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REVIEW

Fibrinogen as a key regulator of inflammation in disease

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Abstract The interaction of coagulation factors with the perivascular environment affects the development of disease in ways that extend beyond their traditional roles in the acute hemostatic cascade. Key molecular players of the coagulation cascade like tissue factor, thrombin, and fibrinogen are epidemiologically and mechanistically linked with diseases with an inflammatory component. Moreover, the identification of novel molecular mechanisms linking coagulation and inflammation has highlighted factors of the coagulation cascade as new targets for therapeutic intervention in a wide range of inflammatory human diseases. In particular, a proinflammatory role for fibrinogen has been reported in vascular wall disease, stroke, spinal cord injury, brain trauma, multiple sclerosis, Alzheimer's disease, rheumatoid arthritis, bacterial infection, colitis, lung and kidney fibrosis, Duchenne muscular dystrophy, and several types of cancer. Genetic and pharmacologic studies have unraveled pivotal roles for fibrinogen in determining the extent of local or systemic inflammation. As cellular and molecular mechanisms for fibrinogen functions in tissues are identified, the role of fibrinogen is evolving from a marker of vascular rapture to a multi-faceted signaling molecule with a wide spectrum of functions that can tip the balance between hemostasis and thrombosis, coagulation and fibrosis, protection from infection and extensive inflammation, and eventually life and death. This review will discuss some of the main molecular links between coagulation and inflammation and will focus on the role of fibrinogen in inflammatory disease highlighting its unique structural properties, cellular targets, and signal transduction pathways that make it a potent proinflammatory mediator and a potential therapeutic target.

Keywords Anticoagulant therapy · Inflammatory disease · Autoimmunity · Plasminogen · Complement receptor 3 · CD11b/CD18 · Blood brain barrier · Macrophages · Microglia · Multiple sclerosis · Atherosclerosis · Stroke · Rheumatoid arthritis · Alzheimer's disease

Introduction: cross-talk between coagulation and inflammation and its implications for human disease

Specific regulatory mechanisms have evolved to maintain the balance between coagulation and inflammation, ensuring protection from a wide spectrum of mechanical, environmental or pathological challenges, and ultimately survival. When either coagulation or inflammation is inefficient or becomes excessive, either spontaneously or after a challenge, new pathological manifestations emerge and preexisting ones become exacerbated. In most cases, these two processes are being studied as functioning independently of each other; numerous studies have identified the molecular players and characterized their specific functions that affect the hemostatic balance or the inflammatory cascade. However, coagulation and inflammation are activated by the same types of challenges and correlate both temporally and spatially in the same tissues,

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organs, and pathologies. These observations imply a more intricate interdependence between these two complex pathways.

Deregulated hemostatic incidents that cause the formation of occlusive blood clots are central in cardio/cerebrovascular diseases such as myocardial infarction and stroke, two of the leading causes of morbidity and mortality worldwide. Moreover, several key players of the coagulation system have been implicated in diseases with an inflammatory component. The involvement of coagulatory and fibrinolytic mechanisms to disease pathogenesis is not limited to diseases directly caused by vaso-occlusion but also extends to diseases that involve vascular rupture or leakage of blood components in the damaged tissue [1]. In addition to bleeding after physical injury, numerous vascular, infectious, inflammatory, autoimmune, or cancerous diseases involve disruption of vascular walls, rearrangement of the local vasculature, angiogenesis, and increased permeability resulting in leakage of plasma proteins in the perivascular space. Thus, the interaction of coagulation factors with the perivascular environment can affect the development of disease in ways that extend beyond the acute hemostatic cascade to control bleeding and could significantly influence the containment of infection, the extent of local or systemic inflammation, and the efficiency of tissue repair [1]. Epidemiological data have revealed the association between coagulation markers and the respective affected organs or tissues and have identified them as risk factors for disease development. Moreover, in the last two decades, the generation of a wide array of mouse mutants for molecules involved in coagulation and fibrinolysis has significantly expanded our understanding of the role that coagulation factors play in disease pathogenesis [2]. For example, fibrinogen, the end product of the coagulation cascade, has been identified as a significant risk factor and also as a modulator of inflammatory processes in several pathologic conditions. This review will discuss some of the main molecular links between coagulation and inflammation and will focus on the role of fibrinogen in inflammatory disease, highlighting some of its unique molecular and functional properties that make it an ideal target for therapeutic intervention.

Coagulation: molecular players

Coagulation is an intricate cascade of events that leads to blood clot formation. The primary purpose of coagulation is hemostasis, the cessation of hemorrhage, usually from a damaged blood vessel to the surrounding tissue. Platelets and the damaged endothelial cells of the vessel walls are the main cell types involved in the coagulation cascade. The key molecular players that initiate, amplify, propagate, and terminate this cascade are either freely circulating factors in the blood (e.g., inactive zymogens) or cell-bound on platelets, or endothelial cells of the vessel wall. Through the course of evolution, coagulation in vertebrates involved the interplay of more than two dozen genetically encoded factors [3]. The concept of the cascade/stepwise nature of the coagulation process was first described in 1964 [4, 5]. Although it was initially categorized in two distinct pathways, the *intrinsic* and the *extrinsic* pathways eventually converged into a third *common* pathway. Current views support an interconnected and interdependent relationship between factors of these pathways [6, 7]. Tissue factor (TF), a transmembrane glycoprotein extensively expressed in perivascular tissues, is considered the main initiator of the coagulation cascade following vascular damage and blood leakage in the perivascular space. TF forms a complex with factor VII to activate factor X either directly (*extrinsic* pathway) or by activating factor IX (*intrinsic* pathway). The two pathways intersect with the activation of factor Xa which mediates the cleavage of prothrombin to thrombin. Thrombin then cleaves fibrinogen into fibrin monomers, which upon polymerization form a fibrin clot. The availability of thrombin, calcium, and a negatively charged phospholipid membrane (usually that of platelets) leads to a self-amplified reaction that propagates very quickly by more thrombin and fibrin generation, additional recruitment of platelets, and the formation of clots of sufficient size to stop the hemorrhage from the injured vessel. The entire cascade is very tightly regulated by anticoagulant proteins and a series of natural inhibitors with several regulatory checkpoints that function in a typical positive or negative feedback loop way [8]. In addition, the fibrinolytic system regulates the dissolution of the fibrin clot to ensure no extensive or untimely fibrin formation, which can lead to severe thrombotic complications.

Coagulation factors with proinflammatory roles Coagulation factors play important biological roles not only in hemostasis but also in reproduction, tissue repair, and inflammatory responses related to infection or disease. Some of the most prominent ones like tissue factor, thrombin, and fibrin(ogen) have been significantly implicated in inflammation. For example, the role of tissue factor in inflammation ranges from affecting levels of inflammatory mediators such as interleukin (IL)-6 and IL-8 to activating protease-activated receptor (PAR) pathways either directly or through thrombin and thus contributing to inflammatory processes in disease models such as endotoxemia, sepsis, and ischemia–reperfusion (I/R) [9, 10].

Thrombin's role in inflammation has been extensively investigated since the 1970s [6, 11, 12]. Briefly, thrombin induces production of monocyte chemoattractant protein-1

(MCP-1), tumor necrosis factor (TNF)-α, and IL-1 and IL-6 in fibroblasts and endothelial cells and enhances leukocyte migration [13, 14]. In response to thrombin, endothelial cells become activated and upregulate E-selectin, MCP-1, IL-8, plasminogen activator inhibitor-1 (PAI-1), and IκB-α [15]. Pharmacologic inhibition in vitro and in vivo by hirudin, a specific thrombin inhibitor, underscored thrombin's importance in IL-6 and IL-8 production by fibroblasts and monocytes, as well as in driving leukocyte migration after a peripheral inflammatory challenge [15–17]. Most cellular functions of thrombin are mediated by PARs and especially PAR-1, which is widely expressed in platelets, vascular endothelial and smooth muscle cells, and leukocytes [18]. Thrombin-induced cytokine and chemokine production, platelet activation, vasodilation, leukocyte attraction, and other inflammatory responses have been attributed to PAR signaling [6]. Genetic depletion of PARs in mice and pharmacologic inhibition of thrombin and PAR signaling have been performed in numerous animal models of viral or bacterial infection like human immunodeficiency virus (HIV) encephalitis, endotoxemia and sepsis, and models of inflammatory disease like arthritis, glomerulonephritis, encephalomyelitis, dermatitis, colitis, asthma, and allergic hypersensitivity [19]. Depending on the experimental model, the receptor, and the stage of inflammatory disease, these studies have shown effects that range from protection to exacerbation of inflammatory processes, underscoring the complexity of the interplay between coagulation and inflammation at the molecular level. Since the end product of the coagulation cascade is fibrin, at least some of the effects of tissue factor, thrombin, and the other coagulation factors on inflammatory responses are inadvertently linked to those of fibrin(ogen).

