



# The Coagulation Factors Fibrinogen, Thrombin, and Factor XII in Inflammatory Disorders—A Systematic Review

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**Background:** The interaction of coagulation factors has been shown to go beyond their traditional roles in hemostasis and to affect the development of inflammatory diseases. Key molecular players, such as fibrinogen, thrombin, or factor XII have been mechanistically and epidemiologically linked to inflammatory disorders like multiple sclerosis (MS), rheumatoid arthritis (RA), and colitis.

**Objectives:** To systematically review the evidence for a role of coagulation factors, especially factor XII, fibrinogen, and thrombin in inflammatory disorders like MS, RA, and bowel disorders.

**Methods:** A systematic literature search was done in the PubMed database to identify studies about coagulation factors in inflammatory diseases. Original articles and reviews investigating the role of the kallikrein–kinin and the coagulation system in mouse and humans were included.

**Results:** We identified 43 animal studies dealing with inflammatory disorders and factors of the kallikrein–kinin or the coagulation system. Different immunological influences are described and novel molecular mechanisms linking coagulation and inflammation are reported.

**Conclusion:** A number of studies have highlighted coagulation factors to tip the balance between hemostasis and thrombosis and between protection from infection and extensive inflammation. To optimize the treatment of chronic inflammatory disorders by these factors, further studies are necessary.

**Keywords:** coagulation factors, neuroinflammation, contact system, fibrinogen, factor XII, thrombin

## INTRODUCTION

The coagulation system is a highly regulated cascade that ultimately leads to blood clot formation. The primary purpose of coagulation is hemostasis, i.e., to stop bleeding from a damaged blood vessel.

The concept of a stepwise process or cascade of the coagulation system was first described in 1964 (1). While this traditional model described two separate pathways, the intrinsic and the extrinsic pathway, which culminate in a common pathway, current views support an interconnected

relationship between the two (2, 3). The contact pathway (also called the kallikrein–kinin system) is composed of three zymogens [factor XII (FXII), plasma kallikrein (PK), and high-molecular-weight kininogen (HMWK)]. *In vitro*, the initial triggering event leads to clot formation through the activation of FXII on an artificial surface. However, *in vivo*, the activation of this factor is still under discussion, so that current studies consider tissue factor (TF), a transmembrane glycoprotein expressed in perivascular tissue, to be the main initiator of *in vivo* blood clotting (4). TF forms a complex with factor VII (FVII) to activate factor X (FX) either directly or by activating factor IX (FIX). Both pathways interlace with the activation of FX, which leads to the cleavage of prothrombin (factor II) to thrombin (factor IIa). In the last step, thrombin mediates the cleavage of fibrinogen to fibrin monomers that, upon polymerization, form a fibrin clot and stop bleeding. The formation of these clots is dependent on the availability of thrombin, calcium, and negatively charged phospholipid membranes. The whole coagulation cascade is very tightly regulated with several checkpoints that function in a positive or negative feedback loop (5).

However, in recent years, significant evidence has emerged implicating coagulation factors also in tissue repair and inflammatory responses. In line with this, several of the major coagulation factors, like TF, thrombin, or fibrinogen, are described as potential drivers of inflammation in disease models, such as sepsis, endotoxemia, encephalomyelitis, or multiple sclerosis (MS) (2, 6–8). Thereby, these factors not only enhance inflammation in the bloodstream, but also within tissues. Furthermore, it is known that FXII triggers the release of bradykinin (BK) from HMWK through cleavage by PK, leaving two chain HMWK behind, which has numerous adhesion-regulatory properties (9, 10) including inhibitory activity on the interaction between fibrinogen and the leukocyte integrin CD11b/CD18 (11). Binding of BK to the BK receptors can activate proinflammatory pathways that induce chemotaxis of leukocytes and increase vascular permeability (12).

A proteomic analysis has been performed on human brain material from individuals with MS identifying a dysregulation of several proteins of the coagulation cascade, such as TF or protein C inhibitor (13). Furthermore, in an animal model of MS, i.e., experimental autoimmune encephalomyelitis (EAE), it could be shown that other factors, such as FXII or thrombin, are upregulated in the central nervous system (CNS) (14, 15). It has been demonstrated that this dysregulation of the coagulation system is not restricted to the CNS but can also be found in the peripheral blood (15). Both FXII and thrombin are highly upregulated in the plasma of patients with MS (16). Moreover, dysregulation of BK receptors was found to be relevant in MS (17, 18).

Although further studies using animal models of MS are required, the available data indicate that the interplay between coagulation factors and immune cells and/or brain endothelial cells may modulate initiation and/or the course of neuroinflammatory disorders.

In this review, we summarize key links between inflammation and coagulation, with a specific focus on the molecular

roles of the clotting factors FXII, fibrinogen, and thrombin in neuroinflammation as well as in neuroinflammatory disorders. The role of coagulation factors in non-neurological inflammatory disorders is also discussed. The evidence presented here suggests that manipulation of components of the coagulation system could be potentially therapeutically exploitable not only in inflammatory disorders of the CNS but also in autoimmune diseases in general.

