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MINI-REVIEW

Regulatory T cell therapy for multiple sclerosis: Breaching (blood-brain) barriers

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune disorder causing demyelination and neurodegeneration in the central nervous system. MS is characterized by disturbed motor performance and cognitive impairment. Current MS treatments delay disease progression and reduce relapse rates with general immunomodulation, yet curative therapies are still lacking. Regulatory T cells (Tregs) are able to suppress autoreactive immune cells, which drive MS pathology. However, Tregs are functionally impaired in people with MS. Interestingly, Tregs were recently reported to also have regenerative capacity. Therefore, experts agree that Treg cell therapy has the potential to ameliorate the disease. However, to perform their local anti-inflammatory and regenerative functions in the brain, they must first migrate across the blood-brain barrier (BBB). This review summarizes the reported results concerning the migration of Tregs across the BBB and the influence of Tregs on migration of other immune subsets. Finally, their therapeutic potential is discussed in the context of MS.

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Introduction

Multiple sclerosis (MS) is an autoimmune disease characterized by demyelinating lesions and neurodegeneration in the central nervous system (CNS). MS affects close to 2.8 million people worldwide, with women having a higher prevalence than men (3:1 ratio).[1–3] Demyelination results from chronic neuroinflammation caused by autoreactive immune cells and CNS resident cells.[4] Symptoms of the disease manifest as a heterogeneous spectrum covering motor, sensory, visual, autonomic, and cognitive features depending on the spatio-temporal distribution of the lesions.[2,5] Current therapies are found effective in delaying disease progression and decreasing relapse rates while symptomatic treatments manage acute relapses and symptoms related to neuroinflammation. These disease-modifying treatments (DMT) are available, yet these are associated with many side effects and are not curative.[5,6] In addition, they generally focus on the inflammatory component, which is mainly present in early, relapsing-remitting MS (RR-MS).[7] Indeed, the only DMT available for people with primary progressive MS (PP-MS) is ocrelizumab.[8] Siponimod is the only approved treatment for secondary progressive MS (SP-MS).[9] Therefore, research about novel therapeutic strategies for MS is of great importance and the topic of extensive research. Since regulatory T cells (Tregs) suppress autoreactive T cells, they are currently under investigation as a novel cell therapy for MS.[10,11] Furthermore, they show interesting reparative properties such as remyelination,[12–15] mediated by growth-regulatory protein cellular communication network factor 3 (CCN3),[12] and neural stem cell proliferation.[16] In addition, they suppress neurotoxic astrogliosis[17] and microglia.[18] However, to exert their functions at the site of inflammation, these cells must first cross the blood-brain barrier (BBB). The migratory capacity of Tregs and their influence on migration of other immune cells to the brain are discussed in this review, in light of their therapeutic potential for MS.

MS immunopathology

An important hallmark of the early inflammatory phase of MS is breakdown of the BBB.[19] The BBB mainly consists of endothelial cells, connected by tight junctions; the glia limitans, produced by astrocytic endfeet; and pericytes. This structure normally prevents the entry of immune cells and nonspecific molecules into the CNS, while tightly controlled exchange of nutrients and waste products is ensured.[20] Due to the neuroinflammation in MS, the BBB becomes leaky. Junctional proteins are disturbed and adhesion molecules are upregulated (Figure 1).[21] Subsequently, immune cells infiltrate the CNS and effector CD4+ T cells are locally reactivated by antigen-presenting cells (APCs) presenting their cognate autoantigen. The main effector cells are the CD4+ T helper cell (Th) subsets Th1 and Th17, and CD8+ cytotoxic T cells. Th1 cells were found to be increased in people with MS, from which the myelin-reactive compartment was mostly memory cells.[22,23] This indicates a previous activation of these Th1 cells reactive to myelin components. Increased frequencies of Th17 cells and interleukin (IL)-17 were detected in MS lesions, which were also correlated with disease activity.[24–26] Other Th subsets also contribute to MS pathology. Th1-like Th17 cells are increased in the CNS of EAE and people with MS[27] and the blood during a relapse.[28] This T cell population expresses both interferon gamma (IFN-γ) and IL-17 and possibly contributes to disease

