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THE action of TH17 cells on blood brain barrier in multiple sclerosis and experimental autoimmune encephalomyelitis

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ABSTRACT

Th17 cells, known as a highly pro-inflammatory subtype of Th cells, are involved very early in numerous aspects of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) neuropathology. A crucial event for the formation and accumulation of MS lesions is represented by the disruption of the blood brain barrier (BBB) in relapsing-remitting MS. Th17 cells also contribute to the progression of MS/EAE. These events will allow for the passage of inflammatory cells into the brain. Secondary to this, increased recruitment of neutrophils occurs, followed by increased protease activity that will continue to attract macrophages and monocytes, leading to brain inflammation with sustained myelin and axon damage.

This review focuses mainly on the role of Th17 cells in penetrating the BBB and on their important effects on BBB disruption via their main secretion products, IL-17 and IL-22. We present the morphological aspects of Th17 cells that allow for intercellular contacts with BBB endothelial cells and the functional/secretory particularities of Th17 cells that allow for intercellular communications that enhance Th17 entry into the CNS. The cytokines and chemokines involved in these processes are described. In conclusion, Th17 cells can efficiently cross the BBB using pathways distinct from those used by Th1 cells, leading to BBB disruption, the activation of other inflammatory cells and neurodegeneration in MS patients.

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating and neurodegenerative disease that is heterogeneous, not only clinically and therapeutically, but also immunologically. This pathology is considered to be a T cell–driven inflammatory disease that consists of focal immune cell infiltration, demyelination and neuro-axonal degeneration [1].

Experimental autoimmune encephalomyelitis (EAE) is the animal model of demyelination in the CNS. EAE resembles MS in many aspects, serving also as a model for autoimmune disease of the central nervous system (CNS). Many EAE studies have focused on blood–brain barrier (BBB) breakthrough and the complex involvement of Th17 cells in this process [2,3].

In healthy subjects, BBB's structure preserves the CNS' homeostatic ease of metabolic support and impedes the entry of peripheral immune cells, different pathogens and other neurotoxic elements. The correct function of this highly complex structure depends on a multitude of interactions between its components: endothelial cells (the innermost layer), astrocyte end-feet and pericytes [4]. The "immune privilege" ensures that the external interferences are kept to a minimum, but a crucial need for constant surveillance is imposed [5].

Loss of BBB integrity signalises the first step of altered immune cell traffic into the CNS, representing the pathological hallmark of relapsing-remitting MS (RRMS). The extravasation of leukocytes through the BBB is facilitated by the conjunction of overexpression of cell adhesion receptors and chemotactic constituents. Leukocytes and endothelial

Abbreviations: BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; MCAM, melanoma cell adhesion molecule; MS, multiple sclerosis; MOG, myelin oligodendrocyte glycoprotein; ROS, reactive oxygen species; TGF-β, transforming growth factor beta

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cells suffer major morphological and functional changes due to genetic and environmental factors. Pathological events that determine the BBB malfunction in RRMS include immune cell egress, hypoxia and protein deposition [4,6].

Since the description of Th17 cells in 2005, numerous pathogenic actions have been attributed to this group of cells; specifically, they have been shown to initiate and maintain the immune attack in MS [7,8]. Th17 cells have a central role in the pathogenesis of MS. In MS and other immune-mediated diseases, naïve T cells have been shown to differentiate into IL-17-producing cells under the influence of IL-1β and IL-6, while transforming growth factor-beta (TGF-β) apparently suppresses Th17 differentiation as reported by some studies [9]. In a recent study on a number of 32 RRMS patients treated with IFNβ1a, the role of TGF-β is demonstrated as being titer-dependant, thus, high serum levels are associated with an inhibition of IL-17 secretion [10]. This extremely proinflammatory subset of cytokines is firstly subjected to the activity of IL-23, but this cytokine appears not to have a direct effect on the naïve T cell differentiation. Both TGF-β and IL-6 carry the potential to induce T cell differentiation into Th17 cells, depending on the present state of the innate immune response [11,12]. TGF-β up-regulates the generation of Foxp3+ in the presence of IL-6 and while the increasing concentrations of TGF-β augment the levels of Foxp3, by stimulating the differentiation of naïve Th cells into a regulatory subtype, rather than Th17, the activity of IL-6 overcomes the suppressive effect of Foxp3+, and subsequently induces the expression of IL-17 [13,14]. Durelli et al. reported in 2009 that, in the peripheral blood of active RRMS patients, Th17 cells expand and increase [15]. The main secretion product of Th17 is IL-17, a proinflammatory cytokine involved in maintaining the systemic inflammation. Together with the cascade of Th17 differentiation, IL-17 keeps together a proinflammatory environment essential for the genesis of immune-mediated inflammation, being actively involved in EAE induction and MS pathophysiology [14]. Th17 cells can efficiently cross the BBB in different ways from Th1 cells; they promote its disruptions, induce the activation of other inflammatory cells in the CNS and negatively influence the remyelination of demyelinated axons [2].

Th17 cells contribute to the onset but also to the progression of MS/EAE. In the periphery, Th17 cells activate the bone marrow neutrophils that trigger the migration of immature monocytes into the bloodstream [16]. Disruption of the BBB is crucial for the formation and accumulation of MS lesions. Secondary to this, increased recruitment of neutrophils occurs, followed by increased protease activity that continues to attract macrophages and monocytes at the level of brain inflammation with sustained myelin and axon damage [1,17].

Th17 cells have stunning plasticity, that is, ability to transition (using transcription factors T-bet and ROR) between different phenotypes. A significant proportion of Th17 cells that initially secrete IL-17 convert into interferon gamma (IFNγ)-producing T cells. IL-23-mediated reprogramming is partially responsible for this phenomenon, but this pathway is now partially limited due to an increasing evidence of Th17 differentiation under the activity of TGF-β and IL-6. Bettelli et al., demonstrated on murine models that the administration of IL-23, associated either with IFNγ or with TGF-β and IL-6 did not significantly influence the differentiation of Th17 population, nor the IL-17 production. The activity that IL-23 possesses rather impacts the already present Th17 cells to produce IL-17. Moreover, taken separately, neither TGF-β nor IL-6 stimulate the Th17/IL-17 production, but when added together, a significant increase of IL-17 Th producing cells was noted. Therefore, the innate immune system is responsible for conferring a reciprocity in the development of Th17 cells and Treg cells, dependant on the activity of certain pro-inflammatory cytokines, secreted in the acute phase, such as IL-6 [11]. The encephalitogenic effect of IL-6, together with TGF-β in an IL-23 dependant pathway was widely described by Yang et al. The differentiation and activation of Th17 cells secondary to IL-6/TGF-β exposure is obvious, but the newly obtained subset of Th17 cells was unable to pass through the BBB, into the CNS. This unstable phenotype gains encephalitogenic properties dependant on the transcription factors, in this case the T-bet, and not due to the cytokine secretion as previously thought [18]. A very aggressive subset of Th17 cells also expresses both IL-17A and IFN-γ, from Th1 and Th17 cells respectively [19,20].

