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Role of Immune Cells in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system (CNS) characterized by varying degrees of demyelination of uncertain etiology, and is associated with specific environmental and genetic factors. Upon recognition of CNS antigens, the immune cells initiate an inflammatory process which leads to destruction and deterioration of the neurons. Innate immune cells such as macrophages, dendritic cells and natural killer cells are known to play critical roles in the pathogenesis of MS. Also, the activation of peripheral $CD4^+$ T cells by CNS antigens leads to their extravasation into the CNS causing damages that exacerbates the disease. This could be accompanied by dysregulation of T regulatory cells and other cell types functions. Experimental autoimmune encephalomyelitis (EAE) is a mouse model used to study the pathophysiology of MS disease. In this review, we highlight the roles of innate and adaptive immune players in the pathogenesis of MS and EAE.

Keywords : Multiple sclerosis, Experimental autoimmune encephalomyelitis, Innate immune cells, Adaptive immune cells.

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1. Introduction

Multiple sclerosis (MS) is chronic autoimmune disabling diseases of the central nervous system (CNS) characterized by varying degrees of demyelination of uncertain etiology that is thought to be associated with specific environmental and genetic factors [1]. The pathological characteristics of MS and its progression include an interplay between specific mechanisms mainly inflammation, which is thought to be the triggering point in the course of the disease, demyelination, axonal damage, and gliosis [2].

Multiple sclerosis has four clinically defined subtypes, with relapsing-remitting multiple sclerosis (RRMS) being the most common. RRMS is characterized by periods of acute neurological deterioration known as relapses, followed by recovery phases of varying degrees known as remissions. Remission could vary from full recovery to a sequela with a residual CNS deficit. However, RRMS is distinguished by its stable course between the attacks, but this could shift to a progressive stage, known as secondary-progressive multiple sclerosis (SPMS) [3]. On the other hand, the primary-progressive multiple sclerosis (PPMS) subtype is associated with neurological deterioration in function from the onset of the disease and is characterized by a continuously worsening baseline with no defined relapses. The other form of MS is the, progressive-relapsing multiple sclerosis (PRMS), which shows distinct relapses throughout its course [3].

Although the exact etiology of MS is unknown, infiltration of lymphocytes and macrophages into the CNS in addition to pre-existing environmental and genetic factors leading to the development of the disease [4,5]. As shown in **figure 1**, activated Th1 cells are known to release inflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which increase the expression of surface receptors on nearby antigen presenting cells (APCs) and

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lymphocytes. As a result, the presence of genetically susceptible MS antigens such as myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) cause binding of the activated immune cells in the CNS to the MS antigens leading to a variable and continuous inflammatory process of destruction and deterioration in the CNS [6]. Experimental autoimmune encephalomyelitis (EAE) is an animal model of induced autoimmune disease that usually resembles MS and has been widely used to study the pathophysiology of MS [7].

