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Inflammation in multiple sclerosis

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Stefanie Haase and Ralf A. Linker

***Abstract*:** Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that is characterised pathologically by demyelination, gliosis, neuro-axonal damage and inflammation. Despite intense research, the underlying pathomechanisms driving inflammatory demyelination in MS still remain incompletely understood. It is thought to be caused by an autoimmune response towards CNS self-antigens in genetically susceptible individuals, assuming autoreactive T cells as disease-initiating immune cells. Yet, B cells were recognized as crucial immune cells in disease pathology, including antibody-dependent and independent effects. Moreover, myeloid cells are important contributors to MS pathology, and it is becoming increasingly evident that different cell types act in concert during MS immunopathology. This is supported by the finding that the beneficial effects of actual existing disease-modifying therapies cannot be attributed to one single immune cell-type, but rather involve immunological cooperation. The current strategy of MS therapies thus aims to shift the immune cell repertoire from a pro-inflammatory towards an anti-inflammatory phenotype, involving regulatory T and B cells and anti-inflammatory macrophages. Although no existing therapy actually exists that directly induces an enhanced regulatory immune cell pool, numerous studies identified potential net effects on these cell types. This review gives a conceptual overview on T cells, B cells and myeloid cells in the immunopathology of relapsing-remitting MS and discusses potential contributions of actual disease-modifying therapies on these immune cell phenotypes.

Keywords: B cells, immune network, immune regulation, inflammation, myeloid cells, relapsing-remitting multiple sclerosis, T cells

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) mainly affecting young adults. Despite intense research, the pathology of MS still remains incompletely understood. Traditionally, MS is considered as autoimmune disorder characterised by the infiltration of peripheral autoreactive immune cells into the CNS accompanied by the activation of innate immune mechanisms.[1] The most widely accepted working model on the pathogenesis of MS starts with the escape of autoreactive T cells from clonal deletion in the thymus and dysfunctional regulatory mechanism in the periphery, before (re-)activation of these cells in lymphoid tissues by as yet unknown triggers. Finally, these autoreactive cells cross the blood–brain–barrier into the CNS, facilitating the damage of myelin and oligodendrocytes, ultimately resulting in gliosis, neuro-axonal damage and inflammation.[2] The later stages of the disease are accompanied by compartmentalised inflammation, contributing to continuous inflammatory and degenerative changes in the CNS, hence driving disease progression.[3,4] The autoimmune character of MS may be disputed by the Koch's postulate that the diagnosis of an autoimmune disease requires the definitive identification of the autoantigen. Yet, the specific targets of autoreactive immune cells during MS are still lacking, and some studies only indicate myelin antigens as prominent candidates.[5] Moreover, based on the observation that newly forming MS lesions spare inflammatory immune cells proposed an alternative idea of disease pathology, challenging the traditional concept of MS being an autoimmune disorder.[6,7] The so-called 'inside-out hypothesis'

Correspondence to:
Stefanie Haase
Neuroimmunologie, Klinik und Poliklinik für Neurologie, Universitätsklinik Regensburg, Franz-Josef-Strauss Allee, Regensburg, 93053, Germany
stefanie.haase@ukr.de

Ralf A. Linker
Department of Neurology, University Hospital Regensburg, Regensburg, Germany

claims that the initial loss of oligodendrocytes and myelin in the absence of peripheral inflammation leads to the release of CNS antigens. This triggers the development of autoimmune reactions against myelin components, ultimately resulting in neuroinflammation.[8] Yet, the etiology of MS is unknown, and factors that either induce a primary inflammatory disease onset or a primary oligodendroglial pathology followed by inflammation are still not identified. MS etiology is multifactorial and seems to involve complex interactions of genetic and environmental factors. Particularly the prevalence of the MS-risk allele HLA-DR15 and many single nucleotide polymorphisms of genes that are important for the differentiation or effector function of pathogenic T cells strengthens the concept of an immune-mediated disease pathology.[9,10] Moreover, extensive studies provide compelling evidence for a role of environmental factors in MS. The most consistent risk factors are childhood obesity, cigarette smoking and the infection with Epstein-Barr virus (EBV),[11–13] whereas increased vitamin D levels and sunlight exposure are considered as beneficial factors in MS.[14] Interestingly, the beneficial effects of vitamin D and its active metabolite are attributed to their immunomodulatory capacities affecting innate and adaptive immune cells.[15,16] Moreover, EBV is suggested to cause MS in genetically susceptible individuals by infecting autoreactive B cells,[17] linking environmental and genetic factors on the one hand, and highlighting the importance of the immune system in MS pathology on the other. Adding to this, there are various disease-modifying therapies available that significantly reduce relapse rates and the development of new brain lesions in relapsing-remitting MS (RRMS) patients, mainly by modulating peripheral immune cell activation or CNS infiltration. This immunomodulatory capacity of available MS drugs strengthens the concept that MS is an autoimmune disease where the initial event takes place outside the CNS, especially in the relapsing-remitting disease course. The most important immune cells targeted by disease-modifying therapies are T cells, B cells and, as a side-effect, also myeloid cells. This review thus focusses on the role of T cells, B cells and myeloid cells in the immunopathology of RRMS.

