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Inflammation in adult and neonatal stroke

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Abstract

This chapter will discuss the current knowledge of the contribution of systemic and local inflammation in acute and sub-chronic stages of experimental stroke in both the adult and neonate. It will review the role of specific cell types and interactions among blood cells, endothelium, glia, microglia, the extracellular matrix and neurons – cumulatively called "neurovascular unit" – in stroke induction and evolution. Intracellular inflammatory signaling pathways such as nuclear factor kappa beta and mitogen-activated protein kinases, and mediators produced by inflammatory cells such as cytokines, chemokines, reactive oxygen species and arachidonic acid metabolites, as well as the modifying role of age on these mechanisms, will be reviewed as well as the potential for therapy in stroke and hypoxic-ischemic injury.

Keywords

cerebral ischemia; hypoxia-ischemia; neurovascular unit; leukocyte; microglia; cytokine; chemokine; adhesion molecules; integrin

1. INTRODUCTION

For a long time the central nervous system was considered to be “immunologically privileged” due to shielding from access by immune cells by the blood-brain barrier (BBB) [1]. However, it is becoming apparent that leukocytes [2], as well as cytokines and chemokines [3], can cross the intact BBB and that there is a substantial cross-talk between peripheral and local immune components [4,5]. It is well known that stroke triggers a robust inflammatory reaction characterized by peripheral leukocyte influx into the cerebral parenchyma and activation of endogenous microglia [6–11]. Following adult stroke, the generation of reactive oxygen species (ROS) and intracellular calcium accumulation in neurons and other brain cells triggers immune responses ultimately leading to inflammatory cell activation and infiltration. Ischemic cells, even ischemic neurons, secrete inflammatory cytokines that cause, among other things, adhesion molecule upregulation in the cerebral vasculature which leads to peripheral leukocyte recruitment. Brain cells are also capable of secreting chemokines, leading to further inflammatory cell chemotaxis into the ischemic lesion. Once activated, inflammatory cells can release a variety of cytotoxic agents including cytokines, matrix metalloproteinases (MMPs), nitric oxide (NO) and more ROS. These substances can contribute to more cell damage as well as disruption of the BBB and

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extracellular matrix [12–15]. Figure 1 demonstrates the conceptual framework for the contribution of inflammatory mechanisms to stroke. Studies in experimental stroke have also shown that the extent, timing and consequences of these processes are profoundly affected by the presence of reperfusion, by gender, genetic background, and by age.

The field of perinatal and neonatal stroke is emerging [16,17]. Strikingly, the incidence of arterial stroke in newborns, about 1/4,000 term babies [18], is similar to that in the elderly. Although basic mechanisms of neurodegeneration are shared across all age groups, immaturity critically affects brain susceptibility and response to ischemia-related insults (reviewed in [19–22]). The component of injury is not an exception (see recent reviews [21,23,24]). While signs of inflammation are rapid after both hypoxic encephalopathy and focal stroke in term babies [16,17], data for this age group suggests different than in adult involvement of local inflammation, leukocyte-mediated injury, involvement of the BBB and enhanced susceptibility to ROS. While more limited than in adult, the amount of data on various aspects of inflammation after ischemic injury warrants more study. Known differences between adult and neonate are indicated by asterisks in Figure 1. While blocking various aspects of the inflammatory cascade has shown to ameliorate injury from experimental stroke [22,25], this has yet to be successfully translated to human clinical trials.

2. ACUTE INJURY AFTER STROKE & INFLAMMATION

2-1. SYSTEMIC ACUTE-PHASE RESPONSE

Ischemic brain injury can be amplified by the hepatic release of regulatory acute-phase proteins [26]. The systemic acute-phase response, characterized by hepatic acute phase protein synthesis, is associated with leukocyte mobilization, and changes in serum levels of glucocorticosteroids and cytokines, events that occur even before there is any evidence of an inflammatory response in the brain [4,26]. Accumulating data suggest that leukocytosis depends on the pattern of cytokine expression in the brain. The CXC chemokine cytokine-induced chemoattractant protein –1 (CINC-1), which amplifies the hepatic response by initiating a dose-dependent neutrophil recruitment to the brain, is one of the first acute-phase proteins to be released from the liver in response to interleukin IL-1β microinjection into the brain, whereas injection of tumor necrosis factor TNF-α induces monocytes rather than neutrophils. The contribution of an acute-phase response as an injury amplifier to stroke in humans has yet to be demonstrated [27,28].

2-1. LEUKOCYTES

Ischemic injury to the neural parenchyma leads to local production of pro-inflammatory cytokines [29–31] and chemotactic molecules [32,33], a subsequent increase in numbers of leukocytes in the systemic circulation [34,35], secondary changes in the adhesion properties of the surrounding vascular endothelium, and site-specific chemotaxis of leukocytes. In adults, neutrophils [8,31,36,37] accumulate in ischemic brain tissue as early as 4–6 hours after ischemia onset. Evidence that neutrophils potentiate ischemic injury include numerous studies documenting improved neurological outcome following neutrophil depletion and inhibition of adhesion molecules which facilitate neutrophil entry into injured brain (reviewed in [38,39]). Neutrophils can enhance injury via several mechanisms, including ROS production [40], and release of MMP-9 [14], acting both from within the peripheral circulation and after extravasation into injured tissue. The existence of cytokine-specific patterns of monocytes and neutrophil recruitment [35,41,42] has been demonstrated by direct intracerebral injections of IL-1β, TNFα, IL-8, or NMDA. IL-1β injection triggered recruitment of neutrophils, but very few monocytes. TNFα injection induced predominantly monocyte infiltration, rather than neutrophils [41]. An excitotoxic lesion produced by

NMDA, in turn, profoundly increased IL-1β, but not TNFα, and the corresponding selective infiltration of neutrophils, not monocytes [41]. Macrophage accumulation, which typically occurs within one day after stroke [31,36], will be discussed later in this chapter. While neutrophils are the earliest leukocyte subpopulation in the brain after stroke, lymphocytes have also been documented [43]. Whether lymphocytes play an active role in ischemic brain pathogenesis is unclear. In a study of cultured primary neurons, isolated neutrophils, but not lymphocytes, potentiated neuronal injury due to excitotoxin exposure [44]. However, preventing lymphocyte trafficking into ischemic brain ameliorated injury, suggesting that, like neutrophils, lymphocytes play a deleterious role [45].

In contrast to stroke in the adult, neutrophils do not transmigrate into brains of postnatal day 7 (P7) rats following H-I within 42 h [46] or are present in the parenchyma only briefly [47]. Rather, neutrophils accumulate within vessels. Lack of transmigration is unlikely to be due to an intrinsic inability of neutrophils to transmigrate into the brain, as they do transmigrate following permanent middle cerebral artery (MCA) occlusion [48]. Circulating or marginated neutrophils, however, can contribute to hypoxic-ischemic (H-I) injury in the newborn period as was shown if neutrophils were depleted prior to H-I [49]. Recent studies [35,50–52] have shown that the degree and spatial distribution of BBB disruption during the neonatal period is dependent on neutrophils in response to intra-striatal injection of IL-1β [4].