Th17 cells have been found to acquire brain-homing capability. Th17 cells are elevated in the cerebrospinal fluid (CSF) of RRMS or clinically isolated syndrome patients during relapses, in comparison to patients in the remission phase, and Th17 cells are more abundant in the perivascular CNS tissue during relapses and in the brain lesions of MS patients [21,22]. Serum and CSF levels of IL-17A (the most proinflammatory secretion product from Th17 cells) correlate with MS severity measured by the Expanded Disability Status Scale and with the number of gadolinium-active lesions on magnetic resonance imaging. The role of Th17 cells and their secreted cytokines, such as IL-17F have been thoroughly linked to disease progression. In 2017, a study by Arellano et al. demonstrated that higher levels of IL_17F were found in patients that progressed from clinically isolated syndrome to MS. [23] Furthermore, Th17 cells might have a role in progressive forms of MS, together with the activated B cells and follicular Th cells, being promoters and mediators of systemic inflammation [15,24].

Pikor et al. showed that Th17 cells induce the formation of tertiary lymphoid tissue within the meninges that is associated with local demyelination during EAE. This provides a link with B-cell pathology [25].

The BBB is a key modulator of cellular trafficking into the CNS. Apart from maintaining the homeostasis of the CNS by down-regulating the inflammatory processes occurring past the endothelial layer, the BBB is actively involved in the early pathogenesis of CNS inflammation. Ifergan et al. demonstrated that the BBB endothelial cells induce differentiation of dendritic cells CD209, and subsequently secrete IL-12p70, TGF-β and IL-6, thus inducing the differentiation of T naïve populations into Th1 and Th17 [26]. The encephalitogenicity of Th17 cells is supported by the fact that higher levels of IL-17, contrasting to low titers of TGF-β are present during relapses in CSF of the RRMS patients [27]. O'Connor et al., after analysing the differences between the CSF levels of IL-17 and IFNγ, formulated a hypothesis according to which the Th1 subset is essential for Th17 trafficking into the CNS [28] this being further regulated by the BBB affinity for selecting pro-inflammatory T cell populations [20].

This review will mainly focus on the role played by Th17 cells in BBB disruption, as many anatomo-pathological studies have shown that these cells and their main secretion product, IL-17, are actively involved in the initial phase of the immune cascade in MS [2,9,10,11]. Evidence implicating Th17 cells in the pathophysiology of MS within the active plaques from brain of MS patients was found in numerous anatomo-pathological studies. Increased evidence shows that T cells actively penetrate the CNS parenchyma [26,29,30].

In the last decade, Th17 cells have captivated the interest of scientists, simultaneously with the discovery of a pathogenic Th17 phenotype that was found in the small intestinal mucosa. This proinflammatory intestinal environment was found to promote and to be associated with brain autoimmunity in MS patients [31].

For Th cells with proinflammatory properties and their cytokine responses to be effective, maintaining MS as an active, chronic disease, the involvement of an important number of co-stimulatory and co-inhibitory molecules is needed [16]. Critical components and active players in the inflammatory processes of MS, cytokines are produced by different subtypes of Th cells. All these changes may lead in severe MS cases to the disease evolving from a dominant Th1 response to a Th17 response [23].

Blocking a certain receptor or cytokine from the Th17 family, or providing certain antibodies, may alleviate EAE symptoms. We can be hopeful that progress will be made in clinical treatment of MS.

