# Immunology of Stroke

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## Abstract

Stroke, a multifactorial disease, has distinct pathophysiologic mechanisms, among which inflammation plays a pivotal role. Various types of inflammatory cells, substances, and molecules emerge in the ischemic stroke. Neutrophils, Tcell subtypes, macrophages, microglial cells, dendritic cell, mast cells, asrocytes, as influential cell, tumor necrosis factor_α, interleukin-17, interleukin-10, as released substances, and vascular cell adhesion molecule-1 (VCAM-1), leukocyte very late antigen-4 (VLA-4), and glial fibrillary acidic protein (GFAP), as cellular adhesion molecules. Lymphocytes' invasion to the ischemic brain tissue occurs as the result of VLA-4 −VCAM-1 interaction. Regarding Tcell subtypes, CD4+ cells have known detrimental effects in the ischemic area, while natural killer T cells (NKT cells) and γδ T cells have minor importance in the early stage of ischemia. While some studies proved the cerebroprotective impact of T regulatory cells, others refuted this by presenting a prominent harmful role of them. Bcells have important protective function by releasing IL-10. Neutrophils along with microglial cell, appearing as the first inflammatory cell in the ischemic tissue, and also macrophages deteriorate ischemia. Mast cells and dendritic cells are of great value in stroke progression. The resting astrocytes are neuroprotective, whereas the activated ones present detrimental function in the ischemic region by expression of GFAP. Hence, stroke consequences occur as the result of systemic inflammatory response. The more activation of this system, the poorer neurological outcomes would be observed. As expected, anti-inflammatory interventions in the experimental stroke in animals, have revealed successful results as less infarct size and attenuated neurological damages.**[GMJ.2016;5(Supp.1):10-17]**

**Keywords:** Stroke; Immunity;Immune System; Immune Response; Cerebral Ischemia.

## Introduction

Stroke is a multifactorial disease which has different kinds of mechanisms bringing about its pathogenesis. Among these factors, inflammation plays an important role, especially in the pathophysiology of ischemic stroke and other forms of ischemic brain damage. When the brain reacts to ischemic injury through an acute and prolonged inflammatory process, multiple events occur such as infiltration of various types of inflammatory cells (including neutrophils, different subtypes of T cells, monocytes/macrophages, and other cells), rapid activation of resident cells (mainly microglia), and production of proinflammatory mediators including tumor necrosis factor α and interleukin-1 , in micro vessels

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and ischemic cerebral parenchyma and cause ischemic brain injury [1] .

When blood perfusion to the brain stops, the ischemic neurons become energy deprived and necrosis happens. This event potentiates immune system activation causing accumulation of inflammatory cells in the vicinity of ischemic region. By happening of reperfusion after blood flow cessation, which can be due to the contribution of collateral vessels, or spontaneous or therapeutic recanalization, reactive oxygen species (ROS) would be produced as a result of inflammatory cell activity or receiving oxygenated blood again. This process leads ischemic cells to secrete inflammatory cytokines and chemokines causing at first adhesion molecule over-expression and then in result infiltration of immune cells. Therefore the exponential release of different detrimental agents such as more cytokines, matrix metalloproteinases (MMPs), nitric oxide (NO) and even more ROS will be occurred. Thus, the consequences are blood-brain barrier (BBB), extracellular matrix, and more cellular destruction. Damage to BBB integrity facilitates more cytotoxic substances and agents invasion to ischemic brain tissue and consequently secondary brain injury. Afterwards, hemodynamic modulation, post-ischemic microvascular stasis and brain edema happens which ultimately leading to hypoperfusion and post-ischemic injury and inflammation [2].

There is another inflammatory response happening simultaneously, that is activation of intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM) which are responsible for adhesion of leukocytes to the activated endothelium and therefore the migration of activated T cells across the blood–brain barrier and subsequent brain invasion after cerebral ischemia [1,3].

Stroke patients and the their subsequent prognosis are influenced by systemic inflammatory response [4,5], and the ones with systemic inflammation exhibit clinically poor neurologically outcomes [6,7,8]. Meanwhile, studies showed inhibiting the inflammatory response, leads to less infarct size and attenuates neurological damage in experimental stroke [9,10]. As expected, anti-inflammatory

interventions have shown success in animal models [1,11,12,13].