T cells in the immunopathology of MS

Considerable evidence from studies of multiple MS patients and the most commonly used animal model, experimental autoimmune encephalomyelitis (EAE),[1] has contributed to the common view that MS is a T cell-mediated disease. This is in part due to the association of MS risk with variants in genes that are important for either the differentiation of pathogenic T cell subsets or the modulation of their effector function. Amongst the identified genes are, for instance, the interleukin (IL-) 2 and IL-7 receptor subunits IL-2RA and IL-7RA.[9] In addition, variations in MHCII alleles provide a strong susceptibility to MS, possibly reflecting the presentation of specific CNS autoantigens to autoreactive, MHCII restricted CD4+ T cells.[10] Myelin protein-derived antigens, such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), have been hypothesized to be the main autoreactive targets. Yet, these antigens were shown to be recognized by circulating CD4+ T cells in MS patients but also in healthy individuals, and there is conflicting evidence regarding potential differences in the frequency and avidity of these cells between the two groups.[18,19] It has been shown that healthy individuals are likely to maintain regulatory mechanisms that keep these autoreactive T cells under control, a function that seems to be impaired in MS patients.[20]

The invasion of autoreactive CD4+ T cells into the CNS is considered to be the initial step of MS pathology, initiating inflammatory reactions and consequently neurodegenerative processes. Indeed, CD4+ T cells are found within CNS lesions and in the cerebrospinal fluid (CSF) of patients with MS.[21] Classically, MS was thought to be a T helper (Th) 1-mediated autoimmune disease, while IL-4 producing Th2 cells were considered to exert a modulatory function with a protective role. This observation was supported by the finding of increased numbers of Th1 cells and elevated concentrations of the signature cytokine interferon gamma (IFN-γ) in CNS lesions of MS patients.[21,22] Numerous studies in EAE,[23–26] together with the finding that the administration of IFN-γ to MS patients exacerbated the disease,[27] supported an important role of IFN-γ and Th1 cells in both EAE and MS pathogenesis. Moreover, Th1 cells express high levels of the α4β1 integrin VLA-4 that enables their infiltration into the CNS *via* VCAM-1 interaction.[28] Blocking VLA-4 with the anti-α4 antibody natalizumab is a highly effective therapy in early MS, indicative of a pivotal role for Th1 cells in RRMS.[29] However, further

