Chapter

Innate Immunopathological Mechanisms in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a progressive disease that affects the central nervous system. The core features of MS are demyelination and inflammation. Demyelination refers to degeneration of myelin that covers the neurons and helps facilitate neuronal impulses. Loss of myelin results in inability to conduct impulses, which causes core symptoms of MS such as unsteadiness, weakness, numbness, and tingling. Inflammation is observed at the site of demyelination in the form of scars, and hence, the term sclerosis. Innate immunity is that part of the immune system that is present from birth. Over the years, adaptive immunity has been extensively studied with respect to MS in human and experimental disease models. However, recent evidence has increasingly pointed to significant involvement of innate immune mechanisms in the pathogenesis of MS. This chapter reviews the latest evidence regarding innate immune components such as blood–brain barrier, microglial cells, and complement system, and their role in MS pathogenesis.

Keywords: innate immunity, complement system, neuroinflammation, multiple sclerosis, blood–brain barrier, microglia

1. Introduction

Multiple sclerosis (MS), a progressive neurological disease, is a lifelong illness. The course of the disease can be heterogenous, with reversible neurological deficits seen in clinically isolated syndrome and relapsing–remitting type MS; progressive form of MS results in chronic progression of clinical deficits, and is termed as primary progressive MS. The complexity and heterogeneity of clinical presentation make it imperative to understand the aetiopathogenesis of MS in order to help understand the disease and develop effective treatment modalities. Developing MS means a lifelong process for a person and till date, there has been no known cure for the disease.

The MS pathogenesis has traditionally been considered to be autoimmune in nature. In this regard, myelin proteins, such as myelin basic protein, myelin-associated glycoprotein, and myelin oligodendrocyte glycoprotein, have been extensively studied and used in animal models to induce paralytic and demyelinating disease resembling MS called as experimental autoimmune encephalomyelitis (EAE) [1].

Some of the immunopathological changes observed in MS include breakdown of blood–brain barrier (BBB), neuroinflammation, demyelination, gliosis,

oligodendrocyte degeneration, and gliosis [2]. This chapter will focus on neuroinflammation involving innate immune components such as BBB, microglial cells, and the complement system.

2. Role of blood: brain barrier in MS

BBB is a tightly regulated barrier that is known to facilitate homeostasis of CNS allowing for controlled exchange of metabolic substances and prevent the entry of pathogens into the CNS, thereby acting as a basic first line of defense for the CNS. It is formed of cerebral endothelial cells tightly joined to each other and dynamically interacting with astrocytes, pericytes, and basement membrane (together known as neurovascular unit) [3]. In MS, BBB has been shown to be compromised as the first sign of disease pathogenesis, preceding infiltration of immune cells into CNS and demyelination. Some of the core changes observed include BBB disruption, perivascular astrogliosis, and increased expression of endothelial cell adhesion molecules [4]. Neuroimaging studies have revealed that gadolinium (a marker for detecting BBB disruption) is seen with active inflammation in MS lesions and is a key diagnostic sign. In fact, BBB disruption has now been observed in normal-appearing white matter before enhancing lesions. Furthermore, in few patients, optic neuritis can be the earliest sign of MS, and permeability of BBB has been shown to be predictive in progression from optic neuritis to MS [5]. This highlights the heterogeneity involved in initial MS pathogenesis in the context of BBB breakdown. Another interesting feature observed is that during the initial phases of illness, that is, in the first year of the disease, gadolinium-positive lesions are observed on MRI scans indicative of high permeability of BBB, and this is associated with frequent relapses. As time goes by, the course of illness changes to that of less BBB breakdown and more of an intrinsic CNS inflammation, which occurs as a result of the influx of leucocytes and other adaptive immune components as a part of autoimmune processes, adding to the complexity in devising effective disease management strategies [6].

Several metabolic changes are observed in BBB of MS patients. In vitro studies using sera from relapsing–remitting type MS patients have shown that BBB undergoes significant metabolic dysfunction such as reduced expression of proteins, such as occludin and cadherin, which maintain junctional integrity, reduced glycolysis in cells, and increased pro-inflammatory status indicated by higher release of reactive oxygen species from endothelial cells. These changes cause increased BBB permeability and lead to increased susceptibility to disease progression [7].