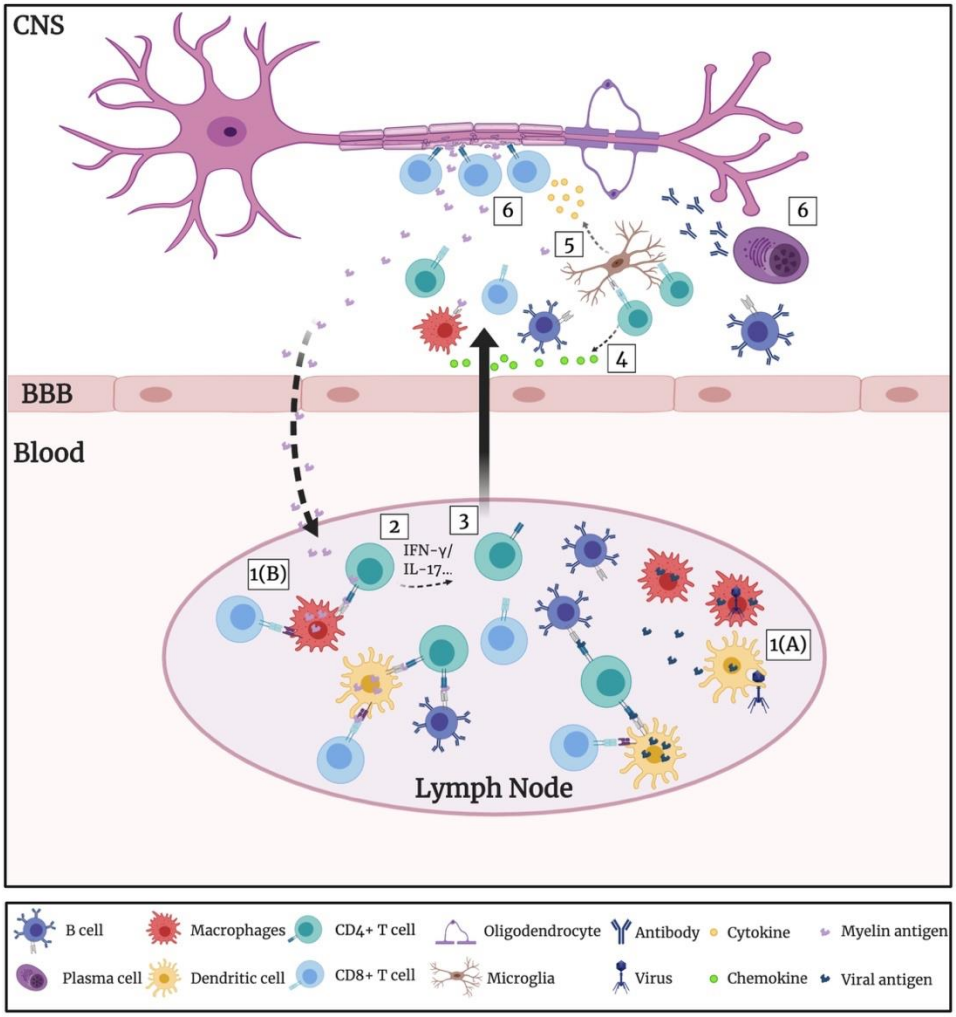

Figure 1. Various immune players in the pathogenesis of multiple sclerosis.

(1) Two main hypotheses for the initial trigger of MS autoimmune response: A) Infectious antigen (mostly viral) that has a molecular similarity (mimicry) with CNS antigen is engulfed by

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DCs or macrophages and presented to $CD4^+$ T cells in the periphery. B) Stress causes damage to the myelin protein of the neurons axons, releasing myelin antigens that bypass the blood-brain barrier (BBB) and induce an autoimmune response. (2) Activated $CD4^+$ T cells produce inflammatory cytokines and differentiate into Th1 that produce IFN-γ, or Th17 cells producing IL-17 or IL-22. (3) Activated T cells up-regulate the expression of surface integrins (e.g. VLA-4), which aid them in crossing the BBB. (4) Released chemokines attract other immune cells including B cells and monocytes/macrophages into the CNS through the permeabilized BBB. (5) The migrating immune cells encounter the cognate myelin antigen, presented by CNS resident cells or migrating antigen-presenting cells (APCs). (6) Myelin antigen presented by APCs activate autoreactive $CD4^+$ and $CD8^+$ T cells which induce direct and indirect damage to the CNS, while migrating B cells differentiate into plasma cells, producing autoantibodies against myelin protein, which can bind and activate complement or induce antibody-dependent cytotoxicity.

1.1 Diagnosis of MS Disorder

There are no specific tests to diagnose MS [8]. As symptoms vary widely, the disease may not be recognized in its early stages. Ultimately, MS is diagnosed based on clinical measures and tests such as magnetic resonance imaging (MRI) of the brain. An MRI scan can detect brain and spinal damages identifying myelin loss. Meanwhile, the examination of the cerebrospinal fluid (CSF), withdrawn from the spinal canal, may indicate a defect or a specific problem related to MS, such as abnormal levels of white blood cells or the presence of proteins. Additionally, blood tests can be used to exclude the presence of viral and other infectious diseases that may elicit neurological symptoms similar to those observed in MS patients [9,10].

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2. Innate Immunity in MS

The innate immunity is the first defense system in vertebrates. It generates a fast, non-specific, inflammatory response to all types of pathogens and tissue damage. Cells of the innate immune system recognize foreign antigens by means of pathogen associated molecular patterns (PAMPs), as well as danger associated molecular patterns (DAMPs), released from damaged or dying cells during injury, via pattern recognition receptors (PRRs) such as toll like receptors (TLRs) and NOD-like receptors (NLRs). Cells of myeloid and lymphoid origins are activated in response to PRR signals [11].The innate immune responses are mediated through cell-dependent mechanisms such as phagocytosis and cytotoxicity, or through secreted factors, such as antimicrobial peptides, complement proteins, cytokines and chemokines [12].

Perturbation of PRRs induces the activation of macrophages, dendritic cells (DCs), neutrophils, and other innate immune cells. However, PRRs can also be expressed on non-immune CNS-resident cells. Many PRRs show increased gene expression in MS patients where genome-wide association studies (GWAS) described some PRRs variants to be associated with increased risk of the disease [13].