Lymphocytes are generally thought to play a negative role in ischemic brain pathogenesis even though there is also conflicting data. In adult rodents after focal ischemia, T and B cells are seen days after injury [53,54], whereas in neonates infiltration of these cells following neonatal H-I and focal stroke may be less profound [47,55] or even transient [48]. Following permanent middle cerebral artery occlusion (MCAO) in rats, lymphocytes were elevated in the ischemic lesion after neutrophils [43,56]. Preventing lymphocyte trafficking into ischemic brain ameliorated injury, suggesting that like neutrophils, lymphocytes also play a deleterious role [45]. However, in a study of cultured primary neurons, isolated neutrophils, but not lymphocytes potentiated neuronal injury due to excitotoxin exposure [44].

There is increasing evidence for the role of mast cells after neonatal H-I and excitotoxic injury [57,58]. Injurious effects of mast cells appear to depend on TGF-β and IL-9 [59]. Agents that inhibit histamine release or mast cell degranulation have been shown to diminish excitotoxic brain injury in P5 rats [57,58]. Consistent with these findings, mast cell-deficient mice suffer less injury than WT controls [58].

2-3. ADHESION MOLECULES

Leukocyte extravasation is separated into discrete steps, which are associated with the interaction of selectins and their ligands, then integrins and other adhesion molecules, and finally chemokines and chemokine receptors. Adhesion molecules may represent important therapeutic targets (see reviews [60–62]), as inhibiting leukocyte adhesion by targeting various adhesion molecules, thus preventing leukocytes from entering ischemic brain, results in reduced neurologic injury [63–66]. The interaction between leukocytes 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) [61,62,67]. Furthermore, adhesion molecules appear most relevant in ischemia with reperfusion, as several anti-adhesion molecule approaches were ineffective when reperfusion did not occur [68–72].

2-3-1. SELECTINS—Selectins mediate the first step of leukocyte infiltration, rolling on the endothelium. P-selectin was originally described on platelets and then described on

endothelial cells, and E-selectin was originally described on endothelial cells [73]. L-selectin which was originally described on lymphocytes, is present on all leukocyte populations [74,75]. While E- and P-selectin are involved in leukocyte rolling and recruitment during the early stages of activation, L-selectin acts as a guide for unstimulated leukocytes [76]. In the presence of activated endothelium, rolling stops and L-selectin is shed from the cell membrane by proteolytic cleavage, followed by endothelial transmigration [77–79].

The induction of P- and E-selectins has been documented in different experimental stroke models [77,80–83]. E- and P-selectin upregulation appears to be involved in promoting ischemic inflammatory responses and injury exacerbation after ischemic stroke. Mice deficient in P-selectin have smaller infarcts and less neutrophil infiltration after transient MCA occlusion compared to wildtype (WT) littermates [84]. Conversely, mice overexpressing P-selectin have larger infarcts than WT. Furthermore, treatment with antibodies or inhibitors against P- and E-selectin was similarly associated with improved neurological outcome in rodents [83–85] and baboons [86]. The role of L-selectin in brain ischemia is less clear, as preventing its shedding by a blocking antibody, and presumably its ability to mediate leukocyte transmigration, does not appear to significantly influence stroke outcome [87,88].

Since E-selectin is almost exclusively upregulated in activated endothelium, tolerance to E-selectin could lead to suppression of immune responses and reduce trafficking of peripheral leukocytes to ischemic brain, the notion confirmed by induction of immune tolerance to brain antigens and the consequent reduction of the extent of ischemic injury by exposing animals to E-selectin intranasally [89,90] [89,90], or even prevention of stroke [91]. Thus, it is conceivable that this approach could lead to the development of a vaccine against stroke.

Developmental differences in E- and P-selectin may underlie the disparities observed in post-ischemic neutrophil infiltration between adult and immature brain. While some studies have shown similar levels of E- and P-selectin in immature and adults rats [92], lower surface expression of P-selectin on endothelial cells or less efficient adhesion of neonatal neutrophils to P-selectin have been described by others [93,94] thus leaving open the question on whether selectins are accountable for the minimal in leukocyte transmigration through the BBB.

In humans, polymorphisms in the P-selectin gene were found to be independent predictors of thrombo-embolic stroke [95], and P-selectin, but not necessarily E-selectin, has also been documented in the serum of stroke patients [96], P-selectin may persist in the circulating blood for up to 90 days after stroke [97]. Soluable sE- and sL-selectin have also been detected in stroke patients [98].

2-3-2. INTEGRINS—Integrins are a family of adhesion molecules that are heterodimers consisting of a common β subunit and a variable α subunit [99]. Of the α subunits, there are three subfamilies, denoted α1–3. Members of the α1 subfamily (or VLA, very late activation) bind collagen, laminin and fibronectin and are involved in the structure of the extracellular matrix, whereas α2 integrins (CD18) are involved in leukocyte cell adhesion. β3 integrins, also known as the cytoadhesins, include the platelet glycoprotein IIb/IIIa (αIIb/α3) and the vitronectin receptor (αv/β3), factors involved in clot generation and stabilization.

Leukocyte integrins are transmembrane cell surface proteins activated by chemokines, cytokines, and other chemoattractants. To bind to activated endothelium, integrins must be expressed on the leukocyte's surface in order to recognize endothelial cell adhesion molecules [100]. The α2 integrins bind to receptors of the immunoglobulin gene superfamily, and contain a common α2 chain with one of 3 distinct α chains (CD11a,

CD11b, or CD11c). The CD11a/CD18 integrin is also referred to as LFA-1, whereas CD11b/CD18 is also called Mac-1. Of the α chains, CD11b has been the most studied in stroke models. Leukocytes and monocytes also express the α4β1 (very late antigen-4,VLA-4, CD49d) integrin, which binds to VCAM-1 and ligands of the subendothelial matrix [79].

Blocking CD11b [101–103], CD18 [104–107] or both [107–109] reduces injury from experimental stroke and is associated with decreased neutrophil infiltration. Similarly, neutrophils from mice that lack CD18 exhibited reduced leukocyte adhesion to endothelial cell monolayers, and when these mice were subjected to experimental stroke, they had improved cerebral blood flow and less neurological injury and neutrophil accumulation compared to the wildtype phenotype [68]. Blocking integrins essential for lymphocyte and monocyte trafficking may also limit damage due to reperfusion injury. Antibodies against VLA-4 given 2 h after a 3 h period of temporary MCA occlusion followed by reperfusion decreased infarct size [45].