Fibrinogen Fibrinogen is a soluble 340-kDa glycoprotein synthesized by hepatocytes in the liver [20]. Three separate genes encode for three distinct polypeptide chains called Aα, Bβ, and γ [21]. The three chains make an elongated molecule which binds to a second identical molecule by disulfide bonds, forming the homodimeric fibrinogen molecule that circulates in the blood [22–24]. Although the molecular structure of fibrinogen was elucidated in the 1970s, it was not until recently that X-ray crystallography was successfully performed on human and chicken fibrinogen as well as on fibrin fragments [25–29]. The resolution of these crystal structures provided a fundamental structural explanation for the pleiotropic biological functions of fibrinogen, fibrin, and their derivatives [30]. Indeed, several distinct binding sites have been identified on different parts of the fibrinogen molecule that are uniquely required for its binding to different receptors or adhesion molecules, expressed on the surface of cells of the hematopoietic, immune, and nervous systems.

Under physiological conditions, plasma concentrations of fibrinogen range from 2 to 4 g/L, and fibrinogen has a half-life of approximately 4 days [21]. However, in pathological conditions, such as after injury, or disease associated with vascular disruption, infection, or inflammation, the blood concentration of fibrinogen increases several fold [31]. Thus, fibrinogen is considered an acute-phase reactant. Upon activation of the coagulation cascade, thrombin cleaves off fibrinopeptides A and B from the fibrinogen molecule, exposing several polymerization sites and allowing spontaneous formation of fibrin fibrils [24]. After a series of crosslinking events that involve factor XIIIa, the insoluble fibrin clot is stabilized against mechanical, chemical, and proteolytic insults [20, 32]. The formation of a typical blood clot requires the involvement of platelets and fibrinogen acts as a substrate for platelet plug formation [33]. The C terminus of fibrinogen's γ-chain binds to a site on the $\alpha_{IIb}\beta_3$ integrin receptor on the platelet surface, thereby mediating the formation of bridges between platelets and facilitating their aggregation [34–36].

Although the activation of the coagulation pathway is essential for stopping a potentially lethal hemorrhage, fibrin deposition within tissues is carefully regulated in space and in time. Fibrin is considered a provisional matrix as its prompt removal usually precedes and is required for tissue healing. This is achieved by the fibrinolytic system, which counterbalances the abundant procoagulatory signals that drive coagulation at the site of tissue injury. The protease plasmin is the primary mediator of fibrin degradation and clot dissolution [37]. Local generation of plasmin is regulated by two serine proteases called plasminogen activators (PA), the tissue PA (tPA), and the urokinase type PA [38]. The proteolytic degradation of fibrin begins with binding of plasmin and tPA to fibrin and results in the generation of diffusible and soluble fibrin fragments, such as fragments D and E and D-dimer [39].

Under physiological conditions, fibrin formation and degradation are perfectly counterbalanced, while deregulation of either process results in profound clinical consequences as those seen in human diseases associated with hemorrhagic or thrombotic incidents as well as tissue fibrosis [40]. Moreover, numerous polymorphisms or mutations of fibrinogen result in either reduced fibrinogen levels (hypofibrinogenemia) or structural deficits that affect fibrinogen function (dysfibrinogenemia) [41]. Mouse mutants of the coagulation or the proteolytic pathway underline the importance of fibrinogen and the ability to form and degradate fibrin properly. For example, plasminogen deficiency in mice results in sustained deposition of fibrin in tissues and leads to high mortality, wasting, spontaneous gastrointestinal ulceration, rectal prolapse, severe thrombosis, and delayed wound healing [42].

Moreover, plasminogen deficiency results in exacerbation of inflammatory and traumatic diseases, such as collagen induced arthritis (CIA) [43] and peripheral nerve injury [44]. Remarkably, these abnormalities were rescued in mice genetically deficient for both plasminogen and fibrinogen [37] or following pharmacological fibrin depletion in plasminogen knockout mice [43, 44]. Overall these studies identified important biological roles for fibrin(ogen) as a molecular mediator of disease pathogenesis not only in the bloodstream but also within tissues [30].

Fibrinogen and inflammation

A constantly increasing body of evidence supports a prominent role for fibrin(ogen) and degradation products in regulating the inflammatory response in several target tissues [45]. Even before extravasation into the perivascular space, increased fibrinogen content in the blood is considered an indicator for a proinflammatory state and a high-risk marker for developing vascular inflammatory diseases, such as hypertension and atherosclerosis. Similarly, elevated levels of fibrin degradation products, such as D-dimer, are extensively used in clinical practice as indicators for inflammation, markers for increased coagulation activity, and risk predictors for thrombotic events [46]. In addition, the peptides released as a part of fibrin formation like fibrinopeptide B, which is cleaved from fibrinogen by thrombin, can act as chemoattractants for leukocytes and thus independently modulate inflammatory responses [47].

In most cases, the proinflammatory functions of fibrin(ogen) and its derivative peptides are associated with their ability to bind to and activate a wide range of immune cells through distinct ligand–receptor interactions [31]. Importantly, these proinflammatory functions are a product of fibrinogen signaling through binding sites that are non-overlapping with those involved in the coagulation cascade. The CD11b/CD18 integrin receptor (also termed Mac-1, complement receptor 3, or $\alpha_M\beta_2$ [48]) is a representative example. This receptor is expressed by leukocytes of the innate immune system, mostly circulating monocytes, tissue-specific macrophages, and central nervous system (CNS)-resident microglia [48]. A series of biochemical, mutational, and binding studies have identified multiple regions of the fibrinogen molecule with binding affinity to this receptor, such as the C-terminal regions of fibrinogen's β and γ-chain as well as that of a specific AαE splicing variant [49–51]. Further characterization identified the region between residues 377–395 (termed the P2 region) and in particular that between residues 383 and 395 on the C terminus of the fibrinogen γ-chain as the most critical region for binding to the CD11b/CD18 integrin receptor [52]. Importantly, this is a cryptic epitope in the blood-circulating conformation of the fibrinogen molecule, which becomes exposed and biologically active after a conformational change that occurs upon fibrinogen immobilization and formation of insoluble fibrin [53]. Fibrin(ogen) signaling through CD11b/CD18 has been shown to activate proinflammatory pathways, such as NF-κB, which results in local production of inflammatory cytokines, such as TNF-α and IL-1β [54–56].

A genetic mutation of residues 390–396 on the fibrinogen γ-chain provided a very elegant in vivo demonstration of the specificity of this region for regulating the inflammatory response by binding to CD11b/CD18 [57]. Indeed, substituting these residues with alanines in the $Fib\gamma^{390-396A}$ mouse failed to support adhesion of primary neutrophils, macrophages, and CD11b/CD18-expressing cell lines in vitro and severely compromised the leukocyte clearance of *Staphylococcus aureus* inoculated into the peritoneal cavity. Importantly, the $Fib\gamma^{390-396A}$ mutation had no effect on clotting functions, supported platelet adhesion, and did not develop spontaneous hemorrhagic events during development or in adulthood [57]. The binding of fibrinogen to the CD11b/CD18 integrin receptor is also important for the regulation of innate immune cell activation following implantation of biomaterials [58], as well as in diseases with an inflammatory component, such as muscle injury [59], multiple sclerosis (MS) [60], rheumatoid arthritis (RA) [61], colitis, and colitis-associated cancer (CAC) [62] (Table 1).

