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Molecular and Cellular Immune Responses to Ischemic Brain Injury

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Abstract

Despite extensive research into stroke pathology, there have not been any major recent advancements in stroke therapeutics. Animal models of cerebral ischemia and clinical data have been used to investigate the progressive neural injury that occurs after an initial ischemic insult. This has lead researchers to focus more on the peripheral immune response that is generated as a result of cerebral ischemia. The therapies that have been developed as a result of this research thus far have proven ineffective in clinical trials. The failure of these therapeutics in clinical trials is thought to be due to the broad immunosuppression elicited as a result of the treatments and the cerebral ischemia itself. Emerging evidence indicates a more selective modulation of the immune system following stroke could be beneficial. The spleen has been shown to exacerbate neural injury following experimental stroke and would provide a strong therapeutic target. Selecting facets of the immune system to target would allow the protective and regenerative properties of the immune response to remain intact while blunting the pro-inflammatory response generated towards the injured brain.

Keywords

spleen; interferon gamma; stroke; T cells

Introduction

One of the emerging targets for stroke therapeutics is the inflammatory response originating from the spleen after ischemic injury. However, the exact signaling mechanism from the spleen that contributes to the increased neural injury following stroke is unknown. Molecular and cellular targets have been studied following stroke and none of these studies have yielded results which have aided in the development of new stroke therapies. The various factors in determining the specific cell types and cytokines/chemokines to target, as well as the timing at which they should be targeted, have played a major role in the inability to translate preclinical research into effective stroke therapies to date.

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The Initial Cascade of Neural Death following Stroke

Ischemia produces a hypoxic and glucose deprived environment that leads to cellular dysfunction and cell death through necrosis or apoptosis. In an attempt to keep up with the high energy demands in the brain, neural cells switch to anaerobic cellular respiration. Cell membranes become damaged from the resulting build up of reactive oxygen and nitrogen free radicals, which leads to cellular edema and necrosis. Additionally, as neurons and astrocytes die, there is a release of glutamate that compromises more neurons through glutamate excitotoxicity. The activation of glutamate receptors leads to excessive intracellular calcium release, edema, and caspase activation, which results in apoptosis [1]. All of these mechanisms lead to early cell death in the core of the infarct, the area directly perfused by the occluded artery. These events occur within the first few hours of stroke and targeting the molecules in this early phase has proven impractical for therapeutic intervention.

However, there is a delayed cellular death that occurs several hours to days after stroke. The initial activation of matrix metalloproteinases (MMPs) opens the blood-brain-barrier (BBB) transiently by MMP-2. Later, at 48 h post MCAO, up regulation of MMP-9 leads to a prolonged disruption of the BBB [2]. This break down in the BBB allows neural antigens into the peripheral circulation. The leaky BBB contributes to enhanced neural injury by increasing edema, as intracranial pressure builds from the influx of excess fluid. This BBB dysfunction also allows the peripheral immune system to come in contact with these neural antigens and generate an immune response to the brain, which results in further injury.

The Role of the Spleen in Ischemic Injury

The spleen is secondary immune organ that stores both red and white blood cells. Recent studies have indicated this organ is an active participant in the body's immune reaction to ischemic injury. In 1996, the first study reporting the splenic immune response was associated with increased tissue damage following liver ischemia [3], but has since been reported in the pathophysiology of stroke [4–6]. This review delves into the cellular and molecular actions of the spleen, in concert with other immune organs, reacting to ischemic injury to the brain.

The spleen is a highly vascularized secondary peripheral lymphoid organ and is organized into the white pulp and the red pulp. The white pulp consists of T cell zones, or periarteriolar lymphoid sheaths, and B cell follicles; while the red pulp contains B cells, natural killer cells (NK cells), and monocytes/macrophages which are in close proximity to the vasculature. This allows monocytes/macrophages to filter the blood for dying red blood cells, hemoglobin, and antibody covered bacterial pathogens. Plasma cells, or antibody producing B cells, are the specific type of B cells found in the red pulp. This location in the spleen allows for rapid delivery of antibodies into circulation [7]. NK cells in the red pulp resemble NK cells found in circulation [8]. The white pulp is split into two areas: one for T cells and another for B cells. The T cell zones allow naïve T cells to be in close proximity to the arteriole blood supply. In addition to T cells, dendritic cells are present in the T cell zones to

present naïve T cells with antigens the dendritic cells find in the blood. Once activated, T cells leave the spleen and initiate an immune response [7].