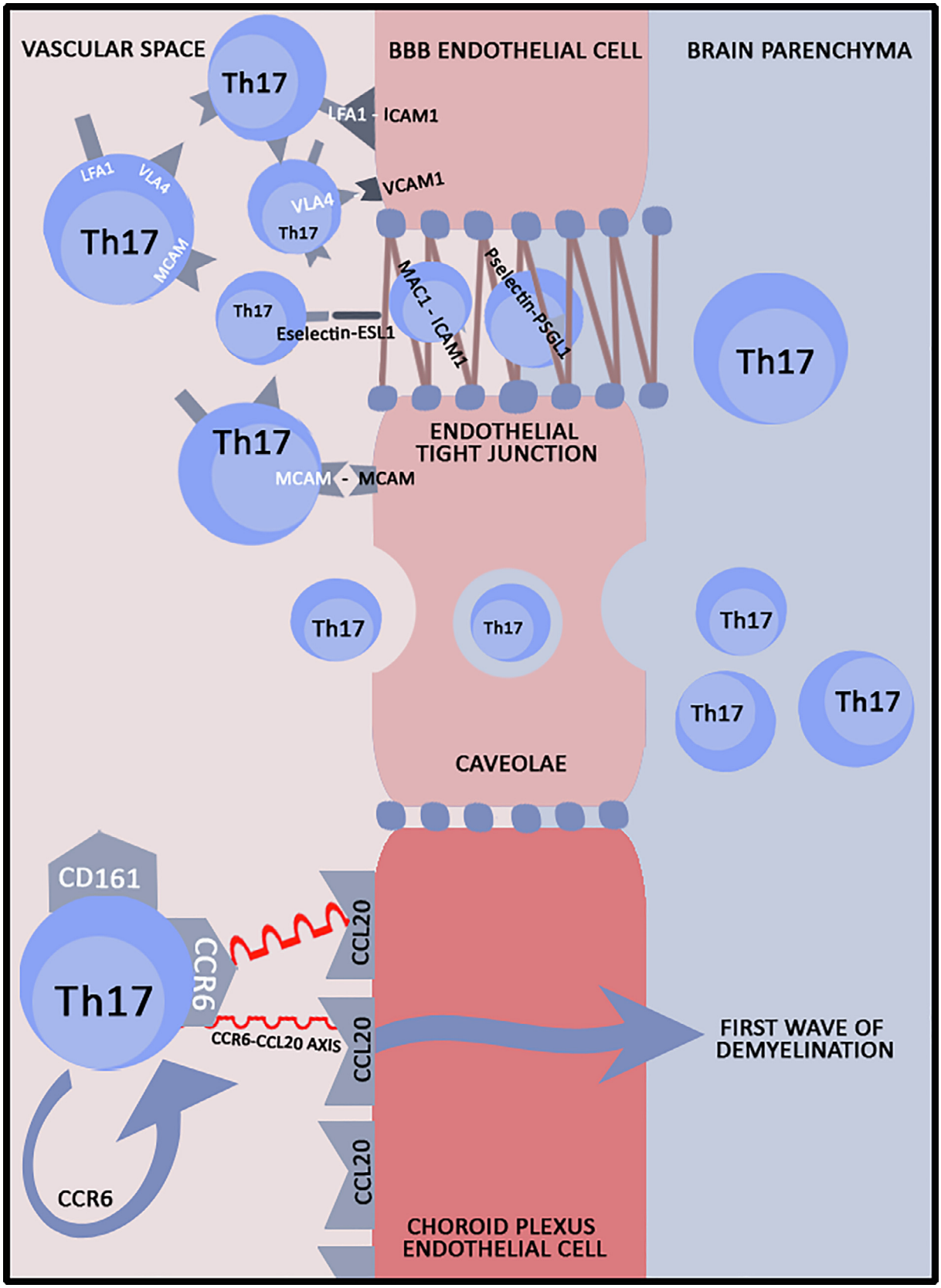

Fig. 1. Different passages of activated Th17 lymphocytes into the brain parenchyma.

2. Effect of Th17 on the BBB

CNS dysfunction and disease activity in RRMS and EAE might be promoted by Th17 cell actions. In vitro studies have shown that human Th17 cells are more efficient in migrating through the BBB than Th1 cells, exhibiting neurotoxic effects [32].

The differentiation of Th17 cells needs more stages: initially, IFN-γ and IL-12 drive naïve T cells into the Th1 pathway; IL-12 finalizes differentiation of Th1 cells; Th1 $CD4^+$ cells and naïve T cells differentiate to Th17 cells in the presence of IL-23 that is essential for stabilizing these cells; Th17 cells produce IL-17, as a family of cytokines IL17A-IL17F, in the presence of IL-6 and TGF-β; then a member of the IL-2 cytokine family—IL-21, produced in large quantities by mature Th17 cells—can, together with TGF-β, amplify Th17-cell differentiation. In short, an autocrine loop for the differentiation of Th17 cells is orchestrated by stimulating cytokines (TGF-β, IL-1β, IL-6, IL-21, IL-23) and inhibiting cytokines (IL-4, IL-12, IL-10, IL-27) [2,7,9,16,23].

IL-23 is the key player in chronic inflammatory autoimmune responses in numerous immune diseases, including MS. This heterodimeric cytokine comprises two subunits, p19 and p40, the latter being common with IL-12. Apart from stimulating the production of interferon-γ (IFN-γ) from T cells, IL-23 stimulates Th17 lymphocytes to promote the disruption of BBB, driving the CNS tropism of Th17 cells through the expression of necessary factors. This cytokine is needed for the final differentiation and expansion of a highly encephalitogenic group of Th17 cells on antigenic-specific stimulation, in contrast with less pathogenic Th17 cells that, stimulated with TGF-β and IL-6, secrete IL-17A and IL-10 (less aggressive cytokines with anti-inflammatory properties) [10,23,33]. IL-23 is also required for the survival of Th17 cells and the maintenance of IL-17 production. This group of Th17 cells efficiently transfers immune and inflammatory processes through the BBB using different mechanisms: penetrating the BBB, inducing neuronal death and recruiting other CD4+ lymphocytes, thus promoting further CNS inflammation. The cytokine microenvironment in the periphery is the main factor for promoting plasticity of Th17 subsets. Entire generations of MS-pathogenic Th17 cells develop in the presence of proinflammatory cytokines such as IL-23 and in the absence of TGF-β [33]. IL-23 acts as a link in the microglia–T cell interaction, as well as in recruitment and activation of inflammatory cells such as Th17. Th17 cells, once migrated beyond the glia limitans basement membrane, initiate tissue destruction in the parenchymal CNS white matter. In animal models, Th17 and IL-23 are essential for the induction of EAE [34,35]. However, the use of ustekinumab, a IL12/23 p40 neutralizing antibody, had disappointing results in RRMS treated population [36]. This event was not without precedent in the history of emerging disease modifying therapies for MS. The use of infliximab, in 1996, a TNFα blocker used routinely for the treatment of rheumatoid arthritis was associated with a worsening of the disease, by amplifying the CNS demyelinating processes [37]. This phenomenon might be explained by the acute involvement of IL-6 in the generation of pathogenic Th17 cells, rather than IL-23, as described in the previous chapter.

Another theory states that, while the integrity of BBB is maintained, the specific neutralizing antibodies have no means to penetrate into the CNS, and while, in theory, they might carry inhibiting effects on the Th17 lineage, the access to the inflammation source is constrained [38]. Another limitation might be that, due to the lack of a specific receptor on the surface of BBB endothelial cells, this type of neutralizing antibody is by default inaccessible to the CNS.

Arguments for a high pathogenic potential of Th17 compared to Th1 cells in MS immune dysregulation include their higher proliferative capacity, reduced susceptibility to suppression, greater plasticity of function, expression of an adhesive molecule melanoma cell adhesion molecule (MCAM) or CD146, and more efficient migration across the BBB [19,21,39,40].

2.1. Morphological aspects of Th17 cells that enhance their entry into the CNS and allow intercellular contacts with BBB endothelial cells

Due to their morphological characteristics, Th17 cells compared to Th1 are more susceptible to suppression and have a higher proliferative capacity [21]. Th17 cells enter the CNS before Th1 cells in EAE, and use three different mechanisms to cross the BBB: via caveolae, by crossing disrupted tight junctions, or using MCAM-MCAM interaction (Fig. 1).

