Published in final edited form as:
Semin Immunopathol. 2023 May ; 45(3): 329–346. doi:10.1007/s00281-022-00975-z.

Regulatory T lymphocytes as a therapy for ischemic stroke

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Abstract

Unrestrained excessive inflammatory responses exacerbate ischemic brain injury and impede post-stroke brain recovery. $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells play important immunosuppressive roles to curtail inflammatory responses and regain immune homeostasis after stroke. Accumulating evidence confirms that Treg cells are neuroprotective at the acute stage after stroke and promote brain repair at the chronic phases. The beneficial effects of Treg cells are mediated by diverse mechanisms involving cell-cell interactions and soluble factor release. Multiple types of cells, including both immune cells and non-immune CNS cells, have been identified to be cellular targets of Treg cells. In this review, we summarize recent findings regarding the function of Treg cells in ischemic stroke and the underlying cellular and molecular mechanisms. The protective and reparative properties of Treg cells endorse them as good candidates for immune therapy. Strategies that boost the numbers and functions of Treg cells have been actively developing in the fields of transplantation and autoimmune diseases. We discuss the approaches for Treg cell expansion that have been tested in stroke models. The application of these approaches to stroke patients may bring new hope for stroke treatments.

Keywords

regulatory T cell; neuroprotection; brain repair; immunoregulation

1 Introduction

The regulatory T (Treg) cell population was first described 50 years ago in an endeavor to investigate the mechanisms for thymectomy-induced autoimmune disease (1, 2). In the years

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to follow, the molecular features of these cells were further characterized. A combined expression of CD4, CD25 and a specific transcription factor Foxp3 has been used to define Treg cells in rodents (3). Treg cells play important immunosuppressive functions in maintaining immune homeostasis and curbing inflammatory responses in different diseases. Treg cell deficiency or functional impairments are associated with autoimmune diseases and chronic inflammatory diseases. In addition, Treg cells have been shown to function beyond immunomodulation and outside the lymphatic system, expanding the breadth of phenotypic and functional diversities of Treg cells (4).

Activation of resident immune cells such as microglia, and infiltration of peripheral immune cells, including neutrophils, T cells, B cells, dendritic cells (DC), and macrophages, are two major pathological changes following stroke (5). These cells are known to be important in restricting brain damage and promoting brain recovery (6). However, post-stroke inflammation must be well-controlled to avoid secondary inflammatory injury to the ischemic brain. For example, overactivation of $CD4^+$ and $CD8^+$ effector T cells after stroke is detrimental due to their pro-inflammatory properties (7, 8). Recent research highlights the importance of Treg cells in reestablishing immune balance and creating a reparative environment in an ischemic brain by targeting various types of immune cells, including $CD4^+$ and $CD8^+$ T cells, DCs, macrophages, microglia, and neutrophils (9, 10). In addition, Treg cells directly interact with CNS residential cells (11). Therefore, increasing Treg cell number and/or function may represent a new immunotherapeutic strategy for CNS disorders. In this review, we discuss the effects of Treg cells in stroke, the mechanisms underlying Treg cell's action in the ischemic brains, and the evolving strategies to boost the numbers and functions of Treg cells for stroke treatment.

2 Changes in Treg cells in stroke patients

It has long been noticed in clinical patients that the number of circulating Treg cells fluctuates after ischemic stroke, especially at the early stage after stroke onset (Table 1). A dramatic decrease in the absolute number of circulating Treg cells is observed after stroke onset (12–14). Indeed, massive apoptosis of lymphocytes, including T helper lymphocytes, cytotoxic T lymphocytes, and B lymphocytes in the spleen and peripheral blood has been observed from the early phases of stroke until weeks after stroke onset (15), resulting in long-lasting lymphopenia. Interestingly, the reduction in the number of Treg cells is relatively mild compared to other lymphocytes during post-stroke lymphopenia, leading to an increase in the percentages of Treg cells among total lymphocytes or among $CD4^+$ lymphocytes after stroke (16–18). Further studies show that changes in the percentages of Treg cells among $CD4^+$ lymphocytes early after stroke might be determined by the size of brain infarct. The Treg cells/$CD4^+$ lymphocytes ratio is decreased in patients with big infarct size but remains unchanged in patients with smaller infarct size on day 1 after stroke (19). Conflicting results are shown in another study, which suggests a higher Treg cells/$CD4^+$ lymphocytes ratio in patients with severe ischemic stroke (NIH Stroke Score (NIHSS) >5) than those with mild ischemic stroke (NIHSS < 5) 24h after the stroke (20). This discrepancy may be attributed to the differences in approaches used to define stroke severity. At the late stage of stroke, Treg cells/$CD4^+$ lymphocytes ratio is increased regardless of infarct size (19).

The changes in Treg cell number and frequency after stroke are accompanied by declines in their immunosuppressive functions. For example, Treg cells collected from patients at 7 and 14 days after stroke exhibit reduced capacities to inhibit proliferation of autologous responder cells at low ratios of Treg to responder cells (18). Such functional decline is more obvious in Treg cells from female stroke patients (18). In addition, Treg cells from stroke patients show a reduced ability to inhibit the activation-induced upregulation of CD154 on the surface of effector T cells (21). Since CD154 plays critical roles in immunoregulation and inflammatory responses by providing costimulatory signaling to B cells, antigen-presenting cells, and other types of immune cells (22), the reduced ability to inhibit CD154 upregulation may result in overactivation of effector T cells and exacerbate post-stroke inflammation.

The quantity and function reductions in Treg cells after stroke provide a rationale for Treg augmentation in stroke patients. Indeed, the Treg cell frequency early after stroke is independently associated with good functional outcome 3 months after stroke (17). Patients with lower Treg cell frequency at 2 days after stroke show higher incidence of neurological deterioration and higher risk of infections (17). Therefore, early supplementation or boosting of Treg cell number may improve long-term recovery after ischemic stroke.

3 Signals for Treg cell mobilization in an ischemic brain

3.1 Treg cell recruitment:

There are very few numbers of Treg cells in a healthy brain. Similar to other T cells, Treg cells infiltrate into the ischemic brain in a relatively delayed manner compared to innate immune cells such as neutrophils and macrophages. Several chemokines are upregulated in the ischemic brain to recruit Treg cells. We find an early increase in the expression of CCR5 in circulating Treg cells after transient middle cerebral artery occlusion (tMCAO). Meanwhile, the expression of CCL5, a ligand of CCR5, increases along the endothelium, providing a docking site for Treg cell-neutrophil interaction (23). Other factors such as sphingosine-1-phosphate gradient (24) and CXCL14 (25) are also important for Treg cell recruitment in the acute stage of stroke. The accumulation of Treg cells in the chronic stage after stroke is mediated by a different set of chemoattractants. Chemokine receptors CCR8 and CCR6 are highly expressed on brain infiltrating Treg cells at 7-14 days after stroke, which is accompanied by increased expression of CCR8 ligand CCL1 on astrocytes and CCR6 ligand CCL20 on oligodendrocytes in the ischemic brain (11). Therefore, the chemokine gradients change dynamically at different stages of stroke, steering Treg cell movement towards the brain lesion.

3.2 Treg cell proliferation and differentiation

It is noted that the number of Treg cells escalates in the ischemic brain over time (11, 26). IL-2 is a cytokine that is important for the expansion of Treg cells. The IL-2 neutralizing antibody decreases the numbers of infiltrating Treg cells and deteriorates neurological functions after stroke (11). However, as a general inducer for T lymphocytes, the function of IL-2 on Treg cell expansion is not cell specific. The amplification of Treg cells is also dependent on IL-33 expression in the ischemic brain and its engagement to ST2 (suppressor

of tumorigenicity 2) receptors on the Treg cells. ST2 or IL-33 deficiency or treatment with an anti-ST2 antibody during the chronic phase of stroke reduces the number of brain infiltrating Treg cells (11). Conversely, IL-33 treatment increases the number of Treg cells in the brain after ischemic stroke (27) or traumatic brain injury (28) with a concomitant upregulation of ST2 expression on Treg cells. A myriad of cytokines and immune mediators within the brain microenvironment may work together to maintain and expand the number of Treg cells in an injured brain.

