Immunity in Stroke: The Next Frontier

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Abstract

Translational stroke research has long been focusing on neuroprotective strategies to prevent secondary tissue injury and promote recovery after acute ischemic brain injury. The inflammatory response to stroke has more recently emerged as a key pathophysiological pathway contributing to stroke outcome. It is now accepted that the inflammatory response is functionally involved in all phases of the ischemic stroke pathophysiology. The immune response is therefore considered a breakthrough target for ischemic stroke treatment. On one side, stroke induces a local neuroinflammatory response, in which the inflammatory activation of glial, endothelial and brain-invading cells contributes to lesion progression after stroke. On the other side, ischemic brain injury perturbs systemic immune homeostasis and results in long-lasting changes of systemic immunity. Here, we briefly summarize current concepts in local neuroinflammation and the systemic immune responses after stroke, and highlight two promising therapeutic strategies for poststroke inflammation.

Keywords
- stroke
- inflammation
- immunomodulatory drugs

Introduction

Stroke is the second largest cause of death after ischemic heart disease worldwide, with ischemic stroke accounting for over 70% of cases depending on regional epidemiology.[1–3] Currently, thrombolysis with recombinant tissue plasminogen activator and endovascular thrombectomy given in the hyperacute phase after ischemic stroke onset are still the only effective therapies.[4,5] Due to the narrow therapeutic time window and safety concerns, the clinical indications for thrombolysis and mechanical thrombectomy are limited and most stroke patients do not receive a specific acute stroke treatment.[6] In fact, so far no specific therapies have been proven efficient when administrated beyond 24 hours after stroke onset. Post-ischemic inflammation, which persists for a prolonged time period of days to weeks after stroke onset, is considered a potential strategy in expanding the time frame for treatment. The immune system has been consistently proven to play a critical role in stroke pathophysiology.[7,8] Therefore, inflammatory mediators and immune cells have received increasing attention as promising therapeutic targets for stroke treatment.

The Neuroinflammatory Response to Stroke

After the onset of ischemic stroke, the lack of oxygen and energy failure in the ischemic tissue triggers a series of deleterious cellular and molecular events.[9] In the acute phase, blood platelets adhere and become activated at the site of ischemic vascular injury. Activated platelets interact with T cells and neutrophils to promote thrombus formation and trigger thromboinflammation through the activation of the kallikrein–kinin system.[10,11] As the ischemic cascade progresses, brain cells undergo necrosis in the injured area and release various intracellular components into the extracellular space. Danger-associated molecular patterns (DAMPs) are a diverse group of immunoactive molecules, including high-mobility group box 1 (HMGB1), adenosine triphosphate (ATP), nucleic acids, and peroxiredoxin (Prx) family proteins as well

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as many other nuclear and cytoplasmic molecules, which are important triggers for sterile inflammation after tissue injury.[12] DAMPS are secreted from necrotic and stressed cells but also actively secreted from immune cells as well as the endothelium and neurons.[12] These danger signals activate purinergic receptors and pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), receptor for advanced glycation end-products, and scavenger receptors, which are widely expressed on immunocompetent brain cells such as microglia, border-associated macrophages, and brain endothelial cells.[13,14] In murine stroke models, ATP, HMGB1, and Prx family proteins are major DAMPs involved in post-ischemic inflammation.[15–17] High levels of extracellular nucleotides (ATP, UTP) released from injured brain cells are recognized by purinoceptors and function as "find-me" signals for phagocytic cells.[18] HMGB1, an intracellular DNA-binding protein, is released early after stroke and its plasma concentrations remain elevated for months after stroke.[19] Recognized by several membrane-bound and intracellular PRRs, HMGB1 is a potent mediator of sterile inflammation which can result in various disease-relevant pathophysiological processes such as cytokine-induced sickness behavior, leakage of the blood–brain barrier (BBB), and activation/recruitment of systemic immune cells.[20–22] Similar to HMGB1, Prx family proteins released from necrotic cells trigger the production of inflammatory cytokines and promote the activation of infiltrating macrophages through activation of PRR pathways.[16,23,24] In addition, other endogenous molecules from damaged tissues, such as nucleic acids, lipids, and extracellular matrix, can also be recognized by PRRs and induce a sterile inflammatory response.[13,14]

