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REVIEW

# Fibrinogen and Neuroinflammation in the Neurovascular Unit in Stroke

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**Abstract:** Stroke remains a leading cause of death and disability worldwide. Recent evidence suggests that stroke pathophysiology extends beyond vascular dysfunction to include complex interactions within the neurovascular unit (NVU), particularly involving fibrinogen. This blood-derived protein accumulates in the brain following blood-brain barrier (BBB) disruption and plays crucial roles in neuroinflammation and tissue repair. Through its unique structural domains, fibrinogen interacts with multiple cellular components, including astrocytes, microglia, and neural stem cells, thereby modulating inflammatory responses and neural repair mechanisms. This review examines fibrinogen's structure and its diverse functions in stroke pathophysiology, focusing on its interactions with vascular cells, glial cells, and peripheral immune cells. We also discuss emerging therapeutic strategies targeting fibrinogen-mediated pathways and the challenge of translating experimental results into effective clinical treatments.

**Keywords:** fibrinogen, neuroinflammation, stroke, neurovascular unit, neurological disorders

## Introduction

Neurological disorders, including stroke, traumatic brain injury (TBI), multiple sclerosis (MS), and Alzheimer's disease (AD), are major causes of mortality and disability, posing significant challenges to healthcare systems and society.[1] Stroke remains the leading cause of death in China. In 2020, nationally representative data showed a stroke prevalence of 2.6%, with incidence and mortality rates of 505.2 and 343.4 per 100,000 person-years, respectively. Additionally, an estimated 17.8 million adults had a history of stroke, and 12.5% of survivors experienced disability.[2,3] These statistics underscore the urgent need for improved stroke prevention strategies in China.

Stroke induces brain injury through pathological processes such as neuroinflammation, which is exacerbated by glial activation, immune cell infiltration, and heightened inflammatory responses. The neurovascular unit (NVU), a concept introduced during a National Institutes of Health workshop in 2001, integrates neuronal, glial, and vascular components and has since guided stroke research.[4] A key pathological change in the NVU during brain injury is the increased permeability of the blood-brain barrier (BBB), allowing plasma proteins to enter the central nervous system (CNS).[5] Among these proteins, fibrinogen, a clotting factor, plays a pivotal role in neuroinflammation and neurodegeneration. Following BBB disruption during stroke and other neurological disorders, fibrinogen infiltrates the brain and interacts with neurons and glial cells, influencing inflammatory pathways. Understanding the role of fibrinogen in stroke-induced neuroinflammation is crucial for developing novel therapeutic strategies. Targeting its inflammatory actions could reduce brain damage and improve stroke recovery.

This review examines the multifaceted roles of fibrinogen in neuroinflammation, its interactions with nervous system cells, and the signaling pathways involved in disease progression. We begin by discussing fibrinogen's structural properties that enable its involvement in inflammatory responses and pathological states. Next, we explore its binding to vascular endothelial cells, which affects vascular permeability, and its subsequent interactions with neuronal and glial

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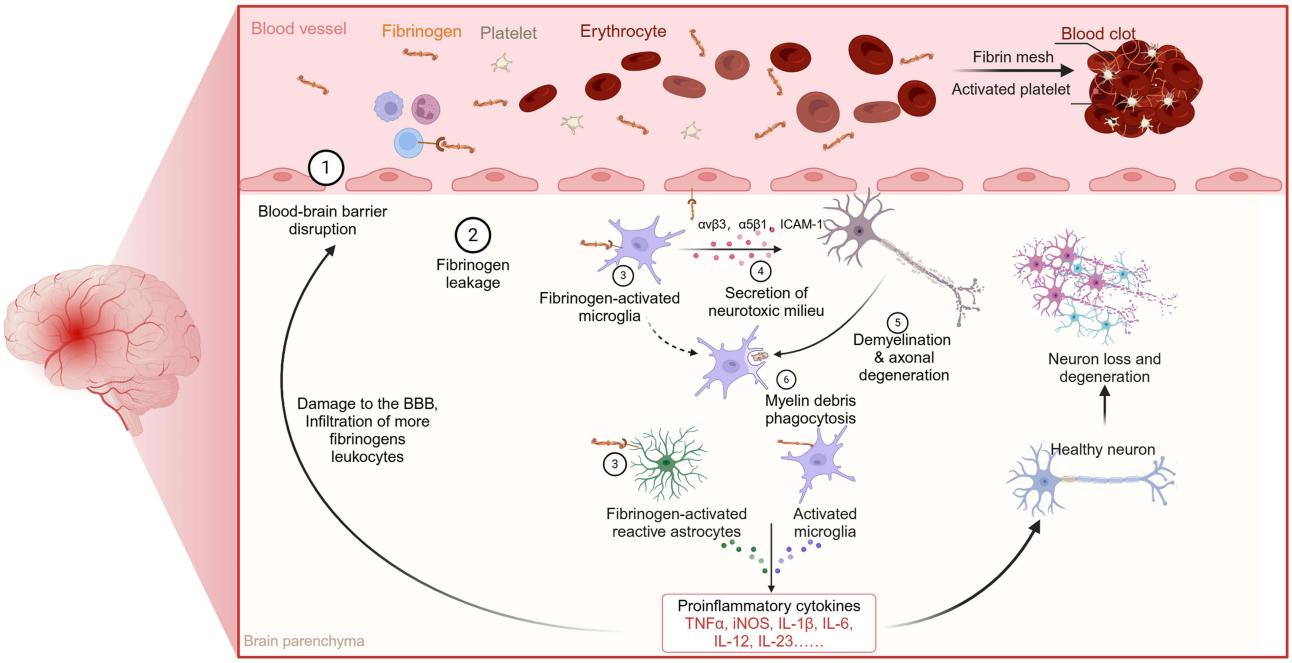
**Graphical Abstract**




cells. We then highlight fibrinogen's contributions to inflammation, neurodegeneration, and recovery in various central nervous system (CNS) conditions. Finally, we discuss therapeutic strategies aimed at targeting fibrinogen in neurological disorders, with a focus on preserving its essential role in blood clotting.