Another structurally similar receptor that also binds fibrinogen is the CD11c/CD18 integrin ($\alpha_X\beta_2$, p150,95) which is expressed mostly on dendritic cells but also on monocytes, macrophages, neutrophils, and some B cells [49, 63]. This receptor appears to bind the same regions on the fibrinogen molecule as the CD11b/CD18; however, the biological role of the fibrinogen–CD11c/CD18 interaction remains under-characterized [51]. A third example of a distinct integrin binding to fibrinogen is that of the $\alpha_{IIb}\beta_3$, which activates mast cells by binding the $\gamma^{408-411}$ epitope of the fibrinogen γ-chain [64]. Mast cell activation by fibrinogen-related homologous C-terminal peptides (haptides) modulates systemic blood pressure [65]. This is particularly important for regulating hypertension, a classic vascular proinflammatory condition for which elevated levels of fibrinogen are a risk factor [66].

Finally, fibrinogen signals either directly or indirectly through a number of other receptors, adhesion molecules, and cell-surface proteins that are involved in inflammatory processes. For example, the toll-like receptor 4 (TLR-4) has been implicated with the induction of macrophage activation and the release of several chemokines and cytokines, such as MCP-1, macrophage inflammatory protein-1 (MIP-1) α and β, IL6, IL8, TNF-α, matrix metalloproteinase (MMP) 1, and

Table 1 Summary of the studies that have employed fibrinogen mouse mutants and their effects on inflammatory processes in different disease models

Mouse line	Disease (animal model)	Function in Inflammation	References
Fib−/−	Atherosclerosis (*LDLR−/−/APOBEC1−/−*)	Increased thrombin generation and platelet activation	[101]
	Myocardial infarction/ischemia–reperfusion (occlusion of left anterior descending coronary artery)	Reduced IL-10 and TNF-α plasma levels and infarct size	[113, 114]
	Infection/endotoxemia (i.p. LPS osmotic pump)	Delayed neutrophil binding to endothelial cells, infiltration in lung capillaries, and expression of inflammatory cytokines	[210]
	MS (Tg [GFAP-TNF])	Reduced inflammation and inflammatory demyelination	[165]
	Colitis-associated cancer (AOM/DSS)	Reduced inflammation-driven adenoma formation	[62]
	Tumor metastasis (Lewis lung carcinoma)	Increased clearance of micrometastatic foci by natural killer cells	[204]
	Rheumatoid arthritis (CIA)	Reduced synovial inflammation and secondary leukocyte recruitment in joints Reduced mRNA levels of TNF-α, IL-1β, and IL-6	[61]
	Duchenne muscular dystrophy (*mdx*)	Reduced macrophage infiltration and activation in muscle	[59]
	Muscle injury (cardiotoxin)	Reduced macrophage infiltration in muscles	[59]
	Pulmonary fibrosis (bleomycin)	Altered neutrophil infiltration and/or clearance in lungs	[207]
	Crescentic glomerulonephritis (anti-GBM)	Reduced number of crescents and accumulated macrophages in kidneys	[209]
Fib+/−	AD (TgCRND8)	Reduced neurovascular pathology	[187]
Fibγ$^{390–396A}$	MS (EAE)	Reduced microglial activation and number of inflammatory demyelinating lesions in brain and spinal cord	[60]
	Colitis early (DSS)	Reduced expression of IL-6, IL-1β, TFN-α, IFN-γ, and macrophage infiltration in colon	[62]
	Colitis chronic (DSS)	Reduced expression of IL-6, IL-1β, macrophage, and neutrophil infiltration in colon	[62]
	Colitis-associated cancer (AOM/DSS)	Reduced inflammation-driven adenoma formation, proliferation, and size	[62]
	Muscle injury (cardiotoxin injection)	Reduced macrophage infiltration in muscle	[59]
	Rheumatoid arthritis (CIA)	Reduced synovial inflammation Reduced mRNA levels of TNF-α, IL-1β, and IL-6	[61]
	Bacterial infection (acute *S. aureus* peritonitis)	Defective activation of leukocyte antimicrobial functions and impaired bacterial clearance	[57]

MMP9 [67, 68]. The numerous interactions of fibrinogen with endothelial cells, platelets, smooth muscle cells, and circulating leukocytes through vascular endothelial cadherin (VE cadherin), ICAM-1, and several integrin receptors will be discussed in the context of their importance for vascular wall disease.

Fibrinogen in human diseases with an inflammatory component

Atherosclerosis and vascular wall disease Atherosclerosis is a chronic inflammatory condition that involves the gradual buildup of lipids in the vessel wall, the infiltration of immune cells, such as macrophages, T cells and mast cells, and the local proliferation of vascular smooth muscle cells [69]. In advanced atheromatous plaques, the excessive buildup of materials, including extracellular matrix proteins such as fibrin and collagen, leads to the formation of the *fibrous cap*, a local swelling of the vascular wall that further restricts the diameter of blood vessels and represents the core of atheromatous lesions [69, 70]. Increasing evidence from epidemiological studies and animal models implicates hemostatic factors in the early development of atherosclerosis and the very genesis of vessel wall-associated disease in general. Peripheral vascular disease (or peripheral arterial disease (PAD)) is a marker of systemic atherosclerosis and is associated with increased incidence of thrombotic disease, either cerebrovascular (stroke) or cardiovascular (myocardial infarction) [71]. Both atherosclerosis as the chronic underly-

ing condition and the vaso-occlusive/thrombotic diseases share similar risk factors and pathogenic mechanisms. Some of the common mechanisms that link coagulation and inflammation in the context of atherosclerosis and vascular diseases will be discussed here.

The most common hemostatic factors associated with the development of atherosclerosis and vascular disease are fibrinogen, von Willebrand factor, tPA, PAI-1, and factors VII and VIII [72]. Among these factors, consistent epidemiological evidence supports a causal relationship for fibrinogen and primary vascular disease, making it one of the best-established risk factors for PAD, along with smoking, hypertension, diabetes mellitus, and hyperlipidemia [71]. In addition, several studies link inflammatory processes to the development of PAD [73, 74]. Besides fibrinogen, C-reactive protein (CRP) and IL-6 are also common inflammatory markers with significant links to PAD [74, 75]. IL-6 is a proinflammatory cytokine that induces the secretion of acute-phase proteins from hepatocytes, such as CRP and fibrinogen [76–78]. Interestingly, a specific polymorphism in the IL-6 gene (the G(−174)C IL-6 polymorphism) appears to promote the development of PAD in patients with type-2 diabetes (a high-risk condition for PAD) in correlation with elevated plasma fibrinogen and CRP [79].

Fibrinogen is a central element of hemostasis and coagulation [72, 80, 81]. Of relevance for atherosclerosis and vascular disease pathology are its effects on plaque composition, blood viscosity, endothelial and smooth muscle cell activation, platelet aggregation and activation, and immune cell recruitment. Elevated fibrinogen levels can cause an increase in blood viscosity and red blood cell aggregation [82]. Significantly increased levels of fibrinogen and blood viscosity are commonly found in patients with transient ischemic attacks, suggesting that fibrinogen levels are elevated even before thrombotic incidents occur and are thus an important risk factor for stroke [83, 84]. Increased blood viscosity and local cell aggregation inevitably increase blood flow shear stress [85], a process that activates endothelial cells [86] and platelets [87]. Disturbances in blood flow with associated reciprocating low shear stress result in upregulation of endothelial cell genes and proteins that promote atherogenesis [88] and could lead to tissue ischemia, heart disease, stroke, or a range of pathological conditions, known as the *hyperviscosity syndrome* [89]. Local activation of endothelial cells especially in areas of altered blood flow results in upregulation of adhesion molecules and integrin receptors that control physiological changes in the vasculature, ranging from vasodilation to vasoconstriction. Fibrinogen has vasoactive effects. It signals through ICAM-1 and causes vasoconstriction through extracellular signal-regulated kinase-1/2 signaling and increased production of endothelin-1 [90]. Fibrinogen signaling through either the $a_5\beta_1$ or the $a_v\beta_3$ integrin also has vasoactive effects, often with opposite results [66]. Integrin and ICAM-1 signaling affect the endothelial cell layer integrity and vascular permeability in a fibrinogen-dependent manner [66]. Binding of fibrinogen to ICAM-1 increases the formation of filamentous actin and endothelial layer permeability, primarily by altering the expression of actin-associated endothelial tight junction proteins, such as occludin and zonula ocluden-1 and zonula ocluden-2 [91, 92]. Altering the physiological properties of the endothelial cells and the integrity of the vascular wall sets the stage for the development of hypertension and/or atherosclerosis, two proinflammatory conditions that are considered of high risk for more severe clinical manifestations, such as sudden cardiac death, myocardial infarction, and stroke.

