Chapter 2
The Critical Roles of Immune Cells in Acute Brain Injuries

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Abstract Acute brain injuries elicit prompt and robust immune responses characterized by the activation of local glial cells and mobilization of peripheral leukocytes. The activation of immune cells originally aims to clear the brain of cellular debris and promote brain repair; however, the immune system can also propel and propagate neuronal cell death when overactivated. Understanding the function of each type of immune cells in the acute brain injuries and their mechanisms of action promises to unveil effective immunomodulatory therapies that beneficially regulate post-injury immune responses. In this chapter, we discuss in detail how immune cells are recruited and/or activated in the injured brain and how they contribute to the evolvement of brain damage.

Introduction

A pivotal role of immune responses in the pathogenesis of acute brain injuries has emerged in recent years. Once an injury occurs, the brain and the immune system influence each other in specific and profound ways. Bidirectional or reciprocal neuro-immune communication presumably evolved to clear the brain of infections and dead cellular debris. However, in addition to its essential role in protecting the organism from harmful microbes and the necrotic spillage of intracellular contents, the immune system can also propel and propagate neuronal cell death when overactivated. In order to elicit activation of the immune system, injured neurons and other central

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nervous system (CNS) cells release ATP and neuronal antigens. Other small molecules, such as cytokines, chemokines, and adhesion molecules, also convey an activation signal to the immune system. In addition, the autonomic nervous system (ANS) releases neurotransmitters such as acetylcholine (Ach) and norepinephrine from the compromised brain. The innate and adaptive immune systems promptly respond to these signals and participate in the development of secondary inflammatory brain injury. Therefore, revealing the paths of communication between the brain and immune system promises to unveil effective immunomodulatory therapies that break the vicious positive cycle of neuroimmune activation. In this chapter, we discuss the intricate dialogue between the brain and immune system after acute brain injuries, with an emphasis on acute ischemic stroke. How immune cells such as microglia and dendritic cells (DCs) are activated and how monocytes, neutrophils, natural killer (NK) cells, T cells, and B cells infiltrate deep into the brain and contribute to neuroinflammatory damage will all be discussed in detail.

The Response of the Innate Immune System Following Cerebral Injury

The innate immune system is the first line of defense that recognizes and responds to pathogens or injuries in a nonspecific manner. Unlike the adaptive immune system, it does not provide long-lasting protection to the host. The cells involved in the innate immune response to the cerebral injury include macrophages, neutrophils, DCs, NK cells, and γδT cells (Fig. 2.1).

Bone Marrow Monocytes

Microglial Activation

Microglia are a specialized type of macrophage residing in the CNS. Unlike other CNS cells, which are derived from neuroectoderm, microglia are derived from bone marrow monocytes and enter the CNS after birth. Therefore, microglia inextricably link the CNS with the immune system.

Microglial activation is the initial step in CNS inflammatory responses induced by acute brain injuries. For instance, activation of microglia after cerebral hemorrhage occurs much earlier than infiltration of neutrophils in and around the hematoma; the former occurs within 1 h, whereas the latter occurs after 4–5 h [1]. In response to insults, microglia, which are normally ramified in appearance, become activated and assume an amoeboid morphology. Meanwhile, the expression of surface markers, such as MHC-II, Iba-1, CD11, etc., is also dysregulated. Both the morphology and the surface markers of microglia are virtually indistinguishable from infiltrating macrophages/monocytes, reflecting their common heritage.