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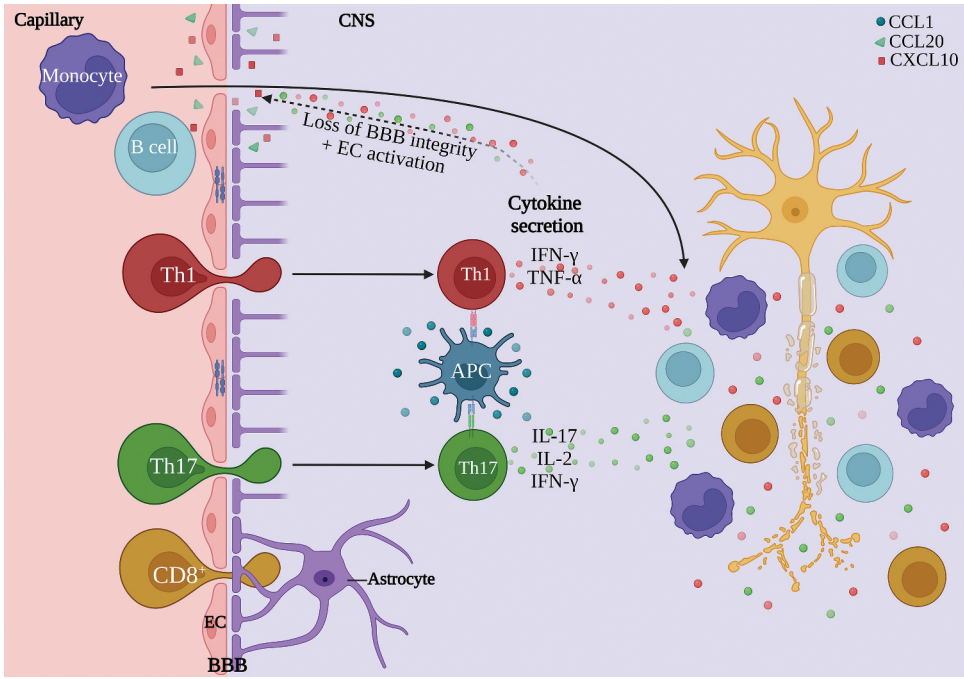

Figure 1. Summary of the immunopathology of MS. Peripherally-activated CD4+ T cell subsets Th1 and Th17, together with CD8+ T cells migrate over the BBB. Here, CD4+ T cells are reactivated by APCs. They secrete pro-inflammatory cytokines responsible for BBB breakdown and endothelial cell activation. Activated endothelial cells will produce inflammatory cytokines and chemokines, which will enhance neuroinflammation. Additionally, B cells and macrophages are recruited and activated. Altogether, this neuroinflammatory response causes demyelination and neurodegeneration in the CNS. APC, antigen-presenting cell; BBB, blood-brain barrier; CCL, C-C motif chemokine ligand; CNS, central nervous system; CXCL, C-X-C motif chemokine ligand; EC, endothelial cell; IFN-γ, interferon-gamma; IL, interleukin; Th, T helper; TNF-α, tumor necrosis factor-alpha. Figure created with Biorender.com.