## METHODS

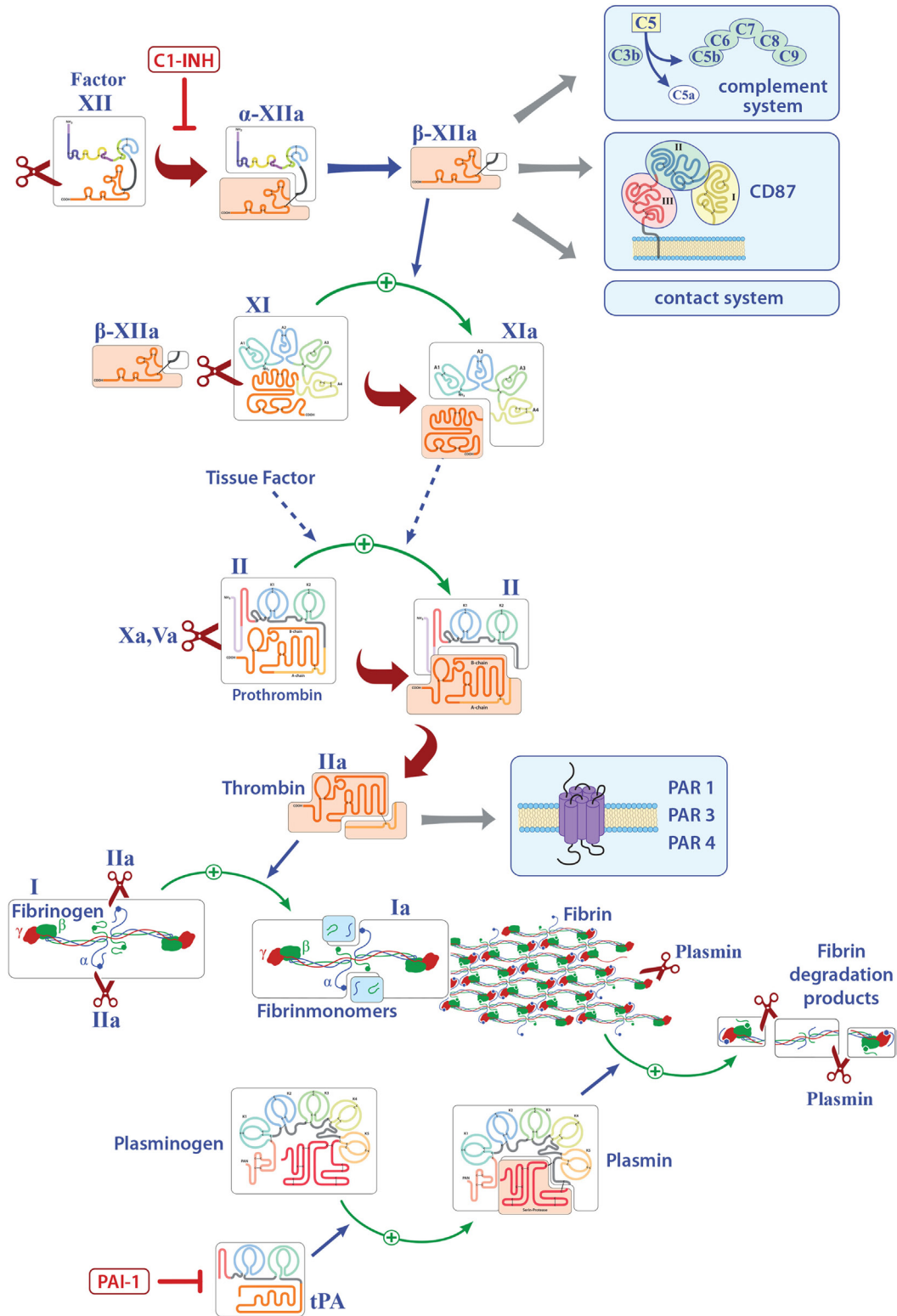
A literature review was done in December 2017 searching the PubMed database using the search items: BK, coagulation factors, colitis, complement, Crohn's disease, EAE, FXII, fibrinogen, inflammatory bowel disease, kallikrein–kinin system, thrombin, MS, and rheumatoid arthritis (RA). The search terms were used in different combinations and plural forms, and the search was limited to articles in English. References were screened for additional articles. Studies in mouse and human were included.

