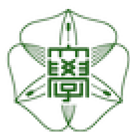

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Inflammatory responses in Brain Ischemia

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1. Abstract

Brain infarction causes tissue death by ischemia due to occlusion of a cerebral artery and recent work has shown that inflammatory responses following ischemia play important roles in the development of ischemic cascade. Because secondary damage by brain inflammation seems to have longer therapeutic time window comparing to the rescue of primary damage by arterial occlusion, controlling the inflammation would be a promising therapeutic target and large amount of experimentally progresses have been made in the past years. However, it is quite difficult to elucidate the precise mechanisms of the inflammatory responses following ischemic stroke because inflammation is a dynamic process involving a complicated set of interactions among various inflammatory cells and molecules, and they could be either detrimental or beneficial. This review will focuses on some recent advances regarding the different key players in neuroinflammation regulating inflammatory signaling pathways, the detrimental effects of post-ischemic inflammation, and the potential molecular targets for ischemic stroke therapy. A better understanding of the roles of the different immune cells and their temporal profile of damage versus repair will help to clarify more effective modulation of inflammation post stroke.

2. Introduction

Stroke is a broad term that includes conditions caused by occlusion and/or rupture of blood vessels in the brain. It is one of the most frequent causes of death and disability worldwide, and has significant clinical and socioeconomic impact. Ischemic strokes represent more than 80% of all cases of stroke and are characterized by the occlusion of a brain arterial blood vessel due to a thrombus or embolus[1]. Besides its high incidence, approved effective therapies are limited[1, 2]. Although different mechanisms are involved in the pathogenesis of stroke, there is increasing evidence that post stroke inflammation plays an key role for its progression, at least acutely[3-5]. Brain inflammation has been implicated as a secondary injury mechanism following ischemia and stroke (Fig. 1). Brain ischemia likely triggers inflammatory responses due to a variety of factors, such as the presence of necrotic cells and generation of reactive oxygen species (ROS), although many factors have yet to be precisely identified. These initiators lead to microglial activation which produce more cytokines causing upregulation of adhesion molecules in the cerebral vasculature within 24 hours of the ischemic insult[6-8]. Chemokine upregulation leads to inflammatory cell chemotaxis to ischemic brain especially around the penumbra. Adhesion molecules mediate adhesion of circulating leukocytes to vascular endothelia not only causing microvascular occlusion but also infiltration of immune cells into the brain parenchyma[9, 10]. Once activated, inflammatory cells can release a variety of cytotoxic agents

including more cytokines, matrix metalloproteinases (MMPs), nitric oxide (NO) and more ROS. These substances may potentiate brain cell damage , and lead to disruption of the blood-brain barrier (BBB) and extracellular matrix[11]. BBB disruption can further potentiate brain tissue injury and contribute to secondary ischemic brain damage by permitting serum elements and blood to enter the brain resulting brain edema and hemorrhagic transformation. Brain ischemia is also thought to influence immune cells in the circulation possibly through increased activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis (HPA),. This in turn may lead to fewer circulating immune cells, and increase the risk of infectious complications[12]. Leukocyte plugging of the brain's microvasculature has also been implicated in microvascular stasis leading to hypoperfusion. Blocking various aspects of the inflammatory cascade has shown to ameliorate injury from experimental stroke[13], although this has yet to be demonstrated at the clinical level.

However, what makes these inflammatory processes difficult to understand is that some of these responses may be detrimental by facilitating cell death, but also beneficial to the ischemic brain by secreting neurotrophic factors and by scavenging necrotic debris allowing for the reorganization of a new environment for neural repair[14-17].

During the past few years, there has been a significant progress regarding the roles of inflammatory signaling molecules, cells and proteins in the process of initiation and development of post-ischemic inflammation. This review focuses on

current findings as they largely pertain to innate immune responses and mechanisms, and provides an update on the understanding of post-ischemic inflammation and possible therapeutic potentials.

3. Cells involved in post stroke inflammation

Inflammation is characterized by the accumulation of inflammatory cells and mediators in the ischemic brain. Accumulation of circulating immune cells begins when ischemia damages and activates endothelial cells with rapid upregulation of adhesion molecules [18]. Circulating immune cells can then gain access into the injured brain and elaborate immune molecules that exacerbate ischemic cell death. Ischemic brain cells also activate endogenous immune cells such as microglia. A few studies implicate astrocytes as another brain resident immune cell which can also activate in response to ischemia.

3.1 Cerebral Endothelial cells and Blood Brain Barrier (BBB)

Cerebral endothelium is highly specialized, and forms the blood brain barrier (BBB) which is made up of tight junctions, pericytes and astrocyte end-foot processes[19, 20]. The interconnection of the cells is mediated by integrins[21]. Endothelial cells are actively engaged in processes of microvascular stasis and are the first cells which face the impact of ischemia. When damaged by ischemic stimuli, endothelial cells swell or detachment from the underlying basement membrane, leading to compromises in barrier function. This leads to increased BBB permeability causing protein extravasation and interstitial edema as well as entry of immune molecules and cells [18, 22]. Hypoxia also activates the arachidonic acid cascade leading to the generation of both vasoactive and pro-inflammatory

molecules, including prostaglandins, leukotrienes, and platelet-activating factor (PAF) [23]. These metabolites induce platelet and neutrophil activation and adhesion, changes in local cerebral blood flow and blood rheology, and increases in BBB permeability[7, 23-25]. Ischemic endothelial cells have also been shown to express and release bioactive inflammatory cytokines and chemokines, including IL-1β, IL-8, TNF-α, and MCP-1. Many of these mediators in ischemic in vitro and in vivo models have been shown to up-regulate the expression of inflammatory mediators such as selectin and immunoglobin superfamilies and subsequently facilitate leukocyte adhesion and transmigration into the brain[26, 27].

3.2 Leukocytes

Leukocytes promote cerebral ischemic injury in several different ways. First, adhesion of leukocytes to the endothelium can impair the flow of erythrocytes through the microvasculature causing the cerebral no-reflow phenomenon and additional ischemic injury[22]. Second, activated leukocytes at the surface of the endothelium produce toxic reactive oxygen species (ROS), proteases, gelatinases, and collagenases, and damage potentially salvageable blood vessels and brain tissues. Third, phospholipase activation in leukocytes results in the production of biologically active substances like leukotrienes, eicosanoids, prostaglandins, and platelet-activating factor, which can cause vasoconstriction and increase platelet aggregation. Finally, infiltrated leukocytes elaborate proinflammatory cytokines and other immune molecules in around the penumbra surrounding the infarct core causing further neuronal injury[28-30].

3.2.1 Neutrophils

Following ischemia, circulating leukocytes adhere to vessel walls, leading to migration and accumulation into ischemic brain tissue with subsequent release of proinflammaroty mediators. Neutrophils are generally considered the first leukocyte subtype recruited to the ischemic brain and have been observed at about 4-6 h in models of transient focal ischemia [31, 32], and at around 12 hours after permanent focal ischemia[33].. They may potentiate injury by directly secreting deleterious substances and other inflammatory mediators[5, 34]. In transient ischemia, several studies have shown that infarct volume is significantly reduced when neutrophil infiltration is inhibited[30, 35-39]. Some immune mediators, while not directly cytotoxic, may be involved in the destruction of necrotic and neighboring viable tissue. Evidence to support the notion that neutrophils potentiate ischemic injury include numerous studies documenting improved neurological outcome when neutrophil entry into the ischemic brain is prevented or inhibited [40].

3.2.2 Lymphocytes

While the data are conflicting, lymphocytes have largely been shown to play a negative role in ischemic brain pathogenesis. Like neutrophils, lymphocytes are also sources of pro-inflammatory cytokines and cytotoxic substances, such as ROS. A few studies in stroke models have shown that lymphocytes are elevated in the ischemic brain later than neutrophils (3 to 6 days post stroke) [41, 42]. Preventing

lymphocyte trafficking into ischemic brain ameliorated injury suggesting that like neutrophils, lymphocytes also play a deleterious role.[43] T lymphocytes, but not B lymphocytes are now considered to be the central to the development of inflammation in stroke models[44, 45]. Several recent studies have evaluated the role of T lymphocyte deficient mice in the transient focal ischemia model, and have consistently reported a smaller infarct volume and improved functional outcome than in control groups[45-48]. Kleinschnitz et al. have shown that protection of lymphocyte-deficient mice subjected to stroke is due to the lack of T lymphocytes, and not B lymphocytes, as the reconstitution of B lymphocytes does not alter the protection observed in mice which lack both T and B lymphocytes ($Rag1^{-/-}$,). By contrast, when T lymphocytes are transplanted back in to $Rag1^{-/-}$ mice, this protection was lost[45, 47, 48]. Although, T lymphocytes play an integral role in transient ischemic model, the role of T lymphocytea in permanent focal ischemia models seems different. Saino el al. [49] failed to see significant differences infarct size between immunodeficient mice (deficient in both T and B lymphocytes) and wildtype. The reason for these differences is unclear.

However, not all T lymphocytes subtypes are detrimental to acute stroke outcome. A recent study showed that neither □□T lymphocytes nor natural killer (NK) cells contribute to stroke injury[45]. Furthermore, the role of regulatory T (T_{reg}) lymphocytes is still in question. Liesz et al. [50] showed that infarct volume and neurological deficit were significantly increased in mice given an antibody to neutralize T_{reg} lymphocytes compared to controls. They also suggested that IL-10

signaling may be essential for this immunomodulatory effect. However, Ren et al. [51] could not find any modulatory effect of T_{reg} cells. In addition, there is now evidence that resident NK cell function in the liver is profoundly impaired due to augmented sympathetic neurotransmission following stroke, and that this loss of invariant NK (iNK) cell activity substantially contributes to the immunosuppression and susceptibility to infections that occur following stroke[52].

Clinical studies also demonstrate that lymphocytes have strong pro-inflammatory and tissue-damaging properties, and the upregulation of circulating lymphocytes are correlated to an increased risk of stroke recurrence and death[53]. However, in a study of cultured primary neurons, isolated neutrophils potentiated neuronal injury due to excitotoxin exposure, whereas lymphocytes were not neurotoxic and actually increased astrocyte proliferation [54].

The precise mechanisms of lymphocyte-mediated brain injury are currently unclear. Classically, T lymphocytes kill bacteria- and virus-infected cells either by the release of cytokines, or cytotoxins[55], and similar actions probably occur at the site of ischemic injury. Alternatively, T lymphocytes may cause cell death via interaction with the Fas receptor[56], and a few groups showed that neutralization of T lymphocyte-derived cytokines (IL-17, IL-12, IL-23, interferon gamma) reduced infarct volume and improved neurological function in experimental stroke models [47, 50, 57]. Moreover, stroke in perforin-deficient mice show a significant neuroprotection, suggesting that the cytotoxin perforin, released by T lymphocytes also contributes to ischemic damage[58]. In addition to these studies, the recent

evidence showed that T lymphocytes may contribute to oxidative tissue injury following stroke, potentially via NADPH oxidase type 2 (Nox-2)-derived superoxide. T lymphocytes are known to contain a functional Nox-2 oxidase and, soon after the ischemic stroke, circulating T cells produce 7 to 15 fold greater amounts of Nox2-derived superoxide than from the control mice[59].

3.3 Microglia/Macrophages

Microglia represent anywhere from 5-20 % of the total glial population and are key modulators of the immune response, immunocompetent and phagocytic activity in the brain[60], and serve as scavenger cells in the event of infection, inflammation, trauma, ischemia, and neurodegeneration[61]. Once activated, microglia can undergo morphologic transformation from a resting state referred to as "ramified" to an "amoeboid" state, where they become virtually indistinguishable from circulating macrophages[60, 62]. Therefore, activated microglia are often called resident brain macrophages. Through its phagocytosic properties, microglia will clear foreign organisms as well as injured neurons[31, 63-65]. Cerebral ischemia also induces microglial activation, however, the precise mechanisms of its activation following ischemia are not completely understood. Accumulating data shows that CD14 receptors, followed by toll-like receptor 4 (TRL4) have been documented in activated microglia in the infarct brain and could be one of the mechanisms involved in its activation[66-68]. Activated macrophages can be detected as early as 2 hours after ischemia, whereas blood-derived macrophages do not enter the brain before 10 hours. By 22-46 hours after the insult, activated

microglia and macrophages are distributed throughout the entire lesion and are detectable up to 1 week after the insult[32, 69-72].

Once activated, microglia are thought to release a variety of inflammatory and cytotoxic mediators contributing to cell damage and cell death[63, 73]. Edaravone, a novel free radical scavenger, significantly reduced the infarct volume and improved the neurological deficit scores for ischemic mice by reducing microglial activation[74]. In spontaneously hypertensive rats with permanent MCAO, repetitive hyperbaric oxygen (HBO) treatment reduced infarct volume by suppressing microglia activation[75]. In transient MCAO, phagocytic microglia were documented in the cerebral cortex of the ischemic hemisphere[64, 76]. Minocycline, a tetracycline family antibiotic, was shown to provide significant protection against brain ischemia by inhibiting microglial activation and proliferation[77, 78]. Direct evidence supporting a damaging role of microglia/macrophages was demonstrated when their direct application potentiated neuron cell death[64, 68, 79, 80]. However, microglia are also a major producer of the growth factorTGF-β1, indicating that some microglial products are also neuroprotective[63, 81]. When microglial proliferation was inhibited in transgenic mice, infarct size was increased following ischemia, and suggests that proliferating microglia cells exert a beneficial role[82]. There are some possible mechanisms underlying these observations. First, microglia produce neurotrophic factors which stimulate neurogenesis and plasticity. Secondly, phagocytosis of neutrophils by activated microglia may prevent the release of toxic mediators[83, 84]. Finally,

resident macrophages scavenge and remove necrotic debris and other potentially harmful substances[84].

3.4 Astrocytes

Astrocytes exert many active roles in brain homeostasis, including the regulation of immune reactions. Aside from traditional inflammatory cells, astrocytes are also known to express different kinds of inflammatory mediators[85, 86]. Following ischemia, brain astrocytes are activated and lead to increased glial fibrillary acidic protein (GFAP) expression. Astrocytes also contribute to reactive gliosis and glial scar formation[87]. Astrocytic gliosis can be destructive after injury[31, 88]. A massive astroglial response starts in the core of the lesion from 4 hours to 1 day after the insult, and reaches a peak around 4 days and is observed up to 28 days after stroke onset[89, 90]. This glial scar has both neurotoxic and neurotrophic properties. The scar can function as a barrier which prevents axonal ingrowth and reinnervation, thus impending recovery. However, while this scar also isolates damaged tissue from viable tissue, and prevents additional damage to the surrounding brain[89]. Astrocytes also participate in brain inflammation by expressing major histocompatibility complex (MHC) and costimulatory molecules, developing Th2 (anti-inflammatory) immune responses and suppressing interleukin-12 (IL-12) expression, although this has yet to be demonstrated in ischemia models[91]. Astrocytes are also capable of secreting inflammatory factors such as cytokines, chemokines and inducible nitric oxide synthase (iNOS)[91, 92]. Following 10 min of transient global ischemia, iNOS expression was detected in

reactive hippocampal astrocytes [92]. Furthermore, iNOS in astrocytes has been shown to potentiate ischemia-like injury to neurons[93]. Newly studied protein tumor necrosis factor like weak inducer of apoptosis (TWEAK), a member of the tumor necrosis factor superfamily, is thought to be produced by neurons, astrocytes and endothelial cells, and can stimulate proinflammatory molecule production by interaction with its Fn14 receptor found on astrocytes[94-96]. Expression of TWEAK and Fn14 has been documented in a murine model of stroke, and a soluble decoy to Fn14 markedly reduced infarct volume[95]. These data may suggest that while astrocytes normally play important roles in neuron maintenance and function, activated astrocytes have the potential to pose harm to ischemic brain.

4. Adhesion molecules

Adhesion molecules involved in acute inflammatory responses permit interactions between endothelial cells, platelets, leukocytes, and lymphocytes, and subsequently play a pivotal role in the infiltration of immune cells into the brain parenchyma after stroke [97]. Three major steps, rolling, adhesion, and transendothelial migration of immune cells are involved in order for leukocytes to enter the brain through the endothelial wall. Activated leukocytes, especially early phase neutrophils, result in further damage to ischemic lesions through reperfusion or secondary injury mechanisms[98]. The interaction between immune cells and the vascular endothelium is mediated by three main groups of cell adhesion

molecules: selectins (P-selectin, E-selectin, and L-selectin), the immunoglobulin superfamily (intercellular adhesion molecules, e.g. ICAM-1, 2 and vascular cell adhesion molecule-1, or VCAM-1) and integrins (CD11a-c)[99, 100]. Several reports have shown that inhibiting leukocyte adhesion by targeting various adhesion molecules, preventing leukocytes from entering ischemic brain, resulted in reduced neurologic injury[36, 101]. Furthermore, animals deficient in adhesion molecules have reduced infarct volume after transient focal cerebral ischemia[102-104]. Although several adhesion molecules have been upregulated in both permanent and transient MCAO[105-107], inhibiting these targets mainly appears to be effective when reperfusion occurs[101, 108, 109].