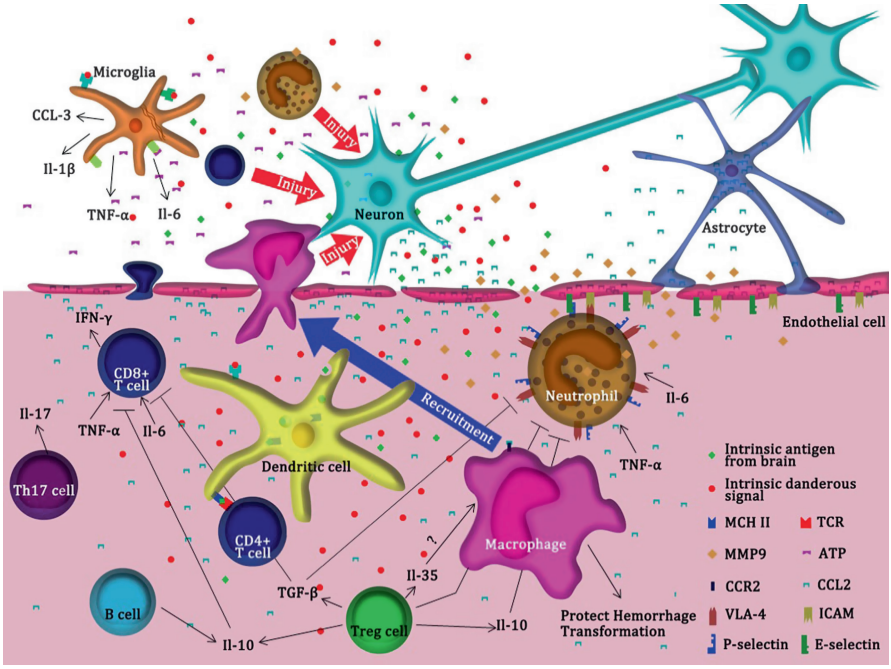


Fig. 2.1 Innate and adaptive immune response after stroke. In response to ischemic brain injury, a variety of danger signals are released from neurons, microglia, and astrocytes. These signals include ATP, cytokines, and chemokines, among other molecules. These signals activate microglia and induce their activation and further release of inflammatory cytokines, such as TNF-α and IL-6. Activated microglia can also release chemokines, such as CCL2 and CCL3, to recruit leukocyte infiltration. The CCL2 and CCR2 axis is responsible for the recruitment of macrophages. The infiltration of macrophages has been implicated as both detrimental to ischemic injury and protective against hemorrhagic transformation. In the ischemic brain, inflamed endothelial cells express adhesion molecules, such as ICAM-1, P-selectin, and E-selectin, which recruit leukocyte adhesion and infiltration. Following stimulation by IL-6 and TNF-α, neutrophils are activated and release MMP-9, which degrades matrix proteins of the blood–brain barrier and stimulates peripheral leukocyte infiltration. Brain-derived antigens can be processed by antigen-presenting cells, such as dendritic cells, and presented by MHC molecules on the cell surface. T-cell receptors on the surface of T cells can recognize the MHC and brain–antigen complex. Subsequently, the adaptive immune system is activated. $CD4^+$ T-helper cells, $CD8^+$ cytotoxic T cells, B cells, and Th17 cells all have been reported to contribute to the pathogenesis of neuroinflammation and neuronal cell death following stroke. Regulatory T cells (Tregs) and a special subset of regulatory B cells have been demonstrated to be neuroprotective through their release of IL-10. IL-35 and TGF-β are two other anti-inflammatory soluble factors which can be secreted by Tregs. Tregs can also inhibit MMP-9 production from neutrophils in a cell–cell contact manner

Microglial activation is not a uniform and monotonous state, as the morphological and gene expression changes associated with microglial activation vary enormously with the nature, strength, and duration of the stimulus [2]. In particular, in vitro stimulation with lipopolysaccharide (LPS) and IFN-γ promotes the differentiation of "classically activated" M1 microglia that typically release destructive

pro-inflammatory mediators [3]. In contrast, interleukin (IL)-4 and IL-10 induce an "alternatively activated" M2 phenotype that possesses neuroprotective properties [4–6]. The dualistic roles of distinctly polarized microglial populations have been reported in several CNS injuries, including ischemic stroke [7] and spinal cord injury [4]. For instance, microglia assume the M2 phenotype at early stages of ischemic stroke but gradually transform into the M1 phenotype at the sites of injury. In vitro studies reveal that ischemic neurons prime the polarization of microglia towards M1. This is thought to enhance injury because M1-polarized microglia exacerbated oxygen glucose deprivation (OGD)-induced neuronal loss. In contrast, maintaining microglia in the M2 phenotype protected neurons against OGD [7]. These findings reveal a Janus-faced nature of microglial responses and reflect the well-known shift between short-term protective effects of early immune activation and long-term detrimental effects of chronic immune activation. Furthermore, this time-dependent polarity mimics the traditional view of stress, as described by Hans Selye in the early half of the twentieth century. Selye first described that acute stress elicits resistance but that chronic stress weakens defenses [8].