One of the early features of increased permeability of BBB in MS is infiltration of neutrophils into the CNS. Neutrophils play an important role in the MS pathogenesis and in EAE models. MS patients show higher peripheral neutrophil count as compared to healthy controls [8]. In EAE mice model, neutrophils have been shown to increase in number before and during the onset of clinical EAE and accumulate in the meninges [9, 10]. Depletion of neutrophils in EAE mice using antibody against neutrophils, prior to disease onset has been found to inhibit the early stages of disease and future relapses, with prevention of breakdown of BBB considered to be a significant factor in this process [11–13]. Migration of neutrophils across the BBB has been found to induce production of interleukin (IL)1β, which are known to, leads to increased production of Granulocyte Macrophage Colony-stimulating factor (known to promote expansion and enhance release of bone marrow-derived neutrophils), thereby further exaggerating neuroinflammation in EAE [14, 15]. Activated microglia and macrophages are known to produce enzymes such as myeloperoxidase (MPO), which are known to

activate and promote accumulation of neutrophils in the CNS. Postmortem brain studies of patients with MS when compared to healthy controls, show elevated MPO level which associates significantly with demyelination [16]. Neutrophils are considered to promote disruption of BBB via release of MPO; inhibition of MPO using a specific peptide called as N-acetyl lysyltyrosylcysteine amide in EAE model caused reduced migration of neutrophils to CNS, reduced breakdown of BBB, and attenuation of the EAE severity [17].

3. Role of microglia in MS

Microglia are innate immune cells of the CNS. These resident macrophages of CNS are responsible for various homeostatic functions such as synaptic pruning, secretion of neuronal growth factors, phagocytosis of cells in developing nervous system, and maintaining vascular tone of the BBB [18]. Microglial cells show 'ramified' appearance when in resting or homeostatic state surveying the CNS as an innate immune cell, while activated microglial cells tend to reveal a more 'amoeboid' appearance [18].

Microglial cells can form about 45% of the pool of macrophage-like cells in MS lesions, as measured by marker TMEM119, which is present on microglia and not on macrophages. In addition, microglia in MS lesions show reduction in specific marker P2RY12 that is expressed only in resting or homeostatic microglia and not in active microglia, thus showing presence of activated microglia in MS lesions [19]. In areas of active demyelination, microglia show proinflammatory-type phenotype, also known as M1 type polarization that is associated with neuroinflammation and neurotoxicity (characterized by markers such as CD86, CD68, p22phox, and MHC Class II antigens). Lesions of later or inactive stages are associated with microglial cells that show anti-inflammatory phenotype, also known as M2 polarization, which is associated with resolution of neuroinflammation and neuroprotection (characterized by markers such as CD206, CD163, and ferritin) [19]. Clinically, magnetic resonance imaging (MRI) is the first choice to detect focal inflammatory lesions. However, in progressive type of MS, plaques that are associated with chronic and progressive forms of disease are characterized by 'slowly evolving/expanding' type of lesions, also known as smoldering lesions that are represented by a 'rim' of microglia and macrophages, and ongoing demyelination and loss of axons [20]. To increase specificity of detecting activated microglia, positron emission tomography (PET) is done using tracers that target a specific protein called translocator protein (TSPO)[1], which is expressed on the outer mitochondrial membrane of microglia. This is considered to be a more specific marker for neuroinflammation and progression of MS, along with assessing the effects of treatment in MS [21].