4.1 Selectins

Selectins are calcium-dependent, transmembrane glycoproteins that bind to carbohydrate residues (sialyl-Lewis^{x}). Selectins mediate cell-cell adhesion, and rolling of leukocytes on the endothelium of postcapillary venules. Three kinds of selectins have been identified: E-selectin, P-selectin, and L-selectin[10, 33, 110]. There is very little E-selectin[105, 107], or P-selectin[9, 111] expression in normal brain microvessels, but they are expressed on the outer cell membrane immediately upon activation. While E- and P-selectin are involved in leukocyte rolling and recruitment during the early stages of activation (reaches peak around 4 hours to 24 hours and return to baseline at around 72-96 hours) [9, 105, 107, 112], L-selectin acts as a guide for unstimulated leukocytes[113].

The expression of P- and E-selectin have been documented in different experimental stroke models and their upregulation appears to be involved in promoting ischemic inflammatory responses and increases injury due to the ischemic stroke[9, 93, 105, 114-116]. In animal studies, mice overexpressing P-selectin had exacerbation of infarcts, whereas treatment with antibodies or inhibitors against P- and E-selectin was associated with improved neurological outcome[114, 117, 118]. The effects of P-selectin in ischemic stroke appear different in focal and global ischemia. In focal cerebral ischemia, neutrophils accumulated in the ischemic cortex of wild-type mice more abundantly than in P-selectin knockout mice[102]. Moreover, P-selectin deficient mice demonstrated smaller infarct volumes and improved survival compared with wild type mice. Functional blockage of P-selectin using a monoclonal antibody also improved early reflow and stroke outcome, with reduced cerebral infarction even when the blocking antibody was administered after ischemia onset[102]. However, P-selectin may play a different role in global cerebral ischemia. Antibody blockage of P-selectin, while reducing leukocyte rolling, paradoxically reduced survival[119]. The reason for these contrasting outcomes remains unclear, but suggests that the inflammatory response and its significance after focal and global ischemia may be quite different. The role of L-selectin in brain ischemia is less clear. Although L-selectin mediates leukocyte transmigration, it does not appear to significantly influence stroke outcome. Treating rabbits exposed to transient focal brain ischemia with an L-selectin antibody did not affect stroke outcome.[120]. Recent work has also shown that

exposing animals to E-selectin intranasally can induce immune tolerance to brain antigens, and consequently reduce the extent of injury and even prevent their occurrence[121, 122]. Since E-selectin is exclusively upregulated in stimulated endothelium, tolerance to E-selectin could lead to suppression of immune responses and prevent peripheral leukocyte trafficking into the brain. Thus, it is conceivable that intranasal E-selectin might lead to the development of a vaccine against stroke.

4.2 Immunoglobulin superfamily

To date, there are several members of the immunoglobulin superfamily known: ICAM-1, ICAM-2, VCAM-1, platelet-endothelial cell adhesion molecule-1 (PECAM-1), the mucosal vascular addressing cell adhesion molecule 1 (MAdCAM-1), and activated leukocyte cell adhesion molecule (ALCAM). ICAM-1 (also referred to as CD54) is constitutively present in low levels on cell membranes of endothelial cells, leukocytes, epithelial cells, and fibroblasts[7]. These molecules contribute to the inflammatory response by attaching immune cells tightly to the endothelial wall for facilitating and even stimulating diapedesis thorough the vessel wall to the site of injury[34, 123]. Its expression increases soon after activation by ischemic insult and cytokines[7, 11, 124]. ICAM-2 (CD102) is an endothelial cell membrane receptor that does not increase after stimulation, whereas VCAM-1 (CD106) is induced by TNF-alpha and IL-1. PECAM-1 (CD31) is involved in the attachment of endothelial cells to each other, and leukocyte transmigration across the endothelium. MAdCAM-1, acting as an endothelial cell ligand for leukocyte homing receptors L-selectin and $\alpha_4\beta_7$ integrin, is an adhesion protein expressed on

endothelium in mucosal tissues that has been shown to play an important role in the selective homing of lymphocytes to intestinal mucosa and associated lymphoid tissue.

Among the immunoglobulin members, ICAM-1 and VCAM-1 have been the most extensively investigated in cerebral ischemia[7]. Prior work has shown increased expression of ICAM-1 in ischemic brain within hours after stroke onset, peaking at about 12-46 h, and precedes leukocyte infiltration and returned to near base line at around 1 week[7, 11, 24, 69, 124]. Several studies have now shown that blocking ICAM-1 with antibodies[30, 58, 125-127] or inhibiting ICAM-1 mRNA with antisense oligonucleotides improves leukocyte infiltration and outcome from experimental stroke[7]. Similarly, mice deficient in ICAM-1 had smaller infarcts compared to wild-type mice[37, 103]. Not only inhibitors of ICAM-1 but also nitric oxide donors prevented ischemia-induced ICAM-1 expression and also led to neuroprotection[116]. In diabetic rats, ICAM-1 expression was higher after ischemia compared to non-diabetic rats, suggesting that ICAM-1 may, in part, explain why stroke is exacerbated under conditions of hyperglycemia[128].

The role of VCAM-1 in stroke is less clear. While increases in VCAM-1 mRNA and protein after cerebral ischemia have been observed[129, 130], others have failed to observe significant changes[127]. In a study of global cerebral ischemia in the rat, ONO-1078, a potent leukotriene receptor antagonist, improved neurological deficits and reduced neuron death by inhibiting the ischemia-induced upregulation of VCAM-1 in the hippocampus of ischemic rats[131]. One study

showed that unfractionated heparin led to reduced infarct size in experimental stroke, and was associated with a reduced inflammatory response including decreased VCAM-1 expression[132]. Leisz el al.[58] also found that administration of VCAM-1 small interfering RNA demonstrated reduced T lymphocyte infiltration in to the brain and infarct volume after ischemic insult. However, there are conflicting results, and other studies did not show any beneficial outcome using anti-VCAM-1 antibodies treatment[58, 130].

At the clinical level, increased soluble ICAM-1 and VCAM-1 (sICAM-1 and sVCAM-1, respectively) have been documented in the plasma and cerebral spinal fluid of subjects with recent cerebral ischemic patients, and correlated to stroke severity[11, 129]. VCAM-1 expression has been observed in autopsied brains of stroke victims within cerebral vessels and astrocytes[129].

4.3 Integrins

Integrins are transmembrane cell surface proteins. They are activated by chemokines, cytokines, and other chemoattractants, which composed of heterodimeric combination of various α and β subunit molecules. In order for leukocytes to bind to activated endothelium, integrins must be expressed on the cell surface so as to recognize endothelial cell adhesion molecules[133]. Almost all leukocytes express CD11a/CD18 (leukocyte function–associated antigen-1 [LFA-1]) and CD11b/CD18 (Mac-1), which are integrins that contain a common β_2 chain (CD18) and are thus known as β_2 integrins. These integrins allow the cells to bind

to endothelial ICAM-1 and -2 and migrate through the vessel. In addition to the β_2 integrins, lymphocytes and monocytes express $\alpha_4\beta_1$ (CD49d/CD29) and $\alpha_4\beta_7$ (CD49d/CD103). Because these integrins contain a common α_4 chain, they are referred to as α_4 integrins. CD49d/CD29, or $\alpha_4\beta_1$, is also known as very late activation antigen-4 (VLA-4), and CD49d/CD103, or $\alpha_4\beta_7$, is also known as lymphocyte-Peyer's patch adhesion molecule-1 (LPAM-1). Lymphocytes bind to the endothelium through the interaction of α_4 integrins with either VCAM-1 or MAdCAM-1[134].

In an in vitro study, hypoxia caused an increase of neutrophil CD11b expression compared to normoxia, and this injury was protected by aprotinin which reduced the upregulation of CD11b[135]. Treatment with 3-aminobenzamide (3-AB), a PARP inhibitor, appeared to protect by reducing expression of CD11b and other proinflammatory molecules[136]. Blocking CD11b and/or CD18 also reduces injury from experimental stroke and is associated with decreased neutrophil infiltration[35, 39, 58, 137]. Similarly, mice lacking CD18 exhibited reduced leukocyte adhesion to endothelial cell monolayers, and improved cerebral blood flow and less neurological injury and neutrophil accumulation when subject to transient ischemic stroke model[108]. Blocking integrins essential for lymphocyte and monocytes trafficking may also limit damage due to reperfusion injury. Relton el al. and Becker et al reported 31 % to 51 % smaller infarct volumes in animals treated with anti-α_4 integrin antibody[43, 138] and Becker also reported that small infarction was accompanied by lesser neurological deficit[43].

5. Inflammatory mediators

A number of immune mediators have been studied in stroke models, and several have been specifically studied for potential therapeutic value.

5.1 Cytokines

Cytokines are upregulated in the brain after a variety of insults including stroke, and are expressed not only in cells of the immune system, but production by resident brain cells, including glia and neurons, have been observed in animal ischemic models[46, 139] and in human[140]. The most studied cytokines related to inflammation in stroke are interleukin-1 (IL-1), TNF-α, interleukin-6 (IL-6), interleukin-10 (IL-10) and transforming growth factor-ß (TGF-ß)[13]. Among those cytokines, IL-1 mediates ischemic, excitotoxic and traumatic brain injury, probably through multiple actions on glia, neurons and the vasculature, while TNFα might contribute to neuronal injury and exert protective effects, and IL-6, IL-10 and TGF-ß may be neuroprotective[141].

5.1.1 IL-1

IL-1 has been strongly implicated in the pathogenesis of ischemic brain as a neurotoxic mediator. IL-1's two isoforms, IL-1α and IL-1ß and its endogenous inhibitor, IL-1 receptor antagonist (IL-1ra) have been deeply studied in experimental stroke[142]. IL-1ß, rather than IL-1α is considered to be more engaged in the ischemic pathogenesis[143]. IL-1β mRNA elevations have been documented within 15–30 min after ischemia[124, 144] with increased protein a few

hours later starting from the infarct core and spreads to the peri-infarct area in the course of time[46, 145]. Following 20 min transient global cerebral ischemia in rats, IL-1β mRNA and protein expression were increased not only during early reperfusion (1 h), but also at later times (6–24 h) indicating that its expression is biphasic [146]. Consistent with a potential damaging effect, increased brain damage occurred when IL-1β was administered to rats[147], and mice deficient in IL-1 had smaller infarcts compared to wildtype[143]. Reperfusion in diabetic rats also demonstrated higher protein level of IL-1 compare to wild type and may contribute to the worsening of ischemic damage by diabetes[128]. IL-1β generation results from conversion from its pro-form by IL-1beta converting enzyme (ICE-1, a member of the caspase family) to its active form[148]. IL-1 has two receptors, IL-1R1 and IL-1R2, but only the former is involved in signal transduction[149]. Inactivating or knocking out the IL-1R1 decreased the extent of damage caused by a hypoxic-ischemic (H/I) insult and preserve neurological function[150]. Overexpression or treatment with the endogenous inhibitor of IL-1's receptor, IL-1ra reduced infarct size[151, 152]. Similarly, IL-1ra deficient mice exhibited a dramatic increase in ischemic damage[142]. Moreover, basal and NMDA- or AMPA-induced cells death was significantly higher in glial-neuronal co-cultures from IL-1ra deficient mice than from wildtype mice [142].

5.1.2 TNF-α

TNF-α is also upregulated in the brain after ischemia with similar expression patterns as IL-1β. Initial increases are seen 1–3 h after ischemia onset and peaks at

12 h[124], and, like IL-1beta, has a biphasic pattern of expression with a second peak at 24–36 h [153, 154]. TNF-α expression has been observed in neurons[139], astrocytes[155] as well as in the peripheral immune system[154] in stroke models. However, it is important to make a distinction between soluble and membrane-bound TNF-α, since there seems to be a functional difference[156].

Although TNF-α and IL-1β often work synergistically, TNF-α seems to have pleiotropic functions in the ischemic brain with both neurotoxic and neuroprotective effects, while IL-1β seems generally neurotoxic [157, 158]. Inhibition of TNF-α reduces ischemic brain injury[159], and administration of recombinant TNF-α protein after stroke onset worsens ischemic brain damage[160]. However, TNF-α may also protect the brain under certain circumstances. TNF-α appears to be involved in the phenomenon of ischemic tolerance[161], and mice deficient in TNF receptors have larger infarcts[162]. TNF-α released in the striatum leads to neurodegeneration, while release in the hippocampus may promote neuroprotection[163]. TNF-α stimulates apoptosis of endothelial cells and contributes to vasogenic edema and infiltration of circulatory immune cells are stimulated by this BBB breakdown. On the other hand, TNF-α activates repair processes of the cerebral microvasculature and mediates neuronal plasticity[163]. The reasons for this disparity are still unknown, and several hypotheses have been proposed. First, TNF-α 's actions may depend on the timing. It appears to contribute to detrimental effects in the early phase of the inflammatory response, but may have more beneficial effects at a later stage[164]. But this does not explain

why TNF-α seems to underlie the phenomenon of tolerance. Another hypothesis relates to the receptors to which TNF-α binds. Soluble TNF-α which binds to TNF receptor 1 primarily leads to detrimental effects, whereas membrane bound TNF-α which binds to TNF receptor 2 leads to neuroprotection[63]. Other studies suggest that TNF receptor 1 signal pathways are also neuroprotective[165].

5.1.3 Other inflammation-related cytokines

The precise role of IL-6 in the ischemic stroke has not been clearly identified. IL-6 has been shown to increase its expression continuously up to 24 hours after ischemia onset[124]. However, ischemic brain damage was not attenuated in IL-6 deficient mice or in IL-6 receptor antagonist treated mice compared to wildtype, and suggests that it probably does not participate in ischemic pathogenesis[166, 167]. Yet, Herrmann et al. [168] reported a beneficial effect of IL-6, while Smith et al. [169] showed detrimental effects. There is also reports showing strong correlation between serum IL-6 levels and in-hospital mortality rates in stroke patients[169, 170]. There has recently been a new focus on brain derived IL-6 where it appears to contribute to neoangiogenesis and neuronal survival through STAT3 activation and manganese-superoxide dismutase[171, 172].

IL-10, an anti-inflammatory cytokine, acts by inhibiting proinflammatory cytokines such as IL-1 and TNF-α and also by suppressing cytokine receptor expression and activation. It is mostly produced by microglia and astrocytes, and is upregulated in experimental stroke[173]. Both exogenous administration[174] and

gene transfer of IL-10[175] in cerebral ischemia models appear to have beneficial effects. Patients with acute ischemic stroke have elevated numbers of peripheral blood mononuclear cells secreting IL-10[176] and elevated concentrations in cerebrospinal fluid[177]. Furthermore, subjects with low IL-10 levels have an increased risk of stroke[178].

TGF-β1 has been observed in microglia and astrocytes, with low levels in neurons[179]. Over expression of TGF-β1 using an adenoviral vector protected mouse brains from ischemic stroke and reduced the accompanying inflammatory response[180]. A recent study showed that cultured neurons may be protected from ischemia-like insults by microglia-secreted TGF-β1[181].

5.2 Chemokines

Chemokines are chemotactic cytokines and, together with their receptors expressed on leukocytes, they play a crucial role in the extravasation and migration of leukocytes under inflammatory conditions. Chemokines are expressed by injured neurons, astrocytes, microglia, and endothelial cells, as well as circulating immune cells.[46, 86, 182, 183] Different classes of chemokines are differentiated by their structures, the main classes being CXC or CC, C, CX3C. The "Cs" refer to the two N-terminal cysteine residues, and the classes are divided depending on whether there is an amino acid between them (CXC), or whether they lie adjacently (CC) with which chemokines act through specific and shared receptors belonging to the superfamily of G-protein-coupled receptors[184, 185]. The CXC subfamily can be

further split into ELR+ or ELR− groups based on whether the glutamate–leucine–arginine motif is present between the N-terminus and the first cysteine [185, 186]. Members of the chemokine superfamily tend to bind to several receptors, and a chemokine receptor possibly binding multiple ligands[187]. However, the ELR+ CXC chemokine subfamily are thought to be mainly neutrophil chemoattractants, whereas the CC chemokines more typically attract monocytes and T lymphocytes[46, 188]. Several chemokines in CXC group have been shown to participate in stroke pathogenesis[44, 189] and are thought to have a deleterious role by increasing leukocyte infiltration[100]. Thus, chemokine ligands and receptors are potential therapeutic targets. Brait el al. [44] showed large increases in expression (by 10- to 300-fold) of key members of the ELR+ CXC chemokine subfamily, the neutrophil receptor CXCR2, and its ligands CXCL1 and CXCL2 which are mostly reached maximum at 24 to 72 hours. As predicted, pharmacological inhibition of CXCR2 following transient focal ischemia prevented the increases in the expression of these genes as well as neutrophil infiltration into the brain; however, this treatment had no effect on functional outcome, infarct or edema volume at 72 h after stroke.

Recent data also showed that CC chemokines such as monocyte chemoattractant protein-1 (MCP-1, CCL2), macrophage inflammatory protein-1α (MIP-1α, CCL-3), regulated on activation, normal T-cell expressed and secreted (RANTES, CCL5), and macrophage inflammatory protein-3 alpha (MIP-3α) are induced in animal models of focal cerebral ischemia[86, 124, 190-192]. At baseline, MCP-1 mRNA expression was almost absent, but ischemia led to a significant

increase in MCP-1 mRNA expression in the ischemic cortex after either permanent or temporary MCAO around 12 h to 2 days and remained elevated up to 5 days [182, 183]. Consistent with a deleterious role, their inhibition or deficiency is associated with reduced injury[38], and Chen et al. [190] found that over-expression of MCP-1 in the brain exacerbated ischemic injury, and was correlated to recruitment of inflammatory cells.

