CHAPTER

30

Multiple Sclerosis

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OUTLINE

INTRODUCTION

General Definition

Multiple sclerosis (MS) is a chronic inflammatory demyelination disease of the human central nervous system (CNS) that affects young adults and over subsequent decades transforms into a progressive neurodegenerative disorder associated with major clinical disabilities.[1] The main pathological lesions of MS are multiple sclerotic plaques displaying demyelination of white and gray matter in the brain and spinal cord.[2] Within these focal lesions the myelin sheaths and the oligodendrocytes are destroyed after myelin protein components are recognized by cells of the immune system.[3] This inflammatory reaction includes the activation of myelin-specific $CD4^+$ autoreactive T cells and their differentiation into a pro-inflammatory T-helper-1 (Th1)-like phenotype, and also $CD8^+$ T cells, B cells secreting myelin-directed antibodies and cytokines, and other factors of the immune system.[4,5]

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During the initial state of the inflammatory response, activated lymphocytes recruited from the periphery to the CNS cross the blood–cerebrospinal fluid (CSF) barrier and the blood–brain barrier (BBB).[6,7] The presence of activated immune cells, as well as immunoglobulins and cytokines in the CSF of MS patients compared with control donors, suggests the participation of the immune system in MS pathogenesis.[6,7] The resulting pathological features in the CNS are the (chronic) demyelinated plaques, which consist of a well-demarcated hypocellular area characterized by the loss of myelin, relative preservation of axons, and formation of astrocytic scars. Lesions can be observed in the optic nerves, the periventricular white matter, brainstem, cerebellum, and spinal cord white matter, and can also involve gray matter.[1,2] Within MS lesions, myelin, oligodendrocytes, and axons are destroyed, and axonal dysfunction is likely to result from nerve conduction disorders following demyelination.

The concept of MS as an autoimmune disease is confirmed by experimental autoimmune encephalomyelitis (EAE), the most widely used animal model of MS. EAE is typically induced in animals either by injection of an emulsion containing a fragment of a myelin membrane protein or a homogenate of spinal cord tissue, or by injection of myelin antigen-specific T cells. Following disease induction, activated T cells cross the BBB and initiate a disease course similar to that observed in MS (inflammation, demyelination, and axonal degeneration).[4] Genetic susceptibility to MS is associated with several genes of the major histocompatibility complex (MHC) regions, for example the *HLA-DRB1* gene on chromosome 6p21; most of them are likely to have immune response functions.[6]

In MS pathogenesis, demyelination results in a progressive loss of structure and function of neurons, namely neurodegeneration, due to impaired propagation of action potentials across the demyelinated axons. No explanation has yet been given for the temporal relationship between inflammation, demyelination, and axonal degeneration, which could be a key point in understanding MS pathogenesis. It is still unclear whether inflammatory reactions cause neurodegeneration through demyelination, or whether MS is a primary neurodegenerative disease with secondary inflammation and demyelination. The accumulation of demyelinated lesions may lead to the neurological features of MS and explain the heterogeneity of the disease. During early stages of MS, spontaneous remyelination – the process by which oligodendrocyte progenitor cells (OPCs) re-ensheath demyelinated axons – helps to restore axonal conduction. However, over time, remyelination fails to compensate for the progression of inflammation-driven demyelination and consequent axonal damage/dysfunction leads to permanent, irreversible neurological decline.[2,8]

Different Forms of Multiple Sclerosis

The current classification of MS clinical forms distinguishes at least four clinical patterns.

- *Relapsing remitting multiple sclerosis (RRMS)* is the most common form (about 85% of cases) and is characterized by discrete attacks (relapses) over days or weeks, described as episodes of acute worsening of neurological functions. These are followed by complete or partial recovery periods (remissions) over months or years, supposedly without any disease progression.
- *Secondary progressive multiple sclerosis (SPMS)* is characterized by gradual neurological decline with occasional minor recoveries. Most cases of RRMS (50% within 10 years of the initial diagnosis) evolve into SPMS (Table 30.1 and Fig. 30.1).
- *Primary progressive multiple sclerosis (PPMS)* affects about 15% of patients with MS. This clinical pattern of disease is characterized by a steady functional worsening from the onset of symptoms, without identifiable relapses or remissions.
- *Progressive relapsing multiple sclerosis (PRMS)* is also characterized by steadily worsening symptoms from the onset, although with clear acute relapses with or without recovery observed. Heterogeneity of clinical features within these patterns increases the difficulty in understanding the pathogenic mechanisms responsible for the onset of the disease.[9]

Therapeutic agents that have been proposed so far primarily target the immune system and help to slow the progressive deteriorating effects of the disease, but do not cure it or reverse the damage.

Epidemiology

MS usually affects young adults, between 20 and 40 years old, with a later onset of disease for PPMS than for RRMS.[10] MS displays different incidence depending on gender, age, geographic distribution, and ethnic origin. Women are more susceptible to MS, the ratio women to men approaching 2:1 to 3:1, which suggests the possible involvement of sex hormones in susceptibility to MS.[11] However, the clinical pattern of PPMS does not show a female predominance.[10]

Pediatric MS accounts for 3–4% of cases and corresponds to symptom onset before age 18. The initial disease course of MS in children and adolescents is similar to that of adults affected by RRMS, with similar symptoms including cognitive deficits. Despite the variability in studies, it seems that pediatric MS patients reach the progressive stage of the disease later than patients with adult-onset MS (within 20 years from onset). Limited data are available regarding the immunopathogenesis

