

REVIEW ARTICLE

Crosstalk between Inflammation and the BBB in Stroke

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Abstract: The blood-brain barrier (BBB), which is located at the interface between the central nervous system (CNS) and the circulatory system, is instrumental in establishing and maintaining the microenvironmental homeostasis of the CNS. BBB disruption following stroke promotes inflammation by enabling leukocytes, T cells and other immune cells to migrate *via* both the paracellular and transcellular routes across the BBB and to infiltrate the CNS parenchyma. Leukocytes promote the removal of necrotic tissues and neuronal recovery, but they also aggravate BBB injury and exacerbate stroke outcomes, especially after late reperfusion. Moreover, the swelling of astrocyte endfeet is thought to contribute to the ‘no-reflow’ phenomenon observed after cerebral ischemia, that is, blood flow cannot return to capillaries after recanalization of large blood vessels. Pericyte recruitment and subsequent coverage of endothelial cells (ECs) alleviate BBB disruption, which causes the transmigration of inflammatory cells across the BBB to be a dynamic process. Furthermore, interneurons and perivascular microglia also make contacts with ECs, astrocytes and pericytes to establish the neurovascular unit. BBB-derived factors after cerebral ischemia triggered microglial activation. During the later stage of injury, microglia remain associated with brain ECs and contribute to repair mechanisms, including postinjury angiogenesis, by acquiring a protective phenotype, which possibly occurs through the release of microglia-derived soluble factors. Taken together, we reviewed dynamic and bidirectional crosstalk between inflammation and the BBB during stroke and revealed targeted interventions based on the crosstalk between inflammation and the BBB, which will provide novel insights for developing new therapeutic strategies.

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1. INTRODUCTION

The blood-brain barrier (BBB) was first thought to be a physical barrier separating the central nervous system (CNS) from the surrounding environment. However, further research found that the BBB is a dynamic metabolic interface that can bi-directionally regulate the transport of fluids, solutes, and cells [1]. The BBB is positioned along the blood vessels of the CNS and plays critical roles in regulating the metabolism of the brain and coordinating the functions of peripheral organs. Anatomically, brain microvascular endothelial cells (ECs) connected by individual junctional components confer the barrier protection function of the BBB. ECs interact closely with surrounding cells, giving rise to the concept of the neurovascular unit (NVU). Among these

surrounding cells, astrocytes signal to ECs through their endfeet, and pericytes cover and connect with ECs *via* gap junctions. The specialization and intercellular signaling of these cells was shown to depend on the extracellular matrix (ECM) [2, 3].

It is clear that stroke may lead to severe damage to the BBB, limiting the use of tissue plasminogen activator (tPA), which remains the main therapy currently used for acute ischemic stroke [4]. Besides, BBB disruption permits the significant inflow of hematogenous fluid into the brain tissues, leading to the progressive elevation of brain water content and tissue swelling [5]. It is generally believed that the permeability of the BBB after a stroke changes over time [6]. Furthermore, the activation of immune cells after a stroke also has obvious temporal characteristics [7, 8]. Here, we focus on the dynamic and bidirectional crosstalk between immune cells and the BBB in stroke-induced inflammation. In addition, we discuss potential immunotherapies that target BBB damage.

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2. BBB DISRUPTION AND THE PROMOTION OF INFLAMMATION

The BBB shows an increase in permeability within an hour post-stroke, and after a brief reduction, the permeability increases again at approximately 24 h after stroke. Multiple studies have shown that the permeability of the BBB reaches its peak in the acute/subacute phase of stroke, which is followed by a reduction in permeability. However, the hyperpermeability of the BBB could last for several weeks, indicating that there can be long-term disruption of barrier function [9, 10]. As the main component of the BBB, ECs have been shown in various studies to produce different pathological changes at different stages after stroke, resulting in changes in BBB permeability [11]. The accumulation and infiltration of immune cells and molecules in the brain parenchyma is considered to be the cause of the further worsening of BBB dysfunction after stroke.