2.1 Role of TLRs in MS

TLRs represent a group of receptors located either on the surface of the cell or in endosomes of both nonimmune and immune cells. Activation of the immune system via TLRs is chiefly mediated by APCs such as macrophages, DCs, and B cells [14]. TLRs are involved in recognizing and binding to PAMPs and DAMPs, resulting in transcriptional activation of proinflammatory cytokines, chemokines, costimulatory and major histocompatibility complex

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(MHC) molecules [15,16]. In the brain, DAMPs released after a spinal cord injury induce the activation of TLR2 and TLR4 [17].

TLRs have crucial roles in MS or EAE brain lesions. For instance, a study by Sloane et al. has shown that TLR2 is expressed in oligodendrocytes and upregulated in MS lesions [18]. Additionally, hyaluronan, a low-molecular-weight glycosaminoglycan, is a major component of extracellular matrix broken down by hyaluronidases into hyaluronan oligomers that block oligodendrocyte precursor cells maturation and remyelination through TLR2-MyD88 signaling [18]. Elevated levels of hyaluronan are observed in both EAE lesions and areas of complete demyelination in MS. This could be attributed to increased stimulation of TLR2 on oligodendrocytes, altered hyaluronan synthesis, or partial hyaluronan degradation, resulting in the remyelination blockade [19,20].

Likewise, MyD88-dependent signaling through TLR2, and other TLRs such as TLR4, TLR7, and TLR9, was found to be associated with MS progression [21,22]. In contrast, TLR3 signaling induced neuroprotective responses and was found to suppress demyelination in EAE mice [23]. Furthermore, TLR7 induces the maturation and differentiation of B lymphocytes into immunoglobulin secreting plasma cells. Studies have shown that TLR7 deficiency stimulated IgM and IgG production in MS patients, and this could be associated with worsening the disease state corroborated with compromised immune responses against TLR7-recognized RNA viruses and infections [24, 25].

2.2 NOD-like Receptors in MS

NOD-like receptors (NLRs, nucleotide-binding domain, leucine-rich repeat-containing family) are essential sensors of cellular stress induced by infection, tissue damage, or cell death

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[26]. NLR proteins such as NLRP1, NLRP3, NLRC4, as well as the absent in melanoma 2 protein (AIM2) are activated by PAMPs and DAMPs. This activation results in the recruitment of the inflammasome-adaptor protein apoptosis-associated speck-like protein containing a caspase recruiting domain (ASC or PYCARD), and procaspase-1 [27]. Other NLR proteins in addition to ASC and procaspase-1 form the inflammasome multiprotein complex, the proximity of these proteins induces the autoactivation of caspase-1 which subsequently cleaves pro-IL-1β and pro-IL-18 to the pro-inflammatory mature IL-1β and IL-18 cytokines [28].

The NLRs are expressed on the nuclear membrane or in the cytoplasm of immune cells such as monocytes, DCs, macrophages, neutrophils, and cerebral endothelial cells. NLRs are also expressed as a secreted form in granulocytes, monocytes, B, and T cells [29,30]. In the CNS, the NLRP1 inflammasome is expressed mainly by pyramidal neurons and oligodendrocytes [31], while the NLRP3 inflammasome is expressed by microglia [32].

2.3 Role of Innate Immune Cells in MS Disease

Macrophages and dendritic cells are the main constituents of the innate immunity. Foreign antigens are phagocytosed by these immune cells, which present the antigen on their surfaces, to be recognized by T cells of the appropriate specificity. This leads to the activation and proliferation of T cells and their cytokine secretion [33]. In MS, the recognized antigen is believed to be an epitope on myelin proteins. Activated T cells infiltrate through the BBB into the nervous system where cytokines are secreted. For instance, Th1 cells secrete inflammatory cytokines such as IFN-γ, whereas Th17 secrete IL-17 which plays an important role in host defenses [34]. These cytokines mobilize macrophages and phagocytic cells in the periphery and microglia in the CNS, which consequently induce the MS attacks [35].