Many receptors and signaling pathways, such as Fc receptors, chemokine receptors, purinergic receptors, and receptors for neurotransmitters, all mediate microglial function following brain injury. These microglia-regulating signals can transmit either "Off" or "On" signals [9]. Off signals are usually constitutively operative in resting microglia. The loss of Off signals or the gain of On signals initiates microglial activation. This context-dependent activation orchestrates the complex and variable microglial responses to stresses.

Monocyte/Macrophage Infiltration

Monocytes are produced by bone marrow from monoblasts and mature into different types of macrophages or DCs. It is well established that peripheral monocyte/macrophage cells are attracted to infarct areas after cerebral ischemia reperfusion [10]. A recent study showed that the shift of spleen monocytes to a less pro-inflammatory state attenuated infarct volume after transient focal ischemia, suggesting a role for peripheral monocytes in the pathogenesis of cerebral ischemia [11]. Similarly, within 24 h of stroke, immature monocytes infiltrated into the infarct border zone and differentiated into mature phagocytes within the lesion compartment [12]. However, infiltrating macrophages are morphologically and functionally similar to reactive resident microglia that transform into a phagocytic phenotype [13, 14]. To distinguish the roles and distributions of microglia and peripheral macrophages in ischemic brain injury, some studies used bone marrow chimeric mice generated by transplanting green fluorescent protein (GFP) transgenic bone marrow into irradiated wild-type recipients [13–16]. Although the infiltration of hematogenous macrophages into the brain was shown to occur 1–2 days after focal cerebral ischemia, their number was much lower than the resident population of activated microglia [13, 17]. Peripheral macrophages were most abundant in the ischemic brain tissue 3–7 days after transient focal cerebral ischemia and

decreased thereafter [14, 18]. Anatomical studies revealed that, at 7 days after transient ischemia, many process-bearing ramified blood-derived macrophages were distributed in the peri-infarct area, while phagocytic cells were detected in the core area of the infarction [13]. The majority of phagocytes in the infarct area were derived from local microglia, which were rapidly activated 1 day after ischemia and reached peak numbers within 2 days [15]. Taken together, most studies indicate that local microglial activation precedes and predominates over peripheral monocyte/macrophage infiltration for the first few days following cerebral ischemic injury, whereas blood-derived macrophages contribute to the delayed post-ischemic inflammation and brain injury. Mechanistically, monocyte chemoattractant protein-1 (MCP-1) and its receptor CC chemokine receptor 2 (CCR2) are involved in the migration of hematogenous inflammatory cells, including macrophages, following cerebral ischemia. Either MCP-1- or CCR-2-deficient mice exhibit reduced numbers of infiltrating macrophages as well as neutrophils [19]. More recently, it was shown that MCP-1/CCR-2 double deficiency virtually abrogates the recruitment of hematogenous macrophages [20], indicating a predominant role of the MCP-1/CCR-2 axis in chemotaxis of monocyte/macrophages after cerebral ischemia. MCP-1 is also implicated in macrophage recruitment into the damaged parenchyma after TBI. For example, sustained elevation of MCP-1 was detected in the cerebrospinal fluid (CSF) of severe TBI patients for 10 days after trauma and in cortical homogenates of C57Bl/6 mice subjected to closed head injury (CHI), peaking at 4–12 h [21].

Neutrophils

The infiltration of neutrophils into lesioned areas of the ischemic brain has been demonstrated not only in animal models [13, 18] but in stroke patients as well [22]. Neutrophil accumulation in injured parenchyma correlated with infarct expansion [22]. Higher peripheral neutrophil counts were associated with more severe stroke outcomes [23]. In rodent models of transient focal ischemia, depletion of neutrophils by treatment with anti-neutrophil monoclonal antibody (RP3) significant reduced the size of infarct as well as the formation of brain edema [24, 25], indicating an important role of neutrophil infiltration in post-ischemic brain injury.