activity.[29] Th22 cells are a major source of IL-22 production.[30,31] This cytokine is increased in the serum[32] and active lesions[33] of people with MS while it is reduced during the recovery phase in EAE.[34] It is also involved in BBB disruption and CNS inflammation.[35] All of these cell types cause an inflammatory cascade in the CNS by releasing chemokines and pro-inflammatory cytokines such as IFN-γ, IL-2, tumor necrosis factor alpha (TNF-α) and oncostatin M (OSM).[2,36,37] As a consequence, the BBB becomes inflamed and other immune cells like B cells and macrophages are recruited and activated. In MS, activated B cells were shown to excessively produce the cytokines lymphotoxin, TNF-α and granulocyte macrophage-colony stimulating factor (GM-CSF), next to their extensive antibody production marking the myelin for phagocytosis.[38,39] Additionally, B cells present antigens that induce proliferation of autoreactive CD4+ T cells.[40] However, the most convincing evidence for the importance of B cells in MS pathology is the efficacy of B cell depleting therapies in MS. Ocrelizumab, a humanized monoclonal antibody that selectively targets and depletes CD20+ B cells, is approved for the treatment of active RR-MS and PP-MS.[41] This therapy depletes B cells in most stages, thereby eliminating B and T cell interactions and reducing T cell activation and proliferation.[40,42] B cell depletion is associated with significantly lower rates of disease activity and progression, suggesting a key role of B cells in MS.[8,43] Macrophages infiltrating the inflamed CNS were shown to predominantly adopt the highly inflammatory M1 phenotype.[44] Here, they have an increased secretion of inflammatory cytokines and toxic mediators, hence contributing to disease severity and progression.[45,46] Mast cells are also involved in MS pathology as they promote demyelination, present myelin antigens to T cells and disrupt the BBB.[47] This immune cascade ultimately cumulates in extensive demyelination, axonal and neuronal injury and eventually brain atrophy (Figure 1).[36]

Treg (dys)functionality in MS

Thymus-derived Tregs (tTregs) are CD4+ CD25+ Forkhead box P3+ (FOXP3+) cells that, in healthy conditions, suppress effector T cells and other immune cells from reacting against self-antigens (reviewed by Janssens et al.[48]). They maintain peripheral self-tolerance by expressing co-inhibitory molecules and secreting anti-inflammatory cytokines. When transforming growth factor beta (TGF-β) and IL-2 are available in the microenvironment, Tregs can develop in the periphery (pTregs) from CD4+ T cells.[49] One subtype of pTregs is Tr1 cells, which are characterized by high levels of IL-10 secretion without expressing FOXP3.[50] Under inflammatory circumstances, Tregs can adapt a Th17 phenotype and become exFOXP3 effector cells.[51] For example, IL-17 producing FOXP3+ Tregs have undergone inflammatory conversion and either transiently or stably express FOXP3 together with IL-17.[50,52] These CD4+ Treg subtypes demonstrate the extensive phenotype plasticity of Tregs (reviewed by Baeten et al.[10]). Another Treg subset, CD8+ Tregs, have a regulatory function by killing antigen-activated CD4+ T cells by the use of perforin.[53] Lastly, regulatory B cells (Bregs) have a similar function to Tregs, suppressing the inflammatory immune response in healthy individuals.[54] In this review, the focus is on CD4+CD25+FOXP3+ Tregs. The suppressive capacity of Tregs can also differ due to alternative splicing of FOXP3.[55]

There are two main isoforms, a full-length FOXP3 and a FOXP3 lacking exon 2.[56] Alterations in splice variants can have implications in the development of autoimmunity and MS.[56,57] For instance, Tregs from people with MS have a reduced expression of the epitope encoded by exon 2, which normally prevents the development of Th17 cells.[56] Tregs expressing this FOXP3 lacking exon 2 were therefore found to be unstable and less suppressive.[58]

It was shown that Treg numbers and function are disturbed in people with MS. Lower numbers of Tregs have been detected in MS peripheral blood samples.[59–61] Depending on the treatment or disease status, Tregs could be identified in some, yet not all MS lesions.[26,62] In contrast, in the cerebrospinal fluid (CSF) of people with MS, an increased number of Tregs was identified, although these were mostly apoptosis-prone $CD95^{high}$ cells.[61–63] Tregs from people with MS are less immunosuppressive compared to healthy controls, unleashing autoreactivity and inflammation in the CNS.[61–67] One crucial factor related to this suppressive dysfunction is the decreased expression of the transcription factor FOXP3 in Tregs.[68] Besides, dysfunction of Bregs has been indicated in MS.[69] This is characterized by reduced IL-10 production *in vitro* and a reduced number of IL-10-producing Bregs.[69,70] Th1 effector cell suppression by Bregs was also dysfunctional in people with MS.[71] Accordingly, Breg dysfunction possibly contributes to MS development or maintenance.