observations in mice revealed contradictory data, weakening the paradigm of Th1 cells in MS.[30–33] Moreover, with the identification of IL-23 in EAE, IL-17-producing Th17 cells were also added to the list of factors potentially involved in disease pathogenesis.[34,35] During past years, several studies in MS patients have provided evidence for a pivotal role of Th17 cells in disease pathogenesis. RRMS patients and patients with active disease show higher frequencies of IL-17-producing Th17 cells in the blood and active MS lesions,[36–38] potentially correlating with disease progression.[39] Furthermore, Th17 cells from MS patients show a highly pathogenic phenotype, with higher expression of costimulatory molecules and higher resistance to suppression.[40] Th17 cells may also gain a Th1-type phenotype, co-expressing IFN-γ and IL-17.[41] These Th1-like Th17 cells were found in CNS tissue from MS patients as well as peripheral blood and CSF of RRMS patients during relapse.[42,43] Yet, the exact pathological role of these cells still needs to be identified. Numerous research studies have addressed the actual cause of increased autoreactive Th1 and Th17 cells in patients with MS. It was first suggested that MS pathology involves an abnormal balance between CNS-reactive effector T cells and regulatory T cells (Treg). The critical involvement of these Treg cells in MS was initially indicated by studies in EAE, showing that the adoptive transfer of Treg cells was sufficient to ameliorate the disease.[44] In contrast, the depletion of Treg cells worsened EAE symptoms. However, MOG-specific Treg cells isolated from mice at different time points during EAE did not suppress MOG-specific effector T cells, either *in vivo* or *in vitro*, indicating an impaired suppressive capacity.[45] Interestingly, this has also been demonstrated for MS patients, showing that Treg frequencies do not differ compared with healthy controls, whereas their suppressive capacity was shown to be impaired.[20,46] The suppression of an augmented differentiation of pathogenic cells by Treg cells can either be induced *via* cell–cell contact mechanisms, modulation of antigen-presenting cells or *via* the secretion of anti-inflammatory cytokines, including IL-10.[47] Yet, Treg cells from MS patients were shown to secrete less IL-10 but higher amounts of IFN-γ.[48] This conversion to IFN-γ-producing Th1-like Treg cells might be one possible mechanism for the functional failure of Treg cells in MS patients. This change in phenotype and function can be induced by the pro-inflammatory cytokine IL-12, which is up-regulated in MS.[49] IFN-γ-producing Treg cells are increased in the blood of MS patients compared with healthy controls.[48] Moreover, *in vitro* data displayed a decline in their suppressive activity, as blocking IFN-γ in co-culture with Treg cells derived from RRMS patients restored their suppressive capability.[48] Restoration of Treg function and a decrease of pathogenic T cells thus represents interesting targets in the therapy of MS. Indeed, some of the approved disease-modifying therapies target T cells. Yet, no drug is approved, either one that directly acts *via* Treg cell modulation or *via* specific Th1/Th17 depletion. In contrast, dietary factors have been shown to directly modulate this T cell balance during EAE and MS.[50,51] While limiting the induction of Th17 cells during EAE, the short-chain fatty acid propionic acid (PA) increases the number of functionally active Treg cells, thereby ameliorating the disease.[50] Of potential interest, supplementation of PA to therapy-naive MS patients and as an add-on to MS immunotherapy increased functionally competent Treg cells significantly. In line with this observation, MS patients receiving PA showed a reduced annual relapse rate together with reduced brain atrophy and a stabilisation of disability.[51] These data support the relevance of Treg suppressive capacity during MS pathology and reveal short-chain fatty acids as interesting targets for the treatment of MS and potentially other autoimmune diseases. Moreover, various approved disease-modifying therapies also act *via* modulation of Treg cells, although most likely indirectly. For instance, glatiramer acetate, a first-line therapeutic for RRMS, was shown to increase Treg frequencies, correlating with an increased regulatory potential.[52] Moreover, memory T cell numbers were shown to be reduced by dimethyl fumarate (DMF) treatment, whereas an increase of Treg cells was observed in the peripheral blood of RRMS patients treated with DMF.[53] In addition, IFN-β therapy may shift the balance from an inflammatory Th1 phenotype to a more anti-inflammatory phenotype, characterized by an increase of Treg cells.[54,55] Yet, the induction of Treg cells seems to be mediated *via* dendritic cells rather than *via* directly acting on CD4+ T cells, indicating the important relevance of other immune cells during the pathogenesis of MS. This is further supported by the notion that daclizumab, a monoclonal antibody targeting CD25 on activated T cells and Treg cells, is associated with an increased risk of secondary immune reactions. Although inhibiting the proliferation of activated T cells, daclizumab did not affect cells that express the low-affinity IL-2

receptor, such as natural killer cells,[56] a fact that is now considered to contribute to severe immune reactions.

While providing a basic understanding of autoimmune pathomechanisms, a CD4+ T cell-centred model might be insufficient to describe the pathogenesis of MS. CD8+ T cells make up the majority of T cells in CNS infiltrates and at the edge of CNS lesions.[57] CD8+ T cells can secrete IL-17, forming so-called Tc17 cells, which were shown to be increased in active lesions of MS patients. Interestingly, DMF treatment decreases the frequency of Tc17 cells instead of Th17 cells,[58] indicating an important role for these cells in MS pathology. Yet, studies in EAE and MS patients indicate that Tc17 cells act *via* supporting Th17 cell pathogenicity,[59] further strengthening the important role of Th17 cells in MS pathology. In summary, the actual concept of MS immunopathology suggests an imbalance of pro-inflammatory Th1, Th17 and Tc17 cells and a defective regulatory T cell pool in the periphery. This imbalance involves direct (cell–cell contact) or indirect (enhanced pro-inflammatory cytokine secretion) interaction with antigen-presenting cells, including macrophages, dendritic cells and B cells, strengthening the concept that different cell types act in concert during MS immunopathology.