The splenic capsule is made up of smooth muscles that express α_1 adrenergic receptors. Activation of the α_1 receptors leads to contraction of the smooth muscles and a subsequent decrease in spleen size. In addition to being an immune cell reservoir, the spleen is also reservoir for red blood cells. During times of physical stress, the spleen has been shown to contract, releasing red and white blood cells into systemic circulation [9, 10]. The spleen is the largest reservoir of undifferentiated tissue-nonspecific monocytes and contains half of the monocytic cell population. These cells have been shown to be released following myocardial infarction (MI) and have detrimental effects on the damaged tissue, as well as preventing tissue healing [11].

As stated previously, the spleen generates a pro-inflammatory response to various ischemic injuries. Splenectomy is protective in other ischemic injuries including the liver, intestines, kidneys, and heart. The initial report of cellular protection following splenectomy showed that removal of the spleen immediately prior to ischemic/reperfusion (IR) injury of the liver reduced the infiltration of polymorphonuclear cells into the liver following IR [3]. A later study found hepatic IR was also associated with injury to other organs including the kidneys, lungs, and intestines. Splenectomy reduced cell apoptosis and caspase 3 signaling in all four organs [12].

Intestinal IR has been linked to acute lung injury. Kupffer cells in the liver become inflammatory in response to intestinal IR. Blocking Kupffer cell activation using gadolinium chloride or splenectomy prior to intestinal IR resulted in significantly decreased injury to the lungs [13]. Renal IR also induces an inflammatory phenotype in Kupffer cells. Again, the gadolinium chloride or splenectomy decreased histopathological changes caused by renal IR [14]. The intestinal and renal IR studies both concluded that splenectomy was protective due to the removal of a large reservoir of monocytes. Monocytes have also been found to play an important role in exacerbating injury following MI. Sequestering splenic monocytes in the spleen with enalapril, an angiotensin-converting enzyme (ACE) inhibitor, or their removal via splenectomy prior to experimental MI, decreased inflammation and infarct size [15].

Monocytes have been shown to play a detrimental role in ischemic pathology in many other organs. As the spleen contains a majority of the monocytes in the body, this suggests these cells are responsible for IR organ damage. This has lead to the conclusion that the spleen is an important mediator of post IR injury tissue damage. Additionally, blocking Kupffer cell activation with gadolinium chloride was as efficacious as splenectomy in renal and intestinal IR. These studies indicate that the spleen activates Kupffer cells to a pro-inflammatory state resulting in increased tissue damage following IR injuries.

Removal of the spleen is also protective in variety of brain injury models: permanent middle cerebral artery occlusion (pMCAO), transient middle cerebral artery occlusion (tMCAO), intracerebral hemorrhage (ICH), and traumatic brain injury (TBI) [4–6, 16–18]. Splenectomy prior to pMCAO in rats decreases infarct volume, the number of neutrophils and activated microglia [4], interferon gamma (IFN γ) levels, and infiltrating immune cells in

the brain [19]. In mice, splenectomy prior to tMCAO decreased infarct volume, brain IFN γ levels, and did not increase post stroke infections [5]. Brain water content was significantly lower in splenectomized animals compared to intact animals prior to ICH [6]. Splenectomy immediately after TBI was found to decrease neural injury in two different models of experimental TBI [18, 16, 17], both of which create areas of ischemia from vessel damage and edema following TBI. As an alternative to splenectomy, irradiation of the spleen 4 h post tMCAO decreases infarct volume in rats similar to the effects of splenectomy prior to pMCAO. Splenic irradiation causes a temporary decrease in splenocytes which does not result in wide spread immunosuppression [20]. Microglia, like Kupffer cells, are tissue-specific macrophages which indicates the spleen is influencing microglia in a similar manner, leading to increased neural injury following ischemic brain injury.

Stem Cell Therapies and the Spleen

Further insight into the role of the spleen has been provided by studies using cellular therapies in experimental models of brain ischemia. Human umbilical cord blood (HUCB) cells [21–23], hematopoietic stem cells (HSC) [24], bone marrow stem cells (BMSC) [25], and neural stem cells (NSC) [6] have all been shown to reduce neural injury in experimental models of stroke. All stem cells are more efficacious when administered systemically, compared to intracerebral administration. When administered systemically, stem cells migrate to the injured brain and the spleen, and in some cases have been shown to modulate the immune response to stroke [6, 24, 25, 21], which might be one of the reasons this injection route is more efficacious. Ninety-five percent of BMSC are found in the spleen following systemic administration after MCAO [25]. Even NSCs migrate to the spleen following ICH and are not as efficacious when combined with splenectomy. NSCs were found to be in direct contact with CD11b⁺ splenocytes [6], which suggests that part of the neuroprotection provided by NSCs involves interacting with the spleen. HUCB cells are another cell type that has been shown to interact with splenocytes. Systemic administration of HUCB cells 24 h post pMCAO results in altered splenic T cell responses to concavalin A. Splenic T cells had decreased cell proliferation and decreased production of inflammatory cytokines TNF α and IFN γ , with an increase in the production of the anti-inflammatory cytokine IL-10. HUCB cells also prevent the decrease in spleen size seen at 48 h in rats. This effect is thought to be mediated by HUCB cells sequestering immune cells in the spleen following MCAO, preventing their release into systemic circulation [26]. These experiments suggest stem cell therapies work in part by modulating the immune response to stroke, specifically at the level of the spleen.

