Editorial

Immunity in Stroke: the next Frontier

Ting Li[1,2] & Arthur Liesz[1, 3*]

1 Institute for Stroke and Dementia Research (ISD), University Hospital, LMU Munich, Munich, Germany

2 Gansu Key Laboratory of Biomonitoring and Bioremediation for Environmental Pollution, School of Life Sciences, Lanzhou University, Lanzhou, China

3 Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

*To whom correspondence should be addressed: arthur.liesz@med.uni-muenchen.de

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 post-stroke inflammation.

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 (rt-PA) 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 thrombo-inflammation 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), ATP, nucleic acids, and peroxiredoxin (Prx) family proteins as well 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 (RAGE), 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][21][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 towards 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 pro-inflammatory 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 induce also 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 pro-inflammatory 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 crosstalk with microglia to enhance the inflammatory response, and produce various pro-inflammatory 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 MMPs produced by pericapillary astrocytes results in dysfunction of BBB and vasogenic edema after the ischemic insult[40, 41]. Thus, similar to microglial cells, also reactive astrocytes 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 pro-inflammatory 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, release pro-inflammatory 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 (DCs) increase in the brain parenchyma[48]. Compared to 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 to 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 pro-inflammatory phenotypes after stroke exhibit detrimental effects of aggravating brain injury by secreting pro-inflammatory cytokines, including IL-2, IL-17, IL-23 and IFN-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 pro-inflammatory T cell cytokines) would be more efficient than the previously tested approaches aiming to rather unspecifically 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 in 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 1-2 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 TNF-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 post-stroke complications and post-stroke 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, 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 three 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 - 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].

In order 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 3000 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. In order to provide novel candidates and therapeutic targets for this relevant pathomechanisms, 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. 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 neurological outcomes to reducing post-stroke

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 CNS and the immune system in order to design specific and highly efficient therapies.

Figure legend

Figure 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 post-stroke 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.

Acknowledgements

The authors would like to thank Antonia Weingart for help in graphical illustration of the figure. This study was supported by the European Research Council (ERC-StGs 802305), the National Natural Science Foundation of China (#31600831 and #81771324) and the German Research Foundation (DFG) under Germany´s Excellence Strategy (EXC 2145 SyNergy – ID 390857198).

Declaration of interests

The authors declare to have no competing interests.

References

1. Collaborators GS. Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2019; 18: 439-458.
2. Barthels D, Das H. Current advances in ischemic stroke research and therapies. Biochimica et biophysica acta Molecular basis of disease 2020; 1866: 165260. DOI: 10.1016/j.bbadis.2018.09.012
3. Wang W, Jiang B, Sun H et al. Prevalence, Incidence, and Mortality of Stroke in China: Results from a Nationwide Population-Based Survey of 480 687 Adults. Circulation 2017; 135: 759-771. DOI: 10.1161/circulationaha.116.025250
4. Hankey GJ. Stroke. Lancet 2017; 389: 641-654.
5. Phipps MS, Cronin CA. Management of acute ischemic stroke. BMJ (Clinical research ed) 2020; 368: l6983. DOI: 10.1136/bmj.l6983
6. Powers WJ. Acute Ischemic Stroke. The New England journal of medicine 2020; 383: 252-260.
7. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med 2011; 17: 796-808. DOI: 10.1038/nm.2399
8. Jian Z, Liu R, Zhu X et al. The Involvement and Therapy Target of Immune Cells After Ischemic Stroke. Frontiers in immunology 2019; 10: 2167. DOI: 10.3389/fimmu.2019.02167
9. Levine SR. Pathophysiology and therapeutic targets for ischemic stroke. Clin Cardiol 2004; 27: II12-24.
10. De Meyer SF, Denorme F, Langhauser F et al. Thromboinflammation in Stroke Brain Damage. Stroke 2016; 47: 1165-1172. DOI: 10.1161/STROKEAHA.115.011238
11. Stoll G, Nieswandt B. Thrombo-inflammation in acute ischaemic stroke - implications for treatment. Nat Rev Neurol 2019; 15: 473-481. DOI: 10.1038/s41582-019-0221-1
12. Gong T, Liu L, Jiang W et al. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat Rev Immunol 2020; 20: 95-112. DOI: 10.1038/s41577-019-0215-7
13. Gelderblom M, Sobey CG, Kleinschnitz C et al. Danger signals in stroke. Ageing Res Rev 2015; 24: 77-82. DOI: 10.1016/j.arr.2015.07.004
14. Shichita T, Ito M, Yoshimura A. Post-ischemic inflammation regulates neural damage and protection. Frontiers in cellular neuroscience 2014; 8: 319.
15. Wang W, Hu D, Feng Y et al. Paxillin mediates ATP-induced activation of P2X7 receptor and NLRP3 inflammasome. BMC biology 2020; 18: 182. DOI: 10.1186/s12915-020-00918-w
16. Shichita T, Hasegawa E, Kimura A et al. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. Nat Med 2012; 18: 911-917. DOI: 10.1038/nm.2749