## Fibrinogen's Structure and Inflammatory Function

Fibrinogen, a multifunctional glycoprotein produced by the liver, circulates in the bloodstream as a soluble homodimer,[6] playing key roles in blood clotting, inflammation, and tissue healing.[7,8] Each of the fibrinogen's two identical disulfide-bonded subunits consists of three polypeptide chains, named Aα, Bβ, and γ.[9] The three polypeptide chains are interconnected by two sets of disulfide bonds (Figure 1). These chains are not uniformly distributed; the primary peptide structural domains are concentrated at the extremities, creating an expanded globular formation, while the central structural domains are established through the polymerization of the connected disulfide bonds.[8] Upon the initiation of the coagulation cascade, thrombin targets the binding sites on fibrin peptides A and B along with the Aα and Bβ chains,

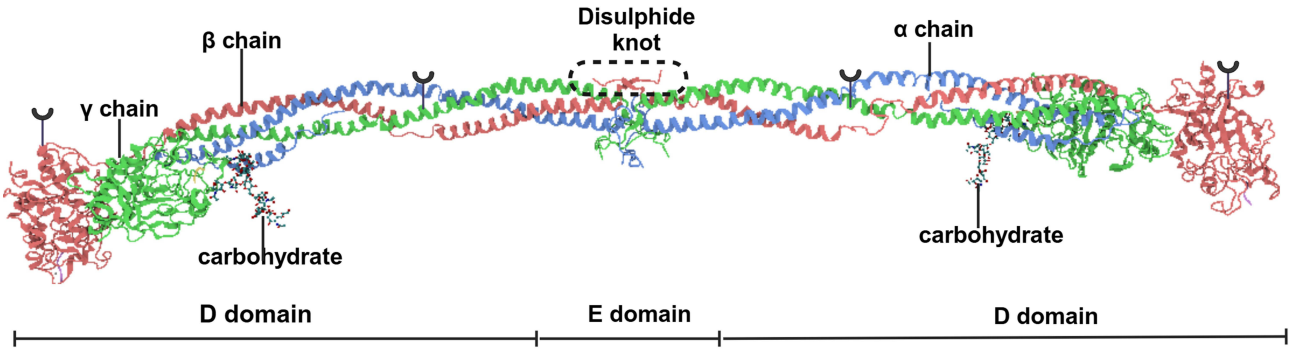



**Figure 1** The structure of fibrinogen.

removing peptides A and B and converting fibrinogen into insoluble fibrin.[10] Serving as the principal protein component of the clot, fibrin adheres to activated platelets, providing a scaffold for the coagulation cascade to proceed. The breakdown of the fibrin clot is facilitated by fibrinolytic enzymes, produced from fibrinogen in the presence of tissue plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA).[11]

Fibrinogen turns into fibronectin mainly via the globular region at its ends, called fibrinogen-related domains (FReDs),[12] highly conserved. After thrombin cleavage, fibrinogen changes shape, allowing FReDs on β- and γ-chains to anchor to another fibrinogen molecule, forming fibrin. FReDs are found in most species, playing key roles in defense and signaling, especially in innate immunity and inflammation.[8] However, only vertebrate FReDs are involved in coagulation, showing FReDs have diverse biological roles beyond clotting, including in disease states, signaling, and tissue repair.[13]

In converting fibrinogen to fibronectin, it's enzymatically split, keeping key FReDs for cellular interactions.[13] Aα binds to fibrinogen, tPA, fibronectin, and integrins α5β1, αvβ3, αvβ8. Bβ links with vascular endothelial cadherin (VE-cadherin), very low-density lipoprotein receptor (very low-density lipoprotein), and amyloid-β (Aβ). γ binds similarly, but plus CD11b/CD18 (also known as integrin receptor αMβ2) through γ-chain's peptide 377–395, and αIIbβ3.[13,14]

Fibrinogen is key in the neurovascular matrix, linking to cell receptors and playing a vital role in inflammation, neurodegeneration, and healing in the nervous system. It reacts with nervous system cells, exposing key sites like the γ-chain 377–395 on surfaces, crucial for αMβ2 integrin receptor activation, triggering neutrophils, and microglia. Beyond clotting, fibrinogen's domains significantly impact CNS, highlighting the need for research on its role in CNS disorders (Figure 1).

## Fibrinogen Enters the Diseased CNS

The BBB's anatomical foundation is the NVU, which includes endothelial cells, pericytes, and astrocytes linked by tight structural and functional relationships, along with noncellular elements like cellular junctions and the basement membrane (BM).[10,11] This barrier is crucial for regulating nutrient flow to the brain and shielding it from toxins, pathogens, and inflammatory cells. An intact blood-brain barrier is essential for maintaining nerve cell metabolism and balance, its compromise can lead to nerve cell pathology and brain damage.[6,15–20] The disruption of the BBB is a common characteristic of various CNS pathologies, including traumatic brain injury, stroke, and MS.[21–24] When BBB is compromised, fibrinogen leaks into the nervous system, becoming insoluble fibrin through perivascular tissue factor and procoagulant proteins, which multiply post-injury, turning fibrinogen into insoluble fibrin.[22,25] Accumulating evidence suggests that BBB disruption and fibrin deposition occur before demyelination and neural damage in many of these conditions.[5,21] This indicates that BBB disruption contributes to the initiation and/or progression of the disease. The normal concentration of fibrinogen in the blood is close to 2 g/L, with a half-life of roughly 4 days.[6] Fibrinogen levels are increased in several pathological states characterized by injury and inflammation. It has been discovered that patients with ischaemic stroke had a significantly increased mean level of fibrinogen (> 4g/L) which also has a positive relationship with ischemic lesions.[26] Thus, it has been used as a reliable indicator of inflammation.[27] In general, fibrinogen stays in the circulation and is prevented from entering the brain parenchyma by the blood-brain barrier (BBB).[27,28] However, when the BBB is compromised, fibrinogen gains access to the CNS and accelerates vascular-neural damage, which can lead to neuroinflammation and poor prognosis.[29]

Patients with inflammatory conditions such as stroke,[30] TBI,[31] AD,[32] and multiple sclerosis[33] have been found to have fibrin and fibrinogen depositions in their brains. It has been demonstrated in animal models of these diseases that the depletion of fibrinogen decreases their severity and development.[27,34,35] In this respect, it became apparent that fibrinogen is not only a biomarker but may also contribute to the development and/or exacerbation of neurodegenerative and neuroinflammatory diseases discussed above.[7,31]

The breakdown of the BBB is important for the development of neurological damage. This occurs when blood elements like fibrinogen build up in the brain tissue, causing local immune reactions and contributing to the barrier's dysfunction. The activation of inflammation, which includes stimulating microglia, increasing inflammatory mediators, and disrupting tight junction proteins, is a key factor in disrupting the blood-brain barrier. As a result, neurovascular damage from the ischemic cerebral area intensifies the breakdown of the BBB further causing physical harm to the brain

parenchyma through swelling. Fibrinogen triggers and amplifies the inflammatory response in the nervous system following cerebral ischemia via multiple pathways.[26,36] It promotes the secretion of pro-inflammatory cytokines and chemokines, along with the release of reactive oxygen species(ROS), by attaching to microglia/macrophage receptors. This process leads to the transformation of microglia into an M1-like state, intensifying the nervous system's inflammatory response, which in turn worsens the disruption of the blood-brain barrier and escalates brain damage.[26,36] Fibrinogen also engages with intercellular adhesion molecule-1, boosting the permeability of the endothelial cell layer and cerebral blood vessels via the activation of extracellular signal-regulated kinase 1/2 signaling.[37] This activation heightens matrix metalloproteinase-9 activity, further dismantling the tight junctions among endothelial cells and undermining the endothelial layer's integrity, leading to blood-brain barrier disruption.[38] As a result, the leakage of fibrinogen after this disruption amplifies the local inflammatory response and blood-brain barrier damage, thus worsening secondary brain injury.