Fractalkine (CX3CL1), a neuronally expressed chemokine, acts through its G-protein-coupled receptor CX3C. Following ischemia, its expression has been localized to viable neurons in the infarct periphery as well as some endothelial cells. Interestingly, expression of its receptor, CX3CR1 was observed only on microglia/macrophages suggesting that fractalkine is involved in neuron-microglial signaling[193]. Furthermore, fractalkine deficient mice have smaller infarct sizes and lower mortality after transient focal cerebral ischemia, suggesting that fractalkine somehow exacerbates cell death[194].

In addition to chemotactic properties, chemokines were found to directly affect BBB permeability. The addition of MCP-1 enhanced 17-fold the permeability of an in vitro BBB (cocultures of endothelial cells and astrocytes) model and caused alterations in tight junction (TJ) proteins, suggesting that MCP-1 may play a role in 'opening' the BBB[195].

With recent interest in the area of cell based therapy for stroke, chemokines may also play an important role in homing stem cells to regions of injury[196].

MCP-1 and SDF-1 and/or their receptors have been observed at the interface of ischemic tissue and cell transplants[197]. MCP-1 and other chemokines seem to be involved in marrow derived stromal cell migration into ischemic brain[198-200]. Manipulation of these signals may be important in the successful application of such therapies.

5.3 Arachidonic acid metabolites

Downstream of immune cell activation, the arachidonic acid (AA) cascade is initiated via the release of phospholipase A2 (PLA2)[23]. Energy failure due to cessation of blood flow can result in calcium accumulation in brain cells. This high concentration calcium activates PLA2 which hydrolyses glycerophospholipids to release AA. Following transient MCAO, PLA2 activity significantly increased[201]. As potent mediators, AA metabolites contribute to post-ischemic brain inflammation and circulatory disorders[202]. Consistent with a damaging role of this pathway, PLA2 deficient mice had smaller infarcts and developed less brain edema with fewer neurological deficits than their wild type littermates[203]. AA is metabolized through two different pathways via Cyclooxygenase (COX) pr lipoxygenase (LOX).

5.3.1 Cyclooxygenase pathways

Arachidonic acid released from brain phospholipids during ischemia/reperfusion is converted to prostaglandin H2 (PGH2) by cyclooxygenase (COX). There are two isoforms of COX. COX-1 is constitutively expressed in many

cells types, including microglia and leukocytes during brain injury[204]. COX-1 deficient mice have increased vulnerability to brain ischemia, and would support a protective role possibly due to an effect on maintaining cerebral blood flow[205]. However, conflicting data exist. In transient global cerebral ischemia, pharmacologic inhibition of COX-1 with valeryl salicylate increased the number of healthy neurons in the hippocampal CA1[206]. These discrepancies may be due to differences in the immune responses between focal and global cerebral ischemia models.

COX-2, a rate-limiting enzyme for prostanoid synthesis, is upregulated and present at border of the ischemic territory following ischemia[207]. In postmortem specimens of ischemic stroke patients, COX-2 is upregulated not only in regions of ischemic injury[208], but also regions remote from the infarct area[209]. The roles of various COX metabolites are protean, but accumulated data suggest that those downstream of COX-2 are likely deleterious. Recent work has shown that prostaglandin E (2) EP1 receptors may be the downstream effectors responsible for neurotoxicity in ischemic stroke[210]. Several studies have now shown that treatment with COX-2 inhibitors improve neurological outcome after stroke[207, 211]. Furthermore, COX-2 deficient mice have reduced injury after N-methyl-D-aspartate (NMDA) exposure[212], whereas COX-2 over expression exacerbates brain injury[213]. Interestingly, COX-2 mediates its toxic effect through PGE2 rather than ROS, even though COX-2 can generate both[214].

5.3.2 5-Lipoxygenase pathway

Compared to the COX pathway, there is limited knowledge about the role of the lipoxygenase pathway in brain ischemia. AA can be converted to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) by 5-lipoxygenase (5-LOX) which is metabolized to leukotriene A4 (LTA4), a precursor of cysteinyl leukotrienes (cysLTs). LTC4 is a potent chemoattractant that has been implicated in the BBB dysfunction, edema and neuronal death after ischemia/reperfusion. During brain ischemia/reperfusion, biphasic AA and LTC4 elevations have been documented and appear to correspond to biphasic patterns of BBB disruption[215]. 5-LOX has also been documented in autopsied ischemic human brains, with 5-LOX localizing to perivascular monocytes [216]. Pretreatment with a 5-LOX inhibitor, AA861 resulted in significant attenuation of LTC4 levels and reduction in brain edema and cell death[217]. Furthermore, OGD-induced PC12 cell death was attenuated by 5-LOX inhibitor caffeic acid[218]. However, 5-LOX deficient mice had similar infarct sizes 6 days after both permanent and transient MCAO to wild type mice [219]. These conflicting observations may reflect the different times when assessments were made, but clearly, more work is needed in this area.

5.4 Nitric oxide/nitric oxide synthase

Oxidative stress can damage the organism if the physiological balance between oxidants and anti-oxidants is disrupted in favor of the former. Nitric oxide (NO) is an important signaling molecule involved in physiological processes such as neuronal communication, host defense, regulation of vascular tone, and inhibitor of platelet aggregation and leukocyte adhesion. This relatively stable gas readily

diffuses into cells and cell membranes where it reacts with molecular targets. Three nitric oxide synthases (NOS) isoforms exist; endothelial NOS (also known as eNOS, type III, NOS-III and NOS-3), neuronal NOS (also known as nNOS, type I, NOS-I and NOS-1), and inducible NOS (also known as iNOS, type II, NOS-II and NOS-2) are made from the L-arginin. Among these three isoforms, iNOS is especially relevant to inflammatory cells and may contribute to ischemic injury via NO. In fact, iNOS expression is thought to be restricted to cells involved in inflammatory responses such circulating leukocytes, microglia and astrocytes. In the brain, ischemia-induced upregulation of iNOS mRNA and protein is associated with increases in iNOS enzymatic activity and NO production[220-222]. NO may cause DNA damage in cerebral ischemia through the formation of peroxynitrite[223-225]. Pharmacological inhibition of iNOS reduces infarct volume by about 30%[221], and iNOS-null mice have smaller infarcts and better neurologic outcomes than wild-type control animals[226]. Consistent with a damaging role, protection by hypothermia is associated with reduced microglial generation of both NO and iNOS[227], and ischemic brain protection by estrogen and progesterone appears to be through modulating post-ischemic iNOS expression[228, 229].

5.5 Reactive oxygen species

Generation of reactive oxygen species (ROS) by inflammatory cells occurs via several enzyme systems. Superoxide is generated via COX, xanthine dehydrogenase, xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, whereas myeloperoxidase (MPO) and monoamine oxidase (MAO) generate

hypochlorous acid and H_2O_2. Among all the oxidants in the brain parenchyma after MCAO, superoxide anion is a major one, causing direct injury to ischemic brain or by reacting with NO to generate peroxynitrite[230].

NADPH oxidase (NOX) was originally identified in immune cells as playing an important microbicidal role. NOX is a multi-component enzyme comprising a cytoplasmic subunits ($p45^{phox}$, $p67^{phox}$, and $p40^{phox}$ and Rac2) and upon phosphorylation, these subunits can form a complex and translocate to the plasma membrane to dock with the plasma membrane subunits ($p91^{phox}$, $p22^{phox}$)[231] in order to transfer electrons from NADPH to oxygen to form superoxide. NOX has been documented to increase in the brain after experimental stroke[232] and we have shown that NOX derived from circulating cells contributes significantly to stroke pathogenesis compare to the brain resident cells[233]. Walder el al. [234] has shown that mice deficient in the gp91 subunit of NOX2 have smaller infarcts than wild type mice and recent work has also shown that microglia potentiate injury to the blood brain barrier due to superoxide produced by NOX2 in brain ischemia models[235]. NOX also appears to be an important factor in exacerbating stroke outcome due to hyperglycemia[236].

Myeloperoxidase (MPO), an enzyme in leukocytes such as neutrophils and monocytes, is thought to mediate bactericidal killing through H_2O_2 and hypochlorous acid. MPO activity is normally used as a marker of polymorphonuclear neutrophil infiltration, and its upregulation in blood may predict the early risk of infarction. MPO has been documented in both permanent

and transient MCAO[38]. However, after focal cerebral ischemia, infarct size was increased in MPO deficient mice[237], suggesting a beneficial role. MPO deficient mice also had increased products of nitrosylation within the ischemic brain and suggested that MPO's protective effect may be due to its ability to scavenge nitrotyrosine (a by product of peroxynitrite reactions) in the presence of glutathione (Takizawa et al., 2002). Therefore, it is possible that MPO may actually limit the extent of ROS-mediated tissue injury.

5.6 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are essential proteases that break down components of extracellular proteins, such as collagen. Physiologically, these proteases are involved in tissue development, wound healing, cone growth, ovulation and angiogenesis, however, after brain ischemia, MMPs are upregulated, activated, and involved in neuroinflammatory response and also in extracellular matrix remodeling. MMPs are normally found in the cytosol in a pro- or inactivated state, but are cleaved by proteases such as plasmin or other MMPs to their active state[238]. The only evidence-based treatment available for stroke is recombinant tissue plasminogen activator (r-tPA), and tissue plasminogen activator (tPA) has been shown to disrupts components of the BBB following MMP-9 activation resulting in hemorrhagic transformation [239, 240]. As a major source of the MMPs following ischemia, microglia are also necessary to stimulate astrocytes to generate active MMPs[241]. In models of focal ischemia, MMP-9 activity increases at early time points (15-48 h) and returns to baseline around 15 days, followed by MMP-2

(peaks at 5 days and return to baseline around 15 days)[242, 243]. Functionally similar to IL-1β, MMPs induce apoptotic neuronal cell death by TNF-α and FasL processing.

In experimental stroke models, MMP inhibition reduces infarct size, brain edema and hemorrhage[244]. Mice deficient in MMP-9 had smaller infarcts compared to wildtype controls[245]; however, such an effect was not observed in MMP-2 deficient mice[246], suggesting that MMP-9 may be primarily involved with edema, while MMP-2 may correlate more with neovascularization[247]. Peripheral inflammatory cells, rather than brain derived MMP-9 may contribute significantly to ischemic brain injury as mice transplanted with bone marrow from MMP-9 deficient mice suffer less injury and less BBB disruption than mice transplanted with marrow containing intact MMP-9[248]. Current work also showed that MMPs may be involved in endogenous mechanisms of neurogenic migration because a broad spectrum MMP inhibitor GM6001 was found to significantly decrease the migration of doublecortin-positive cells that extend from the SVZ into the striatum in transient focal cerebral ischemia in mice[249].

Interestingly, MMPs seem to play a different role in the later phases of cerebral ischemia, and may participate in plasticity and recovery. For instance, MMPs are known to associate with factors involved in angiogenesis such as vascular endothelial growth factor (VEGF). In one study, treatment with the MMP inhibitor FN-439 7 d post MCAO suppressed neurovascular remodeling, increased

ischemic brain injury and impaired functional recovery at 14 days. This was also associated with reduced VEGF signaling resulting from MMP inhibition[250].

6. Transcriptional regulation of inflammation

It is now well recognized that cerebral ischemia upregulates gene expression. Activation of several transcription factors has been documented in experimental stroke models. Some of these transcription factors are particularly involved in the inflammatory response, and will be discussed here.

6.1 Nuclear factor □B (NF-□B)

Nuclear factor-kappaB (NF-□B), involved in the regulation of inflammation[251], is a dimeric transcription factor consisting of subunits of the Rel family, which includes five Rel forms: Rel (cRel), RelA (p65), RelB, NF-κB1 (p50 and its precursor p105) and NF-κB. The most common form of NF-κB is a heterodimer composed of Rel A (p65) and p50. NF-κB is normally located in the cytoplasm bound to its endogenous inhibitor protein, known as IκB, a family of proteins consisting of IκB-α, IκB-β, IκB-γ and IκB-ε. Phosphorylation of IκB-α at serines 32 and 36 by an upstream IκB kinase (IKK) leads to IκB phosphorylation, ubiquitination and degradation in the 26S proteasome. This liberates NF-κB and allows it to translocate to the nucleus, and bind to κB sites, specific domains within the promoters of downstream genes to activate their transcription. Many genes involved in inflammation contain functional κB sites, such as tumor necrosis factor-α (TNF-α), intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), iNOS

and interleukin-6 (IL-6). Han et al. found that IKK and NF-κB activation were correlated to the anti-inflammatory effect of mild hypothermia following experimental stroke[252]. However, the function of NF-κB in stroke is still controversial[253]. Mice deficient in NF-κB's p50 subunit are protected from experimental stroke[254], consistent with a death-promoting role of NF-κB in focal ischemia. Similar observations were made by inhibiting activation of NF-κB with the treatment of S-nitrosoglutathione (GSNO), an NO modulator[116]. After deleting IkappaB kinase complex IKK, the central component of NF-kappaB activation, inhibition of IKK activity markedly reduced infarct size in a mouse model of stroke[255]. In contrast, constitutive activation of IKK2 enlarged the infarct size[255]. In global ischemia, neuronal damage was significantly attenuated by employing the nuclear factor-kappa B decoy oligodeoxynucleotides into rat brain neurons through the carotid artery[256]. However, rats given diethyldithiocarbamate (DDTC), a NF-κB inhibitor, had enhanced neuronal DNA fragmentation and larger infarct sizes compared to controls, suggesting a beneficial role[257], and. The reasons for these discrepancies are not clear, but could be due to the cell type in which NFkB is activated, the experimental model studied, or a lack of specificity of pharmacological inhibitors.

6.2 Mitogen-activated protein kinase (MAPK)

Mitogen-activated protein kinases (MAPK) play an important role in transducing stress-related signals by a cascade of intracellular kinase phosphorylation and transcription factor activation that regulate inflammatory

gene production among other functions[3, 258]. During cerebral ischemia, three interlinked signaling pathways have been documented: the stress-activated protein kinases/c-Jun N-terminal kinases (SAPK/JNK), the p38 MAPKs and extracellular signal-regulated kinases (ERKs)[258-260]. p38 MAPK promotes the stabilization and enhanced translation of mRNAs encoding proinflammatory proteins[261]. In forebrain ischemia, MLK3-MKK4-JNK activation was rapidly increased with peaks both at 30 min and 3 days of reperfusion[262]. Intracerebroventricular infusion of POSH (plenty of SH3s) antisense oligodeoxynucleotides (AS-ODNs) not only significantly decreased POSH interactions with MLK3, MKK4 and phospho-JNKs, but also attenuated the activation of the JNK signaling pathway as well as significantly increased the neuronal density in the CA1 region. This type of protective effect of POSH AS-ODNs on ischemic injury might be through a mechanism of inhibiting the MLK3-MKK4-JNK signaling pathway and c-Jun activation, which strongly suggests the involvement of MAPK. In a model of in vitro ischemia in astrocytes, introduction of bone marrow stromal cells activated MAPK, and subsequently exerted protection to astrocytes[263]. In permanent MCAO, CDP-choline, a major neuronal membrane lipid precursor, showed a key role in recovery after ischemic stroke by a notable reduction in the phosphorylation of MAP-kinase family members, ERK1/2 and MEK1/2, as well as Elk-1 transcription factor. Following forebrain ischemia in rodents, phosphorylated p38 MAPK was detected in the hippocampus within neuron-[260] and microglia-like[264] cells, suggesting its role in the endogenous inflammatory response. Furthermore, p38 MAPK inhibitors

have been shown to reduce brain injury and neurological deficits in focal cerebral ischemia as well as ischemia-induced cytokine expression[3].

7. Initiation of innate immune responses

The initiation of the immune response following stroke is still not fully clear, but recent studies have focused on various pro-inflammatory factors elaborated by the ischemic brain that might act on receptors involved in innate immune responses. The two main groups of innate immune receptors studied in brain ischemia are found on microglia and circulating immune cells, and include the Toll-like receptors (TLRs) and purinergic receptors. The ischemic brain is thought to generate extracellular nucleic acid following cell lysis. These and other ligands are often referred to as danger associated molecular pattern molecules (DAMPs). When bound to their respective ligands, an inflammasome is formed consisting of nucleic acids such ATP, UTP, adenosine and other pro-inflammatory molecules such as caspase 1, leading to the maturation and elaboration of pro-inflammatory cytokines and a full blown inflammatory response [14, 265] Toll-like receptors (TRLs) and purinergic receptors are widely expressed on microglia.[14, 266].

7.1 Toll-like receptors

Toll-like receptors (TLRs) are pattern recognition receptors that recognize exogenous pathogen-associated molecular patterns (PAMPs) and endogenous DAMPs. They have been the focus of recent investigation, and are considered to

play critical roles in the initiation of the immune response in stroke and related injuries[267, 268]. TLRs have traditionally been found on immune cells, but they have also been described in various cell types of the central nervous system (CNS), including microglia, astrocytes, neurons, and cerebral vascular cells[269, 270]. Reports in the brain ischemia literature indicate that TLRs are most likely activated by DAMPs, such as heat shock proteins, high mobility group box 1 protein (HMGB1) [271], extracellular peroxiredoxin[272] and nucleic acids[14].

