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Harnessing the power of dynamic immune responses in CNS disorders: The promise and limitations of immune cell-based therapies

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Abstract

The healthy immune system maintains natural checkpoints that temper pernicious inflammation, including regulatory T cells, regulatory B cells, regulatory dendritic cells, and microglia/macrophages/monocytes. Here, we highlight recent discoveries on the beneficial functions of regulatory immune cells and their mechanisms of action, and evaluate their potential use as novel cell-based therapies for brain disorders. Regulatory immune cell therapies have the potential to not only mitigate the inflammatory exacerbation of brain injury, but also promote an active post-injury brain repair program. By harnessing their reparative properties, we may reduce over-reliance on medications that mask clinical symptoms but fail to impede or reverse the progression of brain disorders. Although recent discoveries encourage further testing and genetic engineering of regulatory immune cells for the clinical management of neurological disorders, a number of challenges must be surmounted to improve their safety and efficacy in humans.

Introduction

Research conducted over the last three decades has forced a revision of the century-old concept that the central nervous system (CNS) is isolated from the peripheral immune system and immunologically inert. Rather, the brain and spinal cord are under continuous immune surveillance and regulation. A strong consensus has emerged that the activation and recruitment of immune cells during the course of CNS diseases or injury are critical for pathogen eradication, debris clearance, resolution of inflammation, and neurorestoration. However, excessive or indiscriminate immune responses harbor the potential to exacerbate brain damage and impair its capacity for self-repair. The ability of immune sentinels to maintain or upset immune equilibrium presents us with new opportunities to mitigate tissue

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Conflict of Interest

None.

damage and expedite restoration of the neurovascular unit.^{1, 2} In this perspective article, we propose that these therapeutic goals might be achieved by boosting natural immune regulatory mechanisms using cell-based approaches. Various types of immune cells, including regulatory T cells (Tregs),^{3, 4} regulatory B cells (Bregs),⁵ regulatory dendritic cells (DCregs),⁶ and microglia/macrophage/monocyte⁷ are known to alleviate inflammation and promote brain debris clearance. Intriguingly, these cells also execute unique regenerative functions during brain repair and regeneration, such as oligodendrocyte differentiation, myelin restoration, neural stem cell proliferation, neurovascular remodeling, and rewiring of neural circuitry.^{8–10} Extensive preclinical testing and promising early clinical trials in autoimmune diseases and transplantation have kindled great interest in adoptive immune cell therapies, particularly for their ease of delivery, ability to naturally home in on target tissues, and potential to change disease course. In this article, we present recent discoveries on the functions of several beneficial immune cell populations in the compromised CNS, their mechanisms of anti-injury and/or pro-repair actions, and their use as cell-based therapies for CNS diseases or injuries. We conclude the review with a discussion of the technical barriers and challenges that remain to be solved before these approaches can be transformed into mainstream clinical regimens.

Regulatory lymphocytes:

Regulatory T Cells

Functions in CNS disorders: Tregs are a naturally-occurring, albeit rare specialized T lymphocyte subpopulation characterized by the expression of cell surface markers CD4 and CD25 (IL-2Ra), and the transcription factor forkhead box p3 (Foxp3) (Box 1). The major functions of Tregs include suppression of the activities of other immune cells, maintenance of immune homeostasis, and mediation of antigen-specific immune tolerance. As excessive neuroinflammation can amplify CNS pathologies, the immunosuppressive properties of Tregs are expected to mitigate the impact of multiple diseases. Thus, the effects of Tregs have been widely investigated in multiple sclerosis (MS), a common inflammatory demyelinating disease of the CNS. As expected, genetic or pharmacological depletion of Tregs exacerbates disease severity, and this is accompanied by local inflammation in the experimental autoimmune encephalomyelitis (EAE) model of MS.¹¹ Conversely, intravenous infusions of isolated Tregs, especially those derived from the CNS of EAE mice, significantly alleviate demyelination and delay the progression of EAE.¹² In addition to MS, the immunomodulatory effects of Tregs have been shown to confer protection in preclinical *in vivo* models of stroke,^{3, 4} Parkinson's disease,¹³ Alzheimer's disease (AD),¹⁴ and amyotrophic lateral sclerosis (ALS).¹⁵

Although the bulk of evidence supports beneficial effects of Tregs in CNS diseases, some studies nevertheless recount detrimental effects of Tregs. For example, Treg-mediated immunosuppression of phagocytes may impair plaque clearance in AD.¹⁶ Another study showed that Treg depletion reduced stroke lesion volume and improved neurological functions.¹⁷ These discrepancies may have arisen due to the diverse and dynamic roles of Tregs in different disease stages, and the complexities of Treg interactions with other immune and non-immune cells.