Among all immune cells, brain resident microglial cells are activated first. They may participate in recruiting peripheral immune cells, including macrophages, to the brain within an hour post-stroke, and these macrophages participate in BBB disruption in the early stage [12]. Soon after, immune cells in peripheral blood are recruited into the damaged brain at approximately 24 h post-stroke. Macrophages, neutrophils, mast cells, and T cells migrate to the damaged area and interact with components of the BBB [11, 12]. The immune response reaches a peak at approximately 72-96 h after stroke, which leads to severe damage to the BBB [13, 14]. Subsequently, the immune response is weakened gradually, but it still lasts for several weeks (Fig. 1).

3. INNATE IMMUNITY AND THE BBB

3.1. Microglia/Macrophages and the BBB

Microglia are macrophages that reside in the CNS and display little turnover under physiological conditions. Microglia continuously scan the extracellular environment; therefore, they can be recruited immediately after necrotic neurons release proinflammatory cytokines and further show an active inflammatory response after brain damage [15, 16]. Thus, microglia may participate in recruiting peripheral immune cells, including macrophages, to the brain within an hour post-stroke, in which their level reaches its peak within 24-96 h and then gradually decreases to baseline levels until 28 days post-stroke [17]. It has been proposed that the NVU might serve as a dynamic interface between the innate immune system that is controlled by microglia [18]. In addition, it has been reported that an increased number of microglia were observed in rat brain tissues up to 16 weeks after ischemia [19]. Hypoxia in brain tissue triggers the upregulation of vascular endothelial growth factor (VEGF) [20] and also induces quiescent vessels to sprout [21]. This process increases the permeability of the BBB, causing the leakage of serum proteins into the brain parenchyma thus inducing activated microglia to migrate to the injured area [22]. Therefore, microglia/macrophages are continuously activated throughout the course of stroke, and microglia/macrophages may have different effects on the structure of the NVU and BBB as a result of pathophysiological processes since they can switch to the M1/M2 phenotype.

3.1.1. Macrophages/Microglia and Astrocytes

As an important component of the BBB, astrocyte endfeet form an envelope around blood vessels and are tightly