At the clinical level, two studies examined the potential of anti-integrin therapies in acute stroke patients. In one phase III trial, a humanized CD11/CD18 antibody was administered to patients within 12 h symptom onset [110]. The second trial was a phase IIb dose escalation study of a non-antibody peptide, recombinant neutrophil inhibiting factor (rNIF) in stroke patients (Acute Stroke Therapy by Inhibition of Neutrophils or ASTIN) [111] administered within 6 h of symptom onset. Both studies were stopped prematurely due to a lack of effect on predetermined endpoints. Although both compounds appeared to be effective in preclinical studies [107,109,112], 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 recanalization) 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, while many integrins are upregulated after stroke in humans, CD11b is actually decreased [113]; therefore, some anti-adhesion molecule approaches may not be appropriate. Regardless, it is clear that more work and possibly improved trial design are needed.

2-3-3.IMMUNOGLOBULIN SUPERFAMILY—Firm adhesion of leukocytes to the endothelial cells as well as leukocyte activation is mediated by receptors of the immunoglobulin superfamily. These molecules cause stronger attachment of leukocytes to the endothelium than the selectins. This family includes 5 molecules that are expressed by endothelial cells: ICAM-1 and ICAM-2, VCAM-1, platelet-endothelial cell adhesion molecule-1 (PECAM-1), and the mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) [114]. These molecules are involved in leukocyte adhesion at relatively low shear forces. ICAM-1 (CD54) is constitutively present in low levels on the cell membranes found on endothelial cells, leukocytes, epithelial cells, and fibroblasts. Its expression greatly increases upon stimulation by cytokines. ICAM-2 (CD102) is an endothelial cell membrane receptor that does not increase after stimulation, whereas VCAM-1 (CD106) is induced by TNF-α and IL-1. PECAM-1 (CD31) is involved in the attachment of endothelial cells to each other, and leukocyte transmigration across the endothelium.

ICAM-1 has been the most studied target within immunoglobin superfamily adhesion molecules in cerebral ischemia. Increased expression of ICAM-1 in ischemic brain occurs within hours of stroke onset, peaks at about 12–24 h, and appears to precede leukocyte infiltration [115–117]. Several studies have now shown that blocking ICAM-1 with antibodies [103,106] or inhibiting ICAM-1 mRNA with antisense oligonucleotides [118] improves outcome from experimental stroke. Consistent with these findings, mice deficient in ICAM-1 had smaller infarcts compared to wildtype mice [119–121]. Furthermore,

combination treatment with recombinant tissue plasminogen activator (rt-PA) and anti-ICAM-1 antibodies led to reduced infarct size compared to rt-PA treatment alone [71]. Anti-ICAM-1 treatment appears to generally require reperfusion to be effective [72], but one study in ICAM-1 deficient mice demonstrated smaller infarcts in both permanent and transient MCA occlusion [119].

The role of VCAM-1 in stroke is less clear. While increases in VCAM-1 mRNA after cerebral ischemia have been observed by some investigators [122], others have not [118]. One study [123] 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. However, treatment with anti-VCAM-1 antibodies did not have any effect on stroke outcome [124], suggesting that VCAM-1 may not play a significant role in ischemic brain injury.

In humans with stroke, elevated soluble intercellular and vascular cell adhesion molecules-1 (sICAM-1 and sVCAM-1) have been documented [98], and VCAM-1 expression has been observed in autopsied brains of stroke victims within cerebral vessels and astrocytes [122]. Furthermore, stroke patients treated with unfractionated heparin were found to have blunting of the typical rise in serum sVCAM-1 [125]. However, anti-ICAM therapy for stroke was not only ineffective in one phase III clinical trial, but it significantly worsened outcome [126]. The interpretation of this study may have been confounded by the use of a murine antibody in humans, with subsequent neutrophil and complement activation [127].

3. THE NEUROVASCULAR UNIT & INFLAMMATION

The concept of the “neurovascular unit” includes the brain microvasculature, glia, neurons and the extracellular matrix. It has emerged from the understanding that focusing on mechanisms of neuronal death without taking into account the microenvironment which provides support for neurons and their connections, may have hampered successful translation of animal data to human stroke. This concept is especially relevant to the understanding of inflammation and ischemic brain injury.

3-1. BLOOD-BRAIN BARRIER (BBB)

The integrity of the BBB is controlled by a number of different and partially independent components, including the presence of an extracellular matrix, tight junctions, pericytes and astrocyte endfeet [128–130], which provides exclusion of circulating cells and macromolecules. Tight junctions, together with adherens junctions, form junctional complexes and play a central role in the control of paracellular permeability and maintenance of cell polarity [130]; endothelial cells have a poor capacity for pinocytosis precluding trans-cellular movement of solutes in the absence of endothelial fenestrae. As a result, trafficking of leukocytes [2] and solutes [131] occurs in the controllable way. It is well known that in the adult brain, the BBB is disrupted after stroke, with the extent of temporo-spatial damage dependent on both the systemic and local inflammatory reactions. Peripheral leukocytes, especially neutrophils, can contribute to the opening of the BBB [14,36,37] and can infiltrate through the disrupted barrier and produce injury by releasing toxic mediators [8,132]. Many factors affect BBB breakdown [11,133]. Besides leukocyte activation [131,134], increased levels of adhesion molecules and degradation of components of the basal lamina by MMPs [135] leads to disruption of the BBB after adult stroke. Microglia [136] and locally and systemically produced cytokines [10,137] also potentiate damage to BBB constituents.

Emerging evidence suggests that the early postnatal BBB is not as permeable as commonly thought. Although mechanisms of BBB function in the fetus are different than those in adult

[138], tight junctions are present early in embryonic development [139], restricting entrance of proteins into the brain in a controllable fashion, and by birth the BBB is functional with no fenestrations [140]. Studies using intra-striatal injections of inflammatory cytokines IL-1β or TNFα [35,50–52] have shown that regulation of the BBB is age-dependent even during the first 3 weeks of life, with the BBB being more permeable at postnatal day 21 (P21) than in adult or one day old pups, demonstrating that the relationship between BBB integrity and brain maturation is not linear. Importantly, the BBB remains more integrant in P7 than in adult rats at least within 24 hr post-transient MCA occlusion [141]. Mechanisms that keep the BBB relatively preserved are not well understood but may be related to the very limited [46,47,49] transmigration of neutrophils in the injured parenchyma during the neonatal period.