Recent data support the notion that neutrophils infiltrate into the injured brain more rapidly than other types of peripheral inflammatory cells. This pattern in the brain closely mimics the leukocyte response to peripheral injuries, where neutrophils lead the way out of blood vessels into damaged tissue and are known as the first responders. Neutrophil extravasation into damaged peripheral tissues is followed by monocyte/macrophages, also called the second responders. In contrast to monocytes, which are most abundant in the injured parenchyma 3–7 days after ischemia, neutrophils are observed within a few hours and peak at 1–3 days after cerebral ischemia in animal models of stroke [26]. In stroke patients, neutrophil recruitment was also demonstrated to occur within 24 h of symptom onset [22]. Cellular adhesion molecules, including ICAM-1 and P-selectin, appear to be

involved in the transendothelial migration of neutrophils into the brain similar to their role in peripheral tissues. Either ICAM-1-deficient or P-selectin-deficient mice exhibit less neutrophil infiltration as well as smaller infarct volumes following acute stroke compared with wild-type mice [27–29]. The MCP-1/CCR-2 chemotactic axis is also thought to contribute to post-ischemic neutrophil infiltration, as deficiency in either one or both significantly decreases the numbers of neutrophils recruited to the site of injury [19, 20]. These findings reveal many commonalities between peripheral and central immune responses.

Recruitment of neutrophil granulocytes is also characteristic of the early inflammatory response following human TBI. Neutrophil recruitment has been shown to increase over the first 24 h after experimental TBI and is dependent on both leukocyte CD11/CD18 and ICAM-1. Neutrophil recruitment is also involved in edema formation, cell death, and tissue loss following TBI in mice [30]. Recently, Victor Friedrich and his colleagues implicated neutrophils in early microvascular injury after subarachnoid hemorrhage (SAH) and showed that treatments which reduce neutrophil activity can increase survival after SAH and limit microvascular injury [31]. These studies support the notion that the neuroimmune responses to stroke and TBI are somewhat mechanistically similar and play the same negative roles in pathological outcome.

Infiltrating neutrophils, as well as microglia and macrophages, are able to release toxic amounts of nitric oxide (NO) via the inducible nitric oxidase (iNOS) isoform. The release of this free radical gas may contribute to post-ischemic brain damage [10] via multiple mechanisms including activated matrix metalloproteinase-9 (MMP-9) [32]. Many studies have highlighted the involvement of MMPs in ischemic pathophysiology. For example, MMP-9 is strongly implicated in the disruption of the blood–brain barrier (BBB) and hemorrhagic transformation following ischemic injury both in rodent models [33, 34] and in stroke patients [35, 36]. MMP-9 is elevated after stroke both in plasma and brain tissue [37] and participates in the neuroinflammatory response to stroke [38]. Importantly, current research suggests that neutrophils might be the main source of MMP-9 [37]. Neutrophils greatly contribute to elevations in MMP-9 following cerebral ischemia [39], which, in turn, causes BBB breakdown, promotes hematogenous leukocyte infiltration, and ultimately results in neuronal injury [40]. These results indicate that neutrophil inhibition might be a reasonable peripheral target for stroke treatment in the clinic.

Natural Killer Cells

Natural killer (NK) cells are cytotoxic lymphocytes critical to the innate immune system. An early study indicated the presence of $CD4^-/CD8^+$ NK cells at the infarct edge following permanent focal cerebral ischemia [41]. However, a recent study showed no significant change in the numbers of infiltrating NK cells 3 days after transient focal cerebral ischemia, although there was infiltration of neutrophils, macrophages, T lymphocytes, and even NKT cells [42]. In contrast, there is

a significant decrease in blood NK cell counts [43], as well as peripheral NK cytotoxicity [44] in stroke patients. However, no change in NK cell counts was observed in gut-associated lymphoid tissue after experimental stroke in animal models [45]. Together, these studies indicate a role for NK cells in stroke-induced systemic immunosuppression rather than in the pathogenesis of post-ischemic brain injury.