Microglia and astrocytes are the key brain resident cell population which participate in the initial inflammatory response to the ischemic brain injury. Activated microglia undergo a rapid phenotypic change toward a reactive cell state and promote further inflammatory response.[25] Activated microglia develop a de-ramified morphology with significantly enhanced migratory capacity, phagocytosis, and production of proinflammatory mediators.[25] During the early phase after stroke, microglia remove cellular debris from damaged tissue through phagocytosis mediated by receptors, including TLRs, triggering receptor expressed on myeloid cells 2 (TREM2), purinergic receptors, and the Tyro3, Axl, and Mer (TAM) tyrosine receptor kinases.[26,27] Yet, the ischemic activation of microglia can also induce a further inflammatory exacerbation of lesion progression by various deleterious mechanisms. Reactive microglia not only phagocytose necrotic cells but also engulf surviving neurons in the perilesional tissue-at-risk.[28] Additionally, perivascular microglia have been demonstrated to engulf vascular endothelial cells which can further promote dysfunction of cerebrovascular integrity.[29] DAMPs in the extracellular space result via activation of PRR pathways in the increased secretion of cytokines and chemokines by microglia. On one hand, activated microglia release large amounts of proinflammatory mediators that contribute to neuronal apoptosis, and signals that recruit peripheral immune cells to exacerbate inflammation. On the other hand, microglia produce various anti-inflammatory mediators and neurotrophic factors that play an important role in neurogenesis, particularly in the tissue repair process during the chronic phase after ischemia.[30,31] Overall, activated microglia perform a complex and diverse role in the inflammatory response following cerebral ischemia; many studies have substantiated this "dual function." However, a recent study proposes that an absence of microglia leads to dysregulated neuronal network activity and results in exacerbated stroke outcome, which implies neuroprotective function of microglia despite the plethora of inflammatory cytokines they produce.[32]

Astrocytes, the most abundant glial cells in the brain, are activated in response to signals released from damaged neurons or activated microglia and undergo reactive astrogliosis after ischemic stroke. Reactive astrocytes form a glial scar in the peri-infarct area, which isolates the lesion and restricts the spread of neuroinflammation but also hinders axonal regeneration.[33] Reactive astrocytes cross-talk with microglia to enhance the inflammatory response, and produce various proinflammatory mediators and free radicals that cause severe secondary damage to neurons.[33,34] However, reactive astrocytes also show neuroprotective effects by releasing neurotrophic factors, taking up extracellular excitotoxic glutamate, releasing antioxidant endogenous glutathione, and stabilizing extracellular fluid and ionic homeostasis.[35–38] Moreover, astrocytes are essential for maintaining vascular integrity and correct function of the BBB. The astrocytic endfeet wrap around blood vessels and are tightly attached to the outer surface of the basal lamina.[39] However, in turn, cytokines and matrix metalloproteinases produced by pericapillary astrocytes result in dysfunction of BBB and vasogenic edema after the ischemic insult.[40,41] Thus, similar to microglial cells, reactive astrocytes also exert a dualistic role in the immune responses to stroke.

As another functional component of the BBB, the cerebral endothelium is an important component of the inflammatory reaction after stroke. Since their unique position at the boundary between blood circulation and brain parenchyma, endothelial cells play a vital role in initiating and regulating the recruitment of peripheral inflammatory cells after stroke.[39] When stimulated either directly by hypoxia, DAMPs, or cytokines derived from immune cells, endothelial cells express substances with vasoactive and proinflammatory properties as well as upregulate cell-adhesion molecules (CAMs) which can facilitate the recruitment of circulating leukocytes to the injured brain.[42] Among the large group of CAMs, three groups of CAMs have been shown to be particularly relevant for the transvascular leukocyte invasion at the BBB: selectins (p-selectin, e-selectin, and l-selectin), cellular adhesion molecules (ICAM-1 and -2, VCAM-1, and PECAM-1), and integrins.[43] Selectins have been shown to mediate the initial cell–cell adhesion and rolling of leukocytes on the endothelium, while leukocyte integrins interact with cellular adhesion molecules expressed on endothelial cells to make firm attachment and induce the transmigration.[44] Previous studies have demonstrated that inhibition or deficiency of adhesion molecules leads to decreased intracerebral leukocyte

accumulation, reducing ischemic injury, and improving neurological outcome.[45–47]