17. Liesz A, Dalpke A, Mracsko E et al. DAMP signaling is a key pathway inducing immune modulation after brain injury. J Neurosci 2015; 35: 583-598.
18. Cockram TOJ, Dundee JM, Popescu AS et al. The Phagocytic Code Regulating Phagocytosis of Mammalian Cells. Frontiers in immunology 2021; 12: 629979. DOI: 10.3389/fimmu.2021.629979
19. Schulze J, Zierath D, Tanzi P et al. Severe stroke induces long-lasting alterations of high-mobility group box 1. Stroke 2013; 44: 246-248. DOI: 10.1161/STROKEAHA.112.676072
20. Nishibori M, Wang D, Ousaka D et al. High Mobility Group Box-1 and Blood-Brain Barrier Disruption. Cells 2020; 9.
21. Schuhmann MK, Kollikowski AM, März AG et al. Danger-associated molecular patterns are locally released during occlusion in hyper-acute stroke. Brain, behavior, & immunity - health 2021; 15: 100270.
22. Ye Y, Zeng Z, Jin T et al. The Role of High Mobility Group Box 1 in Ischemic Stroke. Frontiers in cellular neuroscience 2019; 13: 127. DOI: 10.3389/fncel.2019.00127
23. Kuang X, Wang LF, Yu L et al. Ligustilide ameliorates neuroinflammation and brain injury in focal cerebral ischemia/reperfusion rats: involvement of inhibition of TLR4/peroxiredoxin 6 signaling. Free Radic Biol Med 2014; 71: 165-175. DOI: 10.1016/j.freeradbiomed.2014.03.028
24. Riddell JR, Wang XY, Minderman H et al. Peroxiredoxin 1 stimulates secretion of proinflammatory cytokines by binding to TLR4. Journal of immunology (Baltimore, Md : 1950) 2010; 184: 1022-1030. DOI: 10.4049/jimmunol.0901945
25. Xiong XY, Liu L, Yang QW. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. Prog Neurobiol 2016; 142: 23-44. DOI: 10.1016/j.pneurobio.2016.05.001
26. Jia J, Yang L, Chen Y et al. The Role of Microglial Phagocytosis in Ischemic Stroke. Frontiers in immunology 2021; 12: 790201. DOI: 10.3389/fimmu.2021.790201
27. Leitner GR, Wenzel TJ, Marshall N et al. Targeting toll-like receptor 4 to modulate neuroinflammation in central nervous system disorders. Expert opinion on therapeutic targets 2019; 23: 865-882. DOI: 10.1080/14728222.2019.1676416
28. Neher JJ, Emmrich JV, Fricker M et al. Phagocytosis executes delayed neuronal death after focal brain ischemia. Proc Natl Acad Sci U S A 2013; 110: E4098-4107.
29. Jolivel V, Bicker F, Biname F et al. Perivascular microglia promote blood vessel disintegration in the ischemic penumbra. Acta neuropathologica 2015; 129: 279-295. DOI: 10.1007/s00401-014-1372-1
30. Xu S, Lu J, Shao A et al. Glial Cells: Role of the Immune Response in Ischemic Stroke. Frontiers in immunology 2020; 11: 294. DOI: 10.3389/fimmu.2020.00294
31. Ma Y, Wang J, Wang Y et al. The biphasic function of microglia in ischemic stroke. Prog Neurobiol 2017; 157: 247-272. DOI: 10.1016/j.pneurobio.2016.01.005
32. Szalay G, Martinecz B, Lenart N et al. Microglia protect against brain injury and their selective elimination dysregulates neuronal network activity after stroke. Nature communications 2016; 7: 11499. DOI: 10.1038/ncomms11499
33. Liu Z, Chopp M. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. Prog Neurobiol 2016; 144: 103-120. DOI: 10.1016/j.pneurobio.2015.09.008
34. Liddelow SA, Guttenplan KA, Clarke LE et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 2017; 541: 481-487. DOI: 10.1038/nature21029
35. Mahmoud S, Gharagozloo M, Simard C et al. Astrocytes Maintain Glutamate Homeostasis in the CNS by Controlling the Balance between Glutamate Uptake and Release. Cells 2019; 8. DOI: 10.3390/cells8020184
36. Shen LH, Li Y, Chopp M. Astrocytic endogenous glial cell derived neurotrophic factor production is enhanced by bone marrow stromal cell transplantation in the ischemic boundary zone after stroke in adult rats. Glia 2010; 58: 1074-1081.