## Fibrinogen Interacts with NVU

After entering the CNS, the unique molecular structure of fibrinogen offers multiple binding sites for receptors and proteins. When fibrinogen is administered to vascular smooth muscle αvβ3 integrin, it can cause vasodilation; however, it results in vasoconstriction when administered to endothelial α5β1 integrin.[17] Aside from binding to the integrins mentioned above, fibrinogen also binds to intracellular adhesion molecule-1 (ICAM-1).[20,37] At high levels, fibrinogen can cause vasoconstriction,[19] exocytosis of Weibel-Palade bodies which released endothelin-1,[39] and increased EC membrane permeability.[40]

High levels of fibrinogen can compromise microvascular integrity by activating matrix metalloproteinases 9, downregulating an adherence junction protein, vascular endothelial cadherin, and upregulating plasmalemma vesicle-associated protein-1, an integral membrane-associated protein that is found in caveolae.[41] It has been tested that fibrinogen compromises the integrity of the EC layer in cultured brains[42] and activates astrocytes.[43] When mice were infused with nondegraded fibrinogen, pial venular permeability was increased.[41] A recent study has shown that microvesicles released from neutrophils interact with and influence the gene expression of brain microvascular endothelial cells.[44] Consequently, these results suggest that elevations of fibrinogen levels may alter the permeability of cerebral vessels and the EC layer. Thus, in cases of mild to moderate traumatic brain injury and stroke, persistently high fibrinogen levels in the blood heighten the permeability of the brain's blood vessels. This condition facilitates the leakage of large molecular weight proteins, particularly fibrinogen, thereby further augmenting the permeability of cerebral blood vessels.[43]

Some results confirm the conclusion that fibrinogen must cross the vascular wall and deposit in extravascular space in order to affect microglia and/or neurons. In a study, immobilized fibrinogen changes microglia morphology in vitro, whereas soluble fibrinogen has no effect at a concentration of 50 mg/mL, which is lower than the physiological concentration.[45] The same group also showed that intravenous administration of fibrinogen-activated microglia, caused neural dendritic loss, and eliminated dendritic spines.[46] There was evidence that fibrinogen crossed the vascular wall and deposited at the vascular-astrocyte endfeet interface,[47] where it was immobilized and most likely converted to fibrin. As mentioned above, lyophilized fibrinogen was used in the study. If fibrinogen were administered to animals, the blood level of fibrinogen could be increased by no more than 0.5 mg/mL,[46] whereas the blood level of fibrinogen in inflammatory conditions is equal to or greater than double the level in normal circumstances.[48,49] When fibrinogen and fibrin are immobilized, they both bind to their receptors, αMβ2, with high affinity and specificity. In contrast, soluble fibrinogen exhibits comparatively lower affinity and specificity.[50,51] During venous thrombogenesis, two potential receptors on red blood cells (RBCs) have been implicated in fibrinogen-RBC interactions: β3 or a β3-like molecule[52] and the integrin-associated protein CD47.[53] Thus, in some in vitro studies, hirudin (which inhibits thrombin's conversion of fibrinogen to fibrin) was used to determine the effects of fibrinogen prior to its conversion to fibrin.[43,54–56] There is evidence that by using hirudin, and thus inhibiting fibrin formation after the onset of intracerebral hemorrhage, long-term outcomes are improved and neuroinflammation is reduced.[34] In summary, as a component of the perivascular extracellular matrix,[57] fibrinogen directly interacts with all cellular elements of the NVU. It attaches to integrin and non-

integrin receptors, thereby directly controlling various essential functions of glia, neurons, and endothelial cells. Additionally, it plays a role in influencing inflammatory, neurodegenerative, and repair processes in the injured CNS.[7,58]

# Fibrinogen and Innate Immune Cells in the Brain

## Fibrinogen and Astrocyte

Astrocytes, with a star-like shape, are specialized glial cells. They are five times more abundant in the brain than neurons.[59] Astrocytes serve as the link between neurons and the CNS vasculature. In instances of nervous system damage, astrocytes undergo a reactive process called astrocytosis. This process is characterized by an excessive expression of glial fibrillary acidic protein (GFAP), which is an intermediate filament cytoskeletal protein primarily expressed by astroglia.[60] Research has demonstrated that activated astrocytes, identified by a heightened expression of GFAP, experience a decline in functionality and their capacity to sustain neurons. Those astrocytes, known as "A1", exhibit a potent neurotoxin potential.[61] Reactive astrocytes induced by ischemia, referred to as "A2", are thought to have a reparative role through the upregulation of neurotrophic factors.[61,62]

A previous study by one group demonstrated that high-level fibrinogen-induced activation of astrocytes in vitro led to increased expression of ICAM-1 and tyrosine receptor kinase B (TrkB).[43,63] TrkB receptors are present in both the central and peripheral nervous system. Their signaling is crucial for the survival and upkeep of neurons, as well as their synaptic plasticity.[64] Activation of TrkB in astrocytes leads to nitric oxide (NO) production and nitrotyrosine deposition, which may promote neurodegeneration.[65] The level of reactive oxygen species (ROS) in cells is modulated by NO, which is known to result in neurodegeneration.[66] ICAM-1, a kind of transmembrane glycoprotein expressed by many types of cells, including leukocytes and ECs, is abnormally expressed by diseased glia.[67] In vitro, blocking ICAM-1 function resulted in reduced interactions between fibrinogen and astrocytes and increased TrkB expression, suggesting that ICAM-1 can serve as an fibrinogen receptor on the surface of astrocytes.[43] ICAM-1 ligation induced the expression of proinflammatory cytokines on rat astrocytes, including interleukin-1α (IL-1α), IL-1β, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α).[68,69] According to another study, fibrinogen causes astrogliosis by increasing GFAP and neurocan immunoreactivity.[70] Neurocan is a proteoglycan belonging to the chondroitin sulfate family that is often found elevated following CNS injury, and it inhibits axonal and neurite growth.[71,72] Fibrinogen-induced increases in TrkB, ICAM-1, and neurocan expression support the theory that fibrinogen contributes to neuroinflammation (Figure 2). Considering that astrocytes are a type of glial cell, they may possess phagocytic capabilities. A study has demonstrated that astrocytes can remove fibrinogen from Petri dishes that have been coated with fibrinogen.[73] However, astrocytes were activated and died as a result of their uptake of fibrinogen,[73] suggesting toxic effect of fibrinogen. In both animal[74–76] and clinical studies,[77,78] inflammation has been linked to post-traumatic neurodegeneration and functional deficits. Reactive, toxic A1 astrocytes, formed following inflammation, deposit an inhibitory extracellular matrix composed primarily of chondroitin sulfate proteoglycan, which contributes to the formation of gliosis scar.[79] In mild-to-moderate TBI, activation of astrocytes is observed.[47] The findings are corroborated by the elevated expression of complement component 3, a marker of toxic astrocytes,[80] which is significantly upregulated in neurotoxic, A1 astrocytes known to worsen disease pathogenesis, and not in A2 astrocytes, which are known to support neural survival.[81] It is unclear how phenotypic polarization occurs in activated astrocytes. However, lipocalin-2 has been suggested to play a significant role as an autocrine modulator of the functional polarization of astrocytes during classical inflammatory activation.[62] Interestingly, astrocytes activated with LPS or interferon-gamma exhibit opposite responses to astrocytes stimulated with interleukin-4 or interleukin-10. As opposed to the latter two, which protect neurons against excitotoxic and oxidative damage, the former two are neurotoxic.[62] Interestingly, activated microglia generate molecular agonists such as IL-1β, TNF, and complement component 1q, which in turn activate astrocytes.[81]