B cells in the immunopathology of MS

It was long believed that MS is primarily a T-cell-mediated disease with an imbalance of pro- and anti-inflammatory cells driving CNS inflammation. Yet, recent findings also indicate an important role of B cells in disease pathology, including antibody-dependent and -independent effects. B cells were originally thought to contribute to the disease by differentiation towards antibody producing plasma cells after cell–cell contact and the resulting B cell activation. This autoantibody producing role of B cells was supported by the identification of oligoclonal bands (OCB) in the CSF of MS patients. OCB result from elevated immunoglobulin (Ig) G and IgM production by B cells differentiated towards plasma cells and represent a diagnostic hallmark in MS.[60–62] More than 90% of MS patients show IgG OCB, whereas IgM OCB are only found in 30–40% of patients, potentially correlating with disease activity and therapy response.[63–65] Within the CNS, antibody accumulation is associated with complement activation and demyelination, indicating that antibodies are directed against components of the CNS. Indeed, several studies identified numerous antibodies binding CNS structures, including MOG, MBP, neurons (neurofilament), astrocytes (KIR4.1), heat shock proteins and others.[5,66–69] However, some of these autoantibodies can also be detected in healthy individuals, some could not be reproduced and the exact target antigens for antibodies in MS still remain unknown.[70–72] Moreover, recent work identified antibodies that recognize intracellular self-proteins of cell debris, indicating that OCB may result from dead immune cells rather than representing a primary injury.[73] Although the exact pathogenic role of B-cell-associated structures in the CNS remain controversial, increased numbers of B cells forming so-called ectopic lymphoid follicle-like aggregates within the meninges were described as associated with more aggressive forms of MS.[74,75] These B-cell-rich lesions can also contain T cells and follicular dendritic cells that together contribute to increased microglial activation, and neuronal as well as oligodendroglial death in the cortex.[76] The resulting cortical demyelination is now considered as an important contributor to the pathology of progressive MS.[77,78] Interestingly, oligodendroglial and neuronal death was linked to soluble products of B cells.[79,80] Supernatants of *in vitro* stimulated B cells isolated from RRMS patients but not controls induced cell death in rat oligodendrocytes and neurons.[79,80] This effect was even present after the removal of immunoglobulins, suggesting antibody-independent effects of B cells, such as cytokine production and antigen-presentation, in MS pathology.

Normally, the development of autoreactive B cells is controlled by central and peripheral tolerance mechanisms during early B cell development, including suppression *via* Treg cells.[81] Studies in MS patients demonstrated a defective peripheral tolerance in autoreactive B cell control, involving an impaired suppressive capacity of Treg cells in MS patients.[82] This observed interaction of B cells and T cells coincides with the more recent finding that B cells affect MS disease *via* antibody-independent effects. Memory B cells can internalize, process and present different antigens *via* MHC class II molecules to antigen-specific CD4+ T cells.[83,84] T cell activation further requires the interaction with co-stimulatory molecules expressed on B cells, including CD40, CD80 and CD86. Strong interaction of these molecules with their corresponding ligands induces a highly active state in T cells.[85] Interestingly, researchers

identified a higher expression of co-stimulatory molecules on B cells of MS patients compared with healthy controls,[86] suggesting an enhanced antigen-presenting capacity in MS. Moreover, memory B cells mediate the proliferation of autoreactive T cells in a HLA-DR dependent manner, further supporting the pathogenic B cell–T cell interaction in MS.[87] Studies in the EAE model additionally indicate the importance of co-inhibitory molecules expressed on B cells. These co-inhibitory molecules can downregulate T cell responses or induce Treg cell differentiation during EAE, thereby improving the disease.[88,89] The importance of co-inhibitory molecules expressed on B cells, however, needs to be proven in humans. Recent work also identified a subclass of plasma cells with a potential regulatory function during MS independent from T cell interaction. Gut microbiota-specific IgA+ B cells were found to be enriched in the CSF and inflamed tissue of MS patients with active disease, suggesting their migration from the gut to the CNS during relapse.[90] There is some evidence that these cells may exert regulatory functions *via* local IL-10 production, as observed in an EAE model.[91]