37. Guo X, Jiang Q, Tuccitto A et al. The AMPK-PGC-1α signaling axis regulates the astrocyte glutathione system to protect against oxidative and metabolic injury. Neurobiol Dis 2018; 113: 59-69. DOI: 10.1016/j.nbd.2018.02.004
38. Min R, van der Knaap MS. Genetic defects disrupting glial ion and water homeostasis in the brain. Brain Pathol 2018; 28: 372-387. DOI: 10.1111/bpa.12602
39. Langen UH, Ayloo S, Gu C. Development and Cell Biology of the Blood-Brain Barrier. Annual review of cell and developmental biology 2019; 35: 591-613. DOI: 10.1146/annurev-cellbio-100617-062608
40. Shan Y, Tan S, Lin Y et al. The glucagon-like peptide-1 receptor agonist reduces inflammation and blood-brain barrier breakdown in an astrocyte-dependent manner in experimental stroke. J Neuroinflammation 2019; 16: 242. DOI: 10.1186/s12974-019-1638-6
41. Amantea D, Bagetta G, Tassorelli C et al. Identification of distinct cellular pools of interleukin-1beta during the evolution of the neuroinflammatory response induced by transient middle cerebral artery occlusion in the brain of rat. Brain Res 2010; 1313: 259-269. DOI: 10.1016/j.brainres.2009.12.017
42. Stanimirovic D, Satoh K. Inflammatory mediators of cerebral endothelium: a role in ischemic brain inflammation. Brain pathology (Zurich, Switzerland) 2000; 10: 113-126. DOI: 10.1111/j.1750-3639.2000.tb00248.x
43. Engelhardt B, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. Trends Immunol 2012; 33: 579-589. DOI: 10.1016/j.it.2012.07.004
44. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. Journal of neuroimmunology 2007; 184: 53-68. DOI: 10.1016/j.jneuroim.2006.11.014
45. Ma XJ, Cheng JW, Zhang J et al. E-selectin deficiency attenuates brain ischemia in mice. CNS neuroscience & therapeutics 2012; 18: 903-908.
46. Edwards DN, Salmeron K, Lukins DE et al. Integrin alpha5beta1 inhibition by ATN-161 reduces neuroinflammation and is neuroprotective in ischemic stroke. J Cereb Blood Flow Metab 2020; 40: 1695-1708.
47. Liesz A, Zhou W, Mracsko E et al. Inhibition of lymphocyte trafficking shields the brain against deleterious neuroinflammation after stroke. Brain 2011; 134: 704-720.
48. Gelderblom M, Leypoldt F, Steinbach K et al. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. Stroke 2009; 40: 1849-1857. DOI: 10.1161/STROKEAHA.108.534503
49. Ludewig P, Winneberger J, Magnus T. The cerebral endothelial cell as a key regulator of inflammatory processes in sterile inflammation. J Neuroimmunol 2019; 326: 38-44. DOI: 10.1016/j.jneuroim.2018.10.012
50. Wu F, Liu L, Zhou H. Endothelial cell activation in central nervous system inflammation. J Leukoc Biol 2017; 101: 1119-1132. DOI: 10.1189/jlb.3RU0816-352RR
51. Stubbe T, Ebner F, Richter D et al. Regulatory T cells accumulate and proliferate in the ischemic hemisphere for up to 30 days after MCAO. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2013; 33: 37-47. DOI: 10.1038/jcbfm.2012.128
52. Chu HX, Kim HA, Lee S et al. Immune cell infiltration in malignant middle cerebral artery infarction: comparison with transient cerebral ischemia. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2014; 34: 450-459. DOI: 10.1038/jcbfm.2013.217
53. Mracsko E, Liesz A, Stojanovic A et al. Antigen dependently activated cluster of differentiation 8-positive T cells cause perforin-mediated neurotoxicity in experimental stroke. J Neurosci 2014; 34: 16784-16795. DOI: 10.1523/JNEUROSCI.1867-14.2014
54. Filiano AJ, Gadani SP, Kipnis J. How and why do T cells and their derived cytokines affect the injured and healthy brain? Nat Rev Neurosci 2017; 18: 375-384. DOI: 10.1038/nrn.2017.39
55. Gu L, Xiong X, Zhang H et al. Distinctive effects of T cell subsets in neuronal injury induced by cocultured splenocytes in vitro and by in vivo stroke in mice. Stroke 2012; 43: 1941-1946. DOI: 10.1161/strokeaha.112.656611
56. Lei TY, Ye YZ, Zhu XQ et al. The immune response of T cells and therapeutic targets related to regulating the