3-2. MATRIX METALLOPROTEINASES (MMP) AND EXTRACELLULAR MATRIX

The MMP family of zymogen proteases are involved in the degradation and remodeling of the extracellular matrix under normal conditions. MMPs are found in the cytosol in a pro- or inactivated state, and are cleaved by proteases such as plasmin or other MMPs to their active state. MMP-9 appears to be the most relevant studied in stroke. Expression of the pro- and active MMP-9 is elevated within hours to days after focal stroke in rats [128,142], mice [143,144] and humans [145], and is temporally and spatially correlated with a loss of BBB integrity (reviewed in [135,146]). The damaging role of MMP-9 has been established by reduction of postischemic BBB disruption after MMP inhibition [147] and MMP-9 gene deletion [14,144,148]. While postischemic MMP-9 can be produced by various brain cell types, including neurons, astroglia and microglia, and endothelial cells [128,149], neutrophils contain abundant quantities of pro-MMP-9 in secretory granules [150], and can produce large quantities of MMP-9 upon activation and degranulation [14]. Recently, it was shown that neutrophil, rather than parenchyma derived MMP-9 participate in BBB disruption and injury after experimental stroke using bone marrow chimeras. When MMP-9 deficient bone marrow was transplanted into wildtype (WT) mice, these chimeras demonstrated less injury and BBB disruption compared to WT mice transplanted with WT marrow, indicated that only MMP-9 in circulating immune cells are involved in ischemic injury [14]. Microglia, another major source of the MMPs following ischemia, are also necessary to stimulate astrocytes to generate active MMPs [151]. Importantly, upregulation of brain MMP-9 levels by thrombolytic therapy with tissue plasminogen activator (tPA) poses risks of cerebral hemorrhage after ischemic stroke[152,153]. In contrast to MMP-9, MMP-2 seems to have no effect on acute brain injury after focal ischemia [154] but may play a role in recovery. The role of tissue inhibitors of metalloproteinases, TIMPs, in regulating activity of MMPs and their effects on neuronal cell death repair after cerebral ischemia, particularly during angiogenesis, has been recently reviewed [12,146].

3-3. MICROGLIA/MACROPHAGES

Macrophages in ischemic brain lesions derive from three distinct sources, including parenchymal microglial cells, infiltrating macrophages derived from blood monocytes, and perivascular monocytes. Microglia, the resident macrophages of the brain, are the main cell type that provides immuno-surveillance [155]. Microglia populate the developing brain by birth [156] and gradually ramify during the first two weeks of life, as on going programmed cell death during development declines [157]. Microglial cells undergo a graded process of activation in response to injury [158], which involves morphological transformation, increased migratory activity, proliferation, secretion of cytokines, proteases, and other inflammatory mediators, and antigen presentation [158–160]. Different types of insults can activate distinct receptor types and elicit particular subsets of these functions which, in turn, can serve to protect or exacerbate injury [156,161]. While studies in tissue slices, dissociated cells, and animal models suggest that populations of resting or activated microglia are very

diverse morphologically and functionally [160,162,163], it is not clear what distinguishes one subpopulation from the other.

Brain ischemia induces activation of microglia through various mechanisms [114,155]. The number of receptor families on microglia shown to contribute to injury mediated by these cells keeps growing [33,164,165] but interestingly, effects are in part mediated by CD14 and toll-like receptor 4 (TLR4) following experimental[166] and human stroke [167]. Activated microglia secrete or express inflammatory cytokines and chemokines [4,168–172], FAS receptor [55,173], and iNOS [61,174,175], which further activate microglia and propage local inflammatory responses. Microglial cells can damage other cells, including endothelial cells [136], oligodeondrocytes [176], astrocytes [136] and neurons [177,178], in part via superoxide produced by NADPH oxidase. Activation of microglial cells is often associated with proliferation of these cells [179–181], further amplifying microglial-dependent aspects of injury. In vivo data suggest a general rule that a more severe ischemic brain insult is generally associated with higher macrophage densities. Pharmacological inhibition of cytokine accumulation, such as IL-1 receptor antagonist (IL-1ra) [168], or deficiency of inflammatory cytokines, such as IL-18, IL-6, or deletion of the FAS receptor, reduce injury and diminish various signs of inflammation, including microglial activation [168,170,173]. Minocycline, a tetracycline family antibiotic, was shown to provide significant protection against brain ischemia by inhibiting of microglial activation and proliferation in adult [136,182–186] but show mixed results in neonatal rodents after focal stroke or H-I [187–189].

The relative contribution of resident macrophages (fully activated microglia) versus invading peripheral macrophages after ischemia is unclear, and hampered by a lack of tools to differentiate these two cell populations. Invaded monocytes and microglia (which once activated, become virtually indistinguishable from circulating macrophages) are nearly impossible to distinguish, although some studies have used a pattern of common leukocyte antigen CD45 expression to separate the two populations [43,159,190]. Regardless, depletion of peripheral macrophages using liposome encapsulated clodronate failed to affect infarct size [191], suggesting that brain, rather than circulating macrophages are important in ischemia pathogenesis.

Compared to the adult, microglial activation in neonates is much more rapid following transient focal ischemia [192,193] and excitotoxic injury[194], and continues for weeks following focal ischemia [48,189] and excitotoxic injury [194]. While it is still unknown why there are such differences between microglial activation in the mature and immature brain, several studies have suggested that distinct steps of maturation and differentiation [195] and the propensity of neurons to undergo apoptosis [196,197] in the developing brain may contribute to this age-dependency of the microglial response. Activated microglia/macrophages readily accumulate in injured tissue following H-I [47,171,198,199], produce inflammatory cytokines [171], high levels of NO [200] and MMPs [59]. Reduction of microglia/macrophage associated injury is diminished [187,194] [194] if microglial activation is inhibited [189], thus supporting the idea that microglia contribute to ischemic and excitotoxic injury in the immature brain as well.

While microglial cells can adversely affect acute ischemic injury by secreting toxic inflammatory mediators, they may also be involved in repair and neurogenesis [201]. Microglia/macrophages have the potential to actually protect cells by secreting factors such as transforming growth factor-beta1 (TGF-beta1) and glial cell line-derived neurotrophic factor (GDNF) [202,203]. Interestingly, administering isolated microglia intracerebroventricularly improved behavioral and histologic outcome in an experimental stroke model [204].

3-4. ASTROCYTES

Astrocytes play an important role within the neurovascular unit. These cells contribute to neuronal homeostasis and the structure and maintenance of the BBB. It has been increasingly recognized that astrocytes also have immune modulating roles in brain and contribute to brain inflammation by expressing major histocompatibility complex (MHC) and costimulatory molecules, developing Th2 (anti-inflammatory) immune responses and suppressing interleukin-12 (IL-12) expression [205]. After cerebral ischemia astrocytes become a major source of inflammatory mediators such as cytokines, chemokines [206–208] and inducible nitric oxide synthase (iNOS) [209,210]. Death of astrocytes in ischemic core shortly cerebral ischemia may have important implications for excitotoxicity, anti-oxidative defenses and integrity of the BBB. So-called "reactive gliosis" which is characterized by specific structural and functional changes, is observed acutely in ischemic penumbra and, later, in the core [211]. While the function of glial fibrillary acidic protein (GFAP) in brain ischemia is controversial, GFAP-null mice had significantly larger cortical infarct volumes than their wild-type littermates, suggesting a beneficial role of GFAP [212]. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK), a member of the tumor necrosis factor superfamily, can stimulate proinflammatory molecule production by interaction with its Fn14 receptor found on astrocytes [213]. Expression of TWEAK and Fn14 has been documented in a murine model of stroke, and a soluble decoy to Fn14 markedly reduced infarct volume [214]. 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.