## RESULTS AND DISCUSSION

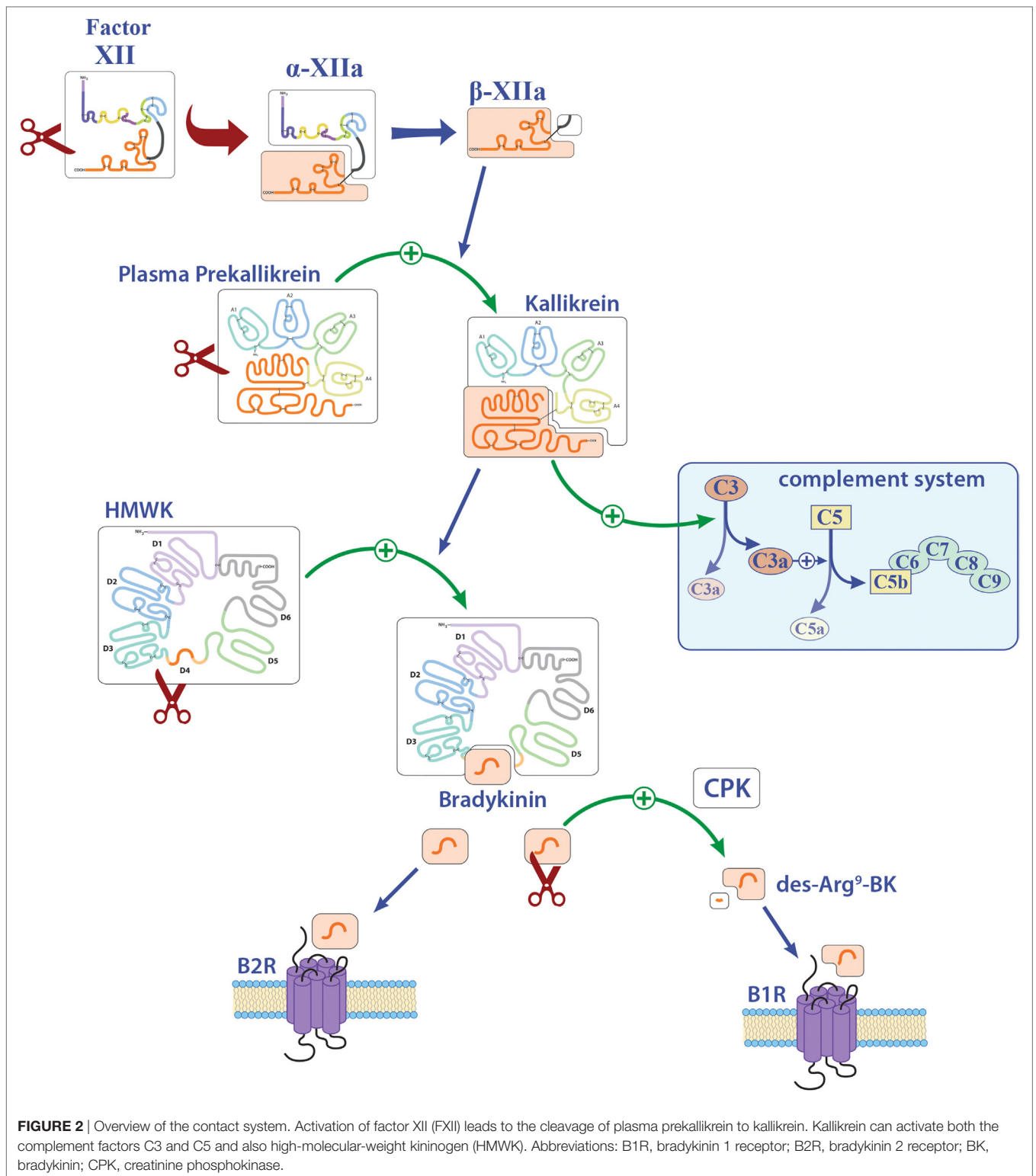
### Factor XII and Neuroinflammation

Factor XII is a soluble zymogen with a molecular weight of approximately 80 kDa that is produced in the liver (3). FXII consists of a heavy chain (353 residues) and a light chain (243 residues) held together by a disulfide bond (**Figure 1**) (19). It has several domains, namely, a leader peptide, a fibronectin type II domain, an epidermal growth factor (EGF)-like domain, a fibronectin type I domain, a second EGF-like domain, a kringle domain, a proline-rich region, and the catalytic domain (**Figure 1**). Proteolytic cleavage of its R353–V354 site converts the zymogen FXII to activated FXII (FXIIa). This cleaved protein circulates as a two-chain protein, a heavy and a light chain, held together by a disulfide bond (19). *In vitro*, FXII can be activated by PK, plasmin, or on negatively charged surfaces, while *in vivo* activation is still under debate (20, 21). FXIIa is suggested to initiate the intrinsic coagulation, the contact, and complement systems (**Figure 1**). Thus, FXIIa leads to the cleavage of PK to generate active PK (contact system, **Figure 2**), triggers fibrin formation through the activation of factor XI (FXI; **Figure 1**), and activates the complement pathway. The serine protease C1 inhibitor (C1-INH) is the major inhibitor of FXII, and thereby controls its proteolytic activity. Besides C1-INH, antithrombin III and plasminogen activator inhibitor I also have FXII-inhibitory capacity. Despite its contribution to fibrin formation *in vitro*, FXII seems not to be essential for hemostasis *in vivo* (21, 22). However, under pathological conditions, FXII participates in thrombus formation and thromboembolic disorders, such as stroke (23).

In terms of neuroinflammation, we have been able to show that FXII deficiency leads to an attenuated disease severity in EAE, accompanied by reduced numbers of interleukin (IL)-17A-producing effector T helper cells (T<sub>H</sub>17). The role of FXII in EAE was mediated by its ability to shift the cytokine profile



**FIGURE 1** | Overview of the known pathways activated by factor XII (FXII) and the extrinsic coagulation system. Only factors that are involved in inflammation are shown. Activated FXII leads to the cleavage of factor XI, activates the intrinsic, the contact, and complement systems and can bind to CD87. Tissue factor finally leads to the release of thrombin (FIIa) that can directly bind several receptors and activates fibrinogen to fibrin. Deposition of fibrin is regulated by plasmin. Abbreviations: C1-INH, serine protease C1 inhibitor; CD87, urokinase-type plasminogen-activator receptor; tPA, tissue plasminogen activator; PAI-1, plasminogen-activator inhibitor 1; PAR, protease-activated receptor.



**FIGURE 2** | Overview of the contact system. Activation of factor XII (FXII) leads to the cleavage of plasma prekallikrein to kallikrein. Kallikrein can activate both the complement factors C3 and C5 and also high-molecular-weight kininogen (HMWK). Abbreviations: B1R, bradykinin 1 receptor; B2R, bradykinin 2 receptor; BK, bradykinin; CPK, creatinine phosphokinase.

of dendritic cells (DC) necessary to induce differentiation of effector T cells (see also **Table 1**) (15). Pharmacologic inhibition of FXII by recombinant human albumin-tagged infestin-4 (24) resulted in decreased EAE severity as well (see **Table 1**). These findings suggest a potential novel link between FXII and

the immune system in neuroinflammation. Strikingly, we also found significantly increased FXII plasma activity in individuals with relapsing–remitting MS and secondary progressive MS, as compared to healthy donors, thus, indicating a role for this factor in human MS pathogenesis (15).

**TABLE 1** | Studies of intrinsic and contact system factors: effects on neuroinflammatory processes in transgenic mice or using pharmacological substances.

Mouse line or treatment	Model (peptide)	Genetic background	Effects in models of autoimmune neurodegeneration	Reference
<i>B1R</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, decreased expression of endothelial adhesion molecules, reduced migration of lymphocytes	(25)
			Earlier EAE onset, severe clinical disease course with enhanced inflammation, demyelination, glial activation, increased migration of CD4 <sup>+</sup> T cells, especially T <sub>H</sub> 17 cells	(26)
			Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, decreased cytokine production of CD4 <sup>+</sup> T cells	(27)
			Delayed EAE onset, reduced clinical deficits, reduced glial activation, decreased release of proinflammatory mediators by astrocytes	(28)
Des-Arg <sup>3</sup> -BK (B1R activator)	Active EAE (MOG)	C57BL/6	No clinical effect	(27)
R838 (B1R agonist)	Active EAE (MOG)	C57BL/6	Aggravated disease course, enhanced inflammation, demyelination, axonal damage	(25)
	Active RR-EAE (PLP)	SJL	Milder clinical deficits Reduced clinical deficits	(26) (26)
Des-Arg <sup>3</sup> -[Leu <sup>25</sup> ]-BK (B1R inhibitor)	Active EAE (MOG)	C57BL/6	Delayed EAE onset, reduced clinical deficits, decreased release of proinflammatory mediators by astrocytes	(28)
			Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, decreased cytokine production of CD4 <sup>+</sup> T cells	(27)
R715 (B1R inhibitor)	Active EAE (MOG)	C57BL/6	Reduced clinical deficits, reduced inflammation, demyelination, axonal damage	(25)
			Accelerated disease onset	(26)
<i>B2R</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	No effect on clinical course, immune cells, cytokine production	(25, 26)
			Moderate reduced clinical deficits, reduced inflammation, reduced leukocyte adhesion, decreased chemokine (CCL2, CCL5) production	(29)
HOE-140 (B2R inhibitor)	Active EAE (MOG)	C57BL/6	Moderate or no effect on clinical disease course, immune cells, cytokine production	(25–28)
			Moderate reduced clinical deficits, reduced inflammation, reduced leukocyte adhesion, decreased chemokine (CCL2, CCL5) production	(29)
<i>F12</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, reduced T <sub>H</sub> 17 cells, decreased cytokine production (IL-6, IL-23) of DC	(15)
rHA-Infestin 4 (FXIIa inhibitor)	Active EAE (MOG)	C57BL/6	Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, reduced cytokine production (IL-6, IL-17A)	(15)
	Active EAE (PLP)	SJL	Ameliorated disease course	(15)
<i>F11</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	No effect on clinical symptoms, inflammation and demyelination	(15)
<i>C1q</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	No effect on clinical symptoms	(30)
<i>C3</i> <sup>-/-</sup>	Active EAE (MOG)	129SVJ/ C57BL/6	Reduced clinical deficits, decreased inflammation and demyelination	(31)
		C57BL/6	No clinical effect, but higher mortality, tendency to enhanced inflammation and demyelination	(32)
<i>C3</i> <sup>-/-</sup>	Active + AT-EAE (MOG)	C57BL/6	Reduced clinical deficits, reduced infiltration of T cells	(33, 34)
<i>C5</i> <sup>-/-</sup>	Active EAE (guinea pig myelin)	B10.D2/oSnJ	Moderate reduction of clinical deficits, narrow zones of inflammation and demyelination, gliosis, reduced remyelination, enhanced apoptosis of oligodendrocytes, axonal damage	(35, 36)
<i>C5a/GFAP</i>	Active EAE (MOG)	C57BL/6	No clinical effect	(37)
<i>Cd87</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	Aggravated disease course, enhanced inflammation, axonal damage, reduced T-cell proliferation and cytokine production	(38)
			Delayed disease onset, reduced clinical deficits, reduced inflammation, enhanced demyelination and axonal damage	(39)
	Active EAE (MOG) in BM-chimera		Reduced clinical deficits with <i>Cd87</i> <sup>-/-</sup> BM, partial protection of <i>Cd87</i> <sup>-/-</sup> hosts	(15)