γδT Cells

γδT cells represent a minor subset of T cells that possess a nonclassic T-cell receptor. The T-cell receptor of γδT cells is composed of two glycoprotein chains, one γ chain and one δ chain. Because they do not require antigen processing and antigen presentation by MHC, γδT cells are considered part of innate immunity. Many studies have demonstrated that γδT cells play a sentinel role in the early host response against many infectious agents [46, 47]. Direct evidence demonstrating their involvement in the progression of ischemic brain damage comes from the observation that depletion of γδT cells significantly ameliorated ischemia–reperfusion injury [48]. It was also shown that IL-17 is the key mediator of γδT cell-mediated delayed brain damage following cerebral ischemia–reperfusion [48]. These results suggest that γδT cells might be a rational therapeutic target for mitigating secondary inflammation-mediated damage following cerebral ischemia. However, it was also shown that the pro-inflammatory response of peripheral γδT cells was acutely diminished in stroke patients and that the subsequent increases in IFN-γ and perforin expression by γδT cells correlated well with clinical improvement [44]. Therefore, therapeutic strategies targeting γδT cells will have to be carefully timed to avoid undesired immunosuppression. This is not an unusual requirement, because all immunosuppressive therapies will have to ultimately ensure that the treatment is not accompanied by undesired side-effects such as increased vulnerability to microbial infections.

The Link Between the Innate and Adaptive Immune Response: APCs

Recently, a novel concept, the immunological synapse, was proposed to describe the structure formed by cytoskeletal molecules that assemble at the zone of contact between antigen-presenting cells (APCs) and T cells. The immunological synapse is associated with surface and cytoplasmic signaling [49]. Studies have shown that several hours of interaction between APCs and T cells were necessary for T-cell activation [50]. Biophysical analyses and two-photon microscopy studies confirmed immunological synapse formation at the site of robust interactions between APCs and T cells [50, 51].

There are professional APCs, including DCs, macrophages, B cells, and certain activated epithelial cells, and nonprofessional APCs, such as glial cells in the brain and vascular endothelial cells. Following stroke, autoimmune responses directed against neuroepitopes, which are not recognized by T cells under normal conditions, may be induced by cerebral ischemic injury. DCs, which have the broadest range of antigen presentation, might play an important role in the primary immune response against neuroepitopes after stroke [52].

There are two lineages of DCs: mDCs (myeloid DCs), which respond to bacteria and fungi and release IL-12, and plasmacytoid DCs (pDCs), which release IFN-α upon viral infection [53]. The precursor cells of both lineages can be detected in blood where these immature DCs patrol the circulation and invade tissue in response to a local infection. Upon migration into tissue, they pick up antigens and acquire the ability to stimulate T cells and subsequently induce the adaptive immune response against antigens not previously encountered [54], such as the cerebral antigens sequestered in the CNS under normal conditions.

A rapid increase in the levels of DCs in the ischemic brain was first observed in a rat model of permanent focal cerebral ischemia [55]. A subsequent study analyzed in detail the temporal dynamics of brain immune cell accumulation in a mouse model of transient focal ischemia [42]. This study revealed a significant increase in DCs in the ipsilateral brain at 1 day after cerebral ischemia/reperfusion, followed by a 20-fold increase on day 3 and a 12-fold increase on day 7 [42]. As there is already a DC population residing in the healthy brain, Felger et al. used radiation chimeras (wild-type hosts restored with CD11c/EYFP transgenic bone marrow or CD11c/EYFP transgenic hosts restored with wild-type bone marrow) to distinguish between brain original and peripheral DC [56]. Their results demonstrated DC infiltration in the ischemic hemisphere beginning 1 day after transient focal ischemia, and further indicated a role of original brain DCs in post-ischemic neuroinflammation [56]. On the other hand, a significant decrease in circulating DC precursors [57], consistent with a reduced costimulatory efficacy of circulating cells [58], was observed after human or experimental stroke. These results suggest that peripheral DCs are recruited from blood into the ischemic brain, which probably either triggers post-ischemic cerebral immune reactions or results in systemic immunosuppression. However, the role of DCs (both peripheral and brain original DCs) as potent mediators of inflammation in stroke has not been sufficiently investigated.