Activation of microglial cells in response to cerebral ischemia is associated with signaling through several TLRs, especially TLR2 and TLR4. Activation of TLRs can cause increased proinflammatory cytokine expression, leading to inflammatory immune responses and neuronal damage. Brain TLR2 expression is increased in transient and permanent focal ischemia models as well as in vitro ischemia models[273-275]. TLR2-related genes with pro-inflammatory and pro-apoptotic capabilities, such as NF-κB, Cyclooxygenase-2 (COX2), IL-1β, IL-17, IL-23, and TNF-α were induced after ischemia[273, 275-277]. Yao el al.[277] showed upregulation of TLR2 and IL-1β expression in an in vitro ischemia model, and this led to neuronal cell death. The ischemic insult induced IL-1β up-regulation, but cell death was abolished in TLR2 knockout mice. However, Hua et al.[278] reported that TLR 2 knockout mice had higher mortality, and increased infarct size compared to wildtype mice. Brain expression of TLR4 in ischemic brain is also up regulated[279, 280]. In some reports, TLR4, but not TLR3 or TLR9, knock-out mice had significantly smaller infarct area and better neurological function compared

with wild-type mice and TLR4 was co-localized with CD11b-positive microglial cells in the ischemic striatum and the number of CD11b-positive microglial cells was smaller in TLR4 KO mice than in wild-type mice[279, 280].

7.2 Purinergic receptors

The purinergic receptors are found in numerous cell types, both in brain and peripherally, and mediate a variety of cellular functions. The P2 purinoreceptors consist of two families: the ionotropic receptors (P2X) contain channels that permit ion flow, whereas the metabotropic receptors (P2Y) are G-protein coupled second messenger systems. In immune cells, they are involved in pro-inflammatory responses, migration, and phagocytosis[281]. The family of purinergic receptors on microglia have recently become of interest because they bind to nucleotides that may be released by injured cells, and may initiate proinflammatory signaling. The role of the purinergic receptors in the microglial inflammatory response has largely focused on P2X7, where it has been shown to modulate microglial activation following experimental brain ischemia and stroke, and its pharmacologic blockade led to decreased ischemic damage[282-285]. However, little has been studied on the Gi coupled ATP receptor, P2Y12[281, 286]. P2Y12 is present on microglia and is expressed on the membrane surface in the resting state and activated by ATP, ADP, or neighboring neurotoxicity. It promotes microglial migration toward the source of these nucleotides and involves in the phosphorylation of Akt[287]. Because P2Y12 is also the target of a widely used antiplatelet agent, clopidogrel, it is an attractive target for modulating the microglial inflammatory cascade. We have recently

shown that P2Y12 participates in ischemia related inflammation by mediating microglial migration and potentiation of neurotoxicity using P2Y12 knockout mice[288].

8. Inflammatory responses to the other organs following ischemic stroke

Recent studies indicate that inflammatory responses following stroke affect the entire body, and not simply the brain. For example, splenectomy has been shown to confer neuroprotection against experimental stroke. The spleen is an important lymphatic organ, and sequesters red and white blood cells. It also synthesizes antibodies in its white pulp and removes antibody coated blood cells from blood and lymph node circulation. Ajmo et al. [289] have shown that removal of the spleen significantly reduced neurodegeneration after brain ischemia. Rats splenectomized 2 weeks before permanent middle cerebral artery occlusion had a >80% decrease in infarction volume and splenectomy also resulted in decreased numbers of activated microglia, macrophages, and neutrophils present in the brain tissue. They concluded that these results demonstrate that the peripheral immune response as mediated by the spleen is a major contributor to the inflammation that enhances ischemic damage after stroke

Yet, evidence suggests that stroke renders the body in a state of immunodepression which could be detrimental. Soon after stroke, circulating immune cells are quickly reduced, thus increasing the risk of developing infections.

This systemic immunodepression occurs as early as 12 hours after ischemic stroke, and may continue out to several weeks[12, 290, 291]. This phenomenon involves reduced numbers of T cells and other immune cells present in the spleen, thymus, liver and lymph node[12, 154, 290-292], and is considered to be mediated by hyperactivity of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis (HPA)[12, 293]. This leads to increased apoptosis of immune cells in these organs and as a result, these secondary lymphatic organs undergo atrophy[12, 154, 290]. However, it has yet to be shown whether the infiltration of these cells into the brain is what contributes to the lower circulating cell numbers.

As a consequence of this phenomenon, infectious complications often arise after stroke, predominantly lung and urinary tract infections in animal models[12, 154, 290] which lead to worsened outcome[294-296]. Hyperactivity of the sympathetic nervous system (SNS) and the hypothalamic pituitary axis (HPA) is thought to underlie this phenomenon, although the precise signals and mechanisms that trigger this immunodepression remain unclear. Blocking the SNS and HPA significantly reversed the percentage of apoptotic splenocytes to control levels and prevented the decrease in circulating lymphocytes, bacterial infections and mortality following stroke in the animal model[12, 293]. Consistent with experimental reports, several recent clinical studies have found evidence that SNS-mediated stroke-induced immunodepression and subsequent susceptibility to post stroke infections also occurs in patients. Chamorro et al.[4] found that acute ischemic stroke is associated with an early activation of the sympathetic

adrenomedullar pathway that lowers the threshold of infection and increases the mortality. Urra et al.[297] reported increased apoptosis and a reduction in the circulating levels of T and B lymphocytes following stroke. They also found a correlation between SNS and HPA activation, lower level of T lymphocytes and infection. In addition to this, another pathway of communication between the nervous and immune systems, known as the vagal cholinergic anti-inflammatory pathway has been indentified. When the vagus nerve is activated by pro-inflammatory cytokines, it releases acetylcholine, which results in inhibition of the release of more pro-inflammatory mediators by macrophages[298, 299]. Experimental studies have shown that vagal nerve signaling inhibits the release of pro-inflammatory cytokines and improves outcomes following different models of ischemic stroke[299]. This vagal cholinergic anti-inflammatory pathway is another potential mediator of immunodepression.

9. Bench to bedsides: trials, tribulations and promising therapies

9.1 Clinical trials

Following several promising preclinical studies, a few clinical trials were carried out to determine whether anti-inflammatory strategies were beneficial (Table. 1). However, some of these trials did not meet with success either due to unanticipated effects of the agents tested or inappropriate study design [300-302]. Yet, with increased knowledge of the complexities of inflammation in stroke, a few treatments may be on the horizon.

9.1.1 Anti-integrin

In order to limit the leukocyte adhesion and migration into the infarct brain, a few clinical studies examined the potential of anti-integrin therapies in acute stroke patients. In one study, a humanized CD11/CD18 antibody (LeukArrest) was given to patients within 12 h of symptom onset[301]. Another trial was a phase IIb dose escalation study of a nonantibody peptide, recombinant neutrophil inhibiting factor (rNIF) in stroke patients (Acute Stroke Therapy by Inhibition of Neutrophils or ASTIN) administered within 6 h of symptom onset[303]. Both studies were terminated prematurely due to a lack of effect on predetermined endpoints. Although both compounds appeared to be effective in rodent stroke models [39, 137], lack of an obvious effect in humans could be due to study design not in line with laboratory data (such as late treatment or lack of documented reperfusion in humans) or the inherent heterogeneity of clinical stroke. Another possibility is that changes in neutrophil integrins are different in acute ischemic stroke patients compared to rodents. For example, CD11b is actually decreased in human stroke [304], but increased after experimental stroke in rats[305]. Therefore, some anti-adhesion approaches may not be appropriate in humans. Regardless, it is clear that more work and possibly improved trial design are needed.

9.1.2 Anti-ICAM1

A phase III clinical trial of anti-ICAM (enlimomab) therapy for stroke indicated that anti-ICAM therapy with Enlimomab was not an effective treatment for ischemic

stroke[300, 302]. In fact, treatment significantly worsened the outcome. However, the interpretation of this study may have been confounded by several explanations. First, this study adopted the use of a murine antibody to humans, which may influence the unnecessary immune reaction with subsequent neutrophil and complement activation. This is also to be said to the above mentioned anti-integrin trials that the therapeutic antibodies interfered with endogenous immunoregulatory defenses to promote the development of clinically significant infections and fever, thereby negating any potential cerebroprotective effects. This deleterious immunomodulation is not entirely unexpected, because ICAM-1 and integrin are critical to numerous host defenses such as leukocyte adhesion, diapedesis, oxidative burst, and selectin expression[99]. Second, proinflammatory microvascular failure leading to "no-reflow" might be important in rodent stroke, yet of limited relevance to primate stroke, due to differences in cerebrovascular collateralization[306].

9.1.3. Minocycline

Minocycline is a member of the tetracycline family of antibiotics with recently recognized anti-apoptotic, anti-inflammatory properties, reduction of microglial activation, MMP reduction, and NO production. The anti-apoptotic properties of Minocycline appear to be due to its ability to inhibit caspase-3[307], whereas its anti-inflammatory effect appears to be due to a mechanism inhibiting MAPK activation in microglia[308]. As such, Minocycline has been shown to protect the brain against ischemic insults and improve functional impairment[78, 309-311].

According to these results, Lampl et al. [312] have conducted a clinical trial using oral Minocycline administration to the ischemic patients and found that both NIHSS and mRS were significantly lower and BI scores were significantly higher in minocycline-treated patients. This pattern was already apparent on day 7 and day 30 of follow-up. Deaths, myocardial infarctions, recurrent strokes, and hemorrhagic transformations during follow-up did not differ by treatment group. However, this study was an open-labeled, evaluator-blinded study and the total number of the patients was relatively small. A second study established safety, dose ranging and feasibility in combination with rt-PA[313]. Recently, this group also reported that intravenous Minocycline administration could decrease the plasma matrix metalloproteinase-9 in the stroke patients[314]. However, clinical studies to determine minocycline's efficacy in stroke and long term recovery (Neuroprotection with minocycline therapy for acute stroke recovery trial, NeuMAST) has recently terminated because interim analysis showed futility.

9.2 Future Therapies

9.2.1 FTY 720

FTY720 (Fingolimod) is a novel immunomodulatory agent, which in its phosphorylated form acts as a high affinity agonist of Sphingosine-1-phosphate (S1P) receptors[328, 329]. It became the first oral drug to be FDA-approved for clinical use in the treatment of multiple sclerosis. FTY720 readily crosses the blood-brain barrier and exerts a number of direct effects in the central nervous

system. FTY720 is phosphorylated by Sphingosine kinase (SphK), mainly by SphK2[330, 331], into the active compound phospho-FTY720, which then acts on 4 of the 5 known S1P receptor subtypes ($S1P_1$, $S1P_3$, $S1P_4$, $S1P_5$), and shows neuroprotective effect against many central nervous system disease including cerebral ischemia[331-335] . Mechanisms include regulation of myelination and microglial activation following injury, proliferation and migration of neural precursor cells toward injury sites, and potentiation of growth-factor regulated neuronal differentiation, survival, and process extension, and also antiapoptotic and anti-inflammatory pathways[334-340]. FTY720 also exerts immunomodulatory actions by affecting lymphocyte production, trafficking, and apoptosis through S1P receptors which induces a depletion of circulating lymphocytes by preventing the egress of lymphocytes from the lymph nodes. Mechanistically, this is due to a down regulation of the S1P type 1 receptor (S1P1). Expression levels of endothelial adhesion molecules such as E-selectin, P-selectin, ICAM-1 or VCAM-1 were shown to be induced by FTY720 treatment, and therefore might contribute to the prevention of early infiltration of neurotrophils and activation of microglia/macrophages. These findings suggest that anti-inflammatory mechanisms, and possibly vasculoprotection, rather than direct effects on neurons, underlie the beneficial effects of fingolimod after stroke. Most of the past reports have shown beneficial effect of S1P in the field of ischemia, but by contrast, Liesz et al.[58] showed opposite results. These authors found that S1P treatment did show a reduction of lymphocyte brain invasion but could not achieve a significant

reduction of infarct volumes and behavior dysfunction[58]. Liu et al.[341] recently published a systematic meta-analysis of the efficacy of FTY720 in animal model of stroke. In this study, they concluded that FTY720 reduced infarct volume and improve functional outcome. However, the authors also indicated that more experimental studies should be performed to evaluate the safety of FTY720 in the future. Thus, taken this recent scientific highlights together, it is obvious that S1P receptor pathways and sphingolipids regulating enzymes are a highly promising target in stroke treatment.

10. Brief Summary

Inflammation following ischemic stroke is increasingly recognized as a key element in its progression. In this review we have focused on many key players which are involved in the inflammatory responses following stroke, such as cellular components including microglia and leukocytes, adhesion molecules, inflammatory mediators and chemokines. Although, early inflammatory responses may potentiate ischemic injury, late responses may be important in recovery and repair. However, the precise mechanisms of the inflammatory responses are still to be elucidated. And future work and better understanding against this field will shed light on new therapeutic methods to the ischemic stroke.

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12. References

[1] Durukan, A.; Tatlisumak, T. *Pharmacol Biochem Behav*, **2007**, *87*, 179-97.
[2] *N Engl J Med*, **1995**, *333*, 1581-7.
[3] Barone, F. C.; Feuerstein, G. Z. *J Cereb Blood Flow Metab*, **1999**, *19*, 819-34.
[4] Chamorro, A.; Hallenbeck, J. *Stroke*, **2006**, *37*, 291-3.
[5] Davies, C. A.; Loddick, S. A.; Stroemer, R. P.; Hunt, J.; Rothwell, N. J. *Exp Neurol*, **1998**, *154*, 199-212.
[6] Becker, K. J. *Curr Opin Neurol*, **1998**, *11*, 45-9.
[7] Stanimirovic, D.; Shapiro, A.; Wong, J.; Hutchison, J.; Durkin, J. *J Neuroimmunol*, **1997**, *76*, 193-205.
[8] Morioka, T.; Kalehua, A. N.; Streit, W. J. *J Comp Neurol*, **1993**, *327*, 123-32.
[9] Suzuki, H.; Abe, K.; Tojo, S.; Morooka, S.; Kimura, K.; Mizugaki, M.; Itoyama, Y. *Neurosci Lett*, **1997**, *228*, 151-4.
[10] Hallenbeck, J. M. *Acta Neurochir Suppl*, **1996**, *66*, 27-31.
[11] Danton, G. H.; Dietrich, W. D. *J Neuropathol Exp Neurol*, **2003**, *62*, 127-36.
[12] Prass, K.; Meisel, C.; Hoflich, C.; Braun, J.; Halle, E.; Wolf, T.; Ruscher, K.; Victorov, I. V.; Priller, J.; Dirnagl, U.; Volk, H. D.; Meisel, A. *J Exp Med*, **2003**, *198*, 725-36.
[13] Han, H. S.; Yenari, M. A. *Curr Opin Investig Drugs*, **2003**, *4*, 522-9.
[14] Iadecola, C.; Anrather, J. *Nat Med*, **2011**, *17*, 796-808.
[15] Spite, M.; Serhan, C. N. *Circ Res*, **2010**, *107*, 1170-84.
[16] Schilling, M.; Besselmann, M.; Muller, M.; Strecker, J. K.; Ringelstein, E. B.; Kiefer, R. *Exp Neurol*, **2005**, *196*, 290-7.
[17] Denes, A.; Vidyasagar, R.; Feng, J.; Narvainen, J.; McColl, B. W.; Kauppinen, R. A.; Allan, S. M. *J Cereb Blood Flow Metab*, **2007**, *27*, 1941-53.
[18] Ishikawa, M.; Zhang, J. H.; Nanda, A.; Granger, D. N. *Front Biosci*, **2004**, *9*, 1339-47.
[19] del Zoppo, G.; Ginis, I.; Hallenbeck, J. M.; Iadecola, C.; Wang, X.; Feuerstein, G. Z. *Brain Pathol*, **2000**, *10*, 95-112.
[20] Dietrich, P. Y.; Walker, P. R.; Saas, P. *Neurology*, **2003**, *60*, 548-54.
[21] Pantoni, L.; Sarti, C.; Inzitari, D. *Arterioscler Thromb Vasc Biol*, **1998**, *18*, 503-13.
[22] Yilmaz, G.; Granger, D. N. *Neurol Res*, **2008**, *30*, 783-93.
[23] Stanimirovic, D.; Satoh, K. *Brain Pathol*, **2000**, *10*, 113-26.
[24] Wang, X.; Siren, A. L.; Liu, Y.; Yue, T. L.; Barone, F. C.; Feuerstein, G. Z. *Brain Res Mol Brain Res*, **1994**, *26*, 61-8.
[25] Stanimirovic, D. B.; Wong, J.; Shapiro, A.; Durkin, J. P. *Acta Neurochir Suppl*, **1997**, *70*, 12-6.
[26] Hess, D. C.; Zhao, W.; Carroll, J.; McEachin, M.; Buchanan, K. *Stroke*, **1994**, *25*, 1463-7; discussion 1468.
[27] Howard, E. F.; Chen, Q.; Cheng, C.; Carroll, J. E.; Hess, D. *Neurosci Lett*, **1998**, *248*, 199-203.
[28] Arvin, B.; Neville, L. F.; Barone, F. C.; Feuerstein, G. Z. *Neurosci Biobehav Rev*, **1996**, *20*, 445-52.
[29] Hartl, R.; Schurer, L.; Schmid-Schonbein, G. W.; del Zoppo, G. J. *J Cereb Blood Flow Metab*, **1996**, *16*, 1108-19.
[30] Chopp, M.; Li, Y.; Jiang, N.; Zhang, R. L.; Prostak, J. *J Cereb Blood Flow Metab*, **1996**, *16*, 578-84.
[31] Wang, Q.; Tang, X. N.; Yenari, M. A. *J Neuroimmunol*, **2007**, *184*, 53-68.
[32] Nilupul Perera, M.; Ma, H. K.; Arakawa, S.; Howells, D. W.; Markus, R.; Rowe, C. C.; Donnan, G. A. *J Clin Neurosci*, **2006**, *13*, 1-8.