Splenic Size Decreases Reacting to Stroke

The spleen has been found to decrease in size following pMCAO in rats [26] and tMCAO in mice [27]. The spleen transiently decreases in size from 24 to 72 h following pMCAO in rats [28]. A catecholamine (CA) surge occurs following damage to the insular cortex, an area mainly perfused by the MCA, which may be responsible for this reduction in size. Activation of the α_1 adrenergic receptors on the splenic smooth muscle capsule results in contraction of the splenic capsule, which leads to the decrease in spleen size. Administration of prazosin, an α_1 adrenergic receptor antagonist, prevents the decrease in spleen size

following pMCAO [29]. Spleen size has also been inversely correlated with infarct volume in rats following pMCAO, with smaller spleen sizes correlating with larger infarcts [26]. The splenic response in mice following tMCAO appears to be different from the response observed in rats following pMCAO. The spleens of mice continually decrease in size following tMCAO out to 96 h. This decrease in spleen size appears to be due to apoptosis of the spleen and a loss of the germinal B cell centers. The only immune cell population that has been shown to decrease in number following tMCAO in mice is B cells [27]. Another study in mice with tMCAO found the spleen continues to decrease in size out to 7 days post tMCAO [30].

There are several reasons that could explain the observed differences in the splenic response to MCAO. The studies were performed in different animal species, mice compared to rats. There are known differences in the mouse immune system compared to other mammals, including rats and humans. One of two of the major differences with mice is that non antigen presenting cells do not up regulate major histocompatibility complex (MHC) II in response to IFN γ , whereas rat and human cells do up regulate MHC II in response to IFN γ [31]. Additionally, activated mice T cells do not express MHC II, unlike rat and human activated T cells [32]. While it is not known why the mouse immune system differs in T cell response, these observations show that rat and human T cell responses are similar. Another similarity between rats and humans is the transient decrease in spleen size observed after stroke.

In order to truly understand the role the spleen plays in patients following stroke, studies are being conducted that examine spleen size in stroke patients. One such study currently being conducted has produced preliminary data indicating that the spleen in individuals who have suffered a stroke decreases in volume initially, < 6 h to 3 days, and slowly begins to increase in volume starting at day 4 and continuing out to 8 days following the stroke. An individual who suffered a severe stroke and ultimately died had spleen volumes that continued to decrease as their NIHSS score also progressively increased, indicating a worsening of neurological symptoms. Two other individuals who had better outcomes had spleens that initially decreased in volume and began to increase in volume as their NIHSS scores decreased. One of the two individuals had their spleen volume measured 90 days following their stroke, and at 90 days the individual's spleen volume was not different from the measurement taken at discharge [33]. A follow-up to this study determined that the spleen returns to size by five days [34] which is similar to the findings in the rat model [28].

Cellular Immune Response

The BBB protects the brain from exposure to anything harmful in the blood, which includes protection from the peripheral immune system under normal healthy conditions. Generally, the only immune cells present in the brain are the endogenous macrophages, microglia. Occasionally, a T cell may enter the brain but due to the decreased expression of MHC molecules in the CNS, the T cell leaves the brain within 24–48 h [35]. This makes the brain an immunoprivileged site, which is beneficial in protecting the brain from systemic inflammation. However, neural antigens can be seen as foreign to the immune system, resulting in immune responses generated against neural antigens that are present in systemic circulation following brain injuries, including stroke.

Both the innate and the adaptive immune systems play an important role in the inflammatory response following ischemic brain injury. The injured cells of the CNS, in combination with glial cells which become activated after a stroke, express chemotactic molecules, which signal to the peripheral immune system that there is an injury to the brain. Various cytokines cause up regulation of vascular adhesion molecules in endothelial cells and on immune cells. This creates a leaky BBB which allows entry of immune cells into the brain [36].