The Adaptive Immune Response

Unlike the innate immune system, adaptive immunity refers to antigen-specific defense mechanisms that require a relatively long time (several days) to become protective. Once the adaptive immune response has been established, more specific defense can be achieved. With the ability to remember specific antigens, the adaptive immune system can mount immediate, strong attacks each subsequent time that it encounters the same antigen. This powerful system of defense appeared first in

jawed vertebrates and is phylogenetically more modern than the ancient innate immune system. The cells of the adaptive immune system include the T and B lymphocytes, both of which are related to the post-injury inflammatory response. Below we discuss the involvement of both cell types in the immune response following acute brain injuries.

T Lymphocytes

T lymphocytes play a central role in cell-mediated adaptive immunity. Accumulating evidence has verified the involvement of T lymphocytes in post-ischemic neuroinflammation and brain damage. Several earlier studies indicated that the infiltration of T lymphocytes into ischemic brain occurred relatively late (3–4 days) post-ischemia, following $CD11b^+$ microglia/macrophages and $Ly6G^+$ neutrophils [59, 60]. However, there is now mounting evidence showing that T-cell accumulation in the post-ischemic brain actually begins within the first 24 h of reperfusion in rodent models [61–63]. Treatment with anti-α4-integrin antibody blocked T lymphocyte recruitment into the post-ischemic brain and reduced infarct volume after transient cerebral ischemia in rats [64, 65]. In addition, mice lacking RANTES (also called CCL5), which plays a critical role in recruiting and activating T lymphocytes, exhibited smaller infarct volumes than wild-type littermates [66]. The most important evidence for the damaging effects of T lymphocytes arise from studies using lymphocyte-deficient mice. Smaller infarct volumes and improved functional outcomes after transient cerebral ischemia were consistently shown in severe combined immunodeficiency (SCID) mice [67] or $Rag1^{-/-}$ mice that lack mature B and T lymphocytes [62, 68]. Moreover, the lymphocyte deficiency-mediated neuroprotection was almost completely abolished when wild-type $CD3^+$ T lymphocytes were transplanted into $Rag1^{-/-}$ mice [62, 68], confirming a detrimental pathophysiologic role for circulating T lymphocytes in brain ischemia/reperfusion injury. Interestingly, a recent study showed that the detrimental effect of T cells neither depends on antigen recognition nor T-cell receptor (TCR) costimulation in the early stages of ischemic stroke, suggesting that classic adaptive immune pathways may be not involved in T-cell-mediated early ischemia/reperfusion injury. Recently, studies of TBI suggest that scavenging of reactive oxygen species (ROS) at the endothelial level dramatically reduced the infiltration of activated T lymphocytes [69]. Thus, targeting T lymphocyte trafficking to the injured brain at the microvascular level is a novel neuroprotective concept in TBI and warrants further exploration [69].

In recent years, there has been an increase in research on the roles of specific T-cell subtypes in brain injuries. Several subtypes, such as $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells, have been implicated in the pathogenesis of brain injury [62]. However, not all T-cell subtypes have detrimental effects after ischemic stroke. For example, neutralizing regulatory T cells with anti-CD25 antibody significantly increased infarct volume and neurological dysfunction, implicating an essential role for regulatory T cells in limiting post-ischemic brain injury [70]. Below, we will discuss the different effects of T-cell subtypes after brain injuries.