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In MS, macrophages induce tissue damage by producing pro-inflammatory mediators. Paradoxically, these cells may also have anti-inflammatory and neuroprotective functions. Macrophages polarization into pro-inflammatory or neuroprotective depends on the mechanism through which they are activated. For instance, monocyte-derived macrophages stimulated with IFN-γ and lipopolysaccharide differentiate into the classical phenotype, M1 macrophages, whereas upon stimulation with IL-4 and IL-33, they differentiate into M2 macrophages [36]. Macrophages in active MS lesions mainly exhibit M1 characteristics and markers, including CD40, CD86, CD64 and CD32 which are abundantly expressed by microglia in the healthy white matter and activated microglia and macrophages in the active MS lesions. On the other hand, M2 markers, such as mannose receptor and CD163 were expressed by myelin-laden macrophages in demyelinating lesions and perivascular macrophages [36]. Another study has shown that in the early phases of MS and EAE, macrophages of the M1 phenotype, producing pro-inflammatory cytokines that contribute to the destruction of the CNS. Nevertheless, in the later phase of the disease, more macrophages in the CNS are of the anti-inflammatory M2 phenotype are present leading to the neuroprotective effects and thus re-establishing homeostasis [37].

It was noted that the number of circulating myeloid DCs isolated from SPMS patients were higher in comparison with RRMS patients and control subjects suggesting that DCs are highly recruited during the transition into the progressive phase of the disease. Also, DCs isolated from SPMS patients were found to produce IL-12 and TNF-α, while DCs from RRMS patients produced higher levels of Th1 cytokines IFN-γ and TNF-α, or Th2 cytokines IL-4 and IL-13 compared to controls subjects. DCs from SPMS patients only promoted a polarized Th1 response [38].

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2.3.1 Role of Neutrophils in MS/EAE

The activation of CNS-specific autoreactive $CD4^+$ T cells in the periphery can result in a dysregulation of the innate immune system [39]. For example, neutrophils and monocytes are expanded in the bone marrow and accumulated in the circulation in response to systemic upregulation of the CXCL1 and granulocyte-colony stimulating factor (G-CSF) in EAE. In MS patients, it was observed that there is an increased number and priming of circulating neutrophils. Furthermore, a correlation between MS relapse phases and G-CSF, CXCL1, CXCL5, or neutrophil elastases, support the role of neutrophils in CNS damage and pathogenesis of MS. It was also demonstrated that peripheral neutrophils can affect the autoreactive T cells activation which contribute to MS pathogenesis [40].

NETosis is a process mediated by neutrophils through which neutrophils form neutrophils extracellular traps (NETs) which are composed of decondensed chromatin and histones forming a large web-like structure. NETs contain factors such as myeloperoxidase (MPO), neutrophil elastase, and proteinase 3, which are released into the surrounding environment, allowing NETs to capture and kill foreign pathogens. This has been previously discussed in studies that showed the significant roles for NETs in MS and EAE, among other autoimmune diseases [41].

In EAE, the depletion of MPO was found to decrease the disease severity reducing the levels of reactive oxygen species (ROS), and leading to improved BBB functions [42-44]. Additionally, the inhibition of neutrophil elastase reduced the infiltration of neutrophils into the optic nerves in EAE mice [45]. Neutrophil elastase and MPO in addition to other neutrophil related factors were increased in the plasma of MS patients and correlated with MS lesion burden [40, 46].

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2.3.2 Role of NK Cells in MS/EAE

Human $CD56^{+}$ $CD3^{-}$ NK cell subsets are defined based on the expression of the cell surface markers CD16 and CD56 [47]. $CD56^{dim}$ $CD16^{+}$ NK cells are present mainly in the peripheral blood and can attack and lyse targeted tumor cells, whereas $CD56^{bright}$ $CD16^{-}$ NK cells are found mostly in the lymphoid organs and can produce large amounts of cytokines but have little ability to kill tumor cell targets [47]. Murine NK cells lack the CD56 marker, and express NK1.1 marker which identifies NK cells obtained from C57BL/6J, and other mice strains [48].

The role of NK cells in MS is not yet resolved [49,50]. It was reported that the CSF of MS patients harbor a distinct subset of NK cells expressing CCR4, releasing IL-17 and IFN-γ and hence, designated as NK17/NK1 cells [51]. Intriguingly, it was reported that most drugs used to treat MS patients activate NK cell lysis of DCs. These include glatiramer acetate (GA, Copaxone) [52], vitamin D_3, calcipotriol, and FTY720 [53]. In addition, monomethyl fumarate (MMF) and dimethyl fumarate (DMF, Tecfidera) enhance NK cell lysis of DCs [54,55]. Further, MMF and vitamin D_3 ameliorated the EAE clinical score in mice [56]. Previously, Munger et al. reported a negative association between the intake of vitamin D and the risk of developing MS in a large female cohort. Women with a high intake of dietary vitamin D (about 700 IU/day) had a 33% lower incidence of MS compared to those with lower intake. In addition, women who used vitamin D supplements (more than or equal to 400 IU) had a 41% reduced risk of developing MS [57]. This was further supported by another study where subjects with serum levels of the circulating form of vitamin D_3, 25-hydroxyvitamin D, greater than 100 nmol/L (40 ng/mL) had a 62% lower chance of developing MS [58].