[33] Kim, J. S. *J Neurol Sci*, **1996**, *137*, 69-78.
[34] Huang, J.; Upadhyay, U. M.; Tamargo, R. J. *Surg Neurol*, **2006**, *66*, 232-45.
[35] Bowes, M. P.; Rothlein, R.; Fagan, S. C.; Zivin, J. A. *Neurology*, **1995**, *45*, 815-9.
[36] Clark, W. M.; Lauten, J. D.; Lessov, N.; Woodward, W.; Coull, B. M. *J Mol Neurosci*, **1995**, *6*, 43-50.
[37] Connolly, E. S., Jr.; Winfree, C. J.; Springer, T. A.; Naka, Y.; Liao, H.; Yan, S. D.; Stern, D. M.; Solomon, R. A.; Gutierrez-Ramos, J. C.; Pinsky, D. J. *J Clin Invest*, **1996**, *97*, 209-16.
[38] Garau, A.; Bertini, R.; Colotta, F.; Casilli, F.; Bigini, P.; Cagnotto, A.; Mennini, T.; Ghezzi, P.; Villa, P. *Cytokine*, **2005**, *30*, 125-31.
[39] Yenari, M. A.; Kunis, D.; Sun, G. H.; Onley, D.; Watson, L.; Turner, S.; Whitaker, S.; Steinberg, G. K. *Exp Neurol*, **1998**, *153*, 223-33.
[40] Zheng, Z.; Yenari, M. A. *Neurol Res*, **2004**, *26*, 884-92.
[41] Li, G. Z.; Zhong, D.; Yang, L. M.; Sun, B.; Zhong, Z. H.; Yin, Y. H.; Cheng, J.; Yan, B. B.; Li, H. L. *Scand J Immunol*, **2005**, *62*, 481-6.
[42] Stevens, S. L.; Bao, J.; Hollis, J.; Lessov, N. S.; Clark, W. M.; Stenzel-Poore, M. P. *Brain Res*, **2002**, *932*, 110-9.
[43] Becker, K.; Kindrick, D.; Relton, J.; Harlan, J.; Winn, R. *Stroke*, **2001**, *32*, 206-11.
[44] Brait, V. H.; Rivera, J.; Broughton, B. R.; Lee, S.; Drummond, G. R.; Sobey, C. G. *Brain Res*, **2011**, *1372*, 169-79.
[45] Kleinschnitz, C.; Schwab, N.; Kraft, P.; Hagedorn, I.; Dreykluft, A.; Schwarz, T.; Austinat, M.; Nieswandt, B.; Wiendl, H.; Stoll, G. *Blood*, **2010**, *115*, 3835-42.
[46] Hurn, P. D.; Subramanian, S.; Parker, S. M.; Afentoulis, M. E.; Kaler, L. J.; Vandenbark, A. A.; Offner, H. *J Cereb Blood Flow Metab*, **2007**, *27*, 1798-805.
[47] Shichita, T.; Sugiyama, Y.; Ooboshi, H.; Sugimori, H.; Nakagawa, R.; Takada, I.; Iwaki, T.; Okada, Y.; Iida, M.; Cua, D. J.; Iwakura, Y.; Yoshimura, A. *Nat Med*, **2009**, *15*, 946-50.
[48] Yilmaz, G.; Arumugam, T. V.; Stokes, K. Y.; Granger, D. N. *Circulation*, **2006**, *113*, 2105-12.
[49] Saino, O.; Taguchi, A.; Nakagomi, T.; Nakano-Doi, A.; Kashiwamura, S.; Doe, N.; Nakagomi, N.; Soma, T.; Yoshikawa, H.; Stern, D. M.; Okamura, H.; Matsuyama, T. *J Neurosci Res*, **2010**, *88*, 2385-97.
[50] Liesz, A.; Suri-Payer, E.; Veltkamp, C.; Doerr, H.; Sommer, C.; Rivest, S.; Giese, T.; Veltkamp, R. *Nat Med*, **2009**, *15*, 192-9.
[51] Ren, X.; Akiyoshi, K.; Vandenbark, A. A.; Hurn, P. D.; Offner, H. *Metab Brain Dis*, **2011**, *26*, 87-90.
[52] Wong, C. H.; Jenne, C. N.; Lee, W. Y.; Leger, C.; Kubes, P. *Science*, **2011**, *334*, 101-5.
[53] Nadareishvili, Z. G.; Li, H.; Wright, V.; Maric, D.; Warach, S.; Hallenbeck, J. M.; Dambrosia, J.; Barker, J. L.; Baird, A. E. *Neurology*, **2004**, *63*, 1446-51.
[54] Dinkel, K.; Dhabhar, F. S.; Sapolsky, R. M. *Proc Natl Acad Sci U S A*, **2004**, *101*, 331-6.
[55] Harrison, D. G.; Guzik, T. J.; Goronzy, J.; Weyand, C. *Curr Cardiol Rep*, **2008**, *10*, 464-9.
[56] Barry, M.; Bleackley, R. C. *Nat Rev Immunol*, **2002**, *2*, 401-9.
[57] Konoeda, F.; Shichita, T.; Yoshida, H.; Sugiyama, Y.; Muto, G.; Hasegawa, E.; Morita, R.; Suzuki, N.; Yoshimura, A. *Biochem Biophys Res Commun*, **2010**, *402*, 500-6.
[58] Liesz, A.; Zhou, W.; Mracsko, E.; Karcher, S.; Bauer, H.; Schwarting, S.; Sun, L.; Bruder, D.; Stegemann, S.; Cerwenka, A.; Sommer, C.; Dalpke, A. H.; Veltkamp, R. *Brain*, **2011**, *134*, 704-20.
[59] Brait, V. H.; Jackman, K. A.; Walduck, A. K.; Selemidis, S.; Diep, H.; Mast, A. E.; Guida, E.; Broughton, B. R.; Drummond, G. R.; Sobey, C. G. *J Cereb Blood Flow Metab*, **2010**, *30*, 1306-17.
[60] Kreutzberg, G. W. *Trends Neurosci*, **1996**, *19*, 312-8.
[61] El Khoury, J.; Hickman, S. E.; Thomas, C. A.; Loike, J. D.; Silverstein, S. C. *Neurobiol Aging*, **1998**, *19*, S81-4.
[62] Thomas, W. E. *Brain Res Brain Res Rev*, **1992**, *17*, 61-74.
[63] Lai, A. Y.; Todd, K. G. *Can J Physiol Pharmacol*, **2006**, *84*, 49-59.

[64] Zhang, Z.; Chopp, M.; Powers, C. *Brain Res*, **1997**, *744*, 189-98.
[65] Schubert, P.; Morino, T.; Miyazaki, H.; Ogata, T.; Nakamura, Y.; Marchini, C.; Ferroni, S. *Ann N Y Acad Sci*, **2000**, *903*, 24-33.
[66] Saito, S.; Matsuura, M.; Tominaga, K.; Kirikae, T.; Nakano, M. *Eur J Biochem*, **2000**, *267*, 37-45.
[67] Beschorner, R.; Schluesener, H. J.; Gozalan, F.; Meyermann, R.; Schwab, J. M. *J Neuroimmunol*, **2002**, *126*, 107-15.
[68] Lehnardt, S.; Massillon, L.; Follett, P.; Jensen, F. E.; Ratan, R.; Rosenberg, P. A.; Volpe, J. J.; Vartanian, T. *Proc Natl Acad Sci U S A*, **2003**, *100*, 8514-9.
[69] Zhang, R. L.; Chopp, M.; Zaloga, C.; Zhang, Z. G.; Jiang, N.; Gautam, S. C.; Tang, W. X.; Tsang, W.; Anderson, D. C.; Manning, A. M. *Brain Res*, **1995**, *682*, 182-8.
[70] Stoll, G.; Jander, S.; Schroeter, M. *Prog Neurobiol*, **1998**, *56*, 149-71.
[71] Dirnagl, U.; Iadecola, C.; Moskowitz, M. A. *Trends Neurosci*, **1999**, *22*, 391-7.
[72] Clausen, B. H.; Lambertsen, K. L.; Babcock, A. A.; Holm, T. H.; Dagnaes-Hansen, F.; Finsen, B. *J Neuroinflammation*, **2008**, *5*, 46.
[73] Wood, P. L. *Neurol Res*, **1995**, *17*, 242-8.
[74] Zhang, N.; Komine-Kobayashi, M.; Tanaka, R.; Liu, M.; Mizuno, Y.; Urabe, T. *Stroke*, **2005**, *36*, 2220-5.
[75] Gunther, A.; Kuppers-Tiedt, L.; Schneider, P. M.; Kunert, I.; Berrouschot, J.; Schneider, D.; Rossner, S. *Eur J Neurosci*, **2005**, *21*, 3189-94.
[76] Yu, Y. M.; Kim, J. B.; Lee, K. W.; Kim, S. Y.; Han, P. L.; Lee, J. K. *Stroke*, **2005**, *36*, 2238-43.
[77] Yrjanheikki, J.; Keinanen, R.; Pellikka, M.; Hokfelt, T.; Koistinaho, J. *Proc Natl Acad Sci U S A*, **1998**, *95*, 15769-74.
[78] Yrjanheikki, J.; Tikka, T.; Keinanen, R.; Goldsteins, G.; Chan, P. H.; Koistinaho, J. *Proc Natl Acad Sci U S A*, **1999**, *96*, 13496-500.
[79] Giulian, D.; Corpuz, M.; Chapman, S.; Mansouri, M.; Robertson, C. *J Neurosci Res*, **1993**, *36*, 681-93.
[80] Huang, W. C.; Qiao, Y.; Xu, L.; Kacimi, R.; Sun, X.; Giffard, R. G.; Yenari, M. A. *Anat Cell Biol*, **2010**, *43*, 325-31.
[81] Watanabe, H.; Abe, H.; Takeuchi, S.; Tanaka, R. *Neurosci Lett*, **2000**, *289*, 53-6.
[82] Lalancette-Hebert, M.; Gowing, G.; Simard, A.; Weng, Y. C.; Kriz, J. *J Neurosci*, **2007**, *27*, 2596-605.
[83] Weston, R. M.; Jones, N. M.; Jarrott, B.; Callaway, J. K. *J Cereb Blood Flow Metab*, **2007**, *27*, 100-14.
[84] Frank-Cannon, T. C.; Alto, L. T.; McAlpine, F. E.; Tansey, M. G. *Mol Neurodegener*, **2009**, *4*, 47.
[85] Benveniste, E. N. *Cytokine Growth Factor Rev*, **1998**, *9*, 259-75.
[86] Che, X.; Ye, W.; Panga, L.; Wu, D. C.; Yang, G. Y. *Brain Res*, **2001**, *902*, 171-7.
[87] Pekny, M.; Nilsson, M. *Glia*, **2005**, *50*, 427-34.
[88] Kadhim, H. J.; Duchateau, J.; Sebire, G. *J Intensive Care Med*, **2008**, *23*, 236-49.
[89] Nowicka, D.; Rogozinska, K.; Aleksy, M.; Witte, O. W.; Skangiel-Kramska, J. *Acta Neurobiol Exp (Wars)*, **2008**, *68*, 155-68.
[90] Zhu, Y.; Roth-Eichhorn, S.; Braun, N.; Culmsee, C.; Rami, A.; Krieglstein, J. *Brain Res*, **2000**, *866*, 286-98.
[91] Dong, Y.; Benveniste, E. N. *Glia*, **2001**, *36*, 180-90.
[92] Endoh, M.; Maiese, K.; Wagner, J. *Brain Res*, **1994**, *651*, 92-100.
[93] Hewett, S. J.; Muir, J. K.; Lobner, D.; Symons, A.; Choi, D. W. *Stroke*, **1996**, *27*, 1586-91.
[94] Donohue, P. J.; Richards, C. M.; Brown, S. A.; Hanscom, H. N.; Buschman, J.; Thangada, S.; Hla, T.; Williams, M. S.; Winkles, J. A. *Arterioscler Thromb Vasc Biol*, **2003**, *23*, 594-600.

[95] Yepes, M.; Brown, S. A.; Moore, E. G.; Smith, E. P.; Lawrence, D. A.; Winkles, J. A. *Am J Pathol*, **2005**, *166*, 511-20.
[96] Saas, P.; Boucraut, J.; Walker, P. R.; Quiquerez, A. L.; Billot, M.; Desplat-Jego, S.; Chicheportiche, Y.; Dietrich, P. Y. *Glia*, **2000**, *32*, 102-7.
[97] Sughrue, M. E.; Mehra, A.; Connolly, E. S., Jr.; D'Ambrosio, A. L. *Inflamm Res*, **2004**, *53*, 497-508.
[98] Guha, M.; Mackman, N. *Cell Signal*, **2001**, *13*, 85-94.
[99] DeGraba, T. J. *Neurology*, **1998**, *51*, S62-8.
[100] Emsley, H. C.; Tyrrell, P. J. *J Cereb Blood Flow Metab*, **2002**, *22*, 1399-419.
[101] Clark, W. M.; Lessov, N.; Lauten, J. D.; Hazel, K. *J Mol Neurosci*, **1997**, *9*, 103-8.
[102] Connolly, E. S., Jr.; Winfree, C. J.; Prestigiacomo, C. J.; Kim, S. C.; Choudhri, T. F.; Hoh, B. L.; Naka, Y.; Solomon, R. A.; Pinsky, D. J. *Circ Res*, **1997**, *81*, 304-10.
[103] Kitagawa, K.; Matsumoto, M.; Mabuchi, T.; Yagita, Y.; Ohtsuki, T.; Hori, M.; Yanagihara, T. *J Cereb Blood Flow Metab*, **1998**, *18*, 1336-45.
[104] Soriano, S. G.; Coxon, A.; Wang, Y. F.; Frosch, M. P.; Lipton, S. A.; Hickey, P. R.; Mayadas, T. N. *Stroke*, **1999**, *30*, 134-9.
[105] Haring, H. P.; Berg, E. L.; Tsurushita, N.; Tagaya, M.; del Zoppo, G. J. *Stroke*, **1996**, *27*, 1386-91; discussion 1391-2.
[106] Suzuki, H.; Abe, K.; Tojo, S.; Kimura, K.; Mizugaki, M.; Itoyama, Y. *Neurol Res*, **1998**, *20*, 463-9.
[107] Zhang, R. L.; Chopp, M.; Zhang, Z. G.; Phillips, M. L.; Rosenbloom, C. L.; Cruz, R.; Manning, A. *J Cereb Blood Flow Metab*, **1996**, *16*, 1126-36.
[108] Prestigiacomo, C. J.; Kim, S. C.; Connolly, E. S., Jr.; Liao, H.; Yan, S. F.; Pinsky, D. J. *Stroke*, **1999**, *30*, 1110-7.
[109] Zhang, R. L.; Chopp, M.; Jiang, N.; Tang, W. X.; Prostak, J.; Manning, A. M.; Anderson, D. C. *Stroke*, **1995**, *26*, 1438-42; discussion 1443.
[110] Carlos, T. M.; Harlan, J. M. *Blood*, **1994**, *84*, 2068-101.
[111] Barkalow, F. J.; Goodman, M. J.; Gerritsen, M. E.; Mayadas, T. N. *Blood*, **1996**, *88*, 4585-93.
[112] Zhang, R.; Chopp, M.; Zhang, Z.; Jiang, N.; Powers, C. *Brain Res*, **1998**, *785*, 207-14.
[113] Bargatze, R. F.; Kurk, S.; Butcher, E. C.; Jutila, M. A. *J Exp Med*, **1994**, *180*, 1785-92.
[114] Huang, J.; Choudhri, T. F.; Winfree, C. J.; McTaggart, R. A.; Kiss, S.; Mocco, J.; Kim, L. J.; Protopsaltis, T. S.; Zhang, Y.; Pinsky, D. J.; Connolly, E. S., Jr. *Stroke*, **2000**, *31*, 3047-53.
[115] Huang, J.; Kim, L. J.; Mealey, R.; Marsh, H. C., Jr.; Zhang, Y.; Tenner, A. J.; Connolly, E. S., Jr.; Pinsky, D. J. *Science*, **1999**, *285*, 595-9.
[116] Khan, M.; Jatana, M.; Elango, C.; Paintlia, A. S.; Singh, A. K.; Singh, I. *Nitric Oxide*, **2006**, *15*, 114-24.
[117] Goussev, A. V.; Zhang, Z.; Anderson, D. C.; Chopp, M. *J Neurol Sci*, **1998**, *161*, 16-22.
[118] Mocco, J.; Choudhri, T.; Huang, J.; Harfeldt, E.; Efros, L.; Klingbeil, C.; Vexler, V.; Hall, W.; Zhang, Y.; Mack, W.; Popilskis, S.; Pinsky, D. J.; Connolly, E. S., Jr. *Circ Res*, **2002**, *91*, 907-14.
[119] Lehmberg, J.; Beck, J.; Baethmann, A.; Uhl, E. *J Neurol*, **2006**, *253*, 357-63.
[120] Yenari, M. A.; Sun, G. H.; Kunis, D. M.; Onley, D.; Vexler, V. *Neurol Res*, **2001**, *23*, 72-8.
[121] Chen, Y.; Ruetzler, C.; Pandipati, S.; Spatz, M.; McCarron, R. M.; Becker, K.; Hallenbeck, J. M. *Proc Natl Acad Sci U S A*, **2003**, *100*, 15107-12.
[122] Takeda, H.; Spatz, M.; Ruetzler, C.; McCarron, R.; Becker, K.; Hallenbeck, J. *Stroke*, **2002**, *33*, 2156-63.
[123] Kalinowska, A.; Losy, J. *Eur J Neurol*, **2006**, *13*, 1284-90.
[124] Yoshimoto, T.; Houkin, K.; Tada, M.; Abe, H. *Acta Neuropathol*, **1997**, *93*, 154-8.
[125] Bowes, M. P.; Zivin, J. A.; Rothlein, R. *Exp Neurol*, **1993**, *119*, 215-9.
[126] Kanemoto, Y.; Nakase, H.; Akita, N.; Sakaki, T. *Neurosurgery*, **2002**, *51*, 1034-41; discussion 1041-2.