The recruitment of peripheral leukocytes to the injured brain after stroke occurs in a well-orchestrated manner with distinct kinetics for the different leukocyte subpopulations.[48] Myeloid cells (monocytes and neutrophils) are recruited to the injury site within hours after stroke. They are involved in the inflammatory response through phagocytosis of necrotic cell debris and production of cytokines and chemokines. This early cerebral leukocyte accumulation, together with reactive microglia, releases proinflammatory cytokines that stimulate endothelial cells to upregulate adhesion molecules, thereby facilitating further leukocyte influx to the brain parenchyma.[49,50] Activated neutrophils produce inflammatory factors which exacerbate endothelial damage and neuronal cell death. As the neuroinflammatory reaction aggravates, dendritic cells increase in the brain parenchyma.[48] Compared with these innate immune cell populations, lymphocytes infiltrate with delayed kinetics after only several days but can then persist for more than 30 days in the injured brain.[48,51] The first T cell subset invading the ischemic tissue are CD8+ cytotoxic T cells, which cause neuronal death and exacerbation of brain damage.[52,53] Most infiltrated T cells are CD4+ helper T cells, which differentiate into different subtypes (e.g., Th1, Th17, or Treg) and then produce pro- or anti-inflammatory cytokines.[48,54] Despite the relatively small number of T cells compared with innate immune cells in the brain, this cell population has been consistently demonstrated to be a major contributor to stroke pathophysiology.[55,56] Infiltrating helper T cells that acquire either the Th1 or Th17 proinflammatory phenotypes after stroke exhibit detrimental effects of aggravating brain injury by secreting proinflammatory cytokines, including interleukin (IL)-2, IL-17, IL-23, and interferon-γ.[47,54] In contrast, regulatory T cells show a protective role in neuroinflammation at a more delayed stage through the secretion of anti-inflammatory factors and cell–cell contact-dependent mechanisms.[57–59] Therefore, it is very likely that future therapeutic approaches targeting only cellular subpopulations (such as pro- versus anti-inflammatory T cells) or specific inflammatory mechanisms (such as neutralizing proinflammatory T cell cytokines) would be more efficient than the previously tested approaches aiming to rather nonspecifically block the—at least in part seemingly beneficial—neuroinflammatory response to stroke.

Systemic Immunity in Stroke

In addition to the local neuroinflammatory response to tissue injury in the brain, stroke causes also a profound alteration in systemic immune homeostasis. The systemic immune response to stroke can be divided into several phases with a distinct immunological phenotype ranging from early immune activation to subsequent immunosuppression and chronic low-grade inflammation. In the hyperacute phase of cerebral ischemia, the peripheral immune system is over-activated and characterized by a rapid and extensive increase in cytokines from activated splenocytes and lymphoid tissue.[60] Moreover, stroke activates hematopoietic stem cells in the bone marrow, leading to a myeloid-biased emergency hematopoiesis and an increasing output of neutrophils and inflammatory monocytes to the circulation.[61,62] However, this early activation of systemic immunity lasts only for 1 to 2 days before severe systemic immunosuppression occurs. Immunosuppression in the subacute phase after ischemia is characterized by lymphopenia, reduced functional activity of monocytes, and splenic atrophy.[63,64] These immunological changes make patients susceptible to infections, which is a key factor to the morbidity and mortality of stroke patients during the first month after stroke.[65] Over-activation of the immune system in the hyperacute stage of stroke results in functional exhaustion of mature monocytes which leads to apoptosis of lymphocytes.[17] We have recently demonstrated that the activation of innate immune cells via brain-released alarmins and activation of the inflammasome complex in circulating cells is the mechanistic link between early immune activation and subsequent lymphopenia.[17,66] In the chronic phase after stroke, systemic immune dysfunction can still be observed for several months.[19] The low-grade chronic inflammation can also be observed in stroke patients as a sustained increase in inflammatory blood biomarkers such as C-reactive protein, IL-6, IL-8, and tumor necrosis factor-α.[67–69] The persistence of inflammatory factors is associated with cognitive decline and stroke recurrence in patients. Moreover, a long-term increase in circulating leukocytes and changes of lymphocyte subsets are found for several months after stroke.[19,70] Considering that stroke patients are in a large proportion multimorbid patients with several comorbidities such as atherosclerosis, diabetes, hypertension, and others, the contribution of the long-term chronic inflammation to underlying comorbidities, the development of poststroke complications, and poststroke recovery warrants an in-depth analysis of currently unknown mechanisms and therapeutic targets.

