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 (FXIla). 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). FXIla is suggested to initiate the intrinsic coagulation, the contact, and complement systems (Figure 1). Thus, FXIla leads to the cleavage of PK to generate active PK (contact system, Figure 2), triggers fibrin formation through the activation of factor XI (FX; 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 1 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(H)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 (IIa) 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.
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).
| Mouse line or treatment | Model (peptide) | Genetic background | Effects in models of autoimmune neurodegeneration | Reference |
|------------------------|-----------------|--------------------|-----------------------------------------------|-----------|
| **B1R**<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<br>Earlier EAE onset, severe clinical disease course with enhanced inflammation, demyelination, glial activation, increased migration of CD4<sup>+</sup> T cells, especially Th17 cells<br>Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, decreased cytokine production of CD4<sup>+</sup> T cells<br>Delayed EAE onset, reduced clinical deficits, reduced glial activation, decreased release of proinflammatory mediators by astrocytes | (25) |
| Des-Arg<sup>9</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<br>Milder clinical deficits | (25) |
| Active R838-EAE (PLP) | C57BL/6         | Reduced clinical deficits | (26) |
| Des-Arg<sup>9</sup>-[Leu<sup>8</sup>]-BK (B1R inhibitor) | Active EAE (MOG) | C57BL/6            | Delayed EAE onset, reduced clinical deficits, decreased release of proinflammatory mediators by astrocytes<br>Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, decreased cytokine production of CD4<sup>+</sup> T cells | (25) |
| R715 (B1R inhibitor)  | Active EAE (MOG) | C57BL/6            | Reduced clinical deficits, reduced inflammation, demyelination, axonal damage<br>Accelerated disease onset | (25) |
| **B2R**<sup>−/−</sup>  | Active EAE (MOG) | C57BL/6            | No effect on clinical course, immune cells, cytokine production<br>Reduced clinical deficits, reduced inflammation, reduced leukocyte adhesion, decreased chemokine (CCL2, CCL5) production | (25, 26) |
| HOE-140 (B2R inhibitor) | Active EAE (MOG) | C57BL/6            | Moderate or no effect on clinical disease course, immune cells, cytokine production<br>Moderate reduced clinical deficits, reduced inflammation, reduced leukocyte adhesion, decreased chemokine (CCL2, CCL5) production | (25–28) |
| **F12**<sup>−/−</sup>  | Active EAE (MOG) | C57BL/6            | Delayed EAE onset, reduced clinical deficits, reduced inflammation and demyelination, reduced TH17 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) |
| **F11**<sup>−/−</sup>  | Active EAE (MOG) | C57BL/6            | No effect on clinical symptoms, inflammation and demyelination | (15) |
| C1q<sup>−/−</sup>     | Active EAE (MOG) | C57BL/6            | No effect on clinical symptoms | (30) |
| C3<sup>−/−</sup>      | Active EAE (MOG) | 129SV/J/C57BL/6    | Reduced clinical deficits, decreased inflammation and demyelination<br>No clinical effect, but higher mortality, tendency to enhanced inflammation and demyelination | (21) |
| C3<sup>−/−</sup>      | Active EAE (MOG) | C57BL/6            | Reduced clinical deficits, reduced infiltration of T cells | (33, 34) |
| **C5**<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) |
| C5a/GFAP              | Active EAE (MOG) | C57BL/6            | No clinical effect | (37) |
| Cd87<sup>−/−</sup>    | Active EAE (MOG) | C57BL/6            | Aggravated disease course, enhanced inflammation, axonal damage, delayed T-cell proliferation and cytokine production<br>Delayed disease onset, reduced clinical deficits, delayed inflammation, enhanced demyelination and axonal damage<br>Reduced clinical deficits with Cd87<sup>−/−</sup> BM, partial protection of Cd87<sup>−/−</sup> hosts | (38) |

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; Th17, 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 T2-weighted images, but not with gadolinium-enhancing lesions