[127] Vemuganti, R.; Dempsey, R. J.; Bowen, K. K. *Stroke*, **2004**, *35*, 179-84.
[128] Ding, C.; He, Q.; Li, P. A. *J Neuroimmunol*, **2005**, *161*, 61-7.
[129] Blann, A.; Kumar, P.; Krupinski, J.; McCollum, C.; Beevers, D. G.; Lip, G. Y. *Blood Coagul Fibrinolysis*, **1999**, *10*, 277-84.
[130] Justicia, C.; Martin, A.; Rojas, S.; Gironella, M.; Cervera, A.; Panes, J.; Chamorro, A.; Planas, A. M. *J Cereb Blood Flow Metab*, **2006**, *26*, 421-32.
[131] Zhang, L. H.; Wei, E. Q. *Acta Pharmacol Sin*, **2003**, *24*, 1241-7.
[132] Cervera, A.; Justicia, C.; Reverter, J. C.; Planas, A. M.; Chamorro, A. *J Neurosci Res*, **2004**, *77*, 565-72.
[133] Smith, C. W. *Semin Hematol*, **1993**, *30*, 45-53; discussion 54-5.
[134] Sharar, S. R.; Winn, R. K.; Harlan, J. M. *Springer Semin Immunopathol*, **1995**, *16*, 359-78.
[135] Harmon, D.; Lan, W.; Shorten, G. *Eur J Anaesthesiol*, **2004**, *21*, 973-9.
[136] Koh, S. H.; Park, Y.; Song, C. W.; Kim, J. G.; Kim, K.; Kim, J.; Kim, M. H.; Lee, S. R.; Kim, D. W.; Yu, H. J.; Chang, D. I.; Hwang, S. J.; Kim, S. H. *Eur J Neurosci*, **2004**, *20*, 1461-72.
[137] Jiang, N.; Moyle, M.; Soule, H. R.; Rote, W. E.; Chopp, M. *Ann Neurol*, **1995**, *38*, 935-42.
[138] Relton, J. K.; Sloan, K. E.; Frew, E. M.; Whalley, E. T.; Adams, S. P.; Lobb, R. R. *Stroke*, **2001**, *32*, 199-205.
[139] Liu, T.; Clark, R. K.; McDonnell, P. C.; Young, P. R.; White, R. F.; Barone, F. C.; Feuerstein, G. Z. *Stroke*, **1994**, *25*, 1481-8.
[140] Sairanen, T.; Carpen, O.; Karjalainen-Lindsberg, M. L.; Paetau, A.; Turpeinen, U.; Kaste, M.; Lindsberg, P. J. *Stroke*, **2001**, *32*, 1750-8.
[141] Allan, S. M.; Rothwell, N. J. *Nat Rev Neurosci*, **2001**, *2*, 734-44.
[142] Pinteaux, E.; Rothwell, N. J.; Boutin, H. *Glia*, **2006**, *53*, 551-6.
[143] Boutin, H.; LeFeuvre, R. A.; Horai, R.; Asano, M.; Iwakura, Y.; Rothwell, N. J. *J Neurosci*, **2001**, *21*, 5528-34.
[144] Buttini, M.; Sauter, A.; Boddeke, H. W. *Brain Res Mol Brain Res*, **1994**, *23*, 126-34.
[145] Davies, C. A.; Loddick, S. A.; Toulmond, S.; Stroemer, R. P.; Hunt, J.; Rothwell, N. J. *J Cereb Blood Flow Metab*, **1999**, *19*, 87-98.
[146] Haqqani, A. S.; Nesic, M.; Preston, E.; Baumann, E.; Kelly, J.; Stanimirovic, D. *FASEB J*, **2005**, *19*, 1809-21.
[147] Yamasaki, Y.; Matsuura, N.; Shozuhara, H.; Onodera, H.; Itoyama, Y.; Kogure, K. *Stroke*, **1995**, *26*, 676-80; discussion 681.
[148] Hara, H.; Friedlander, R. M.; Gagliardini, V.; Ayata, C.; Fink, K.; Huang, Z.; Shimizu-Sasamata, M.; Yuan, J.; Moskowitz, M. A. *Proc Natl Acad Sci U S A*, **1997**, *94*, 2007-12.
[149] Rothwell, N. J.; Luheshi, G. N. *Trends Neurosci*, **2000**, *23*, 618-25.
[150] Basu, A.; Lazovic, J.; Krady, J. K.; Mauger, D. T.; Rothstein, R. P.; Smith, M. B.; Levison, S. W. *J Cereb Blood Flow Metab*, **2005**, *25*, 17-29.
[151] Mulcahy, N. J.; Ross, J.; Rothwell, N. J.; Loddick, S. A. *Br J Pharmacol*, **2003**, *140*, 471-6.
[152] Yang, G. Y.; Zhao, Y. J.; Davidson, B. L.; Betz, A. L. *Brain Res*, **1997**, *751*, 181-8.
[153] Murakami, Y.; Saito, K.; Hara, A.; Zhu, Y.; Sudo, K.; Niwa, M.; Fujii, H.; Wada, H.; Ishiguro, H.; Mori, H.; Seishima, M. *J Neurochem*, **2005**, *93*, 1616-22.
[154] Offner, H.; Subramanian, S.; Parker, S. M.; Afentoulis, M. E.; Vandenbark, A. A.; Hurn, P. D. *J Cereb Blood Flow Metab*, **2006**, *26*, 654-65.
[155] Uno, H.; Matsuyama, T.; Akita, H.; Nishimura, H.; Sugita, M. *J Cereb Blood Flow Metab*, **1997**, *17*, 491-9.
[156] McCoy, M. K.; Tansey, M. G. *J Neuroinflammation*, **2008**, *5*, 45.
[157] Hallenbeck, J. M. *Nat Med*, **2002**, *8*, 1363-8.
[158] Pan, W.; Kastin, A. J. *Prog Neurobiol*, **2007**, *83*, 363-74.

[159] Yang, G. Y.; Gong, C.; Qin, Z.; Ye, W.; Mao, Y.; Bertz, A. L. *Neuroreport*, **1998**, *9*, 2131-4.
[160] Barone, F. C.; Arvin, B.; White, R. F.; Miller, A.; Webb, C. L.; Willette, R. N.; Lysko, P. G.; Feuerstein, G. Z. *Stroke*, **1997**, *28*, 1233-44.
[161] Ginis, I.; Jaiswal, R.; Klimanis, D.; Liu, J.; Greenspon, J.; Hallenbeck, J. M. *J Cereb Blood Flow Metab*, **2002**, *22*, 142-52.
[162] Bruce, A. J.; Boling, W.; Kindy, M. S.; Peschon, J.; Kraemer, P. J.; Carpenter, M. K.; Holtsberg, F. W.; Mattson, M. P. *Nat Med*, **1996**, *2*, 788-94.
[163] Sriram, K.; O'Callaghan, J. P. *J Neuroimmune Pharmacol*, **2007**, *2*, 140-53.
[164] Amantea, D.; Nappi, G.; Bernardi, G.; Bagetta, G.; Corasaniti, M. T. *FEBS J*, **2009**, *276*, 13-26.
[165] Pradillo, J. M.; Romera, C.; Hurtado, O.; Cardenas, A.; Moro, M. A.; Leza, J. C.; Davalos, A.; Castillo, J.; Lorenzo, P.; Lizasoain, I. *J Cereb Blood Flow Metab*, **2005**, *25*, 193-203.
[166] Clark, W. M.; Rinker, L. G.; Lessov, N. S.; Hazel, K.; Hill, J. K.; Stenzel-Poore, M.; Eckenstein, F. *Stroke*, **2000**, *31*, 1715-20.
[167] Yamashita, T.; Sawamoto, K.; Suzuki, S.; Suzuki, N.; Adachi, K.; Kawase, T.; Mihara, M.; Ohsugi, Y.; Abe, K.; Okano, H. *J Neurochem*, **2005**, *94*, 459-68.
[168] Herrmann, O.; Tarabin, V.; Suzuki, S.; Attigah, N.; Coserea, I.; Schneider, A.; Vogel, J.; Prinz, S.; Schwab, S.; Monyer, H.; Brombacher, F.; Schwaninger, M. *J Cereb Blood Flow Metab*, **2003**, *23*, 406-15.
[169] Smith, C. J.; Emsley, H. C.; Gavin, C. M.; Georgiou, R. F.; Vail, A.; Barberan, E. M.; del Zoppo, G. J.; Hallenbeck, J. M.; Rothwell, N. J.; Hopkins, S. J.; Tyrrell, P. J. *BMC Neurol*, **2004**, *4*, 2.
[170] Rallidis, L. S.; Vikelis, M.; Panagiotakos, D. B.; Rizos, I.; Zolindaki, M. G.; Kaliva, K.; Kremastinos, D. T. *Atherosclerosis*, **2006**, *189*, 193-7.
[171] Jung, J. E.; Kim, G. S.; Chan, P. H. *Stroke*, **2011**, *42*, 3574-9.
[172] Gertz, K.; Kronenberg, G.; Kalin, R. E.; Baldinger, T.; Werner, C.; Balkaya, M.; Eom, G. D.; Hellmann-Regen, J.; Krober, J.; Miller, K. R.; Lindauer, U.; Laufs, U.; Dirnagl, U.; Heppner, F. L.; Endres, M. *Brain*, **2012**, *135*, 1964-80.
[173] Strle, K.; Zhou, J. H.; Shen, W. H.; Broussard, S. R.; Johnson, R. W.; Freund, G. G.; Dantzer, R.; Kelley, K. W. *Crit Rev Immunol*, **2001**, *21*, 427-49.
[174] Spera, P. A.; Ellison, J. A.; Feuerstein, G. Z.; Barone, F. C. *Neurosci Lett*, **1998**, *251*, 189-92.
[175] Ooboshi, H.; Ibayashi, S.; Shichita, T.; Kumai, Y.; Takada, J.; Ago, T.; Arakawa, S.; Sugimori, H.; Kamouchi, M.; Kitazono, T.; Iida, M. *Circulation*, **2005**, *111*, 913-9.
[176] Pelidou, S. H.; Kostulas, N.; Matusevicius, D.; Kivisakk, P.; Kostulas, V.; Link, H. *Eur J Neurol*, **1999**, *6*, 437-42.
[177] Tarkowski, E.; Rosengren, L.; Blomstrand, C.; Wikkelso, C.; Jensen, C.; Ekholm, S.; Tarkowski, A. *Clin Exp Immunol*, **1997**, *110*, 492-9.
[178] van Exel, E.; Gussekloo, J.; de Craen, A. J.; Bootsma-van der Wiel, A.; Frolich, M.; Westendorp, R. G. *Stroke*, **2002**, *33*, 1135-8.
[179] Flanders, K. C.; Ren, R. F.; Lippa, C. F. *Prog Neurobiol*, **1998**, *54*, 71-85.
[180] Pang, L.; Ye, W.; Che, X. M.; Roessler, B. J.; Betz, A. L.; Yang, G. Y. *Stroke*, **2001**, *32*, 544-52.
[181] Lu, Y. Z.; Lin, C. H.; Cheng, F. C.; Hsueh, C. M. *Neurosci Lett*, **2005**, *373*, 159-64.
[182] Kim, J. S.; Gautam, S. C.; Chopp, M.; Zaloga, C.; Jones, M. L.; Ward, P. A.; Welch, K. M. *J Neuroimmunol*, **1995**, *56*, 127-34.
[183] Wang, X.; Yue, T. L.; Barone, F. C.; Feuerstein, G. Z. *Stroke*, **1995**, *26*, 661-5; discussion 665-6.
[184] Bajetto, A.; Bonavia, R.; Barbero, S.; Florio, T.; Schettini, G. *Front Neuroendocrinol*, **2001**, *22*, 147-84.
[185] Rossi, D.; Zlotnik, A. *Annu Rev Immunol*, **2000**, *18*, 217-42.
[186] Bizzarri, C.; Beccari, A. R.; Bertini, R.; Cavicchia, M. R.; Giorgini, S.; Allegretti, M. *Pharmacol Ther*, **2006**, *112*, 139-49.

[187] Gerard, C.; Rollins, B. J. *Nat Immunol*, **2001**, *2*, 108-15.
[188] Rollins, B. J. *Blood*, **1997**, *90*, 909-28.
[189] Semple, B. D.; Kossmann, T.; Morganti-Kossmann, M. C. *J Cereb Blood Flow Metab*, **2010**, *30*, 459-73.
[190] Chen, Y.; Hallenbeck, J. M.; Ruetzler, C.; Bol, D.; Thomas, K.; Berman, N. E.; Vogel, S. N. *J Cereb Blood Flow Metab*, **2003**, *23*, 748-55.
[191] Terao, S.; Yilmaz, G.; Stokes, K. Y.; Russell, J.; Ishikawa, M.; Kawase, T.; Granger, D. N. *Stroke*, **2008**, *39*, 2560-70.
[192] Terao, Y.; Ohta, H.; Oda, A.; Nakagaito, Y.; Kiyota, Y.; Shintani, Y. *Neurosci Res*, **2009**, *64*, 75-82.
[193] Tarozzo, G.; Campanella, M.; Ghiani, M.; Bulfone, A.; Beltramo, M. *Eur J Neurosci*, **2002**, *15*, 1663-8.
[194] Soriano, S. G.; Amaravadi, L. S.; Wang, Y. F.; Zhou, H.; Yu, G. X.; Tonra, J. R.; Fairchild-Huntress, V.; Fang, Q.; Dunmore, J. H.; Huszar, D.; Pan, Y. *J Neuroimmunol*, **2002**, *125*, 59-65.
[195] Stamatovic, S. M.; Shakui, P.; Keep, R. F.; Moore, B. B.; Kunkel, S. L.; Van Rooijen, N.; Andjelkovic, A. V. *J Cereb Blood Flow Metab*, **2005**, *25*, 593-606.
[196] Newman, M. B.; Willing, A. E.; Manresa, J. J.; Davis-Sanberg, C.; Sanberg, P. R. *Stem Cells Dev*, **2005**, *14*, 576-86.
[197] Kelly, S.; Bliss, T. M.; Shah, A. K.; Sun, G. H.; Ma, M.; Foo, W. C.; Masel, J.; Yenari, M. A.; Weissman, I. L.; Uchida, N.; Palmer, T.; Steinberg, G. K. *Proc Natl Acad Sci U S A*, **2004**, *101*, 11839-44.
[198] Wang, L.; Li, Y.; Chen, J.; Gautam, S. C.; Zhang, Z.; Lu, M.; Chopp, M. *Exp Hematol*, **2002**, *30*, 831-6.
[199] Wang, L.; Li, Y.; Chen, X.; Chen, J.; Gautam, S. C.; Xu, Y.; Chopp, M. *Hematology*, **2002**, *7*, 113-7.
[200] Shichinohe, H.; Kuroda, S.; Yano, S.; Hida, K.; Iwasaki, Y. *Brain Res*, **2007**, *1183*, 138-47.
[201] Adibhatla, R. M.; Hatcher, J. F.; Larsen, E. C.; Chen, X.; Sun, D.; Tsao, F. H. *J Biol Chem*, **2006**, *281*, 6718-25.
[202] Sanchez-Moreno, C.; Dashe, J. F.; Scott, T.; Thaler, D.; Folstein, M. F.; Martin, A. *Stroke*, **2004**, *35*, 163-8.
[203] Bonventre, J. V.; Huang, Z.; Taheri, M. R.; O'Leary, E.; Li, E.; Moskowitz, M. A.; Sapirstein, A. *Nature*, **1997**, *390*, 622-5.
[204] Schwab, J. M.; Beschorner, R.; Meyermann, R.; Gozalan, F.; Schluesener, H. J. *J Neurosurg*, **2002**, *96*, 892-9.
[205] Iadecola, C.; Sugimoto, K.; Niwa, K.; Kazama, K.; Ross, M. E. *J Cereb Blood Flow Metab*, **2001**, *21*, 1436-41.
[206] Candelario-Jalil, E.; Gonzalez-Falcon, A.; Garcia-Cabrera, M.; Alvarez, D.; Al-Dalain, S.; Martinez, G.; Leon, O. S.; Springer, J. E. *J Neurochem*, **2003**, *86*, 545-55.
[207] Nogawa, S.; Zhang, F.; Ross, M. E.; Iadecola, C. *J Neurosci*, **1997**, *17*, 2746-55.
[208] Iadecola, C.; Forster, C.; Nogawa, S.; Clark, H. B.; Ross, M. E. *Acta Neuropathol*, **1999**, *98*, 9-14.
[209] Sairanen, T.; Ristimaki, A.; Karjalainen-Lindsberg, M. L.; Paetau, A.; Kaste, M.; Lindsberg, P. J. *Ann Neurol*, **1998**, *43*, 738-47.
[210] Kawano, T.; Anrather, J.; Zhou, P.; Park, L.; Wang, G.; Frys, K. A.; Kunz, A.; Cho, S.; Orio, M.; Iadecola, C. *Nat Med*, **2006**, *12*, 225-9.
[211] Sugimoto, K.; Iadecola, C. *Brain Res*, **2003**, *960*, 273-6.
[212] Iadecola, C.; Niwa, K.; Nogawa, S.; Zhao, X.; Nagayama, M.; Araki, E.; Morham, S.; Ross, M. E. *Proc Natl Acad Sci U S A*, **2001**, *98*, 1294-9.
[213] Dore, S.; Otsuka, T.; Mito, T.; Sugo, N.; Hand, T.; Wu, L.; Hurn, P. D.; Traystman, R. J.; Andreasson, K. *Ann Neurol*, **2003**, *54*, 155-62.