In addition to endothelial cells, fibrin(ogen) also interacts with several other cell types that participate in lesion formation such as smooth muscle cells, platelets, and leukocytes and exerts its atherogenic effects in multiple ways [1]. Indeed, fibrinogen and fibrin degradation products have been detected within atherosclerotic plaques [93], where they can induce migration, proliferation, and secretion of proinflammatory cytokines, such as IL-6 and TNF-α by smooth muscle cells [94, 95]. In addition, fibrinogen causes platelet aggregation by binding to the platelet $\alpha_{IIb}\beta_3$ integrin [96]. It is widely accepted that the increased local accumulation of platelets within atherosclerotic lesions has detrimental consequences after sudden lesion disruption and release of a thrombus with vaso-occlusive potential.

However, the degree to which platelets are also involved with the initial stages of atherosclerosis is actively debated, given that platelets are separated from the vascular intima—the primary niche for atheromatous lesion initiation—by a continuous layer of endothelial cells [97]. Fibrinogen binding on both the endothelial cells (altering the vessel wall permeability) and to platelets (causing their local aggregation) could thereby act as the central mediator of atherosclerosis within the vascular wall. Indeed, the adherence of platelets to the endothelial cells is mediated primarily by P-selectin, and fibrinogen is required for the maintenance of P-selectin expression in platelets [98]. Experiments performed in fibrinogen- and β3 integrin-deficient mice elegantly demonstrated the requirement of both β3 integrin engagement and fibrinogen for the maintenance of platelet intracellular and cell-surface P-selectin expression [98]. The adherence of platelets to the endothelial cell wall involves not only P-selectin but also the integrins $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ and induces their activation [99]. In the context of atherosclerosis, increased blood cholesterol levels are also known to increase the aggregability and the sensitivity of platelets through lipoprotein particles such as low density lipoprotein (LDL). LDL sensitizes platelets via binding of apolipoprotein B-100 to

a receptor on the platelet surface and through transferring of lipids to the platelet membrane [100]. Fibrinogen deficiency resulted in an accelerated initiation of LDL cholesterol-driven atherosclerosis via thrombin generation and platelet activation in mice genetically predisposed to develop LDL-C–dependent atherosclerosis [101]. Platelets are the main cell type involved in the coagulation cascade, and after their activation, they exert a proinflammatory role by releasing cytokines and chemokines, such as IL-1β, CD40 ligand, and RANTES [102–104]. IL-1β synthesized by activated platelets signals endothelial cells and induces polymorphonuclear cell adhesion to them in a β3 integrin-dependent manner [102]. Moreover, IL-1β acts in concert with P-selectin to induce NF-κB activation and cyclooxygenase-2 promoter activity during the tethering of monocytes on activated platelets [105]. The binding of fibrinogen to integrin $\alpha_{IIb}\beta_3$ rapidly activates platelets to release CD40 ligand. In turn, this promotes chemokine secretion and adhesion molecule upregulation in endothelial cells, thereby promoting the accumulation and extravasation of leukocytes at the site of lesions [103, 106]. Finally, RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium in a P-selectin-dependent manner [104, 107].

Fibrinogen and the fibrin degradation products also attract circulating leukocytes to the vessel wall, through ligand–receptor interactions both on the side of the endothelial cell layer and on the side of the leukocytes. Leukocyte–endothelium interactions are a key event in vessel wall disease and are implicated in the pathophysiology of reperfusion injury. This refers to the extensive tissue damage that occurs in the myocardium when the blood flow in the heart is restored after an ischemic incident, which is distinct from the damage caused by the ischemia per se [108]. Fibrinogen binds to endothelial cells through ICAM-1, and this interaction affects leukocyte–endothelial adhesion and transmigration in vitro [109–111]. In addition, fibrin(ogen) binds to VE cadherin (CD144) on the endothelial cell surface. This binding has been mapped on the fibrinogen β-chain (residues β15–42), a site which is also cryptic in the soluble form of the fibrinogen molecule and is only exposed after cleavage of fibrinopeptide B by thrombin [112]. As discussed above, fibrinogen also binds to the CD11c integrin receptor, which is widely expressed on several cell types of the leukocytic lineage. However, VE cadherin and CD11c have distinct binding sites on the fibrinogen molecule. Interestingly, binding sites for both receptors are found on the same fibrin degradation product (fragment E1), which is a product of plasmin-mediated fibrinolysis and is elevated after ischemic incidents [108]. Petzelbauer et al. used a small peptide with exactly the sequence required for fibrin binding to VE cadherin (peptide $B\beta^{15-42}$), to block E1 fragment-induced transmigration of leukocytes through an endothelial cell monolayer in vitro [113]. A similar effect was achieved with a blocking fibrin-derived peptide for the CD11c integrin receptor or blocking antibodies against either the receptor of the endothelial cells (VE cadherin) or the receptor on the leukocytes (CD11c). Moreover, significantly reduced infarct size, as well as IL-10 and TNF-α plasma levels, were shown after myocardial reperfusion injury in fibrinogen knockout mice [113, 114]. In a series of follow-up studies, the $B\beta^{15-42}$ peptide reduced infarct size both ex vivo (in perfused hearts) and in vivo (in acute and chronic ischemia–reperfusion models simulating myocardial infarction or heart transplantation conditions) in mice, rats, or pigs [113–116] (Table 2). Treatment of these animals with the $B\beta^{15-42}$ peptide was followed by reduced leukocyte accumulation, scar formation, and plasma levels of proinflammatory cytokines such as TNF-α, IL-1, IL-6, IL-10, and IL-12 [113–116]. Furthermore, the protective effects of the $B\beta^{15-42}$ peptide were also shown in multiple organs in a pig model of hemorrhagic shock that involved extensive blood withdrawal and resuscitation [117]. Compared to vehicle-treated animals, $B\beta^{15-42}$ peptide treatment resulted in reduced accumulation of myeloperoxidase-positive cells (neutrophils, monocytes, and macrophages) in myocardium, liver, small intestine, and lower IL-6 plasma levels [117]. These results indicate that fibrin(ogen) and its derivative fragments are involved in mechanisms regulating systemic inflammatory signals, as well as the transmigration of mostly neutrophils and monocytes through endothelial cell surfaces and their accumulation in organs throughout the body, and could thus be explored as pharmacological targets with significant therapeutic potential for ischemia–reperfusion injury.

Overall, the role of fibrinogen in atherosclerosis and related vascular pathologies extends from a structural component of the atherosclerotic plaque to a key proinflammatory player within the affected vascular bed. Fibrin(ogen)'s pro-atherogenic role can be manifested through its contribution to the actual lesion matrix and collateral damage on the vessel wall, activation of endothelial and smooth muscle cells, recruitment of platelets, and accumulation of circulating monocytes, thereby orchestrating a multi-cellular inflammatory cascade with potentially lethal long-term consequences (Fig. 1). As a result, epidemiological studies have now established fibrinogen as an indicator of the severity of PAD, an early predictor for future development of PAD, and thus a high-risk indicator for ischemic cardiovascular or cerebrovascular incidents [71, 118].