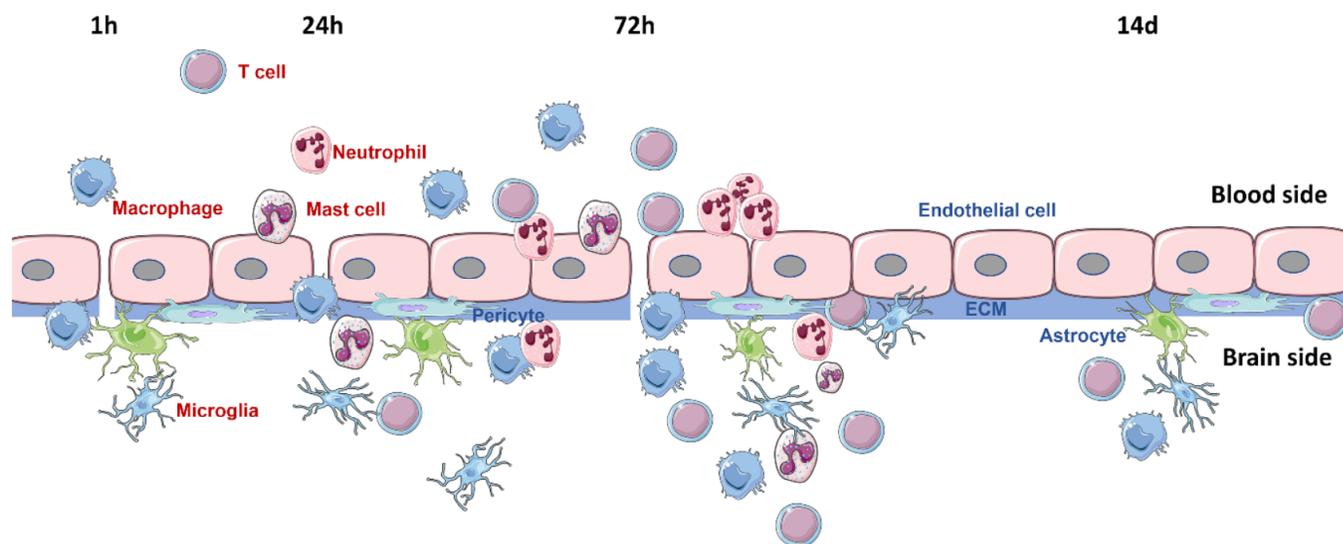


Fig. (1). BBB disruption promotes inflammation by enabling immune cells. Microglia/macrophages are recruited into the brain tissue within an hour, reach a peak in 24-96h and then gradually decrease to baseline level until 28 days post-stroke; Neutrophils are trapped within the confines of the neurovascular unit and the leptomeningeal spaces during the early phase of reperfusion, however, neutrophils can pass through the junctions of the barrier and being detected after 48h post-stroke; Mast cells increase significantly in the brain tissues in 24h after stroke; T cells are identified in the postischemic brain as early as 24 h and are at peak 72-96h after reperfusion; the presence of T cells persists as late as 7 weeks post-stroke. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

attached to the basement membrane [23]. Ischemic injury may disrupt the contact of astrocyte endfeet with the basement membrane by increasing swelling, which further leads to BBB damage [24]. It has been demonstrated that astrocytic factors such as macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) could cause changes in microglial morphology without physical contact [25]. In normal brain tissue under physiological conditions, astrocytes make contact with microglia in the local microenvironment and maintain the microglia in a dormant state [26]. However, microglial activation induced by stroke could produce high levels of neurotoxic mediators, thereby activating neighboring astrocytes. Existing research has shown that the inflammatory cytokines interleukin 1 beta (IL-1 β), IL-6, and tumor necrosis factor alpha (TNF- α) released by microglia induce the upregulation of aquaporin 4 (AQP4), thus resulting in the swelling of astrocyte endfeet [27]. Conversely, neuroinflammation post ischemia induced the differentiation of two different types of reactive astrocytes, which are referred to as “A1” and “A2”, using terminology that parallels the “M1” and “M2” macrophage nomenclature, respectively. Microglia induce the differentiation of the more toxic “A1” reactive astrocyte by releasing IL-1 α , TNF- α , and complement component subunit 1q (C1q), thus preventing the regulation of microglia by astrocytes [28].

3.1.2. Macrophages/Microglia and Pericytes

Among the nonendothelial components of the BBB that regulate microglial activation, pericytes have emerged as key mediators of this process. Pericytes are perivascular cells located on the abluminal side of ECs lining the microvasculature in all organs. In the CNS, pericytes surrounding the endothelium make contacts with perivascular microglia/macrophages [29]. Using a pericyte-reporter mouse model, a recent study showed that activated pericytes that leave the blood vessel wall proliferate and adopt a microglial phenotype after ischemic brain injury. Pericytes upregulate mRNAs specifically associated with activated microglial cells under hypoxic conditions *in vitro*, indicating that pericytes may transform into microglia-generating multipotent vascular stem cells after experimental cerebral ischemia and are involved in perivascular microglial proliferation in stroke [30, 31]. Further research confirmed that one week after ischemic injury, the pericytes that migrated to the parenchymal tissue showed a microglial morphology and expressed several microglia-specific markers as well. Importantly, similar cells were not detected in the contralateral brain tissue. Overall, the results show that pericytes are capable of acquiring a microglial phenotype with phagocytic capacity in the late stage of ischemic stroke [30].

3.1.3. Macrophages/Microglia and ECs

The BBB mainly consists of the ECs of the brain microvasculature. These cells coordinate cell-cell interactions and signaling from glial cells to maintain the barrier properties of the BBB. The crosstalk between these cells plays an important role in the context of the penumbra region [32]. Pathophysiologically, soon after reperfusion, activated microglia were shown to extend cellular protrusions toward adjacent blood vessels [33] and later to adhere to the endo-

thelial surface, which was mediated by the adhesion receptor macrophage-1 antigen (Mac-1) [34, 35]. As observed, blood serum proteins that leaked into brain tissue allowed microglia to be recruited into blood vessels, where they began to engulf ECs, and this caused the activation of the local endothelium and contributed to the disintegration of blood vessels at 24 h after reperfusion [36]. ECs are closely connected through tight junction proteins (TJPs), and IL-1 β and TNF- α released by microglia could downregulate TJP expression in ECs, which may have caused the dysfunction of ECs [37, 38]. In the process of neurovascular remodeling, although ECs are still immature, the inhibition of microglia may still affect TJPs and the functions of the BBB [39]. Moreover, in response to the damaging effects on the BBB, purinergic receptor P2Y (P2RY12)-mediated activation of microglial processes causes the formation of a physical barrier that temporarily seals the damaged BBB, permitting the microglia to accelerate endothelial cell repair of the damage to the BBB [40].