These results suggest that a possible mechanism of action for these drugs is potentiating the cytolytic activity of NK cells against DCs, which impede the latter from presenting autoantigens to autoreactive T cells. In this regard, it was reported that NK cells isolated from MS patients treated with GA express high cytolytic activity against DCs [59]. Collectively, these findings

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support the notion that drugs used to treat MS patients promote NK cell lysis of DCs, and consequently, impede activation of autoreactive T cells.

2.3.3 Role of NKT Cells in MS/EAE

Natural killer T (NKT) cells are lymphocytes that are a part of the innate immune system. They express T cell receptor (TCR) α/β) as well as NK cell markers such as CD161 and CD94. However, the TCR of NKT cells does not interact with that of peptide antigens presented by MHC-class I or class II molecules like the conventional T cells, but this TCR recognizes glycolipids presented by CD1d, which is a non-classical antigen-presenting molecule expressed by APCs [58]. The NKT cells bind to their cognate antigens resulting in the polarization of IFN-γ and/or IL-4, resulting in regulation of the adaptive immune responses [60]. NKT cells have important regulatory effects in autoimmunity and immune responses to infections and tumors through secreting high levels of IL-4 and IFN-γ. The role of NKT cells in MS was investigated in studies of EAE mice. As an analog of the synthetic glycolipid α-galactosylceramide (GalCer) binds to CD1d on APCs and induces NKT cells to produce IL-4, thus ameliorating EAE severity [61].

2.3.4 Role of γδ T Cells in MS/EAE

γδ T cells mediate host defense and immunoregulatory functions. These cells represent a small population of T cells that express a unique TCR composed of γδ chains instead of αβ chains. Also, they lack a precise MHC restriction, acting as a bridge between the innate and adaptive immune systems. γδ T cells are present mainly in peripheral blood, lymph nodes, spleen, intestine, and skin [62]. Previous findings using EAE mice discussed the role of γδ T cells in MS pathogenesis. In EAE mice, γδ T cells were found in low numbers in the spleen, but

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their concentration in the CNS rises to above 10% at the peak of the relapse phase and fall back during the remission phase [63]. Moreover, depletion of γδ T cells during different stages of EAE resulted in decline of disease worsening, suggesting that these cells play a vital role in the EAE pathogenesis [64].

2.3.5 Role of MAIT Cells in MS/EAE

Mucosal-associated invariant T (MAIT) cells are a subset of innate-like lymphocytes that represent about 25% of $CD8^+$ T cells in healthy subjects. These cells recognize conserved microbial ligands and act against bacterial and fungal pathogens [65]. Furthermore, MAIT cells display a semi-invariant TCR and are restricted by the nonpolymorphic MHC-related molecule-1 (MR1). In order for these cells to accumulate in the periphery, B cells, commensal flora, and the MR1 molecule are needed. In humans, the MAIT cells expand quickly after birth and acquire a memory phenotype, whereas in mice they remain few in number and stay in a naive state [66].

Recent studies suggested their potential role in chronic inflammatory diseases, such as MS, through IFN-γ and/or IL-17 production [65]. However, contradictory observations have been reported indicating an immunoregulatory behavior of MAIT cells in MS through inhibiting Th1 pathogenic responses [67].

2.4 Role of Complement System in MS

Complement system has an important role in the antimicrobial defense. The brain is considered an immune-privileged site and separated from the periphery by the blood brain barrier (BBB). Yet, all major CNS cells produce most of the complement proteins where

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astrocytes are the main source of CNS complement, indicating their involvement in immune defenses against pathogens, and their contribution to tissue destruction [68].

The demyelination process results from an autoreactive immune response against myelin through the complement classical pathway, in addition to the direct activation of complement upon binding to myelin. It is well known that myelin that is purified from the CNS can activate the complement classical pathway [69]. Additionally, mature oligodendrocytes from rats were found to be lysed *in vitro* by complement in the absence of anti-myelin antibodies [69]. MOG may be able to bind and activate the C1q component of complement because it has a similar domain to the C1q-binding sequence of IgG antibodies. These interactions between myelin-specific protein and Clq have consequential effects in inflammatory diseases that affect the CNS such as MS [70].

3. Role of Adaptive Immune Cells in MS

3.1 Role of $CD4^+$ T Cells in MS and EAE

Adaptive immunity is evident in acute and active chronic lesions of MS. This has been reported and confirmed by various studies [71]. The consensus for the pathology of MS is that disease is initiated by activation of CNS antigen specific $CD4^+$ T cells in the periphery [72]. This proposed theory stems from the similarities between MS and the EAE model, as EAE can be induced by immunization with CNS derived peptides or myelin and is mainly driven by autoreactive $CD4^+$ T cells specific for CNS peptides [73].