$CD8^+$ Cytotoxic T Cells and $CD4^+$ Helper T Cells

The unique expression profiles of specific cell-membrane proteins can be used to subtype different T-cell groups. The $CD3^+$ T lymphocytes mainly encompass both $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells in approximately equal proportions [71]. As the name suggests, the $CD8^+$ cytotoxic T cells are capable of directly inducing the death of intercellular pathogen-infected somatic cells or tumor cells either through the release of cytotoxins, perforin, and granzymes, or via cell–surface interactions such as Fas–FasL pathway [72, 73]. The $CD8^+$ cytotoxic T cells can also produce several cytokines, including IFN-γ and TNF-α, which serve to promote immune and inflammatory response [74]. In a rat TBI model, significant $CD8^+$ cell accumulation was observed 3 days post-injury. The $CD8^+$ cells were strictly distributed in the pan-necrotic areas and around the pan-necrotic perimeter [75]. The accumulation of activated antigen-specific T cells at traumatic injury sites, in addition to antigen-containing areas, could amplify local inflammatory processes in the CNS [75].

Although both $CD4^+$ and $CD8^+$ T cells were recruited to the ischemic hemisphere following stroke, there are greater numbers of $CD4^+$ helper T cells than $CD8^+$ cytotoxic T cells [42, 76]. The $CD4^+$ helper T cells have no cytotoxic activity themselves, but instead help to activate and direct other immune cells including $CD8^+$ cytotoxic T cells [77]. They can be further differentiated into several types according to their cytokine secretion profiles. Th1 and Th2 cells are two classic subgroups of helper T cells. Importantly, either $CD4^+$ or $CD8^+$ T-cell-deficient mice exhibited significant smaller infarct volumes than wild-type controls [62]. Mice deficient in these T-cell subtypes showed comparable reductions in neurological deficits at 24 h after ischemia, although the difference did not reach statistical significance [62]. This was confirmed by a recent study showing that selective antibody-mediated depletion of $CD4^+$ or $CD8^+$ T lymphocytes significantly reduced infarct volumes in mice with permanent cerebral ischemia [78]. Therefore, these findings demonstrate a detrimental role for both $CD4^+$ helper and $CD8^+$ cytotoxic T cells in the development of brain injury following stroke.

Stroke induces similar changes in peripheral $CD4^+$ helper and $CD8^+$ cytotoxic T cells. There is a stroke-induced reduction in numbers of both T-cell subtypes in spleen, thymus, lymph nodes, and gut-associated lymphoid tissue [45, 79–82]. This phenomenon may be partially due to an increased apoptosis of either $CD4^+$ or $CD8^+$ T cells in lymphatic organs following stroke and is mediated by the SNS [82]. Moreover, there is a shift from Th1 to Th2 cytokine production [82, 83], which also may suppress pro-inflammatory immune responses [84]. These findings indicate that T-cell subtypes are involved in stroke-induced immunosuppression.

Regulatory T Cells

The regulatory T cell (Treg), identified by expression of CD25 and the transcription factor FoxP3, is an important $CD4^+$ T-cell subtype that accounts for about

10 % of peripheral $CD4^+$ T cells [85]. The mechanisms by which Tregs exert their immunosuppressive function are still not clear; however, Tregs generally possess a diverse arsenal of inhibitory mechanisms, ranging from secretion of inhibitory cytokines, such as TGF-β, IL-10 [70], and a novel anti-inflammatory cytokine, IL-35 [86, 87], to the expression of inhibitory surface molecules, such as CTLA-4 and GITR [88]. With their vigorous immunosuppressive function, Tregs play critical roles in the maintenance of immunologic self-tolerance and negative control of a number of physiological and pathological immune responses [89]. For example, Tregs may be a critical factor in controlling experimental autoimmune encephalomyelitis and Parkinson's disease. Expansion of Tregs by prophylactic infusion of immunoglobulin prevented the development of experimental autoimmune encephalomyelitis and the protection was associated with increases in peripheral Treg number and function. Depletion of Tregs abrogated the protection of intravenous immunoglobulin, which strongly suggested that Tregs mediated the protection [90]. Adoptive transfer of Tregs to MPTP-intoxicated mice attenuated Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration and provided significant protection of the nigrostriatal system [91, 92].