Similar to that observed in adult ischemia models, GFAP immunoreactive astrocytes are rarely seen within the ischemic core but are abundant in penumbra regions 24 hr after transient focal ischemia in P7 rats [192]. While mechanisms of astrocytic death in the immature post-ischemic brain are not well understood, at least a subpopulation of these cells is dying in a caspase-3 dependent manner [48,215]. Caspase-3-dependent death of GFAP – immunoreactive astrocytes is seen early after permanent MCA occlusion and transient CCA occlusion in P7 rats [48,215] and is associated with increased BAX expression [48], cytochrome c release from mitochondria, DNA fragmentation and PARP cleavage [216,217]. The fate of astrocytes has been shown to be affected by microglial activation after H-I in immature rodents [218].

4. MEDIATORS OF INFLAMMATION

4-1. CYTOKINES

Cytokines are upregulated in the brain after a variety of insults including stroke. They are expressed not only in cells of the immune system, but also through endogenous production by resident brain cells, including glia and neurons [219,220]. The most studied cytokines related to inflammation in stroke are IL-1, TNF-α, IL-6, IL-10 and transforming growth factor- β (TGF- β) [25].

4-1-1. IL-1—IL-1 is a 17-kDa polypeptide and exists two isoforms, IL-1α and IL-1β and its endogenous inhibitor, IL-1 receptor antagonist (IL-1ra) [10]. In experimental stroke, IL-1beta mRNA elevations have been documented within 15–30 min after ischemia [221,222], with protein detected a few hours later [223]. Much of the data to date suggests that IL-1 potentiates brain injury in experimental stroke [224]. Increased brain damage occurred when IL-1beta was administered to rats [219], and mice deficient in IL-1 have smaller infarcts compared to wildtype mice. In rat models of focal ischemia, administration of IL-1ra [225] or its overexpression by adenoviral vectors [226] has been shown to reduce neurologic deficits and infarct size. IL-1 has two receptors, IL-1R1 and IL-1R2, but only the

former is involved in signal transduction [223]. However, IL-1β does not appear to act through IL-1R1 in brain ischemia, as IL-1β administration increased infarct volume even in IL-1R1 deficient mice [224,227]. Focal transient ischemia in neonates induces a rapid and sustained increase of IL-1β in circulation and then in the brain [189,228].

4-1-2. TNFα—TNFα is also upregulated in the adult brain after ischemia with similar expression patterns as IL-1β initial increases are seen 1–3 h after ischemia onset [220,229,230]. TNFα expression was initially observed in neurons [220], then later in microglia and some astrocytes [231]. Expression is also biphasic with a second increase appearing at 24 and 48 h [229]. TNFα appears to have pleiotropic functions in the ischemic brain [229]. Inhibition of TNFα reduces ischemic brain injury [29,232], while administration of recombinant TNF-α protein after stroke onset worsens ischemic brain damage [233]. However, TNFα may also protect the brain under certain circumstances. TNFα appears to be involved in the phenomenon of ischemic tolerance [234], and mice deficient in TNF receptors have larger infarcts [235,236].

The reasons for this disparity might be due to different pathways through which TNFα signals. There are at least two TNFα receptors: TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). Most effects induced by TNFα are mediated by TNFR1. TNFR1 contains a death domain (DD) that interacts directly with TNFR1 and may act as a bifurcation point for signaling related to cell death or cell survival. By reacting with Fas-associated death domain (FADD) and caspase-8, TNFα may lead to apoptosis. Reacting with TNF-receptor associating factor 2 and receptor-interacting protein may lead to anti-inflammatory and anti-apoptotic functions (reviewed in [8,9]). Whether and how this applies in brain ischemia has yet to be clearly elucidated.

The pathophysiological role of TNFα in neonatal stroke is assumed but yet to be proven. While neonatal mice lacking functional Fas death receptors are resistant to H-I brain injury [173], perhaps in part due to a dependence on TNFα, but no change in TNFα levels was observed in injured ischemia-reperfused brain of animals of the same age [189].

4-1-3—IL-6 is largely thought of as a pro-inflammatory cytokine, but whether it plays a significant role in ischemic stroke is far from clear. IL-6 deficient mice have similar sized infarcts compared to wildtype suggesting that it does not participate in ischemic pathogenesis [237]. However, other studies suggest either a beneficial [238] or detrimental role [239]. IL-6 appears transiently several hours after ischemia [169,170], and blocking its actions protects the neonatal brain [168].

4-1-4—IL-10 is an anti-inflammatory cytokine and acts by inhibiting IL-1 and TNFα and also by suppressing cytokine receptor expression, and inhibiting receptor activation. It is synthesized in the CNS and is upregulated following experimental stroke [240]. Exogenous administration [241,242] and gene transfer of IL-10 [243] in cerebral ischemia models both appear to have a protective effect. It also reduced excitotoxic brain lesions in neonatal mice [244]. This pleotrophic Th2 cytokine can act on both hematopoietic and non-hematopoietic cells and can reverse injury caused by IL-1β, TNF-a and IL-6 [244]. Importantly, protection is seen only when IL-10 is administered post-insult, whereas treatment prior to or at the time of insult is ineffective [244]. IL-10 also reduces macrophage accumulation in the injured brain.

4-1-5—IL-18 is a less studied pro-inflammatory cytokine. IL-18 becomes active upon cleavage by caspase-1 in a way similar to that for IL-1β, and occurs predominantly in activated microglia and monocytes. One study in stroke patients suggested that IL-18 is involved in stroke-induced inflammation and that initial serum IL-18 levels may be

predictive of stroke outcome [245]. In experimental stroke models, IL-18 levels increase in injured tissue [189]. The role of this cytokine is still unclear. Deletion of the IL-18 gene differentially affected injury in adult and neonatal rodents. There was no effect on infarct volume in adults [246], but there was reduced H-I injury in neonates [170].

4-1-6—TGF-β1, a member of a family of multifunctional peptide growth factors, is considered an anti-inflammatory cytokine that can affect survival of cells of the CNS. TGF-β1 is involved in the modulation of cell growth, differentiation, angiogenesis, immune function, extra-cellular matrix production, cell chemotaxis, apoptosis and hematopoiesis [247]. Its expression has been reported in microglia and astrocytes, with low levels in neurons. Overexpression of TGF- 1 using an adenoviral vector protected mouse brains from ischemic stroke and reduced the accompanying inflammatory response [248]. A recent study showed that cultured neurons may be protected from ischemia-like insults by microglia-secreted TGF-β1 [203].