Inflammatory joint disease or rheumatoid arthritis RA is a systemic disorder characterized by chronic inflammation, edema, pronounced angiogenesis, and extensive fibrin deposition in the synovial joints, which progressively

Table 2 Summary of the studies that have employed blocking peptides or pharmacologic agents that either inhibit the interaction of fibrinogen with its receptors, or reduce systemic fibrinogen levels or prevent fibrin formation, and their effects on inflammatory processes in different disease models

Pharmacological agent	Disease (animal model)	Function in Inflammation	References
$\gamma^{377–395}$ peptide	MS (EAE)	Reduced microglial activation (Mac-3 and iNOS expression) and number of inflammatory demyelinating lesions in brain and spinal cord	[60]
$B\beta^{15–42}$ peptide	Myocardial infarction/ischemia–reperfusion (occlusion of left anterior descending coronary artery)	Reduced leukocyte accumulation and scar formation in vivo (rats) and reduced infarct size ex vivo (perfused hearts) and in vivo after acute or chronic reperfusion (rats and mice)	[113]
	Myocardial infarction/ischemia–reperfusion (occlusion of left anterior descending coronary artery)	Reduced plasma levels of TNF-α, IL-1, IL-6, IL-10, and IL-12 after acute reperfusion in vivo (mice) and infarct size after acute or chronic reperfusion (rats and mice)	[114]
	Myocardial infarction/ischemia–reperfusion (occlusion of left anterior descending coronary artery)	Reduced IL-6 plasma levels and infarct size in vivo (pigs)	[115]
	Hemorrhagic shock/reperfusion (blood withdrawal and resuscitation)	Reduced accumulation of myeloperoxidase-positive cells (neutrophils, monocytes, macrophages) in myocardium, liver, small intestine, and reduced IL-6 plasma levels in vivo (pigs)	[117]
	Ischemia–reperfusion injury (heart transplantation)	Reduced number of infiltrating leukocytes during reperfusion and reduced necrosis in myocardial tissue	[116]
Hirudin	Arthritis (AIA)	Reduced synovial IL1β mRNA levels	[125]
	Arthritis (CIA)	Reduced synovial IL1β and IL-12p35 mRNA levels	[126]
	MS (EAE)	Fewer inflammatory foci in brain and spinal cord, decreased immune cell proliferation and IL-6, TNF-α and IL-17 production by splenocytes in vitro production by splenocytes in vitro	[164]
Ancrod	MS (EAE)	Reduced microglial activation and number of inflammatory demyelinating lesions in brain and spinal cord	[60]
	MS (Tg [GFAP-TNF])	Reduced inflammation and inflammatory demyelination	[165]
	AD (TgCRND8)	Reduced microglial activation	[187]
	AD (intra-hippocampal injection of Aβ1–42)	Reduced microglial activation	[188]
	Duchenne muscular dystrophy (*mdx*)	Reduced macrophage infiltration and activation, and TNFα, IL-6, MIP-2, and IL-1β levels in muscle	[59]

degenerate and become dysfunctional [119]. Several lines of evidence point to a link between fibrin(ogen) and disease pathology in RA and related animal models. Within the first weeks of its onset, the most prominent manifestations are pain, swelling, and fibrin deposition in the joints, which persists throughout the progression of the disease [119]. Moreover, fibrin degradation products such as D-dimer are common biomarkers found in patient plasma as well as in synovial fluid [120–122]. The presence of fibrin in the synovial space correlates with the aggregation and morphological activation of intimal cells in the antigen induced arthritis (AIA) model of RA [123]. Following exposure to fibrinogen, synovial fibroblasts secrete IL-8 and growth-related oncogene-alpha and upregulate ICAM-1 in an NF-κB-dependent manner, leading to enhanced adhesiveness of lymphocytes in vitro [124].

A direct demonstration of the proinflammatory role of fibrin(ogen) comes from studies in animal models using pharmacologic and genetic approaches (Tables 1 and 2). Administration of the thrombin inhibitor hirudin resulted in a significant reduction of fibrin formation within joints; this reduction was accompanied by a diminution of inflammation and disease severity in mice with either AIA or CIA [125, 126]. Experiments using fibrinogen-deficient mice in CIA showed fewer affected joints and an overall significant amelioration in disease severity compared to controls [61]. Similarly improved clinical image was reported in the $Fib\gamma^{390–396A}$ mouse line, which also showed a reduction in proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, in a CD11b/CD18-dependent manner [61] (Fig. 1). While fibrin did not affect leukocyte trafficking to joints, it induced local activation of leukocytes that drives the

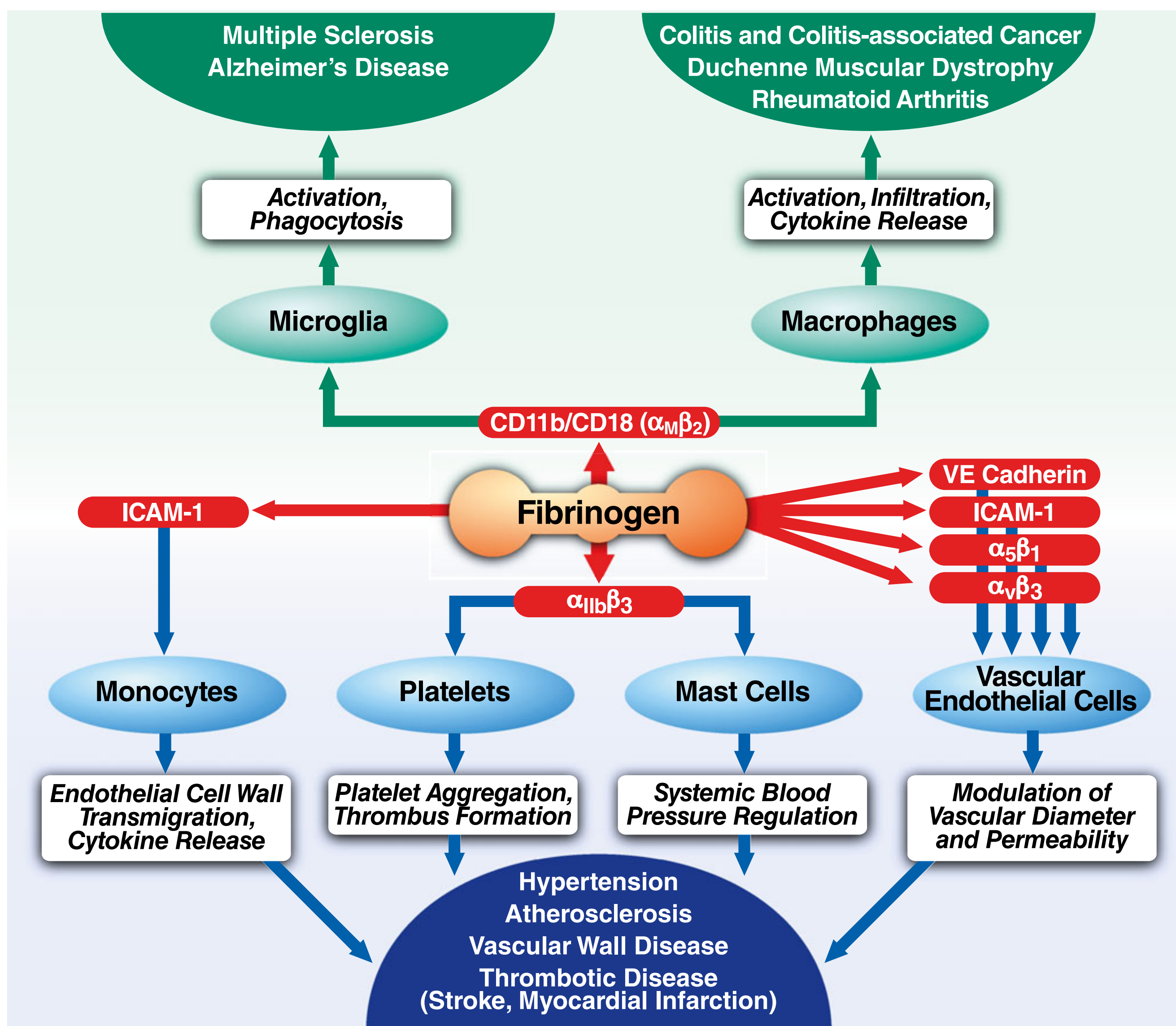

Fig. 1 Fibrinogen as a mediator of inflammatory disease. Fibrinogen acts on different cell types through cell-specific integrin and non-integrin receptors to induce specific inflammatory functions in a variety of diseases with an inflammatory component

proinflammatory cascade within inflamed joints [61, 127]. These findings suggest a central role for fibrin in RA pathogenesis.