In the context of cerebral ischemia, the activation of regulatory T cells appears to be one of the intrinsic mechanisms that the body naturally uses to restrict the cerebral inflammation induced by ischemic stroke. Stroke induces a significant increase in peripheral regulatory T cells several days after the onset of stroke in patients [93] and in experimental models [94]. With regard to cell function, it was recently reported that the suppressive effect of Tregs in mouse and human was unaltered after stroke [58]. Thus, an increase in regulatory T-cell numbers, rather than enhanced function of each cell, may be responsible for the endogenous immunosuppression after stroke.

Expansion of endogenous Tregs might underlie the mechanism of protection afforded by MBP tolerance and E-selectin tolerance [95, 96]. This notion is supported by the following findings. Rats tolerant of MBP were less likely to develop Th1 responses compared to ovalbumin-tolerant rats following experimental stroke [95]. Mucosal tolerance to E-selectin protected against stroke in spontaneously hypertensive rats [96]. The protective effect of endogenous Tregs after stroke occurs by limiting secondary cerebral inflammatory responses and was further confirmed by a recent study showing that depletion of endogenous Tregs profoundly increased delayed brain damage and neurological dysfunction after brain ischemia [70]. Controversial evidence exists showing that Treg depletion failed to affect brain infarct volume [97]. However, our recent study confirmed neuroprotective effects of Tregs in experimental stroke. We showed that delayed post-stroke transplantation of Tregs robustly reduced brain damage and improved long-term neurological outcomes [98]. The neuroprotective effect of endogenous Tregs was previously shown to be associated with their ability to attenuate the activation of resident and invading inflammatory cells via releasing IL-10 [70]. In our study, we demonstrated that adoptively transferred Tregs reduced inflammatory responses both intrinsic and extrinsic to the central nervous system in rodent models of transient focal cerebral ischemia. Moreover, Tregs provided neurovascular protection against stroke by

inhibiting peripheral neutrophil-derived MMP-9. These findings all suggest that Tregs might be promising candidates for cell-based therapies targeting post-stroke inflammatory dysregulation and neurovascular disruption [98].

B Lymphocytes

Currently, there are few studies investigating the role of B lymphocytes in cerebral ischemia/reperfusion injury. In various peripheral organs ranging from the intestines, heart, kidney, to skeletal muscles, the B lymphocyte has been found to be predominantly pathogenic following ischemia/reperfusion injury [99]. However, current data suggest the opposite role for B cells in cerebral ischemic injury. Although T- and B-cell-deficient mice, either $Rag^{-/-}$ [62] or SCID [67], exhibited significantly reduced infarct sizes and neurologic damage when subjected to transient MCAO, mice lacking only B cells failed to show improvement against ischemic injury [62]. Recently, another study showed B-cell deficiency worsened histological damage and functional outcomes after transient cerebral ischemia [100]. Adoptive transfer of B cells to B-cell-deficient mice reduced ischemic infarct size and improved neurological deficits [100]. The authors further identified IL-10-secreting regulatory B cells as a major regulatory cell type in stroke. Notably, the secretion of IL-10 was responsible for the B-cell-mediated neuroprotection [100]. These data suggest that the role of B lymphocytes might be protective rather than pathogenic in cerebral ischemia/reperfusion injury.

Conclusion

In summary, an exquisitely coordinated crosstalk between the CNS and peripheral immune system regulates the fate of the animal after acute brain injuries. In addition to the conventional idea that the brain signals danger to the periphery, recent studies also reveal multiple novel neural circuits through which brain injury evokes a robust immune response. Brain injury-induced immune responses used to be considered innate or antigen nonspecific. Recent work on $CD8^+$ cytotoxic T cells, $CD4^+$ helper T cells, B cells, and regulatory T cells have refreshed scientific thought by revealing that adaptive immunity also plays pivotal roles in secondary brain injury (Fig. 2.1). With these new findings in neuroimmune communication, therapeutic candidates to modulate immune responses and thereby improve stroke outcome may lie on the horizon. Moreover, it seems reasonable to propose that immunomodulation through immune cell transplantation will improve brain injuries more profoundly than immunosuppressive drugs because immune cell transplantation harnesses the protective power of the body's natural defenses. Further research on immunomodulatory therapies that leverage our current knowledge of brain injuries is highly warranted.

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