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Review Article

Cytokines: Their Role in Stroke and Potential Use as Biomarkers and Therapeutic Targets

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ABSTRACT: Inflammatory mechanisms both in the periphery and in the CNS are important in the pathophysiologic processes occurring after the onset of ischemic stroke (IS). Cytokines are key players in the inflammatory mechanism and contribute to the progression of ischemic damage. This literature review focuses on the effects of inflammation on ischemic stroke, and the role pro-inflammatory and anti-inflammatory cytokines play on deleterious or beneficial stroke outcome. The discovery of biomarkers and novel therapeutics for stroke has been the focus of extensive research recently; thus, understanding the roles of pro-inflammatory and anti-inflammatory cytokines that are up-regulated during stroke will help us further understand how inflammation contributes to the progression of ischemic damage and provide potential targets for novel therapeutics and biomarkers for diagnosis and prognosis of stroke.

Key words: Stroke, Cytokines, Biomarkers

Approximately 800,000 Americans suffer from a stroke each year, and the American Heart Association predicts a 5.1 percent increase in stroke cases for Americans between 45 and 64 years of age by 2030. More than 137,000 people die from a stroke each year resulting in stroke being the fourth leading cause of death and leading cause of disability in the United States [1]. Ischemic strokes which result from an interruption of blood flow to the brain due to a clot, account for 87 percent of all stroke cases [1]. Although ischemic stroke is a devastating problem in the United States and worldwide, the only FDA approved treatment is tissue plasminogen activator (tPA), which is a thrombolytic to break up the clot. Unfortunately, less than 5 percent of patients receive tPA due to its limited time window of 4.5 hours; thus, most patients only receive supportive care [2].

An occlusion of a cerebral artery causes deprivation of oxygen, glucose, and lipids resulting in necrotic brain tissue. Breakdown of the blood-brain barrier and activation of inflammatory cells in the brain and in the periphery result in inflammation in the necrotic tissue [3, 4]. Inflammatory mechanisms both in the periphery and in the central nervous system (CNS) are important in promoting brain inflammation by producing inflammatory mediators such as cytokines and clearing away dead tissue after cerebral ischemia [5]. Cytokines are key players in the inflammatory mechanism and contribute to the progression of ischemic damage [6]. This review will focus on the effects of inflammation on ischemic stroke, and the role pro-inflammatory and anti-inflammatory cytokines play on deleterious or beneficial stroke outcome. The discovery of biomarkers and novel

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therapeutics for stroke has been the focus of extensive research recently; thus, understanding the roles of pro-inflammatory and anti-inflammatory cytokines that are produced during stroke will help us further understand how inflammation contributes to the progression of ischemic damage and provide potential targets for novel therapeutics and biomarkers for prognosis of stroke.

Immune Response to Stroke

The complex, multifaceted cascade of events that results from brain deprivation of oxygen, glucose, and other essential nutrients to the brain causes dysfunction of the neurovascular unit [7]. During ischemia, glutamate stored within brain cells is released when cells are hyperactive or die resulting in excitotoxicity [8, 9]. Furthermore, brain and immune cells produce reactive oxygen species (ROS), and restoration of blood flow in the occluded vessel generates additional ROS [10]. ROS activate endothelial cells and cause oxidative stress [11, 12]. Oxidative stress and the induction of the inflammatory cascade leads to the breakdown of the blood-brain barrier allowing activated blood-borne immune cells such as neutrophils and T-cells to infiltrate and accumulate in the ischemic brain tissue [11]. Along with the accumulation of activated immune cells from the periphery, microglia in the brain become activated after cerebral ischemia due to the increase in extracellular ATP from the depolarization of neurons and glia and the release through damaged plasma membranes of dying cells [11]. Activated microglia secrete pro-inflammatory mediators such as cytokines and develop phagocytic and major histocompatibility complex (MHC) class II-restricted antigen presenting characteristics [13]. Microglia activation can be beneficial by producing growth factors such as brain-derived neurotrophic factor and clearing away dead tissue and debris after ischemia; however, the release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and production of reactive oxygen species and nitric oxide after microglia activation is detrimental [14, 15]. ATP is an early danger signal increasing inflammation, but as cells die, molecular signals called danger associated molecular pattern molecules (DAMPs) are generated and activate pattern recognition receptors such as toll like receptors (TLRs) [16]. These pattern recognition receptors are found on endothelial cells and microglia in the brain along with infiltrating leukocytes from the periphery, and activation of pattern recognition receptors increases cytokine release [16]. Moreover as neurons begin to die after ischemia, cell-cell interactions with microglia are lost and further increases inflammatory signaling [17]. Thus acutely after ischemia, hypoxia and oxidative stress induces synthesis of nuclear factor κB (NF-κB), hypoxia inducible factor 1 and many other transcription factors that increase the expression of pro-inflammatory and anti-inflammatory cytokines [18]. As cells die and brain tissue is damaged, molecular danger signals further potentiate the inflammatory response by activating more microglia and infiltrating leukocytes in a feed-forward response producing more deleterious pro-inflammatory cytokines (Figure 1). This increase in expression of cytokines further increases the expression of adhesion molecules on endothelial cells that result in additional recruitment of leukocytes from the periphery [19]. These inflammatory changes after ischemia lead to an increase in neuronal cell death resulting in a larger infract volume and worse neurological outcome. Inflammation is a key player in brain damage during cerebral ischemia; however, inflammation aiding in repair and recovery after cerebral ischemia can be beneficial. Thus, further mechanistic understanding of how inflammation contributes to injury or repair after ischemia is important for discovering potential therapeutic targets for stroke and for using inflammatory mediators such as cytokines as biomarkers for prognosis.