AT, adoptive transfer; B1R, bradykinin receptor 1; B2R, bradykinin receptor 2; BK, bradykinin; BM, bone marrow; CCL, chemokine (C-C) motif ligand; DC, dendritic cells; EAE, experimental autoimmune encephalomyelitis; FXIIa, activated factor XII; IL, interleukin; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; rHA, recombinant human albumin; RR, relapsing-remitting; T<sub>H</sub>17, IL-17A-producing effector T helper cells.

As aforementioned, FXIIa leads to the cleavage of FXI (Figure 1). However, studies from our laboratory indicate that the latter factor has no significant role in EAE (see also Table 1), suggesting that not the entire intrinsic coagulation system is involved, but rather that the effect of FXII in neuroinflammation is dependent on other pathways triggered by FXII (15).

In particular, besides hemostasis, FXII leads to the activation of the contact system and hereby to the release of BK (Figure 2). Reports on the function of BK in MS and EAE remain contradictory. While three reports described a protective role of genetic or pharmacological inhibition of one distinct bradykinin receptor (bradykinin receptor 1, B1R), another study demonstrated enhanced inflammation by B1R blockade (see also Table 1) (25–28). For MS patients, B1R has been shown to have a detrimental effect, as it is upregulated on T-lymphocytes from patients with either secondary progressive MS or relapsing–remitting MS during active relapse (17). Levels of B1R expression on mononuclear cells correlate positively with the Expanded Disability Status Scale, with occurrence of clinical relapses and lesion volumes on T2-weighted images, but not with gadolinium-enhancing lesions (40). Furthermore, a potential role for B1R has been described in the regulation of blood–brain barrier permeability and chemokine production (18), indicating this factor's involvement in neuroinflammation.

FXII has the capacity to activate the classic complement pathway by direct cleavage of C1q (20). However, it has been shown that C1q has no influence on neuroinflammation, at least in terms of clinical symptoms (30). It is known that PK can activate the complement components C3 and C5 (20). However, reports on these members of the complement system in the context of neuroinflammation remain elusive. While three reports showed a significant role of C3, as C3-deficient animals had an attenuated EAE disease course and reduced T-cell infiltration (31, 33, 34), another study showed no clinical differences, but a tendency to enhanced inflammation and demyelination (32). For C5, a dual role in EAE has been suggested: One study revealed that C5 leads to reduced inflammation and tissue repair in acute lesions, while this factor seemed to be responsible for increased axonal damage and enhanced gliosis in chronic lesions (35). Furthermore, it has been shown that C5 can limit oligodendrocyte apoptosis in EAE, thus promoting remyelination (36). Use of transgenic mice that express C5 under the astrocytic-specific glial fibrillary acidic protein promoter revealed no significant contribution to disease development of this component in the CNS (37), so that the role of complement in EAE remains contradictory.

Although most investigations focus on FXII as a serine protease, FXII can interact with cells independently of its enzymatic activity. In line with this, FXII can bind to urokinase plasminogen activator receptor (uPAR, CD87; Figures 1 and 3) (20). Studies from our laboratory have demonstrated high levels of CD87 on DC. In this context, we could show that FXII exerts its immunoregulatory effects directly *via* CD87 and by regulating cyclic adenosine monophosphate (cAMP) and thereby cytokine levels (e.g., IL-6, IL-23) in DC. In contrast, we could rule out the involvement of alternative FXII-triggered pathways, such as the

intrinsic coagulation, the contact and complement systems, for EAE pathogenesis.

However, reports on the relevance of CD87 inhibition, *per se*, in EAE remain contradictory: while two reports indicated a protective role in terms of clinical score and inflammation, when CD87 was missing (15, 39), and another showed enhanced inflammation (38).