Mechanism of neuroprotection or regeneration: Treg-enabled immunosuppression involves multiple mechanisms (Fig. 1). Many types of T effector cells (Teffs) have been implicated in the progression of neurological disorders. For example, CD4⁺ and CD8⁺ T cells promote tissue damage in models of EAE¹⁸ and stroke.¹⁹ IL-17-producing $\gamma\delta$ T cells also play pivotal roles in the evolution of ischemic brain injury and accompanying neurological deficits.^{20, 21} Tregs inhibit the functions of these Teffs by 1) releasing suppressor cytokines TGF β , IL-10, and IL-35,^{22, 23} 2) inducing cytotoxicity toward Teffs through granzyme B and perforin,²⁴ 3) expressing other immunoregulatory molecules such as galectin-1,²⁵ lymphocyte-activation gene 3 (LAG-3),²⁶ CTLA-4,²⁷ and CD39/CD73.²⁸ Tregs also target a variety of other immune cells and convert antigen-presenting cells (APCs), such as dendritic cells (DCs), into cells that exhibit suppressive phenotypes.²⁹ Tregs preserve the integrity of the blood-brain barrier after stroke by inhibition of neutrophil-derived matrix metalloproteinase 9 through PDL1/PD1 interactions.⁴ Membrane-bound TGF β on the surface of Tregs directly inhibits the effector functions of natural killer cells.³⁰ In genetic models of AD, Treg amplification increases the numbers of plaque-associated microglia and restores cognitive functions, suggesting a potential beneficial impact of Tregs on microglial behavior.¹⁴ Another study in an intracerebral hemorrhage model further confirmed that Tregs shift microglia/macrophage responses toward a M2-like anti-inflammatory phenotype through the IL-10/GSK3 β /PTEN axis *in vitro* and *in vivo*.³¹

Tregs possess additional functions beyond immunosuppression in CNS pathologies. For example, interactions between Tregs and endothelial cells through the CCR2-CCL2 axis may protect the blood-brain barrier and mitigate thrombolysis-induced brain hemorrhage.³² Furthermore, Treg depletion inhibits neural progenitor cell migration after stroke,³³ whereas adoptive transfer of Tregs after stroke enhances neural stem cell proliferation in an IL-10-dependent manner.⁸ Tregs can also promote the process of remyelination independent of immunomodulation, by directly enhancing oligodendrocyte progenitor cell differentiation in a CCN3-dependent manner.⁹ Taken together, these findings support the flexible and multifunctional nature of Tregs. However, our understanding of the mechanisms underlying Treg-afforded neuroprotection still lies in its infancy, and the abovementioned mechanisms await confirmation in further preclinical and clinical studies.

Preclinical approaches to boost the number and/or function of Tregs: Brain injuries or diseases often result in peripheral immunosuppression and a reduction in the number of circulating Tregs. The clinical literature on this topic is vast and only a few highlights are presented in Table 1. For example, acute ischemic stroke in humans immediately induces a dramatic loss of Treg numbers, followed by a sustained increase during the recovery phase, although the response varies depending on stroke volume.^{32, 34} These clinical observations prompted the preclinical development of Tregs as a cell therapy for CNS disorders and investigations of the underlying mechanisms (Table 2). To overcome the low frequency and anergic properties of Tregs, investigators developed approaches to boost the number and/or function of Tregs—either via *in vivo* induction or exogenous administration of Tregs after their *in vitro* expansion or activation. Tregs have been successfully expanded *ex vivo* by crosslinking with anti-CD3 and anti-CD28 antibodies in the presence of exogenous IL-2.³⁵ The addition of the serine-threonine protein kinase