levels of T helper cells after ischaemic stroke. J Neuroinflammation 2021; 18: 25. DOI: 10.1186/s12974-020-02057-z

57. Liesz A, Suri-Payer E, Veltkamp C et al. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. Nat Med 2009; 15: 192-199. DOI: 10.1038/nm.1927

58. Liesz A, Hu X, Kleinschnitz C et al. Functional role of regulatory lymphocytes in stroke: facts and controversies. Stroke 2015; 46: 1422-1430. DOI: 10.1161/STROKEAHA.114.008608

59. Shi L, Sun Z, Su W et al. Treg cell-derived osteopontin promotes microglia-mediated white matter repair after ischemic stroke. Immunity 2021; 54: 1527-1542 e1528. DOI: 10.1016/j.immuni.2021.04.022

60. Offner H, Subramanian S, Parker SM et al. Experimental stroke induces massive, rapid activation of the peripheral immune system. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2006; 26: 654-665. DOI: 10.1038/sj.jcbfm.9600217

61. Courties G, Herisson F, Sager HB et al. Ischemic stroke activates hematopoietic bone marrow stem cells. Circulation research 2015; 116: 407-417.

62. Denes A, McColl BW, Leow-Dyke SF et al. Experimental stroke-induced changes in the bone marrow reveal complex regulation of leukocyte responses. J Cereb Blood Flow Metab 2011; 31: 1036-1050. DOI: 10.1038/jcbfm.2010.198

63. Haeusler KG, Schmidt WU, Foehring F et al. Immune responses after acute ischemic stroke or myocardial infarction. Int J Cardiol 2012; 155: 372-377. DOI: 10.1016/j.ijcard.2010.10.053

64. Liu Q, Jin WN, Liu Y et al. Brain Ischemia Suppresses Immunity in the Periphery and Brain via Different Neurogenic Innervations. Immunity 2017; 46: 474-487. DOI: 10.1016/j.immuni.2017.02.015

65. Shim R, Wong CHY. Complex interplay of multiple biological systems that contribute to post-stroke infections. Brain Behav Immun 2018; 70: 10-20.

66. Roth S, Cao J, Singh V et al. Post-injury immunosuppression and secondary infections are caused by an AIM2 inflammasome-driven signaling cascade. Immunity 2021; 54: 648-659 e648. DOI: 10.1016/j.immuni.2021.02.004

67. Boehme AK, McClure LA, Zhang Y et al. Inflammatory Markers and Outcomes After Lacunar Stroke: Levels of Inflammatory Markers in Treatment of Stroke Study. Stroke 2016; 47: 659-667. DOI: 10.1161/STROKEAHA.115.012166

68. Narasimhalu K, Lee J, Leong YL et al. Inflammatory markers and their association with post stroke cognitive decline. International journal of stroke : official journal of the International Stroke Society 2015; 10: 513-518. DOI: 10.1111/ijs.12001

69. Kliper E, Bashat DB, Bornstein NM et al. Cognitive decline after stroke: relation to inflammatory biomarkers and hippocampal volume. Stroke 2013; 44: 1433-1435. DOI: 10.1161/STROKEAHA.111.000536

70. Li S, Huang Y, Liu Y et al. Change and predictive ability of circulating immunoregulatory lymphocytes in long-term outcomes of acute ischemic stroke. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2021; 41: 2280-2294. DOI: 10.1177/0271678X21995694

71. Neumann J, Riek-Burchardt M, Herz J et al. Very-late-antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke. Acta neuropathologica 2015; 129: 259-277. DOI: 10.1007/s00401-014-1355-2