The transmigration of Th17 lymphocytes across the BBB during neuroinflammation is possible due to the remodelling of caveolae-independent tight junctions, with penetration of Th1 lymphocytes into the CNS being caveolae-dependent [41]. This Th17 infiltration precedes Th1 infiltration through caveolar transcytosis in EAE. These first steps are followed by disease propagation.

In a review published in 2010 focused upon the interaction between the T cells and the endothelial cells, Haqqani and Stanmirovic described a number of 116 Th17 membrane proteins and 62 human glycocalyx proteins, responsible for the formation of over 180 interacting pairs, such as ICAM1-LFA1, VCAM1-VLA4, E-selectin-ESL1, ICAM1-Mac1 and P-selectin-PSGL1 [42]. Th17 surface molecules can interact with endothelial cells at the luminal membrane or in the tight junction region.

Brain endothelial cells are more easily crossed by Th17 cells than by Th1 cells, partially due to the presence of CD146 receptor (MCAM) on the surface of Th17 cells. The entry of Th17 cells into the CNS is also facilitated by the high expression of molecules present on their membrane; molecules involved in T cell adhesion to the endothelium such as CCR6 (a chemokine receptor characteristic of Th17 cells), CD6 and CD49d; and the MCAM that can enhance BBB breakthrough [43]. The blockade of MCAM/CD146 prevents infiltration of Th17 cells through the choroid plexus [44]. This remark draws the attention to this adhesion molecule that, when expressed on lymphocytes, increases significantly the expression of GM-CSF and granzyme B on these cells (see the below exhaustive presentation of the role of GM-CSF and granzyme B in Th17 cell–related BBB disruption). MCAM is an adhesion molecule expressed on Th17 lymphocytes and endothelial cells, which has the very important characteristic of being able to interact with itself. Larochelle et al. confirmed that one means of Th17 lymphocytes to traffic through the BBB is the MCAM–MCAM interaction from both BBB endothelial and proinflammatory cells. MCAM seems to be the first adhesion molecule expressed both on T lymphocytes and endothelial cells that stimulates leucocytes homing and diapedesis through a self-interaction [43].

Via the choroid plexus, Th17 cells can traffic into the CNS and infiltrate across the BBB using the CCR6-CCL20 axis. All Th17 cells express the chemokine receptor CCR6, together with CD161 that permits Th17 cells to cross the BBB, as endothelial cells at the level of the choroid plexus are abundant in CCL20. The findings of Reboldi et al. suggest that the immune surveillance of the CNS is performed through the CCR6-CCL20 axis in the choroid plexus [44,45]. Th17 cells produce high levels of CCL20 (the ligand of CCR6), with possible self-maintained feedback. The human Th17 cells that have on their surface the CCR6 receptor secrete 100 times more IL-17 than other cells [46]. Both Liston et al. and Villares et al. demonstrated in EAE murine models that the inhibition of CCR6 decreases the severity of the disease [47,48]. Additionally, Th17 clones exhibit higher basal levels of activation markers such as human leukocyte antigen (HLA)-DR, CD2, CD5, CD69 and CD28-related family compared with Th1 clones [21].

Interactions between Th17 and BBB are possible through IL-17 because high levels of this cytokine induce the release of chemokines CXCL1 and CCL2 on endothelial cells. CXCL1 attracts neutrophils in the early phase of RRMS. IL-17 increases the half-life of CXCL1 by stabilizing its mRNA. Th17 cells induce CXCL1 production via IL-17, resulting in the stimulation of neutrophil penetration in the CNS. CCL2 has a role in the adhesion and traffic of leukocytes through the BBB, resulting in a BBB breakthrough in a positive feedback loop. Some experiments have shown that, during the migration of Th17 through brain

endothelium, the expression of vascular cell adhesion molecule 1 (VCAM-1) is increased [46]. (Fig. 1)

Prolonged interactions between myelin oligodendrocyte glycoprotein (MOG)-specific Th17 cells and neurons were found by Siffrin et al. in EAE brainstem demyelinating lesions, most frequently during exacerbations. This interaction was followed by considerable axonal loss. The Th17 cells induced fluctuations in the neuronal intracellular Ca^{2+} concentration, marking the initial neuronal damage. This finding demonstrated that Th17 cells have a central role in neuronal loss, not only in demyelination [31,45,49].

2.2. Functional and secretory particularities of Th17 cells that enhance entry into the CNS and allow for intercellular communication

Th17 cells communicate with the endothelial cells of the BBB through a network of cell–cell extracellular signals [17]. The signalling molecules identified and studied, mainly on EAE models, are cytokines and chemokines. The endothelial cells secrete cytokines such as CCL2/MCP1, CCL5/RANTES and CCL19, and their respective receptors are found on the Th17 surface. Correspondingly, Th17 cells secrete cytokines such as IL-17, IL-22 and IL-26, which stimulate their receptors on the endothelia of the BBB [50,51].

Th17 cells interact with neutrophils inside the CNS, resulting in their recruitment and activation through an IL-17-dependent pathway using the induction of chemoattractant expression for neutrophils (CXCL1 and CXCL2) and an IL-17-independent pathway, based on Th17 cell production of TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF, see description below) and IFN-γ [52].

Th17 cells also have an indirect role in BBB breakthrough through secreted cytokines, which are more potent activators of proinflammatory cytokines compared to Th1 cells. Thus, Th17 cells secrete numerous cytokines, mainly with proinflammatory effects: IL-17A, IL-17F, IL-6, IL-9, IL-21, IL-22, IL-23, IL-26 and TNF-α [9]. Among these, the capacity of IL-22 and IL-17 to adjust the migration of lymphocytes across the endothelial cells of the human BBB was experimentally studied by Kebir et al., who showed an increased capacity of these cytokines to stimulate the migration of CD4+ lymphocytes. This effect was likely induced by the up-regulation of Th17 cells by CCL2-secreting endothelial cells [33].

IL-17A has the capacity to damage the integrity of the BBB during EAE development. The breakdown of the BBB is the most important function of IL-17 in RRMS pathogenesis. The presence of IL-17 in the CNS amplifies the activation of matrix metalloproteinase-3 (MMP-3) that carries neutrophils to the inflammation sites, and stimulates the secretion of proteases and gelatinases that also participate in BBB disruption and recruitment of macrophages and neutrophils. The process can also occur in reverse, because BBB disruption determines further activation of proteases that generate sustained damage. The final effect is concentrated on sustained damage to both the myelin sheath and axons [8,21].