4-2. CHEMOKINES

Chemoattractant cytokines, or chemokines, and their receptors exert a variety of physiological functions, including control of cell migration, proliferation, differentiation and angiogenesis under normal and disease states [33]. An important role for β (CC), α (CXC) and δ (CXC3) classes of chemokines has been documented in many animal models of neurodegeneration, including stroke [249,250]. They are classified into four classes based on the positions of key cysteine residues (C): C, CC, CXC, and CX3C, and act through specific and shared receptors belonging to the superfamily of G-protein-coupled receptors [32]. While chemokines play a central role in leukocyte physiology by controlling inflammatory cell trafficking, the functional consequences of chemokine receptor activation are not limited to leukocyte chemotaxis, but include cell activation, gene transcription, mitogenic effects, apoptosis, an increase in the respiratory burst, degranulation, phagocytosis, and lipid mediator synthesis (reviewed by Horuk [251,252]). In the CNS, activated glial cells are almost the exclusive source of intra-parenchymal cytokine production [253]. Accumulated data indicate that a variety of chemokines are induced in the animal models of focal cerebral ischemia.

4-2-1. MCP-1—The central role of MCP-1 and its only known receptor, CCR2 [254], in monocyte transmigration into the brain was demonstrated by profound deficits in the recruitment of monocytes to sites of inflammation and injury in CCR2 deleted mice [255,256], but increased brain cytokine production and brain injury in CCR2 overexpressing mice [257]. Increases in MCP-1 expression in the CNS is seen in a variety of acute insults, including ischemia [206,258,259]. A variety of cells has been shown to express MCP-1 after focal ischemia [206]. Consistent with a deleterious role, its inhibition or deficiency is associated with reduced injury [260,261], whereas overexpression of MCP-1 exacerbats injury [259].

In newborn rodents, MCP-1 is expressed following H-I [262], transient focal ischemia [189] and excitotoxic insults [263]. The pathophysiologic role of MCP-1 in neonatal brain injury was evident by protection of the neonatal brain by functional inactivation of MCP-1 post-insult [263] or in mice with depleted intrelukin-1 converting enzyme [262].

4-2-2. MIP-1α—Macrophage inflammatory protein-1α (MIP-1α) is induced in the animal models of focal cerebral ischemia in adults [253,264–266] and in neonates [171]. It is thought to contribute to ischemic injury by regulating monocyte and microglial recruitment and activation and disruption of the BBB [267].

4-2-3. IL-8, CINC-1/KC—These ELR^+ CXC chemokines exhibit potent chemoattractant activities towards neutrophils and show angiogenic activities [268,269]. All ELR^+ CXC chemokines except IL-8 bind to CXCR2 with high affinity, while IL-8 binds to both CXCR1 and CXCR2. While two functional high affinity CXC receptors for IL-8 are identified in human [270,271], only CXCR2 is described in rat [272]. Both CXCR1 and CXCR2 are predominantly expressed in immune cells, and to a lesser extent by a variety of cells, including glial cells [273] and cerebellar and cortical neurons [252,274]. Transient increase of the CXCR2 ligand, CINC-1 has been reported acutely following transient ischemia in adult [250]. IL-8 [42] and CINC-1/KC [35] are thought responsible for the neutrophil-mediated BBB disruption after brain injury.

4-2-4. Fractalkine—The CX3C chemokine, fractalkine is expressed almost exclusively in neurons. Following ischemia, 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 [275]. The significance of these observations are not entirely clear, but fractalkine deficiency is associated with reduced infarct size suggesting that fractalkine may mediate neuron-microglial interactions and cell death [276].

4-2-5. SDF-1—With recent interest in the area of cell based therapy for stroke, chemokines may also play an important role in honing stem cells to regions of injury. Several chemokines such as MCP-1 and SDF-1 and/or their receptors have been observed in the penumbra [132,277] and at the interface of ischemic tissue and cell transplants [132,278]. Inhibiting these chemokines reduced stem cell migration into ischemic tissue [278,279].

4-3. REACTIVE OXYGEN SPECIES

Once activated, the inflammatory cells in the brain generate ROS as a defense mechanism against microbes. Inflammatory cells generate ROS via several enzyme systems. Superoxide is generated via COX, xanthine dehydrogenase, xanthine oxidase and NADPH oxidase, whereas myeloperoxidase (MPO) and monoamine oxidase (MAO) generate hypochlorous acid and H_2O_2. Superoxide anion is a major oxidant generated in the brain parenchyma after MCA occlusiob, and is well known to cause direct injury to ischemic brain as well as to react with NO to generate peroxynitrite [280].

NADPH oxidase is a multicomponent enzyme that consists of two membrane bound subunits, gp91 and p22, and three cytosolic subunits, p67, p47 and p40 plus Rac, a small GTPase [281]. With appropriate stimuli, the cytosolic subunits translocate to the membrane where they interact with the membrane bound subunits to transfer electrons from NADPH to oxygen to form superoxide. Prior work has shown that NADPH oxidase deficient mice have smaller infarcts than wild type mice. Furthermore, when bone marrow from NADPH oxidase deficient mice were transplanted into either wildtype or deficient mice, wildtype mice transplanted with deficient marrow suffered the same amount of injury as wildtype mice transplanted with wildtype marrow. These latter data would suggest that NADPH oxidase generated within endogenous brain cells such as microglia, rather than circulating leukocytes, are responsible for the worsened ischemic damage [282]. Studies in brain slices [283] and co-cultured oligodendrocytes and microglial cells [176] also suggest the direct injurious role of microglial NADPH oxidase.

MPO, found in neutrophils and monocytes but not macrophages, is thought to mediate bactericidal killing through H_2O_2 and hypochlorous acid. However, very little has been directly investigated regarding its role in ischemic brain pathophysiology. One study subjected MPO deficient mice to focal cerebral ischemia and found that infarct size was

paradoxically increased [284]. The authors subsequently found that these MPO deficient mice also had increased products of nitrosylation within the ischemic brain. Subsequent work 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 [284,285]. Related work has also shown that MPO contributes to the termination of neutrophil influx, as MPO-deficient leukocytes can exhibit a stronger and more prolonged respiratory burst [286]. Therefore, it is possible that MPO may actually limit the extent of ROS-mediated tissue injury.

ROS-mediated injury is a major cause of injury in the developing brain [287,288] due to imbalanced brain antioxidative defense mechanisms, high concentrations of unsaturated fatty acids [289], high rates of oxygen consumption, low concentrations of antioxidants, an imbalance between anti-oxidative enzymes, including catalase, CuZn-superoxide dismutase (SOD1), mitochondrial SOD2 and glutathione peroxidase (Gpx) [290–293], and availability of unbound iron. All of these factors contribute to the vulnerability of the immature brain to oxidative damage, as described in several recent reviews [20,22,24,294].