4 The function of Treg cells in ischemic stroke

4.1 Neuroprotective effects of Treg cells early after stroke

The effect of Treg cells in acute ischemic brain injury was first reported in 2009 (29). Dr. Liesz and colleagues report that depletion of Treg cells using a CD25-specific antibody enlarges the brain infarct and worsens neurological functions 7 days after permanent distal MCAO (dMCAO). However, a later study using a DTR mouse model of inducible Treg cell depletion upon diphtherial toxin (DT) injection shows detrimental effects of Treg cells 7 day after tMCAO (30). Another study using the same transgenic mice reports no differences in Treg cell depleted mice or non-depleted mice 3 or 4 days after tMCAO (31). These discrepancies may be attributed to the differences in approaches of Treg cell depletion, the dynamic nature of post-stroke immunity at different days after stroke, and the variance in stroke severity (32).

In contrast to the results in mice with Treg cell depletion, adoptive transfer of allogeneic Treg cells has been shown to provide acute protection in mouse models of stroke (33, 34). Intriguingly, Treg cells are still protective when they are administered as late as 24h after the onset of ischemia, making Treg cell therapy applicable to human patients who enter the clinic many hours after symptom onset. The acute neuroprotective effect of Treg cells is mainly attributed to their immunosuppressive functions through interplay with other immune cells (33, 34). Interestingly, we find that the protective effects of adoptively transferred Treg cells are observed before a massive number of Treg cells infiltrate into the brain parenchyma. Indeed, Treg cells provide prompt CNS protection by regulating activities of neutrophils (34, 35). Therefore, Treg cells can act rapidly by targeting easily accessible peripheral immune cells, without infiltrating into the brain.

4.2 Function of Treg cells in brain repair after stroke

Recent advances in research about Treg cells in the CNS discover their effects in brain repair at the late stage after ischemic brain injury. Significant increases in the number of infiltrating Treg cells start from 5-7d after tMCAO and escalate until months after stroke (11, 26). Depletion of Treg cells impairs functional recovery after stroke. This functional deterioration is not associated with enlarged grey matter tissue loss but is accompanied by exacerbated white matter damage (26) and excessive astrogliosis (11). The impact of Tregs on white matter integrity and behavioral performance is only observed in the late stages of recovery and not in the early stages after stroke, suggesting that the long-term beneficial effects of Treg cells are related to enhanced brain repair rather than a consequence of early tissue preservation (11). In addition, increased white matter damage

and exaggerated glia scar formation are still prominent when Treg cells are removed at the late stage of stroke, whereby their early protective effects are preserved but late effects are deprived (11, 26). Further studies suggest that Treg cells are critical for CNS regeneration, such as oligodendrogenesis and neurogenesis after stroke, as Treg cell depletion inhibits oligodendrocyte precursor cell differentiation (26) and neural progenitor cell migration after stroke (33).

In summary, Treg cells play dual roles of acute neuroprotection and delayed neuro-restoration in the context of ischemic stroke (Figure 1), which renders this cell population a powerful tool that can be leveraged for stroke treatment.

5 Mechanisms of action for Treg cells in context of stroke

A variety of cellular and molecular mechanisms are utilized by Treg cells to exert their functions of immunoregulation and tissue repair/regeneration **(**Figure 2**)** (36).

5.1 Immunosuppressive mechanisms on other immune cells

5.1.1 Effect of Treg cell-derived soluble factors on immune cells—Treg cell-derived cytokines including IL-10, transforming growth factor (TGF)-β, and IL-35 are known to suppress immune responses involving effector T-lymphocytes and other immune cells (29, 37, 38) **(**Figure 2A**)**.

IL-10 is the first cytokine that has been identified to be essential for the neuroprotective effects of Treg cells in a stroke model (29). Deletion of IL-10 in Treg cells negates their immunosuppressive effects on effector T cells and subsequent neuroprotection 7 days after permanent dMCAO. In contrast, IL-10 supplementation rescues the brain tissue after cerebral ischemia in Treg-depleted mice (29). A later study reveals that intestinal antigen presenting DCs reprogram Treg cells in the mesenteric lymph node toward an IL-10 producing anti-inflammatory phenotype, which in turn inhibits IL-17^+ γδ T differentiation (39). This mechanism is a key step along the gut-brain axis and mediates intestinal microbial dysbiosis-induced neuroprotection against acute ischemic brain injury. Interestingly, brain residential Treg cells with high IL-10 expression has been discovered in a healthy brain and seems to be important to counterbalance inflammatory microglia/macrophage responses upon an aversive stimulation (40). Whether these cerebral Treg cells with a memory phenotype are involved in quick responses to ischemic brain injury awaits further exploration. In addition, the number of IL-10^+Foxp3^+ Treg cells increases in the ischemic brain from 1 month onward, suggesting long-term effects of these cells in stroke recovery (41). Consistent with this notion, IL-10 is important for Treg cells to enhance neural stem/progenitor cell proliferation after stroke (33).

TGF-β is another important anti-inflammatory cytokine produced by Treg cells and has been reported to participate in immune cell suppression. For instance, TGF-β produced by Treg cells abrogates the cytotoxicity of tumor-specific CD8 T cells (38). TGF-β also suppresses the proliferation, survival, activation, and differentiation of B cells, natural killer (NK) cells, macrophages, DCs, and granulocytes (42). A significant reduction in serum TGF-β levels has been observed with concomitant decline in the frequency of blood Treg cells in patients

with acute ischemic stroke compared with healthy controls (43). In contrast, treatments that increase Treg cell numbers after stroke can increase the expression of TGF-β in the ischemic brain and in peripheral organs (44). However, the direct evidence confirming the function of Treg cell-derived TGF-β in stroke is lacking. Wang et al. even report in their study that TGF-β has minimum effects on neural stem/progenitor cell proliferation after stroke (33). Further studies are needed to evaluate the influence of Treg cell-derived TGF-β on stroke outcomes.

Interleukin-35, an IL-12 family member, is a potent immune suppressive cytokine preferentially secreted by Treg cells and plays an important role to suppress effector T cells (37). Treg cell-derived IL-35 contributes to T cell exhaustion and inhibits the proliferation of effector T cells. In addition, IL-35 facilitates the polarization of neutrophils or macrophages toward anti-inflammatory phenotypes and impedes DC maturation via reducing the expression of MHCI/II and co-stimulatory molecules (45). Given its immunosuppressive roles, IL-35 might be a therapeutic target for stroke. Indeed, IL-35 has been reported to protect against ischemic brain injury (46). A significant reduction in IL-35 and EBi3 (a major component of IL-35) is observed 6h after stroke and persists for several days. Administration of recombinant IL-35 protein pre- or post-stroke inhibits inflammatory responses, reduces brain infarct volume, and improves neurological deficits early after stroke (46). However, whether Treg cells are the main cellular source of neuroprotective IL-35 in the ischemic brain remains to be confirmed. An *in vitro* study shows that IL-4-induced M2 macrophages can release IL-35 and reduce neuronal cell death upon oxygen glucose deprivation (47), suggesting that multiple types of immune cells, in addition to Treg cells may contribute to IL-35-mediated neuroprotection.