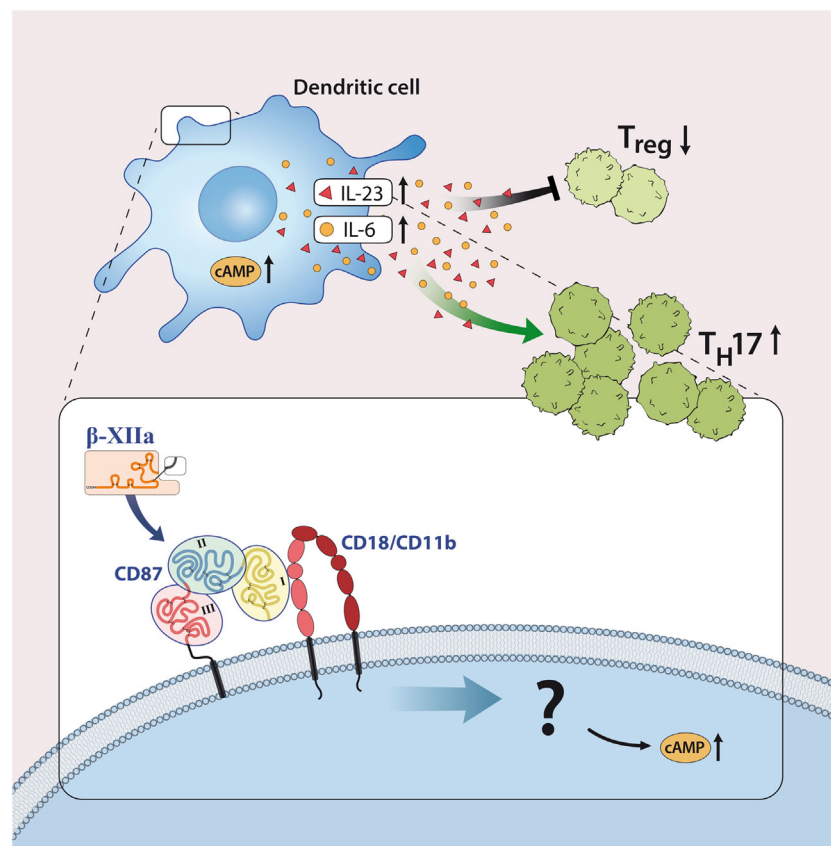
In conclusion, the data so far indicate a significant role of FXII and downstream factors and pathways in neuroinflammation. However, further studies are needed to clarify remaining contradictions.

## Fibrinogen and Neuroinflammation

Fibrinogen (Factor I) is a 340-kDa glycoprotein that is synthesized in the liver (41). It is activated to fibrin by thrombin, exposing several polymerization sites that are crosslinked to an insoluble fibrin clot under the involvement of activated factor XIII (41, 42). Although activation of the coagulation system and thereby fibrin formation is essential for stopping lethal hemorrhage, the deposition of fibrin is carefully regulated to avoid thrombotic incidents (43). This is achieved by the fibrinolytic system in which plasmin especially counterbalances the procoagulatory signals, leads to clot dissolution, and results in the generation of soluble fibrin fragments, such as fragments D and E, and D-dimers (44). Plasmin generation is regulated by two proteases, tissue plasminogen activator (tPA) and uPA (45), which are controlled by plasminogen activator inhibitor-1 (PAI-1; Figures 1 and 4) (46).

Under physiological conditions, the plasma concentration of fibrinogen is between 2 and 4 g/l; however, it is known that this concentration can rapidly increase under pathological conditions (acute phase reactions), such as injury, infection, or inflammation (47, 48). Similarly, elevated levels of fibrin degradation products, such as D-dimer, are used in clinical practice as indicators of inflammation and risk predictors of thrombotic events (49). In the majority of cases, the proinflammatory function of fibrin/fibrinogen is mediated by its ability to bind to different immune cells for instance to the CD11b/CD18 integrin receptor (also termed Mac-1) on macrophages, monocytes, or microglia that induces the release of reactive oxygen species and is required for axonal damage in EAE (8, 47, 50). In this context, it has been shown that binding of fibrin/fibrinogen to the CD11b/CD18 integrin receptor results in activation of proinflammatory cascades, such as nuclear factor  $\kappa$ B, which leads to the release of inflammatory cytokines, like tumor necrosis factor (TNF)- $\alpha$  or IL-1 $\beta$  (51, 52) and can thereby influence diseases such as RA (53) or colitis-associated cancer (Figure 4) (54). In addition, fibrinogen-dependent effects of platelets may also contribute to EAE disease pathogenesis (55).

A detrimental role of fibrin/fibrinogen has also been suggested for neuroinflammation, as fibrin deposition in the CNS correlates with microglial activation in active MS lesions (56, 57). In line with this finding, it has been shown that fibrinogen can directly activate microglia, enhance their phagocytic ability, induce peripheral macrophage recruitment and local CNS activation of myelin antigen-specific T<sub>H</sub>1 cells (58, 59). Moreover, genetic deletion of fibrinogen resulted in reduced inflammation



**FIGURE 3** | Factor XII (FXII) as a mediator of inflammatory disease. FXII acts on dendritic cells through the urokinase-type plasminogen-activator receptor (CD87) to enhance the release of interleukin (IL)-6 and -23. This cytokine shift leads to increased amounts of IL-17A-producing CD4<sup>+</sup> effector T helper cells (T<sub>H</sub>17). Abbreviations: AC, adenylate cyclase; CD11b/CD18, leukocyte integrin adhesion molecule.

and demyelination using a TNF transgenic model of MS (mice that lack the TNF receptor, develop spontaneous clinical symptoms of paralysis, and die by 5 weeks of age; *TgK21fib<sup>-/-</sup>*; see also **Table 2**) (60). Furthermore, inhibition of fibrinogen binding to CD11b/CD18 by genetic mutation of the CD11b/CD18-binding motif (*Fibγ<sup>390-396A</sup>*) (61) or a peptide ( $\gamma^{377-395}$ ) results in reduced microglial activation and an attenuated disease course in EAE (see also **Table 2**) (58).

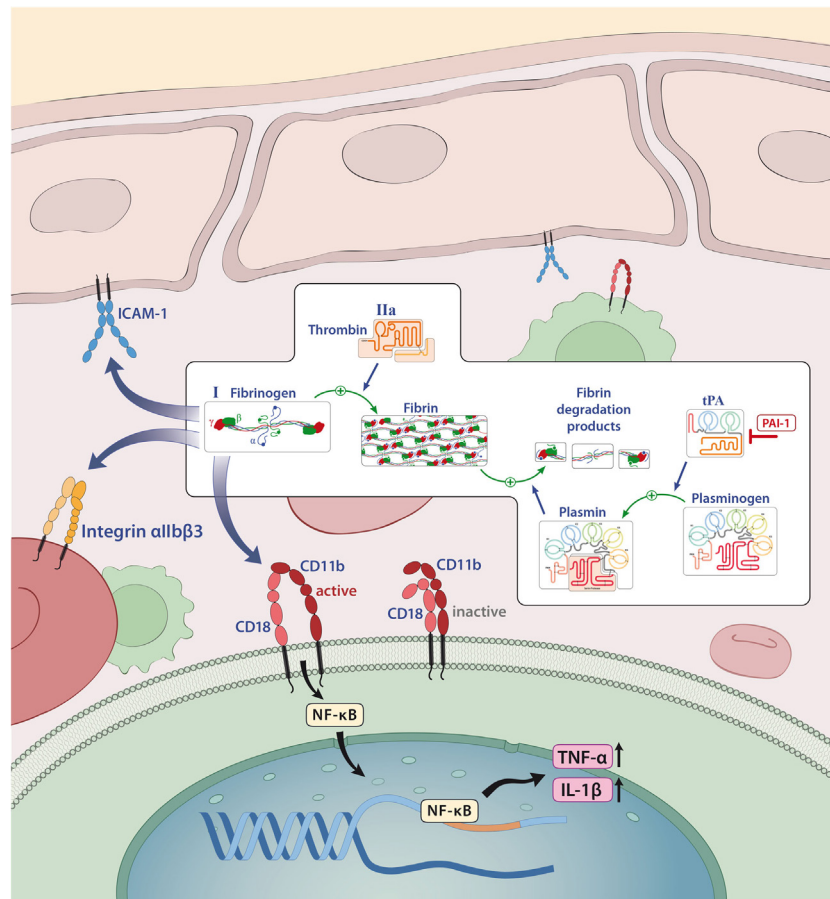
Interestingly, none of these inhibitory approaches interferes with the clotting function of fibrinogen (53, 58). Moreover, staphylococcal-derived extracellular adherence protein, which, among others, interferes with the interaction between CD11b/CD18 and fibrinogen, also suppressed murine EAE disease severity (67), while pharmacological treatment strategies with snake venom-derived defibrinogenating agents, such as anrod or batroxobin, suppress clinical symptoms in different animal models of MS (see also **Table 2**) (60, 62–64, 68).