4-4. NITRIC OXIDE/NITRIC OXIDE SYNTHASE

Nitric oxide (NO) is a small, relatively stable, free-radical gas that readily diffuses into cells and cell membranes where it reacts with molecular targets. NO is produced via conversion of L-arginine by three different isoforms of nitric oxide synthase (NOS). Inducible NOS (iNOS), produced by several cell types following brain injury [295–297] but is acutely found in activated microglia [61,298]. NO generation can have opposing roles in the process of ischemic injury, protective when generated by endothelial NOS (eNOS) and detrimental when produced by neuronal NOS (nNOS) and iNOS [299,300], making nonspecific NOS inhibitors undesirable [301]. NO that accumulates locally in the brain can directly damage cell constituents, produce a number of toxic species, such as peroxynitrate [302], activate COX-2 [303], or activate inflammatory cells [298]. Consistent with the notion that iNOS is injurious in ischemia, deletion of iNOS [304] or iNOS inhibition [296,305,306] is neuroptrotective in neonatal and adult animals. The spatial and temporal patterns of iNOS expression and activity are not uniform following ischemia-reperfusion. While some studies in adult rats found increased iNOS expression/activity in penumbra but not in ischemic core within 6–48 hr post 2 hr MCA occlusion [307], other studies showed a more delayed onset of iNOS up-regulation [308].

The pathophysiological role of iNOS in neonatal brain injury has been established by data that showed both elevated iNOS message and/or protein in rodent models of birth asphyxia [293], neonatal excitotoxic damage [295] and H-I [200,309], as well as the ability to ameliorate ischemic injury and NO synthesis in rat brain by pharmacologic inhibition of iNOS [200,309]. In contrast, following focal ischemia-reperfusion in neonatal rats, iNOS inhibition attenuated activation of mediators of neuronal death without affecting activation or proliferation of microglial cells or reducing injury volume [175]. Inhibition of iNOS, appeared to have deleterious effects in neonatal meningitis model [310].

4-5. ARACHIDONIC ACID METABOLITES

The arachidonic acid (AA) cascade is initiated by the activation of phospholipase A_2 (PLA_2) [311]. Energy failure due to disruption of blood flow can result in calcium accumulation in brain cells. Calcium then activates PLA_2 which hydrolyses glycerophospholipids to release AA. The cytosolic form, cPLA2 is found in reactive astrocytes and microglia following cerebral ischemia [312]. It is an important enzyme in ischemia, as $cPLA_2$ deficient mice had smaller infarcts and developed less brain edema with fewer neurological deficits than their wild type littermates [313]. Arachidonic acid metabolites are potent mediators that

contribute to post-ischemic brain inflammation and circulatory disorders [314]. AA is metabolized through two different pathways via cyclooxygenase (COX) or lipoxygenase (LOX).

4-5-1. Cyclooxygenase pathway—Arachidonic acid released from brain phospholipids during ischemia/reperfusion is converted to prostaglandin H_2 (PGH_2) by cyclooxygenase (COX). Among the two isoforms, COX-1 is constitutively expressed in many cells types, including microglia and leukocytes during brain injury [315] and COX-2 is an inducible isoform increased following ischemia [316,317]. 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 [11]. PGH_2 is further metabolized to a variety of prostaglandins such as prostacyclin (PGI2) and thromboxane A_2.(TXA2). The roles of various COX metabolites are protean, but accumulated data suggest that those downstream of COX-2 are likely deleterious. Several studies have now shown that treatment with COX-2 inhibitors improve neurological outcome after stroke [318–321]. COX-2 deficient mice have reduced injury after N-methyl-D-aspartate (NMDA) exposure [322], whereas COX-2 overexpression exacerbates brain injury [323]. COX-2 activation at least in part depends on iNOS activation [303]. Interestingly, COX-2 mediates its toxic effect through PGE_2 rather than ROS, even though COX-2 can generate both [324].

4-5-2. 5-Lipoxygenase pathway—Compared to the COX pathway, less work has been done to study interventions in the lipoxygenase pathway, but like COX, modulators of lipoxygenase have the potential to improve the stroke outcome as well. 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). cysLTs are proinflammatory lipid mediators and consist of leukotriene C4 (LTC4), D4 (LTD4) and E4 (LTE4). LTC4 synthase is found exclusively in inflammatory cells of hematopoietic origin, and converts LTA2 to LTC4 after conjugation with glutathione [325]. 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 [326]. Pretreatment with a 5-lipoxygenase inhibitor, AA861 resulted in significant attenuation of LTC4 levels and reduction in brain edema and cell death [327]. Although little work has been done to study interventions in the lipoxygenase pathway, but have the potential to improve the stroke outcome.

5. INTRACELLULAR INFLAMMATORY SIGNALING MECHANISMS

Cerebral ischemia upregulates gene expression [328–331], including rapid transcriptional activation of pro-inflammatory factors. Some of these transcription factors are particularly involved in the inflammatory response, and will be discussed here. (Figure 2).

5-1. NUCLEAR FACTOR kappa Beta (NF-kB)

NF-kB, is a dimeric transcription factor consisting of subunits of the Rel family, and are involved in the regulation of inflammation [332,333]. The most common form of NF-kB is a heterodimer composed of Rel A (p65) and p50. NF-kB is normally located in the cytoplasm bound to its endogenous inhibitor protein, known as IkB. Phosphorylation of IkB at serines 32 and 36 by an upstream IkB kinase (IKK) leads to IkB phosphorylation, ubiquitination and degradation in the 26S proteasome. This liberates NF-kB and allows it to translocate to the nucleus. Once in the nucleus, NF-kB binds to κB sites, specific domains within the promoters of downstream genes to activate their transcription. Many genes involved in inflammation contain functional kB sites, such as TNF-α, ICAM-1, COX-2, iNOS and IL-6.

Following experimental stroke, IKK and NF-kB activation have been documented, and these processes appear to be correlated to the anti-inflammatory effect of mild hypothermia [334]. However, the function of NF-kB in stroke is still controversial [335]. Mice deficient in NF-kB's p50 subunit are protected from experimental stroke [336], consistent with a death-promoting role of NF-kB in focal ischemia. However, another study demonstrated that rats given diethyldithiocarbamate (DDTC), a NF-kB inhibitor, had enhanced neuronal DNA fragmentation and larger infarct sizes compared to controls, suggesting a beneficial role [337]. A potential role for IKK inhibitors in stroke therapy has been demonstrated by data where mice that contain a targeted deletion of IkBkb (which encodes IKK2) in mouse neurons and mice that express a dominant inhibitor of IKK in neurons have markedly reduced infarct size, whereas constitutive activation of IKK2 enlarges infarct size [338].