Next to the peripheral effects of Tregs, once migrated through the BBB, they can perform their suppressive properties in the CNS. However, in a preclinical murine model for MS, called experimental autoimmune encephalomyelitis (EAE), Tregs were found to become unstable after entry in the CNS due to the downregulation of FOXP3 expression, leading to a reduced suppressive capacity.[72,73] In a different study, myelin-specific Tregs accumulate in the CNS and do effectively suppress naïve myelin oligodendrocyte glycoprotein (MOG)-specific T cells.[74] CNS-derived encephalitogenic T effector cells, however, were found to be resistant to this suppression during the active phase of the disease.[74] This effect is likely due to the production of IL-6 and TNF-α in inflamed tissue, making T cells less sensitive to Tregs. In contrast, it has been demonstrated that Treg accumulation has suppressive properties in the CNS of EAE mice, mediated by the production of IL-10.[75] This effect is primarily responsible for the remission phase of EAE. It was also shown that Tregs that have entered the CNS are more activated and have a more protective role compared to Tregs derived from the lymph nodes.[75,76] Although studies in EAE models have brought on much knowledge on autoimmune pathology and neuroinflammation, caution should be taken when translating the results to MS. EAE is a mainly T cell driven pathology, hence largely ignoring the role of B cells.[77,78] Additionally, the progressive phases of MS are only limitedly represented by EAE models.[79,80] Despite their disadvantages, EAE and MS share several key features such as neuroinflammation and BBB disruption.[81,82] These animal models are crucial in acquiring knowledge on MS immunopathology and developing novel therapeutics.

In addition to their immunosuppressive properties, Tregs were found to have regenerative abilities as well. Importantly, Dombrowski et al.[12] discovered that Tregs mediate remyelination in the CNS. Whether this process is affected in MS similarly to the suppressive capacity of Tregs, is not known yet. Overall, functional Tregs could have high potential of ameliorating disease in people with MS, even in progressive stages.[12,83] The functional consequences of Treg migration across the BBB remains a black box that urgently needs to be explored.

Diapedesis of immune cells

For immune cells to exert effector functions in the CNS, they must migrate through the BBB; a process called diapedesis. Leukocytes can undergo transendothelial migration through the BBB in a series of sequential steps.[84,85] Mediated by endothelial E- and P-selectins, cells adhere weakly to the vascular endothelial wall and start rolling. Chemokines produced by the endothelial cells increase the affinity of integrins very late antigen (VLA)-4 and leukocyte function associated antigen (LFA)-1 on leukocytes. Binding their ligands on the endothelial cells, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, leads to a complete arrest.[21,36,86] Immune cells start crawling against the blood flow in search for a suitable site to transmigrate,[87] which is also influenced by endothelial cell surface levels of ICAM-1.[88] Infiltrating immune cells enter the perivascular space and specifically T cells are here reactivated by local APCs. Cytokines produced by these T cells trigger BBB dysfunction and expression of matrix metalloproteinases (MMP) to degrade the glia limitans.[21] Together with this cytokine-induced compromised BBB, chemokine-dependent attraction of other immune cells leads to a massive leukocyte infiltration into the CNS parenchyma (Figure 1). In MS, changes in protein expression are found for the adhesion molecule platelet endothelial cell adhesion molecule-1 (PECAM-1) and MMP-9 amongst others, contributing to higher immune cell migration through the BBB.[89,90] Furthermore, research suggests that oxidative stress and nitric oxide play a role in the loss of BBB integrity in MS.[91,92] Lastly, Nishihara et al. recently showed an intrinsic impairment of BBB-EC-like cells derived from people with MS.[93] Altogether, BBB impairment is a key component of MS pathology early in the disease. Full understanding of BBB disturbances and immune cell migration, including interactions between both compartments, is of high importance to develop novel, more specific treatments for MS.