[214] Manabe, Y.; Anrather, J.; Kawano, T.; Niwa, K.; Zhou, P.; Ross, M. E.; Iadecola, C. *Ann Neurol*, **2004**, *55*, 668-75.
[215] Rao, A. M.; Hatcher, J. F.; Kindy, M. S.; Dempsey, R. J. *Neurochem Res*, **1999**, *24*, 1225-32.
[216] Tomimoto, H.; Shibata, M.; Ihara, M.; Akiguchi, I.; Ohtani, R.; Budka, H. *Acta Neuropathol*, **2002**, *104*, 601-7.
[217] Baskaya, M. K.; Hu, Y.; Donaldson, D.; Maley, M.; Rao, A. M.; Prasad, M. R.; Dempsey, R. J. *J Neurosurg*, **1996**, *85*, 112-6.
[218] Song, Y.; Wei, E. Q.; Zhang, W. P.; Zhang, L.; Liu, J. R.; Chen, Z. *Neuroreport*, **2004**, *15*, 2181-4.
[219] Kitagawa, K.; Matsumoto, M.; Hori, M. *Brain Res*, **2004**, *1004*, 198-202.
[220] Iadecola, C.; Zhang, F.; Xu, S.; Casey, R.; Ross, M. E. *J Cereb Blood Flow Metab*, **1995**, *15*, 378-84.
[221] Iadecola, C.; Zhang, F.; Xu, X. *Am J Physiol*, **1995**, *268*, R286-92.
[222] Lakhan, S. E.; Kirchgessner, A.; Hofer, M. *J Transl Med*, **2009**, *7*, 97.
[223] Cui, J.; Holmes, E. H.; Liu, P. K. *J Neurochem*, **1999**, *73*, 1164-74.
[224] Cui, J.; Holmes, E. H.; Greene, T. G.; Liu, P. K. *FASEB J*, **2000**, *14*, 955-67.
[225] Huang, D.; Shenoy, A.; Cui, J.; Huang, W.; Liu, P. K. *FASEB J*, **2000**, *14*, 407-17.
[226] Zhao, X.; Haensel, C.; Araki, E.; Ross, M. E.; Iadecola, C. *Brain Res*, **2000**, *872*, 215-8.
[227] Han, H. S.; Qiao, Y.; Karabiyikoglu, M.; Giffard, R. G.; Yenari, M. A. *J Neurosci*, **2002**, *22*, 3921-8.
[228] Coughlan, T.; Gibson, C.; Murphy, S. *J Neurochem*, **2005**, *93*, 932-42.
[229] Park, E. M.; Cho, S.; Frys, K. A.; Glickstein, S. B.; Zhou, P.; Anrather, J.; Ross, M. E.; Iadecola, C. *J Cereb Blood Flow Metab*, **2006**, *26*, 392-401.
[230] Chan, P. H. *J Cereb Blood Flow Metab*, **2001**, *21*, 2-14.
[231] Groemping, Y.; Rittinger, K. *Biochem J*, **2005**, *386*, 401-16.
[232] Vallet, P.; Charnay, Y.; Steger, K.; Ogier-Denis, E.; Kovari, E.; Herrmann, F.; Michel, J. P.; Szanto, I. *Neuroscience*, **2005**, *132*, 233-8.
[233] Tang, X. N.; Zheng, Z.; Giffard, R. G.; Yenari, M. A. *Ann Neurol*, **2011**, *70*, 606-15.
[234] Walder, C. E.; Green, S. P.; Darbonne, W. C.; Mathias, J.; Rae, J.; Dinauer, M. C.; Curnutte, J. T.; Thomas, G. R. *Stroke*, **1997**, *28*, 2252-8.
[235] Yenari, M. A.; Xu, L.; Tang, X. N.; Qiao, Y.; Giffard, R. G. *Stroke*, **2006**, *37*, 1087-93.
[236] Won, S. J.; Tang, X. N.; Suh, S. W.; Yenari, M. A.; Swanson, R. A. *Ann Neurol*, **2011**, *70*, 583-90.
[237] Takizawa, S.; Aratani, Y.; Fukuyama, N.; Maeda, N.; Hirabayashi, H.; Koyama, H.; Shinohara, Y.; Nakazawa, H. *J Cereb Blood Flow Metab*, **2002**, *22*, 50-4.
[238] Rosenberg, G. A. *Glia*, **2002**, *39*, 279-91.
[239] Kelly, M. A.; Shuaib, A.; Todd, K. G. *Exp Neurol*, **2006**, *200*, 38-49.
[240] Yamashita, T.; Kamiya, T.; Deguchi, K.; Inaba, T.; Zhang, H.; Shang, J.; Miyazaki, K.; Ohtsuka, A.; Katayama, Y.; Abe, K. *J Cereb Blood Flow Metab*, **2009**, *29*, 715-25.
[241] Rosenberg, G. A.; Cunningham, L. A.; Wallace, J.; Alexander, S.; Estrada, E. Y.; Grossetete, M.; Razhagi, A.; Miller, K.; Gearing, A. *Brain Res*, **2001**, *893*, 104-12.
[242] Rosenberg, G. A.; Estrada, E. Y.; Dencoff, J. E. *Stroke*, **1998**, *29*, 2189-95.
[243] Romanic, A. M.; White, R. F.; Arleth, A. J.; Ohlstein, E. H.; Barone, F. C. *Stroke*, **1998**, *29*, 1020-30.
[244] Pfefferkorn, T.; Rosenberg, G. A. *Stroke*, **2003**, *34*, 2025-30.
[245] Asahi, M.; Asahi, K.; Jung, J. C.; del Zoppo, G. J.; Fini, M. E.; Lo, E. H. *J Cereb Blood Flow Metab*, **2000**, *20*, 1681-9.
[246] Asahi, M.; Sumii, T.; Fini, M. E.; Itohara, S.; Lo, E. H. *Neuroreport*, **2001**, *12*, 3003-7.
[247] Montaner, J.; Alvarez-Sabin, J.; Molina, C.; Angles, A.; Abilleira, S.; Arenillas, J.; Gonzalez, M. A.; Monasterio, J. *Stroke*, **2001**, *32*, 1759-66.
[248] Gidday, J. M.; Gasche, Y. G.; Copin, J. C.; Shah, A. R.; Perez, R. S.; Shapiro, S. D.; Chan, P. H.; Park, T. S. *Am J Physiol Heart Circ Physiol*, **2005**, *289*, H558-68.

[249] Lee, S. R.; Kim, H. Y.; Rogowska, J.; Zhao, B. Q.; Bhide, P.; Parent, J. M.; Lo, E. H. *J Neurosci*, **2006**, *26*, 3491-5.
[250] Zhao, B. Q.; Wang, S.; Kim, H. Y.; Storrie, H.; Rosen, B. R.; Mooney, D. J.; Wang, X.; Lo, E. H. *Nat Med*, **2006**, *12*, 441-5.
[251] Baeuerle, P. A.; Henkel, T. *Annu Rev Immunol*, **1994**, *12*, 141-79.
[252] Han, H. S.; Karabiyikoglu, M.; Kelly, S.; Sobel, R. A.; Yenari, M. A. *J Cereb Blood Flow Metab*, **2003**, *23*, 589-98.
[253] Cechetto, D. F. *Prog Brain Res*, **2001**, *132*, 391-404.
[254] Schneider, A.; Martin-Villalba, A.; Weih, F.; Vogel, J.; Wirth, T.; Schwaninger, M. *Nat Med*, **1999**, *5*, 554-9.
[255] Herrmann, O.; Baumann, B.; de Lorenzi, R.; Muhammad, S.; Zhang, W.; Kleesiek, J.; Malfertheiner, M.; Kohrmann, M.; Potrovita, I.; Maegele, I.; Beyer, C.; Burke, J. R.; Hasan, M. T.; Bujard, H.; Wirth, T.; Pasparakis, M.; Schwaninger, M. *Nat Med*, **2005**, *11*, 1322-9.
[256] Ueno, T.; Sawa, Y.; Kitagawa-Sakakida, S.; Nishimura, M.; Morishita, R.; Kaneda, Y.; Kohmura, E.; Yoshimine, T.; Matsuda, H. *J Thorac Cardiovasc Surg*, **2001**, *122*, 720-7.
[257] Hill, W. D.; Hess, D. C.; Carroll, J. E.; Wakade, C. G.; Howard, E. F.; Chen, Q.; Cheng, C.; Martin-Studdard, A.; Waller, J. L.; Beswick, R. A. *Brain Res Bull*, **2001**, *55*, 375-86.
[258] Irving, E. A.; Bamford, M. *J Cereb Blood Flow Metab*, **2002**, *22*, 631-47.
[259] Irving, E. A.; Barone, F. C.; Reith, A. D.; Hadingham, S. J.; Parsons, A. A. *Brain Res Mol Brain Res*, **2000**, *77*, 65-75.
[260] Sugino, T.; Nozaki, K.; Takagi, Y.; Hattori, I.; Hashimoto, N.; Moriguchi, T.; Nishida, E. *J Neurosci*, **2000**, *20*, 4506-14.
[261] Kyriakis, J. M.; Avruch, J. *Physiol Rev*, **2001**, *81*, 807-69.
[262] Zhang, Q. G.; Wang, R. M.; Yin, X. H.; Pan, J.; Xu, T. L.; Zhang, G. Y. *J Neurochem*, **2005**, *95*, 784-95.
[263] Gao, Q.; Li, Y.; Chopp, M. *Neuroscience*, **2005**, *136*, 123-34.
[264] Walton, K. M.; DiRocco, R.; Bartlett, B. A.; Koury, E.; Marcy, V. R.; Jarvis, B.; Schaefer, E. M.; Bhat, R. V. *J Neurochem*, **1998**, *70*, 1764-7.
[265] Martinon, F.; Burns, K.; Tschopp, J. *Mol Cell*, **2002**, *10*, 417-26.
[266] Thauerer, B.; Zur Nedden, S.; Baier-Bitterlich, G. *J Neurochem*, **2012**, *121*, 329-42.
[267] Liew, F. Y.; Xu, D.; Brint, E. K.; O'Neill, L. A. *Nat Rev Immunol*, **2005**, *5*, 446-58.
[268] Piccinini, A. M.; Midwood, K. S. *Mediators Inflamm*, **2010**, *2010*.
[269] Carty, M.; Bowie, A. G. *Biochem Pharmacol*, **2011**, *81*, 825-37.
[270] Kong, Y.; Le, Y. *Int Immunopharmacol*, **2011**, *11*, 1407-14.
[271] Yang, Q. W.; Lu, F. L.; Zhou, Y.; Wang, L.; Zhong, Q.; Lin, S.; Xiang, J.; Li, J. C.; Fang, C. Q.; Wang, J. Z. *J Cereb Blood Flow Metab*, **2011**, *31*, 593-605.
[272] Shichita, T.; Hasegawa, E.; Kimura, A.; Morita, R.; Sakaguchi, R.; Takada, I.; Sekiya, T.; Ooboshi, H.; Kitazono, T.; Yanagawa, T.; Ishii, T.; Takahashi, H.; Mori, S.; Nishibori, M.; Kuroda, K.; Akira, S.; Miyake, K.; Yoshimura, A. *Nat Med*, **2012**, *18*, 911-7.
[273] Ziegler, G.; Harhausen, D.; Schepers, C.; Hoffmann, O.; Rohr, C.; Prinz, V.; Konig, J.; Lehrach, H.; Nietfeld, W.; Trendelenburg, G. *Biochem Biophys Res Commun*, **2007**, *359*, 574-9.
[274] Lalancette-Hebert, M.; Phaneuf, D.; Soucy, G.; Weng, Y. C.; Kriz, J. *Brain*, **2009**, *132*, 940-54.
[275] Tu, X. K.; Yang, W. Z.; Shi, S. S.; Wang, C. H.; Zhang, G. L.; Ni, T. R.; Chen, C. M.; Wang, R.; Jia, J. W.; Song, Q. M. *Neurochem Res*, **2010**, *35*, 1147-55.
[276] Lv, M.; Liu, Y.; Zhang, J.; Sun, L.; Liu, Z.; Zhang, S.; Wang, B.; Su, D.; Su, Z. *Neuroscience*, **2011**, *176*, 162-72.
[277] Yao, H.; Felfly, H.; Wang, J.; Zhou, D.; Haddad, G. G. *J Neurochem*, **2009**, *108*, 835-46.

[278] Hua, F.; Ma, J.; Ha, T.; Kelley, J. L.; Kao, R. L.; Schweitzer, J. B.; Kalbfleisch, J. H.; Williams, D. L.; Li, C. *Brain Res*, **2009**, *1262*, 100-8.

[279] Hyakkoku, K.; Hamanaka, J.; Tsuruma, K.; Shimazawa, M.; Tanaka, H.; Uematsu, S.; Akira, S.; Inagaki, N.; Nagai, H.; Hara, H. *Neuroscience*, **2010**, *171*, 258-67.

[280] Caso, J. R.; Pradillo, J. M.; Hurtado, O.; Lorenzo, P.; Moro, M. A.; Lizasoain, I. *Circulation*, **2007**, *115*, 1599-608.

[281] Skaper, S. D. *CNS Neurol Disord Drug Targets*, **2011**, *10*, 44-56.

[282] Melani, A.; Amadio, S.; Gianfriddo, M.; Vannucchi, M. G.; Volonte, C.; Bernardi, G.; Pedata, F.; Sancesario, G. *J Cereb Blood Flow Metab*, **2006**, *26*, 974-82.

[283] Yanagisawa, D.; Kitamura, Y.; Takata, K.; Hide, I.; Nakata, Y.; Taniguchi, T. *Biol Pharm Bull*, **2008**, *31*, 1121-30.

[284] Arbeloa, J.; Perez-Samartin, A.; Gottlieb, M.; Matute, C. *Neurobiol Dis*, **2012**, *45*, 954-61.

[285] Chu, K.; Yin, B.; Wang, J.; Peng, G.; Liang, H.; Xu, Z.; Du, Y.; Fang, M.; Xia, Q.; Luo, B. *J Neuroinflammation*, **2012**, *9*, 69.

[286] Inoue, K. *Cell Mol Life Sci*, **2008**, *65*, 3074-80.

[287] Irino, Y.; Nakamura, Y.; Inoue, K.; Kohsaka, S.; Ohsawa, K. *J Neurosci Res*, **2008**, *86*, 1511-9.

[288] Webster, C. M.; Hokari, M.; Tang, X. N.; Yenari, M. A. *Stroke*, **2011**, *42*, e296.

[289] Ajmo, C. T., Jr.; Vernon, D. O.; Collier, L.; Hall, A. A.; Garbuzova-Davis, S.; Willing, A.; Pennypacker, K. R. *J Neurosci Res*, **2008**, *86*, 2227-34.

[290] Liesz, A.; Hagmann, S.; Zschoche, C.; Adamek, J.; Zhou, W.; Sun, L.; Hug, A.; Zorn, M.; Dalpke, A.; Nawroth, P.; Veltkamp, R. *Stroke*, **2009**, *40*, 2849-58.

[291] Gendron, A.; Teitelbaum, J.; Cossette, C.; Nuara, S.; Dumont, M.; Geadah, D.; du Souich, P.; Kouassi, E. *Brain Res*, **2002**, *955*, 85-97.