Another interesting aspect of microglial involvement in MS includes its role in lipid metabolism. Triggering receptor expressed on myeloid cells 2 (TREM2) is an immunoreceptor expressed on microglia that helps in lipid metabolism and regulation of lipid transport in CNS, along with recognition of bacterial ligands such as lipopolysaccharide, cardiolipin, sulfatides, as well as physiological ligands such as low-density lipoprotein and apolipoprotein E (apoE) [22]. TREM2 and apoE metabolic pathways are crucial in microglial switching from homeostatic state to a neurodegenerative state; mutations in TREM2 are associated with increased

[1] TSPO ligands are used to target translocator protein found on outer mitochondrial membrane of microglia. This is used as a marker to observe 'real time' activation of microglia under PET scanner.

microglia-mediated neurodegeneration [23, 24]. Soluble TREM2 level in cerebrospinal fluid (CSF) has been proposed to be a useful biomarker for microglial activation in MS, as well as for assessing response to treatment in MS. Increased level of soluble TREM2 is observed in CSF of MS patients when compared to controls, which is reduced to physiological levels following treatment with natalizumab[2] [25, 26]. Postmortem histopathological studies of MS patients also show high expression of TREM2 in demyelinating lesions. Mice deficient in TREM2 show reduced microglial activation and increased accumulation of myelin debris, while antibody-dependent TREM2 activation was found to increase oligodendrocyte production, which sustains and enhances remyelination [27].

Neuroinflammation also promotes lipid peroxidation, which leads to generation of oxidized phospholipids such as oxidized phosphatidylcholines (OxPCs). OxPCs, considered to be mediators of neurodegeneration, are found in the lesions of MS [28]. In MS, OxPCs have been directly implicated in the disease pathogenesis, along with microglia and TREM2. In an elegant study, endogenous OxPCs were found to be formed in a histopathological study on MS patients brain tissue. The authors then showed that OxPCs *in vitro* are toxic to neurons and oligodendrocytes. Direct injection of proinflammatory factors such as IL-1β in EAE mice model showed OxPC deposition in spinal cord lesions, indicating a possible role of caspase-3 pathway in this mechanism. Moreover, direct injection of OxPCs into the spinal cord of mice also resulted in demyelination and loss of oligodendrocytes, while neutralization of OxPCs by antibody showed reduced neurodegeneration. Microglial cells were found to accumulate OxPCs; loss of such microglial cells were found to exacerbate neurodegeneration, thus highlighting a protective role for microglia. TREM2 was shown to directly bind OxPCs; mice lacking TREM2 showed exacerbated neurodegeneration. Thus, TREM2 can bind and clear OxPCs and help in preventing neurodegeneration [29, 30].

4. Role of complement system in MS

The complement system is a major part of the innate immunity and consists of more than 40 serum and membrane-bound proteins. There are three activating pathways, namely (i) classical pathway, which is mainly antibody-mediated with C1q being the first ligand recognition subcomponent; (ii) alternative pathway is activated spontaneously by low-level hydrolysis of C3 to C3(H_2O); (iii) lectin pathway is activated *via* mannan-binding lectin (MBL) and ficolins. Each pathway leads to the generation of target cell lysing membrane attack complex (MAC). For further information on the role of complement system in CNS physiology and pathology, see review by Shastri et al. [18]. Here, we will focus on its role in MS and possible treatment avenues.

Complement proteins, such as C4, C1-inhibitor, and properdin, have been found to be elevated in the CSF of patients with MS. Postmortem immunohistochemistry of MS tissues has shown positive staining for several complement proteins such as C1q and C3; for activation products such as C3b, C4d, MAC; and for regulators such as factor H, clusterin and C1-inhibitor. Complement activation is observed in both white and gray matter lesions, indicating a key role for

[2] Natalizumab is a humanized monoclonal antibody against α4 integrins and is an effective treatment used in relapsing–remitting type of MS. It prevents the migration of leucocytes across the blood–brain barrier.

complement system in the MS pathogenesis [31–33]. Systemic inhibition of MAC by subcutaneous administration of a specific antisense oligonucleotide specifically targeting murine C6 mRNA that blocks formation of MAC, in EAE disease model has been found to successfully limit chronic relapsing symptoms by reducing neuroinflammation and protecting from axonal and neuronal synaptic damage. The key mechanism involved reduced secretion of IL-1β [34]. Lectin pathway activity and MBL-associated serine proteases-2 plasma levels were found to be increased in MS patients' serum when compared to controls [35].