Cells of monocytic origin, CD11b⁺ cell, become activated as early as 18 h and are significantly increased in number out to 96 h post tMCAO in mice [37]. It is not possible to differentiate between microglia and peripheral monocytes/macrophages, as both types of cells express CD11b. Microglia/macrophages become maximally activated in the brain 72 h post pMCAO in rats [38]. Neutrophil numbers are significantly increased in the infarcted hemisphere, beginning at 48 h and remain elevated out to 96 h post tMCAO. As expected with an adaptive immune response, T cells are present in the brain starting 72 h and remain at 96 h post tMCAO [37].

Since the spleen decreases in size after ischemic insult, this tissue appears to be the origin of peripheral immune cells in the brain following stroke. Splenocytes labeled *in vivo* with carboxyfluorescein diacetate succinimidyl ester (CFSE) were traced to the infarct following pMCAO. These labeled splenocytes were also found to decrease in number in the spleen and increase in number within the circulation at 48 h post pMCAO. CFSE positive cells were found only in the injured hemisphere of pMCAO animals, and were not found in the brains of sham pMCAO animals. Additionally, it appears that the CFSE positive cells in the brain remain in the vasculature [28]. This observation suggests that splenocytes are releasing factors at the BBB to influence the immune response to the infarcted area.

Lymphocytes have also been shown to be involved in increasing neural injury after an ischemic stroke. Following tMCAO, Rag^{-/-} mice, which lack functional T or B cells, have decreased infarct volumes compared to wild type (WT) mice. T cell^{-/-} mice, both CD4^{-/-} and CD8^{-/-}, have decreased infarct size compared to WT mice. However, B cell^{-/-} mice had infarcts similar to WT mice, indicating B cells play a minimal role in detrimental post stroke brain inflammation [39]. Additionally, severe combined immunodeficiency (SCID) mice, which lack lymphocytes, have reduced infarcts compared to WT mice. SCID mice also have reduced cytokine levels, except for IL-1 β , in the brain post tMCAO [40].

The reaction of the immune system towards neural antigens, which is orchestrated by Th cells, can be harmful or beneficial following ischemic stroke, depending on how the cells are activated and the environment in which the cells are activated. T cells primed to react with a pro-inflammatory response to myelin oligodendrocyte glycoprotein (MOG) prior to tMCAO had increased infarct volumes in mice [41], and resulted in death following sensitization with myelin basic protein (MBP) prior to tMCAO in rats [42], when compared to animals primed with a non-neural neutral antigen. However, mice with T cells that are tolerized to MBP prior to tMCAO have decreased infarct volume compared to controls [42].

Molecular Immune Response

Cytokines have been extensively studied following experimental stroke, as well as in stroke patients. Most of the data regarding cytokines and stroke have been contradictory, as some cytokines have dual roles in the immune response and can be either protective or detrimental depending on the circumstances. Some cytokines can be inflammatory early after a stroke, but provide trophic support to cells at delayed time points. Other cytokines can have survival or inflammatory effects depending on the receptor to which they bind. Additionally, some cytokines are elevated very early following stroke. All the above stated examples demonstrate why no good therapeutic targets for cytokines have been developed (Table 1).

In mouse models of tMCAO, mRNA levels of IL-1 β , IL-10, and TNF α are elevated 6 h post tMCAO [43, 44]. All of these cytokines are elevated at a time that is outside the therapeutic window to successfully interfere with their signaling. IL-1 β mRNA expression increases early in the brain after an ischemic event and remains elevated out to 96 h post tMCAO [43]. Non-immune cells in the brain express IL-1 β . Primary producers of IL-1 β in the brain are astrocytes and microglia [36]. This could explain why IL-1 β is the only cytokine that does not have decreased expression in SCID mice following tMCAO [40]. IL-10 is considered an anti-inflammatory cytokine and is associated with a Th₂ response. IL-10^{-/-} mice have increased infarct volumes compared to WT mice following tMCAO [45], and mice that over-express IL-10 have decreased infarct volumes compared to controls following pMCAO [46]. This suggests IL-10 may play a beneficial role following brain ischemia.