From Bench to Bedside: Therapeutic Strategies

As far as the current status of ischemic stroke treatment is concerned, effective therapies to treat the acute phase and prevent recurrent events are still very limited. Although many molecules have been reported to be neuroprotective in experimental stroke models, all of them have to date failed to clinically improve neurological outcomes in clinical trials. Despite this so far failed translation of primarily neuroprotective agents, many potential strategies are currently under investigation for stroke treatment, particularly those targeting neuroinflammation in stroke. Accumulating evidence suggests that inhibition of neuroinflammation in the brain has a beneficial effect for stroke outcome. It has been demonstrated that blockade of lymphocyte trafficking reduces infarct volume and thus improves stroke outcomes in experimental stroke models.[47,71] However, several clinical trials for drug repurposing of

compounds already well-established for primary autoimmune brain disorders have failed to prove clinical efficacy in stroke patients. Among them, the functional sphingosine-1-phosphate (S1P) receptor antagonist FTY720 (fingolimod), an immunomodulatory drug established for treatment of multiple sclerosis by reducing the circulation and cerebral T cell infiltration, has attracted great attention. FTY720 significantly reduced ischemic damage and neurological deficits, and promoted recovery in animal models.[72,73] Results from clinical trials show that oral administration of FTY720 for 3 consecutive days after stroke onset reduces microvascular permeability, limits secondary brain injury, and improves neurological outcome in patients.[74–76] Despite the reduction of peripheral T cell circulation by FTY720, clinical data show that FTY720-treated patients have relatively mild infection signs that resolved after a brief treatment of antibiotics. In the meantime, no drug-related serious adverse events are observed, suggesting that FTY720 is safe for patients.[74,75] Therefore, FTY720 is currently one of the most promising therapeutic immunomodulatory drugs for ischemic stroke. However, the actual effectiveness of FTY720 is closely related to the type of stroke, the timing, and route of administration. Therefore, larger clinical trials are required to ultimately confirm its clinical efficacy and safety for ischemic stroke. Besides this one highlighted example of clinical trials for FTY720 in stroke, other immunomodulatory clinical trials have already concluded or are currently undergoing to test the effectiveness of targeting immune cell migration (e.g., by administration of the CD49-specific antibody natalizumab), CD18 antagonists to inhibit neutrophil activation, or use of the immunomodulatory antibiotic minocycline to reduce microglial activation after stroke.[77–79]

Recurrent stroke and other ischemic events are major problems for patients surviving ischemic stroke. Epidemiological data indicate that the stroke recurrence increases over time. The 1-year recurrence rate of ischemic stroke ranges from 6 to 12%, while the 5-year recurrence rate rises to 16 to 22%,[80–84] depending on the patients' age, sex, comorbidities, and stroke subtype. Standard of care for secondary prevention in stroke patients is mainly focusing on optimizing treatment of the metabolic syndrome (obesity, hypertension, diabetes), which is a common comorbidity, cardiovascular risk factor, and often cause of the incident stroke. Therapies for this include antihypertensive, lipid lowering, and thrombocyte aggregation inhibiting medication. This treatment has been proven effective and approved for reducing the long-term risk of recurrent cardiovascular events (stroke, myocardial infarction, and death of any cause). However, currently approved secondary prevention therapies are only insufficiently preventing early cardiovascular disease (CVD) recurrence. This becomes obvious by the fact that the risk for an acute ischemic event is approximately doubled (hazard ratio: 0.67) in the acute phase after a stroke despite current standard of care treatment. Epidemiological data from the Oxfordshire Stroke Project showed indeed that patients with atherosclerotic stroke incidence had the highest recurrence rate in the (currently untreated) acute phase (7-day period) with an odds ratio of 3.3.[85]