Enhanced fibrin deposition is usually counterbalanced by plasmin that is generated by tPA and uPA. Interestingly, uPA as well as PAI-1 are significantly increased in acute MS lesions, while tPA levels are unchanged (69, 70). Results concerning tPA activity remain contradictory; while one report indicates a reduction in tPA activity in normal-appearing white and gray

matter and lesions of individuals with MS (70), others describe a significant increase of activity in lesions and the cerebrospinal fluid of MS patients during the acute, but not the chronic disease phase (71).

When EAE was induced in *uPA<sup>-/-</sup>* mice, these animals displayed an aggravated disease course. This finding was accompanied by enhanced microglial activation (see also **Table 2**) (38). In line with these results, treatment with a PA inhibitor-derived peptide (PAI-1-dp) that increases plasminogen activation ability of uPA, suppressed the development of EAE symptoms (see also **Table 2**) (38). In contrast, another publication using  $\epsilon$ -aminocaproic acid, an inhibitor of plasminogen and trypsinogen activator, reported a suppression of EAE severity (see also **Table 2**) (65).

Results of EAE experiments in tPA-deficient animals remain contradictory: while two publications described increased severity and a delayed recovery with enhanced demyelination and axonal damage after genetic depletion of tPA, disease onset was reported to be either earlier or delayed in the literature (see also **Table 2**) (39, 66). Due to the significant upregulation of PAI-1 in MS patients, EAE induced in PAI-1-deficient animals was shown to have moderate clinical protection with reduced perivascular cuffs, but no difference in terms of demyelination or axonal damage was observed (39).



**FIGURE 4 |** Fibrinogen as a mediator of inflammation. Fibrinogen acts on different cells through integrin and non-integrin receptors to induce specific inflammatory effects. Abbreviations: CD11b/CD18, leukocyte integrin adhesion molecule; ICAM-1, intercellular adhesion molecule-1; IL-1 $\beta$ , interleukin-1 $\beta$ ; NF- $\kappa$ B, nuclear factor  $\kappa$ B; tPA, tissue plasminogen activator; PAI-1, plasminogen-activator inhibitor 1; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ .

Nonetheless, data so far indicate a significant role of local fibrin deposits in neuroinflammation and indicate a promising anti-inflammatory therapeutic potential of targeting this pathway.

## Thrombin and Neuroinflammation

Prothrombin (factor II) is a soluble 72-kDa protein that is produced by the liver. It is activated to thrombin (factor IIa) *via* enzymatic cleavage of two sites by activated FX (FXa). Activated thrombin leads to cleavage of fibrinogen into fibrin monomers that, upon polymerization, form a fibrin clot. Therefore, activation of prothrombin is crucial in physiological and pathophysiological coagulation. For instance, various rare disorders, such as congenital hypoprothrombinemia (a blood disease in which deficiency of prothrombin results in impaired blood clotting) and acquired hypoprothrombinemia (e.g., in autoimmune diseases with lupus anticoagulant) have been described (72, 73).

Beyond its key role in coagulation, thrombin can mediate further effects, e.g., thrombin is a potent vasoconstrictor and is implicated in vasospasms following subarachnoid hemorrhage (74).

In terms of neuroinflammation, thrombin activity was found to be significantly increased in the spinal cord of mice with EAE (14). Thrombin activity precedes the onset of neurological signs and correlates with the amount of fibrin deposition, microglial activation, demyelination, axonal damage, and clinical severity. Interestingly, inhibition of thrombin activity by hirudin leads to a significant improvement of disease severity (13) (see also **Table 2**). This is accompanied by decreased immune cell proliferation and cytokine secretion, as well as a reduction in the number of inflammatory lesions (13). Furthermore, it has been shown that levels of thrombin inhibitors are significantly increased during EAE. For instance, antithrombin III (as well as protease nexin 1) were detected at higher levels in CNS homogenates during EAE compared with controls (75). Additionally, it was recently shown that prothrombin levels are elevated in plasma of patients suffering from relapsing-remitting MS or secondary progressive MS indicating a prominent role of this coagulation factor in neuroinflammation (16).

## Coagulation Factors in Non-Neurological Inflammatory Diseases

An increasing body of evidence also supports a decisive role of coagulation factors in regulating inflammatory responses



**TABLE 2** | Studies of coagulation system factors: effects on neuroinflammatory processes using transgenic mice or pharmacological substances.