Overall, the three key cytokines, IL-10, IL-35 and TGF-β, released from activated Treg cells may contribute to Treg-afforded beneficial effects to stroke to different extents. Their function may extend beyond the canonical anti-inflammatory modality and exert regenerative effects. TGF-β and IL-10 also convert $CD4^+CD25^-$ T cells to Tregs and boost Treg cell functions (48, 49), which may lead to a positive propagation of Treg cell responses after stroke as well as in other CNS diseases.

5.1.2 Effect of cell-cell interaction on Treg-mediated immunosuppression—

Some cell surface molecules expressed on Treg cells, such as programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte–associated protein-4 (CTLA-4), membrane-bound TGF-β, and galectin-1, play important roles in cell-cell communications and are essential for Treg cell–mediated immunosuppression (Figure 2A).

In an ischemic brain, Treg cells relay inhibitory signals to neutrophils through PD-L1/PD-1 interaction, thereby inhibiting matrix metallopeptidase 9 (MMP-9) production and protecting blood brain barrier integrity (35). It is noted that the functions of PD-L1/PD-1 system in the ischemic brain are cell-specific. Studies by Bodhankar et al. show that global PD-L1 knockout or administration of anti-PD-L1 monoclonal antibody alleviates brain injury 4 days after MCAO by enhancing the immunomodulatory function of $CD8^+CD122^+$ suppressor T cells (50, 51). Additionally, it is well-known in tumor studies that PD-L1 expression on Treg cells contributes to effector T cell exhaustion through interacting with its

ligand PD-1 on activated T cells (52). Whether PD-L1 on Treg cells is involved in reducing ischemic injury through limiting effector T cells activation needs further exploration.

CTLA-4 is another key inhibitory receptor on Treg cells. The interaction between CTLA-4 on Treg cells and the CD80/CD86 molecules on the DCs is important for suppression on effector T cells in both physiological and pathological condition (53, 54). Both CTLA-4 and CD28 can bind to CD80/CD86. The binding of CTLA-4 on Treg cells with CD86 on DC leads to CD86 capture by Treg cells, a process called trans-endocytosis, and impairs the co-stimulation *via* CD28 (54). As a result, the DCs fail to activate effector T cells. CTLA-4 has recently been identified as a novel prognostic marker for ischemic stroke in the Chinese hypertensive population (55). In addition, polymerase-1 (PARP-1) inhibitor protects against ischemic stroke through increasing Treg proportions and their CTLA-4 production in stroke patients (56). The exact role of CTLA-4 in Treg cell function after stroke and its mechanism of action, however, are largely unknown and require further investigation.

Cell contact-dependent suppression by Treg cells could also be executed by other molecules, such as surface-bound galectin-1 (57) and TGF-β (58). Galectin-1 is widely expressed in neurons, glial cells, and endothelial cells in the brain. Administration of galectin-1 improves functional outcome following ischemia via many mechanisms, including enhancing astrocytic brain-derived neurotrophic factor (BDNF) production, fostering vascular remodeling, facilitating blood flow recovery, and promoting neurogenesis in the subventricular zone (59–62). Galectin-1 is also expressed on the surface of activated Treg cells. The surface bound galectin-1 on Treg cells can cross-link glycoprotein receptors on responder T cells and inhibit their proliferation (57). It would be interesting to explore whether galectin-1 on Treg cells is one of the mechanisms for the beneficial effect of galectin-1 in stroke outcomes.

Taken together, cell-cell interactions play important roles in the immunoregulation and tissue repair/regeneration effects of Treg cells. However, considering the relatively low number of infiltrating Treg cells in the ischemic brain (11, 26), cell-cell contact seems to be a less efficient way for Treg cells to impact sufficient numbers of target cells to achieve beneficial effects. Highly dynamic interactions between Treg cells and their surrounding cells, or other mechanisms, are required to amplify the Treg cell-relayed signals. For example, the injured endothelial cells express CCL5, which provides docking sites for Treg cells. Once engaged with CCL5, CCR5 is activated in Treg cells and upregulates their expression of PD-L1, which in turn enhances the interaction between Treg cells and blood-borne neutrophils/macrophages (23). In this way, multiple cell types may interact in concert to execute efficient function of Treg cells in an ischemic brain.

5.2 Mechanisms for Treg cells to enhance tissue repair and regeneration

5.2.1 Promoting oligodendrocyte precursor cell differentiation—White matter injury, which is characterized by significant loss of oligodendrocytes and myelin sheaths, is an important contributor to neurological dysfunction after stroke (63). The differentiation of oligodendrocyte precursor cells (OPCs) into new myelin-producing mature oligodendrocytes helps to repair myeline sheath and restore white matter integrity. Thus, promoting OPC differentiation represents a legitimate strategy for successful remyelination after stroke.

It has been reported that Treg cells are required for effective OPC differentiation during myelin regeneration in the CNS. Treg cell-deficient mice exhibit significantly impaired remyelination and oligodendrocyte differentiation, which is rescued by Treg cell adoptive transfer (64). The cellular communication network factor 3 (CCN3) is identified as a Treg cell-derived factor that promotes OPC differentiation and myelination *in vitro* and *ex vivo* (64). A recent study from the same research group shows that CCN3 is predominantly expressed by neurons and is transiently up-regulated following demyelination (65). However, CCN3 is not essential for efficient remyelination in a murine cuprizone model of multiple sclerosis. This discrepancy between *in vitro* and *in vivo* studies might be due to a mismatch between the CCN3 concentrations in different settings. It remains questionable if Treg cell released CCN3 can reach a level high enough to stimulate sufficient OPC in CNS disease models, including stroke.

The transcriptomic comparison between circulating Treg cells and brain-infiltrating Treg cells reveals elevated expression of insulin-like growth factor 1 (IGF-1) and oncostatin M (Osm) in brain infiltrating Treg cells (26). IGF-1 is known to promote oligodendrocyte differentiation from multipotent adult neural progenitor cells (66). Osm is a novel glucocorticoid-dependent neuroinflammatory factor that enhances oligodendrocyte precursor cell activity in demyelinated sites (67). Exogenous OSM can modulate the expression of genes involved in OPC mobilization in a mouse model of ethidium bromide (EtBr)-induced demyelination (67). The potential role of these factors in Treg cell mediated OPC differentiation after stroke, however, remains elusive. It is plausible that multiple Treg cell-released factors work synergistically to promote OPC differentiation in CNS diseases.

5.2.2 Shifting microglia phenotype toward a reparative phenotype.—

Microglia, the key innate immune cell type in the brain, act as the first line of defense against exogenous invaders and endogenous injuries. During ischemic stroke, microglia rapidly migrate toward the site of injury to clear dead or damaged cells. These activated microglia play diverse beneficial roles in the ischemic brain to restrict brain inflammation and to promote brain repair (68, 69). However, overactivated microglia may release multiple inflammatory factors and free radicals and therefore exacerbate brain damage (69). Thus, stroke outcomes could be critically affected by the phenotypic and functional status of microglia. Elucidating the mechanisms that fine tune microglial responses after stroke will provide insights into immunotherapies for stroke.