Cytokine Changes Following Stroke

Cytokines are soluble glycoproteins that are produced by cells in the brain in response to damaged tissue after ischemia and are responsible for regulating the innate and adaptive immune response [20]. Microglia, astrocytes, endothelial cells, and neurons in the brain are able to secrete pro-inflammatory and anti-inflammatory cytokines [20]. An increase in production of pro-inflammatory cytokines and a decrease in production of anti-inflammatory cytokines is correlated with a larger infarct size in animal models and a worse clinical outcome [21]. TNF-α, interleukin (IL)-1β, IL-6, and IL-10 are inflammatory cytokines that have been found to be related to ischemic stroke and have been implicated as therapeutic targets and biomarkers for prognosis. The inflammatory response to ischemia changes with time, and knowing the timing of when these cytokines are increased or decreased and how these cytokines affect infarct volume is important in understanding how cytokines can be utilized clinically. As such, this review will focus on three pro-inflammatory cytokines and one anti-inflammatory cytokine that are associated with stroke and found to be related to outcome. TNF-α and IL-1β are well characterized in experimental stroke studies and human subjects. However, little is known about the temporal profile of IL-6 and IL-10, and fewer experimental stroke investigations have studied IL-6.

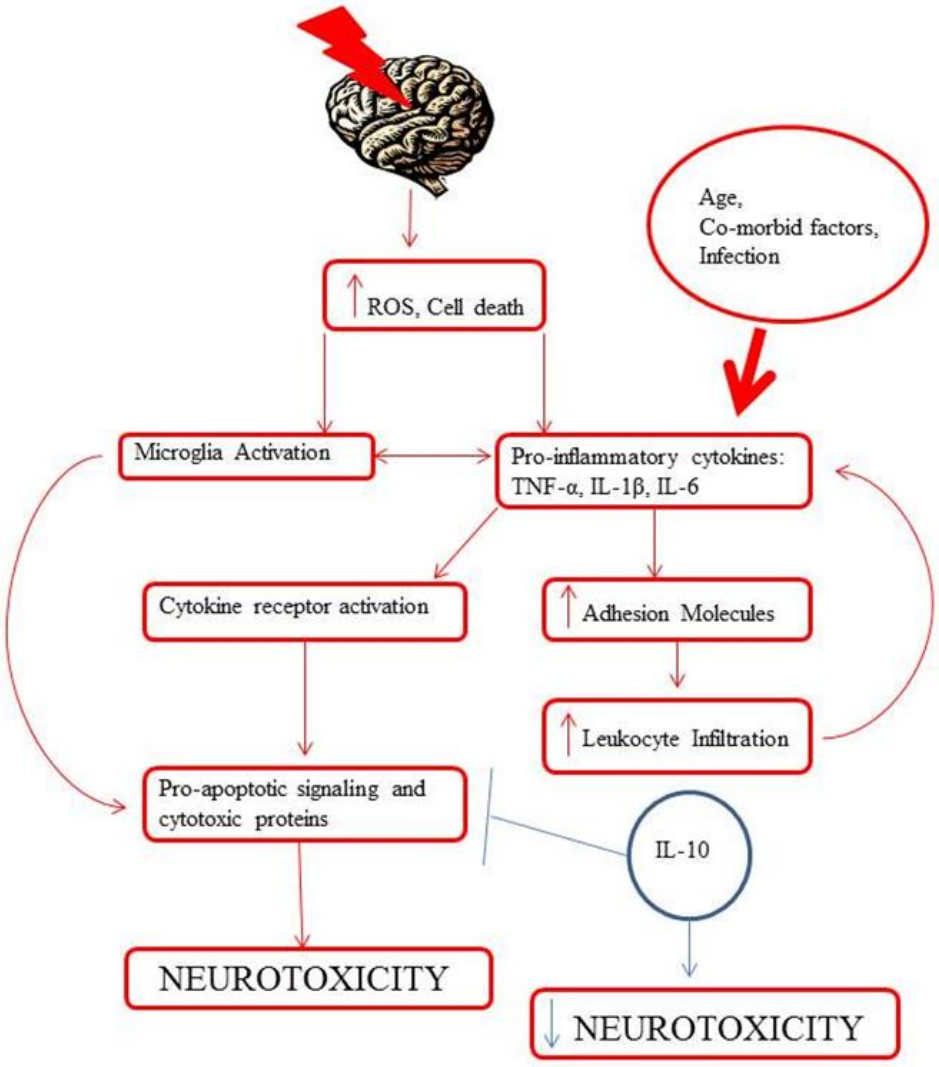

Figure 1: Schematic of the effects of cerebral ischemia and co-morbid factors on pre- and anti-inflammatory cytokines and immune cells and their contribution to neurotoxicity.

TNF-α

Tumor necrosis factor-alpha (TNF-α) precursor is membrane bound and cleaved by TNF-α converting enzyme (TACE) to release the soluble form that can bind to TNFR1 or TNFR2 [22]. Most cells in the brain express TNFR1, and TNFR1 has an intracellular death domain (DD) with which adaptor proteins such as TNFR-associated DD protein (TRADD) interacts, and the interaction of TRADD with the intracellular DD of TNFR1 elicits signaling for cell death or cell survival by recruiting other adaptor proteins [22-24]. The recruitment of adaptor proteins such as caspase-8 and FAS-associating protein with a