3.2. Macrophages/Microglia and ECM

As a dynamic component of the BBB, ECM may degrade, and this may lead to BBB breakdown [41]. Multiple signaling pathways are activated *via* ECM molecules and their receptors; however, when the BBB is damaged, ECM molecules such as fibronectin and vitronectin become available in the environment and activate microglia directly [42]. As indicated by recent research, a phenomenon observed in the early phase of stroke involves the release of cytokine pools trapped by ECM, which thus activate glial cells and ECs and contribute to the recruitment of peripheral immune cells [43]. Subsequently, in the later phase of stroke (48-72 h), microglia may take part in remodeling the ECM by secreting matrix metalloproteinase-3 (MMP-3), MMP-9 and MMP-19 [44].

3.3. Neutrophils and the BBB

The genes that are acutely regulated in the blood of stroke patients are highly expressed in neutrophils and, to a lesser extent, in macrophages [45]. Accordingly, together with macrophages, neutrophils are the first peripheral immune cells to infiltrate the ischemic brain. As reported by some studies, neutrophil infiltration begins at 24 h, peaks at 72 to 96 h and then vanishes at 14 days after ischemia [13, 46]. IL-1 acts on the brain endothelium and attracts neutrophils, and this may be a key mechanism involved in neutrophil infiltration [47]. Neutrophils, in a process mediated by VCAM and ICAM, anchor themselves to and migrate along with ECs and pass through the tight junctions between ECs by reorganizing the cytoskeleton and intercellular connections [48, 49]. Moreover, recent findings have shown that in the setting of stroke, neutrophils showed the ability to polarize toward a beneficial N2 phenotype, which was induced by activation of nuclear peroxisome proliferator-activated receptor (PPAR)- γ [50], and this promoted early neutrophil infiltration into the ischemic core. At the same time, the activity of PPAR- γ also results in neuroprotection and the resolution of inflammation after experimental stroke by increasing neutrophil clearance [50, 51].

However, the vast majority of the attracted granulocytes stayed within the microvessels rather than entering the brain

tissues [52], so most early studies reported that neutrophils were not found in the brain tissues but were trapped within the confines of the NVU and the leptomeningeal spaces during the early phase of reperfusion [52, 53]. This phenomenon was also proven by using MRI and intravital two-photon microscopy, which showed that neutrophils immediately attached firmly to sites of endothelial activation in stroke-affected brain areas [54]. Conventional theories state that neutrophil migration mediates mechanical disruption of the endothelium [55, 56]; however, it has been proposed that neutrophils can pass through the junctions of the barrier in the absence of serum protein extravasation, thus leading to physical disruption of the paracellular pathway [49]. Moreover, activated neutrophils release a mixture of substances, including reactive oxygen species (ROS), proteolytic enzymes, and cytokines, that can impair endothelial barrier function by acting on the endothelial cell cytoskeleton, junctional proteins, and the endothelial glycocalyx. Meanwhile, neutrophils are also able to enhance transendothelial protein exchange by releasing proteases, such as elastase and MMPs, which appear to alter barrier function by disrupting junctional complexes and inducing endothelial cell retraction [57]. Based upon the instrumental effects of neutrophils on the BBB, inhibiting the interaction of neutrophils with deposited platelets suppresses the recruitment of neutrophils, which has been previously shown to limit vascular injury, thereby reducing further damage to brain tissues [58]. However, the interaction between neutrophils and the BBB is dynamic, and a recent study revealed that extravasating leukocytes deposited microparticles on the subendothelium, while passing through the junctions and that microparticle deposition serves to maintain barrier function; inhibition of neutrophil-derived microparticle formation resulted in dramatically increased vascular leakage [59].