The plausible encephalitogenic effectiveness of Th17 cells is by producing numerous mediators. Among them is granzyme B, a serine cytolytic protease secreted together with perforins that determine apoptosis in targeted cells. Kebir et al. showed that in MS patients, cytolytic molecules might be expressed on Th17 cells, and therefore analysed these cells for the expression of granzyme A and B and perforin. Considerable cytolytic activity of granzyme B^+ Th17 cells was found, compared to inactivated T lymphocytes [32]. Ganor et al. described a mechanism by which extracellular granzyme B targets the glutamate receptor Glu3 on the surface of neurons, initiating their destruction [53]. The disruption of BBB tight junctions by Th17 cells is more efficient due to the expression of high levels of the cytolytic enzyme granzyme B, stimulating the recruitment of more CD4+ lymphocytes from the periphery into the CNS. Granzyme B overproduction is a component of the Th17 pathogenic signature in MS [19].

IL-17A stimulates the production of reactive oxygen species (ROS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, resulting in important oxidative stress. Huppert et al. described that, secondary to oxidative stress, endothelial cells contract (secondary to the increased quantity of the phosphorylated myosin light chain that together with the actin cytoskeleton produces contraction) and cause the down-regulation of occludin (a tight junction molecule). As a result, the BBB disrupts. In the same study, the activation of IL-17A by the means of IL-17A endothelial receptor is followed by an increase in the production of ROS, therefore, preventing BBB disruption involved either IL-17A neutralising antibodies or blocking ROS formation [2,54].

One important finding is the abundance of IL-17 T cells in perivascular spaces in acute and chronic active MS lesions, where they account for the majority of CD3+ cells. Inside the CNS, IL-17 is produced by numerous infiltrating T cells (CD4+ and CD8+) and also by activated astrocytes. IL-17 triggers a positive feedback that attracts supplementary proinflammatory cells such as Th1 and Th17 cells [55].

In the CSF of MS patients, numerous chemokines are elevated. Human chemokines, such as CXCL8 together with CXCL1 and CXCL2 (from EAE murine models), form the ELR^+ CXC chemokines, a group of chemokines with a role in the pathophysiology of EAE/MS demyelination and an important contribution to BBB breakdown. Carlson et al. found that IL-17 has a major function in inducing the production of ELR^+ CXC chemokines in the CNS. These authors promoted the possibility that ELR^+ CXC chemokine production in EAE is amplified by IL-17 as the disease progresses [56].

IL-1β, a cytokine produced by Th17 cells, can enhance BBB breakthrough and recruit neutrophils in the brain. Furthermore, Ferrari et al. found that IL-1β activates astrocytes and microglia, which further stimulates the demyelination process [57] (Fig. 2).

GM-CSF is a growth factor, a cytokine that has a proinflammatory role, involved both in Th1 and Th17 and other cell-mediated immune responses. While it's effects were mainly attributed to the Th17 lineage, Noster et al. reported that this cytokine is strongly dependant of the Th1 cells, while the Th17 cells seem to constraint the development of GM-CSF [58]. It is produced mainly by T cells, in response to IL-23 and IL-1β. GM-CSF has a remarkable role in neurotoxicity as it activates dendritic cells, microglia and macrophages (which secrete IL-23 and IL-6). Some authors consider GM-CSF the 'secret weapon' in the Th17 arsenal. GM-CSF has important encephalitogenic properties, enhanced by the presence of CCR6+ that permits the breakage into the CNS at the level of BBB. Novel potential therapeutic targets have been described both by Restorick et al. and Galli et al. Thus, they suggest attacking the CCR6+ Th1 pathway and/or C-X-C chemokine receptor type 4 pathway, in order to diminish the effects that GM-CSF carry upon the BBB. [59,60] The data published by El-Behi et al. showed that GM-CSF induces the secretion of IL-23, consecutively inducing secretion of GM-CSF in Th17 cells. The authors concluded that a positive feedback loop exists: IL-23 induces the production of GM-CSF by Th17 cells, and GM-CSF secreted by Th17 cells stimulates the production of IL-23 by antigen-presenting cells. In the case of autoimmune inflammation in MS, a pathogenic axis GM-CSF/IL-23 with the main actor Th17 cell might be stipulated [61]. Indeed, EAE studies have shown that GM-CSF is crucial for determining Th17 cell–induced encephalitogenicity. The effect is so powerful that it undoubtedly indirectly influences the BBB disruption process. T cells that produce GM-CSF are likely a distinct subset of cells, called Th-GM, but this matter is still debated [19].

A very important approach with evidence both in vivo and in vitro is that in MS, the Th17 population has a different mechanism of self-sustaining, leading to an immune attack mediated by these cells that persists both in the periphery and beyond the BBB. This indicates that, in MS patients, Th17 cells can sustain the disease [19].

The disease-modifying drugs used in MS treatment have complex mechanisms of action, with the purpose of attenuating the effects of Th17 cells [2,10,38]. Hopefully, further studies on the effect of Th17 cells in patients with early and drug-naïve MS will contribute to diminishing the effect of these cells in the immunopathogenic attack.