inhibitor rapamycin further prevents the acquisition of Treg functions and allows selective expansion of Tregs.³⁶ However, the *ex vivo* expansion of sufficient numbers of Tregs usually takes several weeks, rendering it difficult to quickly expand sufficient autologous (self) Tregs from patients with abrupt brain injuries such as stroke or TBI. Fortunately, the expansion of non-autologous (non-self) Tregs derived from banked umbilical cord blood (UCB)³⁷ or from human thymus removed during pediatric cardiac surgery³⁸ provides another avenue for preparation of large numbers of Tregs. Alternatively, Tregs can be expanded *in vivo* using various stimulants, including low dose IL-2 or complexes of IL-2 and a specific anti-IL-2 antibody JES6-1,³⁹ or monoclonal antibodies against DR3 (aDR3).⁴⁰ Another indirect *in vivo* Treg expansion strategy involves administration of Flt3 ligand (Flt3L), a hematopoietic growth factor that stimulates the development of conventional myeloid DCs and non-conventional plasmacytoid (p) DCs. Thus, Flt3L and rapamycin can synergistically induce antigen-specific Tregs via selective expansion of plasmacytoid DCs.⁴¹ Protocols for the generation of antigen-specific Tregs are being developed and may be effective in some CNS diseases without compromising general immune defenses.^{42, 43} The successful genetic engineering of chimeric antigen receptors (CARs) for the generation of alloantigen-specific Tregs may further enhance their clinical translation in transplantation and other diseases.^{44–46} In sum, increasing numbers of preclinical studies support the therapeutic potential and restorative nature of exogenously or endogenously boosted Tregs in CNS diseases.

Regulatory B Cells

Functions in CNS disorders: Bregs include highly differentiated subpopulations in terms of phenotype and distribution. Unlike Tregs, Bregs do not express a unique transcription factor that can be readily exploited. Identification of Bregs relies on their expression of specific cell surface markers and the production of anti-inflammatory factors (Box 1). The most widely investigated Bregs are the IL-10-expressing CD19⁺CD1d^{hi}CD5⁺ Bregs known as “B10 cells”. A number of studies have demonstrated the immunomodulatory functions of B10 cells in CNS diseases.^{47, 48} As expected, depletion of B10 cells increases, while adoptive transfer of B10 cells mitigates the severity of symptoms in animal models of MS^{47, 48} and stroke.⁵ In addition, the number of CD19⁺CD1d⁺CD5⁺ B1a cells and CD19⁺CD23⁺CD21^{hi} transitional 2 marginal-zone (T2MZ) cells are altered during the course of MS treatment,⁴⁹ but the nature of their specific contributions to MS and other CNS diseases awaits further investigation.

Mechanism of neuroprotection: Production of the immunosuppressive cytokine IL-10 is the major mechanism whereby Bregs downmodulate the function of other immune cells.⁴⁸ In addition, IL-35-producing Bregs have recently been identified and shown to regulate CNS autoimmune diseases.⁵⁰ The interactions between Bregs and Tregs may represent another mechanism that can be leveraged for neuroprotection. For example, Breg-derived TGF- β induces Treg generation. Furthermore, glucocorticoid-induced tumor necrosis factor ligand superfamily member 18 ligand and receptor (GITRL/GITR) and PD-L1/PD1 interactions between Bregs and Tregs stimulate Treg expansion and stabilize the Treg phenotype, and are therefore essential for Breg-mediated neuroprotection.^{51, 52}

Preclinical immune cell therapies targeting Bregs: Adoptive transfer of B10 cells is effective in blunting disease progression and improving functional outcomes in models of MS and stroke (Table 2).^{5, 52, 53} Another promising Breg-related therapy is the transient stimulation of bone marrow cells *in vivo* or *in vitro* through Toll-like receptor 9 to generate proB cells (CpG-proBs). When transferred at the onset of neurological symptoms, CpG-proBs can differentiate into IL-10-producing Bregs and interrupt the course of EAE.⁵³ Although Bregs are protective against many neurological diseases or injuries, other non-regulatory B cells may promote disease progression or impair brain recovery.⁴⁷ In addition, the functions of B10 cells might dominate only at specific time points. For example, in an EAE model of MS, B10 cells only retarded the initiation of the disease, whereas Tregs inhibited disease progression at late phases of injury.⁴⁸ Thus, the success of therapeutic strategies targeting Bregs will depend upon their specificity and the timing of treatment, as well as the type(s) of immunosuppressive agents employed.