Migration process of Tregs across BBB

Tregs are crucial in moderating the neuroinflammatory response and show remyelinating functions in MS animal models. Hence, it is interesting to investigate how these cells migrate through the BBB in MS and whether they exert certain effects on the migration of other immune cells through the BBB.

Transendothelial migration of Tregs in MS

Tregs are hypoproliferative *in vitro* and form only a small section of the peripheral CD4+ T cell subset.[94,95] As a result, Tregs must have higher migration rates to the target site for

them to influence effector cells.[96,97] This feature is confirmed in both murine and human Tregs from healthy individuals in non-inflammatory conditions, where Tregs showed enhanced migratory abilities compared to non-Treg cells.[96] Nevertheless, Tregs derived from people with RR-MS showed significantly impaired migration under non-inflammatory conditions, possibly explaining the following unleashed CNS inflammation. In inflammatory conditions, the proportion of migrated Tregs was restored, similar to control conditions. During the remission phase of EAE, Tregs accumulate in the murine CNS.[86,96] In this phase, ICAM-1 was shown to be upregulated (Figure 2).[86] Inhibition of ICAM-1 during this phase aggravated the clinical symptoms in EAE animals, suggesting a role for LFA-1/ICAM-1 interaction in Treg infiltration in the CNS. The molecular interactions between T cells and the BBB are similar for T effector cells and Tregs. Yet, they possibly occur at different stages of the disease course given that inhibition of ICAM-1 at the stage of disease progression had a tempered disease course.[86] Additionally, LFA-1 is highly expressed on FOXP3+ Tregs.[96] Mice deficient in LFA-1 showed an increased demyelination and increased numbers of autoreactive T cells, leading to more severe EAE.[98,99] This suggests that Tregs make use of LFA-1 for their entry into the CNS during EAE.[100]

During inflammation, Tregs express transcription factors that define Th subsets and cytokine production.[101,102] It is suggested that this enables Tregs to also express typical chemokine receptors of Th1 and Th17 cells to follow them to the site of inflammation.[101] Examples are chemokine receptor C-X-C motif chemokine receptor 3 (CXCR3) for Th1 cells and C-C motif chemokine receptor type 6 (CCR6) for Th17 cells. The CXCR3 is a plausible mediator for Treg chemotaxis (Figure 2).[103] In the broad context of inflammation, CXCR3 is expressed on Tregs,[104,105] while one of the ligands for this receptor, C-X-C motif chemokine ligand 10 (CXCL10), is upregulated in EAE and MS lesions.[103] Inhibition of CXCR3 led to a decrease in Tregs present in EAE lesions and failure to recover from the disease. This suggests a role for this receptor-ligand interaction in the migration of Tregs across the BBB. On the other hand, CXCR3 is also an important mediator in the accumulation of Th1 cells in MS, as CXCR3+ T cells are increased in the CSF of people with MS and in active MS lesions.[106–108]

Furthermore, CCR6 is also involved in the migration of Tregs (Figure 2).[108,109] In EAE, both Tregs and Th17 cells express CCR6, attracting them to the inflamed CNS in response to the ligand C-C motif chemokine ligand 20 (CCL20). Indeed, CCR6+ Tregs are effector-memory cells which accumulate in the CNS during inflammation after induction of EAE.[110] CCR6-deficient mice show an abnormal EAE disease course with a delayed onset, yet a higher clinical score in later disease stages.[111] This could be attributed to the decreased Treg migration to the CNS at the peak phase of the disease. These results suggest the role of CCR6 as an important chemokine receptor for Th17 cell recruitment at onset of EAE, while CCR6 is important for Treg migration at later time points. These findings highlight the complexity of these molecular interactions at different stages of the disease.