In our recent transcriptomic analysis, we found that Treg cells are reprogrammed after entering an ischemic brain and show a strong capacity to promote the mobilization and activation of phagocytes, including microglia and macrophages (26). Further, Treg cells exposed to the ischemic brain milieu gain a capacity to drive microglia towards an anti-inflammatory and reparative phenotype to steer white matter repair **(**Figure 2B**)**. Microglia-depletion reduces the capacity of Treg cells to augment post-stroke oligodendrogenesis, supporting a pivotal role of Treg cell-microglia interaction in oligodendrocyte replacement and white matter repair after stroke (26). Consistent with our conclusion about Treg cell-microglia interaction, Treg cells have been reported to modulate microglial functions in neurodegenerative diseases. For example, Treg cells reduce microglial activation and ROS production and ameliorate pathological changes in a model of Parkinson’s disease (70,

71). In addition, Treg cells switch microglia phenotype in the early phase of amyotrophic lateral sclerosis (ALS) and therefore mitigate disease symptoms (72). Treg cell-derived IL-4 suppresses microglial toxicity in the slowly progressing phase of ALS (73). Interestingly, in the Dombrowski study using a mouse model of multiple sclerosis, the involvement of immunoregulation is excluded as a mechanism for Treg cell-enhanced oligodendrogenesis (64). Therefore, the significance of Treg cell-microglia dialog may differ in the context of different diseases.

Osteopotin (OPN), a glycoprotein with immunomodulatory functions (74), is important for the interaction between microglia and Treg cells in a stroke brain (26). Treg cells upregulate their OPN expression after entering an ischemic brain, which is essential for Treg cells to promote the oligodendrogenic activity (the ability to enhance oligodendrocyte regeneration) of microglia. Furthermore, Treg cells enhance OPN production, as well as other trophic factors, in microglia and maintain reparative capacity of microglia **(**Figure 2B**)**. It is also noted that OPN knockout in Treg cells or the blockade of OPN receptors in microglia reduces but does not abolish the capacities of Treg cells to enhance oligodendrogenic activity of microglia, suggesting that the involvement of additional mechanisms in Treg-microglia crosstalk. In line with this observation, other factors, such as PD-1, have been reported for Treg cell-microglia interactions in a model of spinal cord injury (SCI) (75) **(**Figure 2B**)**. PD-1 expression is significantly increased in infiltrating Treg cells as compared to other T cell subsets in the subacute phase of SCI. Moreover, Treg cell-mediated immunosuppression of pro-inflammatory macrophages and microglia relies on PD-1 expression by Treg cells (75). Whether this molecule also participates in Treg cell-microglia crosstalk and drives reparative microglial responses after stroke awaits further investigation.

5.2.3 Inhibiting neurotoxic astrocytic responses—Astrocytes are an important type of glial cell that provide trophic support to neurons, regulate synaptic formation, communicate with parenchymal cells, and maintain brain homeostasis (76, 77). In stroke, astrocytes undergo reactive astrogliosis, in which their morphology and functions alter to form glial scar as a physical barrier between injured and healthy tissue. The glial scar limits the spread of proinflammatory mediators into the healthy tissue and reduces brain damage (78, 79). However, reactive astrocytes and glial scars have detrimental effects in the chronic phase of stroke. The glial scar physically interferes with axonal growth (80), which is further inhibited by chondroitin sulfate proteoglycans (CSPGs) expressed by reactive astrocytes (81). Reactive astrocytes also contribute to a proinflammatory response through nuclear factor κB (NF-κB) activation, exacerbate blood-brain barrier (BBB) breakdown through vascular endothelial growth factor (VEGF)-A signaling, and induce cytotoxic swelling via aquaporin-4 (82–84). Indeed, the reactive astrocytes are not homogenous. The A2 astrocytes play a neuroprotective role, with upregulation of neurotrophic factors (85). In contrast, A1 astrocytes upregulate the expression of “harmful” genes and induce neuronal and oligodendrocyte death (85). Hence, inhibition of neurotoxic astrocytic responses may be a significant therapeutic strategy for neuroprotection in ischemic stroke.

Treg cells play an important role in reducing astrocyte activation after stroke **(**Figure 2B**)**. Treg cell depletion results in neurotoxic reactive astrocytes and concomitant deterioration

of neurological functions (11). By contrast, adoptive transfer of Treg cells into T-cell-deficient $Rag2^{-/-}$ or $Cd3e^{-/-}$ mice reduces astrogliosis after stroke (11). The EGFR ligand amphiregulin (AREG) is important for tissue repair (86, 87). Treg cell-derived AREG acts on astrocytes and inhibits the expression of astrocyte markers associated with impaired neuronal viability. Intraventricular administration of AREG in Treg cell-depleted mice reduces astrogliosis and neuronal apoptosis and attenuates neurological dysfunction after ischemia (11). In a progressive model of multiple sclerosis, nasal administration of a CD3-specific antibody had a therapeutic effect mediated by the induction of IL-10-expressing Treg cells (88). Importantly, astrocyte-specific knockdown of the IL-10 receptor blocks the therapeutic anti-CD3 effects, suggesting an effect of IL-10-producing Treg cells on astrocyte responses. Whether Treg cell-derived IL-10 also inhibits astrogliosis in stroke model remains unknown.

5.2.4 Other mechanisms—Treg cells may be involved in brain recovery via other mechanisms. For example, Treg cells release neurotrophic factors, such as brain derived neurotrophic factor (BDNF) **(**Figure 2B**)**. Although the circulating BDNF levels show no dramatic increase following stroke, the percentage of $BDNF^+$ Treg cells among the total $CD4^+$ population significantly increases in stroke patients compared to controls. Moreover, patients with high percentages of $CD4^+BDNF^+$ Treg cells have improved outcomes compared to those with lower levels (89). Treg cells may deliver BDNF to the site of injury to assist in recovery after stroke.

Accumulating evidence highlights the importance of Treg cells in post-stroke brain recovery. The mechanisms by which Treg cells impact the CNS entity have been widely explored. The surge of new techniques at single cell levels facilitates the investigation in immune cell population and their interactions with other cells. Multifaceted cell-cell cross talks seem to be essential for the minor Treg cell population to exert far-reaching effects in the injured brain. Engineered Treg cells with enhanced mechanism of protection are being developed and are expected to harness the power of Treg cells for treatment (90).

5.3 Tregs in gut-brain crosstalk

5.3.1 The Brain-gut axis after stroke—Both clinical and preclinical studies have shown significant changes in microbiome composition after stroke. The gut microbiome of patients with large-artery atherosclerotic ischemic stroke and transient ischemic attack are significantly different from those of patients with atherosclerotic plaques or healthy controls (91). The stroke patients have more opportunistic pathogens and fewer commensal pathogens, and the differences in proteobacteria are correlated with stroke severity (91, 92). A recent prospective case-control study found disruptions in α and ß diversity of intestinal communities in ischemic and hemorrhagic stroke patients (93). These clinical studies suggest that the intestinal bacterial composition changes after stroke. However, whether the changes in microbiota composition are a consequence of stroke or an indication of stroke susceptibility cannot be determined in clinical studies.

Mouse models of stroke have been utilized to elucidate the causal link between gut microbiota and stroke. The results indicate that ischemic stroke causes alterations

in microbiome diversity (94, 95). Mechanistic studies suggest that a post-stroke catecholaminergic stress leads to gastrointestinal paralysis and an associated overgrowth of intestinal bacteria. The resultant microbiota dysbiosis is causally linked to impaired stroke outcome (94). On the other hand, microbial communities before stroke indeed impact stroke outcome. It is reported that mice with the same genetic background from three different commercial breeders have comparable bacterial load of distinct species and exhibit differences in stroke outcome (96). Therefore, adjusting gut microbial composition may represent a legitimate strategy to improve stroke recovery.