Microenvironmental cues influence the regulatory properties of immune cells

Due to their broad disease relevance, factors that govern the differentiation and functional stability of regulatory immune cells have been a focal point of recent research. Similar to other types of lymphocytes, the generation of regulatory lymphocytes relies on antigen-specific activation of T-cell and B-cell receptors (TCR/BCR) and co-stimulation signals mediated by B7/CD28 family molecules. For example, myelin protein-specific Tregs have been detected in the EAE mouse model for MS, where they play critical roles in neuroprotection.⁵⁴ Similarly, BCR recognition is important for Breg cell function and development in MS.⁵⁵ For most other CNS diseases, the antigens for Treg or Breg activation have not been identified. It is possible that the intracellular contents released from injured CNS cells serve as antigens to activate regulatory immune cells. Interestingly, Tregs may suppress Teffs with various antigen specificities, and the suppression might be even more effective when the Tregs and the suppressed Teffs have the same antigen specificity.⁵⁶

Regulatory immune cells are regarded as intrinsically stable cell populations, and recent concerns regarding intrinsic Treg lineage plasticity and functional instability have been largely attributed to contamination from Foxp3⁺ non-Tregs.⁵⁷ However, some external inputs, especially those in the highly inflammatory microenvironment within the compromised CNS, might perturb the stability of regulatory cells. For example, endothelial cells may directly interact with Tregs to enhance their inhibitory properties,^{58, 59} Human endothelial cells has also been reported to regulate the local inflammatory allogeneic response, by promoting either a Th17 response or a Treg response depending upon the specific cytokines in the microenvironment.⁶⁰ Increased interactions between Tregs and the ischemic brain endothelium may accelerate the platelet accumulation and thereby enhance the formation of microvascular thrombi,¹⁷ which may underlie the detrimental effects of Tregs in ischemic stroke. In addition, TGF β and IL-10 are known to enhance the stability of Tregs,^{61, 62} whereas IL-6 can skew the differentiation of Tregs toward a Th17 cell fate.⁶³ Thus, cytokine accumulation in the neurovascular milieu of the pathological CNS likely influences the stability of infiltrated regulatory immune cells. Notably, cytokines released from gut microbiota and peripheral organs such as the spleen may also modulate Treg or

Breg induction and stability.^{64, 65} Such complexity in Treg and Breg regulation may be a function of their critical roles in immune regulation in pathological conditions.

Regulatory DCs

Functions of CNS DCs:

DCs are a heterogeneous population of professional, bone marrow-derived antigen-presenting cells that link innate and adaptive immunity. They play an important role in the maintenance of self-tolerance in the normal healthy steady state.⁶⁶ DCs are known to travel from the CNS to cervical lymph nodes and dampen anti-CNS immune responses in the periphery.⁶ There is also evidence that CNS plasmacytoid DC (pDCs) regulate the severity of relapsing EAE.⁶⁷ Furthermore, pDCs have been implicated in the modulation of autoimmune Th1 and Th17 priming in secondary lymphoid organs during early phases of EAE development.⁶⁸

DC-based therapies against experimental CNS diseases:

DC-based immunotherapy shows considerable promise for the restoration of tolerance in autoimmune disease. DCs can be modified *ex vivo* to induce stable tolerogenic function and used as cellular ‘vaccines’. They can also be targeted *in vivo* with sophisticated antigen delivery systems. Regulatory DCs induce antigen-specific T-cell tolerance *in vivo* and have therapeutic effects in animal models of autoimmunity. DCs loaded with the brain autoantigen myelin basic protein (MBP) and incubated with mitomycin C confer resistance to subsequent EAE in mice.⁶⁹ Furthermore, transfer of embryonic stem cell-derived DCs expressing myelin oligodendrocyte glycoprotein peptide along with TNF-related apoptosis-inducing ligand (TRAIL) or PD-L1 can prevent EAE.⁷⁰ The effect of DC-based therapy on other CNS diseases or injuries has not yet been investigated.

Clinical trials using immune cell-based therapy — successes and limitations

Successes

Adoptive immune cell therapies were originally based on the anticancer properties of immune cells, such as tumor-infiltrating lymphocytes,⁷¹ and are currently most often employed for the clinical management of tumors, hematological malignancies, transplant rejection, autoimmune diseases, and viral infections. Last year, the FDA approved the first use of genetically engineered T cells with chimeric antigen receptors for relapsing/refractory B-cell precursor acute lymphoblastic leukemia in patients under 25 years of age. This approval built on a long series of historical advances, some of which are highlighted in Box 2. Most of these advances were in peripheral or systemic conditions rather than brain disorders, although progress against CNS glioblastoma has also been made with chimeric antigen receptor T cells.⁷² One of the earliest uses of immune cells to treat brain disorders was by transplantation of autologous bone marrow-derived mononuclear cells. The early exploitation of these versatile cells helped researchers gain confidence in the feasibility, safety, and efficacy of autologous cell infusions. Clinical trials have reported beneficial effects of autologous bone marrow-derived mononuclear cells when delivered by lumbar

puncture after spinal cord injury or intracerebrally into the motor cortex in ALS patients (Box 2),^{73, 74} and clinical trials on bone marrow-derived mononuclear cells have also been initiated on patients with stroke or cerebral palsy.^{75–77}