The most evident implication of antibody-independent contributions of B cells during MS pathogenesis result from the effectiveness of anti-CD20 therapies in patients with RRMS.[92–95] The first B-cell-depleting clinical study testing rituximab – an anti-CD20 chimeric monoclonal antibody – decreased CNS inflammation and limited MS relapses.[93] Since plasma cells or plasmablasts express no or little CD20, this success has been linked to autoantibody independent effects. The efficacy of anti-CD20 therapies seems to be mediated rather by reduced antigen-presentation and cytokine regulation, thereby limiting the stimulation of pathogenic T cells or myeloid cells. CD20 is expressed on a broad range of B cells, including immature, transitional, naïve and memory B cells. These B cells secrete pro-inflammatory cytokines, such as IL-6, IFN-γ, tumour necrosis factor alpha (TNFα) and granulocyte-macrophage colony-stimulating factor (GM-CSF), but also the anti-inflammatory cytokines IL-10, IL-35 and transforming growth factor beta (TGF-β). Interestingly, stimulated B cells isolated from untreated MS patients secrete less IL-10 and higher amounts of the pro-inflammatory cytokines IL-6 and GM-CSF,[96,97] all cytokines that can induce Th1 or Th17 cell differentiation and inhibit Treg cell induction.[98] In EAE, B cell-derived IL-6 increases disease pathogenesis by promoting the activation of Th1 cells and Th17 cells, which can be inhibited by treatment with CD20-depleting therapies.[99] Recent studies identified GM-CSF-producing B cells in humans that are increased in MS patients compared with healthy controls and decreased after anti-CD20 therapy.[100] This GM-CSF production might further enhance the pro-inflammatory response of myeloid cells during MS, highlighting that B cell depleting therapies might act *via* modulations of the B cell cytokine profile and their interaction with other immune cells. Besides reduced pro-inflammatory cytokine secretion, reconstituting B cells of patients treated with anti-CD20 therapy also produce higher levels of IL-10.[96,101] In parallel, pro-inflammatory T cells and myeloid cells are decreased during the reconstitution phase, indicating a regulatory function of IL-10 secreting B cells. This property has already been demonstrated in EAE[102,103] and studies in MS patients also indicate a regulatory function of IL10-secreting B cells in humans.[104,105] Numerous studies identified an increase of these cells after treatment with disease-modifying therapies, including IFNβ, glatiramer acetate, fingolimod, rituximab and alemtuzumab.[96,101,106–109] Whether the beneficial effects of these therapies can be (in part) directly linked to IL10-producing B cells still needs to be proven. Yet, a recent study demonstrated that reappearing B cells after cessation of rituximab treatment show an immature phenotype with high expression of CD25, co-stimulatory molecules and increased pro-inflammatory cytokine secretion, indicating a highly active phenotype.[110] These data suggest that B cell reconstitution is an active process rather than a physiological regrowth of depleted B cells with a similar phenotype, demonstrating the importance of carefully monitoring anti-CD20-treated MS patients. Moreover, this study revealed a long-lasting effect of B cell depletion on T cells, indicating the importance of B cell–T cell interaction while confirming other studies showing a direct effect of rituximab and ocrelizumab on CD20-expressing CD4+ and CD8+ T cells.[111–114] CD20+ T cells represent a highly active cell population characterized by enhanced production of pro-inflammatory cytokines (TNFα, IL1β and IL-17) that were found to be higher in RRMS and primary progressive MS compared with healthy controls.[115] A recent study also identified an increased number of myelin-specific memory CD8+CD20+ T cells in MS patients that

are significantly reduced following anti-CD20 treatment.[114] These data indicate that the efficacy of CD20-depleting therapies are not related solely to antibody-independent functions of B cells but may also involve CD20+ T cell reduction, thus further strengthening the importance of T cells in the pathophysiology of MS. This concept is further supported by the finding that anti-CD20 therapies result in a significant reduction of CD4+ and CD8+ T cells, with a more pronounced effect in ocrelizumab-treated patients compared with rituximab treatment.[116] In summary, B cells affect MS pathology by antibody-dependent and, more importantly, antibody-independent effects. These antibody-independent pathomechanisms include antigen-presentation and cytokine secretion, affecting mainly T cell phenotypes. Moreover, the defective suppression of Treg cells enhances autoreactive B cells during MS and, vice versa, defective Breg cells enhance the pro-inflammatory T cell pool, strengthening the concept of B cell–T cell interaction as important immunopathological factor. Yet, B cells are not considered the sole antigen-presenting cell type relevant in MS pathology and many data additionally point towards the involvement of myeloid cells in MS.

Myeloid cells in the immunopathology of MS

In addition to the established focus on autoreactive T cells and B lymphocytes, substantial evidence additionally points towards the involvement of myeloid cells in MS pathogenesis, including monocytes, macrophages and microglia. This finding is supported by the fact that macrophages are found in high numbers in MS lesions in RRMS, while microglia are present predominantly in progressive phases of the disease.[117,118] Moreover, a recent study identified a lower magnetization transfer ratio (MTR) in white matter lesions associated with a lower density of macrophages, indicating a potential direct contribution of macrophages to tissue damage.[119] In addition, studies investigating the occurrence of monocytes secreting IL-6, IL-12, TNF-α and IL-10 revealed that pro-inflammatory monocytes secreting IL-6 and IL-12 are higher in untreated MS patients compared with healthy controls.[120] The higher percentage of IL-12 secreting monocytes was shown to correlate with disease activity and progression as measured by gadolinium-enhancing MRI and EDSS.[121] These data suggest an important contribution of pro-inflammatory monocytes to MS pathology. On the other hand, studies also identified monocyte-derived macrophages with an anti-inflammatory phenotype in MS brain, potentially suppressing neuroinflammatory processes.[122,123] These data demonstrate the heterogeneity of monocytes/myeloid cells, and add to the importance to determine the phenotype-associated functionality during MS pathogenesis rather than solely considering cell counts.