## Fibrinogen and Microglia

In the central nervous system, microglia serve as macrophages.[82] A model of experimental autoimmune encephalomyelitis (EAE) has been used to test the effects of fibrinogen on microglia.[83,84] When fibrinogen was injected locally into the healthy mouse cortex, microglial responses were significantly higher than when albumin or artificial cerebrospinal fluid

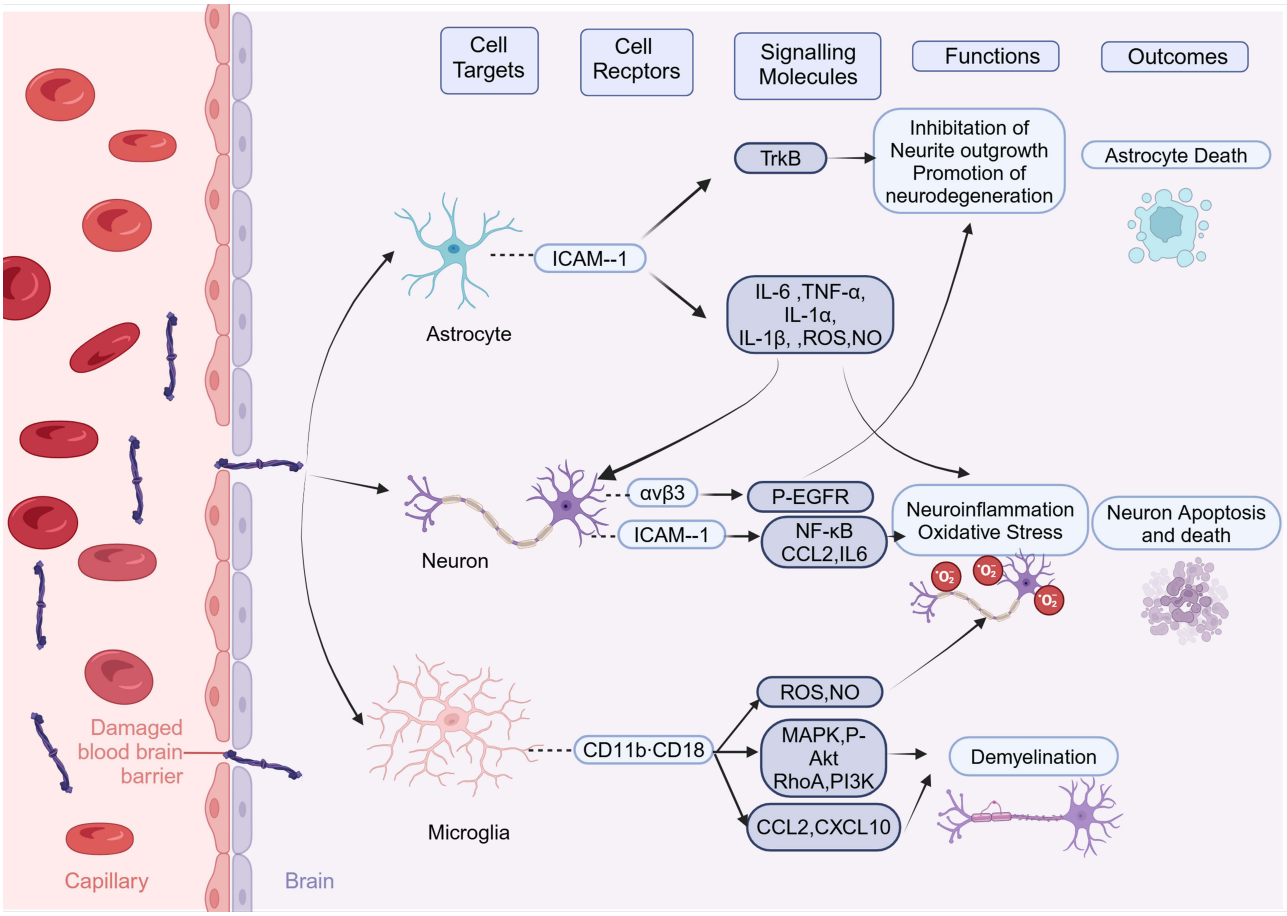



**Figure 2** The fibrinogen's role in neuroinflammation and oxidative stress within the NVU during brain injury. When the blood-brain barrier is compromised, fibrinogen enters the brain and interacts with astrocytes, neurons, and microglia via specific receptors (ICAM-1, αvβ3, and CD11b, CD18). In astrocytes, this interaction activates P-Smad and TrkB, which inhibit neurite outgrowth and induce astrocyte death. In neurons, fibrinogen binding triggers P-EGFR, leading to NF-κB, CCL2, and IL-6 activation, resulting in neuroinflammation, oxidative stress, and neuronal apoptosis. In microglia, the interaction activates P-Akt, RhoA, and PI3K, promoting CCL2 and CXCL10 production and contributing to demyelination. Collectively, these processes worsen brain injury through neuroinflammation and oxidative stress. *Created in BioRender. Chen, Y. (2025)* https://BioRender.com/z28c694.

