Immunopathogenesis

3

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Introduction

Although the precise etiology of multiple sclerosis (MS) remains unknown, three factors are involved (Table 3.1). The first is genetic vulnerability. Over 230 risk/susceptibility genes, along with occasional protection and disease severity genes, are being identified at an increasing pace. They are typically linked to immune/inflammatory rather than central nervous system (CNS) factors. Linked genes are not universal and can vary based on patient racial, ethnic, and geographic background. The second factor involves environmental exposures, which probably occur at critical time points especially earlier in life. These include vitamin D deficiency, Epstein-Barr virus (EBV) infection, adolescent obesity, smoking, and ultraviolet light exposure. The final factor is the host immune system, which damages the CNS. MS is clearly an immune-mediated disease. It appears to be heterogeneous, however, with different pathways leading to disease expression [1]. Studies focused on pathology and immunology allow important insights into MS pathogenesis and pathophysiology. This chapter will begin with a review of the neuropathology of MS and then cover current concepts on major immunologic disease factors

Table 3.1 Proposed etiologic factors in MS

Gene associations
30–50% of MS risk is genetic
20% of risk from gene associations (>230) from GWAS; largely increase susceptibility, especially HLA-DRB1∗1501
Some decrease susceptibility/protect
Rare reports of genes influencing disease severity
5% of risk from low-frequency variants (NLRP8, PRKRA, HDAC7, PRF1)
Environmental factors/lifestyle
Vitamin D deficiency
Epstein-Barr virus (EBV) infection (infectious mononucleosis; high antibody levels to EBV nuclear antigen)
Tobacco exposure
Ultraviolet radiation exposure
Gut microbiota
Adolescent obesity
Organic solvents
Shift work
Immune system factors
Acquired and innate immunity is involved
CNS inflammation (both focal and diffuse), including leptomeningeal inflammation
Changes in CNS components (BBB, glial cells, neurons, axoglial unit, ion channels, synapses)

involved. Distinctions between relapsing and progressive MS will be highlighted.

Pathology

Since there is no true animal model for MS, neuropathologic studies are uniquely informative. Unfortunately biopsy and autopsy materials are

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Table 3.2 Pathologic changes in MS

Increased water content (edema), BBB injury
Endothelial cell injury
Inflammation (lymphocytes, monocytes/macrophages, dendritic cells)
Demyelination
Axonal injury and loss
Oligodendrocyte injury and loss
Neuronal injury and loss
Dendrites, synapses affected
Microglial activation
Astrocytosis
Remyelination
Focal macroscopic plaques (typically centered around venules) in white matter and gray matter (cortex and deep nuclei)
Widespread microscopic injury

limited and subject to the criticism that they may not be representative of MS in general. Nevertheless, such studies have provided novel insights.

Abnormal pathology in MS is confined to the CNS. There are two major pathological processes. The first is focal inflammation leading to formation of macroscopic plaques, visualized initially as contrast-positive lesions on neuroimaging. This reflects major focal breach of the blood-brain barrier (BBB) and is a hallmark of relapsing MS. The second pathologic process is neurodegeneration, with microscopic injury to axons, neurons, synapses, and dendrites and subsequent tissue volume loss. This is believed to be the neuropathologic substrate of progressive MS [2]. These two key pathologic processes, resulting in macroscopic and microscopic lesions, involve a spectrum of changes that can vary over time, as well as between patients (Table 3.2). Studies indicate progression is age dependent, which might support neurodegeneration as a truly independent process from focal inflammation [3]. In this setting, transition to progressive MS might reflect critical loss of CNS reserve.

Macroscopic Injury

Multifocal lesions referred to as plaques occur in waves and can occur throughout the course of MS, but especially in the early years. They result from focal inflammation. About 80–85% of MS plaques are centered around small veins. They show sharp margins. Plaque pathology involves edema and inflammation early, variable degrees of myelin loss and axonal injury/loss, oligodendrocyte and neuronal loss including via programmed cell death (apoptosis), myelin pallor or vacuolization, normal or aberrant remyelination, microglial activation, and reactive astrocytosis. Programmed necrosis (necroptosis) has been suggested as a neuronal cell death mechanism in MS via microglial activation [4, 5]. Early on there is infiltration of cells with marked BBB breakdown, identified by contrast enhancement on magnetic resonance imaging (MRI). This breakdown likely reflects direct effects of proinflammatory cytokines and chemokines, as well as indirect WBC-related injury [6]. This is followed by a local immune cascade, with proinflammatory cytokine and chemokine release, local cell activation, and injury to myelin, underlying axons, and oligodendrocytes. There is disruption of blood vessel walls, with deposition of perivascular serum albumin, fibrinogen, and immunoglobulins [7]. The edema and influx of serum components lead to nerve conduction block at nodes of Ranvier [8]. Over time inflammatory cells clear, leaving a permanent area of damage surrounded by an astrocytic scar. These macroscopic lesions are visualized on MRI as hyperintense foci on T2/fluid-attenuated inversion recovery (FLAIR) sequences. When there is marked tissue matrix damage, they will also appear as chronic hypointense black holes on T1 sequences.

Plaques form in preferential areas, including corpus callosum, periventricular white matter, optic nerves, cortical gray matter, juxtacortical white matter, brain stem/cerebellum, and spinal cord. They always seem to be close to blood or cerebrospinal fluid (CSF), raising the issue of diffusible humoral factors playing a role in their occurrence. White matter plaques are typically most apparent, but gray matter plaques and gray matter demyelination can be extensive. Neocortical lesions have been divided into leukocortical (Type I), intracortical (Type II), or subpial (Type III). Most cortical lesions are subpial

[7]. Macroscopic injury, along with the microscopic damage discussed below, leads over time to discernable atrophy of the brain and spinal cord. The corpus callosum thins, and the lateral ventricles expand. In 5–10% of patients, there is severe hydrocephalus ex vacuo [7].

Microscopic Injury

MS CNS shows diffuse global injury. Much of the normal-appearing CNS, in between the macroscopic plaques, is microscopically abnormal [9]. Changes include BBB disturbances, low-grade (CD8+ T cell) inflammation, gliosis, microglial activation, axonal injury, and damage to the nerve fiber layer of the retina [10]. This has been documented using imaging techniques such as magnetization transfer imaging, diffusion tensor imaging, magnetic resonance spectroscopy, and optical coherence tomography and confirmed with careful pathologic studies. Inflammatory cuffs are often seen in normal-appearing white matter. There is variable axonal injury, characterized by axonal spheroids and terminal swellings. Cellular and molecular changes point toward axonal transport disturbances, hypoxic injury, and loss of neurites and synapses [7]. Progressive MS patients in particular show both perivascular and parenchymal inflammatory infiltrates (see "Progressive MS" section). This microscopic injury is independent of macroscopic pathology.

Plaque Pathology

Formation of the MS lesion goes through stages with distinct differences. In an autopsy study of very early MS, the pathologic changes that preceded myelin phagocytosis involved marked loss of oligodendrocytes, often by apoptosis; marked microglial activation; myelin pallor without myelin loss; and virtually no systemic inflammatory cells [11]. The authors suggested these very early prephagocytic lesions, characterized by oligodendrocyte loss and microglial activation, preceded systemic inflammation. They interpreted this as most consistent with a primary in situ disturbance at the level of the oligodendrocyte and/or microglial cell, provoking a secondary systemic inflammatory response. This supports an "inside-out" hypothesis for MS and has important implications for the role of the systemic immune system in MS, which will be discussed later. More recent reports suggest abnormalities in astrocyte foot processes may also be a very early lesion feature [12].