5-2. MITOGEN-ACTIVATED PROTEIN KINASE (MAPK)

Mitogen-activated protein kinases 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 (for reviews, see [339-342]). There are three well characterized, interlinked signaling pathways which have been documented following brain ischemia: the stress-activated protein kinases/c-Jun N-terminal kinases (SAPK/JNK), the p38 MAPKs and extracellular signal-regulated kinases (ERKs) [340,342–344]. Of these, p38 MAPK appears to be the best studied in terms of promoting inflammatory signals in the ischemic brain [339]. p38 MAPK promotes the stabilization and enhanced translation of mRNAs encoding proinflammatory proteins [342]. p38 plays a role in the induction of the inflammatory and immune response via the regulation of NF-kB recruitment to selected targets [345]. It is activated by inflammatory cytokines such as IL-1 and TNF, both of which have been documented in the ischemic brain. Following activation and phosphorylation by its upstream kinase, MAPK kinase (MKK), p38 phosphorylates and activates the transcription factor, activating transcription factor (ATF2), whereas phosphorylation of JNK induces c-Jun. The role of JNK in neurodegeneration was recently reviewed [346].

Following forebrain ischemia in rodents, phosphorylated p38 MAPK was detected in the hippocampus within neuron- [344] and microglia-like [347] cells, suggesting its role in the endogenous inflammatory response. Furthermore, p38 MAPK inhibitors reduced brain injury and neurological deficits in focal cerebral ischemia as well as ischemia-induced cytokine expression. SB239063, a second generation inhibitor also reduced lipopolysaccharide (LPS)-induced plasma TNF production [348].

While p38 inhibitors showed some promise in adult stroke models, the role of this kinase is less clear in neonatal ischemic injury. While increased p38 phosphorylation was observed after HI in neonatal rats brain [349], profound reduction of p38 phosphorylation was observed in injured brain after transient focal ischemia [189].

5-3. ACTIVATOR PROTEIN -1 (AP-1)

AP-1 is a heterodimer comprised of bZIP transcription factors (e.g., c-Jun and JunD), activating transcription factor 2 (ATF2) and c-*Fos*. Upstream activation of AP-1 components is mediated through the JNK/SAPK cascade. c-*Fos* was found to be up regulated as early as 30 minutes after stroke onset [350]. The Fos protein contains a DNA binding region and a leucine zipper. The leucine zipper forms an α-helix which can align with other proteins (such as Jun) containing like structures to form dimers. These dimers bind to specific DNA regions known as the AP-1 domain, which regulates the expression of a number of target genes (collectively referred to as late response genes). Combinations of *c-Fos* and *c-Jun* family proteins form different dimers consisting of various subunits

depending on the circumstances. The composition of the dimer may determine whether the late response gene is turned on or off [351]. ATF2 is a member of the cAMP response element binding protein (CREB) subfamily of bZIP transcription factors. AP-1 heterodimers containing ATF transcription factors can bind both to the TPA (12-O-tetradecanoylphorbol-13-acetate) responsive element (TRE) and to the cAMP response element (CRE) [352]. AP-1 is an important activator of a number of inflammatory genes including the genes for IL-1 and TNF. In addition, AP-1 participates in the transcriptional induction of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and E-selectin which are involved in leukocyte recruitment [342,352,353].

6. SUMMARY

Stroke triggers a robust inflammatory response in both adult and neonatal brain. An overwhelming number of publications have linked post-ischemic inflammation to the progression of brain damage. Thus, anti-inflammatory therapies may be effective against stroke. However, this has yet to be shown at the clinical level. Furthermore, inflammation is also a host defense against pathogens, and inflammation plays a role in angiogenesis, tissue remodeling and regeneration [201,354]. Though the potential benefits of post-ischemic inflammation have not been well documented, at least some cytokines or other inflammatory mediators produced by inflammatory cells showed to be neuroprotective after stroke. Whether inflammation is destructive or protective may depend on how severe the ischemia is, how inflammatory cells are activated and the stage of stroke in which inflammatory responses contribute. For the developing brain, studies are also needed to determine whether anti-inflammatory agents have adverse effects on the long-term brain development. Future work should address the optimal timing of inflammation modulating interventions as well as elucidating how the immune system moves from damaging to protective/restorative responses and how these stages are affected by genetic background, age or gender.

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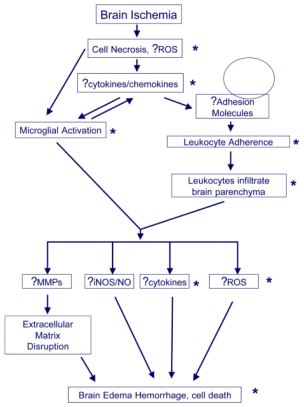

Figure 1. Inflammation following stroke

Brain ischemia triggers inflammatory responses due to the presence of necrotic cells, generation of reactive oxygen species (ROS) and production of inflammatory cytokines even within neurons. These initiators lead to microglial activation which produce more cytokines causing upregulation of adhesion molecules in the cerebral vasculature. Chemokines lead to inflammatory cell chemotaxis to ischemic brain. Adhesion molecules mediate adhesion of circulating leukocytes to vascular endothelia and infiltration into the brain parenchyma. Once in the brain, activated leukocytes and microglia produce a variety of inflammatory mediators such as matrix metalloproteinases (MMPs), inducible nitric oxide synthase (iNOS) which generates nitric oxide (NO), cytokines and more ROS which lead to brain edema, hemorrhage and ultimately, cell death. MMPs are thought to mediate extracellular matrix disruption, a key event in brain edema and hemorrhage. Asterisks (*) are used to indicate the described differences in events in neonatal compared to adult stroke.

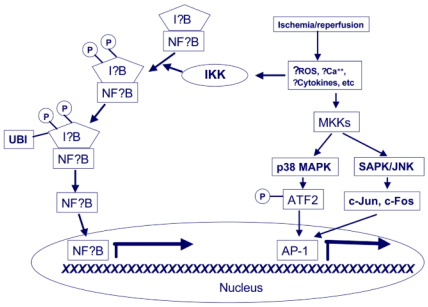

Figure 2. Transcriptional activation of inflammation following ischemia

Brain ischemia leads to the generation of a variety of substances that are capable of activating various inflammatory transcription factors (see text section 3 for details). Ischemia triggers the activation of the following inflammation relevant transcription factors: nuclear factor kappa B (NFkB), the p38 mitogen activated protein kinase (p38 MAPK), and stress activated protein kinase (SAPK/JNK). NFkB is normally contained in the cytosol by its inhibitor protein (IkB). When phosphorylated by its kinase, IKK (P), IkB is ubiquitinated (UBI) and degraded in the proteasome. Once liberated from IkB, NFkB translocates to the nucleus where it binds to consensus DNA sequences. p38 MAPK and SAPK/JNK are activated in a tiered fashion by MAPK kinase (MKK). Activated p38 MAPK phosphorylates activating transcription factor (ATF2), while SAPK/JNK leads to c-Jun and c-Fos expression. These three factors make up the transcription factor, activator protein-1 (AP-1).