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-κB, which leads to the release of inflammatory cytokines, like tumor necrosis factor (TNF)-α or IL-1β (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 T41 cells (58, 59). Moreover, genetic deletion of fibrinogen resulted in reduced inflammation
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−/−; see also Table 2) (60). Furthermore, inhibition of fibrinogen binding to CD11b/CD18 by genetic mutation of the CD11b/CD18-binding motif (Fibγ390–396) (61) or a peptide (γ377–395) 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 ancrod 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−/− 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 ε-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).
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 |
|----------------------------------|-----------------|------------------------------|--------------------------|-----------|
| γ277–355S                        | Active RR-EAE (PLP) | SJL | Reduced clinical symptoms, decreased inflammation, reduced microglial activation | (58) |
| Ancrod                          | Active RR-EAE (PLP) | Tg6072 TNF transgenic mice | Reduced clinical symptoms, decreased microglial activation and demyelination, no impact on immune cell proliferation | (58) |
| Batroxobin                     | Active EAE (MOG) | TgK21fib | Reduced demyelination, downregulation of MHC-I | (60) |
| AT-EAE (GP-MBP)                 | Active EAE (MOG) | Rat | Reduced clinical symptoms, decreased inflammation and fibrin deposition | (62) |
| Fibrin−390–396A                 | Active EAE (MOG) | C57BL/6 | Reduced clinical symptoms, decreased inflammation | (63) |
| Hirudin                        | Active EAE (PLP) | SJL/J | Improvement of clinical deficits, reduced inflammation, decreased immune cell proliferation and cytokine production | (13) |
| PAI-1−/−                        | Active CREAE (spinal cord homogenate) | ABH | Reduced incidence, moderately delayed onset, reduced inflammation, no effect on demyelination and axonal damage | (39) |
| PAI-1-dp (uPA activator)        | Active EAE (MOG) | C57BL/6 | Attenuated disease course, reduced T cell proliferation and cytokine production | (38) |
| TgK21fib−/−                     | TNF-transgenic model (no peptide, spontaneous) | C57BL/6 | Increased lifespan, delayed onset of clinical symptoms, reduced inflammation and demyelination | (60) |
| tPA−/−                          | Active EAE (MOG) | C57BL/6 | Aggravated disease course, enhanced demyelination, axonal damage, fibrin deposition | (39) |
| uPA−/−                          | 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-1, plasminogen activator inhibitor I; PAI-1-dp, plasminogen activator inhibitor I-deprived; 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, have been suggested in RA or inflammatory joint disease as 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 |
|-------------------------|-----------------|-----------------------------|---------------------------------------|-----------|
| B1R/B2R/−/−             | 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) |
| Fib/−/−                 | CIA (type II collagen) | DBA/1J | Reduced incidence, severity, joint destruction, synovial inflammation | (53) |
| Fib−/−                  | Colitis-associated cancer (DSS) | C57BL/6 | Reduced inflammation-driven adenoma formation | (54) |
| Fib−/−                  | CIA (type II collagen) | DBA/1J | No effect on incidence and severity | (53) |
| Fib−/−                  | CIA (type II collagen) | DBA/1J | Reduced incidence, severity, joint destruction, synovial inflammation | (53) |
| Fib−/−                  | Colitis (DSS) | C57BL/6 | Diminished inflammatory disease, reduced ulceration, cytokine levels and neutrophil infiltration | (54) |
| Fib−/−                  | 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 | (83) |
|                         | CIA (type II collagen) | DBA/1J | Decreased disease incidence, severity, reduced loss of articular cartilage, inflammation, fibrin deposition and PAR-1 expression | (81) |
| HMWK/−/−                | 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) |
| Plg−/−                  | AIA (mBSA) | C57BL/6 | Enhanced inflammation, bone erosion, synovial thickness, fibrin deposition | (90) |
| Plg−/−                  | CIA (type II collagen) | C57BL/6xDBA/1 | No clinical symptoms of disease, no signs of inflammation No clinical symptoms of disease, no signs of inflammation Enhanced synovial thickness Reduced synovial thickness | (91) (91) (92) (92) |
| Tg197Fib−/−             | 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) |
| uPA−/−                  | AIA (mBSA) | Ola129xC57BL/6 | Enhanced joint inflammation, bone erosion, synovial thickness, fibrin deposition | (90) |
| uPA−/−                  | Monoarticular arthritis (mBSA/IL-1) | C57BL/6 | Enhanced arthritis, fibrin deposition, increased numbers of macrophages | (93) |
|                         | CIA (type II collagen) | C57BL/6 | Reduced clinical symptoms, decreased inflammation, cartilage destruction, bone erosion, reduced cytokine production | (94) |
| CAIA (M2139, CitC1 antibodies) | No clinical signs of disease | (95) |
| AIA (mBSA)              | Enhanced proteoglycan loss, inflammation, bone erosion | (92, 96) |
| uPA−/−                  | 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)
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 druggability 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.