To target this remaining therapeutic window in recurrent stroke prevention, anti-inflammatory therapies have come into focus of translational stroke research.[86] We have previously demonstrated that stroke results in exacerbation of atherosclerotic plaques in experimental stroke models—probably contributing to early recurrent stroke events—via the systemic inflammatory response to brain injury.[87] These observations particularly emphasize the possible contribution of inflammatory mechanisms to early CVD recurrence after ischemic stroke.

A promising, currently tested approach for reducing CVD recurrence is the treatment with colchicine—an anti-inflammatory drug used for decades, primarily for treatment of acute gout. Recent meta-analyses provide evidence that colchicine administration significantly reduces the stroke risk in patients with high cardiovascular risk.[88,89] Colchicine is a microtubule inhibitor with anti-inflammatory properties that attenuates inflammasome assembly, IL-1β activation, inflammatory cell motility, and cytokine secretion.[90,91] The ongoing CONVINCE (Colchicine for prevention of Vascular Inflammation in Non-CardioEmbolic stroke) is a randomized phase III clinical trial of secondary stroke prevention investigating the efficacy and safety of daily low-dose colchicine on the prevention of recurrent stroke and major vascular events. Over 3,000 patients in 17 countries will be enrolled in CONVINCE, with clinical trials due to be completed by 2023.[92] Despite the obvious medical need to prevent recurrent ischemic events due to residual inflammatory risk, no other drug candidates are currently in development for this indication to the best of our knowledge. To provide novel candidates and therapeutic targets for this relevant pathomechanism, more insights into the mechanisms of systemic immune modulation after stroke and its impact on post-stroke comorbidities are required.

Conclusion

Over the past decades, there has been a massive increase in data which improved our understanding of the immune response to stroke. The crucial role of immunity in the pathological development of stroke has been widely recognized and the immune system has emerged as a key target for therapeutic intervention in stroke (►**Fig. 1**). Extensive data from clinical and experimental studies suggest DAMPs released from brain-injured tissue as initiators of sterile inflammation following ischemic stroke. These danger-signaling molecules cause activation of innate immune cells in the brain and recruitment of circulating immune cells, which have a profound effect on neuronal damage and recovery. A complex and prolonged systemic immune response induced through the neuro-immune axis ensues, especially immunosuppression that may cause life-threatening systemic infections. Many elements of the immune system have partially opposing roles in ischemic stroke with both beneficial and deleterious phenotypes, which may be time-dependent. Targeting such immunological mechanisms after stroke provides an expanded time window of opportunity and a wide range of applications for therapeutic strategies, from improving