was injected.[84] Axonal injury and the release of reactive oxygen species were induced by perivascular clusters of activated microglia in response to fibrinogen.[84] The employment of transgenic mice Fibγ3940–396A, which lack the binding site for the CD11b/CD18 receptor, confirmed the essential role of fibrin signaling through this receptor in axonal damage and microglial activation. When fibrinogen binds to the CD11b/CD18 integrin receptor on leukocytes, innate immune responses are activated, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K), v-Akt Murine Thymoma Viral Oncogene (Akt), and Rho signaling.[84–86] These in turn lead to adhesion, migration, chemotaxis, and phagocytosis of leukocytes, macrophages, and monocytes.[84–86] As a result of fibrin interacting withc CD11b/CD18 receptors, CNS immune cells essential for innate immunity, such as microglia and perivascular macrophages, become activated. In a similar manner to macrophages that exhibit a well-characterized M1 and M2 polarization, their CNS counterparts, microglia, may display an "M1-like" pro-inflammatory phenotype that is accompanied by upregulation of IL-1 beta and TNF. A "M2-like" immunosuppressive phenotype is characterized by the upregulation of chitinase-3 (Chil3), frizzled class receptor-1 (Fzd1), and arginases-1 (Arg1).[87] The activation in response to fibrinogen leads to a heightened expression of CD86, IL-12, and major histocompatibility complex class II, indicating the activation of antigen-presenting cells in reaction to infection and inflammation.[88] Extravascular fibrin deposition may activate TLRs and stimulate chemokines, including C-X-C motif chemokine 10 (CXCL10) and C-C motif chemokine 2 (CCL2), indicating that fibrin deposition mediates the recruitment of peripheral macrophages into the central nervous system. As a result, neuroinflammation and related damage, such as demyelination, are intensified.[7,28] Merlini et al

discovered that in AD mice, fibrinogen triggers the elimination of spines and fosters cognitive deficits through the activation of CD11b-CD18 microglia.[83] Furthermore, they found that genetically inhibiting the binding of fibrinogen to CD11b enhances cognitive function in AD mice.[83] Decreasing fibrinogen levels through genetic or therapeutic approaches diminishes microglial activation[83] (Figure 2).

### Fibrinogen and Neuron

The brain and nervous system are based on neurons.[89] Despite some reports that neurons innervate brain vessels,[90] it is generally accepted that neurons' action on microvessels is mediated by astrocytes. In a study, cortical neurons cultured in a conditioned medium from astrocytes treated with 2.5 mg/mL fibrinogen showed inhibited neurite growth.[70] However, when exposed to conditioned media from astrocytes pre-treated with a transforming growth factor beta (TGFβ) inhibitor, this inhibition of neurite outgrowth was reversed. This indicates that the receptor plays a role in activating astrocytes, leading to the subsequent inhibition of neurite growth by neurons.[70]

Neurotoxicity is associated with fibrinogen.[91] The fibrinogen treatment exhibited a dose-dependent increase in cell death, as evaluated by the 3-(4,5-dimethylthiazol-2-yl) 2–5,-diphenyltetrazolium bromide reduction method. After 24 hours of incubation, cell death was around 10% with a treatment of 5 μg/μL fibrinogen, but it rose to nearly 50% when the concentration was increased to 10 μg/μL fibrinogen.[91] Although the study did not involve culturing primary neurons, the SH-SY5Y neuroblastoma cells utilized originated from a metastatic bone tumor biopsy and demonstrated neural properties.[92] Similarly, fibrinogen treatment elevated caspase-3 expression after 45 minutes, suggesting that fibrinogen induces neural apoptosis.[93] At the time of this writing, we do not know of any studies that have tested the direct effect of fibrinogen on neurons. It may still be necessary to investigate this interaction because, in nature, apart from scenarios where fibrinogen deposits in the NVU cause astrocyte activation and death, leading to neuroinflammatory pathologies, a significant accumulation of fibrinogen/fibrin could lead to direct contact with neurons.

## Fibrinogen and Blood-Derived Immune Cells in the Brain

Under disease conditions, fibrinogen not only interacts with cells in the central nervous system but also engages with various external immune cells circulating in the periphery. Fibrinogen has been found to interact with neutrophils in the brain through various mechanisms. It binds to the leukocyte integrin Mac-1, which is present on the surface of neutrophils, and this interaction is mediated by a unique recognition site.[94] Fibrinogen also inhibits the binding of high molecular weight kininogen to neutrophils, suggesting a potential role in modulating neutrophil function.[95] Fibrinogen association with its astrocytic receptors induces the release of pro-inflammatory cytokines, resulting in oxidative stress, and ultimately neuronal death.[96] Platelet interaction with neutrophils via P-selectin and glycoprotein Ibα modifies neutrophil function, playing a role in the damage linked to ischemic stroke. This involves triggering the release of neutrophil extracellular traps, which are neurotoxic and promote clot formation, resulting in worsened stroke outcomes.[95] Platelet-neutrophil interactions significantly contribute to the pathophysiology of ischemic stroke brain injury[97] and neutrophil extracellular traps (NETs) form a composite network within thrombi. These interactions are of particular interest in the context of vascular diseases, as fibrinogen has been implicated in the recruitment of leukocytes at the site of vascular injury.[94] Additionally, fibrinogen has been found to inhibit neutrophil activation and delay apoptosis, further highlighting its role in modulating neutrophil function.[94] These studies together highlight the necessity for a more profound comprehension of the processes driving the interaction between fibrinogen and neutrophils in the brain, especially regarding neuroinflammatory diseases and ischemic stroke.

Several research findings emphasize the importance of fibrinogen in macrophage movement and differentiation. The team led by Silva et al has shown that plasmin-induced fibrinolysis facilitates macrophage migration,[98] while the group under Tanaka et al has found that fibrin hydrogels enhance the attraction of anti-inflammatory macrophages.[99] However, this interaction can also lead to adverse outcomes, as demonstrated in the research by Dzikowski et al,[96] which showed that fibrinogen enlarged the spheres of brain tumor-initiating cells in culture, intensified astrocytic inflammatory responses, and ultimately resulted in neuronal death.

## Fibrinogen, Fibrin and Inflammation

Is fibrinogen merely a marker of BBB disruption, or does it contribute to the onset and progression of neuroinflammatory diseases? Fibrinogen plays a critical role in orchestrating immune and oxidative stress responses where the BBB is compromised. It triggers neuroinflammation by stimulating microglia and facilitating the recruitment and activation of peripheral inflammatory macrophages into the central nervous system.[45,84,100] When fibrinogen is converted to fibrin, it reveals a previously concealed epitope at the end of the fibrinogen γ-chain (amino acid sequence γ377–395). This epitope then bonds strongly to the CD11b-I domain of CD11b/CD18.[51,58] Fibrin binding to the CD11b/CD18 receptor on microglia and infiltrating macrophages triggers multiple signal transduction pathways that stimulate inflammatory responses.[58] Fibrin activates nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K) pathways to facilitate adhesion, migration, chemotaxis, and phagocytosis.[51] Additionally, fibrin activates the serine/threonine-protein kinase AKT and Rho-family GTPases in microglia via CD11b/CD18, leading to an increase in cell body size, actin cytoskeleton rearrangements, and enhanced phagocytosis.[45] In addition to changes in shape and phagocytosis, fibrin initiates a unique transcriptional M1-like activation of microglia and macrophages. This activation is associated with the stimulation of antigen presentation, the production of ROS, and the release of the leukocyte-attracting chemokines monocyte chemoattractant protein 1 (MCP1; also called CCL2) and CXC-chemokine ligand 10 (CXCL10).[84,100] Fibrin may act as a CD11b/CD18 ligand, influencing Toll-like receptor 4 (TLR4) -mediated signaling in innate immune cells in the CNS. Since the well-established crosstalk between TLR4 and CD11b/CD18 signaling further supports this connection.[101–103] Research involving macrophages from mice lacking TLR4 supports the idea that TLR4 plays a role in starting NF-κB activation and the expression of cytokine genes when exposed to fibrinogen.[104]