In addition to the local proinflammatory role that fibrin deposition appears to play in joints, another role for citrullinated fibrinogen (a specific post-translationally modified form of fibrinogen) has emerged, positioning it as a strong autoantigen candidate in RA pathology [128]. Indeed, both in arthritis patients and in animal models of RA, elevated levels of citrullinated fibrinogen as well as antibodies against it have been reported in the plasma and within the joints [129–132]. Moreover, immunization in mice transgenic for the RA-associated MHC class II molecule DRB1*0401 (DR4-IE tg mice) with citrullinated, but not unmodified human fibrinogen induced a progressive arthritic condition, a transient appearance of citrullinated proteins and swelling in the joints but limited inflammatory cell infiltration [133]. In addition, mice tolerized with a citrullinated peptide showed significantly reduced disease severity and incidence compared with controls [134]. Importantly, the same study demonstrated that antibodies specific to citrullinated fibrinogen were sufficient to enhance arthritis and bound targets within the inflamed synovium of mice with CIA [134]. These results demonstrate that antibodies against citrullinated fibrinogen could play a central role in the pathogenesis of autoimmune arthritis.

Furthermore, human naturally citrullinated fibrinogen was successfully used to develop a novel model of experimental arthritis in specific mouse strains [135]. The recently developed fibrinogen-induced arthritis (FIA) model

presents with fibrinogen-reactive T cells that produce IL-6, IL-17, TNF-alpha, and IFN-gamma and can be adoptively transferred to healthy mice with either plasma or fibrinogen-specific T cells from diseased mice [135]. Although FIA was limited to specific joints and mouse strains, these results demonstrated that fibrinogen is arthritogenic in mice and that the pathogenesis of FIA is mediated by both autoantibodies and fibrinogen-reactive T cells [135]. Importantly, the mouse strains that are susceptible to FIA express a full array of markers characteristic of the human disease, highlighting another proinflammatory function for fibrinogen that involves the development of autoimmunity, at least in a mouse model of RA-like disease [127].

Neurologic diseases with BBB disruption

In healthy conditions, fibrinogen circulates through the brain and the spinal cord vasculature without entering the CNS due to the elaborate architecture of the vascular walls in the CNS that form the *blood brain barrier* (BBB). The BBB is a physical barrier, attributed primarily to the tight junctions between the endothelial cells of the cerebral vasculature [136, 137]. The BBB restricts entry of potentially harmful substances, plasma proteins, and immune cells from the blood stream to the CNS, while supplying it with essential nutrients for proper function [138]. However, several pathological conditions that involve either acute hemorrhage (such as brain or spinal cord injury and hemorrhagic stroke) or chronic disruption of the BBB (such as MS, Alzheimer's disease (AD), brain glioblastoma, HIV encephalitis, and bacterial meningitis) result in the deposition of fibrin(ogen) in the CNS [45, 136, 137, 139].

Stroke and brain or spinal cord injury After brain trauma or spinal cord injury (SCI) or stroke, the sudden leakage of blood in the CNS parenchyma initiates the coagulation cascade that stops the hemorrhage to prevent further tissue damage or death. However, the coagulation cascade also results in an extensive deposition of fibrin in the CNS that can impede regenerative and local tissue repair mechanisms and modulate local inflammation [31, 45, 140]. Indeed, the deposition of blood proteins and fibrin(ogen) in particular is extensive after brain trauma or SCI [141–143], as well as after hemorrhagic but also ischemic stroke [137, 144]. Traumatic cerebrovascular injury causes ischemia in the tissue that is left without sufficient oxygen supply and therefore results in acute tissue necrosis; however, a secondary wave of tissue damage is associated with the inflammatory response that follows CNS trauma [137, 144, 145]. Importantly, fibrinogen knockout mice had a significantly improved cerebral reperfusion in the absence of fibrin(ogen) deposition and a marked reduction in brain infarction after ischemia–hypoxia [144].

Our previous studies identified several distinct cellular targets, as well as novel molecular mechanisms through which fibrin(ogen) inhibits the recovery and repair mechanisms after injury in the peripheral and the central nervous systems. In brief, fibrinogen binding to $\alpha_V\beta_3$ directly inhibits neurite outgrowth in the CNS [142], while binding to CD11b/CD18 activates microglia [60]. Fibrinogen prevents remyelination and axonal regeneration after peripheral nerve injury [146] and regulates glial scar formation after brain trauma [143]. These studies revealed new cellular targets for fibrin in the nervous system and described mechanisms that prevent physiological recovery processes following a traumatic insult.

In addition to cell activation by fibrinogen binding to cellular receptors, we recently discovered that fibrinogen in the bloodstream serves as a carrier for latent transforming growth factor-β (TGF-β) [143]. Following stab wound injury, fibrinogen gets deposited in the brain and the latent form of TGF-β, which is carried along by fibrinogen, interacts with astrocytes, leading to the formation of active TGF-β and activation of downstream signaling pathways essential for glial scar formation in the injured brain [143]. Moreover, since TGF-β is also an established regulator of inflammation, fibrinogen's role in carrying the latent form of TGF-β into tissues could also represent a potentially important mechanism for the regulation of local inflammatory processes. Indeed, TGF-β has potent immunosuppressant functions and is mostly known for controlling immune cell proliferation and for regulating the development of adaptive immune responses [147, 148]. In addition, TGF-β can suppress the activation of macrophages, dendritic, and natural killer cells [148]. For example, TGF-β inhibits the induction of inducible nitric-oxide synthase (iNOS) and MMP-12, as well MyD88-dependent TLR signaling pathways in macrophages [149, 150]. The delivery of a potent regulator of both innate and adaptive immune responses within tissues with fibrin deposition could represent a novel role for fibrinogen in the regulation of inflammation with important therapeutic implications for treatment after injury in the CNS and possibly in other target organs as well.

Recently, a novel mechanism of fibrin(ogen) extravasation in the brain was identified, involving the formation of a thrombus that obstructs the circulation in cerebral blood vessels, like those that primarily cause ischemic strokes. After a non-lethal thrombotic incident, the primary mechanism for re-establishing blood flow, restoring delivery of oxygen and glucose, and limiting the extent of ischemic damage in the brain is that of the activation of the fibrinolytic system. Importantly, administering recombinant tPA, which initiates the fibrinolytic cascade, is the only treatment for acute ischemic strokes approved for use in the clinic [151, 152].

Through this mechanism plasmin degrades fibrin-based thrombi to soluble degradation products [39], leading to recanalization of the occluded vessels, a process that also depends on the hemodynamic forces at the site of the occlusion [153]. However, spontaneously formed microemboli, such as small fibrin clots or atheromatous fragments, could occlude capillaries and terminal arterioles as a result of their small diameter and the reduced flow velocity through them. Lam et al. showed that exogenous fibrin or cholesterol microemboli that were injected intravenously in mice but were not cleared by fibrinolysis or hemodynamic forces had undergone extravasation and the flow in capillaries was restored within a few days [154]. The authors elegantly combined repetitive in vivo two-photon microscopy to decipher the mechanism of extravasation and electron microscopy to confirm it. Surprisingly, this phenomenon required envelopment of intravascular microemboli by an extending endothelial membrane and subsequent translocation of the microemboli to the perivascular space within 2–5 days [154]. Mechanistically, the perivascular translocation of the emboli required MMP2/9 and resulted in the formation of a new vascular wall and restoration of the blood flow, while the extravasated clots were taken up by perivascular microglia [154]. In stroke patients, a significant percentage of ischemic strokes undergo a hemorrhagic transformation [155]. This mechanism of thrombus extravasation together with increased BBB permeability might contribute to the extensive fibrin deposition and the other signs of hemorrhage that have been described in several stroke models [156–158].