According to previous studies, there is much emphasis on the effect of immune cells in post stroke inflammation and secondary brain injury:

CD4+ and CD8+ T cells take part in the inflammatory and thrombogenic responses, brain infarction, and neurological deficit associated with experimental stroke [14]. Other papers stated that other cell types such as dendritic cells, natural killer cells, mast cells, neutrophils, macrophages and microglia also have immunologic impacts [15].

Post stroke inflammation and secondary brain injury by means of different types of immune cells [16]:

**Lymphocytes**

On Day 3 post stroke, the same amount of both types of lymphocytes (Tcell and Bcell) in brain tissue can be diagnosed.

By Day 3, every six lymphocytes found ipsilateral to the lesion, had a CD4/CD8 phenotype's cells encompass 2 major subgroups ,defined by the expression of the CD4 or CD8 molecule.

There was no significant increase of lymphocytes contralateral to the lesion [1]. Careful investigations made clear that in lymphocyte subsets, T cells, but not B cells, have harmful impact during stroke, whereas underlying molecular mechanisms are unknown [1,16].

Cerebral invasion of lymphocytes doesn't occur unless the important interaction of the leukocyte very late antigen-4 (VLA-4) with vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells happens. VLA-4 (integrin alpha4beta1) is an integrin heterodimer consisting of an alpha chain (integrin). One report implies the key underlying mechanism in reduction of lymphocyte-mediated cytotoxicity on secondary tissue injury after experimental brain ischemia is inhibition of the VLA-4–VCAM-1 axis [17].

**T Cells**

After reaching 24 hours of reperfusion, T cells have been detected in the post ischemic brain. Abs (Antibodies), directed against vascular adhesion receptors expressed on the

brain endothelium or leukocyte very late antigen-4 (VLA-4) expressed on lymphocytes, inhibited T-cell transmigration and reduced tissue damage in models of stroke [18]. Due to a state of protection seen in T cell-deficient mice after ischemic stroke, which was not related to adaptive immune pathways, and due to the emerging thrombus in RAG1_/_ mice, for elucidating the harmful impact of T cell in stroke other mechanisms should be taken into consideration. The vessel endothelium of brain participates in these processes with rapid over-expression of some adhesion receptors, including lymphocyte function antigen-1 (LFA-1), macrophage antigen-1 (Mac-1), and very late antigen-4 (VLA-4) which are attachment elements of detrimental T cell activity leading to microvascular dysfunction, impaired capillary reperfusion, and worse outcome in the vicinity of ischemic stroke [17].

The effects of different T-cell subtypes in stroke pathophysiology is less obvious, nevertheless the detrimental role of T cells in stroke progression is well known. According to adoptive cell transfer in Rag1−/− mice, CD4+ subtype of T cells is an important part of the inflammatory axis of stroke. It has been demonstrated that two other subtypes of T cells, naming natural killer T cells (NKT cells) and γδ T cells do not cause early ischemic damage after transient middle cerebral artery occlusion (tMCAO) in the brain tissue [17,23]. One recent study stated that there is a low possibility of antigen processing and MHC presentation for peptide epitopes for both NKT cells and γδ T cells. Meanwhile, a study introduced an interestingly new part for γδ T cells during the late stage of infarct progression by means of IL-17 secretion. This study was in line with previous ones about the roles of NKT cell and γδ T cells in early stages of stroke development which are of minor importance [17]. Regulatory T cells (Tregs) and conventional T helper cells are two distinguished subtypes among the population of CD4+ T cells.

**Tregulators**
There are two markers that help us to identify Regulatory T cells (Tregs); the one is CD25 and the other is intracellular transcription factor Forehead box P3 (FoxP3). Tregs are usually prototypic of anti-inflammatory cells that limit antigen-specific immune diseases. Although Tregs have been shown to be beneficial in inflammatory diseases of the CNS. Their pathologic role in ischemic stroke, which is regarded a primary non immunologic disease, should be more discussed. One study declared Tregs as crucial part of non immunologic disease state: ischemic neurodegeneration. Results of their survey showed within 24 hours after transient MCAO, frequencies of expressions of FoxP3 by Tregs in the brain among the total number of CD4+ T cells was more than two times in comparison with sham operated mice and moreover it increased until day 3 [19].