The main advantages of adoptive cell therapies include avoidance of the toxicity associated with drug regimens and the potential for reversal of the underlying disease process. Adoptive cell therapies can be viewed as a “living treatment” if the transferred cells continue to proliferate in the patient.⁷⁸ This is particularly important for neurological disorders, as most are progressive and have a prolonged course. Importantly, the therapeutic time windows for immune cell therapies are expected to be far wider than classic neuroprotectants, as immune cells mainly target immunomodulation and CNS repair over the long term. Additionally, the therapeutic targets for immune cell therapies include not only CNS cells, but also peripheral immune cells. Indeed, the immunomodulatory effects of regulatory immune cells may not be achieved by entering the CNS. For example, Tregs cells may provide CNS protection by ameliorating the deleterious activities of other peripheral immune cells.^{4, 79} This feature greatly improves the clinical feasibility of peripheral Treg manipulation as a non-invasive means of targeting the human CNS. Recent discoveries on the influence of the microbiome on Treg populations and the resulting effects on neurological conditions further support the indirect protective effects of peripheral Tregs on the brain,^{65, 80} and raise the possibility that orally delivered agents might be developed in the future to modify or genetically engineer the gut microbiome to release factors that noninvasively and indirectly influence the CNS and impede the progression of brain disorders.

A wealth of promising preclinical studies has translated to clinical trials showing that Tregs can be isolated and expanded *ex vivo*, infused into patients, and tolerated at high doses. The original clinical trials on *ex vivo*-expanded Tregs revealed their immunosuppressive power in mitigating graft-versus-host disease (GVHD) and promoting transplant tolerance (Box 2).^{81, 82} Since then, a large body of work demonstrates the promise of Tregs in treating leukemia relapse, type 1 diabetes, chronic GVHD, and lupus.^{82–84} Combination therapies of allogeneic Tregs with cytokines such as low-dose IL-2 have also been successful.⁸⁵ Methods to enrich and expand Tregs from ALS patients and restore their immunoregulatory activity for prospective clinical testing have been reported,⁸⁶ and CD4⁺CD25⁺FoxP3⁺CD127_{low} Tregs have been expanded *ex vivo* to decrease relapses and disability in MS patients.⁸⁷ Treg clinical trials completed or underway as of October 2016 have been reviewed by Romano *et al.*⁸⁸ Although there are no clinical studies of adoptive Breg cell therapy, IL-21 has been shown to induce granzyme-B expression in human Bregs and may promote Breg infiltration of solid human tumors.⁸⁹

Extensive preclinical studies demonstrate the efficacy of adoptively transferred DCregs in inhibiting GVHD, promoting long-term organ allograft survival/transplant tolerance, and suppressing autoimmune disorders.⁹⁰ In addition, local injections of immature, monocyte-derived DCs induce antigen-specific T cell tolerance to cognate antigens in healthy human volunteers.⁹¹ These findings led to phase I clinical trials of autologous (either unpulsed or pulsed with autoantigen) DCregs in type 1 diabetes, rheumatoid arthritis, and Crohn's disease.⁹⁰ These studies provided early evidence of the safety and biological activity of DCreg-based therapy.⁹² Additionally, stable antigen-specific T-cell hyporesponsiveness has

been induced *in vitro* by DCregs generated from MS patients for prospective clinical testing.
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Concerns and challenges

Despite early advances in adoptive cell therapies, serious concerns and challenges remain today. To ensure future successes, it will be necessary to 1) learn how to steer the influence of adoptive cells on endogenous immune cells, their interactions with normal spleen function, and their potential to damage organs, 2) monitor potential adoptive cell transformation into malignancies and attenuate the prevention of tumor control by other immune cells, 3) assuage adoptive cell-induced inability to control runaway infections, and 4) weaken the likelihood of kindling cytokine release syndrome (*e.g.* IL6 and IFN γ), which results from activation of large numbers of leukocytes and can intensify into cytokine storms that demand pressor or ventilator support and sometimes culminate in death.⁹⁴ One creative method of managing these risks is to engineer safety switches by incorporating a conditional suicide gene into the adoptive cells, such as the gene encoding herpes simplex virus thymidine kinase, which thwarts DNA synthesis but is somewhat delayed in action, or the newer approach of inserting inducible caspase-9 into adoptive cells.^{95, 96} Induced caspase-9 directly activates the terminal effector caspase-3 of the mitochondrial apoptotic pathway within hours and rapidly kills >99% of adoptive cells. In addition, age and sex may also influence the effect of immune cell therapies. Sex differences in regulatory immune cells have been reported in experimental stroke; increased levels of Bregs and anti-inflammatory CD11b⁺CD206⁺ microglia/macrophages have been observed in the ischemic brain of female mice.⁹⁷ Aging is also associated with increased Treg numbers and activities.⁹⁸ However, the impact of these factors on the effectiveness of immune cell therapies has not been explored.