Multiple sclerosis MS is a chronic inflammatory disease of the CNS characterized by perivascular inflammatory lesions that show extensive fibrin deposition, demyelination, and axonal damage [159–161]. The disruption of the BBB can be detected in human MS patients before the onset of clinical signs [162] and persists throughout the course of disease [163]. A thorough proteomic analysis was performed on human brain material that was laser-microdissected to isolate MS lesions at different disease states [164]. This analysis identified a prominent dysregulation of several proteins of the coagulation cascade within MS lesions, such as tissue factor and protein C inhibitor [164]. Moreover, inhibition of the coagulation cascade with the thrombin inhibitor hirudin in experimental autoimmune encephalomyelitis (EAE), the classic animal model for MS, produced a significant amelioration in disease severity and suppressed the expression of Th1 and Th17 cytokines in astrocytes and immune cells [164]. This confirmed prior studies where genetic or pharmacologic depletion of fibrin (using snake venom-derived defibrinogenating agents like ancrod or batroxobin from the pit vipers *Agkistrodon rhodostoma* and *Bothrops atrox*, respectively) suppressed clinical symptoms in EAE [60, 165–167]. For example, we previously showed that fibrin(ogen) can regulate the inflammatory response in neuroinflammatory disease using transgenic animals, where TNF is specifically expressed in the CNS (TNF-transgenic mice). In the TNF-transgenic animal models for MS, mice spontaneously develop chronic inflammatory demyelinating disease. Introducing fibrinogen deficiency in one such model [168] increased the lifespan and delayed clinical symptom onset, while it significantly decreased inflammation and demyelination [165]. In accordance, administration of ancrod in another TNF-transgenic mouse model [169] also delayed the onset of inflammatory demyelination and diminished immune responses in the CNS [165]. These studies highlight the potential for exploring molecules of the coagulation cascade as targets for pharmacologic intervention with promising anti-inflammatory and overall therapeutic potential (Tables 1 and 2).

Increased overall inflammatory activity as well as prominent microglial activation correlates with fibrin deposition in active demyelinating MS lesions [170, 171]. Interestingly, BBB disruption, fibrin deposition, and microglial activation are the earliest histopathological findings that can be detected even in normal appearing white matter, namely in areas with no signs of demyelination [172, 173]. These findings suggest a potential link between fibrin deposition and the onset of inflammation in MS. Indeed, studies from our lab showed that microglia, the resident immune cells of the CNS, are a direct cellular target for fibrinogen upon its leakage in the CNS [60]. We and others have also identified microglia as the first responders to local parenchymal or vascular damage in the CNS, and their rapid process extension toward injured blood vessels has been proposed as a possible response to the disruption of the BBB [174, 175]. Fibrinogen can directly activate microglia in vitro and increase their phagocytic ability [60]. As discussed above, conversion of fibrinogen to insoluble fibrin exposes the cryptic epitope $\gamma^{377-395}$, and binding to the integrin receptor CD11b/CD18, which is specifically expressed by microglia in the CNS, becomes possible. In vivo, genetic or pharmacologic inhibition of this specific ligand–receptor interaction showed a significant reduction in microglial activation that was correlated with a suppression of paralysis and relapse incidence in EAE [60]. The fibrinogen mutant $Fib\gamma^{390-396A}$ [57], or administration of a $\gamma^{377-395}$ peptide that had previously been shown to inhibit fibrinogen binding to CD11b/CD18 [52], significantly reduced microglial activation during the course of EAE [60]. Importantly, neither of these inhibitory approaches interfered with the clotting function of fibrinogen [45, 57]. This study demonstrated a requirement for fibrinogen's binding to CD11b/CD18 for its downstream proinflammatory effects on microglia and identified fibrinogen as a target for pharmacologic intervention in inflammatory demyelinating disease, like MS (Fig. 1). To date, anticoag-

ulant treatment has been used by several different groups in four animal models of MS-like disease, which all showed significant amelioration in clinical scores, and some further identified a reduction in the inflammatory processes in the CNS [60, 164–167] (Tables 1 and 2). The potential advantages as well as the considerations concerning the adverse hemorrhagic effects of prolonged anticoagulant therapy for the treatment of MS have been discussed in more detail elsewhere [45]. In addition to specific epitope targeting for fibrinogen's ligand–receptor interactions, further exploration of new-generation inhibitors of the coagulation pathway with decreased hemorrhagic side effects might prove beneficial for the treatment of chronic inflammatory diseases, such as RA and MS.

Alzheimer's disease AD is a neurodegenerative disease with prominent BBB disruption. In AD, the proteolytic processing of the amyloid precursor protein results in an increased production and extracellular secretion of a 40–42-amino acid peptide called *amyloid β* (Aβ) [176]. The increased burden of Aβ in the AD brain exists either in a soluble form or as aggregates that form in the brain parenchyma, called *amyloid plaques* [177, 178]. Aβ also aggregates in the form of fibrils that deposit in the walls of mostly arterial, small- to medium-sized blood vessels, causing a condition called *cerebral amyloid angiopathy* [179, 180].

Disruption of the BBB has been documented in human patients [181–184] and animal models of AD [185–187]. Several coagulation factors that leak from the disrupted vasculature have been identified in AD brains, especially in advanced stages of the disease, including prothrombin, factor VIII, von Willebrand factor, and fibrinogen [184, 188]. Increased fibrinogen levels have been associated with an increased risk for the development of dementia and AD [189, 190]. Indeed fibrinogen deposition increases in parallel with Aβ load in the brain as the AD pathology progresses [187, 188, 191].

Two studies demonstrated the close spatial relationship between Aβ and fibrinogen deposition in the AD brain as well as their proinflammatory role in activating microglia, using both human material and animal models for AD (Table 2). In the first study, Paul et al. used both a genetic and a pharmacologic approach to impair fibrin formation or fibrinolysis and study their effects in an animal model for AD (TgCRND8) [187]. They found that while AD mice bearing one copy of the plasminogen gene (TgCRND8; $plg^{+/-}$) had exacerbated neurovascular pathology and BBB disruption, mice heterozygous for the fibrinogen gene (TgCRND8;$fib^{+/-}$) had significantly improved pathology, both compared to TgCRND8 littermates [187]. In accordance, they also demonstrated opposite effects when they pharmacologically depleted fibrin formation or impaired fibrinolysis in the same mouse AD model; while fibrinogen depletion significantly reduced BBB damage and microgliosis, the phenotype was exacerbated when a plasmin inhibitor was used [187]. Interestingly, when the plasminogen mutant AD mice were pretreated with ancrod, the resulting depletion of fibrinogen from the blood inversed their exacerbated phenotype, implying that fibrin deposition in the AD brain was indeed responsible for the observed proinflammatory effects [187]. The second study showed in human AD brains a correlation of increased fibrinogen immunoreactivity with areas of microglial activation [188]. In the same study, the proinflammatory role of fibrinogen was found to have a synergistic effect with Aβ. While intra-hippocampal injection of Aβ increased vascular permeability, microglial activation and neuronal damage, co-injection of fibrinogen together with Aβ further enhanced these effects in vivo [188]. These results were rescued when ancrod or a blocking antibody for the CD11b/CD18 integrin receptor were used, implying that the neurodegenerative effect of Aβ in the brain could at least in part involve the proinflammatory function of fibrinogen on microglia [188] (Fig. 1).

Additional studies indicate that Aβ associates with fibrinogen, leading to formation of abnormal fibrin clots that are resistant to fibrinolysis and are therefore more potent activators of proinflammatory mechanisms that can lead to neurodegeneration [192, 193]. While the original amyloid hypothesis that attributed the AD pathology primarily to amyloid plaque deposits in the brain parenchyma is increasingly being challenged by the AD field [194, 195], new avenues need to be explored for potential treatments. Since there is a prominent cerebrovascular pathology associated with AD [182, 183, 196], a therapeutic strategy against the fibrinogen-induced inflammatory and neurodegenerative functions could be very beneficial against the onset and progression of this debilitating disease [197, 198].