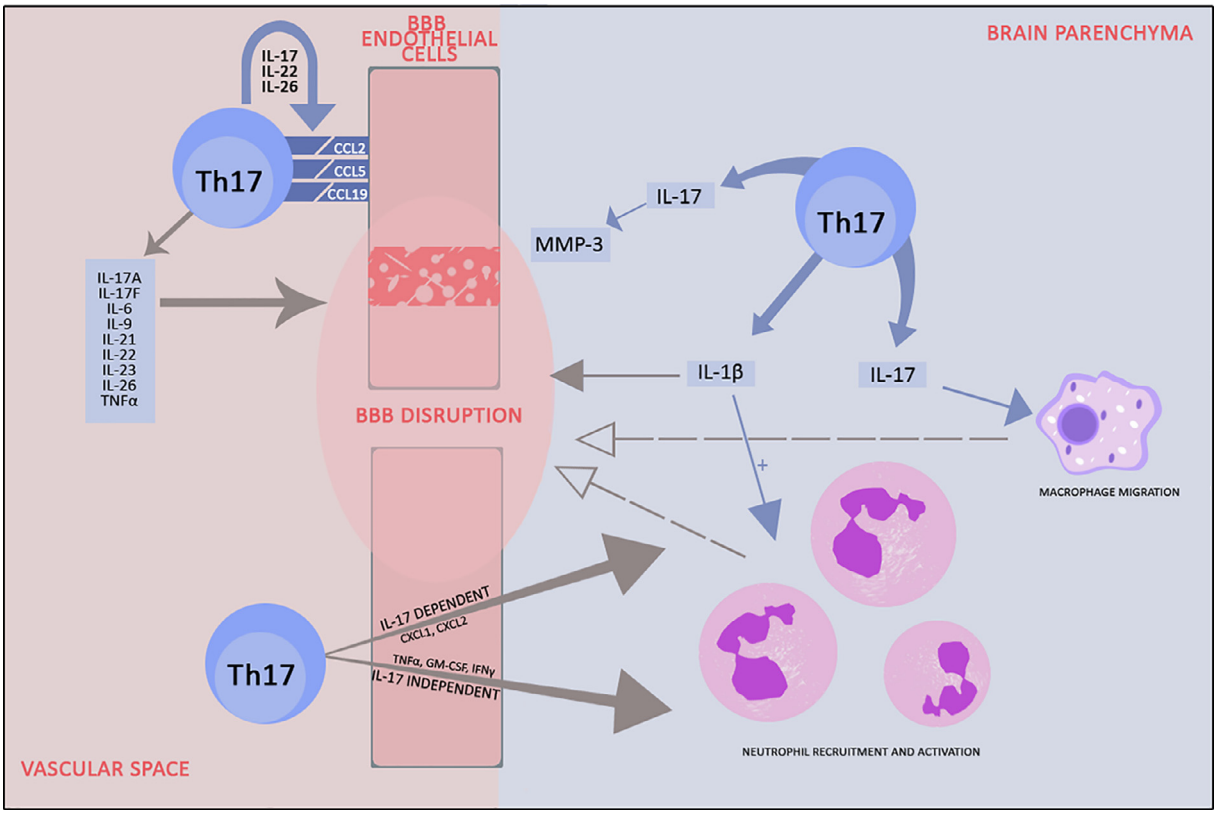

Fig. 2. The effectors of BBB disruption and Th17 involvement.

3. Conclusion

The literature underlines that Th17 cells are implicated in early MS pathology. Their high pathogenic potential in MS/EAE has been found in both clinical and experimental trials, notably in BBB breakthrough. Numerous phases need to occur for Th17 to penetrate the BBB, including IL-23 stimulation of Th17, the production of GM-CSF, high expression of receptors and chemokines on BBB/Th17 membranes, and the presence of mediators such as granzyme B. The BBB in the region of the choroid plexus is the main gate of Th17 entry into the CNS due to the CCR6-CCL20 axis. Numerous morphological aspects of Th17 cells that allow intercellular contacts with BBB endothelial cells, and the functional or secretory particularities of Th17 cells that allow intercellular communication, contribute to enhanced Th17 entry into the CNS. The most interesting interaction is the MCAM–MCAM adhesion molecule that contributes to CNS homing by Th17 cells. After a certain point, this process is self-maintained. Once reaching the CNS, Th17 cells have a neurotoxic effect. Th cell migration through the BBB is a very complex process that is partially self-sustained by the secretion of IL-17. Th17 cells might represent an essential link joining the inflammatory and neurodegenerative aspects of MS.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] A. Willing, J. Jäger, S. Reinhardt, N. Kursawe, M.A. Friese, Production of IL-17 by MAIT cells is increased in multiple sclerosis and is associated with IL-7 receptor expression, J. Immunol. 200 (3) (2018) 974–982.
[2] A. Rostami, B. Cilic, Role of Th17 cells in the pathogenesis of CNS inflammatory demyelination, J. Neurol Sci. 333 (1–2) (2013) 76–87.
[3] A. Jäger, V. Dardalhon, R.A. Sobel, E. Bettelli, V.K. Kuchroo, Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes, J. Immunol. 183 (11) (2009) 7169–7177.
[4] J.I. Spencer, J.S. Bell, G.C. DeLuca, Vascular pathology in multiple sclerosis: reframing pathogenesis around the blood-brain barrier, J. Neurol. Neurosurg. Psychiatry 89 (1) (2018) 42–52.
[5] R.M. Ransohoff, B. Engelhardt, The anatomical and cellular basis of immune surveillance in the central nervous system, Nat. Rev. Immunol. 12 (5) (2012) 623–635.
[6] G.G. Ortiz, F.P. Pacheco-Moisés, M.A. Macias-Islas, L.J. Flores-Alvarado, M.A. Mireles-Ramírez, E.D. González-Renovato, et al., Role of blood-brain barrier in multiple sclerosis, Arch. Med. Res. 45 (8) (2014) 687–697.
[7] L.E. Harrington, R.D. Hatton, P.R. Mangan, H. Turner, T.L. Murphy, K.M. Murphy, et al., Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from theT helper type 1 and 2 lineages, Nat. Immunol. 6 (11) (2005) 1123–1132.
[8] R. Balasa, C. Bianca, V. Septimiu, S. Iunius, H. Adina, A. Sebastian, et al., The matrix metalloproteinases panel in multiple sclerosis patients treated with natalizumab: a possible answer to natalizumab non-responders, CNS Neurol Disord DrugTargets. 17 (6) (2018) 464–472.
[9] F. Jadidi-Niaragh, A. Mirshafiey, Th17 cell, the new player of neuroinflammatory process inmultiple sclerosis, Scand J Immunol. 74 (1) (2011) 1–13.
[10] R. Balasa, S. Maier, S. Voidazan, A. Hutanu, Z. Bajko, A. Motataianu, et al., Assessment of interleukin-17A, interleukin-10 and transforming growth factor-beta1 serum titers in relapsing remitting multiple sclerosis patients treated with avonex, possible biomarkers for treatment response, CNS Neurol. Disord. Drug Targets 16 (1) (2017) 93–101.
[11] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, et al., Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells, Nature 441 (7090) (2006) 235–238.
[12] M. Veldhoen, R.J. Hocking, R.A. Flavell, B. Stockinger, Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease, Nat. Immunol. 7 (2006) 1151–1156.
[13] L. Zhou, J.E. Lopes, M.M. Chong, I.I. Ivanov, R. Min, G.D. Victora, et al., TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function, Nature 453 (2008) 236–240.
[14] C. Dong, Differentiation and function of pro-inflammatory Th17 cells, Microbes Infect. 11 (5) (2009) 548–588.
[15] L. Durelli, L. Conti, M. Clerico, D. Boselli, G. Contessa, P. Ripellino, et al., T-helper