Following a brain injury, immune mediators are released, initiating a cascade of inflammatory events within minutes. Six hours post-CCI, levels of IL-1β, IL-6, and TNF-α were significantly elevated.[105] It has been observed that the ICAM-1 protein on the surface is expressed at a low level in primary rat astrocytes. However, upon exposure to the cytokines TNF-α or IL-1β, the ICAM-1 level in astrocytes increases, peaking within 12 to 16 hours after treatment with both cytokines.[106] In a distinct investigation, triggering ICAM-1 ligation in rats with monoclonal antibodies induced the mRNA expression of IL-1α and IL-1β via the activation of extracellular signal-regulated kinases 1/2 (ERK 1/2). Moreover, this process stimulated the expression of IL-6 through the activation of both ERK 1/2 and p38 MAPK. This study highlights the critical role of ICAM-1 in managing pro-inflammatory cytokines and the importance of certain signaling pathways in this process.[69,107] TNF-α was also induced, albeit to a lesser extent. These findings indicate that the activation of ICAM-1 may be either a consequence of or a contributor to the ongoing inflammatory processes.[69,107] As previously demonstrated, a high level of fibrinogen induces an increase in the expression of ICAM-1 in cultured astrocytes[43] and in mouse brain microvessels in vivo.[41]

It is essential to identify the exact functions of known inflammatory receptors in the signaling processes of fibrin and fibrinogen and to find new cellular receptors for fibrin and fibrinogen.

## Fibrinogen Aggravates Inflammatory Response in Injured Brain

### Fibrinogen in Stroke

Blood clots have the potential to obstruct cerebral blood vessels, resulting in significant harm caused by insufficient oxygen and nutrients.[108] The association between fibrinogen levels and stroke severity can be attributed to its role in promoting thrombus formation.[100] Higher levels of fibrinogen may contribute to the formation of larger and more occlusive clots, resulting in more severe ischemic stroke and worse patient outcomes.[109,110] Several studies have provided evidence supporting the use of fibrinogen as a prognostic marker for stroke severity.[26,111] Zoppo et al[112] present the link between high fibrinogen levels and functional recovery after ischemic stroke, based on placebo data from two well-known clinical trials on the use of the defibrinogenating agent ancrod in acute ischemic stroke.[113,114] In summary, the authors found that patients with lower initial fibrinogen levels (<4.5 g/L) had better functional outcomes, even after accounting for age and initial stroke severity.[112] This finding was reaffirmed in a study that also identified a negative correlation between the clinical progression of ischemic stroke patients and fibrinogen levels.[26] They also

confirmed an independent relationship between fibrinogen and prognosis, regardless of other cardiovascular risk factors and stroke severity.[26,112] These findings highlight the potential of fibrinogen as a reliable biomarker for assessing the severity of stroke, aiding in treatment decisions, and predicting patient outcomes. However, small sample sizes in some studies (40 and 50 stroke patients) suggest a need for larger cohorts to strengthen the results. The dynamics of serum fibrinogen levels were assessed up to day 14 after the incident in one study, indicating a potential limitation in terms of long-term follow-up. Recently, a study found that post-stroke inflammation induced by fibrinogen is primarily driven by neutrophils and can be ameliorated using the fibrin-derived γ377-395peptide.[115] This suggests that targeting the fibrinogen-mediated neuropathological process could be a promising strategy for protecting the brain after a stroke, while still allowing fibrinogen to perform its essential function in blood clotting. It potentially provides a more targeted and effective form of neuroprotective therapy for stroke patients.

Pous et al discovered that fibrinogen leaked into the distant subventricular zone (SVZ) stem cell niche environment where neural stem/precursor cells (NSPC) interact with local cells following cortical injury, prompting NSPC differentiation into astrocytes.[116] This was evidenced by a heightened presence of GFAP+, Aldh1l1+, and Aqp4+ cells, alongside an increase in GFAP, Aqp4, and Aldoc mRNA and protein expression by astrocytes.[116] By using mouse models for cortical ischemic stroke (photothrombotic ischemia) and cortical brain trauma (stab wound injury), the author shows genetic or pharmacologic depletion of fibrinogen in vivo reduced NSPC differentiation into astrocytes.[116]

In hemorrhagic stroke, a recent clinical study has shown that fibrinogen levels in the circulatory system are significantly lower than those observed in ischemic stroke patients.[117] This decrease in fibrinogen levels is likely due to acute blood loss, which leads to hemodilution, increased fibrinogen consumption, and activation of the coagulation cascade. However, despite the systemic reduction in fibrinogen, the disruption of the BBB in hemorrhagic stroke facilitates the rapid infiltration of fibrinogen into brain tissue. As a highly concentrated plasma protein, even reduced systemic levels of fibrinogen can result in sufficient infiltration to create a high local concentration within the brain.

At the hemorrhagic site, the substantial leakage of fibrinogen strongly activates TLR-4, which subsequently upregulates the expression of pro-inflammatory cytokines such as IL-1α and IL-33, thereby amplifying neuroinflammatory responses.[118] Additionally, fibrinogen binds to CD11b/CD18, forming an insoluble matrix that directly activates microglia and macrophages, further exacerbating the inflammatory response. Interestingly, fibrinogen-induced neuroinflammation in hemorrhagic stroke may share similar mechanisms with those observed in TBI. However, there is currently a lack of specific research investigating the precise mechanisms of fibrinogen in hemorrhagic stroke models.

Numerous studies have demonstrated the efficacy of fibrinogen inhibitors in reducing the severity of stroke.[116,119,120] These inhibitors work by either directly blocking the binding sites on fibrinogen or inhibiting the enzymatic activity responsible for its conversion into fibrin.[120] Clinical trials evaluating the use of fibrinogen inhibitors have shown promising results. In one study, patients treated with a fibrinogen inhibitor exhibited a significant reduction in the size of the infarcted area, indicating a decreased severity of stroke.[119,121] Additionally, these inhibitors have been shown to enhance neurological outcomes and decrease the risk of recurrent stroke. The potential of fibrinogen inhibitors in stroke management is further underscored by their favorable safety profile, with few adverse effects reported in clinical trials. However, additional research is required to refine dosing regimens and ascertain the long-term effects of these inhibitors.