The theory of molecular mimicry indicates a similarity between a peptide from a foreign agent and an autoantigen. This was introduced by Fujinami and Oldstone by immunizing rabbits with hepatitis B virus peptides that shared 6 amino acids with the myelin basic protein (MBP), after

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which EAE was induced and T cells were involved with cross reactivity against the peptides [74]. Another hypothesis for the development of MS disease is that autoreactive $CD4^+$ T cells are activated in the circulation after cross-recognition of peptides derived from a foreign antigen, such as that of a virus [75].

Studies are still investigating the antigens responsible for activating autoreactive $CD4^+$ T cells in the periphery. Reports from studies on animal models demonstrated that immunization with MBP results in the peripheral activation of MBP-specific $CD4^+$ T cells in the draining lymph nodes as one of the initial events in the development of EAE [76]. After activation, these $CD4^+$ T cells produce inflammatory cytokines and may be differentiated into Th1 or Th17 cells producing cytokines such as IFN-γ or IL-17, respectively [77].

Activated $CD4^+$ T cells upregulate integrins including the lymphocyte function-associated antigen (LFA-1) and the very late antigen-4 (VLA-4) which enable them to cross the BBB. Upon encountering the antigen, autoreactive T cells are reactivated and differentiated leading to cytokine production, which consequently activates neighboring immune and neural cells, further attracting inflammatory cells into the CNS (Figure 1). This activation was observed in macrophages that are thought to indirectly and directly damage the CNS [78]. Also, these CNS antigen-specific $CD4^+$ T cells are the only immune cells that upon transferred to immunocompetent recipient animals, induce the EAE disease. Furthermore, transgenic mice that expressed TCRs derived from human $CD4^+$ myelin-specific T cells and the appropriate human leukocyte antigen (HLA) class II molecule developed EAE disease [7].

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3.2 Role of CD8$^+$ Cells in MS

CNS antigen specific CD8$^+$ T cells are involved in CNS damage during the relapse phases and in the chronic phase of MS. These CD8$^+$ T cells are activated by APCs presenting CNS-derived peptides [79] and are more abundant in MS lesions [80]. Moreover, axons and neurons express MHC-class I and not class II molecules, allow CD8$^+$ T cells to recognize their cognate antigen on axons/neurons and directly attack and damage them [81].

3.3 Role of Regulatory T Cells (Tregs) in MS or EAE

Tregs play crucial roles in maintaining self-tolerance, immune balance in the periphery, and immune privilege in the CNS [82]. Dysregulation of suppressive and migratory markers on Tregs has been associated with MS disease. For example, genetic abnormalities in Treg suppressive markers CTLA-4 and CD25 have been found in some MS patients whereas others had a decreased FoxP3 and IL-10 levels [83]. However, studies in animal models indicated that the transfer of isolated Tregs from naive mice into CNS injured immune-competent Balb/c mice decreased their neuroprotective effect [84]. Conversely, the exogenous transfer of the same Treg cells resulted in a neuroprotective phenotype in C57BL/6 mice. These findings indicate that Tregs might lead to either neuro-destruction or neuroprotection effects in mice with different genetic backgrounds [85].

On another note, Treg cells are affected by various host factors such as the gut microbiota, which was shown to play a vital role in the pathogenesis of MS through altering the host's immune system, and the BBB functions thus leading to autoimmune demyelination [86]. This was supported by analysis of the microbiomes of 71 MS patients which showed that the presence of specific bacterial populations was highly associated with MS. For instance, *Akkermansia*

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muciniphila and *Acinetobacter calcoaceticus*, were found to be increased in MS patients where they induced proinflammatory effects in monocolonized mice and human peripheral blood mononuclear cells. On the other hand, *Parabacteroides distasonis*, was reported to be lower in MS patient. In mice models, the presence of this bacteria species was found to enhance the anti-inflammatory IL-10–expressing human $CD4^+CD25^+$ T cells and $IL\text{-}10^+FoxP3^+$ Tregs activities. Furthermore, transplanting microbiota from MS patients into germ-free mice induced more severe EAE and reduced proportions of $IL\text{-}10^+$ Tregs compared with mice transplanted with microbiota from healthy subjects [87].

Another host related factor is melatonin, the hormone of sleep, which was found to have protective effects against EAE and MS through inducing the protective effects of IL-10 producing T regulatory cells in addition to blocking pathogenic Th17 cells and inhibiting their production of IL-17 [88].