death domain (FADD) results in apoptosis, and recruitment of TNF-receptor associating factor 2 (TRAF2) and receptor-interacting protein (RIP) activate NF-κB resulting in activation of genes for anti-inflammatory, anti-apoptotic proteins along with pro-inflammatory, apoptotic, and cytotoxic proteins [24]. Therefore, the structure of the TNF-signaling complex

enables TNF-α to induce inflammation and cell death after stroke or induce tolerance to ischemia.

Experimental Stroke Studies

Consequently in experimental stroke studies, it is not surprising that TNF-α has been shown to be either neurotoxic or neuroprotective. In a mouse distal permanent middle cerebral artery occlusion (pMCAO) electrocoagulation ischemic model, intracisternal injection of TNF-α 48 hours before occlusion decreases infarct volume [25]. Furthermore, using the same ischemia model TNF-α, TNFR1, and TNFR2 knock-out mice show larger infarct size compared to control mice suggesting that TNF-α is neuroprotective [26]. However, multiple approaches suggest that TNF-α is neurotoxic in stroke. I.p. or i.v. injection of TNF-α binding protein immediately after occlusion and topical administration of TNF-α binding protein immediately and 1 hour after occlusion in the distal pMCAO electrocoagulation ischemic model causes a dose-dependent decrease in infarct volume at 1 hour, 24 hours, and 2 weeks [27, 28]. Also, in the one hour distal transient middle cerebral artery occlusion (tMCAO) filament ischemia mouse model, i.c.v. injection of anti-TNF antibody decreases infarct size [29]. In the rat ischemia model using the distal tMCAO and distal pMCAO where the vessel is occluded and cut, treating with TNF-α 24 hours before occlusion is neurotoxic and increases infarct volume [30]. Also, in the distal pMCAO thromboembolic model, inhibiting TACE decreases infarct volume. In agreement with the mouse model, administrating anti-TNF antibody and TNF-α binding protein in the rat ischemia model decreases infarct volume [31, 32]. The neurotoxic versus neuroprotective action of TNF-α is most likely due to signaling through nuclear factor κB producing both pro-inflammatory and anti-inflammatory mediators and the timing of administration of TNF-α in experimental stroke models.