Mouse line (genetic background)	Model (peptide)	Genetic background or species	Neuroinflammatory effect	Reference
$\gamma^{377-395}$	Active RR-EAE (PLP)	SJL	Reduced clinical symptoms, decreased inflammation, reduced microglial activation	(58)
Ancrod	Active RR-EAE (PLP)	SJL	Reduced clinical symptoms, decreased microglial activation and demyelination, no impact on immune cell proliferation	(58)
	TNF-transgenic model (no peptide, spontaneous)	Tg6072 TNF transgenic mice	Reduced demyelination, downregulation of MHC-I	(60)
	Active EAE	Rat	Reduced clinical symptoms, decreased inflammation and fibrin deposition	(62)
Batroxobin	Active EAE (MOG)	C57BL/6	Reduced clinical symptoms, decreased inflammation, demyelination, no effect on axonal damage	(63)
	AT-EAE (GP-MBP)	Rat	Attenuated disease course, reduced fibrin depositions, no effect on inflammation and immune cell proliferation	(64)
$\epsilon$ -Aminocaproic acid	Active EAE	Rat	Attenuated disease course	(65)
<i>Fib</i> $\gamma^{390-396A}$	Active EAE (MOG)	C57BL/6	Reduced clinical symptoms, decreased inflammation, demyelination, reduced microglial activation	(58)
Hirudin	Active EAE (PLP)	SJL/J	Improvement of clinical deficits, reduced inflammation, decreased immune cell proliferation and cytokine production	(13)
<i>PAI-1</i> <sup>-/-</sup>	Active CREAE (spinal cord homogenate)	ABH	Reduced incidence, moderately delayed onset, reduced inflammation, no effect on demyelination and axonal damage	(39)
<i>PAI-1</i> -dp (uPA activator)	Active EAE (MOG)	C57BL/6	Attenuated disease course, reduced Tcell proliferation and cytokine production	(38)
<i>TgK21fib</i> <sup>-/-</sup>	TNF-transgenic model (no peptide, spontaneous)		Increased lifespan, delayed onset of clinical symptoms, reduced inflammation and demyelination	(60)
<i>tPA</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	Aggravated disease course, enhanced demyelination, axonal damage, fibrin deposition	(39)
			Delayed disease onset, aggravated disease course, delayed, but prolonged demyelination and axonal damage, reduced microglial activation	(66)
<i>uPA</i> <sup>-/-</sup>	Active EAE (MOG)	C57BL/6	Aggravated disease course, enhanced inflammation and microglial activation	(38)

AT, adoptive transfer; CREAE, chronic relapsing experimental allergic encephalomyelitis; EAE, experimental autoimmune encephalomyelitis; MHC-I, major histocompatibility complex class I; MOG, myelin oligodendrocyte glycoprotein; PAI-I, plasminogen activator inhibitor I; PAI-I-dp, plasminogen activator inhibitor I-depleted; PLP, proteolipid protein; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; RR, relapsing–remitting.

in non-neurological inflammatory diseases. For instance, a substantial contribution of different coagulation factors has been suggested in RA or inflammatory joint disease as fibrin depositions can be found in the joints of patients with RA (76). Moreover, the degradation products of fibrin, such as D-dimer, are used as common biomarkers for disease activity (77, 78). *In vitro*, it was shown that fibrinogen can enhance IL-8 secretion and intercellular adhesion molecule 1 expression from human synovial fibroblasts, leading to enhanced lymphocyte adhesiveness (79). A further direct proinflammatory role of fibrin/fibrinogen was suggested in RA pathogenesis as its genetic depletion in mice leads to an improvement in the clinical symptoms in animal models of RA and results in decreased synovial inflammation (see **Table 3**) (53). Interestingly, it was shown that the interaction of fibrinogen with immune cells *via* CD11b/CD18 is the relevant partner for this effect. Furthermore, pharmacological inhibition of thrombin *via*

hirudin resulted in a significant reduction in synovial inflammation and disease severity in two different animal models of RA (see **Table 3**) (80, 81). In this context, it could be shown that the plasmin activity is decreased, while PAI-1 levels are increased in both blood and inflamed joints of mice with collagen-induced arthritis (CIA) (82).

While treatment with uPA and tPA improves plasmin activity and removes fibrinogen depositions in joints, disease severity remains unchanged, challenging the pathophysiological role of fibrinogen in this context (82). Nonetheless, a significant contribution of uPA could be seen in other studies, but this remains contradictory for different arthritis models: in monoarticular models, uPA-deficient mice had an aggravated disease course (90, 93). In contrast, other studies using polyarticular animal models demonstrated resistance to or suppression of disease and reduced inflammation in animals lacking uPA, indicating a distinct role of uPA in different types of arthritis (91, 92, 94–96).

**TABLE 3** | Studies of coagulation system factors: effects on inflammatory processes using transgenic mice or pharmacological substances.

Mouse line or treatment	Model (peptide)	Genetic background or species	Effect in arthritis and colitis models	Reference
<i>B1R/B2R<sup>-/-</sup></i>	CAIA (anti-CII antibodies)	C57BL/6	Decreased clinical symptoms, reduced inflammation and cytokine levels	(83)
C11C1 (HMWK antibody)	Reactive arthritis (PG-PS) Spontaneous arthritis	Lewis rats HLA-B27 transgenic rats	Reduced joint diameter, local inflammation Reduced joint destruction, inflammation	(84) (85)
<i>Fib<sup>-/-</sup></i>	CIA (type II collagen)	DBA/1J	Reduced incidence, severity, joint destruction, synovial inflammation	(53)
<i>Fib<sup>-/-</sup></i>	Colitis-associated cancer (DSS)	C57BL/6	Reduced inflammation-driven adenoma formation	(54)
<i>Fib<sup>Δ5</sup></i>	CIA (type II collagen)	DBA/1J	No effect on incidence and severity	(53)
<i>Fibγ<sup>390-396A</sup></i>	CIA (type II collagen)	DBA/1J	Reduced incidence, severity, joint destruction, synovial inflammation	(53)
<i>Fibγ<sup>390-396A</sup></i>	Colitis (DSS)	C57BL/6	Diminished inflammatory disease, reduced ulceration, cytokine levels and neutrophil infiltration	(54)
<i>Fibγ<sup>390-396A</sup></i>	Colitis-associated cancer (DSS)	C57BL/6	Reduced inflammation-driven adenoma formation	(54)
Heparin	CIA (type II collagen)	DBA/1	No effect on clinical score	(82)
Hirudin	AIA (mBSA)	C57BL/6	Reduced knee joint inflammation, fibrin deposition	(80)
	CIA (type II collagen)	DBA/1J	Decreased disease incidence, severity, reduced loss of articular cartilage, inflammation, fibrin deposition and PAR-1 expression	(81)
<i>HMWK<sup>-/-</sup></i>	Reactive arthritis (PG-PS)	Rats	No clinical signs of arthritis, absence of inflammation	(86)
MEN16132 (B2R antagonist)	Inflammatory arthritis (carrageenan)	Rats	Reduced knee diameter and myeloperoxidase activity	(87)
P8720 (PK inhibitor)	Reactive arthritis (PG-PS)	Lewis rats	Decreased joint swelling, reduced inflammation	(88)
PKSI-527 (PK inhibitor)	CIA (type II collagen)	DBA/1	Reduced severity of arthritis, reduced PK and HMWK plasma levels	(89)
<i>Plg<sup>-/-</sup></i>	AIA (mBSA)	C57BL/6	Enhanced inflammation, bone erosion, synovial thickness, fibrin deposition	(90)
<i>Plg<sup>-/-</sup></i>	CIA (type II collagen)	C57BL/6xDBA/1	No clinical symptoms of disease, no signs of inflammation	(91)
	CAIA (anti-CII antibodies)		No clinical symptoms of disease, no signs of inflammation	(91)
	AIA (mBSA)		Enhanced synovial thickness	(92)
	CIA (type II collagen)		Reduced synovial thickness	(92)
<i>Tg197Fib<sup>-/-</sup></i>	TNF-transgenic model, spontaneous polyarthritis		No effect on incidence, severity, inflammation, joint destruction	(53)
uPA	CIA (type II collagen)	DBA/1	No effect on clinical score, reduced fibrin deposits in joints, decreased plasma D-dimer level	(82)
<i>uPA<sup>-/-</sup></i>	AIA (mBSA)	Ola129xC57BL/6	Enhanced joint inflammation, bone erosion, synovial thickness, fibrin deposition	(90)
<i>uPA<sup>-/-</sup></i>	Monoarticular arthritis (mBSA/IL-1)	C57BL/6	Enhanced arthritis, fibrin deposition, increased numbers of macrophages	(93)
	CIA (type II collagen)		Reduced clinical symptoms, decreased inflammation, cartilage destruction, bone erosion, reduced cytokine production	(94)
	CAIA (M2139, C1C1 antibodies)		No clinical signs of disease	(95)
	AIA (mBSA)		Enhanced proteoglycan loss, inflammation, bone erosion	(92, 96)
<i>uPA<sup>-/-</sup></i>	CIA (type II collagen)	C57BL/6xDBA/1	Reduced clinical symptoms, no histological changes	(91)
	K/BxN serum transfer arthritis (K/BxN serum)		No clinical signs of disease, reduced inflammation, bone erosion, fibrin deposition	(95, 96)
tPA	CIA (type II collagen)	DBA/1	No effect on clinical score, reduced fibrin deposits in joints	(82)