TNF α is also expressed early in the brain, and is primarily responsible for the activation of the immune system and recruitment of other immune cells. In addition to being expressed early, TNF α is known to have different effects following brain ischemia. Administration of TNF α has been shown to exacerbate infarct volume in both tMCAO and pMCAO in a dose dependant manner [47]. However, TNF α ^{-/-} mice had increased infarct volumes compared to WT mice following pMCAO [48]. This suggests TNF α plays a protective role following ischemic stroke. Though, when there is a decrease in TNF α production, by inhibiting TNF α converting enzyme (TACE), prior to and following pMCAO TACE-inhibited rats demonstrated decreased infarct volume and reduced neurological deficits compared to control rats [49]. In addition, blocking TNF α with a neutralizing antibody injected intracerebroventricularly (i.c.v.) 15 min post tMCAO in mice decreased infarct volume, however, administration of the same antibody 3 days post tMCAO did not decrease infarct volume [45]. TNF α has also been linked to the up regulation of manganese superoxide dismutase (Mn-SOD), an important anti-oxidant enzyme that is believed to play a role in ischemic preconditioning in stroke [50]. All of the contradictory results in experimental stroke with TNF α could be due to the two different TNF α receptors and the subsequent cellular processes induced by these receptors.

The two TNF α receptors, when activated, can result in different cellular responses depending on the cell type or the presence of both receptors on the same cell. TNF α can initiate a response resulting in apoptosis and the production of cytokines, or be protective by preventing apoptosis. The two different receptors, TNF α R1 and TNF α R2, result in a combination of different cellular responses. TNF α R1 has an intracellular death domain that

can divert the cellular response to TNF α in a Fas-associated protein with a death domain (FADD) towards apoptosis, or the binding of TNF-receptor associated protein 2 (TRAP2), which leads to the transcription of anti-inflammatory factors. FADD signals to activate caspase 8, leading to apoptosis. TRAP2 leads to the activation of NF κ B and c-JUN which induce anti-apoptotic, anti-inflammatory and cellular protective proteins [50]. TNF α R1 is expressed on all cells, while TNF α R2 is expressed only on oligodendrocytes, astrocytes, T cells, myocytes, endothelial cells, thymocytes, and human mesenchymal stem cells. TNF α R2 does not contain an intracellular cytoplasmic death domain and activation leads to the recruitment of TNF receptor-associated factor 2 (TRAF2). TRAF2 acts similarly to TRAP2 and activates NF κ B, AP1, and mitogen-activated protein kinase (MAPK). The activation of these pathways leads to inflammation, cellular proliferation, and cell survival [51]. In pMCAO experiments with TNF α R knockout mice, TNF α R1^{-/-} was associated with neuroprotection, while TNF α R2^{-/-} was not associated with neuroprotection [48]. Studies using cuprizone toxicity as a model of white matter injury found that TNF α is important in the recruitment of oligodendrocyte progenitors to remyelinate axons. This TNF α signaling is mediated through the TNF α R2 [52]. The diverse effects of TNF α can be contributed to many factors, including timing of TNF α signaling and the receptors it signals through; and the contradictory outcomes in animal experiments indicate TNF α is not a good therapeutic target for stroke. Additionally, it is initially elevated outside therapeutically relevant time points to treat stroke. Therefore, despite the fact that there are several FDA approved anti-TNF α therapies, none of them are being used to treat stroke.

Another cytokine that has been researched extensively in experimental stroke is IL-6. One of the primary reasons a lot of emphasis has been placed on IL-6 is because it is detectable in the serum of stroke patients. Serum IL-6 levels in stroke patients have been shown to be the strongest independent predictive variable of in-hospital mortality following stroke [53]. IL-6 mRNA levels are elevated early in the brain following tMCAO in mice [44]. Despite evidence that IL-6 might be playing a detrimental role in stroke pathology, there has been very little investigation into blocking IL-6 as a therapeutic for stroke. This is likely due to the confounding data from animal studies looking at IL-6 and experimental stroke. IL-6^{-/-} mice had infarcts that were not significantly different than their WT or heterozygous littermates following tMCAO [54]. However, IL-6 is known to have pyrogenic activity and IL-6^{-/-} mice were found to have lower body temperatures compared to WT mice. Body temperature is known to play an important role following any brain injury, including stroke. When IL-6^{-/-} mice had their body temperatures monitored and adjusted to match the WT mice, the IL-6^{-/-} had significantly increased infarct volumes and increased neurologic deficits compared to the WT mice following MCAO [55]. An additional study found i.c.v. administration of recombinant IL-6 prior to pMCAO in rats significantly decreased infarct volume, suggesting IL-6 is directly neuroprotective [56]. A different study using IL-6^{-/-} mice found increased infarct volumes compared to WT mice following tMCAO, out to four weeks. This study suggested the loss of IL-6 was important for angiogenesis during the recovery phase following stroke [57].