Circulating monocytes represent a heterogeneous cell population, which are divided into two main groups depending on the expression of the LPS receptor CD14 and the low-affinity FcγRIII CD16.[124] Classical monocytes are defined as CD14++ CD16− cells, whereas non-classical monocytes are defined as CD14++ CD16+ cells.[125] In addition, cells showing lower expression of CD14 but high expression of CD16 (CD14+ CD16++) are referred to as intermediate monocytes that, together with non-classical monocytes, make up around 10% of total monocytes in peripheral blood.[125] It was shown in EAE that each monocyte population has distinct functionalities in the peripheral immune system and CNS pathology during neuroinflammation. Mouse Ly6C^{hi} monocytes represent the equivalents of human CD16− classical monocytes, which are considered key players in monocyte subpopulations in MS.[125,126] Probably due to the expression of the chemokine receptor CCR2, these cells emigrate from the bone marrow towards sites of inflammation,[127] where they can differentiate towards pro-inflammatory macrophages or dendritic cells.[128] Mice lacking CCR2 are resistant to EAE induction, which was linked to missing monocyte infiltration in the CNS and reduced antigen-induced T cell activation.[129,130] In contrast, CCR2 negative but CX_3CR1^{hi} Ly6C^{low} monocytes, the counterparts of human CD16+ monocytes, may harbour a patrolling function in the peripheral immune compartment.[131,132] Ly6C^{low} cells adhere mainly to endothelial surfaces, scanning for damage or the presence of pathogens and coordinating inflammatory processes (reviewed in Guilliams *et al.*[133]). This patrolling function has also been suggested to occur at the brain microvascular endothelial interface, indicating a potential importance at the blood–brain barrier and hence during MS pathology.[134] In an *in vitro* transmigration assay, CD16+ monocytes were found to be enriched in the fraction adhering to the brain microvascular endothelium. Moreover, the CD16+ monocyte subset promoted CD4+ T cell trafficking *via* the endothelial barrier, suggesting that CD16+ monocytes

contribute to the breakdown of the blood–brain barrier by promoting T cell entry into the CNS.[134]

These data confirm former studies demonstrating that monocyte frequencies are reduced in the CSF of RRMS patients but are increased at the meninges and the inflamed parenchyma.[135,136] The importance of CD16+ monocytes during MS was further shown by the identification of increased expression of co-stimulatory markers such as CD40, CD86 and HLA-DR on CD16+ monocytes, and *in vitro* stimulation with LPS induced higher secretion of IL-6 and IL-12.[137] Moreover, CD16+ cell numbers are increased in the peripheral blood of MS patients compared with healthy controls,[138] although CD16+ monocyte frequencies seem to be influenced by disease-modifying therapies or disease duration.[134,139] In contrast to treated MS patients, treatment-naïve patients had reduced frequencies of CD16+ monocytes in the peripheral blood,[134] with therapy-naïve patients showing an early active MS phenotype compared with treated MS patients with a long disease duration. These data further add to the above-mentioned importance of determining monocyte functionality or phenotype rather than solely analysing cell counts. Moreover, circulating monocytes can differentiate towards macrophages upon entry into different tissues. Brain infiltrating monocytes/macrophages contribute to MS pathology *via* different mechanisms, which, together with the CNS resident monocytes, named microglia, may adopt a neuroinflammatory or neuroprotective role. Microglia can be found early in MS brains, forming so-called pre-active lesions that lack infiltrating leukocytes and demyelination.[140] However, these microglial clusters were also found in healthy controls, albeit in lower numbers, and later studies identified that the lack of a homeostatic microglia population coincides with lesion and disease activity.[141]

Another study showed that, in active demyelinating MS lesions, although macrophages and activated microglia displayed predominantly pro-inflammatory characteristics, the majority of these cells co-expressed the markers of pro-inflammatory and anti-inflammatory macrophages, suggesting an intermediate activation status.[142] These data indicate that the phenotype or activation state of microglia/macrophages is very diverse and contribute differentially to MS pathology. During early neuroinflammation, microglia/macrophages are suggested to conduct a beneficial function that later turns into a deleterious role with neurodegenerative contribution. This pro-inflammatory role has been linked to the antigen-presenting capacity of microglia/macrophages, which may re-activate CNS-infiltrating T cells after ingesting myelin and axonal components. This uptake promotes the expression of MHCII and co-stimulatory molecules,[143] which, together with the secretion of pro-inflammatory cytokines and neurotoxic molecules, results in neuroinflammation and demyelination. In addition, pro-inflammatory macrophages can also suppress the expansion of Treg cells, thus inhibiting anti-inflammatory or regulatory processes during MS pathology and indicating the importance of monocyte/T cell interaction.[144] However, phagocytosis of myelin debris is also essential to facilitate CNS repair and anti-inflammatory macrophages are necessary for efficient remyelination.[145–147] In addition, the secretion of anti-inflammatory cytokines and neurotrophic factors by macrophages/microglia suppresses the disease-promoting activity of astrocytes and autoreactive T cells, thereby promoting remyelination processes and tissue repair.[148–150] It is thus of high interest to shift the macrophage/monocyte pool from a pro-inflammatory towards and anti-inflammatory phenotype, suppressing neuroinflammation and promoting CNS repair.