5.3.2 Treg cells in brain-gut axis—Gut microbiotas play a critical role in T cell priming in the intestine and thereby affect stroke outcomes. Investigations with germ-free mice have shown that gut microbiomes modulate lymphocyte-driven immune reactions in response to brain injury (95). The intestinal microflora modulates the activity of intestinal DCs, which in turn induces IL-10 producing Treg cells. These Treg cells inhibit IL-17$^+$ γδ T cells within the intestine and improve stroke outcome without penetrating the brain (39). Therefore, dysbiotic microbiota induces a greater proinflammatory Th1 and Th17 response in gut after experimental stroke in mice (95) **(**Figure 2B**)**. These inflammatory T cells migrate from the intestine to the periinfarct tissue and enlarge brain lesion size. Treating dysbiotic microbiota through fecal transplantation after stroke increases the number of Treg cells in the peripheral immune organs and ischemic brain, which is associated with improved stroke outcomes (95). Antibiotic treatment prior to ischemic injury can also have a major impact on infarct volume and functional recovery (39, 97). C57BL/6 mice given amoxicillin and clavulanic acid for two weeks prior to ischemic brain injury exhibit reduced gut bacterial α diversity, an increased number of Treg cells and a decreased number of IL-17$^+$ γδ T cells in the small intestine, thereby leading to reduced brain infarct and improved functional recovery (39). These studies show that a balanced Treg cell and effector T cell response is important for the gut-brain cross talk and mediates the impact of gut microbiota on ischemic brain injury.

6 Age and sex differences in Treg cells

6.1 Aging in Treg cells

Aging is a multifaceted process with significant functional and structural alterations in the immune system (98). Aging-related immune dysregulation likely contributes to increased risk of infection, systemic autoimmune/inflammatory diseases, malignancy, and CNS diseases, and therefore increases morbidity and mortality in afflicted elderly (98, 99). Mounting evidence has shown that Treg cell dysfunction and dysregulation are pivotal components in age-related immune alteration (100–103). The number of blood Treg cells are substantially increased in aged mice and humans (100, 104) despite decreased thymic production and differentiation of Treg cell with aging (105). This phenomenon might be explained by the anti-apoptotic characteristics, such as the loss of pro-apoptotic Bim and enhanced expression of anti-apoptotic Bcl-2 in aged Treg cells (106, 107). Concomitant increases in the number of blood Treg cells and antigen experienced memory CD4$^+$Foxp3$^+$CD45RO$^+$ Treg cells have been observed in the elderly and are associated with neurodegenerative diseases, such as Alzheimer's Disease (AD) (102). Enrichment of

memory-like Treg cells and decreased production of naive $CD4^+$ T cells in the elderly might be responsible for an imbalance between suppressive and proinflammatory responses, leading to immune dysregulation upon environmental challenges, such as infection, vaccination, and malignancy (98, 108).

The impacts of aging on the functions of Treg cells are heterogenous across the literature. Some studies report more potent suppressive effects of Tregs in aged hosts. For example, aged Treg cells express a higher level of Foxp3 due to hypomethylation of CpG sites in the Foxp3 enhancer, leading to a phenotype with greater ability to suppress effector T cell proliferation than young Treg cells (103). Treg cells from aged mice also express a higher level of IL-10 and more potently inhibit CD86 expression on DCs than young Treg cells (101, 103). However, other studies suggest comparable functions of Treg cells in aged vs young animals (109). It seems that some specific functions are altered in Treg cells with aging. It is reported that aged Treg cells are much less efficient in downregulating IL-2 and IL-17 production by surrounding non-regulatory T cells compared to young Treg cells, but are as potent as young Treg cells in suppressing APC activation, T cell proliferation and IFN-γ production (110). In addition, aging impairs the tissue repair capacity of $CD4^+$ Treg cells. Treg cells do not accumulate effectively in muscle due to insufficient IL-33 production in aged tissue, resulting in inadequate repair after acute muscle injury (111).

The effects of aging on post-stroke Treg cell responses remain unclear. A clinical study demonstrates increased expression of CD39 with aging in Treg cells of healthy people, and this difference diminishes in stroke patients, suggesting the importance of immunosenescence in Treg cell functions after stroke (21). However, the majority of studies that investigate the effects of Treg cells in stroke models are only performed in young animals. Considering the dramatic changes in the number and properties of aged Treg cells, further studies using aged mice are critical to validate the protective effects of this immune cell in clinically relevant age groups.

6.2 Sexual dimorphism in Treg cells

Sexual dimorphisms in Treg cell-mediated immune responses have been reported and seem to be complicated under physiological and different pathological conditions. Human studies show higher number of circulating Treg cells in healthy adult males compared with age-matched females (112). However, animal studies in different disease models yield organ-specific and phase-specific results regarding Treg cell frequencies in males and female. Specifically, the number of circulating Treg cells decreases significantly in both male and female patients within one day after stroke onset and shows no obvious sex difference (14). A study in neonatal hypoxic-ischemic (HI) brain injury documents higher levels of Treg cell infiltration into the brain in 9-day-old female mice compared with male littermates 24h after HI (113). In contrast, significantly higher levels of infiltrating Treg cells are detected in the brain of aged male mice compared to female mice 2 weeks after stroke (114). These results suggest age-dependent sex differences in Treg cell response in different phases after stroke.

The functional differences in Treg cells between males and females have been intensively investigated due to the fact that many autoimmune diseases such as multiple sclerosis (115), and asthma (116) are more prevalent in women compared with men. The sexual differences

in Treg cells seem to be hormone dependent. Androgens signal through the nuclear androgen receptor and stabilize the suppressive function of Treg cells by reducing IL-33–induced ST2 expression in lung Tregs, providing a mechanism for the lower prevalence of asthma in males vs. females (116). Treg cells also express the estrogen receptor and progesterone receptor, which are critical in the sex differences in multiple sclerosis pathophysiology (117). Estrogen enhances Treg cell growth and function in females (118). Impaired estrogen signaling results in loss-of-function in Treg cells and subsequent chronic intestinal inflammation (118). However, as noted in many studies, the effect of estrogen in immune responses are complicated. It not only promotes Treg cell function but also enhances Th2 responses (119), macrophage activation (120), and IFN-γ production from NKT cells (121). Therefore, the ultimate function of estrogen in a specific disease relies on its collective effects on different immune cells. Interestingly, a recent study in neonatal HI brain injury documents that Treg cells from neonatal female mice provide neuroprotection, while Treg cells from male mice increase post-HI neurodegeneration (113). Furthermore, isolated Treg cells from female neonate mice display enhanced anti-inflammatory activity compared with male neonate mice. This study in mice before puberty suggests hormone-independent mechanisms underlying the sex dimorphism in Treg cells.

With our increased knowledge about age and sex differences in Treg cells, age- and sex-stratified clinical and preclinical analyses of Treg cells are imperative to develop Treg cell-targeted therapeutic strategies for stroke.

7 Expanding Treg cells for stroke treatment

7.1 The potential of Treg therapy in neurological disorders

Based on promising rodent studies, including testing of human Treg cells in humanized mice, strategies to selectively augment endogenous Treg cells (122, 123) and Treg cell-based adoptive cell therapies (124) are currently undergoing early phase clinical trials for the treatment of autoimmune disorders, organ transplant rejection, and graft-versus-host disease following hematopoietic stem cell (bone marrow) transplantation. Although Treg cells represent only a small proportion of peripheral blood T cells (5-10% of the $CD4^+$ T cell population) and only a small population in the ischemic brain, they can be expanded for stroke treatment either by the ex vivo expansion of naturally-occurring, thymic-derived autologous Treg cells, or by the expansion or induction of Treg cells *in vivo* using various biological or pharmacological agents. In addition, newly-emerging Treg cell genetic engineering approaches (125) to augment the function, specificity and delivery of Treg cells have been developed, with clinical trials in transplantation getting underway. It is envisaged that successful testing of Treg cells in transplantation and autoimmunity will provide further justification for and encourage clinical evaluation of Treg cell therapy in neurological disorders, such as stroke. Here, we discuss methods for expansion of Treg cells either *in vivo* or *ex vivo,* with the aim of reducing stroke-induced injury, including emerging approaches for generation of genetically-engineered Tregs.