3.4. Mast Cells and the BBB

Mast cells (MCs) are brain-resident immune cells that enter the CNS by penetrating blood vessels during development, and ultrastructural studies have shown that MCs are located in the perivascular space surrounding the brain parenchymal vessels and in the dura mater of the meninges [60]. However, mature MCs are also able to move to the brain and can change their number and distribution in response to a variety of pathological stimuli [61]. MCs sense alarm signals from injured parenchymal cells and become activated within a few minutes [62]. This immediate phase response is mediated by the release of MC granule contents (such as histamine and TNF- α). Histamine, a prominent MC-derived mediator that has been shown to rapidly disrupt cell-cell and cell-ECM interactions, affected the stability of the endothelium and basal connective tissue [63]. The late phase response of MCs was characterized by the effects of other MC mediators, such as leukotrienes, prostaglandins, cytokines, and additional chemotactic factors. A significant increase in the number of MCs after 24 h in the ischemic brain in a rat model was observed [62]. There are hundreds of metachromatic granules containing biologically active mediators in the cytoplasm of MCs. Therefore, given the location of MCs and mediators they secrete, at the onset of ischemia, MCs play a potential role in the early phase of ischemic damage [64].

After acute ischemic damage, activated MCs secrete proteolytic gelatinase-positive granules, which target the NVU and lead to the degradation of the microvascular wall, especially the tight junctions and basement membrane thus, they contributed to brain swelling in a rat middle cerebral artery occlusion (MCAO) model [65]. In an intracerebral hemorrhage (ICH) model, mice stereotactically injected in the basal ganglia with collagenase exhibited MCs activation [66]. Pharmacological MCs inhibition significantly decreased the mortality rate and improved neurologic outcomes by mitigating neuroinflammation and BBB disruption [24, 66]. In addition, it has been confirmed that MCs degranulation aggravates neutrophil infiltration and further inhibits angiogenesis [67, 68].

4. THE BBB AND ADAPTIVE IMMUNITY

It was once believed that T cells could not successfully penetrate the intact BBB to reach the CNS parenchyma under quiescent conditions. However, later studies confirmed that activated T cells readily cross the undamaged BBB to enter the CNS [69]. Recent research has demonstrated that a group of leukocytes, including T cells, are present in normal human cerebrospinal fluid and are probably recruited across the choroid plexus [70]. These T cells could contribute to immune surveillance and respond to recurrent pathogen exposure in the CNS [71]. Acute cerebral injury activates not only innate immune responses but also the adaptive immune response. Damage to the BBB leads to the exposure of the adaptive immune system to dangerous molecules, such as α -synuclein, that are typically excluded by the BBB under physiological conditions. T cells can be identified in the postischemic brain as early as 24 h after reperfusion and are mostly located in infarction boundary zones. It was suggested that the accumulation of T lymphocytes peaks at 3–4 days after stroke in mouse models, and the presence of T cells persists 7 weeks poststroke [13].

Both helper Th1 and Th17 T cells produce proinflammatory cytokines (IL-2, IL-12, IFN- γ , TNF α , and IL-17), which can damage the BBB and activate microglia, neutrophils, and brain ECs to contribute to the pathology of stroke. IL-17 plays a role in BBB disruption, which occurs in patients and rodents after ischemic stroke. There are several mechanisms by which IL-17 can cause BBB damage. Migration of Th17 cells across the BBB into the brain can increase IL-17 secretion and thus generate a pro-inflammatory response in the brain to increase lymphocyte recruitment and cytokine secretion [72, 73]. However, Th2 cells secrete anti-inflammatory cytokines, which further suppress Th1 cells (TGF- β 1, IL-4, IL-5, IL-10, and IL-13) to exert neuroprotective effects [74].

The first cellular structure T cells encounter after penetrating the blood vasculature would be the endfeet or processes of astrocytes. However, there is currently insufficient *in vivo* evidence demonstrating the direct interaction between astrocytes and T cells. Fortunately, some *in vitro* studies have provided clues regarding the effect of astrocytes on T cells. Culturing mouse CD4 T-cells on mouse primary astrocytes was found to modify T-cell polarization into the Th1 phenotype and regulatory T cell (Treg) subtypes without the addition of supplemental cytokines [75], and the effects of

this could be diminished by the inflammatory activation of astrocytes [71].