(Continued)

TABLE 3 | Continued

Mouse line or treatment	Model (peptide)	Genetic background or species	Effect in arthritis and colitis models	Reference
<i>tPA</i> <sup>-/-</sup>	Monoarticular arthritis (mBSA/IL-1)	C57BL/6	Enhanced arthritis, fibrin deposition, increased numbers of macrophages	(93)
	CIA (type II collagen)		Aggravated clinical symptoms, enhanced inflammation, cartilage destruction, bone erosion, fibrin deposition	(94)

AIA, antigen-induced arthritis; B1R, bradykinin receptor 1; B2R, bradykinin receptor 2; CAIA, type II collagen mAb-induced arthritis; CIA, collagen-induced arthritis; CII, collagen type II; DSS, dextran sulfate sodium; HLA, human leukocyte antigen; HMWK, high molecular weight kininogen; mBSA, methylated bovine serum albumin; PAR-1, protease-activated receptor; PG-PS, peptidoglycan-polysaccharide; PK, plasma kallikrein; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator.

The same result was observed in plasminogen-deficient animals (90–92). In contrast to uPA, studies using tPA-deficient animals have so far indicated an aggravated disease course with enhanced inflammation (93, 94).

A substantial role for the contact system in arthritis has been discussed. For instance, FXIIa levels were significantly increased in RA patients compared with healthy controls (97). Furthermore, pharmacological blockade of PK by different inhibitors revealed reduced disease severity and inflammation in different models of arthritis (88, 89). In line with these findings, genetic or pharmacological inhibition of HMWK leads to an attenuation of PK–kinin system activation, local and systemic inflammation, indicating a therapeutic potential in RA (84–86). Moreover, arthritis severity is significantly attenuated in mice lacking B1R and B2R (83) or by treatment with a B2R antagonist (87, 98).

In addition to RA, potential drugability of the coagulation system and its factors is under consideration for the treatment of inflammatory bowel disease. Interestingly, it was shown that patients with Crohn's disease have significantly higher levels of C1-inhibitor and intestinal tissue kallikrein, while plasma levels of prekallikrein, FXI, and HMWK are unaltered (99). Furthermore, inflammatory bowel disease in humans is associated with higher plasma levels of fibrinogen, prothrombin, factor V, factor VIII, plasminogen, and platelets (100). In line with these findings, animal models of colitis have demonstrated reduced inflammation in animals with a genetic disruption to the binding of fibrinogen to the CD11b/CD18 integrin receptor (see Table 3) (54). Since a link between chronic inflammation and tumor development, e.g., colitis and colorectal cancer, could be established (101), it is interesting that both fibrinogen-deficient mice and mice with a genetic disruption of the interaction between fibrinogen and the CD11b/CD18 integrin receptor develop significantly fewer adenomas (54).

Collectively, these results demonstrate a clear role of the coagulation system, not only in neuroinflammation, but also in other autoimmune and inflammatory disorders.

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## CONCLUSION

In this review, we have discussed the links between coagulation and inflammation, focusing on the role of different coagulation factors in neuroinflammatory disorders. Overall, it becomes increasingly clear that the deposition of different coagulation factors in the CNS tissue may trigger exacerbation of inflammation, thereby limiting regenerative mechanisms. A prominent role was especially described for fibrinogen, thrombin, and factor XII. As novel molecular and cellular binding partners are identified, the role of coagulation factors is evolving from hemostasis regulators to multi-faceted signal molecules, which control the balance between immune defense mechanisms and extensive inflammation.

Interestingly, the binding of coagulation factors to their cellular targets requires distinct non-overlapping epitopes and is usually independent of their protease function. Taking advantage of this knowledge, targeted inhibition of coagulation factors that facilitate disease pathogenesis without affecting their protease activity represents an ideal strategy for pharmacological intervention in different neuroinflammatory disorders without unwarranted side-effects like bleeding. Therefore, future studies are needed to elucidate the exact contribution of blood proteins to autoimmune neurodegeneration.

## AUTHOR CONTRIBUTIONS

KG drafted the manuscript. SE, HW, TC, CK, and SM extensively revised the manuscript. KG and TC funded the study. All authors provided substantial input throughout the process.

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