An involvement of complement system in MS is quite evident in EAE disease model studies. Mice deficient in either C3 or factor B showed significantly reduced severity of disease and protection from demyelination [36]. Another study showed an increased level of C1q and C3 in EAE mice; C3 deficiency was shown to protect mice from synaptic loss and reduced level of microglial activation [37]. In an elegant study, it was found that in patients with MS and as well as EAE animal model, significant loss of synapses occurs along with engulfment of presynaptic terminals by microglial cells associated with activation of C3. Blockage of C3 by viral overexpression of C3-inhibitor Crry restored the demyelinating function, thus indicating a key role for complement interaction with microglial cells in MS [38]. C3 levels are also increased the dentate gyrus (a key region of hippocampus involved in episodic memory) of EAE disease model, with microglial cells being the main source of C3 in the region. Inhibition of C3 function using rosmarinic acid, which blocks C3b attachment to complement-activating surface, showed reduced loss of synapses and improved memory performance in EAE mice [39]. C1q level has also been found to be increased in MS patients and EAE model. Inhibition of C1q function by knockdown of C1s subunit of C1 was found to reduce demyelination and improve neurological function in EAE mice [40].

Recent studies have assessed the usefulness of measuring complement activation as a potential biomarker for MS progression. For example, neuromyelitis optica (NMO) is another autoimmune demyelinating disorder; it can be hard to differentiate NMO from MS especially in the early stages of the disease due to similar clinical presentation. In a study that included CSF analyses of patients with MS and NMO, a statistical model involving six complement proteins namely C3, C9, factor B, C1q, factor I, and properdin was able to differentiate between MS and NMO [33]. Response gene to complement-32 (RGC32) is a molecule induced by activation of complement; RGC32 mRNA expression is significantly decreased during relapse and increased in responders to a specific treatment called as glatiramer acetate therapy. Predictive statistical model is considered to be about 90% accurate in detecting relapses and about 85% accurate in detecting response to therapy [41]. It is also worth noting that phase 3, randomized, double-blind clinical trials using eculizumab, a monoclonal antibody against C5, has been found to be significantly effective in relapse prevention in NMO [42].

As mentioned earlier, the progressive form of MS is characterized by smoldering lesions represented by microglial cells. Absinta et al. [43] identified that white matter from healthy individuals consisted of mainly oligodendrocytes, while those from MS lesions contained immune cells such as microglia, macrophages, monocytes, dendritic cells and astrocytes, along with reduced oligodendrocytes. The authors further studied microglial cells in MS lesion edges and found an increased expression of C1 complex (C1q, C1r, and C1s) genes. Further analysis of a cohort of more than a thousand MS patients revealed that complement protein risk variants (C1QA, CR1, and C3) were associated with clinically significant lesions observed on MRI scans. The authors then induced EAE in a conditionally knocked

out C1q mice model that specifically ablated C1q in microglia, which attenuated microglia activation, suggesting the importance of C1q-mediated microglial activation in MS. Blocking C1q in EAE mice reduced density of microglial cells in white matter lesions [43, 44].

5. Role of other pattern-recognition receptors

Apart from complement system, a number of innate immune pattern-recognition receptors (PRRs) have also been implicated in the pathogenesis of MS. Toll-like receptors (TLRs) are type 1 membrane proteins and contain an extracellular leucine-rich domain involved in pathogen-associated molecular pattern (PAMP) recognition and a cytoplasmic Toll/IL1 receptor (TIR) domain, which is involved in signaling pathway. It is well-known that TLRs are expressed on microglia and other CNS cells such as neurons and astrocytes [18]. Upon PAMP receptor (PRR) binding with ligand, adapter protein recruitment takes place as part of the signaling pathway. Adapter proteins include myeloid differentiating factor 88 (MyD88), MyD88 adapter-like protein, TIR domain-containing adapter inducing interferon-β (TRIF), TRIF-related adapter molecule, and sterile-α and armadillo-motif- containing protein. These adapter proteins activate microglia that ultimately lead to release of chemokines and proinflammatory cytokines such as IL-1β, tumor necrosis factor α (TNFα), and IL-6 [18]. TLR2 levels are increased in the serum of MS patients. An enhanced activation of TLR2 was observed in peripheral blood mononuclear cells (PBMCs) of MS patients when stimulated with TLR2 ligand [45, 46]. In another study involving PBMCs from MS patients, a lower baseline level of TLR8 was found when compared to healthy controls, and transcriptional response of proinflammatory cytokine IL-12β was also found to be impaired in serum of MS patients [47]. TLR and MyD88 activation pathways influence adhesion molecules of BBB, thereby playing a role in BBB disruption and subsequently in MS pathogenesis [47, 48]. Furthermore, TLR4 is considered to play a dual role with its involvement in remyelination as well as demyelination processes, which remains unclear. EAE studies have shown that TLR4-deficient mice develop more severe symptoms, while other studies show that TLR4-deficient mice develop less severe symptoms [48, 49]. This discrepancy is possibly explained by the method of induction of EAE, which varied in both these studies, and a difference in using MOG peptide by Kerfoot et al. [48] as compared to MOG protein by Marta et al. [49], which show a difference in induction of B and T cell response, thereby having an impact on demyelination process. Modulation of TLR9 activity in MyD88-deficient mice was found to render it resistant to developing EAE [50].

Nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) are intracellular PRRs and contain a central nucleotide-binding and oligomerization (NACHT) domain and C-terminal leucine-rich repeat (LRR). NLRs can be further divided based on their N-terminal component into caspase activation and recruitment domain, pyrin domain, and baculovirus inhibitor of apoptosis protein repeat, respectively, called NLRC, NLRP, and NLRB. Binding of NLR to ligand leads to a signaling process causing formation of inflammasomes and ultimately cause release of proinflammatory cytokines such as IL-1β and IL-18 [18]. Clinically, a homozygous variant of NLRP1 gene has been found to be associated with a familial type of MS [51]. Also, in MS patients who respond to treatment, NLRP3 expression is increased, as compared to those who do not respond to treatment [52]. In EAE, deficiency of NLRP3 [53] or NLRP12 [54] is associated

with reduced severity of the disease. Inhibition of NLRP3 inflammasome activity was found to reduce production of IL-1β and diminish response of T-cells, thereby reducing severity of disease [55].

6. Conclusions

MS can be described as being heterogeneous in terms of clinical presentation, complexity, and progression of disease (summarized in **Figure 1**). This is largely due to numerous pathophysiological changes occurring in the patients. Adaptive immunity has been studied extensively over the years, but less emphasis had been placed on innate immune changes that occur in MS. This notion has changed, and now there is an increasing number of studies that are looking at the key role of innate immune components in the pathogenesis of MS. One of the challenges in this regard is recruitment of patients at different stages of illness and replicating such findings to arrive at a robust and reliable conclusion. Other useful aspects of studying innate immune components are to understand and establish their role in facilitating predictive,

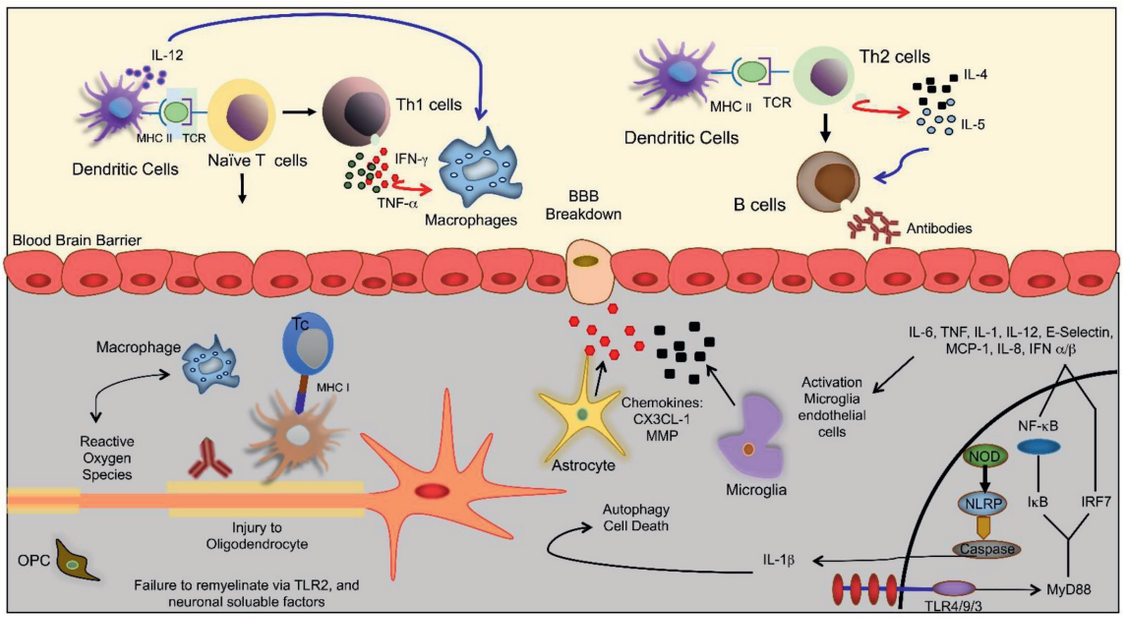

Figure 1.
Pathophysiology of multiple sclerosis: Contact in early childhood with a pathogen and other susceptibility factors, such as racial and demographic background, can elicit reactivation, triggering innate immune mechanisms via toll-like receptors (TLRs), which signal downstream through MyD88 (myeloid differentiation primary response 88) and phosphorylated IκB, allowing nuclear translocation of NF-kB and the transcription of IL-6, TNF, IL-1, IL-12, and E-selectin. IFN/transcription is signaled by TLR via IRF7 (interferon regulatory factor 7). Another significant signal is provided by NOD receptors (nucleotide-binding oligomerization domain), which are activated by potassium efflux-inducing substances such as ATP and TLR stimulation; pathogen associated molecular patterns (PAMPs) toxins, danger, or stress activate the inflammasome through nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing (NLRP), which forms a complex with ASC (apoptosis-associated speck-like protein containing a CARD) and caspase-1, triggering IL-1β. All of these proinflammatory cytokines and growth factors stimulate microglia and endothelial cells, upregulating the expression of adhesion molecules such as E-selectin and increasing the movement of T cells into the CNS. Matrix metallo proteases (MMP) degrade the blood–brain barrier (BBB), hence facilitating the migration of autoreactive T lymphocytes and macrophages via proinflammatory cytokines (CX3CL-1). The Th1 response induced by IL-12 and IFN-stimulates macrophages, activating $CD8^+$ T cells. Th2 response mediated by IL-6 primarily increases B cell maturation and autoantibody production. Cytotoxic oligodendrocyte destruction results in myelin loss and axon exposure to reactive oxygen species that delay or stop action potentials and the formation of neurological symptoms. OPCs (oligodendrocyte precursor cells) are intended to remyelinate these lesions, but neuronal factors such as TLR2 impede their migration.

diagnostic, and prognostic markers in the clinical setting. Further understanding of innate immune components in MS would also aid future research using animal or experimental models that incorporates innate immune aspects as a part of studies in order to justify the heterogenous nature of MS pathophysiology.

In this regard, considerable progress has been made in establishing role of BBB, PRRs, and microglial cells. There is considerable evidence to suggest that BBB breakdown is a key stage in MS pathogenesis, along with complement activation. Experimental studies have been successful in attenuating severity of MS by blocking activated complement proteins. More evidence continues to accumulate to highlight the possible protective role of microglial cells in association with lipid metabolism and myelination. There is still a long way to go in terms of developing clinically useful biomarkers, better research disease models, and effective and safer treatment strategies to benefit patients and improve their overall quality of life.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

MS	multiple sclerosis
EAE	experimental autoimmune encephalomyelitis
CNS	central nervous system
BBB	blood–brain barrier
MPO	myeloperoxidase
TREM2	triggering receptor expressed on myeloid cells 2
apoE	apolipoprotein E
CSF	cerebrospinal fluid
OxPC	oxidized phosphatidylcholine
MBL	mannan binding lectin
MAC	membrane attack complex
NMO	neuromyelitis optica
TLR	toll-like receptor
MyD88	myeloid differentiating factor 88
TRIF	TIR domain-containing adapter inducing interferon-β
NOD	nucleotide-binding and oligomerization domain
NLR	NOD-like receptor

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