## Fibrinogen in Traumatic Brain Injury

In the event of a TBI, immediate and delayed dysfunction of the BBB is observed. Fibrinogen is transported into the extravascular space as a result, inducing high levels of fibrinogen.[31,122] Widespread perivascular fibrin deposition in both acute and chronic phases after TBI.[36] After 2 days, the level of fibrinogen rises to above 4 g/L in human patients and remains high for up to 14 days.[36] In mice with cortical contusion injury (CCI), a model for TBI, higher fibrinogen deposition was observed at the vasculo-astrocyte interface after 14 days.[123] In the further experiment, due to the increase of fibrinogen in NVU, neuronal degeneration is deteriorating by expressing less neuronal marker NeuN compared to the sham group.[124]

Studies indicate that microglia activation plays a crucial role in disrupting neural homeostasis following TBI, and all TBI-associated functional and behavioral impairments were prevented by microglial depletion.[125,126] In response to brain injury, fibrinogen induces microglial activation and ROS production.[46,58] Induced nitric oxide synthase expression and

NO production are also enhanced in reactive astrocytes when CNS homeostasis is disrupted during TBI.[127] An increase in ROS production during inflammatory diseases, such as TBI, typically coincides with an increase in NO, leading to the generation of potent reactive nitrogen species.[128] Increased ROS production leads to neural injury and axonal damage, thought to result from mitochondrial dysfunction and reduced energy availability to neurons.[129] Degeneration of neural cells has been shown to be caused by NO exposure to axons.[130] As a result, neurodegeneration may result from fibrinogen-activated astrocytes.

TrkB expression on astrocytes is also associated with NO production and neurodegeneration.[107] TrkB-T1 truncated isoform expression is increased in MS lesions from human patients, which is confirmed by immunohistochemistry, mRNA expression, and flow cytometry.[107] It is well known that brain-derived neurotrophic factor (BDNF) is an agonist of the TrkB receptor.[131] The primary role of BDNF is to modulate activity-dependent plasticity at excitatory synapses in the CNS and to control neural synaptic transmission.[132] Additionally, BDNF activates astrocytes in a dose-dependent manner, and the application of conditioned media to neurons leads to neural degeneration.[65] NO was responsible for initiating neurodegeneration when activated astrocytes treated with 25 ng/mL of BDNF produced significantly higher NO than the controls who produced 5 ng/mL of NO.[65]

The PrPC in astrocytes is another source of ROS production. Well-established research indicates that PrPC has dual effects in the brain: it offers neuroprotection when unaltered, but induces neurotoxicity when ligated.[133] The PrPC is crucial in maintaining cellular redox homeostasis by generating ROS through Nicotinamide adenine dinucleotide phosphate oxidase and extracellular regulated kinase 1/2 signaling.[134] Consequently, PrPC-induced ROS formation causes oxidative stress and neural toxicity in brain cells.[134] The combination of fibrinogen and PrPC has been discovered to form a complex in the brain's extravascular space during TBI.[47,135] Consequently, the formation of the fibrinogen-PrPC complex may alter PrPC properties, potentially serving as a mechanism for neurodegeneration, which could result in short-term memory loss, as observed in a study.[47,135] Together, these findings imply that elevated PrPC expression in astrocytes and its linkage with leaked fibrinogen could result in the creation of the fibrinogen-PrPC complex, potentially impairing memory in diseases triggered by brain inflammation, such as TBI.

Additionally, the generation of ROS activated the protein kinase B signaling pathway through Ras homolog family member A, leading to increased permeability of the brain endothelial layer.[136] Concurrently, ROS production in ECs could sufficiently promote fibrinogen extravasation, as previously discussed.

## Fibrinogen in Multiple Sclerosis (MS)

Fibrinogen recognized as a pivotal protein essential for the coagulation of blood, plays a significant role in the onset and progression of MS, a complex autoimmune condition.[137] The presence of elevated fibrinogen levels in the plasma during episodes of MS exacerbation suggests a potential link between this protein and the mechanisms underlying the disease's dynamics, as reported by Acuña[138] in 2017. This observation points towards fibrinogen's involvement in the inflammatory processes associated with MS flare-ups. However, it is intriguing to note that, according to a study conducted by Ehling[139] in 2011, the concentration of fibrinogen in the cerebrospinal fluid of individuals diagnosed with MS does not show a marked difference when compared to that in healthy individuals. This finding introduces a layer of complexity in understanding fibrinogen's role in MS.

Furthermore, the association of fibrinogen with the severity of lesions observed in MS[140] adds another dimension to its involvement in the disease. This correlation suggests that fibrinogen may contribute to the pathological changes seen in the nervous system of MS patients. Additionally, recent research by Willis in 2019 has shed light on a novel aspect of fibrinogen's role in MS.[141] The study demonstrates that fibrinogen can influence the formation of encephalitogenic CD8+ T cells, which are pivotal in the development of MS, through the mediation of extracellular vesicles in a mouse model of MS.[141] This discovery underscores the complex interplay between fibrinogen and the immune system in the context of MS, suggesting that fibrinogen could be a key player in the disease's pathogenesis. Despite significant advancements in research, challenges persist with the limitations of animal models in accurately replicating disease pathogenesis and the complexities in modeling spontaneous CD8+ T cell responses in EAE models.

These findings collectively emphasize the intricate and multifaceted role of fibrinogen in multiple sclerosis. They highlight the necessity for more in-depth and comprehensive research efforts aimed at unraveling the full spectrum of

fibrinogen's impact on the disease. Understanding the precise mechanisms through which fibrinogen contributes to MS could pave the way for the development of novel therapeutic strategies and improve the management of this challenging autoimmune disorder. The insights gained from such research could significantly enhance our comprehension of MS and lead to more effective treatments that target the underlying causes of the disease, ultimately benefiting individuals affected by MS.

### Fibrinogen in Alzheimer's Disease (AD)

Fibrinogen is also implicated in the onset of AD through its interaction with amyloid-β (Aβ) and its role in abnormal clot formation.[142] Studies have shown that fibrinogen accumulates abnormally in the brains of AD patients, where it binds to Aβ, forming fibrin clots that are resistant to degradation.[142,143] These clots exacerbate neuroinflammation in AD models by promoting the activation of microglia and the release of pro-inflammatory mediators such as TNF-α and IL-1β.[84] These inflammatory factors exert direct neurotoxic effects on synapses, contributing to synaptic loss and neurodegeneration. Beyond its role in promoting inflammation, fibrinogen accumulation and impaired clearance disrupt iron homeostasis, resulting in iron deposition within neural cells and triggering oxidative stress, a defining feature of neurodegenerative diseases.[144] Oxidative stress further amplifies neuronal damage and cell death, compounding the progression of AD pathology. Moreover, fibrinogen's interactions with neurons, astrocytes, and endothelial cells within the NVU contribute to blood-brain barrier (BBB) dysfunction, microglial activation, and cerebral amyloid angiopathy, all of which are key features of AD.

Importantly, reducing fibrinogen levels has been shown to improve cognitive function in AD mouse models by mitigating BBB permeability, cerebral amyloid angiopathy, and microglial activation.[143] Targeting the fibrinogen-Aβ interaction represents a promising therapeutic strategy, as studies have demonstrated that lowering fibrinogen levels alleviates AD-related pathologies and enhances cognitive performance in preclinical models.[145,146] These findings underscore the potential of fibrinogen as a therapeutic target for slowing or halting AD progression.