To avoid rushing prematurely into clinical trials on brain disorders, Walsh and Kipnis argued that we do not sufficiently understand the complexity of Treg-Teff interactions in the brain and their distinct effects in immune-competent *vs* compromised hosts.⁹⁹ A superior understanding of the kinetics of endogenous immune cell responses after human brain injury and their correlations with positive *versus* negative clinical outcomes might also reinforce the likelihood of success in future clinical trials. Other problems that remain to be addressed are the identification or optimization of the most appropriate source of immune cells, isolation and expansion strategy, donor antigen specificity, routes of administration, timing of infusion, cell dose, cell stability, cell homing, and cell fate. Limited information is available to address these issues. For example, the routes of immune cell administration vary across studies and are difficult to evaluate in parallel. For regulatory lymphocyte adoptive therapy, the less invasive intravenous route is commonly used. However, there has been no systematic comparison of the effectiveness of different routes of administration. Table 2 summarizes preclinical studies that employed regulatory lymphocyte adoptive transfer as interventions in models of neurological disorders. Although the dose-response profile of Treg transfer has been explored in animal models,^{4, 32} its application to human patients needs further development.

Aside from the abovementioned issues, additional formidable barriers include standardization, quality control, and identification of specific disease biomarkers and cell

markers. For example, studies of Bregs are hampered by lack of specific markers, and Tregs may lose FoxP3 expression and convert to conventional T cells.¹⁰⁰

For the future success of personalized cell therapies in brain disorders, the approaches must exhibit scalability and reasonable potency and efficacy. They must also have sustained effects without serious adverse events. Maus and colleagues named three facets of adoptive immunotherapeutics that must be optimized for clinical efficacy—the soil (host immune system), the fertilizer (growth factors), and the seed (immune cell).⁷¹ Given the highly heterogeneous human population, the complicating influences of biological variables such as age, sex, gut microbiome, and comorbidities must also be considered carefully. Frequent monitoring of efficacy, safety, and validated biomarkers will be necessary to refine and adjust the treatment according to the unique, dynamic immune status of each individual. We envision that immune cell-based therapies for the CNS will have to be personalized according to the individual patients' immune condition and readjusted during the natural progression of injury and recovery. Nevertheless, given the remarkable success of adoptive cell therapies in systemic conditions, we are cautiously optimistic about their potential to decelerate or perhaps even reverse the typically progressive nature of brain disorders.

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Box 1.**Identification of regulatory lymphocytes in mice and humans****Tregs**

Tregs can be categorized into CD4⁺ and CD8⁺ subpopulations. Of the CD4⁺ subsets, CD4⁺CD25⁺Foxp3⁺ Tregs are the main players in CNS diseases and are identified by expression of the transcription factor Foxp3. However, intranuclear staining of Foxp3 requires cell fixation/permeabilization and the selected cells are killed in the process, which is incompatible with *in vivo* adoptive transfer. Recently, low expression of the surface marker CD127 in the CD4⁺CD25⁺ population has been widely used to identify and select human Tregs. A highly significant correlation between the percentages of CD4⁺CD25⁺CD127⁻ cells and CD4⁺CD25⁺Foxp3⁺ cells has been reported for human Tregs under both physiological and pathological conditions.¹⁰¹ Notably, CD4⁺FoxP3⁻ Tregs have also been identified, which have two major subsets with distinct cytokine profiles. Those Foxp3⁻ Tregs that predominantly produce TGF- β are defined as Th3,¹⁰² whereas those that mostly secrete IL-10 are defined as Tr1.¹⁰³ The CD8⁺ Tregs are characterized by CD122 expression. CD8⁺CD122⁺ T cells were originally recognized as antigen-specific memory T cells.^{ENREF_108} However, recent evidence shows that a specialized population of CD8⁺CD122⁺ T cells also performs non antigen-specific regulatory functions.^{ENREF_108} These CD8⁺CD122⁺ T cells are distinguished from memory CD8⁺CD122⁺ T cells by the expression of CD49d¹⁰⁴ and PD-1,¹⁰⁵ and named CD8⁺ memory-like Tregs (CD8⁺ Tregs). The CD8⁺ Tregs may exert regulatory functions by releasing protective factors such as IL-10 and/or through direct cell-cell interactions.