Tregs are a double-edged sword concerning BBB damage caused by stroke. Treg cells may induce microvascular dysfunction by enhancing the Treg-endothelial interaction *via* integrin leukocyte function-associated antigen (LFA)-1 on lymphocytes and endothelial intercellular adhesion molecule (ICAM)-1 in inflamed microvessels [76]. However, Tregs were reported to suppress excessive immune responses and ameliorate BBB injury after cerebral ischemia and thus alleviate thrombolytic treatment-induced hemorrhage in stroke victims mediated by CCL2 and MMP9 [77]. Moreover, Tr1-type Tregs could produce IL-10, which deactivated astrocytes and macrophage-like cells and thus limited their involvement in the inflammatory process and reduced damage to the BBB. Furthermore, IL-10 also inactivated NF-κB to protect the BBB against glutamate-induced cytotoxicity [78].

The crosstalk between the BBB and immune cells is complex, and the improvement of the understanding of this crosstalk is of great significance for developing targeted therapies for stroke (Table 1).

In addition to the crosstalk between immune cells and the BBB, the increased permeability of the BBB is also related

to the increase in the diameter of cerebral lymphatic vessels. In addition, fluorescent tracer dye that penetrates the brain tissues through the damaged BBB, as well as the CSF, can be absorbed by the lymphatic vessels and transported to the deep cervical lymph nodes [79]. Therefore, the clearance function of the lymphatic system may be one of the key mechanisms involved in the regulation of BBB permeability [80]. The dural lymphatic vessels absorb the cerebrospinal fluid in the subarachnoid space and interstitial fluid through the lymphatic system and transport it to the deep cervical lymph nodes, indicating that the meningeal lymphatic vessels may be involved in the steady-state transport of immune cells. Moreover, around the dura, scientists have observed some insubstantial accumulation of immune cells, such as lymphocytes, monocytes and meningeal macrophages, that might be transported through the lymphatic vessels and participate in the immune regulation of the CNS. However, the specific role of lymphatic vessels in BBB injury is unclear and requires further research.

5. IMMUNOMODULATORY THERAPEUTIC STRATEGIES FOR STROKE THAT TARGET THE BBB

The importance of immunity at all stages of stroke has been recognized, and a series of stroke immunomodulatory

Table 1. Crosstalk between immune cells and BBB after stroke.

Immune Cells	Crosstalk	BBB Component		
Microglia/macrophages	<ul style="list-style-type: none"> ✦ Alter microglia morphology (M-CSF, GM-CSF) [25] ✧ Cause swelling of astrocyte endfeet (IL-1β, TNF-α) [27] ✧ Attenuates the regulatory function of astrocytes (IL-1α, TNF-α, C1q) [28] 	Astrocytes		
	<ul style="list-style-type: none"> ✧ Migrate and obtained the microglia morphology [30] ✦ Turn into microglia-generating stem cells [31] 	Pericytes		
	<ul style="list-style-type: none"> ✧ Engulf endothelial cells [36] ✧ Downregulate tight junction proteins expression in ECs (TNF-α, IL-1β, IL-6) [37, 38] ✧ Accelerate endothelial cell to repair the BBB (P2RY12) [39] 	Endothelial cells		
	<ul style="list-style-type: none"> ✦ Activate microglia (fibronectin, vitronectin) [41] ✧ Remodel the ECM (MMP-3, MMP-9, MMP-19) [43] 	Extracellular matrix		
	Neutrophils	<ul style="list-style-type: none"> ✧ Impair endothelial barrier function (ROS, proteolytic enzymes, cytokines) [55] ✧ Lead to physical disruption of endothelial [55] ✧ Maintain barrier function [57] 	Endothelial cells	
		Mast cells	<ul style="list-style-type: none"> ✧ Decompose the microvascular wall (proteolytic gelatinase enzymes) [63] ✧ Inhibit angiogenesis of the microvascular (degranulation) [65, 66] 	Endothelial cells
			Helper T cells	<ul style="list-style-type: none"> ✧ Disrupt BBB tight junctions [72] ✧ Promote vascular permeability (IL-2, IL-12, IFN-γ, TNFα, and IL-17) [73]
<ul style="list-style-type: none"> ✦ Modify T-cell polarization [69] 	Astrocytes			
<ul style="list-style-type: none"> ✧ Th2 suppress TH1 cells (TGF-β1, IL-4, IL-5, IL-10, and IL-13) [72] 	Indirect effect			
Regulatory T cells	<ul style="list-style-type: none"> ✧ Induce microvascular dysfunction (LFA-1, ICAM-1) [74] 	Endothelial cells		
	<ul style="list-style-type: none"> ✧ Suppress excessive immune responses (IL-10) [76] 	Astrocytes		
	<ul style="list-style-type: none"> ✧ Alleviate thrombolytic treatment-induced hemorrhage [75] 	Indirect effect		