## Targeting Fibrinogen as a Potential Therapeutic Approach

Since fibrinogen leakage through a compromised BBB exacerbates CNS diseases, therapies aimed at limiting its entry into the CNS offer a promising treatment approach. For instance, activated protein C enhances endothelial barrier function, thereby reducing fibrinogen accumulation in the CNS and mitigating inflammation.[147] Interestingly, many disease-modifying drugs currently in clinical use for MS have BBB-stabilizing properties. In addition to their immunomodulatory effects, interferon-β, dimethyl fumarate, fingolimod and laquinimod all stabilize the BBB, often through actions on endothelial tight-junction proteins.[36]

Several studies on CNS diseases have demonstrated that ancrod, a defibrinogenating agent sourced from Malayan pit viper venom, effectively reduces fibrinogen levels in the blood.[27,70,120,145] However, ancrod also lowers the levels of other high molecular weight proteins, including fibronectin, von Willebrand factor, and globulins. Thus, ancrod should not be considered a substance that solely targets the level of fibrinogen in the bloodstream. In rodents, the drug fenofibrate (brand name Tricor) inhibited the expression of the fibrinogen gene in the liver, leading to reduced blood levels of the protein.[112] However, fenofibrate is primarily used to lower low-density lipoproteins and triglycerides.[148] In this context, it cannot be regarded as a specific inhibitor of fibrinogen synthesis. In the majority of studies concerning the effects of fibrinogen, fibrinogen gene knockout (fibrinogen−/−) mice were utilized.[149] According to the findings, additional proteins, like fibronectin, might have a role in coagulation and/or thrombiogenesis akin to that of fibrinogen.[150] Therefore, all the previously mentioned methods mask the impact of fibrinogen in the bloodstream. In contrast, using fibrinogen antisense oligonucleotide (fibrinogen-ASO) specifically and effectively decreases fibrinogen production, thereby reducing its concentration in the blood of mice.[151] Currently, using fibrinogen-ASO is considered the most promising therapeutic approach for reducing fibrinogen levels in animal models and may also benefit individuals with TBI, AD, and MS.

Targeting microglial activation triggered by fibrinogen offers a promising therapeutic strategy while preserving its essential role in blood clotting. Fibrinogen promotes pro-inflammatory (M1) microglial activation either directly or indirectly by inducing the release of inflammatory mediators, contributing to neurodegeneration and vascular cognitive

impairment. Recent studies have shown that the fibrin-derived γ377-395 peptide can block the interaction between fibrinogen and CD11b/CD18, thereby inhibiting fibrinogen-induced microglial activation and neutrophil recruitment both in vitro and in vivo.[45,115] Additionally, preclinical models and stroke patient studies suggest that shifting microglial polarization toward the anti-inflammatory M2 phenotype, using agents such as osteopontin[152] or the CSF1R inhibitor PLX3397[153] reduces tissue damage and promotes functional recovery, including cognitive improvements.[154] Furthermore, case reports indicate that perispinal administration of the TNF inhibitor etanercept can alleviate functional deficits even years after stroke, likely by suppressing microglial activation.[155]

Given the complex interactions between fibrinogen and microglia within the NVU, further development of fibrinogen-targeted therapies is warranted. These therapies could focus on selectively targeting harmful fibrinogen domains in the CNS, employing structure-based drug design, or utilizing high-throughput chemical and genomic screening to identify potential treatments through fibrinogen-activated nervous system cells. Importantly, fibrinogen's binding sites in the CNS are distinct from its coagulation sites, allowing for the development of selective CNS inhibitors without affecting its role in blood clotting. Biochemical studies and crystallographic analysis of fibrinogen complexes with CNS targets can guide structure-based drug design Finally, unbiased screening methods, such as small-molecule libraries, RNAi, and CRISPR–Cas9, could help identify inhibitors of fibrinogen-activated pathways in nervous system cells.

Interactions between the NVU and blood-derived fibrinogen present valuable opportunities for developing stroke therapies. However, many of these concepts have been predominantly explored in rodent models, making their translation into effective clinical treatments challenging. A key obstacle lies in the biological differences between humans and rodents, particularly in the properties of the NVU. For instance, there are significant differences in the expression of marker genes associated with specific cerebrovascular cell types and vessel subtypes, such as arteries, arterioles, and capillaries.[4] Additionally, most experimental studies have been performed on young animals, even though stroke is primarily an age-related condition affecting older individuals. Another important factor is the presence of comorbidities: stroke patients often present with multiple vascular risk factors and associated conditions, such as hypertension, hyperlipidemia, hyperglycemia, obesity, and neurodegenerative disorders. These comorbidities profoundly influence stroke pathophysiology, lesion development, and recovery, yet they are rarely accounted for in experimental models, limiting the applicability of preclinical findings to human stroke. However, experimental models rarely incorporate these comorbidities, which limits the applicability of their findings to human stroke cases.[156,157]

To address the challenges of translating findings from rodent models to humans, the development of multicellular in vitro models of the NVU using human-induced pluripotent stem cells, such as 3D organ-on-a-chip systems and brain organoids, offers valuable opportunities.[158,159] These models allow for detailed studies of the interactions between fibrinogen and the NVU, as well as drug testing in a human-specific context. Patient stratification could further support clinical development. Additionally, fluid biomarkers of the coagulation cascade, noninvasive fibrinogen imaging to detect fibrinogen deposits in the CNS, and metagenome-wide association studies to explore genetic links to coagulation or fibrinolysis genes could play a key role in advancing fibrinogen-targeted therapies from proof-of-concept to clinical application for stroke and other neurological diseases.

## Conclusion

The studies reviewed here support the hypothesis that elevated fibrinogen levels during inflammatory events in stroke contribute to its extravasation and deposition at the NVU, where it interacts with neural cells, triggering neuroinflammation and exacerbating neurological damage. BBB disruption, a hallmark of many brain injuries such as ischemic stroke and TBI, facilitates fibrinogen's entry into the NVU, disrupting the normal functions of astrocytes, microglia, and neurons. Additionally, fibrinogen engages with peripheral immune cells, amplifying inflammatory responses and contributing to the progression of neurological disorders, including stroke, MS and AD. Emerging therapeutic strategies targeting fibrinogen-neural cell interactions in the NVU show promise. These include defibrinogenating agents such as fibrinogen-ASO, which reduces fibrinogen levels in the bloodstream, and fibrin-derived γ377-395 peptides, which inhibit fibrinogen-microglia interactions. However, significant challenges remain in translating these approaches into clinical practice. Future research should focus on developing advanced multicellular in vitro models of the NVU, novel biomarkers for fibrinogen activity, non-invasive fibrinogen imaging techniques, and metagenome-wide association

studies to explore genetic links to fibrinogen-related pathways. These efforts will be critical for advancing fibrinogen-targeted therapies and improving outcomes in stroke and other neurological disorders.

## Ethics Approval and Consent to Participate

The authors confirm that this review was performed in accordance with relevant guidelines and regulations.

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