17 cells expand in multiple sclerosis and are inhibited by interferon-beta, Ann. Neurol. 65 (5) (2009) 499–509.
[16] N. Chihara, Dysregulated T cells in multiple sclerosis, Clin. Exp. Neuroimmunol. 9 (2018) 20–29.
[17] M. Pelletier, L. Maggi, A. Micheletti, E. Lazzeri, N. Tamassia, C. Costantini, et al., Evidence for a crosstalk between human neutrophils and Th17 cells, Blood 115 (2) (2009) 335–343.
[18] Y. Yang, J. Weiner, Y. Liu, A.J. Smith, D.J. Huss, R. Winger, et al., T-bet is essential for encephalitogenicity of both Th1 and Th17 cells, J. Exp. Med. 206 (7) (2009) 1549–1564.
[19] G.R. Dos Passos, D.K. Sato, J. Becker, K. Fujihara, Th17 cells pathways in multiple sclerosis and neuromyelitis optica spectrum disorders: pathophysiological and therapeutic implications, Mediators Inflamm. 2016 (2016) 5314541, https://doi.org/10.1155/2016/5314541.
[20] H. Kebir, I. Ifergan, J.I. Alvarez, M. Bernard, J. Poirier, N. Arbour, et al., Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis, Ann. Neurol. 66 (3) (2009) 390–402.
[21] V. Brucklacher-Waldert, K. Stuerner, M. Kolster, J. Wolthausen, E. Tolosa, Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis, Brain 132 (Pt 12) (2009) 3329–3341.
[22] M. Kostic, I. Stojanovic, G. Marjanovic, N. Zivkovic, A. Cvetanovic, Deleterious versus protective autoimmunity in multiple sclerosis, Cell Immunol. 296 (2) (2015) 122–132.
[23] G. Arellano, E. Acuña, L.I. Reyes, P.A. Ottum, P.D. Sarno, L. Villarroel, et al., Th1 and Th17 cells and associated cytokines discriminate among clinically isolated syndrome and multiple sclerosis phenotypes, Front. Immunol. 8 (2017) 753.
[24] J.R. Christensen, L. Börnsen, R. Ratzer, F. Piehl, M. Khademi, T. Olsson, et al., Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression, PLoS One 8 (3) (2013) e57820.
[25] N. Pikor, J.L. Astarita, L. Summers-Deluca, G. Galicia, J. Qu, L.A. Ward, et al., Integration of Th-17 and lymphotoxins signals meningeal-resident stromal cell remodeling to propagate neuroinflammation, Immunity 43 (6) (2015) 1160–1173.
[26] I. Ifergan, H. Kebir, M. Bernard, K. Wosik, A. Dodelet-Devillers, R. Cayrol, et al., The bloodbrain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells, Brain 131 (Pt 3) (2008) 785–799.
[27] L.J. Edwards, B. Sharrack, A. Ismail, H. Tumani, C.S. Constantinescu, Central inflammation versus peripheral regulation in multiple sclerosis, J. Neurol. 258 (2011) 1518–1527.
[28] R.A. O'Connor, C.T. Prendergast, C.A. Sabatos, C.W. Lau, M.D. Leech, D.C. Wraith, et al., Cutting edge: Th1 cells facilitate the entry of Th17 cells to the central nervous system during experimental autoimmune encephalomyelitis, J. Immunol. 181 (6) (2008) 3750–3754.
[29] M. Pashenkov, Y.M. Huang, V. Kostulas, M. Haglund, M.M. Söderström, H. Link, Two subsets of dendritic cells are present in human cerebrospinal fluid, Brain 124 (Pt 3) (2001) 480–492.
[30] R. Liblau, D. Gonzales-Dunia, H. Wiendl, F. Zipp, Neurons as targets for T cells in the nervous system, Trends Neurosci. 36 (6) (2013) 315–324.
[31] I. Cosorich, G. Dalla-Costa, C. Sorini, R. Ferrarese, M.J. Messina, J. Dolpady, et al., High frequency of intestinal TH17 cells correlates with microbiota alterations and disease activity in multiple sclerosis, Sci. Adv. 3 (7) (2017) e1700492.
[32] H. Kebir, K. Kreymborg, I. Ifergan, A. Dodelet-Devillers, R. Cayrol, M. Bernard, et al., Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation, Nat. Med. 13 (10) (2007) 1173–1175.
[33] F. Tahmasebinia, A. Pourgholaminejad, The role of Th17 cells in auto-inflammatory neurological disorders, Prog. Neuropsychopharmacol. Biol. Psychiatry 79 (Pt B) (2017) 408–416.
[34] M.W. Teng, E.P. Bowman, J.J. McElwee, M.J. Smyth, J.L. Casanova, A.M. Copper, et al., IL-12 and IL-23 cytokines: from discovery to targeted therapies for imune-mediated inflammatory diseases, Nat. Med. 21 (7) (2015) 719–729.
[35] A. Mehdizadeh, V. Shaygannejad, M. Amidfar, S.J. Hasheminia, M.M. Ghasroldasht, Increased mRNA expression of IL-23 in the peripheral blood of patients with multiple sclerosis, J. Immunol. Sci. 2 (4) (2018) 1–4.
[36] B.M. Segal, C.S. Constantinescu, A. Raychaudhuri, L. Kim, R. Fidelus-Gort, L.H. Kasper, Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study, Lancet Neurol. 7 (2008) 796–804.
[37] B. van Oosten, F. Barkhof, L. Truyen, J. Boringa, F. Bertelsmann, B. von Blomberg, et al., Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2, Neurology 47 (6) (1996) 1531–1534.
[38] E.E. Longbrake, M.K. Racke, Why did IL-12/IL-23 antibody therapy fail in multiple sclerosis? Expert Rev. Neurother. 9 (3) (2009) 319–321.
[39] R. Bălaşa, A. Huţanu, Z. Bajko, C. Feier, I. Pascu, Does the serum IL-17 titer influence the efficacy of interferon-β treatment in multiple sclerosis patients? Rev. Romana Med. Lab. 19 (4) (2011) 381–389.