✧ Represent for the effect of BBB component on immune cells; ✦ Represent for the effect of immune cells on BBB component.

therapies are being developed. The key objective of these therapies is to target the pro-inflammatory cytokines, MMPs, and infiltrating leukocytes to reduce the initial brain cell toxicity to maintain the steady-state of the BBB. On the other hand, the BBB is also a barrier that prevents drugs from accessing brain tissue. Therefore, there have been some studies dedicated to developing methods of regulating immune cells to increase the permeability of the BBB at the appropriate time so that drugs can bypass or cross the BBB (Table 2). Although these methods have achieved good results in experimental stroke models, unfortunately, only some of them have shown satisfying results in clinical trials.

Minocycline is known not only as a member of the tetracycline antibiotic family but also as an inhibitor of microglia/macrophages, and its effects have been corroborated by several preclinical studies and clinical trials [81, 82]. Minocycline acts by inhibiting the MMP-9 and p38 mitogen-activated protein kinase (p38 MAPK) signal transduction pathways to prevent the microglial generation of glutamate, IL-1 β and NO [83], thereby improving BBB viability and integrity and protecting the brain after ischemic stroke [84]. To date, minocycline has been incorporated into two clinical trials involving ischemic stroke patients, demonstrating that minocycline administration (both alone and in combination with fibrinolysis) improved neurological functional outcomes following stroke [85]. Additionally, therapy with minocycline may extend tPA treatment time windows in ischemic stroke [86]. Other than minocycline, many other brain protectants were proven to exert neuroprotective effects by inhibiting the activity of microglia and maintaining

the integrity of the BBB. The glucagon-like peptide-1 receptor (GLP-1R) agonist exendin-4 (Ex-4) is known to suppress microglial activation and neutrophil infiltration by attenuating pro-inflammatory cytokine expression levels, therefore ameliorating warfarin-associated hemorrhagic transformation post cerebral ischemia [87].

In contrast, some groups have promoted the concept that microglial cells are important for recovery from ischemic damage. Some *in vitro* and *in vivo* studies have shown the role of microglia in promoting neuroprotection and neuroregeneration [88]. A series of experiments demonstrated that selective ablation of proliferating resident microglia within the first 72 h after ischemic injury resulted in a significant increase in the size of the infarction and the destruction of the BBB, which is associated with an increase in the number of apoptotic cells, especially neurons. Conversely, stimulation of microglial proliferation after cerebral ischemia by M-CSF partly reverses the injury by increasing the number of microglia [89]. Besides, Kitamura and colleagues demonstrated in a rat model that the intracerebroventricular injection of microglia protected the BBB and neurons against focal brain ischemia [90]. The mTOR pathway could drive pro-inflammatory process *via* metabolic reprogramming. As a classic mTOR inhibitor, rapamycin is encapsulated in microthrombus-targeting micelles, which were generated to mediate the polarization of M2 microglia, thus leading to the remodeling of the NVU and BBB preservation and enhancing neuroprotection and blood perfusion in ischemic stroke [91]. Nicotinamide phosphoribosyl transferase (NAMPT) exerts a neuroprotective effect by changing the microglial

Table 2. Immunomodulatory therapies in acute ischemic stroke.