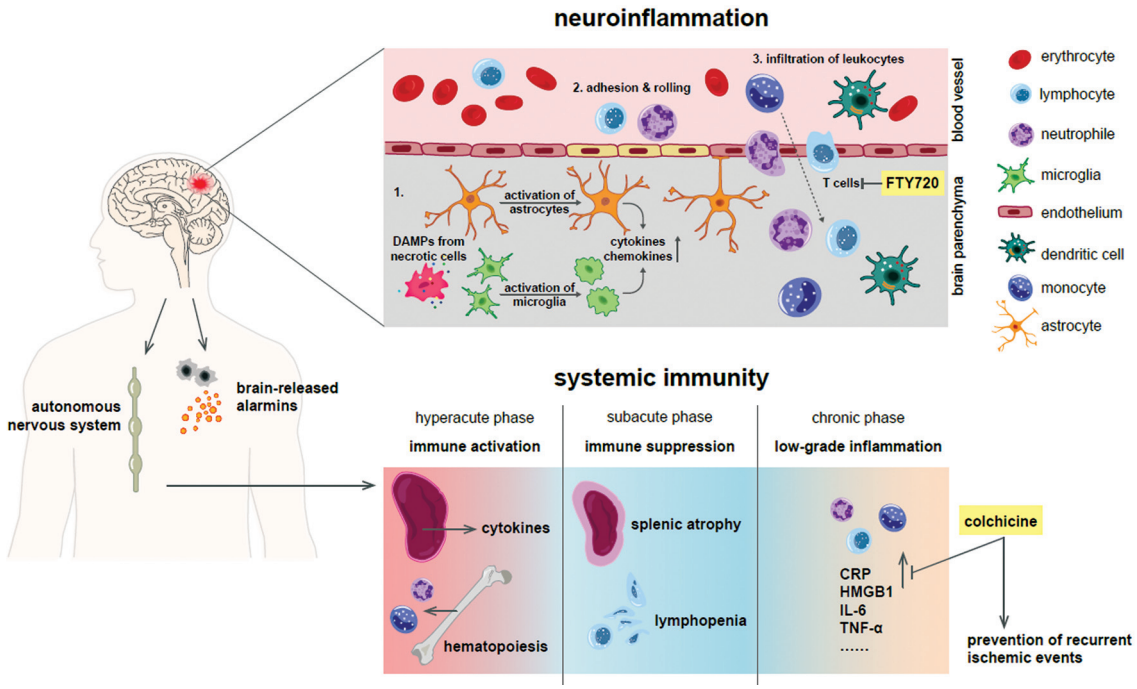

Fig. 1 Overview on key brain–immune interactions after stroke. Ischemic brain injury induces a local neuroinflammatory response as well as long-lasting changes in systemic immune homeostasis. Cerebral neuroinflammation includes activation of resident glial cells and infiltration of circulating leukocytes. Initial ischemic events lead to the release of DAMPs from necrotic cells which cause activation of microglia and astrocytes in the brain, resulting in secretion of various inflammatory cytokines and chemokines (1). The activated endothelium upregulates adhesion molecule expression, facilitating the recruitment of circulating leukocytes to the injured brain (2). Blood-derived inflammatory cells such as monocytes, neutrophils, dendritic cells, and lymphocytes infiltrate the ischemic area in a well-orchestrated manner, further promoting neuroinflammation (3). The intracerebral injury also induces a multiphasic systemic immune response through brain-released alarmins and the autonomous nervous system. In the hyperacute phase, immune activation is characterized by an increase in cytokine secretion and emergency hematopoiesis resulting in increased counts of circulating monocytes. In the subacute phase, the immune reaction turns to an immunosuppressive phenotype, characterized by lymphopenia, splenic atrophy, and monocyte exhaustion, increasing the susceptibility of stroke patients to infections. In the chronic phase, low-grade inflammation is clinically manifested by a long-lasting change in immune cell function and elevation of inflammatory biomarkers including CRP, HMGB1, IL-6, and TNF-α. The figure highlights two exemplary therapeutic approaches for poststroke immunomodulation with promising results. FTY720 alleviates stroke injury by inhibiting leukocyte infiltration into brain tissue, and colchicine reduces systemic inflammation and prevents recurrent ischemic events. Both drugs are currently investigated in ongoing clinical trials for ischemic stroke patients. CRP, C-reactive protein; DAMP, danger-associated molecular pattern; HMGB1, high-mobility group box 1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

neurological outcomes to reducing poststroke systemic infections, and preventing cognitive decline. Thus, "single-target" therapies may be insufficient to deal with the injuries following ischemia. Effective treatments are most likely to selectively target several cell types in different post-ischemic phases to promote protection and recovery. The ultimate effectiveness of immunomodulatory drugs in treating stroke will depend on further improving our understanding of the bidirectional communication between the central nervous system and the immune system to design specific and highly efficient therapies.

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Conflict of Interest
None declared.

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