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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REFERENCES

1. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. Nature (1964) 202:498–9. doi:10.1038/202498a0
2. Schoenmakers SH, Reitsma PH, Spek CA. Blood coagulation factors as inflammatory mediators. Blood Cells Mol Dis (2005) 34(1):30–7. doi:10.1016/j.bcmd.2004.09.001
3. McMichael M. New models of hemostasis. Top Companion Anim Med (2012) 27(2):40–5. doi:10.1053/j.tcam.2012.07.005
4. Gailani D, Broze GJ Jr. Factor XI activation in a revised model of blood coagulation. Science (1991) 253(5022):909–12. doi:10.1126/science.1652157
5. Lippi G, Falavolo EJ, Franchini M, Guidi GC. Milestones and perspectives in coagulation and hemostasis. Semin Thromb Hemost (2009) 35(1):9–22. doi:10.1055/s-0029-1214144

Table 3

| Mouse line or treatment | Model (peptide) | Genetic background or species | Effect in arthritis and colitis models | Reference |
|-------------------------|-----------------|-----------------------------|--------------------------------------|-----------|
| tPA<sup>−/−</sup>      | Monoarticular arthritis (mBSA/IL-1) | C57BL/6 | Enhanced arthritis, fibrin deposition, increased numbers of macrophages | (93) |
| CIA (type II collagen)  | Enhanced 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-FS, peptidoglycan-polysaccharide; PK, plasma kallikrein; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator.
6. Strukova SM. Thrombin as a regulator of inflammation and reparative processes in tissues. Biochemistry (Mosc) (2001) 66(1):8–18. doi:10.1023/A:1002669301080

7. Mackman N. The many faces of tissue factor. J Thromb Haemost (2009) 7(Suppl 1):136–9. doi:10.1111/j.1538-7836.2009.03368.x

8. Davalos D, Akassoglou K. Fibrinogen as a key regulator of inflammation in disease. Semin Immunopathol (2012) 34(1):43–62. doi:10.1007/s00281-011-0290-8

9. Chavakis T, Boeckel N, Santoso S, Voss R, Isoridia-Salas I, Pixmap RA, et al. Inhibition of platelet adhesion and aggregation by a defined region (Gly-486-Lys-502) of high molecular weight kinogen. J Biol Chem (2002) 277(26): 23157–64. doi:10.1074/jbc.M205292V0

10. Chavakis T, Pixmap RA, Isoridia-Salas I, Colman RW, Preissner KT. A novel antithrombotic role for high molecular weight kinogen as inhibitor of plasminogen activating inhibitor-1 function. J Biol Chem (2002) 277(36): 32677–82. doi:10.1074/jbc.M20401020

11. Chavakis T, Kanse SM, Pixmap RA, May AE, Isoridia-Salas I, Colman RW, et al. Regulation of leukocyte recruitment by polypeptides derived from high molecular weight kinogen. FASEB J (2001) 15(13):2365–76. doi:10.1096/fj.01-0201com

12. Leeb-Lundberg LM, Marceau F, Muller-Esterl W, Pettibone DJ, Zuraw BL. Annulling the fibrinogen receptor on T lymphocytes in active multiple sclerosis. J Neuropathol Exp Neurol (1999) 58(9):2087–92. doi:10.1093/jnen/58.9.2087

13. Davalos D, Baeten KM, Whitney MA, Mullins ES, Friedman B, Olson ES, et al. Early detection of thrombin activity in neuroinflammatory disease. Ann Neurol (2014) 75(2):303–8. doi:10.1002/ana.24078

14. Gobel K, Pankratz S, Asaridou CM, Herrmann AM, Bittner S, Merker M, et al. Blood coagulation factor XII drives adaptive immunity during neuroinflammation via CD87-mediated modulation of dendritic cells. Nat Commun (2016) 7:11626. doi:10.1038/ncomms11626

15. Gobel K, Kraft P, Pankratz S, Gross CC, Korsukewitz C, Kwiecien R, et al. Prothrombin and factor X are elevated in multiple sclerosis patients. Ann Neurol (2016) 80(6):946–51. doi:10.1002/ana.24807

16. Prat A, Weindorfer L, Becher B, Rozier J, Dupasquette P, Rutten R, et al. Bradykinin B1 receptor expression and function on T lymphocytes in active multiple sclerosis. Neurology (1999) 53(9):807–12. doi:10.1212/WNL.53.9.807