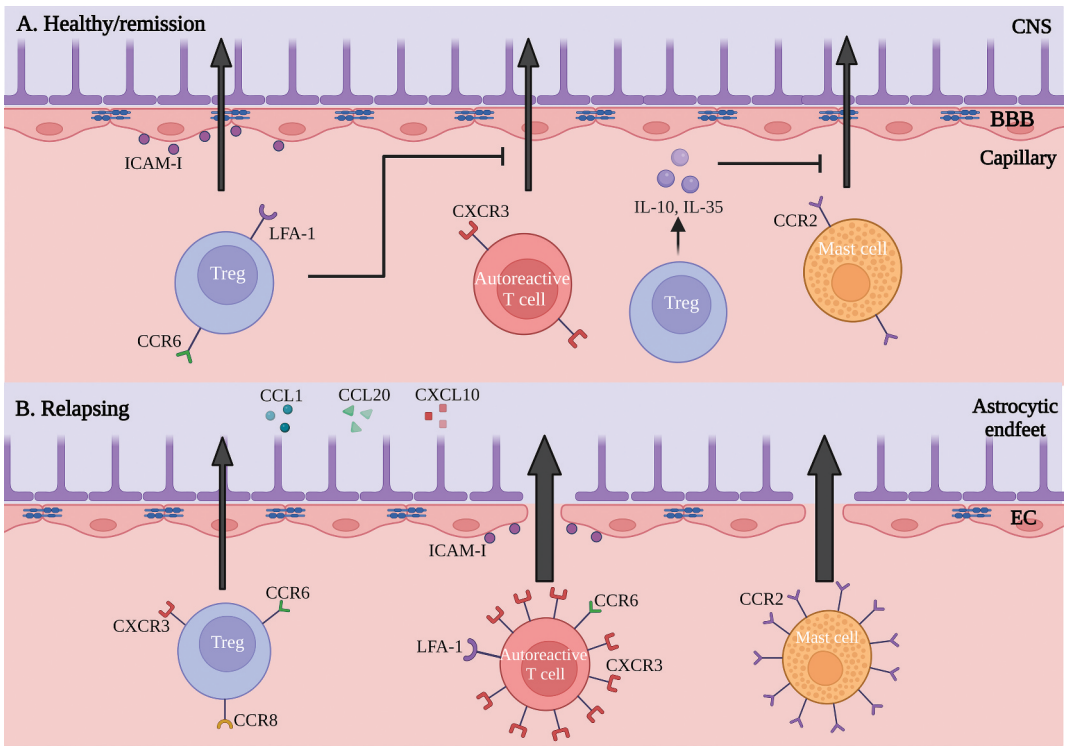

Figure 2. Summary of Treg migration in healthy/remission state vs. relapsing/progressive MS. (a) in healthy conditions or remitting MS, Tregs cause decreased autoreactive T cell migration by downregulating the CXCR3 receptor. Additionally, Treg IL-10 and IL-35 downregulate the CCR2 receptor on mast cells and consequently cause decreased mast cell transendothelial migration. Tregs mainly migrate through interactions of the LFA-1 and CCR6 receptor. (b) in relapsing MS, autoreactive T cells and mast cells show an increased migration. Tregs migrate in response to chemokines derived from the inflamed CNS (CCL1, CCL20, CXCL10). CCR, C-C chemokine receptor; CNS, central nervous system; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; EAE, experimental autoimmune encephalomyelitis; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; LFA-1, leukocyte function associated antigen-1; MS, multiple sclerosis; Treg, regulatory T cell. Figure created with Biorender.com.