Drugs	Mechanism	Animal Model	Clinical Trials
Minocycline (microglia/macrophages inhibitor) [81-86]	➤ Inhibit MMP-9 and p38MAPK	tMCAO Rat; pMCAO Mouse	Clinical trial Phase I and II
Exendin-4 (GLP-1R agonist) [87]	➤ Attenuate pro-inflammatory cytokine expression	tMCAO Mouse	/
Macrophage colony-stimulating factor [89]	➤ Increase the number of microglia	tMCAO Mouse	/
Microglia (intracerebroventricular injection) [90]	➤ BBB remodeling	tMCAO Rat	/
Rapamycin (mTOR inhibitor) [91]	➤ Mediate polarize of M2 microglia	tMCAO Rat	/
Nicotinamide phosphoribosyltransferase [92]	➤ Mediate polarize of microglia	tMCAO Mouse	/
ECM gelation [94]	Mediate polarize of M2 macrophage	tMCAO Rat	/
Interleukin-1 receptor antagonist (stroke prevention) [95]	➤ Reduce microglial activation, neutrophil infiltration	tMCAO Rat	/
Fingolimod [96-98]	➤ Inhibit the infiltration of peripheral immune cells	tMCAO Mouse	Open labeled trial; Clinical trial Phase II
Adeno-associated virus (vaccine) [99]	➤ Increase the permeability of the BBB, promoting the passage of autoantibodies	endothelin-1 MCAO Rat	/
Phosphatidylserine-modified microbubbles (open the BBB by ultrasound) [100]	➤ Target activated microglia for inflammatory area	tMCAO Rat	/

polarization of MCAO mice [92]. All these data suggest that microglia might be a possible target of strategies to minimize ischemic damage in stroke patients [93].

Apart from therapeutics targeting microglia, scientists are still exploring new ways to prevent BBB disruption. ECM was injected into the BBB to replace necrotic debris and promote the infiltration of endogenous immune cells post-stroke, which promoted an acute endogenous repair response that could potentially be exploited to treat stroke [94]. Similarly, naturally occurring IL-1 receptor antagonist (IL-1Ra) was able to reduce microglial activation, neutrophil infiltration, and cytokine levels to protect against ischemic brain damage by reducing BBB damage in both healthy animals and animals with multiple risk factors for stroke [95]. Besides, some researchers have applied fingolimod, an immunomodulating drug, to inhibit the infiltration of peripheral immune cells into the brain, significantly reducing BBB permeability and neurological deficits and promoting recovery in animals and stroke patients [96-98].

The BBB is also a barrier that blocks many drugs from entering the brain tissue. Therefore, in addition to research on protecting the structural integrity of the BBB and reducing leakage, there have also been studies focused on how to change the permeability of the BBB quickly and temporarily to allow drugs to enter the damaged tissue. Early oral administration of an adeno-associated virus (AAV) vaccine may induce self-protection to allow the brain to locally increase the permeability of the BBB during stroke, thereby promoting the passage of autoantibodies and repairing damaged brain tissue [99]. For poststroke treatment, prepared phosphatidylserine (PS)-modified microbubbles (PS-MBs) combined with ultrasound-targeted microbubble destruction (UTMD) can safely increase the permeability of the BBB and target activated microglia in areas of increased inflammation in the later stage of ischemia reperfusion [100].

Due to its characteristics of having a clear target and long intervention window, immunomodulatory therapy has achieved satisfactory results in the treatment of experimental stroke and has been widely promoted in clinical trials (Table 2). However, some of these trials did not show benefits, which might partly be due to the unanticipated effects of the drugs or inappropriate study design [101]. The reasons for the absence of a beneficial effect in humans may be manifold; one reason is the heterogeneity of clinical stroke. Another possibility is the species difference between acute ischemic stroke patients and rodents. For example, proinflammatory microvascular failure leading to “no-reflow” might be important in rodent stroke but of limited relevance to primate stroke due to differences in cerebrovascular collateralization [102]. In addition, among various experimental animal models, we chose to utilize adolescents, which lack features that mimic the complications of chronic diseases. Nevertheless, more work in this area, including the improvement of trial design and guidelines for preclinical development, is needed.

CONCLUSION

Following stroke, the impairment of the BBB evokes inflammation by enabling immune cells, including microglia,

neutrophils, T cells and other types, to infiltrate the CNS parenchyma. Although these immune cells promote the removal of necrotic tissues and neuronal recovery, the release of inflammatory factors such as ROS and MMP, especially after late reperfusion, can aggravate BBB injury and exacerbate stroke outcome. The bidirectional and dynamic crosstalk between inflammation and the BBB after stroke showed that obvious temporal characteristics and immune cells play different roles in this pathological process.

In summary, the BBB plays an important role in the manifestation and development of stroke. On this basis, there is great hope for the enhancement of personalized medicine and stroke prevention. However, more basic trials and pre-clinical development work are currently needed.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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