17. Prat A, Biernacki K, Poully S, Nalbantoglou J, Couture R, Antel JP. Kinin B1 receptor expression and function on human brain endothelial cells. J Neuroimmunol Exp Neurol (2000) 99(9):896–906. doi:10.1093/jnen/99.9.896

18. Stavrou E, Schwerzer AH. Factor XII: a key component of complement activation, is not required for the development of myelin oligodendrocyte glycoprotein peptide-induced autoimmune encephalomyelitis in mice. J Immunol (2001) 166(2):723–6. doi:10.1042/0022-1758/166.2.723

19. Szalai AJ, Hu X, Adams JE, Barnum SR. Complement in experimental autoimmune encephalomyelitis revisited: C5 is required for development of maximal disease. Mol Immunol (2007) 44(12):3132–6. doi:10.1016/j.molimm.2007.02.002

20. Smith SS, Ludwig M, Wohler JE, Bullard DC, Szalai AJ, Barnum SR. Deletion of both ICAM-1 and C3 enhances severity of experimental autoimmune encephalomyelitis compared to C3-deficient mice. Neurosurg Lett (2008) 44(2):158–60. doi:10.1055/s-0038-1621907

21. Weerth SH, Rus H, Shin ML, Raine CS. Complement C5 in experimental autoimmune encephalomyelitis (EAE) facilitates remyelination and prevents glialosis. Am J Pathol (2003) 163(3):689–90. doi:10.1016/S0002-9440(10)63466-9

22. Niculescu T, Weerth S, Niculescu F, Cudrici C, Rus V, Raine CS, et al. Effects of complement C5 on apoptosis in experimental autoimmune encephalomyelitis compared to C3-deficient mice. Neurosci Lett (2008) 442(2):158–60. doi:10.1016/j.neulet.2008.07.005

23. Reiman R, Campos Torres A, Martin BK, Ting JP, Campbell IL, Barnum SR. Expression of C5a in the brain does not exacerbate experimental autoimmune encephalomyelitis. Neurosci Lett (2005) 390(3):134–8. doi:10.1016/j.neulet.2005.08.022

24. Gur-Wahnon D, Mizrahi T, Maaravi-Pinto FY, Lourbopoulos A, Grigoriadis N, Higazi AA, et al. The plasminogen activator system: involvement in central nervous system inflammation and a potential site for therapeutic intervention. J Neuroimmunol (2013) 10:124. doi:10.1186/1742-2094-10-124

25. East E, Baker D, Pryce G, Linnor HR, Cuzner ML, Gveric D. A role for the plasminogen activator system in inflammation and neurodegeneration in the central nervous system during experimental allergic encephalomyelitis. Am J Pathol (2005) 167(2):545–54. doi:10.1016/j.ajpath.2004.08.092

26. Prat A, Biernacki K, Saroli T, Oray JE, Guttmann CR, Weiner HL, et al. Kinin B1 receptor expression on multiple sclerosis mononuclear cells: correlation with magnetic resonance imaging T2-weighted lesion volume and clinical disability. Arch Neurol (2005) 62(5):795–800. doi:10.1001/archneur.62.5.795

27. Weisel JW. Fibrinogen and fibrin. Adv Protein Chem (2005) 70:247–99. doi:10.1016/S0065-2323(05)70008-5

28. Fuss C, Palmaz JC, Sprague EA. Fibrinogen: structure, function, and surface interactions. J Vasc Interv Radiol (2001) 12(6):677–82. doi:10.1016/S1051-4043(07)61437-7

29. Mesosson MW. Dysfibrinogenemia and thrombosis. Semin Thromb Hemost (1999) 25(3):311–9. doi:10.1055/s-2007-994933
44. Sidelmann J, Gram J, Jespersen J, Klaft C. Fibrin clot formation and lysis: basic mechanisms. *Semin Thromb Hemost* (2000) 26(6):605–18. doi:10.1055/s-2000-2631216

45. Lijnen HR. Elements of the fibrinolytic system. *Ann N Y Acad Sci* (2001) 936:226–36. doi:10.1111/j.1749-6632.2001.tb03511.x

46. East E, Gveric D, Baker D, Pryce G, Lijnen HR, Cuzner ML. Chronic relapsing experimental allergic encephalomyelitis (CRAE) in plasminogen activator inhibitor-1 knockout mice: the effect of fibrinolysis during neuroinflammation. *Neuraphot Appl Neurobiol* (2008) 34(2):216–30. doi:10.1111/j.1363-9200.2007.00889.x

