Targeting the Peripheral Inflammatory Response to Stroke: Role of the Spleen

Keith R. Pennypacker

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The migration of immune cells into the stroke-damaged brain has been reported for decades [1, 2] but its relationship to neurodegeneration is still not completely understood. Cells of the innate immune system are among the first immune cells to extravasate along with the adaptive immune cells infiltrating at later time points [3]. The inflammatory response elicited from these immune cells directly exacerbates the neurodegeneration in the ischemic area as well as indirectly by activating microglia [4]. This observation has led to the use of anti-inflammatory therapies in stroke treatment. One problem with this approach is the potentiation of the post-stroke immune suppression, which leads to increased infection rates [5]. The clinical trial evaluating anti-ICAM-1 therapy is an example of this issue in which one of the adverse events was increased incidence of pneumonia in the treatment group [6]. Thus, therapies that are more selective or targeted at the neurodegenerative response by the immune system could be administered to inhibit further damage with minimal contribution to the post-stroke immune suppression.

The spleen has been reported to decrease in size as a response to stroke in rodents [7, 8]. This reduction in tissue mass is caused at least in part to the activation of the sympathetic nervous system to induce contraction of the splenic capsule [9]. An initial study examining the spleen size of stroke patients suggests that spleen reduces in size after stroke in humans [10]. Removal of the spleen significantly reduces infarct volume in the rodent brain after experimental stroke [11–13]. Splenectomy has also proven to be beneficial in other types of brain injuries, such as hemorrhagic stroke and traumatic brain injury [14–17]. In fact, the splenic response to ischemic injury is a universal physiological one. Reports concerning a number of organ systems indicate that the

removal of a spleen protects the liver [18], intestine [19], kidney [20], and heart [21] from ischemic injury. Understanding the splenic response to ischemic injury would not only benefit stroke but potentially other ischemic injuries to all other tissues and organ systems.

The splenic humoral response to stroke comprises of a plethora of inflammatory cytokines and chemokines. Messenger RNAs (mRNAs) for IL-1 β [22, 14], TNF α [14, 22, 23], IFN γ [23], and IL-6 [23, 14] have all been reported as upregulated in the spleen following ischemic or hemorrhagic stroke. The mRNAs for chemokines such as CXCL2 and CXCL10 have been found to be elevated following middle cerebral artery occlusion (MCAO) in mice [24, 23]. Increased splenic levels of IFN γ protein following MCAO has been found in rats [25].

Most interesting, the administration of IFN γ reverses the protective effects of splenectomy, [25] suggesting this inflammatory cytokine is mediating the neurodegenerative effects. Blockade of IFN γ signaling, such as the use of IFN $\gamma^{-/-}$ mice [26] or inhibiting IFN γ with a neutralizing antibody injected directly into the brain reduced stroke-induced neurodegeneration [27]. Using antibodies that preclude the IFN γ -producing cells from entering the injured brain also leads to reduced infarct volume [28]. Systemic administration of an IFN γ -neutralizing antibody significantly decreases infarct volume [29] adding further evidence for the negative role of this inflammatory cytokine in stroke. Stroke patients who developed a Th₁ response to brain antigens at 90 days post-stroke were more likely to have a poorer outcome regardless of age or baseline stroke severity [30, 31]. IFN γ is considered a signature cytokine of a Th₁ response, which could implicate IFN γ as being detrimental following stroke in patients when an inflammatory T cell response is generated against brain antigens.

Since IFN γ is not directly neurotoxic [32], its downstream activation of macrophages/microglia appears to be the

K. R. Pennypacker (✉)
University of South Florida, Tampa, FL, USA
e-mail: kpennypa@health.usf.edu

mechanism by which this inflammatory cytokine can indirectly be responsible for delayed neurodegeneration in stroke. Downstream signaling of IFN γ induces the expression of the chemokine interferon-inducible protein 10 (IP-10), also known as CXCL10. This cytokine is pro-inflammatory and drives the Th₁ response by interacting with the CXCR3 receptor [33]. Microglia/macrophages produce IP-10 after IFN γ stimulation [29], which results in the chemotaxis of Th₁ cells to the site of injury. IP-10 prevents the activation of Th₂ cells by competitive antagonism of the CCR3 receptor [33] to further promote the inflammatory state. Thus, an IFN γ -induced feed forward inflammatory cycle is initiated when IP-10 secreted from macrophage/microglia recruits T cells to the infarct. The T cells express and secrete more IFN γ , leading to further expression of IP-10 in macrophages/microglia and additional neurodegeneration.

One recent study used recombinant TCR ligand (RTL) that consists of the specific domains of the MHC II molecule, which blocks T cells from becoming inflammatory and concomitantly deactivates the splenic response. Mice treated with RTL at 4 h after stroke resulted in decreased infarct volume and inflammatory response from the spleen [34]. This study incorporated elderly mice, and this treatment was equally effective despite age of the mice. Most interestingly, the RTL differentially affected components of the immune response in the elderly and young mice but still resulted in a blunted immune response reducing neurodegeneration.

Other potential targets have been reported that could attenuate the inflammatory response to neurological insult. The chemokine, CCL20, originates from the spleen after brain injury and has direct cytotoxic effects on neurons and oligodendrocytes [35]. Researchers are examining the action of signaling through cannabinoid and serotonin receptors [36] and the use of known anti-inflammatory compounds to alter the immune response to stroke [37]. Another interesting strategy is to activate the immune system's anti-inflammatory response to counter the inflammatory one [38]. Studies such as these are slowly unraveling the role of the immune system in responding to brain injury but will shed insight into chronic neurological disorders as well [39].

Ideally, pharmacological intervention that blunts the splenic response to stroke will arrest further neurodegeneration without worsening post-immune suppression. However, such a treatment would probably not enhance cellular survival/repair processes necessary for functional recovery. An optimal therapy would require a combination treatment that prevents further damage while promoting neural cell survival and stimulating repair of the initial damage leading to functional recovery of the brain area adversely affected by stroke. Cellular therapies contain both of these properties and have shown promise in stroke and other rodent models of neurological insult [40–42]. Much work is needed to translate these

exciting findings into creating a treatment for stroke in the clinical setting.

Conflict of Interest None

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