7.2 Ex-vivo expansion of Tregs

At present, there are three principal methods for the expansion of freshly-isolated Treg cells for adoptive cell therapy, all of which incorporate use of IL-2, which controls the homeostasis and function of Treg cells. The most commonly used approach, including that adopted in experimental stroke models, is the expansion of *polyclonal (p)Treg cells* over 2-3 weeks from purified (magnetic bead-isolated or flow-sorted) circulating Treg cells. The cells are expanded with anti-CD3/CD28 beads in the presence of recombinant IL-2 and the mTOR inhibitor rapamycin to reduce proliferation of effector T cell contaminants (124, 126). The second approach, used mainly in transplantation, entails the use of antigen (Ag)-presenting cells (PBMCs, B cells or DCs) from the transplant donor to stimulate specifically donor Ag-alloreactive Tregs from the graft recipient. These *Ag-specific Tregs* have the benefit of reduced off-target immunosuppressive activity compared with pTregs. They are more potent than pTregs, but their yield is comparatively low after expansion. The third approach, also for preparation of Ag-specific Tregs, is the expansion of pTregs that have been genetically-engineered to express a synthetic receptor, either a chimeric Ag receptor (CAR) produced using a viral vector or artificial T cell receptor (TCR), that recognizes the Ag of interest. Using this latter strategy, large numbers of potent, Ag-specific Tregs can be expanded after their generation. CAR Treg cells have shown promise in experimental models including humanized mouse models of transplantation (127, 128) and in experimental autoimmune disease (129). Studies using second generation CAR constructs with a CD28 domain, have reported excellent CAR Treg cell suppressive function. Current efforts to enhance CAR Treg cell function are focused on regulatory cytokine production. Thus, advanced CAR T cells secreting immunomodulatory cytokines upon CAR signaling and known as TRUCKs (T cells redirected for universal cytokine-mediated killing) (130), are under investigation.

Despite the potential advantages of CAR T cell therapy, their application may possibly be associated with some unwanted side effects, such as cytokine storm and neurotoxicity. In early phase clinical trials to date that have focused on the safety and tolerability of pTreg infusion, cell numbers (in the range 10^5 to 7 x 10^7/kg) appear to be well-tolerated, with only mild and isolated adverse effects. There has been no evidence of enhanced risk of infection or malignant disease in short- to medium-term follow-up periods. An additional important consideration is the influence of anti-inflammatory/immunosuppressive agents and specific cytokines on the therapeutic efficacy of adoptively-transferred Treg cells that may impact their viability and proliferative activity. Rapamycin is the only immunosuppressive agent that has been found to selectively enhance Treg cell survival. In humanized mice, it potentiates the ability of adoptively-transferred pTregs to inhibit the development of transplant vasculopathy (131) and the efficacy of adoptively-transferred CAR Treg in a fully immunocompetent vascularized heart transplant model (132). Low dose IL-2 administration in a mouse skin transplant model selectively enhances the proliferation of infused donor Ag alloreactive Tregs (133).

So far, there are no studies that test the therapeutic effect of ex vivo expanded Treg cells in stroke models despite many reports of beneficial roles of adoptively-transferred pTreg cells during the acute (29, 34) and the chronic phase (11, 26) after experimental stroke. One

concern is that the relatively long time needed to generate adequate numbers of Treg cells preclude them as a treatment for acute ischemic stroke. Intriguingly, however, recent studies suggest that Treg cells can promote brain repair even when they are delivered at the late stage of stroke (11, 26). Further studies are warranted to test if delayed treatment with ex vivo expanded Treg cells can be developed into a restorative therapy to improve long-term stroke outcomes.

7.3 In vivo enhancement of Treg cell number and function in stroke models

Administration of numerous agents has been shown to enhance Treg cells in stroke models. Their effects are documented in Table 2. Systemic administration of IL-2/IL-2Ab complexes selectively and markedly expands Treg cells in blood and lymphoid tissues after stroke, enhances Treg cell function, reduces infarct volume and neuroinflammation and improves sensorimotor functions (134). Very recently, astrocyte-targeted gene delivery of IL-2 has been shown to specifically increase brain-resident Treg cell numbers and protect against pathological neuroinflammation in MCAO and photothrombotic stroke, without impacting on the peripheral immune system (135). The latter findings suggest a delivery platform that may be suitable for clinical application. Interestingly, engineered IL-2 biologics (123), such as long-lived IL-2 muteins that selectively target and expand Tregs in vivo to counteract dysregulated inflammatory responses have proven effective in preclinical models including ongoing autoimmunity (136, 137) and may have potential for immunotherapy of stroke.

IL-33 is an IL-1 superfamily member that expands Treg cells and affords neuroprotection after experimental stroke (138, 139). It promotes Th2-type-effects and inhibits Th1 responses after focal ischemic stroke, promoting IL-10-expressing Treg cells and reducing proinflammatory microglia/macrophages in the brain and infarct size. However, IL-33 can also exacerbate systemic immunosupprerssion and post-stroke bacterial lung infection (138).

CD28 agonism is a widely-used approach to expand Treg cells *in vivo* as well as *in vitro*. Superagonist anti-CD28Ab-induced Treg expansion in mice reduces infarct volume after MCAO by inhibiting inflammation, a persistent effect that is mediated by IL-10 (140). However, it has also been reported that CD28 superagonist boosting of Tregs can enhance vascular lesions and cause inflammatory thrombosis and secondary infarction/ischemic neurodegeneration during the acute phase of experimental stroke (141).

Other agents that have been shown to expand Treg cells, exert anti-inflammatory effects and reduce infarct volume in stroke models include the immunosuppressant drug rapamycin, the sphingosine-1-phosphate agonist fingolimod and other molecules listed in Table 2. Overall, these agents constitute a broad range of experimental and established therapeutics for potential clinical assessment in ischemic stroke. However, several factors including sex, age, the dynamic nature of post-stroke inflammation/immunity (142), and the possible differentiation of Treg cells into pro-inflammatory cells, including Th17 cells (143) may complicate the effects of Treg cell treatment and should be considered when we move Treg cell expansion to clinical application.

8 Conclusions

The inhibitory role of Treg cells on immune response and the impact of Treg cells on brain repair render their multifaceted effects of neuroprotection and neurorestoration in ischemic stroke. Although the past decade has seen breakthroughs in the working mechanisms of Treg cells, how this minor peripheral immune cell population exerts significant beneficial effects after CNS injuries, including stroke remains largely unknown. It is apparent that the mechanisms underlying the effects of Treg cells in an ischemic brain are complicate, involving crosstalk between the CNS and peripheral immune system and interactions between Treg cells and many other types of cells. Further research is needed to elucidate the specific mechanisms through which Treg cells exert immunoregulation and promote brain repair. This insight is critical for efforts to develop therapeutic strategies that harness the power of Treg cells to fight against post-stroke neurological deficits.

Financial supports and conflicts disclosure

Xiaoming Hu is supported by a VA grant (I01 BX003651). AWT is supported by NIH grants AI R01 118777, U01 AI136779, and U19 AI 131453. Rimi Hazra is supported by the pilot grants from the Vascular Medicine Institute, the Hemophilia Center of Western Pennsylvania, Vitalant and Winters Foundation of Pittsburgh. The authors declare no competing interests.