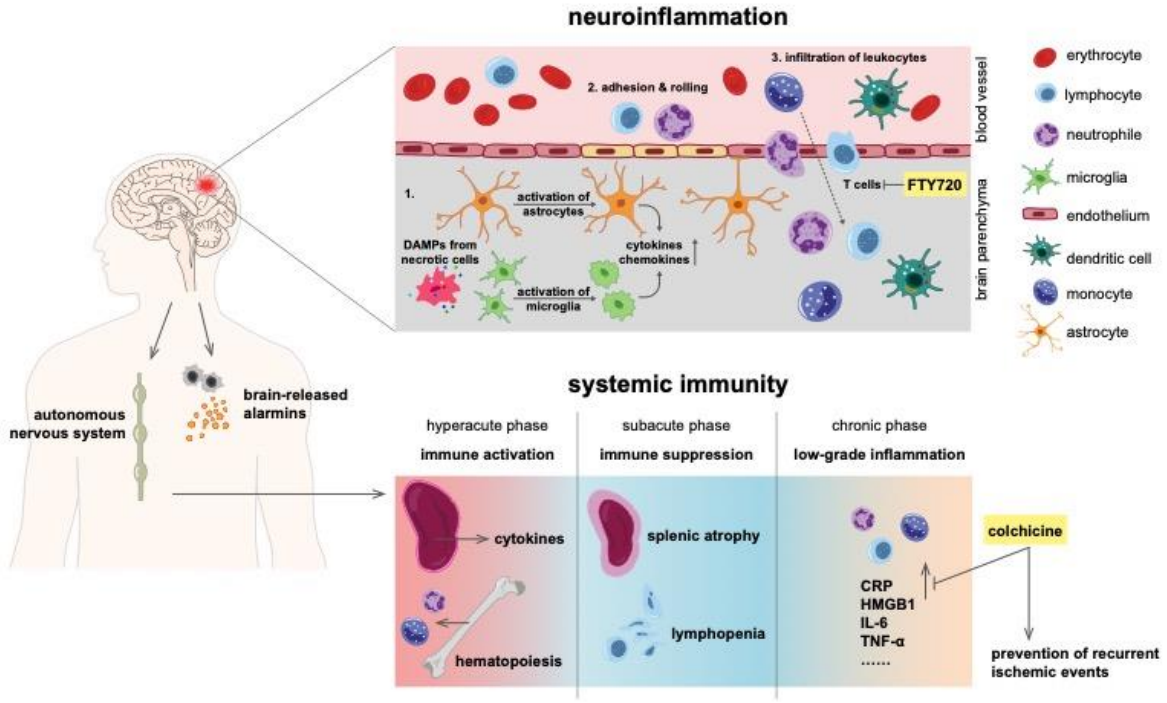
72. Wei Y, Yemisci M, Kim HH et al. Fingolimod provides long-term protection in rodent models of cerebral ischemia. Ann Neurol 2011; 69: 119-129. DOI: 10.1002/ana.22186

73. Qin C, Fan WH, Liu Q et al. Fingolimod Protects Against Ischemic White Matter Damage by Modulating Microglia Toward M2 Polarization via STAT3 Pathway. Stroke 2017; 48: 3336-3346. DOI: 10.1161/STROKEAHA.117.018505

74. Zhu Z, Fu Y, Tian D et al. Combination of the Immune Modulator Fingolimod With Alteplase in Acute Ischemic Stroke: A Pilot Trial. Circulation 2015; 132: 1104-1112. DOI: 10.1161/CIRCULATIONAHA.115.016371

75. Fu Y, Zhang N, Ren L et al. Impact of an immune modulator fingolimod on acute ischemic stroke. Proc Natl Acad Sci U S A 2014; 111: 18315-18320. DOI: 10.1073/pnas.1416166111