Time Course in Experimental Stroke Studies

After stroke onset, TNF-α mRNA expression is induced within thirty minutes after both tMCAO and pMCAO in mice and rats, and TNF-α protein expression is seen within 1 hour in the cortex and striatum after tMCAO in spontaneously hypertensive rats [6]. Levels of TNF-α mRNA and protein peak between 12 and 24 hours in both mice and rats and remain elevated up to 10 days in mice and 6 days in rats [33, 34]. The timing of the expression of TNF-α after ischemia is found to be comparable among different mouse strains after pMCAO, but the level of expression is different [35]. Cells producing TNF-α are found at 12 and 24 hours after stroke in the penumbra of all mouse strains [35]. Interpretation of the time course of TNF-α is complicated in animal models because of the use of different strains of mice and rats, different methods for measuring levels of TNF-α, and the time after stroke when levels are measured.

Time Course in Human Studies

In postmortem brain tissue studies, TNF-α positive cells are observed in all ischemic brains of severe patients 3 days post-stroke and are present up to 15 months post-stroke, and the majority of TNF-α positive cells are microglia and macrophages [36, 37]. TNF-α positive neurons peak between 2 and 3 days post-stroke, TNF-α positive infiltrating immune cells from the periphery peak at day 3, and TNF-α positive astrocytes peak from 15 hours to 14 days [38]. Furthermore, TNF-α levels have been measured in CSF at 0, 3, 7, 9, 21, 26 days and 3 months after stroke, and TNF-α levels are only increased in stroke patients with white matter damage compared to normal controls at 3 months post-stroke [37]. However, TNF-α levels are increased within 24 hours in the CSF [39, 40]. Studies assessing TNF-α in serum of stroke patients have not been conclusive. A few studies found that serum TNF-α levels are not increased at admission or any time between 12 hours and 10 days after stroke [41-43]. However, other studies have shown that TNF-α is increased in the serum of stroke patients compared to controls within 6 hours and stay elevated for 10 days post-stroke [40, 44]. Moreover, studies measuring TNF-α in plasma observed a significant increase of TNF-α in stroke patients at admission and within 24 hours post-stroke [39, 45]. The time after stroke when levels of TNF-α are measured is critical and a contributing factor to the difference in results among and between animal and human studies. Additionally, the severity of stroke may be a key factor in the timing and magnitude of the increase in TNF-α.

IL1-β

IL1-β mRNA and protein is constitutively expressed in the brain at low levels and is regulated by transcription, translation, cleavage and cellular release. IL1-β is synthesized as a large precursor protein and is biologically inactive until it is cleaved by caspase-1 and secreted [46]. Once it is biologically active, it binds to type 1 IL-1 receptor (IL-1R1) which associates with IL-1-receptor accessory protein (IL-1RAcP) that initiates intracellular signaling [47]. IL1-β can also bind to IL-1R2, but IL-1R2 does not initiate downstream signaling because IL-1R2 does not have an intracellular-signaling domain [46, 47]. Furthermore, IL-1R1 and IL-1R2 can exist in soluble forms when they are shed from cell membranes [46-48]. IL-1R2 and the soluble forms of both receptors act as

decoy receptors that prevent a ligand from initiating signaling pathways [48]. During acute ischemia, there is an increase in ATP which promotes the cleavage and cellular release of IL1-β [46]. An increase in IL1-β can cause an increase in calcium entry through NMDA-receptor ion channels resulting in neuronal cell death [49]. Furthermore, IL1-β can potentiate inflammation by activating microglia, and IL1-β increases leukocyte infiltration by increasing the expression of adhesion molecules on endothelial cells and causing breakdown of the blood brain barrier contributing to an increased infarct size and poor clinical outcome [50-52].