3.4 Role of B Cells in MS/EAE

B cells arise from the stem cells present in the bone marrow, where they develop to an immature naïve B cell. In order to be mature, further development occurs in the spleen or lymph nodes in order to give rise to either memory B cell or a plasma cell [89]. Some B cells tend to be autoreactive by possessing capacity to recognize and react to self-antigens. Therefore, the developmental process includes a checkpoint to limit the production of autoreactive B cells. In MS, some autoreactive B cells are able to bypass this checkpoint and escape to the maturity stage. This has been supported by the findings where autoreactive B cells reach the BBB and infiltrate into the CNS, thus contributing to MS pathology through different inflammatory mechanisms. B cells play important roles in both human MS and mouse

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EAE models [90] and are currently considered therapeutic targets for this purpose. For instance, B cells, plasma cells and excess immunoglobulins are known to be present in both lesions and CSF of patients with MS [91]. Studies in animal models demonstrate a complicated role for B cells [92]. Current theories suggest that two probable independent inflammatory processes induce the CNS injury in MS, which possibly involve B cells. It was suggested that *de novo* infiltration of immune cells from the periphery into the CNS correlate with focal inflammation, MRI-detectable lesions, and relapse periods, whereas other studies indicate that chronic progression supposedly driven by CNS-intrinsic inflammation is induced by CNS-resident immune cells in addition to the CNS-trapped B cells [93].

There are several possible mechanisms of B cells associated pathophysiology in MS. One of these mechanisms is antigen presentation, where B cells play an important role in the immune response by recognizing and internalizing specific antigens, followed by intercellular processing to generate fragments of antigens that are eventually expressed on B cell surface. Studies suggest that antigen presentation and co-stimulation of autoreactive B cells in the CNS may activate T cells towards a pro-inflammatory response, where reciprocal inflammatory signals and interactions cause further activation of B cells [90]. On the other hand, autoreactive B cells can differentiate into plasma cells producing antibodies which bind to myelin sheath and oligodendrocyte proteins. These bound antibodies result in the induction and activation of the complement proteins on tissue surfaces promoting injury. Besides, antibodies may activate other immune cells such as NK cells to destroy tissues via antibody cell mediated cytotoxicity [94].

B cells could also promote inflammation in the CNS via cytokine secretion. It has been previously reported that B cells in MS patients tend to produce more pro-inflammatory cytokines and less protective cytokines compared with healthy controls [95]. In the meninges of patients

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with progressive MS, B cells may form ectopic lymphoid structures or germinal centers, which contain activated B cells and follicular DCs in addition to T cells and thus promote ongoing T cell activation within the brain [96]. These ectopic lymphoid structures may be possibly linked to microglial activation, local inflammation and neuronal loss [96].

4. Conclusions

In MS and EAE, several immune cells are involved in the pathogenesis and remission of MS. In this review, we highlighted the role of the innate immune cellular players including macrophages, neutrophils, NK, NKT, γδ T, and MAIT cells as well as the complement system in the inflammatory processes associated with MS and EAE. On the other hand, T cells including $CD4^+$, $CD8^+$ and Tregs are suggested to be crucial contributors to MS, either exacerbating or ameliorating the disease. This is related to their autoreactivity, inflammatory cytokine secretion and their recruitment into CNS inflammatory sites. Furthermore, the adaptive immune B cells are known to be one of the primary causes of MS development, and hence are currently targets for therapeutic agents in MS. The potential protective and pathological roles of the innate and adaptive immune systems in MS/EAE are summarized in **Figure 2**.