In addition, CCR8 is expressed on Tregs, guiding these cells to the CNS in EAE (Figure 2).[112] Ligands of this receptor, e.g. CCL1, are produced by activated T cells and APCs in inflamed tissues, creating a chemotactic gradient. Interactions between CCR8 and CCL1 increase the suppressive capacity of human Tregs by upregulating FOXP3, CD39, IL-10 and granzyme B.[113] When administering a CCL1 antibody fusion protein to the EAE model, CCR8+ Treg proliferation was enhanced, leading to an effective suppression of the disease. This role of CCR8 was also confirmed in graft-versus-host disease (GvHD), where CCR8-/- Tregs showed to be incompetent in the prevention of this disease.[114] Although they are not Treg specific, all of these molecules might be interesting targets for manipulation of Treg migration in future Treg-based therapies.[115]

Finally, it is important to realize that not all Tregs are immunomodulatory. A small part of the Treg population consists of pro-inflammatory CD49d+ cells.[116] CD49d is the α4-β1 integrin receptor, also known as VLA-4, which is an important adhesion molecule for the diapedesis of leukocytes into inflamed tissue.[117] In people with RR-MS, a higher frequency of Tregs in peripheral blood expressed CD49d compared to healthy controls or people with SP-MS.[61] These results might partially explain the therapeutic efficacy of natalizumab in people with RR-MS, an antibody treatment targeted against this α4-β1 integrin receptor.[118–120] This treatment will cease the transmigration of pro-inflammatory effector cells to the inflamed CNS without affecting CD49d- Treg migration, leading to a restored effector/suppressor balance in the CNS.[116]

Tregs influence migration of pathogenic cells in MS

Next to affecting the local immune response, Tregs can also influence other immune cells in the diapedesis process. Tischner et al.[121] describe that polyclonal expansion of Tregs inhibits the infiltration of effector T cells in the CNS of EAE animals without migrating themselves (Figure 2). Tregs rather implemented their suppressive effect in the secondary lymphoid organs, where they suppressed the secretion of IFN-γ by encephalitogenic T cells, reducing their expression of CXCR3. This leads to a compromised migration of these Th1 cells in the CNS. Additionally, with the expansion of Tregs, the BBB was limitedly damaged and remained more intact, indicating a Treg-driven protective effect on the BBB. Enhancement of Tregs also decreases the migration of mast cells through the BBB via a down-regulation of CCR2 and several adhesion molecules.[122] Interestingly, Tregs were shown to inactivate mast cells through cell-to-cell interactions and secretion of IL-10 and IL-35 (Figure 2). In the CNS, mast cells can attract other leukocytes to the site of inflammation by secreting the cytokines IL-1β and IL-8. Hence, an increase in Tregs indirectly leads to a decrease in damaging leukocytes in the CNS and a milder disease course.

Once T effector cells cross the BBB and infiltrate the brain parenchyma, Tregs also influence their local motility. In the absence of Tregs, T effector cells showed a decreased velocity and were more stationary in the CNS of EAE mice.[123] In the presence of Tregs, increased velocities of Th17 cells were observed, indicating decreased Th17 cell-APC interactions.[124]