Experimental Stroke Studies

Numerous studies have shown IL1-β to be neurotoxic in animal ischemia models. In the mouse distal electrocoagulation pMCAO model, injecting an IL-1 receptor antagonist immediately and 4 hours after stroke decreases infarct volume [28]. Moreover in the proximal tMCAO filament model, treating with caspase-1 inhibitors 15 minutes before and immediately after stroke decreased infarct volume, and using caspase-1 knock-out mice result in smaller infarct volume compared to control mice [53, 54]. IL1-αβ knock-out mice also show smaller infarct volumes [55]. Similarly, the rat proximal electrocoagulation and filament pMCAO model exhibit an increase in infarct size in animals treated with IL1-β 30 minutes before and after stroke and a smaller infarct size, improved neurological score, and decreased number of leukocytes in the ischemic tissue when treated with an IL-1 receptor antagonist at the time of occlusion along with injections at 4, 8, 12, and 18 hours after stroke [56-58]. Proximal tMCAO filament models further shows IL1-β neurotoxicity, confirming what was seen in the rat pMCAO and mouse ischemia models. Caspase-1 inhibitors decrease infarct volume in the tMCAO filament model when injected 15 minutes before surgery and after reperfusion, and injecting IL1-β after reperfusion increases infarct size and brain edema [54, 59]. IL-1β is a potent pro-inflammatory cytokine and has a role in activation of numerous inflammatory processes. Using different ischemia models and different treatments to enhance or inhibit IL-1β has resulted in the observation that IL-1β is constantly neurotoxic in experimental stroke models.

Time Course in Experimental Stroke Studies

IL1-β positive microglia cells are found in the cortex 6 to 24 hours after stroke and elevated IL1-β mRNA levels are observed at 10 hours in the brain tissue in both the proximal pMCAO and proximal tMCAO filament mouse model [60, 61]. However, levels of IL1-β mRNA peak later at 18 hours in the pMCAO model compared to peak levels at 10 hours in the tMCAO model. In rat ischemia models, peak levels of IL1-β mRNA are seen at 6 hours in in the proximal tMCAO and distal pMCAO suture model [62, 63] and elevated levels are seen as early as 1 hour after stroke [63, 64]. Thus, both rat and mouse ischemia models show an early increase in expression of IL-1β with peak expression dependent on the occlusion model used. Reperfusion allows for increased access of leukocytes to the brain which may contribute to levels of expression peaking earlier in the tMCAO model.

Time Course in Human Studies

IL1-β is elevated in the CSF of severe stroke patients with peak levels at 2-3 days post-stroke [37, 65]. However, IL1-β in CSF has been shown to be elevated within 6 hours after stroke regardless of its severity [66]. Two peripheral blood studies found elevated levels of IL1-β in plasma and serum of stroke patients [52, 67]. However, all other studies show no increase of IL1-β in serum or plasma [42, 43, 65]. IL-1β has a highly localized role at the site of inflammation, and this localized role may be why IL-1β is not seen in plasma or serum of stroke patients. However, soluble IL-1 receptor decoys are elevated in plasma very early after stroke onset, which suggests an early attempt to regulate IL-1β supporting the important neurotoxic role IL-1β plays in stroke [68].

IL-6

IL-6 is an inflammatory cytokine that binds to class I cytokine receptors [69]. Class I cytokine receptors do not have intrinsic enzyme activity; thus, IL-6 signaling through its class I cytokine receptor requires recruitment of an additional receptor protein gp130 [70]. IL-6 signaling activates intracellular tyrosin-kinases such as Janus Kinase (JAK) which activates signal transducer and activator of transcription (STAT) family of transcription factors and RAS-RAF-MAPK pathways [69]. These transcription factors and pathways can increase astrogliosis and angiogenesis which are important for tissue remodeling and recovery after stroke [71, 72]. Furthermore, IL-6 can inhibit TNF-α, induce apoptosis in neutrophils, and recruit monocytes and T-cells causing the transition between innate and adaptive immune response [73]. However, IL-6 can be detrimental by increasing body temperature which has been shown to increase brain damage after stroke [74].