47. Adams RA, Passino M, Sachs BD, Nuriel T, Akassoglou K. Fibrin mechanisms and functions in nervous system pathology. * Mol Inter* (2004) 4(3):163–76. doi:10.10124/93.4.3.6

48. Tennent GA, Brennan SO, Stangou AJ, O’Grady J, Hawkins PN, Pepsy MB. Human plasma fibrinogen is synthesized in the liver. *Blood* (2007) 109(5):1971–4. doi:10.1182/blood-2006-04-049596

49. Lowe GD. Fibrinogen measurement to assess the risk of arterial thrombosis in individual patients: not yet. *J Thromb Haemost* (2005) 3(4):635–7. doi:10.1111/j.1538-7836.2005.01256.x

50. Solovjov DA, Pluskota E, Plow EF. Distinct roles for the alpha and beta subunits in the functions of integrin alphaMbeta2. *J Biol Chem* (2005) 280(2):1336–45. doi:10.1074/jbc.M406968200

51. Perez RL, Roman J. Fibrin enhances the expression of IL-1 beta by human peripheral blood mononuclear cells. Implications in pulmonary inflammation. *J Immunol* (1995) 154(4):1879–87.

52. Perez RL, Ritzenhaler JD, Roman J. Transcriptional regulation of the interleukin-1beta promoter via fibrinogen engagement of the CD18 integrin receptor. *Am J Respir Cell Mol Biol* (1999) 20(5):1059–66. doi:10.1165/ajrcmb.20.5.3281

53. Flick MJ, Lajeunesse CM, Talmage KE, Witte DP, Palumbo JS, Pinkerton MD, et al. Suppression of experimental autoimmune encephalomyelitis in CD18 integrin deficient mice: the effect of fibrinolysis during demyelination in a tumor necrosis factor transgenic mouse model for multiple sclerosis. *Proc Natl Acad Sci U S A* (2004) 101(17):6698–703. doi:10.1073/pnas.0303859101

54. Flick MJ, Du X, Witte DP, Jirovskova M, Soloviev DA, Busuttil SJ, et al. Leukocyte engagement of fibrinogen via the integrin receptor alphaMbeta2/Mac-1 is critical for host inflammatory response in vivo. *J Clin Invest* (2004) 113(11):1596–606. doi:10.1172/JCI20741

55. Paterson PY. Experimental allergic encephalomyelitis: role of fibrin deposition in immunopathogenesis of inflammation in rats. *Fed Proc* (1976) 35(13):2428–34.

56. Yang Y, Tian SJ, Wu L, Huang DH, Wu WP. Fibrinogen depleting agent baxtroxin has a beneficial effect on experimental autoimmune encephalomyelitis. *Cell Mot Neurobiol* (2011) 31(3):437–48. doi:10.1007/s10571-010-9637-2

57. Inoue A, Koh CS, Shimada K, Yanagisawa N, Yoshimura K. Suppression of cell-transferred experimental autoimmune encephalomyelitis in defibrinated Lewis rats. *J Neuroimmunol* (1996) 71(1–2):131–7. doi:10.1016/0165-5728(96)00150-6

58. Sibley WA, Kiermat J, Laguna JF. The modification of experimental allergic encephalomyelitis with epsilon aminoacapric acid. *Neurology* (1978) 28(9 Pt 2):102–5. doi:10.1212/WNL.28.9_Part_2.102

59. Lu W, Bhasin M, Tsirka SE. Involvement of tissue plasminogen activator in onset and effector phases of experimental allergic encephalomyelitis. *J Neurosci* (2002) 22(24):10781–9. doi:10.1523/JNEUROSCI.22-24-10781.2002

60. Xie C, Alcaide P, Gerisbretch BV, Schneider D, Herrmann M, Preisser NT, et al. Suppression of experimental autoimmune encephalomyelitis by extracellular adherence protein of *Staphylococcus aureus*. *J Exp Med* (2006) 203(4):985–94. doi:10.1084/jem.20051681

61. Paterson PY. Experimental allergic encephalomyelitis-inducing activity of synthetic polyadenyl and polyuridylic homopolymers and complexes in guinea pigs. *J Immunol* (1976) 211(4):48–55. doi:10.1086/308749

62. Akenomi FO, Siren V, Koskimets M, Siimes MA, Teravainen H, Vaheri A. Cerebrospinal fluid activity of tissue plasminogen activator in patients with neurological diseases. *J Clin Pathol* (1996) 49(7):577–80. doi:10.1136/jcp.49.7.577

63. Mazodier K, Arnaud L, Mathian A, Costedoat-Chalumeau N, Haroche J, Frances C, et al. Lupus anticoagulant-hypoprothrombinemia syndrome: report of 8 cases and review of the literature. *Medicine (Baltimore)* (2012) 91(5):251–60. doi:10.1097/MD.0b013e318269971f

64. Lancellotti S, Basso M, De Cristofaro R. Congenital prothrombin deficiency: an update. *Semin Thromb Hemost* (2013) 39(6):596–606. doi:10.1055/s-0033-1384984

65. Hirano K, Hirano M. Current perspective on the role of the thrombin receptor in cerebral vasospasm after subarachnoid hemorrhage. *J Pharmacol Sci* (2010) 114(2):127–33. doi:10.1254/jphs.R092830

66. Bellin O, Karussis DM, Korczyn AD, Garwitz D, Aronovich R, Hantai D, et al. Increased thrombin inhibition in experimental autoimmune encephalomyelitis. *J Neurosci Res* (2005) 79(3):351–9. doi:10.1002/jnr.20270

67. Lee DM, Weinblatt ME. Rheumatoid arthritis. *Lancet* (2001) 358(9285):903–11. doi:10.1016/S0140-6736(00)06075-5

68. So AK, Varisco PA, Kemkes-Matthes B, Herkenne-Morard C, Chobaz-Peclat V, Gerster JC, et al. Arthritis is linked to local and systemic activation of coagulation and fibrinolysis pathways. *J Thromb Haemost* (2003) 1(12):2510–5. doi:10.1111/j.1538-7836.2003.00462.x

69. Inogoni F, Fantini F, Favalli EG, Soldi A, Griffini S, Galbatti V, et al. Immunological and prothrombotic biomarkers in patients with rheumatoid arthritis: effects of tumor necrosis factor-alpha blockade. *Arthritis Rheum* (2008) 51(2):175–9. doi:10.1002/art.21707

70. Liu X, Piela-Smith TH. Fibrinogen-induced expression of ICAM-1 and chemokines in human synovial fibroblasts. *J Immunol* (2000) 165(9):5255–61. doi:10.4049/jimmunol.165.9.5255

71. Varisco PA, Peclat V, van Ness K, Bischof-Delaloye A, So A, Busso N. Effect of thrombin inhibition on synovial inflammation in antigen induced arthritits. *Ann Rheum Dis* (2000) 59(10):781–7. doi:10.1136/ard.59.10.781

72. Marty I, Peclat V, Kirdaite G, Salvi R, So A, Busso N. Amelioration of collagen-induced arthritis by thrombin inhibition. *J Clin Invest* (2001) 107(7):961–70. doi:10.1172/JCI11064

73. Kwiecinski J, Josfesohn E, Jin T. Fibrinolysis is down-regulated in mouse collagen-induced arthritis, but its normalization does not alleviate the course of disease. *Inflamm Res* (2011) 60(11):1021–9. doi:10.1007/s00011-011-0363-0
83. Xie Z, Dai J, Yang A, Wu Y. A role for bradykinin in the development of anti-collagen antibody-induced arthritis. *Rheumatology (Oxford)* (2014) 53(7):1301–6. doi:10.1093/rheumatology/keu155

84. Espinola RG, Uknis A, Sainz IM, Isordia-Salas I, Pixley R, DeLa Cadena R, et al. A monoclonal antibody to high-molecular weight kininogen is therapeutic in a rodent model of reactive arthritis. *Am J Pathol* (2004) 165(3):969–76. doi:10.1016/S0002-9440(10)63358-5

85. Keith JC Jr, Sainz IM, Isordia-Salas I, Agelman A, Liu B, DeLa Cadena RA, et al. Modulation of inflammation by kininogen deficiency in a rat model of inflammatory arthritis. *Arthritis Rheum* (2005) 52(8):2549–52. doi:10.1002/art.21202

86. Sainz IM, Isordia-Salas I, Castaneda JL, Agelman A, Liu B, DeLa Cadena RA, et al. Modulation of inflammation by kininogen deficiency in a rat model of inflammatory arthritis. *Arthritis Rheum* (2005) 52(8):2549–52. doi:10.1002/art.21202

87. Valenti C, Giuliani S, Gialdai C, Tramontana M, Maggi CA. Anti-inflammatory synergy of MEN16132, a kinin B(2) receptor antagonist, and dexamethasone in carrageenan-induced knee joint arthritis in rats. *Br J Pharmacol* (2010) 161(7):1616–27. doi:10.1111/j.1476-5381.2010.09995.x

88. Dela Cadena RA, Stadnicki A, Uknis AB, Sartor RB, Kettner CA, Adam A, et al. Inhibition of plasma kallikrein prevents peptidoglycan-induced arthritis in the Lewis rat. *FASEB J* (1995) 9(5):446–52. doi:10.1096/fasebj.9.5.7896018

89. Fujimori Y, Nakamura T, Shimizu K, Yamamura T, Wanaka K, Okamoto S, et al. Effects of a highly selective plasma kallikrein inhibitor on collagen-induced arthritis in mice. *Agents Actions* (1993) 39(1–2):42–8. doi:10.1007/BF01975713

90. Busso N, Peclat V, Van Ness K, Leatherbury Y, Albert LM, et al. A monoclonal antibody to high-molecular weight kininogen is therapeutic in a rodent model of reactive arthritis. *Am J Pathol* (2004) 160(3):917–26. doi:10.1016/S0002-9440(10)64914-0

91. Li J, Guo Y, Holmdahl R, Ny T. Contrastoring roles of plasminogen deficiency in different rheumatoid arthritis models. *Arthritis Rheum* (2005) 52(8):2541–8. doi:10.1002/art.21229

92. Li J, Ny A, Leonardsson G, Nandakumar KS, Holmdahl R, Ny T. The plasminogen activator/plasmin system is essential for development of the joint inflammatory phase of collagen type II-induced arthritis. *Am J Pathol* (2005) 166(3):783–92. doi:10.1016/S0002-9440(10)62299-7

93. Yang YH, Carmeliet P, Hamilton JA. Tissue-type plasminogen activator deficiency exacerbates arthritis. *J Immunol* (2001) 167(2):1047–52. doi:10.4049/jimmunol.167.2.1047

94. Cook AD, Braine EL, Campbell IK, Hamilton JA. Differing roles for urokinase and tissue-type plasminogen activator in collagen-induced arthritis. *Arq Bras Fis* (2002) 9(5):446–52. doi:10.1096/fasebj.9.5.7896018

95. Cook AD, De Nardo CM, Braine EL, Turner AL, Vlahos R, Way KJ, et al. Urokinase-type plasminogen activator and arthritis progression: role in systemic disease with immune complex involvement. *Arthritis Res Ther* (2010) 12(2):R37. doi:10.1186/ar2946

96. De Nardo CM, Lenzo JC, Pobjoy J, Hamilton JA, Cook AD. Urokinase-type plasminogen activator and arthritis progression: contrasting roles in systemic and monoarticular arthritis models. *Arthritis Res Ther* (2010) 12(5):R199. doi:10.1186/ar3171

97. McLaren M, Alkaabi J, Connacher M, Belch JJ, Valente E. Activated factor XII in rheumatoid arthritis. *Rheumatol Int* (2005) 25(3):143–52. doi:10.1007/s00296-002-0219-6

98. Uknis AB, DeLa Cadena RA, Janardham R, Sartor RB, Whalley ET, Colman RW. Bradykinin receptor antagonists type 2 attenuate the inflammatory changes in peptidoglycan-induced acute arthritis in the Lewis rat. *Inflamm Res* (2001) 50(3):149–55. doi:10.1007/s000110050739

99. Devani M, Cugno M, Vecchi M, Ferrero S, Di Berardino F, Avesani EC, et al. Kallikrein-kinin system activation in Crohn's disease: differences in intestinal and systemic markers. *Am J Gastroenterol* (2002) 97(8):2026–32. doi:10.1111/j.1572-0241.2002.05919.x

100. Dalapcioglu C, Soylu A, Kendir T, Ince AT, Dalapcioglu H, Purisa S, et al. Coagulation parameters in inflammatory bowel disease. *Int J Clin Exp Med* (2014) 7(5):1442–8.

101. Xie J, Itzkowitz SH. Cancer in inflammatory bowel disease. *World J Gastroenterol* (2008) 14(3):378–89. doi:10.3748/wjg.14.378