One reason for the conflicting experimental data could be due to the multiple functions of IL-6. Prior to being named IL-6, this 26 kDa protein was named B-cell stimulatory factor 2, IFN- β 2, hybridoma/plasmacytoma growth factor, hepatocyte stimulating factor, and 26-kDa

protein. Once DNA sequencing was completed, it was discovered that all five proteins were the same molecule [58]. IL-6 signals through one receptor that is a complex of the IL-6R and gp130. The cytoplasmic domain of gp130 contains several signaling motifs that allow IL-6 to signal through ERK or Janus kinases (Jaks) Jak₁ and signal transducers and activators of transcription (STATs) STAT3 or STAT1. STAT3 and STAT1 can form homodimers or a heterodimer once activated. These different signaling pathways allow IL-6 to have diverse effects on a variety of cells and cellular functions. IL-6 is important for liver regeneration, angiogenesis, bone, cartilage, and lipid metabolism, iron homeostasis, and is known to promote cancer cell survival. The immune functions that IL-6 contributes to include promoting neutrophil production and recruitment, enhancing antibody production by B cells, and working with transforming growth factor beta (TGFβ) to increase the production of pro-inflammatory Th₁₇ cells. Th₁₇ cells are thought to play a major role in autoimmune diseases [59]. IL-6 plays many different roles following stroke depending on the exact timing after the stroke, which makes targeting IL-6 as a treatment after stroke problematic.

Out of numerous studies on cytokines in animals and stroke patients, there has yet to be a therapeutic developed for stroke. Many cytokines are part of the innate immune response and increase rapidly following stroke, while other cytokines have dual roles following stroke or serve a potentially protective function. IFNγ is one cytokine that has not been extensively studied and could be promising in the development of a delayed therapeutic option, which could strictly address the pro-inflammatory response. IFNγ is the signature cytokine of the adaptive immune Th₁ response.

Interferon Gamma Signaling following Stroke

IFNγ is a pleiotropic cytokine that can affect cellular processes, ranging from immune cell function to playing a role in vascular leukocyte adhesion. The 34-kDa homodimer is the biologically active form of IFNγ [60]. The half-life of IFNγ in the blood is 1.1 min if it is not bound to heparin or heparin sulfate, and when bound the half-life is increased to 99 min. When IFNγ is injected into the bloodstream, 90% of the protein is proteolytically cleaved at the carboxyl-terminal within 5–10 min, rendering it inactive. The other 10% becomes bound to a heparin molecule, increasing its half-life [61]. The biologically active form of IFNγ binds to the IFNγ receptor complex that is comprised of two pairs of transmembrane proteins which signal through the Jak₁ or Jak₂, and STAT1. Of the two different proteins that make up the IFNγ receptor, the IFNγ R₁, or the α-chain, is the part of the receptor that binds IFNγ. Following binding of IFNγ, the α-chain dimerizes with another α-chain. Then, the second protein IFNγR₂, or the β-chain, dimerizes with another β-chain to the complex. The β-chain is primarily involved in signaling, whereas the α-chain is primarily involved in binding IFNγ. Jak₁ is associated with the α-chain and Jak₂ is associated with the β-chain. Once IFNγ binds, the Jaks become phosphorylated and phosphorylate STAT1α, which homodimerizes and translocates to the nucleus to affect transcription. The IFNγ receptor is expressed on every cell in the body but its density varies from cell type to cell type, with immune cells, monocytes in particular, having the highest expression.

The primary producers of IFNγ are T cells, CD4⁺ Th₁ cells, CD8⁺ T cells, and NK cells. IFNγ production is induced by IL-12, IFNα, and even by IFNγ through positive feedback.

IFN γ induces the production of more IL-12 by macrophages, which further increase IFN γ production. In addition to increasing its own expression, IFN γ causes increased expression of proteins involved in the generation of reactive oxygen species (ROS), chemotaxis of more immune cells to the site of injury, up regulation of MHC molecules, and induces isotype switching in B cells [60]. Indirectly, there is also an increase in cellular adhesion molecules on endothelial cells by IFN γ through up regulating chemokines, including monocyte chemoattractant proteins (MCPs), monokine induced by gamma interferon (MIG), interferon-inducible protein 10 (IP-10), IL-8, and interferon-inducible T cell α chemoattractant (I-TAC). With the recruitment of additional immune cells to the site of injury, IFN γ also primes T cells and cells of monocytic origin towards a pro-inflammatory phenotype. IFN γ and its effector molecules prime naïve Th cells to develop into Th₁ cells, blunting the response of other Th subsets. Microglia/macrophages become activated towards a pro-inflammatory state via the up regulation of ROS production and MHC II expression. The primary role that IFN γ plays in the immune response is to increase resistance to bacteria and viruses, particularly intracellular pathogens. To obtain this immune response, the primary target cells of IFN γ in the immune system are macrophages. These cells are also the primary producers of all the chemokines like IP-10, MIG, and I-TAC [62].