[292] Martin, A.; Aguirre, J.; Sarasa-Renedo, A.; Tsoukatou, D.; Garofalakis, A.; Meyer, H.; Mamalaki, C.; Ripoll, J.; Planas, A. M. *Mol Imaging*, **2008**, *7*, 157-67.

[293] Prass, K.; Braun, J. S.; Dirnagl, U.; Meisel, C.; Meisel, A. *Stroke*, **2006**, *37*, 2607-12.

[294] Aslanyan, S.; Weir, C. J.; Diener, H. C.; Kaste, M.; Lees, K. R. *Eur J Neurol*, **2004**, *11*, 49-53.

[295] Hilker, R.; Poetter, C.; Findeisen, N.; Sobesky, J.; Jacobs, A.; Neveling, M.; Heiss, W. D. *Stroke*, **2003**, *34*, 975-81.

[296] Langhorne, P.; Stott, D. J.; Robertson, L.; MacDonald, J.; Jones, L.; McAlpine, C.; Dick, F.; Taylor, G. S.; Murray, G. *Stroke*, **2000**, *31*, 1223-9.

[297] Urra, X.; Cervera, A.; Villamor, N.; Planas, A. M.; Chamorro, A. *Neuroscience*, **2009**, *158*, 1174-83.

[298] Pavlov, V. A.; Wang, H.; Czura, C. J.; Friedman, S. G.; Tracey, K. J. *Mol Med*, **2003**, *9*, 125-34.

[299] Tracey, K. J. *J Clin Invest*, **2007**, *117*, 289-96.

[300] *Neurology*, **2001**, *57*, 1428-34.

[301] Becker, K. J. *Curr Med Res Opin*, **2002**, *18 Suppl 2*, s18-22.

[302] Schneider, D.; Berrouschot, J.; Brandt, T.; Hacke, W.; Ferbert, A.; Norris, S. H.; Polmar, S. H.; Schafer, E. *Eur Neurol*, **1998**, *40*, 78-83.

[303] Krams, M.; Lees, K. R.; Hacke, W.; Grieve, A. P.; Orgogozo, J. M.; Ford, G. A. *Stroke*, **2003**, *34*, 2543-8.

[304] Caimi, G.; Canino, B.; Ferrara, F.; Montana, M.; Musso, M.; Porretto, F.; Carollo, C.; Catania, A.; Lo Presti, R. *J Neurol Sci*, **2001**, *186*, 23-6.

[305] Campanella, M.; Sciorati, C.; Tarozzo, G.; Beltramo, M. *Stroke*, **2002**, *33*, 586-92.

[306] Del Zoppo, G. J. *J Intern Med*, **1995**, *237*, 79-88.

[307] Wang, X.; Zhu, S.; Drozda, M.; Zhang, W.; Stavrovskaya, I. G.; Cattaneo, E.; Ferrante, R. J.; Kristal, B. S.; Friedlander, R. M. *Proc Natl Acad Sci U S A*, **2003**, *100*, 10483-7.

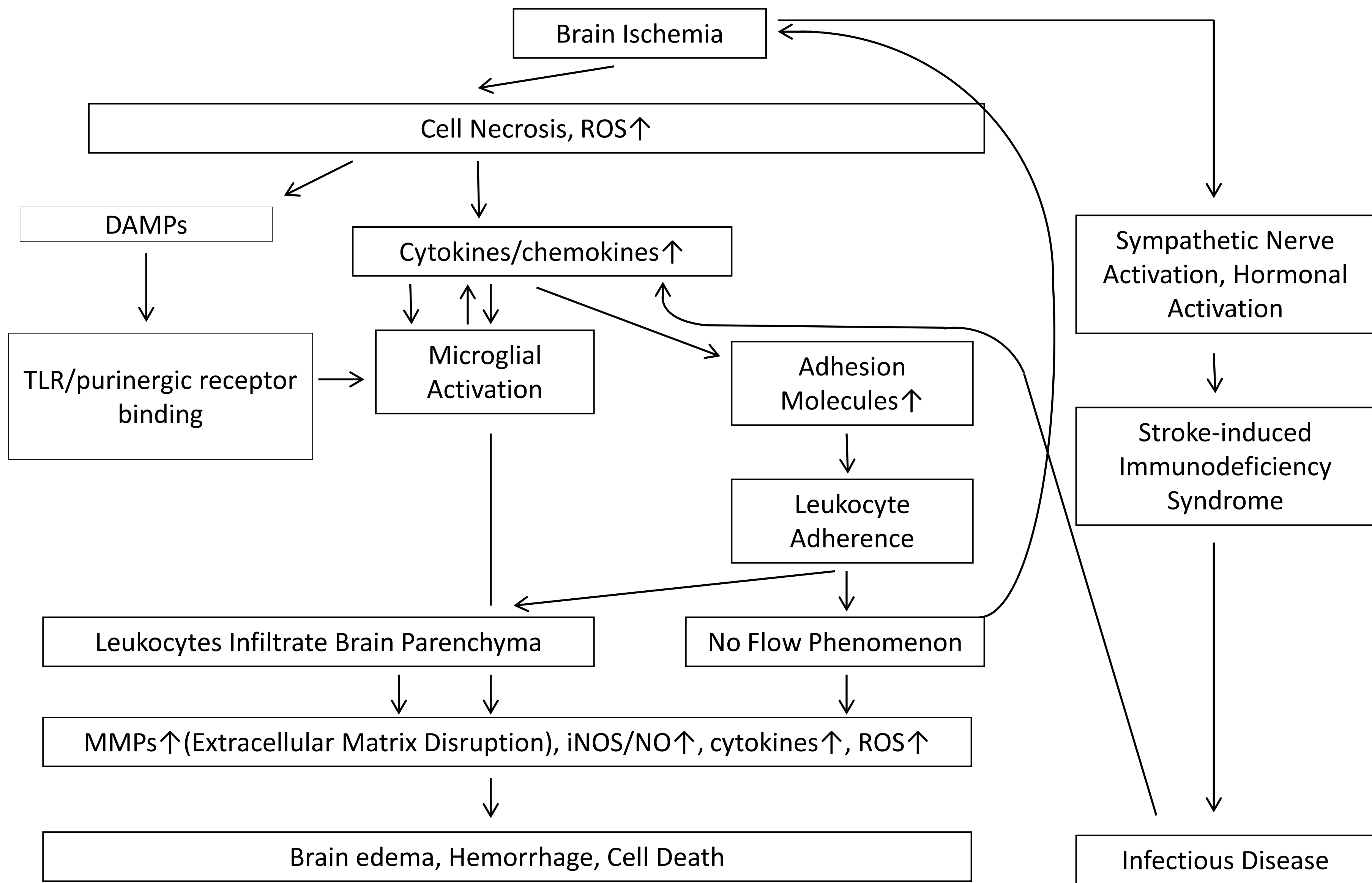
[308] Tikka, T.; Fiebich, B. L.; Goldsteins, G.; Keinanen, R.; Koistinaho, J. *J Neurosci*, **2001**, *21*, 2580-8.

[309] Koistinaho, M.; Malm, T. M.; Kettunen, M. I.; Goldsteins, G.; Starckx, S.; Kauppinen, R. A.; Opdenakker, G.; Koistinaho, J. *J Cereb Blood Flow Metab*, **2005**, *25*, 460-7.
[310] Liu, Z.; Fan, Y.; Won, S. J.; Neumann, M.; Hu, D.; Zhou, L.; Weinstein, P. R.; Liu, J. *Stroke*, **2007**, *38*, 146-52.
[311] Tang, X. N.; Wang, Q.; Koike, M. A.; Cheng, D.; Goris, M. L.; Blankenberg, F. G.; Yenari, M. A. *J Nucl Med*, **2007**, *48*, 1822-8.
[312] Lampl, Y.; Boaz, M.; Gilad, R.; Lorberboym, M.; Dabby, R.; Rapoport, A.; Anca-Hershkowitz, M.; Sadeh, M. *Neurology*, **2007**, *69*, 1404-10.
[313] Fagan, S. C.; Waller, J. L.; Nichols, F. T.; Edwards, D. J.; Pettigrew, L. C.; Clark, W. M.; Hall, C. E.; Switzer, J. A.; Ergul, A.; Hess, D. C. *Stroke*, **2010**, *41*, 2283-7.
[314]C
[315] Yenari, M. A.; Han, H. S. *Nat Rev Neurosci*, **2012**, *13*, 267-78.
[316] Busto, R.; Globus, M. Y.; Dietrich, W. D.; Martinez, E.; Valdes, I.; Ginsberg, M. D. *Stroke*, **1989**, *20*, 904-10.
[317] Edwards, A. D.; Yue, X.; Squier, M. V.; Thoresen, M.; Cady, E. B.; Penrice, J.; Cooper, C. E.; Wyatt, J. S.; Reynolds, E. O.; Mehmet, H. *Biochem Biophys Res Commun*, **1995**, *217*, 1193-9.
[318] Baldwin, W. A.; Kirsch, J. R.; Hurn, P. D.; Toung, W. S.; Traystman, R. J. *Am J Physiol*, **1991**, *261*, H774-81.
[319] Deng, H.; Han, H. S.; Cheng, D.; Sun, G. H.; Yenari, M. A. *Stroke*, **2003**, *34*, 2495-501.
[320] Wang, G. J.; Deng, H. Y.; Maier, C. M.; Sun, G. H.; Yenari, M. A. *Neuroscience*, **2002**, *114*, 1081-90.
[321] Perrone, S.; Szabo, M.; Bellieni, C. V.; Longini, M.; Bango, M.; Kelen, D.; Treszl, A.; Negro, S.; Tataranno, M. L.; Buonocore, G. *Pediatr Neurol*, **2010**, *43*, 236-40.
[322] Meybohm, P.; Gruenewald, M.; Zacharowski, K. D.; Albrecht, M.; Lucius, R.; Fosel, N.; Hensler, J.; Zitta, K.; Bein, B. *Crit Care*, **2010**, *14*, R21.
[323] Webster, C. M.; Kelly, S.; Koike, M. A.; Chock, V. Y.; Giffard, R. G.; Yenari, M. A. *Neurobiol Dis*, **2009**, *33*, 301-12.
[324] Schmitt, K. R.; Diestel, A.; Lehnardt, S.; Schwartlander, R.; Lange, P. E.; Berger, F.; Ullrich, O.; Abdul-Khaliq, H. *J Neuroimmunol*, **2007**, *189*, 7-16.
[325] Choi, J. S.; Park, J.; Suk, K.; Moon, C.; Park, Y. K.; Han, H. S. *Stroke Res Treat*, **2011**, *2011*, 846716.
[326] Matsui, T.; Kakeda, T. *J Neurotrauma*, **2008**, *25*, 709-15.
[327] Truettner, J. S.; Suzuki, T.; Dietrich, W. D. *Brain Res Mol Brain Res*, **2005**, *138*, 124-34.
[328] Pelletier, D.; Hafler, D. A. *N Engl J Med*, **2012**, *366*, 339-47.
[329] Baumruker, T.; Billich, A.; Brinkmann, V. *Expert Opin Investig Drugs*, **2007**, *16*, 283-9.
[330] Billich, A.; Bornancin, F.; Devay, P.; Mechtcheriakova, D.; Urtz, N.; Baumruker, T. *J Biol Chem*, **2003**, *278*, 47408-15.
[331] Pfeilschifter, W.; Czech-Zechmeister, B.; Sujak, M.; Mirceska, A.; Koch, A.; Rami, A.; Steinmetz, H.; Foerch, C.; Huwiler, A.; Pfeilschifter, J. *Biochem Biophys Res Commun*, **2011**, *413*, 212-7.
[332] Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R.; Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R. *J Biol Chem*, **2002**, *277*, 21453-7.
[333] Czech, B.; Pfeilschifter, W.; Mazaheri-Omrani, N.; Strobel, M. A.; Kahles, T.; Neumann-Haefelin, T.; Rami, A.; Huwiler, A.; Pfeilschifter, J. *Biochem Biophys Res Commun*, **2009**, *389*, 251-6.
[334] Hasegawa, Y.; Suzuki, H.; Sozen, T.; Rolland, W.; Zhang, J. H. *Stroke*, **2010**, *41*, 368-74.
[335] Wei, Y.; Yemisci, M.; Kim, H. H.; Yung, L. M.; Shin, H. K.; Hwang, S. K.; Guo, S.; Qin, T.; Alsharif, N.; Brinkmann, V.; Liao, J. K.; Lo, E. H.; Waeber, C. *Ann Neurol*, **2011**, *69*, 119-29.
[336] Kimura, A.; Ohmori, T.; Kashiwakura, Y.; Ohkawa, R.; Madoiwa, S.; Mimuro, J.; Shimazaki, K.; Hoshino, Y.; Yatomi, Y.; Sakata, Y. *Stroke*, **2008**, *39*, 3411-7.

[337] Yagi, H.; Kamba, R.; Chiba, K.; Soga, H.; Yaguchi, K.; Nakamura, M.; Itoh, T. *Eur J Immunol*, **2000**, *30*, 1435-44.
[338] Brinkmann, V.; Pinschewer, D. D.; Feng, L.; Chen, S. *Transplantation*, **2001**, *72*, 764-9.
[339] Jung, C. G.; Kim, H. J.; Miron, V. E.; Cook, S.; Kennedy, T. E.; Foster, C. A.; Antel, J. P.; Soliven, B. *Glia*, **2007**, *55*, 1656-67.
[340] Stessin, A. M.; Gursel, D. B.; Schwartz, A.; Parashar, B.; Kulidzhanov, F. G.; Sabbas, A. M.; Boockvar, J.; Nori, D.; Wernicke, A. G. *Neurosci Lett*, **2012**, *516*, 253-8.
[341] Liu, J.; Zhang, C.; Tao, W.; Liu, M. *Int J Neurosci*, **2012**.

Fig.1 Inflammation following ischemic stroke. Brain ischemia triggers inflammatory responses due to the presence of necrotic cells within the infarct core, penumbra and brain vessels. Necrotic cells can lead to the generation reactive oxygen species (ROS) and inflammatory cytokines. Necrotic cells lyse and release nucleic acids which can act as danger associated molecular pattern molecules (DAMPs). DAMPs and other immune molecules lead to microglial activation which produce more cytokines causing upregulation of adhesion molecules in and around the cerebral vasculature. Chemokines leads to inflammatory cell chemotaxis into ischemic brain. Adhesion molecules then lead circulating leukocytes to bind to vascular endothelium and infiltrate into the brain parenchyma. Once in the brain, activated leukocytes and microglia produce a variety of inflammatory mediators such as matrix metalloproteinases (MMPs) which play important roles in extracellular matrix disruption, inducible nitric oxide synthase (iNOS) which generates nitric oxide (NO), cytokines and more ROS. These mediators lead to brain edema, hemorrhage and ultimately cell death. Brain infarction also affects the rest of the body, leading to a state of immunodeficiency, and is thought to be mediated through sympathetic nerve activation resulting in lowering circulating immune cells. This can subsequently lead to infections which complicates stroke by further increasing immune activation in the brain.

Table. 1 Clinical trials for anti-inflammatory drugs in ischemic stroke


Brain Ischemia
Cell Necrosis, ROS↑
DAMPs
Cytokines/chemokines↑
TLR/purinergic receptor binding
Microglial Activation
Adhesion Molecules↑
Leukocyte Adherence
Sympathetic Nerve Activation, Hormonal Activation
Stroke-induced Immunodeficiency Syndrome
Leukocytes Infiltrate Brain Parenchyma
No Flow Phenomenon
MMPs↑(Extracellular Matrix Disruption), iNOS/NO↑, cytokines↑, ROS↑
Brain edema, Hemorrhage, Cell Death
Infectious Disease
Infarct Core
Infarct Penumbra
Brain Vessels
Body

Agent	Representative Name	Design
Anti-integrin	The HALT Stroke Study	Trial of Hu23F2G anti-adhesion to limit cytotoxic injury in acute ischemic stroke
	ASTIN	Trial of recombinant neutrophil inhibitory factor
Anti-ICAM1	Enlimomab Acute Stroke Trial	Efficacy and safety of enlimomab versus placebo
Minocycline		Trial of 200 mg oral minocycline to evaluate its efficacy
	MINOS	Dose-Finding study of minocycline
	MINOS (Sub-analysis)	Determine the impact of i.v. minocycline of MMP-9 expresion
	NeuMAST	Determine the efficacy of minocyline in long term recovery

Abbreviations; NIHSS, NIH Stroke Scale; mRS, modified Rankin Scale; B.I., Barthel Index

Phase	n	Status	Results	ref
III	–	Complete Aborted	Stopped early for futility: no benefit would occur if the study was completed	301
II	966	Complete Aborted	Stopped early for futility: no benefit would occur if the study was completed	303
III	625	Complete	Not effective, may worsen outcome.	300
Open-Label	152	Complete	Treated patients showed better NIHSS, mRS and B.I.	312
nonrandomized, dose-escalation trial	60	Complete	Showed safe up to dose of 10 mg/kg i.v.	313
nonrandomized, dose-escalation trial	60	Complete	Showed lower MMP-9 expression with the minocyline administration	314
IV	–	Complete Aborted	Stopped early for futility: no benefit would occur if the study was completed	NCT00930020