Experimental Stroke Studies

Few studies have been completed that assess IL-6 and stroke. IL-6 knock-out mice do not show a difference in

infarct size compared to wild types mice in the proximal tMCAO filament model [75]. However, when body temperature of IL-6 knock-out mice is controlled to levels of wild type mice, IL-6 knock-out mice exhibit larger infarcts suggesting IL-6 is neuroprotective [76]. This finding is consistent with the proximal pMCAO electrocoagulation rat model in which IL-6 was injected 30 minutes before and 15 minutes after pMCAO and the infarct size was reduced [77].

Time Course in Experimental Stroke Studies

IL-6 is elevated as early as 3-3.5 hours post-stroke and peaks between 6 and 24 hours depending on the ischemia model. In both mouse proximal pMCAO and tMCAO filament model, levels of IL-6 mRNA are elevated at 10 hours and peak expression is at 18 hours. In rat proximal tMCAO models, elevated IL-6 mRNA and protein levels are seen 3-3.5 hours after stroke and peak expression of IL-6 protein at 12 and 24 hours [78, 79].

Time Course in Human Studies

CSF IL-6 is elevated in stroke patients and increases within 24 hours and peaks 2-3 days after stroke [37, 65]. Vila, et al. [39] and Beridze, et al. [66] found that CSF IL-6 levels are elevated but only in severe stroke patients. Furthermore, IL-6 is elevated in serum and plasma during the first week after stroke in all published studies. However, some studies show IL-6 levels peaking at 10 hours in serum while other studies show levels of IL-6 peaking between 3 and 7 days after stroke [43, 80, 81]. Thus, there is agreement that IL-6 is elevated in stroke patients during the week after stroke onset. However, the timing at which IL-6 levels peak appear to depend on stroke severity and stroke type.

IL-10

IL-10 is an anti-inflammatory cytokine that signals through the IL-10 receptor complex, composed of two chains of IL-10R1 and two chains of IL-10R2 [82]. The two chains of IL-10R1 bind IL-10 and IL-10R2 initiates signal transduction. Jak1 is constitutively bound to IL-10R1 and Tyk2 is constitutively bound to IL-10R2, and when the IL-10 receptor complex is activated by IL-10, two tyrosine residues on the intracellular domain of IL-10R1 are phosphorylated by cross-phosphorylation and Jak1 and Tyk2 become activated [83]. Stat3 interacts with these phosphorylated tyrosines to phosphorylate other Stat proteins that translocate to the nucleus to activate transcription of Stat3-responsive genes [84]. IL-10 signaling blocks pro-inflammatory cytokine production, chemokine secretion, and inhibits antigen presentation by macrophages and microglia [85]. Furthermore, IL-10 can counteract the detrimental effects of TNF-α by inhibiting activation of signaling pathways induced by TNF-α (Figure 1) [86]. IL-10's beneficial anti-inflammatory effects are an attractive target for potential clinical applications in stroke.

Experimental Stroke Studies

Administration of IL-10 into the lateral ventricle 30 minutes and 3 hours after stroke in the pMCAO electrocoagulation rat model significantly decreases infarct size [87]. In the same model, infarct size is significantly decreased when IL-10 is administered 30 minutes post stroke for 3 hours systemically into the tail vein [87]. Furthermore, injecting adenoviral vectors encoding IL-10 into the lateral ventricle 60-90 minutes before photochemical occlusion or bilateral carotid occlusion in rats decreases infarct size and infiltrating leukocytes [85]. In a mouse transgenic model in which astrocytes, microglia, and endothelial brain cells overexpress IL-10, smaller infarcts are observed, and IL-10 deficient mice show larger infarcts after pMCAO [88, 89]. Another study suggesting the beneficial role of IL-10 in stroke, found a subset of regulatory B-cells that secrets IL-10 can decrease infarct size and improve neurological score [90-92]. Collectively, all experimental stroke studies suggest that IL-10 is neuroprotective through suppression of the inflammatory response after ischemia.

Time Course in Experimental Stroke Studies

IL-10 mRNA expression is increased in the ipsilateral cortex of the pMCAO mouse model at 6 hours and peaks at 7 days, and IL-10 receptor mRNA is upregulated after pMCAO [93, 94]. At 1, 4, and 7 days after pMCAO, IL-10 receptor positive cells are found in the subarachnoid space and on the surface of the infarcted cortex, and IL-10 receptor positive astrocytes are found in the infarcted tissue at day 4 [94]. Furthermore in the rat ischemia model, IL-10 is decreased 12 hours after stroke but increases six-fold two days post-stroke [93]. Few studies have examined the temporal profile of IL-10 in experimental stroke models, and these studies measured IL-10 mRNA expression in the brain not in the periphery.