The next stage in very early lesions is detection of macrophages ingesting myelin. Myelin phagocytosis represents an innate response of macrophages, and is not a CD4+ T cell-mediated process [13]. Normal tissue surrounding these active lesions shows microglial activation, except in very acute cases (when the duration is in days). Normal-appearing white matter also shows IgG-positive reactive astrocytes and occasional IgG-positive oligodendrocytes and axons. Very early lesions show CD 209+ dendritic cells in perivascular spaces within and surrounding new lesions, consistent with their being a major antigen-presenting cell (APC) in MS. Proliferating monocytes are present in the Virchow-Robin spaces and adjacent tissues in very early lesions.

CD4+ and CD8+ T cells are seen in perivascular spaces of parenchyma of recently demyelinated tissue, along with B cells and plasma cells and occasional regenerating oligodendrocytes. This has been interpreted as the start of an adaptive/acquired immune response, as opposed to the innate response of the very early lesion.

The tissue bordering active expanding lesions shows early loss of oligodendrocytes accompanied by activated microglia, with little inflammatory infiltrate. There is subsequent accumulation of activated T cells, B cells, and IgG-positive plasma cells, with some oligodendrocyte regeneration.

Early active lesions are marked by heavy infiltration of macrophages that phagocytize myelin fragments. Active plaques are defined by the presence of partially demyelinated axons with myelin-filled macrophages [14]. Male and female MS patients show no inflammation differences in T cells, CD8+ T cells, and macrophages in early MS lesions [15].

The dominant cell in active plaques is the myelin-laden macrophage, which originates from microglia with participation of systemic infiltrating monocytes. They outnumber lymphocytes ten to one. With regard to T cells, clonally expanded CD8+ T cells markedly outnumber CD4+ T cells. B cells and plasma cells are limited. Immunoglobulin and complement products are found on the degenerating myelin sheaths, with variable loss of oligodendrocytes. This inflammatory infiltrate leads to upregulation of proinflammatory cytokines, such as interleukin-1 (IL-1) and IL-2, tumor necrosis factor (TNF), and interferon γ (IFNγ), and activation of endothelial cells, which will express stress proteins, MHC class II and adhesion molecules, and other factors.

Late chronic disease plaques show little inflammation and highly reactive microglia at their rim and can have some macrophages containing myelin debris [7]. Burnt-out chronic inactive plaques are marked by demyelination with little to no inflammation and are surrounded by an astrocytic scar.

Autopsy Specimens

A 2009 study evaluated 67 MS autopsy brains compared to 28 control brains [16]. The MS cohort involved acute MS leading to death within 12 months ($N = 9$); relapsing MS ($N = 5$); secondary progressive MS (SPMS) ($N = 35$); primary progressive MS (PPMS) ($N = 13$); asymptomatic MS ($N = 4$); and benign relapsing MS ($N = 1$). A total of 1148 lesions were evaluated: 378 were active, 222 were slowly expanding (an inactive center, surrounded by a rim of activated microglia and some macrophages at the lesion margin), and 548 were inactive (a sharp lesion border without macrophages and no microglial activation). Detailed quantitative analysis was performed on a subset of 228 lesions (85 active, 50 slowly expanding, 93 inactive). In addition 139 normal-appearing white matter regions, 121 meninges, and 120 control areas were also analyzed from the MS brains.

Several important observations were made. The most marked inflammation was found in acute and relapsing MS brains. T cells were most marked in active lesions (which were most common in the acute and relapsing MS brains), followed by slowly expanding lesions (which were only found in progressive MS). Inactive lesions and normal-appearing white matter showed low T cell numbers. T cells were virtually absent from cortex, but markedly present in meninges. Most of the lesional T cells were CD8+ as opposed to CD4+ cells. B cells showed a similar distribution pattern but were tenfold fewer than T cells. They were predominantly found in perivascular cuffs or meninges; very few were within parenchyma. Cortical lesions have shown limited inflammation at postmortem. They are often associated with leptomeningeal inflammation. They show loss of neurites, decreased synapses, and decreased neurons [7]. Macrophages (HLA-D+) were present in all active lesions, microglia were prominent in slowly expanding lesions, and a ramified microglia-like cell was present in inactive lesions. Plasma cells were mainly found in perivascular and meningeal connective tissue rather than lesions, parenchyma, cortex, or normal-appearing white matter. They were most common in progressive MS. Lymph node-like follicle structures were found only in 22% of the active progressive MS brains.

Acute axonal injury was most marked in active plaques, followed by slowly expanding lesions, inactive plaques, normal-appearing white matter, and cortex. Normal-appearing white matter from progressive MS showed greater axonal injury. Acute axonal injury correlated with inflammation in all MS subtypes, including progressive MS.

An intriguing observation was that in older MS brains (average age was 76 years), inflammation and axonal injury declined to levels consistent with age-matched controls. All lesions seemed inactive, suggesting that perhaps the MS disease process burns out with age. However this concept has not been verified, and ongoing MS damage may be quite marked in elderly individuals. In these brains, there was active remyelination with evidence of shadow plaques.

It could be speculated that clearance of activated microglia permitted resumption of remyelination. These patients could show concomitant vascular and Alzheimer pathology however.

The authors concluded that progressive MS was associated with inflammation, but did not show the degree of endothelial leakiness found in relapsing MS. There was differential cell distribution. T cells were seen in large perivascular cuffs, as well as in brain parenchyma. In contrast, B cells and especially plasma cells accumulated in connective tissue spaces (perivascular spaces and meninges). Plasma cells accumulated later than T and B cells, but persisted long after T and B cells had cleared.

The pathologic data supported a role for multiple cells (CD8+ T cells, B cells, plasma cells, macrophages, monocytes, microglia) in MS, in addition to CD4+ T cells.

In another study of 16 MS brains and 8 controls, focused on the cerebellar dentate nucleus, reduced numbers and density of synapses were found [17]. There was evidence for both a glia-mediated and direct neuronal damage process. In a third study of eight MS brains and eight controls, widespread primary dendrite spine loss was found in MS cortex [18].

Myelocortical MS

A recent study reported on 100 consecutive MS autopsies over a 14-year period [19]. Twelve showed a unique neuropathologic picture. There was no evidence for cerebral white matter myelin loss, despite very abnormal brain MRIs. Cerebral axons were diffusely swollen, with intact myelin. There were activated microglia, astrocytosis, and serum proteins observed in the cerebral regions. The authors referred to this newly recognized disease subtype as myelocortical MS. There was the expected myelin loss in the subpial cortex and spinal cord. Compared to non-myelocortical MS, this subtype had the most profound loss of cortical neurons. Cortical neuron loss did not correlate with cortical demyelination, suggesting independent injury to myelin and neurons. Myelocortical MS patients

had secondary progressive (SP) MS (67%), primary progressive (PP) MS (16.5%), and relapsing MS (16.5%).

Progressive MS

The neuropathology of progressive MS differs from relapsing MS. Progressive MS is believed to represent neurodegeneration, injury to axons, neurons, and synapses. Although both progressive and relapsing MS contain focal inflammatory demyelinating lesions, with variable axonal injury and loss, progressive MS as noted above has been associated with a compartmentalized low-grade diffuse inflammatory process behind a relative intact BBB, with slow expansion of white matter lesions, marked activated microglia, and extensive cortical demyelination. Most focal white matter lesions in progressive MS show slow expansion at the lesion edge or are inactive [20]. The slowly expanding lesions show no oligodendrocyte precursors, and no active remyelination. They appear to reflect mitochondrial injury and implicate an energy disturbance. The diffuse inflammation in progressive MS does not express apoptosis or proliferative markers. It is associated with marked neurodegeneration, with extensive axonal injury and microscopic changes. Such a process could be driven by local antigen exposure or local cytokine production within the CNS microenvironment. There is diffuse injury to normal-appearing brain, along with marked gray matter demyelination within cerebral and cerebellar cortex. Fast axonal transport disturbances, resulting in neurodegeneration, correlate with inflammatory changes in T cells, B cells, and macrophages. Inflammatory lymphocytes predominate in perivascular cuffs. These T cells, B cells, and plasma cells are found largely in the meninges. Despite significant inflammation, progressive MS patients show little to no contrast-enhancing lesion activity. This has been interpreted as progressive MS showing inflammation trapped behind a closed or repaired BBB.

In a recent study of the brain and spinal cord of 34 SPMS patients and 13 PPMS patients, the SPMS patients showed larger brain plaques and

greater demyelination and plaque inflammation, while PPMS showed greater remyelination with more remyelinated shadow plaques [21]. Incomplete remyelination in the spinal cord, but not in brain, correlated with greater disability.

Blood vessels in progressive patients may show thick perivascular infiltrates without leakiness [20]. Progressive patients can also show ectopic lymphoid follicle-like structures, resembling secondary lymphoid tissue, in connective tissue compartments of the CNS. In a subset of SPMS, these lymphoid follicles form within meninges where there is underlying inflammation and demyelination. They also form adjacent to large active subpial cortical lesions. The severity of meningeal inflammation and lymphoid follicles was said to correlate with extent of cortical lesion activity. However, in another study, cortical demyelination did not correlate with meningeal inflammation [22]. The chronically inflamed brain in progressive MS could create a local microenvironment favoring retention of inflammatory cells. In short, the neuropathology supports distinct mechanisms involved in progressive vs. relapsing MS [23].

In a recent study from the Netherlands Brain Bank, progressive MS patients with a mean disease duration of 28.6 years showed marked inflammation: 57% of all lesions were active, and 78% were mixed active/inactive [24]. Progressive MS (vs. relapsing MS) showed higher lesion load ($p = 0.001$) and fewer remyelinated lesions ($p = 0.03$). They also showed higher proportion of mixed active/inactive lesions ($p = 0.006$).

Lesion Heterogeneity

The MS Lesion Project originally proposed four patterns of acute MS plaques: demyelination with abundant macrophages, with or without immunoglobulin and complement deposition; oligodendrocyte apoptosis, with distal dying-back oligodendrogliopathy, and rare primary oligodendrocyte injury [25, 26]. It was suggested that those with immunoglobulin and complement

responded to plasma exchange [27]. To date these patterns have not been confirmed definitively, and have not proven clinically useful.

Remyelination

Remyelination occurs in about 70–75% of MS plaques and is associated with oligodendrocyte progenitor cell (OPC) recruitment. Chronic remyelinated lesions are referred to as shadow plaques. This myelin is thinner than normal [7]. Vessels often show wall dissection and fibrosis and enlarged perivascular spaces (pseudochannels). In the other 25–30% of lesions, remyelination is absent, and oligodendrocyte numbers are limited, suggesting a failure to recruit OPCs [28]. Within a given macroscopic plaque, deeper sections can show signs of repair (remyelination, oligodendrocyte regeneration) even though the edges show continued destructive activity [29]. An important goal of current research efforts involves ways to enhance remyelination.

Axon Pathology

Acute axon injury including transection occurs in early MS, within both active and chronic plaques, as well as normal-appearing brain tissue and periplaque white matter [30–33]. Axon pathology correlates with degree of inflammation. Inflammatory intermediates reduce energy metabolism in demyelinated axons, perhaps by direct mitochondrial effects or by interfering with blood flow resulting in ischemia [8].

More specifically, CD8+ T cell inflammation has been associated with axonal injury [34]. Although there is a symbiotic relationship between myelin and axon, axon changes can occur independent of demyelination [34]. This is emphasized in the myelocortical MS previously discussed. In a study of MS spinal cord tissue, diffuse axonal loss correlated with density of both activated microglia and meningeal T cells [35]. In another recent study of brain tissue from

19 children with early MS, early axonal injury was much more extensive than in 12 adult MS samples [36].

With axon injury, there are sodium influx, activation of calcium-dependent proteases, upregulation of voltage-gated calcium channels, and destruction of the axon cytoskeleton. Small axons (<2.5 μm cross-sectional area) are preferentially lost within MS spinal cord and optic nerve [37, 38] .

Cortical/Gray Matter Pathology

Although MS has been described as a demyelinating white matter disease, pathologic studies document marked gray matter involvement in deep nuclei as well as cortex. Thalamic neuronal loss in MS is estimated at 30–35% [39]. As mentioned earlier, three types of cortical lesions are described. Type I lesions span the cortex and white matter, Type II are completely intracortical, and Type III extend from the pial surface into the cortex, generally to cortical layer 3 or 4, and cover several gyri [40]. Although not visualized on conventional MRI, cortical lesions are common in MS. They are hypocellular compared to white matter plaques, and may not be associated with breakdown of the BBB. Although there are few inflammatory cells and no perivascular cuffs, activated microglia are plentiful. These lesions show loss of axons and neurons. As noted previously, progressive MS patients show more cortical pathology than relapsing patients.

Unusual MS Variants

Tumefactive MS refers to patients who present with an unusually large brain plaque, generally singular, with surrounding edema and mass effect. The lesion mimics a brain tumor or abscess and may lead to urgent biopsy. In rare cases this is the presentation of MS. It can also occur in well-established MS and has been seen in fingolimod- and natalizumab-treated patients [41, 42]. Neuroradiologic features involve size typically >2 cm and mass effect, with edema and/or ring enhancement [43]. The pathology shows active inflammation with myelin loss, reactive gliosis, myelin-laden macrophages, and relative axonal sparing. Prognosis does not differ from classic relapsing MS.

Marburg variant MS refers to a clinically malignant and fulminant disease expression, where patients go on to profound disability or even death within months to a year or 2. Lesion pathology is more destructive [10]. There are many, often large, macroscopic lesions which may become confluent. Active lesions show massive macrophage infiltration, marked myelin loss, severe axon loss, and tissue necrosis. There may be deposition of immunoglobulin and complement activation in some cases. It has been suggested that Marburg variant is associated with increased (>80%) citrullinated myelin basic protein (MBP), a more immature and unstable form of this core CNS myelin component [44, 45].

Balo concentric sclerosis is an unusual demyelinating variant reported as more frequent in the Philippines and Asia. Cognitive features may be prominent. There is often a severe stroke-like onset [46]. The striking pathology involves alternating rings of intact myelin, separated by demyelinated regions. Oligodendrocyte apoptosis along with selective loss of myelin-associated glycoprotein (MAG) has been noted in the demyelinated regions. The demyelinating pattern has been described as similar to what hypoxic injury might produce, with local expression of iNOS and upregulated expression of tissue-preconditioning proteins at the lesion edge [10, 47, 48]. These lesions show defects in mitochondrial respiratory chain proteins [49]. Aquaporin 4 loss without complement or immunoglobulin deposition was extensive in both demyelinated and myelinated regions in four cases of Balo disease [50].

Myelinoclastic diffuse sclerosis (Schilder disease) is a very rare predominantly pediatric disorder, characterized by one or two large (3 × 2 cm) cerebral inflammatory demyelinating lesions [51]. This disorder is typically monophasic and steroid responsive [52]. It behaves like a postinfectious encephalitis [53].

Progressive solitary sclerosis was first described in 1990. It refers to an isolated/single CNS demyelinating lesion which produces progressive motor impairment. In the largest series reported to date ($N = 30$) [54], lesion location was most commonly the cervical spinal cord (60%), followed by cervicomedullary junction/brain stem (20%), thoracic spinal cord (13.3%), and cerebral white matter (6.7%). Patients presented with hemiparesis/monoparesis (80%), quadriparesis (16.7%), or paraparesis (13.3%). The course is typically a slow worsening, but rare presentations are acute to subacute. Median age has been 48.5 (23–71) years, 50% are female, and 50% show CSF abnormalities consistent with MS. Thirteen percent report a first degree relative with MS. Limited studies confirm demyelination, and in rare cases, there is late MRI dissemination. This has been proposed to be a forme fruste of PPMS [55].

Summary

Pathologic studies in MS reinforce several key features. They include the inevitable presence of abnormal inflammation, both focal and diffuse; macroscopic and microscopic pathology; extensive gray matter involvement; neurodegeneration in addition to myelin and oligodendrocyte injury; and distinctive features for relapsing vs. progressive MS. More work is needed to clarify the role of recently described myelocortical MS and whether early in situ pathology triggers systemic inflammatory cell infiltration in a subset of MS or is a more general phenomenon.

Immunology

Traditional MS immunopathogenesis concepts focused on systemic autoreactive CD4+ T cells, sensitized to one or more CNS myelin components, along with proinflammatory cytokine production and a preferential T helper (Th)1 response. This was based on a key animal model discussed below. However, the immunology of MS is now appreciated to be much more com-

plex, with bidirectional interactions between systemic components and resident CNS cells [6]. Mucosal immunity (in particular the gut microbiota) may play a crucial role. In fact it is not even clear that MS is a true autoimmune disorder, since no critical autoantigen target (including any myelin component) has ever been identified. It is more accurate to describe MS as an immune-mediated disorder, with multiple immune system components and factors mediating the key pathologic changes of MS. Changes in systemic immune factors (increase in innate immunity, including myeloid dendritic cells) may contribute to development of SPMS, along with in situ CNS inflammation trapped behind a closed BBB [20, 56, 57]. This CNS-compartmentalized inflammation likely contributes to CNS injury but is poorly targeted by current therapies.

Animal Models

There is no true animal model for MS, but immune-mediated and toxin- and virus-induced models have been studied. The major one is experimental allergic/autoimmune encephalomyelitis (EAE). It is produced in susceptible animal strains by immunizing with CNS whole myelin or myelin components such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), and proteolipid protein. The immunization procedure requires potent adjuvants. Depending on the strain and immunization protocols, clinical expression can involve monophasic, relapsing, or progressive disease. In EAE both cellular (CD4+, CD8+ T cell) and humoral immune responses play a role. The most common model involves CD4+ Th1 cells initiating delayed-type hypersensitivity responses to myelin antigens. Pathogenic CD4+ T cells adoptively transfer EAE, with the brunt of pathology seen in the spinal cord. Myelin-specific CD8+ T cells as well as Th17 cells can also induce EAE [58]. Recent studies suggest three forms of EAE, which can be driven by adoptive transfer of CD4+ Th1, Th17, or Th2/Th9 cells [59, 60]. In the EAE model, inflammation first enters the subarachnoid space and then

the parenchyma. This may be similar to what happens in MS. Although MOG-induced EAE probably comes closest to looking like MS, no EAE model truly duplicates MS. EAE seems to be a truer model for acute disseminated encephalomyelitis (ADEM)/postinfectious encephalitis or encephalomyelitis, which has an immunopathology distinct from MS [61].

Toxin-induced demyelination models include direct injection of gliotoxins (such as ethidium bromide, lysolecithin) into white matter or systemically delivered toxins such as cuprizone [62].

The best infectious animal model for MS involves Theiler's murine encephalomyelitis virus (TMEV), a nonenveloped single-strand RNA picornavirus [58]. This causes an acute mild polioencephalomyelitis in mice, followed by a chronic inflammatory demyelinating spinal cord infection, with virus detectable in glial cells and macrophages. The chronic infection results in an immune-mediated myelopathy with features reminiscent of MS. However, it is relatively easy to document the persistent infection, whereas this has not been shown in MS. Other infection models have included canine distemper virus and mouse hepatitis virus, but none truly recapitulate MS.

MS Immunologic Scenarios

Distinct immunologic scenarios have been proposed for MS (Table 3.3). The most popular "outside-in" hypothesis involves proinflammatory CD4+ T cells, both Th1 and Th17, activated in the periphery by an unknown (likely antimicrobial) antigen. The triggering antigen presumably shares antigenic sequences with myelin or other CNS antigens. These proinflammatory cells attach to the CNS endothelium via adhesion molecules to cross the BBB. This is facilitated by release of proteolytic enzymes such as matrix metalloproteinases (MMPs). Once inside the CNS parenchyma, molecular mimicry results in cross-reactivity, and the misdirected immune attack results in pathologic lesions. Instead of quickly exiting, the infiltrating cells see this shared antigen and are further activated locally to cause injury. This results in further leukocyte

Table 3.3 MS immunologic scenarios [6]

Environmental pathogen(s) or other factor leads to systemic immune response
This response cross-reacts with CNS/possibly myelin antigen
Sensitized systemic cells penetrate the CNS and result in primary tissue damage
Outside-in hypothesis
Intrinsic CNS abnormality (at level of oligodendrocyte, astrocyte, microglia, or neuron) leads to in situ disturbance
Secondary systemic immune cell penetration with secondary tissue damage
Inside-out hypothesis
Progressive vs. relapsing MS
Less focal inflammation; CNS-compartmentalized inflammation
CD8+ T cells, plasma cells; activated microglia, astrocyte inflammatory responses
Accentuated neuro-axonal degeneration (with oxidative stress, mitochondrial dysfunction, extracellular free iron accumulation, loss of myelin trophic support), altered glutamate, proinflammatory microenvironment

recruitment, inflammation, local cell activation, and damage to CNS tissue. There is evidence that immunity to myelin antigen targets can worsen MS. Altered peptide ligands (APL) are created as partial agonists or antagonists to the T cell receptor of autoreactive lymphocytes. In a phase II trial of an APL to MBP 83–99, a subset of patients had marked worsening on MRI with clinical relapse, coincident with a marked expansion of MBP reactive T cells [63].

The inside-out hypothesis is based on a primary in situ CNS abnormality which somehow provokes systemic immune cells to infiltrate, producing secondary inflammatory-mediated damage. This could reflect an abnormality of intrinsic CNS cells (oligodendrocytes, microglia, astrocytes, neurons) or their components (mitochondria, ion channels). The in situ disturbance could be a chronic CNS infection, metabolic, or vascular defect. In this scenario, MS could be a neurodegenerative disorder, with demyelination a secondary issue [64].

Finally, the immunology and damage mechanisms between progressive and relapsing MS may be distinct, with a much more important role for intrathecal mechanisms in progressive MS.

Since MS is most likely heterogeneous, it is quite possible that more than one scenario can result in MS. For example, could disease be driven by persistent CNS infection? It will be important to define distinct MS subsets.

Immune System Cells

T Cells

Although the human immune system does not have true distinct Th1 and Th2 cells, CD4+ Th1-like cells promote proinflammatory cytokines and enhance cellular immunity, while CD4+ Th2-like cells promote antagonistic regulatory cytokines and enhance humoral immunity. CD4+ T cells, depending on whether they are naïve or activated, can show abnormalities in number and/or function in MS patients vs. controls [65].

T regulatory (Treg) cells are immunosuppressive CD4+ T cells that express CD25 and Foxp3. They inhibit autoreactive effector cells [65]. CD4+ CD25+ Treg cells are implicated in development of autoimmune disorders. A number of studies have suggested that Treg number and function are abnormal in MS [66–68]. Transcription factor Foxp3 is the programmer for the suppressive function of Treg cells. Foxp3 mRNA and protein levels are reported as reduced in MS [69]. Recently CD8+ Treg cells were described and found to be decreased in blood and CSF of MS patients who were in an acute attack [70].

CD4+ Th17 cells are distinct from Th1, Th2, and Treg cells and have been associated with inflammation, autoimmunity, and response to extracellular pathogens [71]. Naïve T cells require exposure to transforming growth factor ß (TGFß) and either IL-6 or IL-21 to become Th17 cells, along with exposure to IL-23 produced by macrophages and dendritic cells [72]. IL-23 is a main driver. Th17 cells produce IL-17, which promotes inflammatory responses [68]. IL-17 messenger RNA (mRNA) is elevated in MS patients. These cells also produce IL-21 and IL-22. Th17 cells excel at infiltrating tissues to cause severe inflammation. They express the che-

mokine receptor CCR6 on their surface [66]. Th17 cells are clearly implicated in MS [73–75].

CD8+ T cells function as cytotoxic/regulatory cells. They are activated in the periphery and then enter the CNS. They dramatically outnumber CD4+ T cells within MS lesions at all disease stages [70]. CD8+ T cells show the most profound and reproducible clonal and oligoclonal expansion [76], and memory CD8+ T cells are enriched in both blood and CSF of MS patients [77]. CD8+ T cells are associated with axonal injury in early MS [68]. They interact with autoreactive CD4+ T cells to suppress them. CD8+ T cells are also reduced during MS relapses [69].

A small subpopulation of T cells have a T cell receptor composed of γ/δ polypeptides, as opposed to the usual α/ß polypeptides. They are mainly located in skin and mucosal tissues. These γ/δ T cells are involved in both innate and adaptive immune responses and have been reported as clonally expanded in the CSF of early MS patients [78]. Another subset was associated with very aggressive MS [79]. They are increased in MS lesions and may be involved in oligodendrocyte lysis [80]. γ/δ T cells in the EAE model control inflammatory cell migration into the CNS, promote apoptosis of encephalitogenic T cells, and play a key role in recovery. Their potential role in MS remains to be determined. Terminally differentiated effector memory γ/δ T cells were decreased in the periphery during relapse [81].

There is also a small subpopulation of CD20+ T cells. They make up 3–5% of T cells and are proinflammatory with a high proliferative capacity to CNS antigens. They were reported to be enriched in the blood and CSF of MS patients [82].

B Cells

B cells play a major role in MS (Table 3.4). B cells and plasma cells are present in the brain and CSF of MS patients [83]. There are clonal expansion and somatic mutation of B cell receptor genes, consistent with an antigen-driven response [84, 85]. Healthy controls rarely show B cells in CSF. In contrast, MS patients show clonally

Table 3.4 Role for B cells in MS

B cells and plasma cells are present in CNS tissue
Express hypermutated immunoglobulins (local CNS antigen-driven activation)
Positive response to B cell-depleting anti-CD20 monoclonal antibody
In relapsing MS
In PPMS
CSF oligoclonal bands and intrathecal immunoglobulin production as diagnostic signatures
Data suggests oligoclonal IgM/IgG; elevated IgG index indicates poorer prognosis
Lymphoid follicles in meninges of MS
EBV seropositivity required for development of MS (at least adult onset); EBV persistently infects B cells

expanded CSF memory B cells, centroblasts, and short-lived plasmablasts as the predominant antibody-secreting cell in CSF [86]. When meningeal lymphoid follicles with germinal centers are detected in SPMS patients, they are associated with younger age at MS onset, more severe disability, and more cortical demyelination [87]. Diffusion of antibodies or other soluble factors from the meningeal follicles to the cortex may be responsible for enhanced gray matter lesions. EBV causes persistent infection of B cells, and this results in immunologic changes that might promote development of MS.

Oligoclonal IgG in CSF is a hallmark diagnostic signature in MS. The specificity of these bands is not known, but they are not directed against myelin components and could represent a non-specific polyantigenic exposure response [88]. In a recent study, MS oligoclonal bands were at least partly directed against ubiquitous intracellular autoantigens released during tissue destruction [89]. Oligoclonal IgM (lipid specific) in particular has been suggested to be a poor prognosis marker [90]. Although initial reports suggested anti-myelin antibodies to MBP and MOG might indicate more severe disease, these findings have not been confirmed [91–93]. With a more accurate cell-based assay, anti-MOG IgG appears to be associated with unique non-MS disorders including seronegative NMO spectrum disorder, ADEM, chronic relapsing inflammatory optic neuropathy, and isolated cases of optic neuritis, transverse myelitis, and encephalitis [94].

Elevated IgG index, as a marker of intrathecal immunoglobulin production, is another less specific CSF diagnostic marker.

Perhaps the most impressive data supporting a role for B cells in MS is the success of anti-CD20 monoclonal antibody therapy in relapsing and PPMS [95, 96]. This anti-inflammatory response likely represents an effect on B cells as APCs and T cell regulators, since any effect on humoral antibody production is likely to be delayed for some years.

Plasma Cells

Plasma cells, along with B cells, also show clonal expansion in MS CSF [97]. Plasma cells show a distinct pattern from T and B cells. They accumulate in connective tissue spaces in the meninges and perivascular space [16]. They accumulate in MS CNS tissue later but (unlike T and B cells) will persist. They do not show proliferative markers, suggesting they are long-lived cells [98]. They are most marked in progressive MS. Clonally expanded plasma cells are the presumptive source of CSF oligoclonal bands and intrathecal immunoglobulin production [99].

Monocytes/Macrophages

The mononuclear phagocyte system consists of circulating blood monocytes, tissue macrophages, and dendritic cells [100]. Blood-borne monocytes/tissue macrophages are known to be the major cell type in the perivascular infiltrates in MS. Monocytes enhance T cell migration across the BBB and are the precursor for macrophages. There are three subsets (classic, nonclassical, intermediate) based on expression of CD14 and CD16. MS patients have been reported to show increased levels of nonclassical CD14+ CD16++ monocytes and decreased classic monocytes (CD14++, CD16−) [101]. Monocytes/macrophages along with microglia represent innate immunity. They play an important role in lesion pathogenesis and local tissue injury, in phagocytic removal of debris, as well as in repair processes [102].

Early peripheral blood monocyte count was reported to correlate with MS severity in a Japanese population [103].

Dendritic Cells

Dendritic cells are professional APCs which initiate primary immune responses and develop and maintain immune tolerance [104]. They are the most potent APC. There are myeloid (CD11c+) and plasmacytoid (CD11c^{dim} CD123) types [105]. They are typically elevated in the blood, CSF, and lesions of MS [105, 106]. MS dendritic cells secrete higher levels of proinflammatory cytokines (IL-6, TNF, IL-23) than healthy controls and show decreased expression of maturation markers [105]. There may be deficiencies in the maturation of dendritic cells in PPMS [106, 107].

Recent studies have explored how dendritic cells enter the CNS (BBB, choroid plexus, meninges) and traffic to draining lymph nodes [108]. Tolerogenic DCs have also been proposed as a therapy for MS [109].

Natural Killer (NK) Cells

NK cells, part of innate immunity, are a subset of lymphocytes that are cytotoxic to virus-infected cells and tumor cells; they also secrete cytokines [110]. These distinct functions coincide with two subsets, CD56^{dim} NK cells and CD56^{bright} NK cells. NK cells are found in MS lesions. NK cell abnormalities are described in MS, including certain subpopulations being increased during periods where patients are not actively relapsing [111, 112]. Other subpopulations are reduced in untreated MS, as well as first-attack patients [110, 113]. A number of MS therapies (IFNß, cyclophosphamide, natalizumab, dimethyl fumarate) increase NK cells [110, 114, 115].

Invariant NK-T cells share properties of NK cells and T cells. These cells are reported as decreased in MS, and cell lines isolated from MS patients show higher secretion of IL-4 [66, 110].

It is debated whether enhancing NK cells is beneficial or detrimental in MS [116–118]. Clearly further work is needed.

Mast Cells

Mast cells are innate immune cells that are involved in allergic reactions. They contain granules and can release histamine and cytokines when activated [119]. Mast cells are found in the brain and meninges normally and are certainly present in MS brain. Elevated levels of CSF histamine and tryptase (a specific mast cell enzyme) have been reported in MS CSF [119–120]. Mast cells may play a role in MS meningeal inflammation and neuroinflammation [119, 121] and are the therapeutic target in the MS masitinib trial [122, 123].

Immune System Factors

Cytokines

Proinflammatory Th1 cytokines include IL-17, IL-22, IL-23, granulocyte-macrophage colony-stimulating factor (GM-CSF), TNFα, IL-1, IL-6, IL-8, IL-12, and IFNγ. They are opposed by anti-inflammatory/regulatory Th2 cytokines such as IL-4 and IL-10 [124]. A number of reports find various proinflammatory cytokines elevated or upregulated in MS, while certain regulatory cytokines may be downregulated [124–130]. It has also been noted that there are gene pathways associated with MS that involve cytokine production, in addition to other mechanisms [124]. Several MS disease-modifying therapies are believed to work in part through a Th1 to Th2 shift. The proinflammatory IFNγ cytokine has been reported to worsen MS [131], while IFNβ is used to treat MS. This cytokine picture is not straightforward. Blockade of another proinflammatory cytokine, TNFα, actually worsens MS [132, 133]. Progressive MS patients are reported to show greater IL-12 and IL-18 production by systemic immune cells [56, 134]. Targeted cytokine therapies continue to be therapeutic strategies.

Chemokines

Chemokines are a family of small cytokines involved in chemo-attraction and cell migration. They are also involved in adhesion molecule expression, matrix metalloproteinase and cytokine secretion, T cell activation, and synaptic transmission [135]. They are part of a network which also involves chemokine receptors. Chemokines control the selective CNS recruitment of inflammatory cells in MS [136]. In particular CCL5, CCL2, CXCL10, and CXCL13 are implicated in MS. CCL5 (Rantes) is upregulated at the edge of MS plaques to attract monocytes [137]. CCL2 is expressed by local astrocytes to attract mononuclear cells [138]. MS CSF shows elevated levels of CXCL10 and CCL5, which draws activated T cells, and CXCL13, which draws B cells [135, 139]. CCL18 is reported to be elevated in the blood of MS patients, especially with progressive disease [140]. Chemokines are another potential therapeutic target in MS.

Osteopontin

Osteopontin, early T lymphocyte activation-1, is a proinflammatory cytokine expressed by activated T cells, dendritic cells, and macrophages [59, 141]. It is a member of the small integrin binding-proteins, the SIBLING family [142]. It binds to α4ß1 integrin and modulates Th1 and Th17 cytokines, and studies have reported elevated levels in the blood and CSF during relapses [143–145]. High expression of osteopontin has been found in MS brain lesions using cDNA microarray technology [146]. It has been proposed as a blood and CSF biomarker in MS [147].

Adhesion Molecules

Cell adhesion molecules are located on the surface of cells and mediate binding to other cells or to extracellular matrix via an adhesion process. This is important to cell penetration into the CNS in MS. Adhesion molecules involve four families (immunoglobulin superfamily, integrins, cadherins, selectins). The endothelial cells within MS lesions express elevated levels of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [136]. Activated immune cells express selectins and integrins such as lymphocyte function-associated antigen (LFA)-1 and very late antigen (VLA)-4, which bind to their ligand adhesion molecules on the endothelium. Anti-adhesion molecule therapy (such as natalizumab, a monoclonal antibody directed against ά4 integrin, a component of VLA-4) has been used successfully to treat MS. In a recent study, another immunoglobulin superfamily member, neural cell adhesion molecule (NCAM or CD56), was shed from neural and glial cells [148]. Plasma levels of soluble NCAM correlated with soluble VCAM-1 levels in MS and health controls. In contrast, only MS showed correlated elevations in soluble ICAM-1 and VCAM-1. Levels of soluble NCAM ($p = 0.05$) and VCAM-1 ($p = 0.028$) were higher in progressive MS compared to healthy controls [148].

Matrix Metalloproteinases

MMPs are part of a family of almost 40 endopeptidases, proteolytic enzymes that are involved in extracellular matrix and basement membrane degradation. These proteases include tissue inhibitors of matrix proteases (TIMPs), which downregulate MMPs. Activated immune cells secrete MMPs to help penetrate through the BBB basement membrane and extracellular matrix, to enter the CNS. Matrix proteinases may directly injure CNS cells, as well as activating membrane-bound proinflammatory cytokines, but may also promote CNS repair and regeneration. MMPs are implicated in BBB permeability and CNS inflammation in MS.

MMP-9 is elevated in the serum and CSF of MS, especially during relapses [149]. Elevated serum and CSF MMP-9 levels are reported to correlate with MS disease activity [150]. MMP-2 to TIMP-2 ratio is increased in the CSF and serum of relapsing MS, with evidence of

intrathecal MMP-2 production [151]. Serum MMP-2 and MMP-2/TIMP-1 ratio is said to be elevated in progressive MS [152]. In a recent study, MMP-9 gene expression and protein levels were significantly reduced at baseline in MS patients destined to develop progressive multifocal leukoencephalopathy on natalizumab, compared to healthy controls [153] .

CNS Cells and Components

Microglia

Microglia are the resident CNS macrophages as well as immune cells. They act as APCs, produce cytokines, and are involved in phagocytosis. Along with astrocytes, they modulate CNS inflammation and neurodegeneration [154]. They show plasticity, with neuroinflammatory as well as neuroprotective properties shaped by their CNS microenvironment [155]. In fact, both microglia and macrophages are sometimes classified as M1 (proinflammatory) or M2 (anti-inflammatory), although this division has been challenged [156]. Activated microglia are noted in all MS patients but are especially associated with the progressive phenotypes. Clusters are also found in MS normal-appearing white matter [157].

Activated microglia and macrophages produce cytotoxic molecules including proinflammatory and cytotoxic cytokines, reactive oxygen and nitrogen intermediates, and proteolytic and lipolytic enzymes [102]. Microglia are likely to be an important component of the MS damage process and are particularly involved in axonal injury [158]. It has been suggested that T cells in MS tissue may drive continued activation of microglia [159]. Microglia also promote repair, since they can secrete neurotrophins such as brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and insulin-like growth factor-1 (IGF-1), as well as regulatory cytokines [110]. In a recent study of autopsy brains, on average, 45% of the macrophage-like cells in active lesions were calculated to be derived from resident microglia [156]. Active lesion

microglia showed a proinflammatory phenotype, which changed in later stages to an intermediate phenotype.

Oligodendrocytes and Myelination

Oligodendrocytes show variable degrees of loss in MS. They may be an early target and can die by apoptosis prior to formation of demyelinating plaques [160]. Oligodendrocytes are especially vulnerable to oxidative stress because of their high metabolic rate, high ATP usage to synthesize myelin, large intracellular iron content, high hydrogen peroxide level, high levels of polyunsaturated fatty acid within the myelin, and low levels of antioxidants [161]. It has been suggested that intrinsic apoptosis due to oxidative stress is an important cause for oligodendrocyte loss in MS. Fas/CD95 is expressed on oligodendrocytes in chronic MS lesions; FasL expressed on microglia and inflammatory lymphocytes are likely to play a role in intrinsic oligodendrocyte apoptosis [161, 162].

Remyelination involves generating new mature oligodendrocytes [163]. Oligodendrocytes can be replaced by OPCs, a population of adult CNS stem/precursor cells widely placed within the adult CNS. They are present in white and gray matter at a density similar to microglia. They appear to be the main source of remyelination in MS, as opposed to surviving adult oligodendrocytes. However, in two recent animal models, adult oligodendrocytes did participate in myelination [164]. There is also recent evidence for MS-specific oligodendrocyte lineage cells [165]. At least in animal models, adult neural stem cells (in the third ventrical subventricular zone and the dentate gyrus subgranular zone) contribute to oligodendrogliosis [166]. Remyelination in MS is ultimately inadequate and fails. This is likely to represent in part non-disease-related factors (genetic and immune system background, sex, increasing age) as well as a failure of OPC differentiation and maturation [163, 167, 168].

Along with oligodendrocyte loss, MS involves extensive demyelination. Myelin is stripped and

Table 3.5 Possible basis for myelin and oligodendrocyte injury in MS

Cell based
CD4+ T cells sensitized to myelin antigens
Antigen-specific cytotoxic CD8+ T cells
Astrocyte disturbance
Immune system factors
Proinflammatory cytokines
Antibody-mediated injury
Complement cascade components
Bystander demyelination following infectious superantigen cell activation
Macrophage-mediated injury
Hypoxic/ischemic stress
Reactive oxygen or nitrogen species; oxidative, endoplasmic reticulum stress
CNS tissue infection
Axonal dysregulation with 2° myelin loss
Glutamate excitotoxicity
Excitatory amino acid transporters
Proteolytic, lipolytic enzymes
Fas antigen-ligand interactions
Unstable myelin
Apoptosis (programmed cell death)

phagocytized by macrophages. The basis for myelin and oligodendrocyte injury is unknown and likely multifactorial (Table 3.5). There could even be intrinsic myelin instability.

MBP in MS shows a higher rate of citrullination/deimination (45%) compared to controls (15–20%) [160]. This is developmentally immature myelin, which is less compact and therefore destabilized. An unproven hypothesis is that unstable MBP is a primary factor leading to MS [45, 169, 170].

Astrocytes

Astrocytes are the most abundant CNS cell and are involved in BBB function, glutamate metabolism, weak APC activity, extracellular potassium maintenance, and release of trophic factors for surrounding cells [171]. It is possible that MS could represent a primary disturbance of astrocytes, considering that another CNS inflammatory demyelinating disorder, neuromyelitis optica spectrum disorder, often targets an astrocyte water channel, aquaporin 4

(AQP4). MS patients show changes in sodium channels in reactive astrocytes. There is focal upregulation of the sodium channel Nav 1.5 within, as well as at the edge of, active and chronic MS lesions [172]. This upregulation is not seen in MS NAWM or control brain. It is also seen in astrocytes surrounding brain tumors and cerebrovascular accidents, suggesting it is a compensatory mechanism to CNS damage. Very active MS lesions show structural changes involving astrocytes [173]. Acute MS lesions were reported to show loss of astrocytes along with their food processes that accompanied demyelination [174]. Resolving lesions were repopulated with AQP4-negative stellate astrocytes, but astrocytes were mainly AQP4 positive in older lesions. Decreased levels of creatine kinase B, localized to astrocytes, was reported in the white matter of MS patients but not controls [175]. Activated astrocytes have been shown to promote B cell survival and activation, which may be particularly important in progressive MS [176]. It is clear that the role of astrocytes in MS is not just to form glial scars but to play a role in lesion formation, recruitment of lymphocytes, tissue damage, and tissue repair [177].

Neurons/Axons

Neurons, along with dendrites, axons, and nodes of Ranvier, are damaged and lost in MS [39, 178]. This is not just sequelae of loss of trophic myelin, although demyelinated axons express increased sodium channels and deficits in ATP production, making them more vulnerable to physiologic stressors [179]. The increased expression of sodium channels on the demyelinated axon leads to excess sodium within the axons, requiring increased ATP to correct the sodium concentration. This increased energy demand, along with mitochondrial dysfunction, leads to axonal hypoxia [180]. This appreciation of ion channel changes has led to voltage-gated sodium channel blockade being proposed as a strategy to treat MS [181].

Acute axonal injury is prominent in active inflammatory plaques and correlates with inflammation (CD8+ T cells, macrophages, microglia) [16, 33]. Axonal injury does not require demyelination. Retinal nerve fiber layer, made up of unmyelinated axons, can be evaluated by optical coherence tomography and shows deficits in MS [182]. These include thinning of the retinal fiber layer and thinning of the ganglion cell-inner plexiform layer [183]. Within cortical lesions, neuronal loss is estimated to be at least 20% of the total cell population [184].

The mechanism of axon damage is believed to be multifactorial, mediated by inflammatory cells and soluble factors, loss of trophic support from myelin and glia, Wallerian degeneration, and antibodies. Autoantibodies directed against axo-glial gray matter antigens such as contactin 2, neurofilament light chain, and neurofascin are found in CSF and serum of MS patients [185]. Contactin 2 is a cell adhesion molecule expressed by neuronal subpopulations and juxtaparanodal axon/myelin [180, 186]. Neurofilaments are part of the axonal cytoskeleton. Elevated levels of the light and heavy subunits are reported in the CSF of progressive patients [187]. Neurofilament light protein is also elevated in CSF during acute relapses [188]. Increased CSF neurofilament levels may predict a worse prognosis [189]. Neurofascin is a cell adhesion molecule expressed by oligodendrocytes at the paranode [190, 191]. About 30% of MS patients show antibodies to neurofascin, an axonal component. This is much more common in progressive vs. relapsing MS and seems to enhance axonal injury.

In addition, the neuronal 14-3-3 proteins are reported as elevated in the CSF of patients with more severe disability and disease progression [192]. Abnormally phosphorylated tau, with formation of insoluble tau, has been correlated with transition to secondary progressive MS, implicating tau as a neuronal damage mechanism [2].

Neurofilament light protein (an axon/neuron injury marker) can be measured in blood. It is being proposed as a future prognostic and treatment response biomarker for MS [193].

Blood-Brain Barrier and Vascular System

The BBB involves multiple players that form a neurovascular unit: endothelia, perivascular astrocytes, pericytes, myocytes, neurons, and extracellular matrix components [194]. These endothelial cells lack fenestrations and show reduced pinocytotic activity [195]. There are tight junctions and adherens junctions between cells. The BBB is disrupted in MS at two levels [196]. First, there is marked focal disruption characterized by contrast enhancement on neuroimaging, associated with early focal edema and inflammation, which results in macroscopic plaque formation. This is characteristic of relapsing MS but is also seen in progressive MS. Second, there is a much more subtle but diffuse BBB disturbance with abnormal permeability, tight junction disturbances, and changes in basement membrane and extracellular matrix [196–198]. This is present in normal-appearing brain tissue, and not just the lesion areas. The BBB is also immunologically activated in MS, with upregulation and expression of surface markers as well as secretion of immune factors [194]. Although BBB abnormalities have been thought to be a secondary phenomenon in MS, it is not ruled out that they could reflect a primary disturbance [195].

There are reports of early cerebral blood flow reductions and decreased cerebrovascular reactivity in MS [199]. Deposition of blood-derived fibrin has been proposed as an immunotherapeutic target in MS [200]. Down regulation of claudin-11 is reported at the BBB, blood-spinal cord barrier, and blood-arachnoid barrier in MS [201].

It has also been proposed that the pericyte might offer a new therapeutic target for MS [202].

Excitotoxins

Glutamate is the major excitatory amino acid. Excess glutamate is capable of causing cell death. Glutamate-mediated excitotoxicity involves activation of ionotropic and metabotropic receptors, with calcium cytoplasmic

accumulation leading to cell death. Glutamate is elevated within MS lesions and normal-appearing white matter, as well as CSF. This could be from activated immune cells, astrocytes, or axons [8]. AMPA, NMDA, and kainate receptors are all upregulated. Glutamate transporter expression is also altered [203]. Genetic variation is reported to play a role in glutamate levels [204]. Astrocytes, oligodendrocytes, and myelin all express glutamate receptors. They are all potential targets for excitotoxic damage. Finally, glutamate directly activates T cells [205]. Abnormal CNS glutamate levels and signaling, as well as glutamate activation of T cells and glutamate release by T cells, may all contribute to MS pathophysiology [205].

Nitric Oxide

Nitric oxide (NO) is a free radical implicated as a damage mechanism in MS. It impairs the BBB [206]. Elevated levels of NO will modify function of ion channels, transporters, and glycolytic enzymes, resulting in axonal damage. Both NO and its derivative, peroxynitrite, inhibit neuronal and glial mitochondria and the ability of the axon to generate ATP. NO impairs oligodendrocyte metabolism by damaging cell mitochondria [207]. A key enzyme involved in NO synthesis, NO synthase (iNOS), is upregulated in macrophages and reactive astrocytes in acute MS lesions [208]. NO activity in CSF rises during MS relapses [209–212]. It has been proposed that NO plays a key role in MS by stimulating local inflammation, disrupting the BBB, and increasing permeability, causing neuronal and DNA damage, disrupting axons and mitochondria, and inhibiting myelin formation genes [213].

Mitochondria

Mitochondria produce ATP, control calcium homeostasis, and play a role in apoptosis. They contain nonnuclear DNA which encodes subunits of the mitochondrial respiratory chain complexes. Mitochondrial abnormalities reported in

MS include reduced respiratory chain complex activities, increase in neuron mitochondrial DNA copies, and evidence of oxidative damage to mitochondrial DNA [37, 49].

Mitochondria have been implicated in both conduction block and axonal injury, through calcium-mediated cytoskeletal changes as well as oxidative stress [37]. Mitochondrial abnormalities are believed to play a key role in neurodegeneration [214] and have been proposed as a therapeutic target in MS [215].

Neurotrophic Factors

Neurotrophic factors encompass three families: nerve growth factor (NGF) family, glial cell line-derived neurotrophic factor (GDNF) family ligands, and neuropoietic cytokines [216]. They promote differentiation and survival of CNS cells and their components. They also increase antioxidant enzymes and inhibit free radical formation. They are secreted by activated immune cells and are the basis for the concept of neuroprotective autoimmunity. CNS inflammatory cells may help contain damage and boost repair and cell survival, by releasing these neurotrophic factors in MS brain and spinal cord [217–219]. Those that could have pertinence in MS include NGF, BDNF, NT-3 and NT-4, ciliary neurotrophic factor, and leukemia inhibitory factor [216].

Other Factors

Vitamin D

Vitamin D deficiency is a risk factor for MS, at least among Caucasians. Vitamin D is obtained from synthesis in the skin (triggered by sunlight) and dietary intake. 25-Hydroxy vitamin D is the major circulating metabolite and is the one to measure in blood, while 1,25-dihydroxy vitamin D (calcitriol) is the biologically active metabolite. Biologic effects are mediated via the vitamin D receptor [220], a member of the steroid receptor superfamily. Vitamin D receptor is expressed by monocytes, APCs, and activated lymphocytes.

Vitamin D appears to shift immune responses to a more anti-inflammatory regulatory role and enhances Treg function. Various MS susceptibility genes are located within or near genes associated with vitamin D metabolism [221]. Higher levels may play a role in treating MS, but this is not yet clearly proven [222]. The role of vitamin D in MS is the subject of ongoing research.

Exosomes and MicroRNAs

Exosomes are small (50 nm to 1 µm) extracellular vesicles that provide cell-to-cell communication [223]. They are involved in immune regulation. These vesicles contain proteins, lipids, transcriptional factors, RNA, and DNA [224]. They contain microRNAs (miRNAs), a group of small single-stranded non-coding RNA molecules 21–23 nucleotides in length. They degrade mRNA or repress mRNA translation. They can impact the genetic program of their target cell and influence both innate and acquired immune responses.

Exosomes are increased in the serum and CSF of relapsing MS, particularly during an acute attack [224]. Serum exosomes contain myelin proteins in both MS and healthy controls; exosomes containing MOG correlated with disease activity [223]. Exosome release in MS appears to facilitate immune cell transportation across the BBB.

miRNAs are being studied in the circulation of MS patients, as well as within MS immune cells. This appears to be a promising area to shed further light on the immunology of MS [225–227].

Gut Microbiota

There is a very important gut-brain axis, with bidirectional communication [228]. The gut microbiota influences systemic immunity and inflammation, including within the CNS [229]. There may be a gut microbiota that predisposes to MS, as well as a gut microbiota that can treat MS [230, 231]. This is discussed further in Chap. 5.

Summary

CNS damage in MS is mediated by a number of immune and inflammatory factors beyond the CD4 Th1 cell. Key immune factors may differ based on subsets of MS patients and different stages of the disease. A better appreciation of this immune system complexity is guiding therapeutic developments.

Novel targets are being proposed (ion channels, mitochondria, NO, glutamate) that may lead to improved outcomes. Current personalized medicine emphasizes an individualized approach. Future precision medicine will use validated molecular markers to guide optimal diagnosis and management.

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