Although no actually existing therapy directly addresses the monocyte/macrophage pool or phenotype, numerous data have revealed beneficial effects on these cell populations and further support their contribution to MS pathology. For instance, monocytes isolated from MS patients treated with glatiramer acetate, fingolimod, IFN-β or DMF show a less pro-inflammatory phenotype but enhanced anti-inflammatory characteristics.[151–157] Glatiramer acetate was shown to induce increased IL-10 and TGF-β secretion in MS patient monocytes, but a decreased production of TNFα, IL-12 and IL-1β.[151–153] Moreover, monocytes isolated from fingolimod-treated patients secreted lesser amounts of pro-inflammatory cytokines such as TNFα, IL-1β or IL-6,[154,155] and IFN-β-treated monocytes produce less IL-1β in response to LPS stimulation.[156] The first *in vitro* data with DMF demonstrated suppressed TNFα, IL-6 and IL-10 responses of human monocyte-derived macrophages and microglia to a pro-inflammatory stimulus.[157] This less pro-inflammatory phenotype upon DMF treatment could also be observed *in vivo*, since monocytes

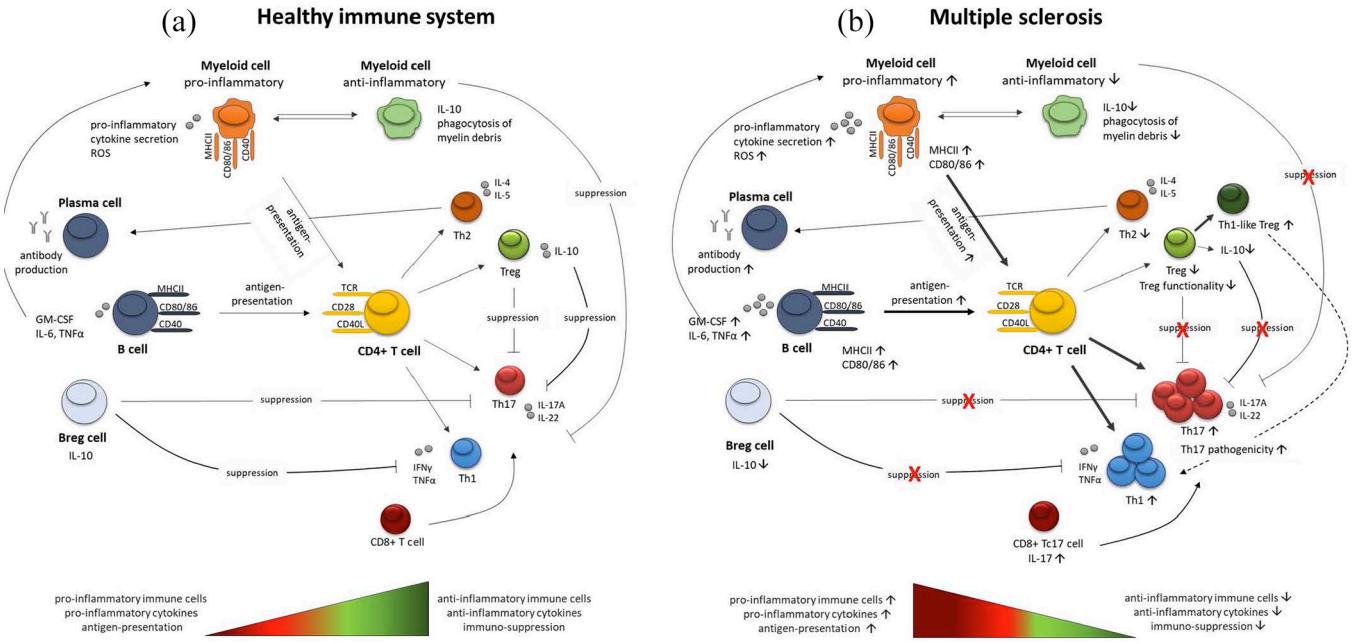

Figure 1. Simplified overview of the immune network during health and disease. (a) Simplified overview of the interaction between T cells, B cells and myeloid cells in a healthy immune system. Upon stimulation, CD4+ T cells can differentiate towards anti-inflammatory Th2 and Treg cells or towards pro-inflammatory Th1 and Th17 cells, depending on the surrounding micro milieu. T cell stimulation can be induced by the interaction with B cells or myeloid cells. Besides their antigen-presenting capacity, B cells also differentiate towards plasma cells, affecting immune responses *via* antibody secretion. A newly identified Breg subset can suppress enhanced pro-inflammatory Th1 and Th17 differentiation *via* IL-10 secretion. Moreover, B cell cytokines can directly affect the myeloid cell phenotype, inducing pro-inflammatory or anti-inflammatory myeloid cells. In a healthy immune system, autoreactive immune responses are suppressed *via* different mechanisms, including IL-10 secretion from Treg cells, Breg cells and anti-inflammatory myeloid cells, maintaining a balance between pro- and anti-inflammatory immune cells. (b) Simplified overview of the interaction between T cells, B cells and myeloid cells in MS. Pro-inflammatory Th1 and Th17 cell responses are increased in MS patients, showing higher secretion of pro-inflammatory cytokines. Moreover, the activation state of pro-inflammatory myeloid cells, secreting high amounts of ROS, as well as autoantibodies produced by plasma cells, and activated B cells are increased in MS patients. This shift towards a pro-inflammatory immune cell pool is induced by disturbed regulatory mechanisms, including defective Treg responses, decreased Breg cells and less anti-inflammatory myeloid cells.
Breg, regulatory B cells; IL, interleukin; MS, multiple sclerosis; ROS, reactive oxygen species; Th, T helper; Treg, regulatory T cells.