Time Course in Human Studies

More studies have assessed the temporal profile of IL-10 in human subjects. Stroke patients show lower levels of IL-10 in plasma within 12 hours after stroke compared to controls, and 24 hours after tPA treatment IL-10 levels are increased [52, 80]. Furthermore, levels of IL-10 are

decreased within 24 hours of stroke and levels increase over 72 hours post-stroke [95]. Studies report a huge variability in the levels of IL-10, which is most likely due to the time the sample was taken and stroke severity. Furthermore, the heterogeneity of co-morbidities among stroke patients could play a role in the variability in levels of IL-10 and other cytokines after stroke.

Effects of Cytokines on Stroke Outcome

The inflammatory response to ischemia plays an essential role in the pathophysiology of stroke, and using inflammatory markers such as the cytokines described above can be useful to predict outcome after stroke. Increased mortality and functional disability occurs in stroke patients who show neurological symptoms that progress acutely after stroke onset. The ischemic penumbra is the tissue that surrounds the infarct core and can survive or be transformed into necrotic tissue and become part of the infarcted tissue due to reduced blood flow and impaired neuronal function [96]. The transformation of the penumbra to infarcted tissue results in worsening of neurological symptoms [97]. Increased levels of IL-6 in both CSF and serum have been associated with worsening of neurological symptoms, increased infarct size and poor functional outcome [37, 39, 66]. It is surprising that IL-6 is associated with poor functional outcome in humans since in experimental stroke studies IL-6 was found to be neuroprotective. However, Ormstad, et al. [42] found no correlation between IL-6 and infarct size, and Sotgiu, et al. [67] found an inverse correlation of IL-6 with infarct size and poor outcome. IL-6 can cause the release of prostaglandin E2 in the brain, and prostaglandin E2 acts on the hypothalamus resulting in an increase in body temperature. Numerous human and experimental stroke studies have shown that fever is correlated with increased infarct size and poor outcome; thus, the early increase in IL-6 and prolonged elevation of IL-6 in the CSF and blood most likely correlates with increased risk of elevated temperature that increases inflammation and tissue damage after stroke. Moreover, there are conflicting findings when using TNF-α as an inflammatory mediator for predicting outcome and infarct size. Increased levels of TNF-α in the serum and CSF of stroke patients is correlated with worsening of neurological symptoms, increased infarct size and poor outcome at 3 months in studies including severe strokes and patients with white matter damage [40, 41, 45, 52, 65]. However, in other studies TNF-α was not correlated with outcome or infarct size [39, 44]. Although IL-1β was neurotoxic in all experimental ischemic animal studies, IL-1β was only found to be correlated with poor outcome in the plasma of patients in one study which is most likely due to the localized role of IL-1β and low levels of IL-1β in the blood or CSF [52]. The anti-inflammatory effects of IL-10 play an important role in preventing neuronal death; thus, it is not surprising that low levels of IL-10 early after stroke onset is correlated with poor outcome and increased infarct size [52]. However, a trend toward increased levels of IL-10 days after stroke and even at admission correlate with poor outcome and an increased risk for infection [98]. Increased IL-10 down regulates TNF-α and inhibits IFN-γ production which can be beneficial in the earlier phases of infarct progression after stroke; however, the increase in IL-10 can induce peripheral immunosuppression resulting in post-stroke infection and infections are the leading cause of death in stroke patients [99].

The variability of the data on peripheral cytokines in human stroke is most likely due to the heterogeneity of stroke in humans. Stroke severity, stroke location, age, co-morbidities, and systemic inflammation prior to stroke may be key factors contributing to the levels of peripheral cytokines seen post-stroke. Furthermore, proper control subjects are important for interpreting cytokine data. In human stroke studies assessing IL-10, TNF-α, IL-1β, IL-6, and other inflammatory mediators, the majority of the studies had age-matched controls but some studies had healthy controls subjects while other studies had controls with co-morbidities such as hypertension or diabetes. In addition, other studies compared cytokine levels based on stroke severity. Thus, it is important to control for co-morbidities and risk factors to determine which and when cytokines are increased due to stroke. Consequently, the use of only one inflammatory mediator to predict outcome or infarct size is not clinically useful due to the heterogeneity of the peripheral cytokine response. Therefore, further understanding of when these cytokines are increased and how they interact with each other can help elucidate how these cytokines can be used clinically as biomarkers to help predict outcome.