76. Zhang S, Zhou Y, Zhang R et al. Rationale and design of combination of an immune modulator Fingolimod with Alteplase bridging with Mechanical Thrombectomy in Acute Ischemic Stroke (FAMTAIS) trial. International journal of stroke : official journal of the International Stroke Society 2017; 12: 906-909. DOI: 10.1177/1747493017710340
77. Krams M, Lees KR, Hacke W et al. Acute Stroke Therapy by Inhibition of Neutrophils (ASTIN): an adaptive dose-response study of UK-279,276 in acute ischemic stroke. Stroke 2003; 34: 2543-2548.
78. Amiri-Nikpour MR, Nazarbaghi S, Hamdi-Holasou M et al. An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: gender-dependent effect. Acta neurologica Scandinavica 2015; 131: 45-50.
79. Elkind MSV, Veltkamp R, Montaner J et al. Natalizumab in acute ischemic stroke (ACTION II): A randomized, placebo-controlled trial. Neurology 2020; 95: e1091-e1104.
80. Amarenco P, Lavallée PC, Labreuche J et al. One-Year Risk of Stroke after Transient Ischemic Attack or Minor Stroke. The New England journal of medicine 2016; 374: 1533-1542. DOI: 10.1056/NEJMoa1412981
81. Zhang Y, Guan Y, Zhang Y et al. Recurrence Rate and Relevant Associated Factors of Stroke among Patients with Small Artery Occlusion in Northern China. Scientific reports 2019; 9: 2834. DOI: 10.1038/s41598-019-39207-0
82. Rücker V, Heuschmann PU, O'Flaherty M et al. Twenty-Year Time Trends in Long-Term Case-Fatality and Recurrence Rates After Ischemic Stroke Stratified by Etiology. Stroke 2020; 51: 2778-2785. DOI: 10.1161/strokeaha.120.029972
83. Pennlert J, Eriksson M, Carlberg B et al. Long-term risk and predictors of recurrent stroke beyond the acute phase. Stroke 2014; 45: 1839-1841. DOI: 10.1161/strokeaha.114.005060
84. Amarenco P, Lavallée PC, Monteiro Tavares L et al. Five-Year Risk of Stroke after TIA or Minor Ischemic Stroke. The New England journal of medicine 2018; 378: 2182-2190.
85. Lovett JK, Coull AJ, Rothwell PM. Early risk of recurrence by subtype of ischemic stroke in population-based incidence studies. Neurology 2004; 62: 569-573. DOI: 10.1212/01.wnl.0000110311.09970.83
86. Kelly PJ, Lemmens R, Tsivgoulis G. Inflammation and Stroke Risk: A New Target for Prevention. Stroke 2021; 52: 2697-2706. DOI: 10.1161/STROKEAHA.121.034388
87. Roth S, Singh V, Tiedt S et al. Brain-released alarmins and stress response synergize in accelerating atherosclerosis progression after stroke. Science translational medicine 2018; 10. DOI: 10.1126/scitranslmed.aao1313
88. Katsanos AH, Palaiodimou L, Price C et al. Colchicine for stroke prevention in patients with coronary artery disease: a systematic review and meta-analysis. European journal of neurology 2020; 27: 1035-1038. DOI: 10.1111/ene.14198
89. Masson W, Lobo M, Molinero G et al. Role of Colchicine in Stroke Prevention: An Updated Meta-Analysis. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association 2020; 29: 104756. DOI: 10.1016/j.jstrokecerebrovasdis.2020.104756
90. Stack J, Ryan J, McCarthy G. Colchicine: New Insights to an Old Drug. American journal of therapeutics 2015; 22: e151-157. DOI: 10.1097/01.mjt.0000433937.07244.e1
91. Leung YY, Yao Hui LL, Kraus VB. Colchicine--Update on mechanisms of action and therapeutic uses. Seminars in arthritis and rheumatism 2015; 45: 341-350. DOI: 10.1016/j.semarthrit.2015.06.013
92. Kelly P, Weimar C, Lemmens R et al. Colchicine for prevention of vascular inflammation in Non-CardioEmbolic stroke (CONVINCE) - study protocol for a randomised controlled trial. European stroke journal 2021; 6: 222-228. DOI: 10.1177/2396987320972566


neuroinflammation
3. infiltration of leukocytes
2. adhesion & rolling
blood vessel
1.
activation of astrocytes
T cells
FTY720
DAMPs from necrotic cells
cytokines chemokines
activation of microglia
brain parenchyma
erythrocyte
lymphocyte
neutrophile
microglia
endothelium
dendritic cell
monocyte
astrocyte
autonomous nervous system
brain-released alarmins
systemic immunity
hyperacute phase
immune activation
subacute phase
immune suppression
chronic phase
low-grade inflammation
cytokines
splenic atrophy
colchicine
CRP
HMGB1
IL-6
TNF-α
.....
lymphopenia
hematopoiesis
prevention of recurrent ischemic events

1