[40] M.J. Butcher, J. Zhu, Plasticity and functionality of Th17 subsets in EAE, J. Immunol. 200 (1) (2018) 163.20.
[41] S.E. Lutz, J.R. Smith, D.H. Kim, C.V.L. Olson, K. Ellefsen, J.M. Bates, et al., Caveolin1 is required for Th1 cell infiltration, but not tight junction remodeling, at the blood-brain barrier in autoimmune neuroinflammation, Cell Rep. 21 (8) (2017) 2104–2117.
[42] A.S. Haqqani, D.B. Stanimirovic, Intercellular Interactomics of human brain endothelial cells and Th17 lymphocytes: a novel strategy for identifying therapeutic targets of CNS inflammation, Cardiovasc. Psychiatry Neurol. 2011 (2011) 175364.
[43] C. Larochelle, R. Cayrol, H. Kebir, J.I. Alvarez, M.A. Lécuyer, I. Ifergan, et al., Melanoma cell adhesion molecule identifies encephalitogenic T lymphocytes and promotes their recruitement to the central nervous system, Brain 135 (Pt 10) (2012) 2906–2924.
[44] J. Breuer, E. Korpos, M.J. Hannocks, T. Schneider-Hohendorf, J. Song, L. Zondler, et al., Blockade of MCAM/CD146 impedes CNS infiltration of T cells over the choroid plexus, J. Neuroinflamm. 15 (1) (2018) 236.
[45] A. Reboldi, C. Coisne, D. Baumjohann, F. Benvenuto, D. Bottinelli, S. Lira, et al., C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE, Nat. Immunol. 10 (5) (2009) 514–523.
[46] D.W. Wojkowska, P. Szpakowski, A. Glabinski, Interleukin 17A promotes lymphocytes adhesion and induces CCL2 and CXCL1 release from brain endothelial cells, Int. J. Mol. Sci. 18 (5) (2017) 1000.
[47] A. Liston, R.E. Kohler, S. Townley, S. Haylock-Jacobs, I. Comerford, A.C. Caon, et al., Inhibition of CCR6 function reduces the severity of experimental autoimmune encephalomyelitis via effects on the priming phase of the immune response, J. Immunol. 182 (5) (2009) 3121–3130.
[48] R. Villares, V. Cadenas, M. Lozano, L. Almonacid, A. Zaballos, C. Martínez-A, et al., CCR6 regulates EAE pathogenesis by controlling regulatory CD4+ T-cell recruitment to target tissues, Eur. J. Immunol. 39 (6) (2009) 1671–1681.
[49] V. Siffrin, H. Radbruch, R. Glumm, R. Niesner, M. Paterka, J. Herz, et al., In vivo imaging of partially reversible th17 cell-induced neuronal dysfunction in the course of encephalomyelitis, Immunity 33 (3) (2010) 424–436.
[50] Z. Shulman, S.J. Cohen, B. Roediger, V. Kalchenko, R. Jain, V. Grabovsky, et al., Transendothelial migration of lymphocytes mediated by intraendothelial vesicle stores rather than by extracellular chemokine depots, Nat. Immunol. 13 (1) (2011) 67–76.
[51] W. Cheng, G. Chen, Chemokines and chemokine receptors in multiple sclerosis, Mediators Inflamm. 2014 (2014) 659206.
[52] D.W. Wojkowska, P. Szpakowski, D. Ksiazek-Winiarek, M. Leszczynski, A. Glabinski, Interactions between neutrophils, Th17 cells, and chemokines during the initiation of experimental model of multiple sclerosis, Mediators Inflamm. 2014 (2014) 590409.
[53] Y. Ganor, V.I. Teichberg, M. Levite, TCR activation eliminates glutamate receptor GluR3 from the cell surface of normal human T cells, via an autocrine/paracrine granzyme B-mediated proteolytic cleavage, J. Immunol. 178 (2) (2007) 683–692.
[54] J. Huppert, D. Closhen, A. Croxford, R. White, P. Kulig, E. Pietrowski, et al., Cellular mechanisms of IL- 17-induced blood-brain barrier disruption, FASEB J. 24 (4) (2010) 1023–1034.
[55] J.S. Tzartos, M.A. Friese, M.J. Craner, J. Palace, J. Newcombe, M.M. Esiri, et al., Interleukin- 17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis, Am. J. Pathol. 172 (1) (2008) 146–155.
[56] T. Carlson, M. Kroenke, P. Rao, T.E. Lane, B. Segal, The Th17-ELR+ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease, J. Exp. Med. 205 (4) (2008) 811–823.
[57] C.C. Ferrari, A.M. Depino, F. Prada, N. Muraro, S. Campbell, O. Podhajcer, et al., Reversible demyelination, blood-brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain, Am. J. Pathol. 165 (5) (2004) 1827–1837.
[58] R. Noster, R. Riedel, M.-F. Mashreghi, H. Radbruch, L. Harms, C. Haftmann, H.-D. Chang, A. Radbruch, C.E. Zielinski, IL-17 and GM-CSF expression are antagonistically regulated by human T helper cells, Sci. Transl. Med. 6 (241) (2014) 241ra80, https://doi.org/10.1126/scitranslmed.3008706.
[59] S.M. Restorick, L. Durant, S. Kalra, G. Hassan-Smith, E. Rathbone, M.R. Douglas, et al., CCR6+ Th cells in the cerebrospinal fluid of persons with multiple sclerosis are dominated by pathogenic non-classic Th1 cells and GM-CSF-only-secreting Th cells, Brain Behav. Immun. 64 (2017) 71–79.
[60] E. Galli, F.J. Hartmann, B. Schreiner, F. Ingelfinger, E. Arvaniti, M. Diebold, et al., GM-CSF and CXCR4 define a T helper cell signature in multiple sclerosis, Nat. Med. 25 (8) (2019) 1290–1300.
[61] M. El-Behi, B. Ciric, H. Dai, Y. Yan, M. Cullimore, F. Safavi, et al., The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF, Nat. Immunol. 12 (6) (2011) 568–575.