Gaps in Knowledge

Since cytokines play an essential role in stroke and have potential clinical utility, it is critical to understand how experimental and human studies contribute to our knowledge on how to effectively use cytokines clinically and how experimental and human studies can be improved to increase the likelihood of clinically translatable results. An important confounding factor in understanding the role of peripheral cytokines in human stroke is that experimental stroke studies observe the cytokine response to stroke in the brain not in the periphery. Furthermore, in experimental stroke studies both permanent and transient MCAO models are invasive and could contribute to the cytokine response especially in stroke models that involves craniectomy. Transient

MCAO mimics occlusion and reperfusion while pMCAO mimics just occlusion. Although reperfusion can attenuate infarction, it allows for increased infiltration of leukocytes into the brain, and the contribution of cytokines from peripheral leukocytes in affecting the progression of infarction and the difference in kinetics of leukocytes in the two ischemia models is not well studied. Thus, further understanding of how reperfusion can affect cytokine levels and their impact on outcome will be beneficial clinically in patients that receive tPA. Although outlined above are signaling mechanisms for each cytokine, few studies have assessed mechanisms of these cytokines in stroke, and there is a substantial lack in understanding of how cytokines balance their neuroprotective and neurotoxic action after stroke. Understanding this balance is crucial for defining a therapeutic time window for using cytokines as biomarkers or therapeutic targets.

Understanding the effects of different experimental stroke models on cytokine expression is important, but equally important is understanding how location of stroke, age, co-morbid diseases and environmental factors affect cytokine expression in human stroke studies. Thus, using clinically relevant stroke models such as aged animals, animals with hypertension, diabetes, etc., and producing strokes in different locations will provide essential information on cytokine expression that can be more easily translated from the bench to the bedside. Additionally, few human stroke studies assess the peripheral cytokine response before 4 hours after stroke which is a critical time in infarct progression, and early inflammatory events have been show to play a role in recovery from stroke. Thus, understanding these early cytokines responses and their effects early post-stroke is critical in using cytokines as biomarkers for prognosis and therapeutic targets. Designing clinical stroke studies to have power to do analysis on cytokine expression controlling for time, location, and co-morbidities and designing pre-clinical stroke studies to assess cytokine expression in peripheral blood as well as brain will be critical for the use of cytokines as prognostic biomarkers for stroke.

Summary

It is clear that cytokines play an important role in the pathophysiology of stroke, and the loss in balance between pro-inflammatory and anti-inflammatory cytokines after stroke affects infarct size and functional outcome. Thus, focusing on one cytokine only as a potential biomarker or a therapeutic target likely will not be advantageous in stroke. Future work needs to elucidate the temporal profile of cytokines in the periphery in human and experimental stroke studies to determine which cells contribute to the elevation of cytokines in the brain and blood and to understand how they work in concert to provide neuroprotection or increase neurotoxicity. Experimental stroke studies aim to produce the same size infarct in the consistent location. Both experimental and human stroke studies show that size and location impact when cytokines are increased and the magnitude of the increase. Thus, future studies should focus on using ischemia models such as the electrocoagulation model and produce strokes in different locations, and additional studies should focus on producing different infarct sizes by adjusting the time of occlusion. Moreover, few human stroke studies have subjects numbers sufficient to do separate analysis based on stroke location or stroke severity, and this lack of power in human studies most likely contributes to the variability in levels of cytokines measured. The crosstalk between the immune system and the brain is still not well understood. Using cytokines as biomarkers or therapeutic targets may be beneficial to understand the post-ischemic immune response and its effects on outcome clinically and to modulate the post-ischemic immune response to limit tissue damage. However, modulation of the immune response can also be detrimental after stroke; thus, it is imperative that further clinical and experimental studies be pursued to better understand the complex interaction between the immune system and the brain after stroke.

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