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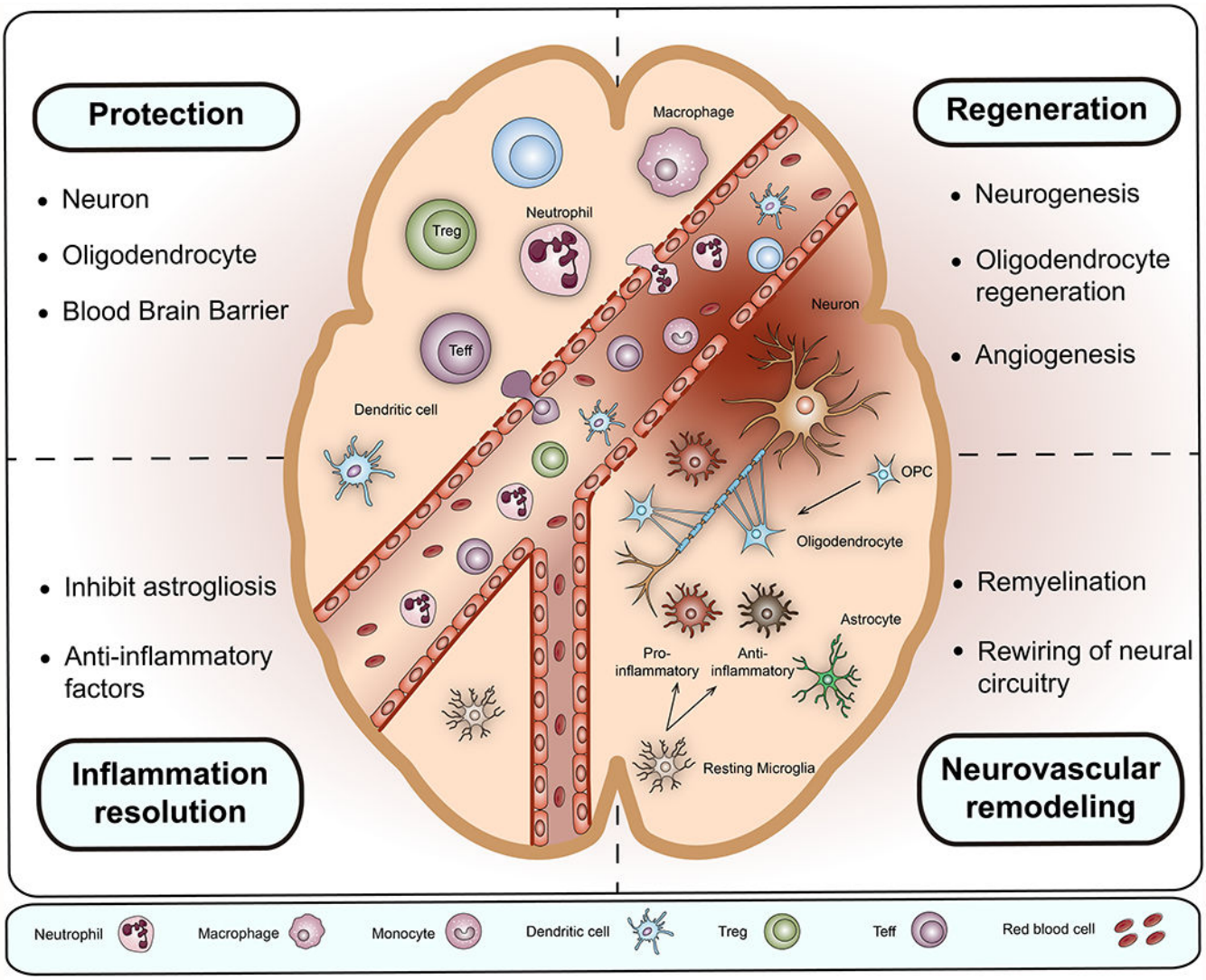

Figure 1. Functions of regulatory T cells in an ischemic brain. Treg cells play diverse functions after ischemic stroke.

1) Protection: Upon ischemic injury, Treg cells are recruited toward the site of injury and protect CNS cells including neurons and oligodendrocytes. Treg cells also preserve blood–brain barrier integrity, which in turn inhibits the cerebral infiltration of peripheral inflammatory cells, such as effector T cells, dendritic cells, neutrophils, and macrophages. **2) Inflammation resolution:** Treg cells participate in inflammation resolution by counteracting proinflammatory peripheral immune cells and proinflammatory microglia in the ischemic brain. Treg cells also inhibit toxic astrogliosis. **3) Regeneration:** Treg cells promote recovery with ability to enhance neurogenesis, oligodendrocytes regeneration, and angiogenesis. **4) Neurovascular remodeling:** Treg cells further enhance remyelination of axons and rewiring of neural circuitry. Consequently, Treg cells promote neurovascular remodeling after stroke.

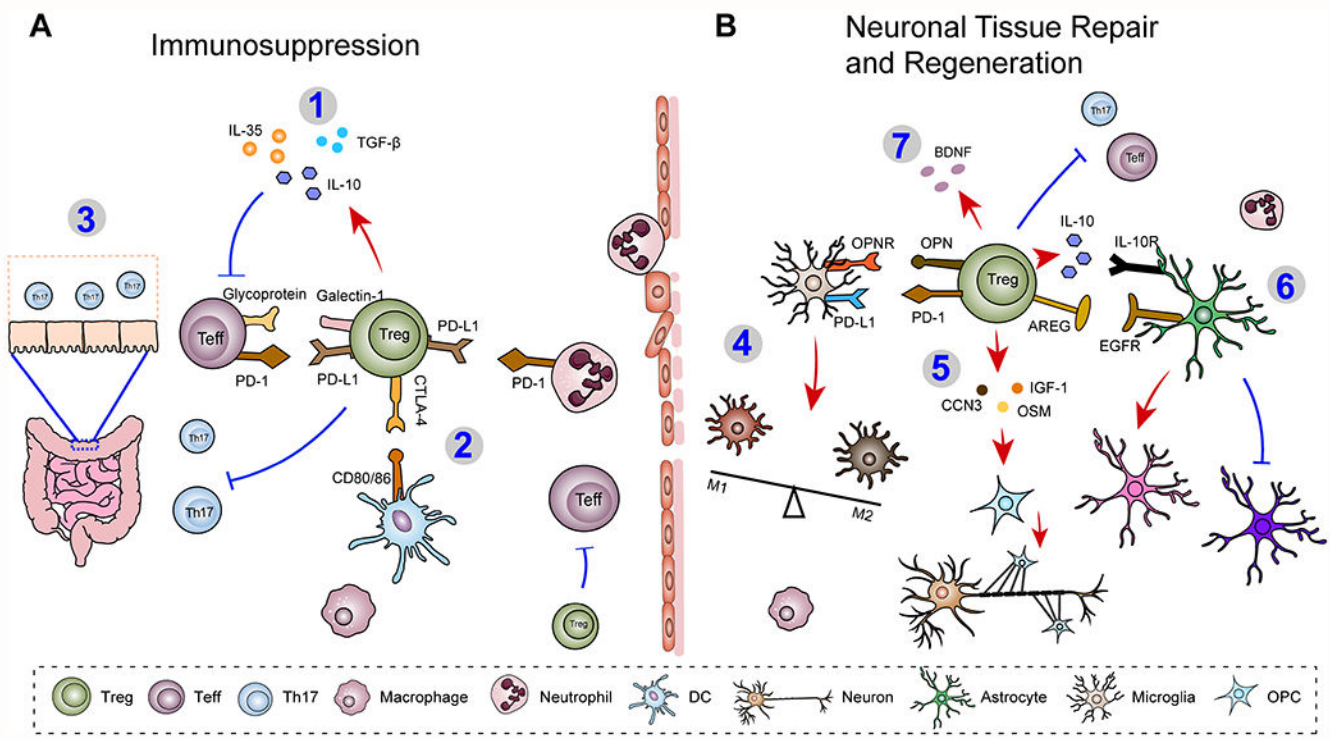

Figure 2. Mechanisms utilized by regulatory T cells to provide neuroprotection and enhance brain repair after ischemic stroke.