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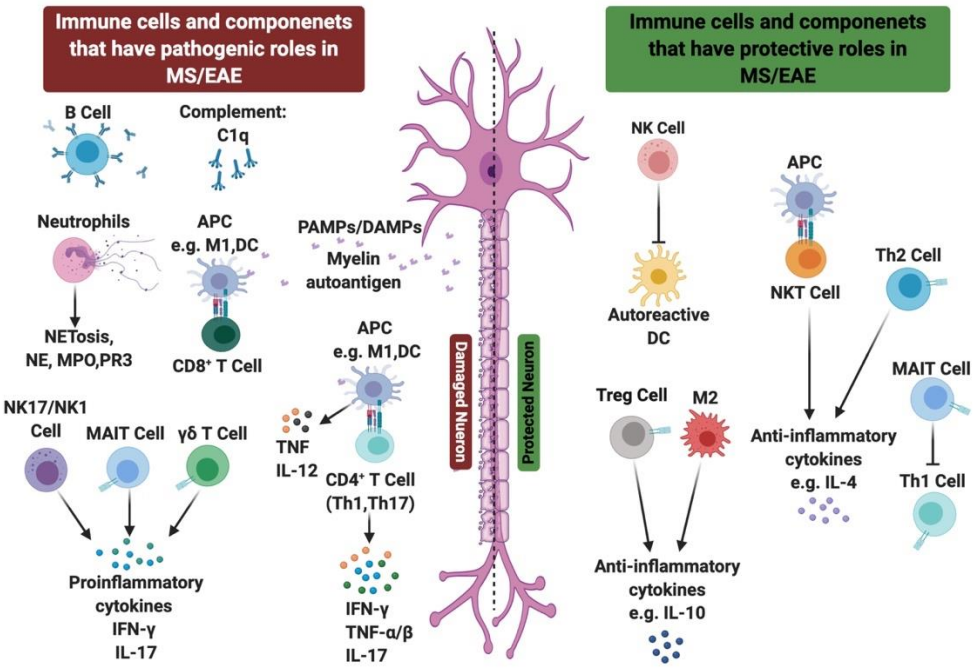

Figure 2. Pathogenic and protective roles of innate and adaptive immune cells and components in MS and EAE.

Innate and adaptive immune cells have important pathogenic roles that lead to the development and worsening of MS and EAE. Antigen presenting cells such as macrophages (in the classical inflammatory phenotype; M1) and DCs phagocytose the DAMPs or PAMPs and the myelin autoantigens released from the damaged myelin in CNS. This leads to the activation of $CD4^+$ T cells such as Th1 and Th17 to secrete pro-inflammatory factors such as IFN-γ, TNF- α/β and IL-17, in addition to the inflammatory factors secreted by macrophages and DCs including TNF and IL-12. Other immune cells (NK17/NK1, γδ T cells, and MAIT cells) release inflammatory cytokines, while B cells secrete antibodies against the myelin autoantigens which bind C1q complement resulting in the activation of the complement classical pathway. Neutrophils start the "NETosis" process and form NETs, releasing factors like NE, MPO. All these inflammatory mediators further exacerbate the inflammatory responses in the CNS of MS patients or EAE animal models. On the other hand, studies have shown that there are several immune cells that

may have protective effects against MS and EAE through the secretion of anti-inflammatory cytokines. These include Treg, M2, NKT, and Th2 cells, or possibly through the lysis of autoreactive DCs by NK cells thus preventing them from acting as antigen presenting cells and inhibiting the inflammatory responses, or through inhibiting pathogenic Th1 cells responses by MAIT cells. APC: antigen presenting cell, MAIT: Mucosal-associated invariant T, MPO: Myeloperoxidase, NETs: Neutrophils' extracellular traps, NE: neutrophil elastase.

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Abbreviations:

AIM2: Absent in melanoma 2 , APCs: Antigen presenting cells, ASC: Apoptosis-associated speck-like protein, BBB: Blood-brain barrier, CNS: Central nervous system, CSF: Cerebrospinal fluid, CTLA-4: Cytotoxic T-lymphocyte-associated protein 4, DAMPs: Danger associated molecular patterns, DCs:Dendritic cells, DMF: Dimethyl fumarate, EAE: Experimental autoimmune encephalitis, G-CSF: Granulocyte-colony stimulating factor, GA: Glatiramer acetate, GalCer: Galactosylceramide,GWAS: Genome-wide association studies, HLA: Human leukocyte antigen, IFN: Interferon, IL-: Interleukin, LFA-1: Lymphocyte function-associated antigen 1, MAIT: Mucosal-associated invariant T, MBP: Myelin basic protein, MHC: Major histocompatibility complex, MMF: Monomethyl fumarate, MOG: Myelin oligodendrocyte

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glycoprotein, MPO: Myeloperoxidase, MR1: MHC-related molecule 1, MRI: Magnetic resonance imaging, MS: Multiple sclerosis, NETs: Neutrophils' extracellular traps, NK: Natural killer, NKT: Natural killer T, NLRs: NOD-like receptors, PAMPs: Pathogen associated molecular patterns, PPMS: Primary progressive multiple sclerosis, PRMS: Progressive relapsing multiple sclerosis, PRRs: Pattern recognition receptors, ROS: Reactive oxygen species, RRMS: Relapsing remitting multiple sclerosis, SPMS: Secondary progressive multiple sclerosis, TCR: T cell receptor, Th: T helper, TLR: Toll like receptor, TNF: Tumor necrosis factor, Tregs: Regulatory T cells, VLA-4: Very late antigen- 4

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