from DMF-treated MS patients express reduced levels of mir-155,[157] a micro-RNA that is known for its pro-inflammatory function. In addition to phenotypic changes, disease-modifying therapies might also affect monocyte/macrophage functionality. For instance, glatiramer acetate was shown to increase phagocytosis in both rat microglia and MS patient monocytes,[153,158] with debris clearance necessary for remyelination.[145] Additional studies suggest that the antigen-presenting capacities of monocytes are affected by disease-modifying therapies. Monomethyl fumarate, the active metabolite of DMF, was shown to inhibit the maturation of myeloid cells *in vitro*, characterized by reduced expression of MHCII and co-stimulatory molecules and a concomitant reduction in their capacity to activate T cells.[159] These data add to the importance of the monocyte/T-cell interaction during MS pathology, and further data will be necessary to discriminate whether the beneficial effects of disease-modifying therapies can be solely linked to direct effects on B and T cells or are rather related indirectly to their side-effects on antigen-presenting cells such as monocytes/macrophages.

Summary

Inflammation in MS is characterised by pathogenic immune responses comprising T cells, B cells and myeloid cells. Depending on distinct activation states and the micromilieu, these different cell types act in concert to amplify or dampen pathogenic immune responses (Figure 1).

The actual concept of MS immunopathology suggests an imbalance of pro-inflammatory immune cells and a defective regulatory immune cell pool in the periphery. This phenomenon is linked to the capacity of immune cells to perform a phenotype-switch, resulting in a defective suppressor-function of regulatory cells, and hence an increased infiltration of autoreactive adaptive immune cells into the CNS. Disease-modifying therapies approved for RRMS target autoreactive immune cells, thereby reducing relapses in early MS. However, if they do not substantially halt the disease, this process may result in a secondary progressive disease course. Such a progressive disease form is linked mainly to neurodegenerative processes. In addition, chronic inflammation by ongoing immune cell infiltration and re-activation of already resident cells within the CNS may enhance this process. Hence, compartmentalisation of inflammation also needs to be considered in progressive forms of MS. A goal for the future treatment of MS may thus be the simultaneous, early targeting of peripheral immune cell function and of CNS-intrinsic inflammation, along with combination therapy with neuroprotective or neuroregenerative compounds. Moreover, first clinical data indicate a potential benefit of dietary supplements as add-on therapies. Besides short-chain fatty acids,[51] anti-oxidative compounds (reviewed in Plemel *et al.*) or coenzyme Q10 may represent potential supplements beneficially affecting MS disease.[160,161] Yet, further clinical studies are needed to prove a relevant effect in clinical practice.

Author contributions

Both authors made a substantial contribution to the data collection and the drafting of the manuscript and reviewed and accepted the contents of the manuscript prior to its submission.

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ORCID iD

Ralf A. Linker https://orcid.org/0000-0002-8740-3106

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