The Detrimental Role of IFN γ following Ischemic Brain Injury

There are several data in animals and stroke patients indicating IFN γ plays a detrimental role in stroke pathogenesis. IFN γ mRNA is up regulated 2 days post pMCAO in the brain of rats [63]. Protein levels of IFN γ are elevated in the brain of rats following pMCAO [19] and mice following tMCAO at 72 h post MCAO [5]. IFN γ protein levels also spike in the spleen 24 h post pMCAO, and splenectomy decreases IFN γ expression in the brain at 72 h [19, 5]. Administration of simvastatin following tMCAO in mice reduced infarct volume and IFN γ levels in the brain [5]. Additionally, IFN $\gamma^{-/-}$ mice have decreased infarcts compared to WT mice and the IFN $\gamma^{-/-}$ mice infarcts are comparable to Rag $^{-/-}$ mice [39]. Delayed administration of antibodies directed against IFN γ decreased infarct volume when injected i.c.v. 3 days post tMCAO, but not earlier [45]. In addition, indirect blocking of IFN γ is neuroprotective. Administration of an anti CD49d (VLA-4) antibody 24 h prior to the MCAO reduced infarct volume and blocked T cells and NK cells from entering the brain following tMCAO. This prevented IFN γ producing cells from entering the injured brain, which decreased IFN γ levels and infarct volume [64].

In stroke patients who developed an infection, particularly pneumonia, within 15 days of having a stroke had a worse outcome compared to individuals who did not develop an infection regardless of stroke severity. Individuals who developed an infection were more likely to have a Th₁ response to MBP and glial acid fibrillary protein (GFAP) at 90 days post stroke, and individuals who generated a higher Th₁ response to MBP at 90 days were more likely to have a poorer outcome regardless of age or baseline stroke severity [65]. IFN γ is considered a signature cytokine of a Th₁ response, which could implicate IFN γ as being detrimental following stroke in patients when an inflammatory T cell response is generated against brain antigens.

The Generation of an IFN γ Driven Response to Brain Antigens following Stroke

IFN γ levels in the spleen and in the brain could become increased due to the elevated levels of circulating catecholamines. Increased levels of circulating norepinephrine (NE) and epinephrine have been found in humans and rats after experiencing a blockage of the MCA [66, 67]. The additional amount of circulating CAs has been attributed to insular cortex damage, which is mainly perfused by the MCA. Damage to this region in patients has also been shown to cause sympathetic dysregulation [66]. Furthermore, NE reduces the ability of Th₁ cells to respond when activated, and this effect is thought to be mediated by the presence of β_2 adrenergic receptors on Th₁ cells [68]. CAs are also thought to induce apoptosis in Th₁ cells leaving stroke patients vulnerable to infections [69]. However, naïve T cells also express β_2 adrenergic receptors. When naïve T cells are exposed to NE, these cells are driven to differentiate into the Th₁ phenotype. Upon reactivation, these cells express 2–4 fold more IFN γ than naïve T cells not exposed to NE [70]. The effect of NE on naïve T cells and Th₁ cells may account for the immune dysfunction to pathogens and the increased levels of IFN γ production observed in the spleen following MCAO.

In addition, the cells that become activated in the presence of catecholamines are also exposed to brain derived antigens that enter circulation following stroke [71, 72]. This could lead to a Th₁ response to brain antigens, which has been shown in animal studies to result in a more severe injury [73]. This response may also be mediated through IFN γ , as IFN γ is considered an indicator of a Th₁ response.

Conclusion

Interferon gamma and the spleen both potentially play important roles in exacerbating neural injury following ischemic stroke. Removal of the spleen has proven that an inflammatory response originating from this organ to stroke is responsible for delayed neurodegeneration. IFN γ levels are increased as part of this splenic inflammatory response and results in delayed activation of microglia/macrophages in the brain that is detrimental to the survival of compromised neural cells. IFN γ is also known to induce several proteins, many of which are chemokines. IP-10 is of particular interest as it plays a role in influencing the differentiation of naïve Th cells to become Th₁ cells, and is a strong chemoattractant for Th₁ cells while subsequently blocking the activation of Th₂ cells. The recruitment of more pro-inflammatory Th₁ cells would result in more IFN γ production and further activation of microglia/macrophages. This would create a feed-forward inflammatory environment in the stroke-injured brain (Fig. 1a). Blocking or interfering with IFN γ signaling could provide a targeted approach to blunting just the pro-inflammatory response and not the whole immune response that is seen with broad immunosuppressants, like cyclosporine (Fig. 1b).