Fibrinogen and inflammation in animal models of disease

Several additional pathologic conditions or diseases with an inflammatory component present with elevated fibrinogen and fibrin degradation products in the blood or within the affected tissues. Some of those in which fibrin(ogen) has been shown to play a role as a proinflammatory mediator include colitis and colitis-associated cancer, tumor metastasis, Duchenne muscular dystrophy, pulmonary fibrosis, crescentic glomerulonephritis, and bacterial infection. Direct demonstration of the link between fibrinogen and the inflammatory processes that are prominent in these

pathologies has come from studies in animal models using mouse mutants for fibrinogen (Table 1), or pharmacological agents which result in significant reduction or depletion of fibrin in tissues (Table 2).

In an animal model of inflammatory bowel disease or *colitis*, genetic disruption of the binding of fibrinogen to the CD11b/CD18 integrin receptor using the $Fib\gamma^{390-396A}$ mice resulted in significant protection both at early stages and at the chronic phase of the disease model [62]. During the early phases of colitis, $Fib\gamma^{390-396A}$ mice showed a marked reduction in proinflammatory cytokine expression (IL-6, IL1β, IFN-γ, TNFα), macrophage infiltration as well as expression of epithelial cell activation markers (cJun, p65) compared to controls [62] (Fig. 1). At the chronic phase of the same colitis model, $Fib\gamma^{390-396A}$ mice were significantly protected from ulceration, edema, macrophage, and neutrophil infiltration, as well as IL-6 and IL1β expression in the colon and IL-6 plasma levels [62].

Chronic inflammation generates an environment that can promote the development of tumors [199], and as such, chronic colitis can cause colorectal cancer [200]. In an animal model of CAC, fibrinogen-deficient mice developed significantly fewer adenomas than wild-type controls [62]. A similarly dramatic improvement was shown in adenoma incidence, size, and proliferation in the colon of $Fib\gamma^{390-396A}$ mice following the same CAC experimental challenge [62]. Collectively, these results demonstrate a role for local fibrin(ogen)–monocyte and neutrophil interactions that promote inflammation at the early stages of disease, exacerbate tissue damage when the disease becomes chronic, and can eventually promote tumor development and growth over time.

Another interesting aspect of fibrinogen's deleterious role in the context of cancer stems from its primary function as a coagulation factor that mediates platelet aggregation and thrombus formation. Fibrinogen has been shown to be an important determinant of the metastatic potential in *lung and skin cancer* models by promoting a stable and sustained adhesion and survival of tumor cells and metastatic emboli after tumor cell intravasation [201, 202]. Interestingly, genetic ablation of fibrin(ogen) or disruption of platelet thrombus formation seems to promote the clearance of micrometastatic foci by natural killer cells [203, 204]. These results reveal a role for fibrinogen as a modulator of local innate immune responses that can determine the dissemination of tumor cells to other organs throughout the body [205, 206].

Duchenne muscular dystrophy (DMD) is a fatal degenerative disease in which skeletal muscle is progressively replaced by fibrotic tissue. Experiments using fibrinogen-deficient mice or ancrod showed a significant reduction in fibrin deposition, fibrosis, macrophage infiltration, as well as muscular degeneration and weakness in the *mdx* animal model for DMD [59]. Ancrod-treated *mdx* mice also had significantly reduced levels of TNFα, IL-6, MIP-2, and IL-1β in muscle extracts compared to untreated mdx controls (Fig. 1). Furthermore, fibrinogen-deficient and $Fib\gamma^{390-396A}$ mice also showed reduced macrophage infiltration and collagen deposition in the cardiotoxin model of experimental muscle injury. Mechanistically, this study identified that fibrinogen acts on infiltrating macrophages to promote synthesis of IL-1β and TGF-β, which in turn induces collagen production by fibroblasts and hence fibrosis in the degenerating muscle of DMD-like disease [59].

A proinflammatory role for fibrin(ogen) has also been shown using fibrinogen-deficient mice in mouse models of other fibrotic diseases such as *pulmonary fibrosis* and *crescentic glomerulonephritis*. Intratracheal administration of bleomycin caused comparable amounts and patterns of collagen deposition in fibrinogen-deficient mice as it did in controls at the later stages of a lung fibrosis model of disease [207, 208]. However, in the acute inflammatory stage of the disease, fibrinogen-deficient mice showed a marked difference in neutrophil population levels in the lungs between days 3 and 5 compared to wild-type controls [207]. These studies showed that fibrinogen was not required for collagen deposition at the later stage of the disease but implied a possible role for fibrinogen in the early acute inflammation stage associated with pulmonary fibrosis [207]. Similarly, in a mouse model of kidney fibrosis, although fibrin(ogen) does not appear to be essential for the development of glomerular crescents, fibrinogen knockout mice developed significantly milder pathology, reduced number of crescents, and accumulated macrophages and an overall improved renal function [209]. These results support the well-established proinflammatory role for fibrinogen in attracting immune cells in tissues with fibrin deposition and thereby driving disease progression and impairing local tissue/organ function.

The role of fibrinogen in the context of *bacterial infection* ranges from beneficial, when it achieves to restrain bacterial growth and promote bacterial clearance by host immune cells, to detrimental when excessive coagulation promotes adhesion to tissues and potentiates pathogen survival and systemic dissemination [45]. As discussed above, genetic disruption of fibrinogen's binding to CD11b/CD18 severely hampered the ability of host leukocytes to clear a *S. aureus* infection from the peritoneal cavity of $Fib\gamma^{390-396A}$ mice [57] (Table 1). In this paradigm, the dramatic in vivo impediment of bacterial clearance was not due to reduced levels of fibrinogen or diminished leukocyte trafficking but rather due to a failure to implement antimicrobial functions [57]. In another study using fibrinogen-deficient mice in a model of acute endotoxemia fibrinogen was shown to be required for the early phase of the systemic

inflammatory response. In this study, the absence of fibrinogen led to delayed binding of neutrophils to endothelial cells, which correlated with their delayed infiltration in lung capillaries, and a generally slower increase in plasma levels of inflammatory cytokines [210] (Table 1). Overall, the initiation of the coagulation cascade and the local formation of fibrin trigger the engagement of the innate immune system and regulates the initiation and progression of bacterial clearance by host defense systems. Indeed, local thrombotic events within the microvasculature appear to be crucial for restricting bacterial dissemination and involve neutrophil-derived serine proteases and nucleosomes [211]. Through the course of evolution, bacteria have evolved elaborate strategies of interaction with host proteins that facilitate their survival and infectivity. For example, a recent study detailed the structural characteristics of the cross-like assembly between two pairs of fibrinogen fragment-D molecules and the streptococcal M1 protein, a major virulence factor of this invasive bacterial strain [212]. Understanding the structural characteristics of these host–pathogen interactions at the intersection between coagulation and inflammation is essential for developing novel antimicrobial strategies.

Conclusions

Here we discussed some of the links between coagulation and inflammation, focusing on the role of fibrin(ogen) in particular, in diseases with an inflammatory component. Overall, it becomes increasingly clear that the persistent deposition of fibrin in the perivascular space in multiple organs creates a deleterious environment that results in exacerbation of the primary damage incurred by physical injury and prevents natural regenerative mechanisms from coming into play. As novel molecular partners and cellular targets are being identified [58, 60, 109, 112, 142, 143, 146], the role of fibrin(ogen) is evolving from a building block of blood clots and a mere marker of vascular rapture, to a multi-faceted signaling molecule with a wide spectrum of functions that can tip the balance between hemostasis and thrombosis, coagulation and fibrosis, protection from infection and extensive inflammation, and eventually life and death (Fig. 1). Importantly, the wide spectrum of molecular targets and cellular partners that have been identified for fibrinogen thus far appears to require distinct non-overlapping epitopes on the fibrin(ogen) molecule. Taking advantage of this unique aspect of fibrinogen's structural/functional segregation has already proven the merit of inhibiting a specific interaction of fibrinogen with a given receptor, without affecting its binding on others, or its vital function in the coagulation cascade. Therefore, the targeted inhibition of fibrinogen's mapped interactions that facilitate disease pathogenesis presents as an ideal strategy for pharmacological intervention, in pursuit of new effective treatments for some of the many inflammatory diseases with fibrinogen involvement.

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