Therapeutic potential of Tregs

Since current therapies are primarily based on delaying disease progression and reducing relapse rate, there is a high need for new and curative treatments specifically targeted at the disease pathology of MS. As previously mentioned, Tregs are of great importance in lowering damaging effects of T effector cells and even show regenerative functions, making them an interesting target for the development of new MS therapies. Most clinical trials using Treg-based therapies have been performed in people with GvHD. Both people suffering from acute and chronic GvHD benefited from this therapy.[125,126] Symptoms of people with chronic GvHD stabilized or even improved after the treatment.[127] People with leukemia experienced reduced post-transplant relapse rates after Treg therapy.[128] For other (auto) immune diseases such as Crohn's disease[129] (and NCT03185000), type 1 diabetes[130,131] (and NCT02691247 and NCT02932826), amyotrophic lateral sclerosis (ALS)[132] (and NCT04055623 and NCT03241784) and coronavirus disease 2019 (COVID-19)[133] (and NCT04468971), Treg-based therapies are also in clinical trials. Results from these studies demonstrated safety and tolerability for the therapy. In the field of MS research, only one clinical trial testing the effect of a Treg-based therapy has been conducted. A phase 1b/2a clinical trial including 14 people with RR-MS was recently performed.[11] Eleven participants received *ex vivo* expanded Tregs intravenously, while three others received freshly isolated Tregs intrathecally. None of them showed adverse effects after administration. Interestingly, disease progression was halted in all people who received Tregs intrathecally. On the other hand, people who received Tregs intravenously still experienced relapses or deterioration of their disability.[11] Although the trial only included a low participant number, these results suggest that in people with MS, Tregs might not get into the brain in high enough amounts regardless the expression of important chemokine receptors and adhesion molecules. This is in line with the observation that not always FOXP3+ Tregs can be found in the brain lesions of people with MS.[62] In addition, Treg functionality in the brain is also still under investigation and might also explain the lack of FOXP3 expression in the CNS. When these questions are resolved, Treg-based therapies could have great potential in treating MS.

Like natalizumab, other therapies inhibiting the migration of B and T cells exist. Ozanimod, a sphingosine-1-phosphate receptor-1 (S1P1) modulator, has recently been approved for the treatment of RR-MS.[134] S1P1 is important in the migration of T and B cells into the blood circulation. Additional research is being conducted, where ozanimod is tested on its ability to prevent migration of immune cells through the BBB and reduce cytokine-mediated breakdown of the BBB (NCT05245344).

A possible approach for Treg-based therapy is to expand and adapt the patient's Tregs *ex vivo*, followed by adoptive transfer of these cells to the patient.[75] A myelin-specific, single-chain Fv chimeric antigen receptor (CAR) was designed which can be expressed by Tregs.[135] These Tregs are specific for myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG). Administration of these CAR Tregs ameliorated disease progression of EAE in mice. Another alternative

is to increase the functionality of Tregs *in vivo*. In preclinical studies, the neutralization of IL-6 was disease-reducing, possibly resulting from a higher Treg activity.[74,136] Many other possibilities of enhancing Treg functionality *ex vivo* have been proposed, including viral vector-mediated approaches and CRISPR-Cas9 gene-editing tools.[10]

Many preclinical studies in EAE have already shown the therapeutic potential of Tregs.[86,121,137] None of these preclinical therapies thus far consider Treg migration across the BBB. To increase the number of Tregs in the brain, one could consider increasing the expression of chemokine receptors (CXCR3, CCR6, CCR8) on Tregs, while ensuring their stability *in vivo*.[10,138] This would result in an increased local T effector cell suppression and increased remyelination.

Conclusion

Not much is known about the specific interactions between the endothelial cells of the BBB and Tregs in MS. This might be interesting when talking about a potential Treg cell-based therapy, since functional Tregs may ameliorate disease course once they have entered the CNS. Some studies indicated a dysfunctional Treg migratory capacity in people with RR-MS. In EAE, an accumulation of Tregs but lack of suppression in the CNS lesions was observed. Different receptors, such as ICAM-1, CXCR3, CCR6, and CCR8 have been proposed regarding the chemotaxis of Tregs, leading them across the BBB. It was also shown that different interactions may play a role in the transendothelial migration of Tregs at different times in the disease course. This leads to the realization that there is still a huge lack of knowledge regarding different phases and time points of the disease and how important these may be in finding novel therapies for MS. Additionally, Tregs have the potential to decrease the migratory capacity of effector cells to the CNS. These results lead to believe that Tregs could be used as a therapeutic opportunity, while bearing in mind the difficulties of Tregs crossing the BBB when administering them intravenously. That being said, many knowledge gaps still exist in this domain, such as elucidation about the migratory capacity of Tregs in MS, the capacity of Tregs to possibly repair the BBB disruption during remitting phases of MS, Tregs affecting B cell migration, and many other topics. Addressing these have great potential to further the quest toward a curative MS treatment.

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