leading to cardiovascular diseases. In contrast, a murine study reported that systemic inhibition of complement by Crry–CR2 reduced development of atherosclerosis (139).

Anti-microbial peptides play a modulatory role in acute inflammation via modulation of cytokine production, recruitment of immune cells to the site of injury, and enhancement of phagocytosis (140). Stimulation with IL-4 or IL-13 – classical Th2 response cytokines – leads to rapid Paneth cell degranulation and subsequent release of anti-microbial peptides (141). Anti-microbial peptides play an important role in maintaining the skin barrier and protection against infections. This has been experimentally underlined by mice deficient for LL-37 (142). In addition, LL-37, HBD-2, and 3 are highly expressed in epidermal keratinocytes in response to injury or infections of the skin (143). It has been further shown that LL-37 prevents sepsis by directly dampening pro-inflammatory signaling initiated by LPS (102). Therefore, it may also play a role in dialysis-related peritonitis where endotoxins are

present. Defects in defensin expression have been shown to contribute to a number of mucosal inflammatory diseases, including necrotizing enterocolitis and inflammatory bowel disease (144). Moreover, differentially regulated expression of epithelial-derived anti-microbial peptides has been shown in acute appendicitis. Arlt et al. (145) showed that the anti-microbial peptide HBD-1 is downregulated in patients with acute appendicitis, whereas HNP1–3, HD5 and HD6, and HBD2 and 3 are upregulated, suggesting that differential regulation of the innate immune system is coincident with altered bacterial diversity.

### THE CASE OF C3a AND OTHER ANTI-MICROBIAL AGENTS

Structural criteria together with functional *in vitro* data suggest that C3a and C4a, but not C5a (all split products of complement activation), may qualify as anti-microbial peptides *per se* (51). C3a (9 kDa), C3a<sub>desarg</sub>, and synthetic peptides derived from C3a were compared to LL-37 (5 kDa when processed) for their inhibitory effect on *E. coli*, *E. faecalis*, and *P. aeruginosa*, their heparin binding, liposome permeabilization and were found to be strikingly similar (146). Structurally, C3a contains  $\alpha$ -helical regions characteristic of anti-microbial peptides, which were found represented in proteolytic fragments generated by the enzymatic activities of cells involved in the acute inflammatory response, such as neutrophils and mast cells (147).

Anti-microbial activity and heparin binding ability are described for histidine-rich peptides (148). Histidine-rich motifs in peptides that relate to anti-microbial activity are conserved (149) and as artificial tags are indeed exploited in subcellular targeting (150). Non-removal of histidine tags after expression of recombinant proteins for the purpose of testing anti-microbial activity bears inherent problems, and findings have to be viewed with utmost caution (151–154). Awareness of this potential pitfall was raised in a very pertinent article in 2013 (155).

By contrast, proteolytic cleavage of high molecular weight kininogen during bacterial infection generates an internal peptide, which has antibacterial activity that compares to LL-37 (156). Similarly, in bovine plasma, activated kallikrein releases from high molecular weight kininogen a histidine-rich fragment (157). Nordahl et al. (152) demonstrated effective antibacterial activity of a histidine-rich peptide generated from high molecular weight kininogen. However, the effect may be potentiated by the presence of the uncleaved histidine tag.

### CONCLUSION

In conclusion, much is to be learnt from cross-specialty comparisons.

Apart from refining one's experimental design (cave histidine tags), greater clarity was gained in the use of the term "anti-microbial peptide." Often, an analog (functionally similar gene product), not homolog (shared ancestry) is meant, and sometimes, a recombinantly expressed or proteolytically generated section only of a protein.

While having important functions in maintaining tissue homeostasis, anti-microbial peptides and complement are both involved in shaping the immune response and transcend from the purely innate immunity realm to adjuvant the adaptive immune response.

In many aspects of health and disease, complement and anti-microbial peptides are remarkably similar in function, sharing certain features and broad range of activities (Figures 1A,B). They may, however, operate at differing preponderance in separate niches, e.g., blood/tissue, epithelial cells/macrophages (Figures 2A,B), supporting the view that two specialist systems are operating in a complementary way. In the context of beneficial activity of immune modulators applied clinically in sepsis, such as Vitamin D (158) and more recently omega-3 fatty acid preparations (159), parallel measurements of, e.g., C3 and LL-37, produced by cells, which express Vitamin D receptor (VDR) and  $\omega$ -3 fatty acid receptor (GPR120), would provide the type of comparative analyses needed to direct this overlapping field.

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