A. Treg cells restrain immune responses through **1)** Soluble factors. Treg cells produce immunosuppressive factors, such as IL-10, TGF-β, and IL-35, thereby inhibiting effector T lymphocytes or other immune cells. **2)** Cell contact-dependent mechanisms. For example, Treg cells relay inhibitory signals to neutrophils or effector T lymphocytes through PD-L1 and PD-1 interaction. The interaction of CTLA-4 on Treg cells with CD80/86 on dendritic cells down-regulate CD80/CD86 expression and thereby depriving co-stimulatory signal of effector T cells. Interaction between surface bound galectin-1 on Treg cells with its receptor on effector T cells can inhibit proliferation of effector T cells. **3)** Treg cells inhibit IL-17^{+} γδ T cells in the small intestine and prevent the infiltration of IL-17^{+} γδ T cells into brain. **B.** Treg cells support tissue repair and regeneration through several different mechanisms. **4)** Treg expressing PD-1 and OPN bind with the corresponding receptors on microglia to **shift microglia towards a reparative phenotype**. **5)** Treg-derived soluble factors CCN3, IGF-1 and oncostatin M promote OPCs differentiation into new myelin-producing mature oligodendrocytes**. 6)** Treg cells act through AREG or IL-10 to inhibit neurotoxic astrocytic responses. **7)** Treg cells promote brain regenerative processes by producing trophic factors such as IL-10 and BDNF.

Table 1.

Clinical studies on Treg cells in stroke patients.

Cohort	Source	Treg timepoint	Result/conclusion	Ref
204 IS[a] patients and 22 HCs[b]	Blood	2, 3 and 7 days after stroke	- IS patients have higher % of circulating Treg among all lymphocytes - Treg at day 2 is independently associated with 3-month outcome - lower Treg % at day 2 is associated with higher frequency of early neurological deterioration and infection - negative correlation between Treg at day 2 and 3 with infarct volume	(17)
77 IS patients and 64 HCs	Blood	Days 1, 7 and 21	- Circulating Treg % among $CD4^+$ cells was significantly increased after IS, particularly in males - Function of Treg to inhibit responder cell proliferation was reduced, particularly in females	(18)
84 IS patients and 115 HCs	Blood	Days 1, 3 and 7	- Number of Treg significantly reduced early after stroke, with a slow recovery until 7d after stroke	(14)
46 IS patients and 12 HC	Blood	3h, 2, 7 and 90 days	- Decline in number of Tregs after stroke with later recovery - Severe stroke on admission and stroke associated infection related to greater decline of T, Th, CTL cells, but not B cell or Tregs	(13)
48 IS patients and 26 HCs	Blood	Days 1, 3, 5, and 7 after stroke	- Treg below control level until day 7 - $CD39^+$ Treg was most strongly reduced in IS patients - On day 3, Treg-mediated inhibition of CD154 upregulation in $CD4^+$ Teff was impaired in IS	(21)
62 IS patients and 30 HCs	Blood	Days 7 and 28	- Proportion of circulating Tregs and levels of IL-10 and TGFβ were decreased after IS	(144)
37 IS patients, 34 TIA patients and 30 HCs. Within 24h onset	Blood	First blood draw after admission	- IS patients had a decline of Treg frequency, Foxp3 expression, suppressive function, and IL-10 and TGF-β1 levels - Serum Oxidized-LDL and inflammatory biomarkers were negatively correlated with the frequency of Treg cells in IS	(145)
40 IS patients, 30 HCs	Blood	Days 1, 5, and 10 after stroke	- Significant reduction in proportion of circulating Treg and levels of TGF-β1 and Foxp3 expression in IS	(43)
25 IS patients, 21 patients with other neurological diseases, 21 HCs	Blood	Day 1, and between 3-6 weeks post stroke	- Percentage of Treg increased in IS patients, compared with controls and patients with other neurological diseases	(12)
139 IS patients	Blood	Days 1, 3, 7, and 14 after stroke	- Increase of Treg/$CD4^+$ T ratio from day 1-14 in group with small infarct volume (< 28.6ml) - Decrease of Treg/$CD4^+$ T ratio on days 1 and 3 and increase of the ration on days 7 and 14 in group with infarct volume > 28.6ml	(19)
118 IS patients, 48 HCs	Blood	Once after admission	- Treg % in severe IS was higher than those in mild IS patients - $CCR5^+$ Treg % was higher in mild IS patients	(20)

[a] IS: ischemic stroke;

[b] HC: healthy control

Table 2.

Strategies to expand Treg cells for stroke treatment

Agent	Species	Model	Observed effect(s)
IL-2/IL-2 Ab complexes	Mouse	MCAO[a]	Tregs increased selectively in blood, spleen and lymph nodes; reduced infarct volume and neuroinflammation; improved sensorimotor function; enhanced Treg function; activation of CD39/CD73 signaling (134)
IL-2/IL-2R Ab	Rat/Human cells	OGD/R[b]	IL-2/IL-2R-treated Tregs but not IL-2/IL-2R treatment alone attenuates death of primary cortical or oligodendrocyte progenitor cells (146)
IL-2 gene delivery (brain-specific)	Mouse	MCAO & photo-thrombotoic stroke	Enhanced IL-2 production by reactive astrocytes; reduced lesion size; no impact on peripheral immune cells (135)
IL-33	Mouse	focal ischemic stroke	Increased IL-10-secreting Tregs in brain (138)
IL-33	Mouse	MCAO	Increased Tregs in spleen; decreased IFNg/enhanced IL-4/IL-10 and TGFb; reduced neurological deficit and infarct volumes (139)
IL-33	Mouse	MCAO	Increased Tregs in ischemic brain; increased ST2[c] receptor expression on Tregs; elevated IL-10 and TGFB in serum and brain; increased levels of AREG[d] and EGFR[e] (27)
IL-33	Mouse	MCAO	ST2-dependent Treg expansion in spleen and brain; reducs infarct volume; Treg depletion enhances infarct size and brain edema (147)
Rapamycin	Rat	focal ischemic stroke	Enhanced anti-inflammatory activity of Tregs; reduced lesion volume; decreased production of pro-inflammatory cytokines by macrophages and microglia (148)
Agonistic CD28 mAb	Mouse; rat	MCAO (2 models)	Increased Tregs in spleen and brain; reduced infarct size; brain-infiltrating Tregs produced IL-10 (140); enhanced vascular lesions/inflammatory thrombosis in the acute phase of stroke(141)
S1P[f] receptor agonist	Mouse	MCAO	Increased frequency of Tregs in blood and spleen and lymph nodes; enhanced number of Tregs in ischemic brain (149)
Resveratrol	Rat	MCAO	Increased frequencies/function of Tregs in spleens and ischemic hemisphere; increased IL-10 /decreased TNF and IL-6 levels in plasma and ischemic hemisphere (150)
CXCL14	Rat	MCAO	Enhanced accumulation of Tregs; reduced infarct volume; CXCL4 promotes IL-2-induced Treg differentiation (25)
PARP-1[g] inhibitor	Human	ischemic stroke	Increased proportions of Tregs in peripheral blood associated with anti-inflammatory effect on cytokine levels (56)

[a] MCAO, middle cerebral artery occlusion;

[b] OGD/R, oxygen glucose deprivation/reoxygenation;

[c] ST2, suppressor of tumorigenicity 2 (IL-33 receptor);

[d] AREG, amphiregulin;

[e] EGFR, epidermal growth factor receptor

[f] sphingosine-1-phosphate;

[g] poly (ADP-ribose) polymerase-1.