Bregs

The recognition of Bregs is more challenging than Tregs due to the lack of cell-specific markers. A wide variety of Bregs are characterized based on the expression of a unique combination of cell surface markers and the production of protective factors. IL-10-expressing CD1d^{hi}CD5⁺ B10 cells, CD1d⁻CD5⁺B1a cells, CD23⁺CD21^{hi} transitional 2 marginal-zone (T2MZ) cells, and CD23⁻CD21^{hi} marginal-zone (MZ) cells all influence the inflammatory milieu in mice.³ In humans, a set of surface markers has been used to identify Bregs. In particular, B10 cells are defined as CD27⁺CD24^{hi}CD148^{hi}CD48^{hi}.¹⁰⁶ Immature Tregs are identified as CD19⁺CD24^{hi}CD38^{hi}.^{ENREF_109}¹⁰⁷ In addition, CD73^{low}CD25⁺CD71⁺ Br1 cells and CD27^{int}CD38⁺ plasmablasts release IL-10 and possess regulatory properties. With the fast pace of immunology research, increasing numbers of Breg subsets are being discovered. Furthermore, current research highlights the intrinsic plasticity of B cell subsets, allowing them to acquire specific profiles in a particular inflammatory microenvironment.

Box 2:**Timeline of some of the seminal findings in the history of adoptive cell therapies.**

The majority of groundbreaking studies in the field of adoptive cell therapies address peripheral or systemic conditions. Key clinical observations that accelerated the development of cell therapies for brain disorders have also been included.

Figure 1. Mechanisms underlying Treg-afforded neuroprotection or regeneration.

Treg-enabled immunosuppression of effector T cells (Teffs) involves multiple mechanisms:

1) Release of suppressor cytokines TGF β , IL-10, and IL-35; 2) cell-cell interactions via CTLA-4, galectin-1, and Lymphocyte-activation gene 3 (LAG-3); 3) cytotoxicity toward Teffs through granzyme B and perforin; and 4) CD39/CD73 mediated production of adenosine. Tregs also regulate the function of other immune cells in the following ways: 1) conversion of dendritic cells (DCs) into cells with suppressive phenotypes; 2) inhibition of neutrophil-derived matrix metalloproteinase 9 (MMP-9) through PDL1/PD1 interactions; 3) direct inhibition of the effector functions of NK cells by membrane-bound TGF β on the surface of Tregs; 4) promotion of an anti-inflammatory phenotype in microglia. In addition, Tregs may directly target non-immune cells by the following means: 1) inhibition of endothelial cell production of CCL2 after ischemia and tissue plasminogen activator (tPA) treatment; 2) promotion of neural stem cell (NSC) proliferation and differentiation; 10) enhancement of oligodendrocyte precursor cells (OPC) differentiation and myelination via CCN3, a growth regulatory protein.

Table 1.

Impact of brain disorders on immune cell populations. This list is not exhaustive.

	Condition	Immune Cell Response	PMID
Lymphocytes	Ischemic stroke	Increased Treg numbers in males Reduced Treg function in males and females	22261543
	Ischemic stroke	Increased BDNF ⁺ Treg cells in CD4 ⁺ population	26298323
	Ischemic stroke	Increased activated T cells, Tregs, and CCR7 ⁺ T cells	19058859
	Ischemic stroke	Decreased Treg numbers	28535201
	Ischemic stroke	Increase in Tregs after small strokes Decrease in Tregs followed by increase after large strokes	28320030
	Traumatic brain injury	Decreases in peripheral T cells, T helper cells, Tregs, and natural killer cells	23061919
	Multiple sclerosis	Decreases in FoxP3 or Treg function	15952173
	Alzheimer's disease	Increased CD4 ⁺ CD25 ⁺ T cells without a Treg phenotype	22153977
	Parkinson's disease	Impairment in Treg ability to suppress T cell proliferation	23054369 17582512
	Multiple sclerosis	No changes in Treg numbers in relapsing-remitting MS	20884014
	Amyotrophic lateral sclerosis	Decreased FoxP3 expression, reduced Treg numbers, and impairments in Treg suppression of T cell proliferation	28289705 23143995
Monocytes	Ischemic stroke and cerebral hemorrhage	Increases in IL-10 secreting mononuclear cells	10362896
	Spinal cord injury	Either M1 or M2 macrophage profiles in peripheral blood	24140737
	Acute ischemic attack	Increase in M1 macrophages in unstable atherosclerotic plaques of carotid artery	23273713