Experimental therapeutics that target the spleen or IFN γ have shown translational promise as stroke treatments. Splenectomy prior to stroke is not a practical translational therapy for stroke. Studies using cellular therapies to treat experimental stroke have shown that this approach is more efficacious when administered systemically relative to directly into the brain. Besides the injured brain, these cells migrate to the spleen and their presence inhibits

its inflammatory response. A recent study demonstrated targeted irradiation of the spleen at 4 h post-tMCAO decreased infarct volume, did not damage surrounding organs, and did not cause severe immune suppression that resulted in post-stroke infections [20]. Another study examined administration of an FDA approved statin, simvastatin, following tMCAO. Administration of a statin at the time of reperfusion decreased infarct volume at 5 days, prevented increased IFN γ levels in the brain at 3 days, and prevented splenic atrophy, which is seen in mice after stroke. Additionally, there was a decrease in post-stroke lung infections despite decreased levels of IFN γ in the brain [5]. These two preclinical studies demonstrate the effectiveness of two different therapeutic strategies that target the spleen and/or IFN γ leading to neuroprotection without suppressing normal immune function. Further investigation into these treatments and treatments similar to the previously mentioned studies is warranted.

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Fig. 1.

The generation of an interferon gamma driven immune response following stroke. After a stroke there is an increase in systemic circulating catecholamines that causes a decrease in spleen size. At the same time there is an increase in splenic interferon gamma ($\text{IFN}\gamma$). There is then a delayed increase in the expression of $\text{IFN}\gamma$ in the brain, which activates microglia and macrophages. Activation of these cells causes increased expression of interferon-inducible protein 10 (IP-10) and increased neural injury. The elevated levels of IP-10 create a pro-inflammatory loop by recruiting more $\text{IFN}\gamma$ producing cells to the brain (a). Blocking or interfering with $\text{IFN}\gamma$ signaling is a potential therapeutic option as blocking $\text{IFN}\gamma$ would decrease microglia/macrophage activation, IP-10 expression, and prevent further increases in $\text{IFN}\gamma$ in the brain resulting in decreased neural injury following stroke (b).

Table 1

Cytokines studied in experimental stroke or stroke patients

Cytokines	
IL-10	
– mRNA initially increases 6 h post-MCAO in the brain	[43, 44]
– IL-10 ^{-/-} mice have increased infarct volumes	[45]
– IL-10 over-expressing mice have decreased infarct volumes	[46]
IL-1β	
– mRNA initially increases 6 h post-MCAO in the brain	[43, 44]
– Lymphocytes are not a major source of IL-1β	[36, 40]
TNFα	
– mRNA initially increases 6 h post-MCAO in the brain	[43, 44]
– Administration of TNFα increases infarct volume post-MCAO	[47]
– TNFα ^{-/-} mice have increased infarct volumes	[48]
– Inhibition of TACE decreases infarct volume and neurologic deficits	[49]
– Administration of a TNFα antibody icv 15 min post-MCAO decreases infarct volume	[45]
– Only TNFαRI ^{-/-} mice, and not TNFαR2 ^{-/-} mice, had decreased infarct volumes	[48]
IL-6	
– mRNA initially increases 6 h post-MCAO in the brain	[43, 44]
– IL-6 ^{-/-} mice have no difference in infarct volumes compared to wild type mice	[47]
– IL-6 ^{-/-} mice with controlled body temperature have increased infarct volumes	[48]
– Administration of IL-6 icv prior to MCAO decreases infarct volume	[49]
– IL-6 ^{-/-} mice have increased infarct volumes at 4 weeks post-MCAO	[45]
– IL-6 can be detected in the serum of stroke patients and predicts outcomes	[48]
IFNγ	
– mRNA initially increases 48 h post-MCAO in the brain	[63]
– Protein levels initially increase 72 h post-MCAO in the brain	[5, 19]
– IFNγ ^{-/-} mice have decreased infarcts	[39]
– Administration of an IFNγ antibody icv 3 days post-MCAO decreases infarct volume	[45]
– Administration of a CD49d antibody prior to MCAO decreases infarct volume and IFNγ levels	[46]
– Splenectomy prior to MCAO decreases infarct volume and IFNγ levels in the brain	[19]