More lately, an admirable study showed significant cerebroprotective immunomodulating role of the Treg cells in acute experimental stroke in mice [18]. The fact about Tregs activity revealed was prevention of secondary infarct growth by acting against over-production of proinflammatory cytokines and modulating lymphocytes invasion and/or activation in the ischemic brain. In the absence of Treg cells obviously delayed brain damage and poor functional outcome were determined. Antagonization of enhanced TNF-α and IFN-γ production, which are responsible for delayed inflammatory brain damage, is another important effect of Treg cells, which is done through Treg-derived secretion of IL-10, the key mediator of cerebroprotection.

Depletion of Treg cells contributed to post ischemic activation of resident and infiltrating inflammatory cells including T cells and microglia, the main sources of cerebral IFN-γ and TNF-α, respectively. TNF-α expression is elevated early after ischemia in the brain, whereas IFN-γ, which is almost absent in normal brain tissue, its expression increases at a later time-point after cerebral ischemia. Considering these facts about a previously unknown role of the Treg cells as cerebroprotective immunomodulators, new insights into the endogenous adaptive immune response after acute brain ischemia have been provided [15]. In contrast to results above, a recent survey demonstrates for the first time that Tregs are prominent detrimental mediators of acute

ischemic stroke. They claimed the deleterious effects of Tregs in the setting of both severe (ie, 60 minutes of tMCAO) and mild (ie, 30 minutes of tMCAO) experimental stroke as well as presence of their harmful behavior in later stages of stroke evolution were observed. It was shown that in the absence of these cells stroke volume after 24 hours was significantly reduced. To compare with resting Tcells, Tregs have more potential and tendency to interact with brain activated endothelium leading to microvascular dysfunction and higher thrombus formation, which are contributed to disruption of cerebral reperfusion after tMCAO [19].

Meanwhile, they declared that immunologic activities of Tregs seem to be not significant in stroke development. According to the literature, Tregs function in experimental ischemic/reperfusion conditions in other organs than brain, such as heart, liver, and kidney, can be obviously presented as an anti-inflammatory activity in the early stages and in ischemic tissue repair in the later stages of ischemia [19]. As described above, every study so far has demonstrated that Tregs have beneficial effects in the ischemic organ systems other than the brain, which is vice versa according to the interestingly novel finding of this paper. They were unable to elucidate exact pathologic mechanisms responsible for this difference, but postulated that it might be due to a specific inflammatory response and the structural characteristics of the blood-brain barrier [19].

**B Cells**
One paper, showing a novel result, stated that B cells have vital protective and beneficial functions in stroke growth. It is demonstrated that B cells attenuate not only infarct progression, but also subsequent neurological damage through inhibition of accumulation of inflammatory cells, including T cells, macrophages and microglia into the ischemic region. As expected, B cell deficient experimental models of mice showed significant infarct progression and neurological damage. They exhibited their novel finding as happening via secretion of IL10 from B cell not Tregs, which in previous studies has received much attention [16,20].

**CD4+ and CD8+ T Cells**
It has been demonstrated that CD4+ and CD8+ T cells contribute to the inflammatory and thrombogenic responses, brain infarction, and neurological deficit associated with experimental stroke [17]. Interestingly , experimental stroke models with depletion of CD4+ cells and CD8+ cells comparing with each other, both exhibited approximately similar less infarct size and cerebral injury, suggesting that there is additional cytotoxic mechanism of CD8+ cells, which could be perforin-mediated. As far as we know, perforin is released via NKT cells as well. To investigate this mechanism in NKT cells, experiment of ischemic stroke was performed in NKT cell-deficient mice. The results showed no major influence on the infarct size and cerebral tissue injury in models of permanent or reversible MCAO [21].

Experimental studies have illustrated that CD4+ T helper 1 (TH1) cells may have a detrimental key role in the pathogenesis of stroke via releasing proinflammatory cytokines, including IL-2, IL-12, IFN-γ, and TNF-α, whereas CD4+ TH2 cells may have a protective role via secretion of anti-inflammatory cytokines such as IL-4, IL-5, IL-10, and IL-13 [17].Some of these cytokines, especially IFN-γ play a crucial role in inhibition of infections, which are a leading cause of death in stroke patients, especially in the post-acute phase of stroke. These infections are mainly due to immuno-depression caused by depletion of circulating T cell and NK cell populations so the antibacterial cytokine IFN-γ in the early reperfusion period take important part and assist the immune system [22,23].

Regarding above data, specific T cells both have protective and harmful actions. Therefore, targeting T cells as a treatment plan must be designed carefully, in regards to when to enhance and when to reduce their actions [15].

**Neutrophils, Macrophages, and Microglia**
Neutrophils, among the various types of leukocytes, are the first to infiltrate ischemic brain (30 min to a few hours of focal cerebral ischemia), peak earlier (Days 1–3), and then disappear or decrease rapidly with time. The infiltrating neutrophils may continue to be

there for more than 3 days after focal cerebral Ischemia/Reperfusion, But under shadow of vast aggravation of activated microglia/macrophages in the inflammatory site after three days, their existence may not be seen [15]. In the rat model of endothelin-1-induced cerebral ischemia, Weston et al observed that neutrophil infiltrates into the brain more at day 1, by having peaks at 3 days, and in spite of decreasing, continues through 7 and 15 days after cerebral ischemia.

From another study's result, it has been observed that small proportion of neutrophils gathered after 12 and 24 hours, whereas their number became way too much at day 3 post reperfusion. Afterwards, significant reduction by day 7 in the ischemic brain area was disclosed. Unexpectedly, by reaching the day 3, the neutrophil amount was much less than macrophages and microglia. The latter 2 started to appear already after 12 hours of ischemia and were greatly increased at 24 hours [6]. It can be suggested that neutrophils and microglia in the infarcted region have similar amount at the peak of inflammation on days 3 and 7. Macrophages accounted for the majority of the remaining cells on day 3 (63%).

More lately, one study remarked that neutrophils' trafficking into the vicinity of ischemic stroke is a subsequent result of microglial cell recruitments and to a lesser degree macrophage, lymphocytes, and DCs. It is stated that activation and infiltration of these inflammatory cells cause to secrete different cytokines such as IL1, IL6, and TNFα from themselves and therefore leading to over-expression of cell adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin. As a consequence of these events, neutrophils recruitments and invasion to the ischemic region can be observed. Noticeably, the consequent neurological damage and tissue injury depend on the severity of neutrophils accumulation, which was in parallel with other studies results [1].

Despite one study failed to show major improvements in neurological outcome and cerebral tissue disruption in the experimental models of neutrophil-deficient mice, many other documents, unravel that inhibition of neutrophil accumulation in the ischemic area directly or with blocking adhesion cell molecules, which are responsible for neutrophil invasion and recruitment, could greatly decrease infarct volume and stroke evolution, thereby remarkable neurological outcome [1,2].

To our knowledge, there are no specific cell markers to distinguish local microglial cells from invading macrophages. Therefore, evaluation of their functional characteristics, whether acting in a similar way or having opposite behavior was faced with a problem. One study found a way to investigate their functional properties in stroke development; the use of MCP-1-deficient mice, an obligatory element of macrophage invasion, showed an obvious reduction of stroke size. In contrast, depletion of microglial cells growth factor CSF-1 in experimental models of mice, demonstrated great increase in the infracted region. Thereafter, cerebroprotective role of microglial cells have proved. Meanwhile, other studies imply that microglial cells, with secretion of the high degree amount of detrimental cytokines and toxic materials in a sustained state of action, play a pivotal role in secondary tissue damage and neurodegeneration disorders like Alzheimer's disease[24,25].

Furthermore, there are other surveys emphasizing on the detrimental effects of microglial cells in the ischemic stroke. They showed their results through various ways: one stated that following ischemia, microglial cells activated via CD14 and toll-like receptor4 (TLR4), however did not elucidate the underlying pathologic mechanisms responsible for activation of these cells due to ischemia. Another study, indicated a novel free radical scavenger naming edaravone that could attenuate microglial cell activation, resulting in smaller stroke size and better neurological outcome. The other study, conducted in hypertensive mice with permanent MCAO, showed treatment with hyperbaric oxygen (HBO) could reduce infarct progression via suppressing of microglial cells function. While the other one utilized minocycline, a member of tetracycline family, which brings about a remarkable protection against ischemia by prevention of microglial cells recruitments [2].

There is no report of significant increase of

microglia, neutrophils, or macrophages in the contralesional region[1].

## Mastcells

Experimental evidence introduces a prominant role of mast cells in cerebral ischemic and hemorrhagic injury. Mast cells (MCs) modulate blood-brain barrier (BBB) permeability, the severity of local neutrophil infiltration, and the formation of brain swelling, In the experimental cerebral ischemic/reperfusion (I/R). The evidence above states that cerebral MCs regulate early ischemic brain edema and neutrophil aggravation in a rat model of transient MCAO [1].

## Dendritic Cells

According to prevoius studies, there is a 20-fold increase in Dendritic cells within 3days and 12-fold increase within 7days in the experimental stroke models and dramatic fall in the number of dendritic precursor cell (DPCs) in blood of stroke patients within the first 24 hours of symptom initiation. The greater the infarct volume is, the greater decrease in DCPs could be occurred [1].

It is stated as well that DCs are present in non-ischemic (sham) brains, but are of low number; while their population rise within one hour of ischemia and exponentially increases till day 6. It can be detected that activated DCs present major histocompatibility complex II (MHC II) which is up-regulated during ischemic injury and if DCs with over-expression of MHC II taking into consideration specifically the increase of their proportion would be markedly higher (100-fold) in ischemic region. So far, direct effect of the DCs increase on stroke size and neurologic deficit has not been completely clarified.

DCs role in experimental ischemic stroke models declared as a key mediator in post-injury activated immune cascade. It was observed that DCs not only over-expressed MHC II but also CD80 but to a much less extent. They claimed DCs have similar amounts of up-regulation of CD80 but not MHC II. They postulated that there should be 2 different groups of DCs based on the degree of MHC II up-regulation, so it might be concluded that these 2 subgroups of DCs might have different immunologic functions in the vicinity of ischemia and may elucidate a clue to explain the correlation of stroke event with sustained deleterious local autoimmune response [1].

## Natural Killer (NK) Cells

Studies in the quantification of NK cells (NK, CD45/NK1.1) demonstrated a remarkable rise in cell numbers of regulatory NKT cells at day 3, but no major changes for NK cells[1].

According to previous studies, it is offered that NKT cells play 2 different roles, one as regulatory cells in autoimmune disease like multiple sclerosis and the other deteriorate post ischemic inflammation [1].

## Astrocytes

Astrocytes participate in the inflammatory process via different pathologic mechanism such as releasing cytokines, chemokines, and over-expression of specific molecules on its surface just like other classic immune cells. The entity "reactive gliosis" is a unique structural and functional change due to activation of astrocytes mediated with astrocyte-expression of glial fibrillary acidic protein (GFAP). It is implied that astrocytes present MHC and other co-stimulatory elements and perhaps leading to TH2 activation and suppression of IL12. Within 10 minutes of ischemia, activated astrocytes of hippocampus release an inflammatory substance, calling inducible nitric oxide synthase (iNOS), which cannot be seen in the resting astrocytes of hippocampal region. This substance is capable of causing ischemic-like neurological damage.

Tumor necrosis factor like weak inducer of apoptosis (TWEAK) is a member of TNF superfamily, which is secreted from neurons, astrocytes, and endothelial cells and can interact with its receptor on astrocytes surface naming Fn14 leading to inflammatory responses. This event was proved when blocking Fn14 receptor on astrocytes, resulting in a notable decrease in the stroke volume. Therefore, from documented data above, while resting asrocytes are indeed obligatory for proper neuron activity, the activated ones have a detrimental function in the cerebral ischemic region [2].

### Conclusion

Stroke is a multifactorial disease with various pathophysiologic mechanisms among which, inflammatory response has garnered great attention obviously. The stroke consequences and the neurological damages could be rooted to activation of the inflammatory and immune response. Many different inflammatory cells, substances, and molecules, have proven to act in the ischemic region with their detrimental impacts ,while activation and infiltration of minority of them have established to be beneficial. Thus, in order to improve stroke prognosis, distinct strategies and measurements ,which could be naming as attenuation and inhibition of detrimental inflammatory factors and enhancing the effects of neuroprotective ones, should be implicate wisely.

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