Table 2. Comparison of studies using regulatory lymphocyte adoptive transfer in neurological diseases

	Animal Model	Dose	Route	Treatment Time	Outcomes and Mechanisms of Protection	PMID
Regulatory T cells	Stroke, mouse, tMCAO (60 min)	2×10 ⁶ (Dose response: 0.5-3×10 ⁶)	iv	2, 6, and 24h after stroke	Reduction of brain infarction and attenuation of BBB disruption and cerebral inflammation via suppression of peripheral neutrophil-derived MMP9. Immunomodulation of peripheral inflammation	23674483, 24092548, 24496394, 28768648,
	Stroke, mouse, tMCAO (60 min)	1×10 ⁵	icv	24h after tMCAO	Promotion of long-term recovery, stimulation of neural stem cell proliferation via IL-10	26441532
	Stroke, rat, tMCAO	3×10 ⁶	iv	2h after tMCAO	Reduction of brain infarction and inhibition of inflammation	24889329
	Hemorrhage transformation, mouse, suture and embolic tMCAO	2×10 ⁶ (Dose response: 0.5-3×10 ⁶)	iv	2h after suture occlusion or 3h after clot occlusion	Reduction of brain hemorrhage and infarction, attenuation of BBB disruption by inhibition of tPA-induced CCL-2 expression in endothelial cells and inhibition of tPA-induced elevation of MMP9	28535201
	MS, mouse, EAE	2×10 ⁶	iv	3d before or simultaneously with EAE induction	Increase in MOG-specific Th2 cells and regulation of T effector cells	12391178
	MS, mouse, lysocleithin-induced demyelination	1×10 ⁶	ip	24h prior to lysocleithin	Enhancement of remyelination/myelination via Treg-derived CCN3	28288125
	MS, mouse, EAE	2.5×10 ⁶	iv	2d before EAE	Suppression of pathogenic T cells, perhaps involving IL-10	14734610
	MS, mouse, EAE	2×10 ⁴	-	1d before or 1d after EAE	Immunomodulation of T effector cells	16116190
	ICH, mouse,	2×10 ⁶	iv	30 min after ICH	Attenuation of edema and BBB permeability, reduction of neuroinflammation	27678140
	SAH, rat,	2×10 ⁶	iv	2h after induction of SAH	Attenuation of cerebral inflammation by suppressing the activation of TLR4/NF-κB axis	26972078
	PD, mouse, MPTP model	1×10 ⁶	iv	12h post-MPTP	Attenuation of neuroinflammation, microglia activation and Th17 cell-induced nigrostriatal dopaminergic neurodegeneration	17675560, 20118279
	Stroke, mouse, tMCAO (60 min)	5×10 ⁶	Iv or ip	24h prior to or 4h, 24h after tMCAO	Reduction in brain infarction, reduction of inflammation in the brain and regulation of peripheral immune responses. Enhancement of the Treg population	23640015, 25537181, 24374817, 21653859
	Stroke, mouse, tMCAO (60 min)	1×10 ⁵	Intrastriatal	24h prior to tMCAO	Reduction in brain infarction	22618587
Regulatory B cells	MS, mouse, EAE	2×10 ⁶	iv	Every 10 days after EAE induction	Improvements associated with expansion of Tregs in the periphery and CNS	27821578
	MS, mouse, EAE	1×10 ⁶	iv	24h before EAE induction	Inhibition of disease initiation by Breg-derived IL-10	20624940

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Abbreviations: IV, intravenous; ICH, intracerebral hemorrhage; ICV: Intraventricular injection; BBB, blood-brain barrier; tMCAO, transient middle cerebral artery occlusion; EAE, experimental autoimmune encephalomyelitis; CNS, central nervous system; MMP9, matrix metalloproteinase-9; SAH, subarachnoid hemorrhage; MS, multiple sclerosis; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease