INTRODUCTION

Inflammation is a complex response to infection or injury with the aim to (i) confine inflammation and/or infection to a limited area, (ii) eliminate noxious stimuli and (iii) restore homeostasis. However, this process is associated with the activation of the coagulation cascade. A wide range of inflammatory conditions including arthritis, multiple sclerosis or sepsis, an uncontrolled activation of the coagulation system contributes to inflammation, microvascular failure and organ dysfunction. Coagulation is initiated by the activation of thrombin, which, in turn, triggers fibrin formation by the release of fibrinopeptides. Fibrin is cleaved by plasmin, resulting in clot lysis and an accompanied generation of fibrin fragments such as D and E fragments. Various coagulation factors, including fibrinogen and/or fibrin (fibrinogen) and also fibrin degradation products, modulate the inflammatory response by affecting leukocyte migration and cytokine production. Fibrin fragments are mostly proinflammatory, however, Bβ15-42 inhibit Rho-kinase activation by dissociating Fyn from Rho and, hence prevents stress-induced loss of endothelial barrier function and also leukocyte migration. This article summarizes the state-of-the-art in inflammatory modulation by fibrinogen and fibrin fragments. However, further research is required in order to gain better understanding of the entire role fibrin fragments play during inflammation and, possibly, disease development.

Cross-talk between Coagulation and Inflammation

An inflammatory response shifts the hemostatic system toward a prothrombotic state, while coagulation also affects inflammation. Two coagulation factors stand out during this cross-talk: tissue factor (TF) and thrombin. TF, the initiator of the coagulation cascade, is strongly induced during inflammation in endothelial cells (ECs) and leukocytes (11), which in turn activates thrombin. Blocking TF using neutralizing antibodies abrogates the inflammatory and coagulopathic response in two experimental in vivo models of sepsis (12) and ischemia/reperfusion (13). When TF is blocked, thrombin generation is also compromised. This is associated with less activation of the inflammatory and coagulation system, suggesting that thrombin is one of the major players in this process. Thrombin activates endothelial and immune cells by binding mainly to protease-activated receptor (PAR)-1, -3 and -4. It induces a strong inflammatory...

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response by enhancing cytokine and chemokine expression as well as by increasing leukocyte recruitment, mainly via PAR-1, but also PAR-4 signaling (6,14–16).

**Fibrinogen and Fibrin Structure**

Fibrinogen is a 340 kDa glycoprotein synthesized in the liver with corresponding plasma levels of 1.5–3 g/L. The protein complex consists of two sets of three polypeptide chains, namely the Aα, Bβ and γ chains. The chains are joined together by disulfide bridges of their N-termini forming the central E globule, whereas the C-termini of Bβ and γ form the two outer D domains connected to the E domain by coiled-coiled rod-like structure of the three polypeptide chains.

Coagulation is initiated by conversion of fibrinogen to fibrin. Thrombin cleaves within the N-termini of the Aα and Bβ chains releasing the fibrinopeptides A (FpA, Aα1–16) and B (Bβ1–15), thereby exposing polymerization sites. The activated fibrin monomers polymerize by binding the exposed polymerization sites to complementary binding pockets within the D domains, therewith forming protofibrils. Finally, thrombin activates coagulation factor XIIa (FXIIa) and stabilizes the fibrin clot by catalyzing the formation of isopeptide bonds between the γ chains of two fibrin molecules (reviewed in 17,18).

Fibrinolysis is facilitated by plasmin. It is synthesized as plasminogen and activated by proteolysis via tissue-type (t) or urokinase-type (u) plasminogen activator (tPA or uPA). Activated plasmin in turn cleaves fibrin at various cleavage sites resulting in X and Y fragments, d-dimers, D and E fragments, Bβ15–42 and smaller fragments mostly derived from the α chain (19–21). Figure 1 schematically depicts fibrinogen, fibrin, its domains, cleavage sites and the resulting plasmin derivatives.

**Inflammatory Potential of Fibrinogen and Fibrin**

During the 1960–70s the first evidence of a potential inflammatory role of fibrinogen in vivo was found. It was demonstrated that fibrinogen and fibrin contribute to inflammation by inducing leukocyte migration (22–24). Later, in vitro and in vivo studies demonstrated that fibrinogen alters inflammation not only by affecting leukocyte migration, but also by directly modulating the inflammatory response of leukocytes and ECs via an increased cytokine/chemokine response. Exposure of ECs to fibrin induces the expression of interleukin (IL)-8 mRNA and protein (25). Fibrinogen has been shown to cause an inflammatory response in peripheral blood mononuclear cells (PBMCs) induced by high levels of reactive oxygen species (ROS) (26), increased cytokine (for example, tumor necrosis factor-α, IL-1β and IL-6) (27,28) and chemokine expression (for example, macrophage inflammatory protein-1 and -2 [MIP-1 and -2] and macrophage chemoattractant protein-1 [MCP-1]) (29).

Fibrinogen-induced chemokine expression was associated with toll-like receptor 4 (TLR-4) signaling, since this effect was absent in C3H/HeJ mice, which express a TLR-4 (29) mutant. In contrast, others have excluded the involvement of TLR-4 signaling pathway in response to a fibrinogen challenge (4). Kaneider et al. showed that fibrinogen reduces clotting time in monocytes and induces matrix metalloproteinase-9 (MMP-9) (30). Analysis of underlying mechanisms revealed that fibrinogen modulates mitogen-activated protein kinase (MAPK) signaling, protein kinase C (PKC) and nuclear factor κB (NFκB) (30–33), a key transcription factor during inflammation (34).

Fibrinogen also binds to various integrins and adhesion molecules such as αMβ2 or αβ3 (CD11c/CD18) (35,36). Current data suggest that the integrin αMβ2 is the “main” fibrinogen receptor involved in (i) ROS production (26), (ii) cytokine expression (28,30–33) and (iii) leukocyte migration. αMβ2 interacts with fibrinogen by recognizing specific sequences (so called P1:383–395 and P2 core recognition motif P2-Cγ390–396) within the γ chain of the D nodule (37–41). Recently, the role of αMβ2 and fibrinogen interaction was addressed under in vivo inflammatory conditions. Converting the αMβ2-binding motif of fibrinogen (fibrinogen-γ390–396) abolished leukocyte adhesion in vitro, while migration in response to S. aureus-induced peritonitis was not affected. However, bacterial clearance in fibrinogen-γ390–396A knock-in mice was impaired significantly, indicating the importance of fibrinogen (42). This is further supported by the fact that fibrinogen deficiency and also fibrinogen-γ390–396A knock-in significantly reduced disease severity in a mouse collagen-induced arthritis model. Interestingly, this effect
was associated with lower cytokine expression, whereas leukocyte trafficking was unchanged (2). Adams et al. demonstrated that fibrinogen activates microglia via \( \alpha_\beta \), but not the TLR-4 pathway. In an experimental autoimmune encephalomyelitis model, inhibition of fibrinogen activation, coagulation was not affected (4). In summary, fibrinogen–\( \alpha_\beta \) interaction strongly modulates the inflammatory response by leukocytes, while trafficking seems to be compensated by other integrins and adhesion molecules.

The intercellular adhesion molecule-1 (ICAM-1) of ECs is another important fibrinogen receptor. By binding to ICAM-1, fibrinogen acts as a bridging molecule enhancing leukocyte–endothelium interaction (43). This interaction is augmented by the vascular endothelial–cadherin (VE-cadherin)-dependent induction of ICAM-1. The exposed Bj15–42 sequence was essential for VE-cadherin binding, since blocking FpB release also prevents binding and ICAM-1 induction (44). Even though there is lot of evidence for fibrinogen-dependent leukocyte adhesion, in vivo studies revealed that leukocytes and platelets do not readily accumulate in fibrin clots (45,46).

Undoubtedly, fibrinogen plays a major role during inflammation. The thrombin inhibitor refludan reduced macrophage adhesion, while fibrinogen knock-out additionally reduced MCP-1 and IL-6 expression in response to thioglycolate (TG) (47). Cunningham et al. demonstrated in a mouse crescentic glomerulonephritis model that administration of the thrombin inhibitor hirudin afforded greater protection against renal injury and inflammation than did PAR-1 deficiency (14). This might indicate the involvement of fibrin fragments inducing inflammation, although, hirudin also inhibits thrombin-dependent activation of PAR-4. Therefore, it cannot be excluded that some protective effects of hirudin in this animal model are due to abrogated PAR-4 signaling. Moreover, platelet PAR-4 plays an essential role in TF-mediated inflammation (16).

Depleting fibrinogen with ancrod showed similar results in a TF-induced inflammation model; TF increased IL-6 levels. This effect was abolished completely when thrombin or FVIIa were blocked or when fibrinogen was depleted by ancrod (16). In a myocardial ischemia/reperfusion model, fibrinogen knock-out mice showed a significant reduction in infarct sizes, when compared with littermates (48). Fibrin deposition seems to contribute to the pathogenesis of Alzheimer disease by increasing blood brain-barrier (BBB) damage and neuroinflammation (3).

In conclusion, fibrinogen augments the severity of various inflammatory conditions. Even though fibrinogen depletion reduces inflammation, it remains unclear if this is solely due to fibrin or also to fibrinogen degradation products. We hypothesize that fibrin fragments also contribute to the pathophysiology of inflammation.

Fibrin Fragments

Various fibrinogen degradation products are generated during coagulation and fibrinolysis. Initiation of clotting involves the release of FpA and B. Plasmin leads to the generation of \( \alpha_\beta \)-dependent leukocyte adhesion, in vivo studies revealed that leukocytes and platelets do not readily accumulate in fibrin clots (45,46).

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Fibrinopeptides A and B. Various groups reported proinflammatory effects of fibrinopeptides, in particular its function as a chemoattractant for neutrophils, monocytes and macrophages (51–53). Analysis of exudates from animals injected with purified FpA and FpB revealed that the numbers of leukocytes and MCP-1 levels were increased in rat air pouches. Since both fibrinopeptides have been injected in combination, single effects of each peptide remains to be elucidated (54). In contrast, one study revealed opposite effects of fibrinopeptides. In a carrageenan-induced inflammatory rat model, FpA and FpB reduced paw swelling, suggesting anti-inflammatory potential. In this particular study, thrombin-dependent effects were abolished by the use of heparin (55).

Since Singh et al. showed that FpB but not FpA attracts macrophages (53), involvement of thrombin to the proinflammatory response could be excluded. As a result of these conflicting reports, further investigation is warranted.

**D fragments.** \( \alpha_\beta \)-dimers generated by plasmin digestion of fibrin are used as markers for fibrinolysis and disseminated intravascular coagulation (DIC) in man. Exposure of \( \alpha_\beta \)-dimers or D monomers on the human promonocytic leukemia cell line, NOMO-1, increased the levels of IL-1\( \alpha \) and \( \beta \), uPA, TF and plasminogen activator inhibitor-2 (PAI-2) (56). In addition, \( \alpha_\beta \)-dimers triggered the release of IL-1\( \beta \), IL-6 and PAI in peripheral blood monocytes (57). Others have shown that \( \alpha_\beta \)-fragments have neither pro- nor antiinflammatory effects on monocytes/macrophages (58,59).

**Fibrin fragment E (FnE).** So far, little is known regarding the inflammatory potential of FnE. Various groups reported that FnE has angiogenic activity (60–62), while fibrinogen fragment E (FgnE) inhibits angiogenesis (62,63). In rat peritoneal macrophages, FnE as well as FgnE induced IL-6 production, which was supposed to be mediated by binding to CD11c (58). In the human monocytic cell line, THP-1–induced IL-1\( \beta \) production was increased by adherent FnE, but not by fragment D or FgnE (59). In addition to the studies on physiological FnE, the
biological activity of FnE is often associated with a fragment named NDSKII (N-terminal disulfide knot II). NDSKII is generated synthetically via the cleavage of fibrinogen by cyanogen bromide and thrombin digestion. The resulting product is similar—but not identical—to the physiological FnE (FnE: Aα17–78, Bβ15–122 and γ1–62; NDSKII: Aα17–51, Bβ15–118, γ1–78) (19,64). Bach et al. studied the interaction of NDSKII using human umbilical vein endothelial cells (HUVECs). Binding assays demonstrated that the interaction of NDSKII is dependent on the Bβ15–42 region, since NDSK (generated after cyanogen bromide digestion without thrombin cleavage) does not expose Bβ15–42 and therefore did not show any affinity to HUVECs. Moreover, NDSKII binds to VE-cadherin but not to PECAM-1, ICAM-1 or various integrins (65) and facilitates leukocyte migration (48). In detail, lymphocyte migration depends on VE-cadherin and is inhibited by Bβ15–42, while monocyte and neutrophil migration is mediated by the binding of the α chain (NDSKII to CD11c). ICAM-1 antibody only partially inhibited migration of all three cell types (48).

Bβ15–42. Bβ15–42, a fragment of the N-terminal β chain, is generated by plasmin cleavage of fibrin. In contrast to earlier studies, Skogen et al. demonstrated that the fragment Bβ1–42 is a potent chemotactant for neutrophils and fibroblasts, independent of the FpB sequence (66). Bβ15–42 also was capable of inducing IL-8 expression in human oral squamous cell carcinoma cells. Antibodies against Bβ15–42 inhibited fibrin-induced IL-8 expression, while a peptide representing a sequence of the N terminus of the region (GHRP) mimicked fibrin exposure (67). However, later studies mainly reported the immunosuppressive potential of Bβ15–42 being protective in various pathologic conditions such as ischemia-reperfusion injury.

Thus, Bβ15–42 protects the myocardium against ischemia-reperfusion injury by reducing infarct size and leukocyte accumulation. Interestingly, the protective effect was abrogated in fibrinogen-deficient mice, suggesting that Bβ15–42 reduces fibrinogen-dependent inflammation. Furthermore, Bβ15–42 reduced NDSK II–induced leukocyte recruitment by competing with it for binding to VE-cadherin (48). This work has been translated into a multicenter phase IIA clinical trial investigating the effects of Bβ15–42 (FX06) on myocardial infarct size. In summary, Bβ15–42 significantly reduced the size of the necrotic core zone of infarcts while total late enhancement was not significantly different between control and Bβ15–42–treated groups (68).

In a pig model of hemorrhagic shock, Bβ15–42–treated animals showed improved pulmonary and circulatory function. Bβ15–42 reduced plasma IL-6 and neutrophil influx into the myocardium, liver and small intestine, protecting these organs from shock (69). Moreover, Bβ15–42 functions as a signaling molecule. In two different shock models—Dengue shock syndrome and LPS–induced shock model, Bβ15–42 preserved endothelial barrier function by inhibiting stress-induced opening of EC adherens junctions (70).
mainly by VE-cadherin, which in turn is under the control of RhoGTPases regulating actin dynamics and junction stability. Rho-kinase is activated in response to stress, and causes loss of function of the endothelial barrier. B15–42 prevented Rho-kinase activation by dissociating Fyn from VE-cadherin, which in turn associates to p190RhoGAP. B15–42–treated animals had improved survival rates and reduced hemoconcentration and fibrinogen consumption (70). In a cardiac transplant model, B15–42 also attenuated the ischemia-reperfusion injury (71). Thus, B15–42 is a promising therapeutic agent; however, the full mechanism of action is still under investigation.

Other fragments. Staton et al. proposed a potential role for a 24-amino acid fragment derived from the N terminus of the α chain of FgN-E named alphastatin. This peptide exhibited antiangiogenic effects by inhibiting growth factor-mediated migration, proliferation and tubule formation of human dermal microvascular ECs. In a syngeneic tumor model, B15–42 also attenuated the ischemia–reperfusion injury (71). Thus, B15–42 is a promising therapeutic agent; however, the full mechanism of action is still under investigation.

CONCLUSION

It is undisputed that inflammation activates the coagulation system and vice versa. Our current knowledge suggests that most players within the coagulation cascade have proinflammatory properties. In contrast, the peptide B15–42 mediates potent antiinflammatory effects. Therefore, any modulation of the coagulation system may reduce inflammation on one side, but on the other side may induce bleeding. Thus, targeting the proinflammatory aspects of coagulation, without affecting coagulation itself, might represent a novel therapeutic strategy within the treatment of inflammatory conditions.

High levels of fibrin fragments in septic patients with organ dysfunction suggest an increased fibrin turnover. Disease severity and outcome have not been correlated with fibrin fragments. Therefore, it might be of further interest to evaluate potential implications of fibrin degradation products as biomarkers of inflammatory conditions.

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DISCLOSURE

The authors declare that they have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.

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