TABLE 30.1 Clinical Forms of Multiple Sclerosis

Clinical Form	Disease Course
Relapsing–remitting multiple sclerosis (RRMS)	85% of cases
	Age of onset between 20 and 30 years
	Characterized by discrete episodes of acute worsening of a given neurological function (relapses) over days to weeks, followed by complete or partial recovery periods (remissions) over months or years
	Female to male ratio between 2:1 and 3:1
	Early clinical symptoms: weakness, diminished coordination of limbs, optic neuritis, and sensory disturbance
Secondary progressive multiple sclerosis (SPMS)	Evolution of 50% of RRMS cases within 10 years of the initial diagnosis
	Characterized by gradual neurological decline with occasional minor recoveries
	Clinical features: inability to walk, progressive paralysis, brain and spinal cord atrophy
Primary progressive multiple sclerosis (PPMS)	About 15% of cases
	Later onset than RRMS (about 10 years)
	Characterized by steady functional worsening from the onset of the disease, without identifiable relapses or remissions
	Female to male ratio: 1:1
	Clinical features: starts with clinical disability, brain and spinal cord atrophy
Progressive relapsing multiple sclerosis (PRMS)	May be a variant of PPMS
	Characterized by steadily worsening disease from the onset, with clearly superimposed acute relapses with or without recovery

Source: Lublin and Reingold. Neurology. *1996;46(4):907–911.*[9]

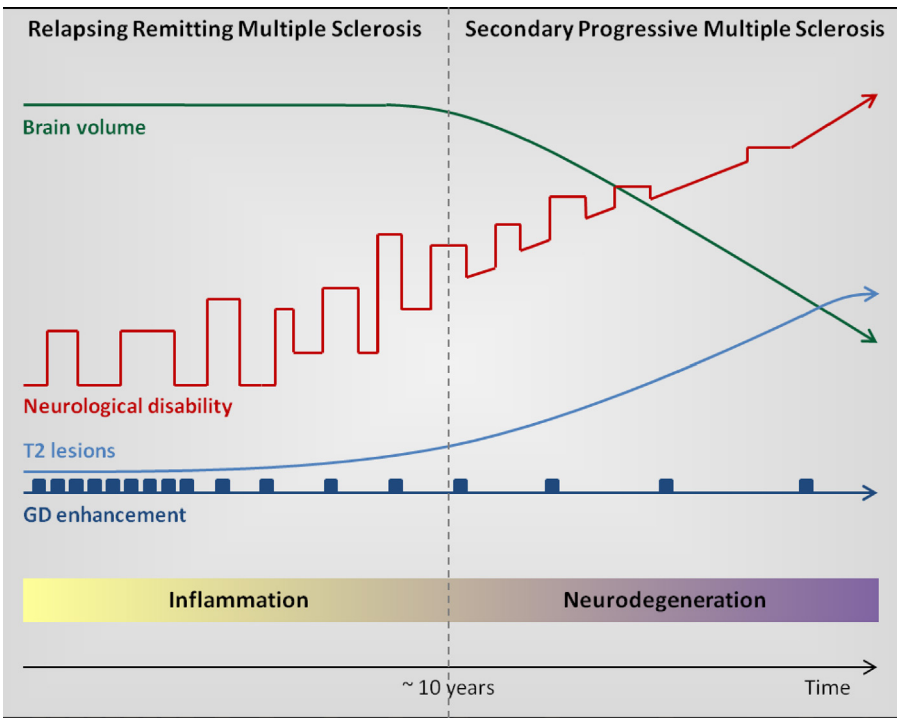

FIGURE 30.1 Schematic description of the clinical evolution of relapsing–remitting into secondary progressive multiple sclerosis over the decades. The graph shows neurological disability, brain atrophy, frequency of inflammatory events [T1 lesions with gadolinium (GD) contrast enhancement showing blood–brain barrier breakdown] and global level of tissue damage (T2 lesions).

of MS with childhood onset. However, pediatric MS patients display an increased activation of the innate immune system, at a higher level than observed in adult MS patients, whose pathophysiology is more dominated by the activation of the adaptive immune response. Magnetic resonance imaging (MRI) of pediatric MS cases shows fewer T2-weighted lesions and a higher frequency of large diffuse MS lesions than in adult MS cases. The first acute episode in MS at an early age has to be distinguished from a single neurological event, such as acute disseminated encephalomyelitis symptoms, which include those of optic neuritis.[12]

MS affects approximately 300,000 people in the USA and about 1 million worldwide,[13] raising critical socio-economic, health, and care issues. Population- and family-based studies have observed a north to south gradient in disease prevalence in the northern hemisphere and the opposite in the southern (Fig. 30.2). The prevalence of MS per 100,000 population is higher than 60 in Europe and North America,[14] whereas the risk of developing MS in Asia and Africa is low. The geographic distribution of MS may be explained by environmental factors such as sunlight exposure or climate, as well as genetic susceptibility among populations. Migration studies have shown that the risk of developing MS is low if an individual migrated from a region with a high prevalence rate to one with a low prevalence rate before age 15, whereas it did not change after the age of 15, supporting the hypothesis that individuals born in low-risk areas benefit form long-lasting protection without transmission to their children. It has been suggested that ultraviolet light exposure may negatively influence disease development through its suppressive effects on the immune system, or through its involvement in the biosynthesis of vitamin D, one metabolite of which may have a role in inflammation.[11] Other behavioral or lifestyle factors such as industrialization, urban living, pollution, smoking habits, or diets may explain the disease distribution and the increasing worldwide prevalence over recent decades.[7]

ETIOLOGY OF MULTIPLE SCLEROSIS

Genetic Factors

MS is thought to emerge in genetically susceptible individuals who encounter external factors that trigger the inflammatory reaction against self antigens in the CNS. Different strategies – population-based association studies, family-based linkage methods, systematic genome screens – have been used to identify candidate genes that may have a role in the initiation of the disease.[15] Several family studies in different European regions have shown that first, second, and third degree relatives of affected

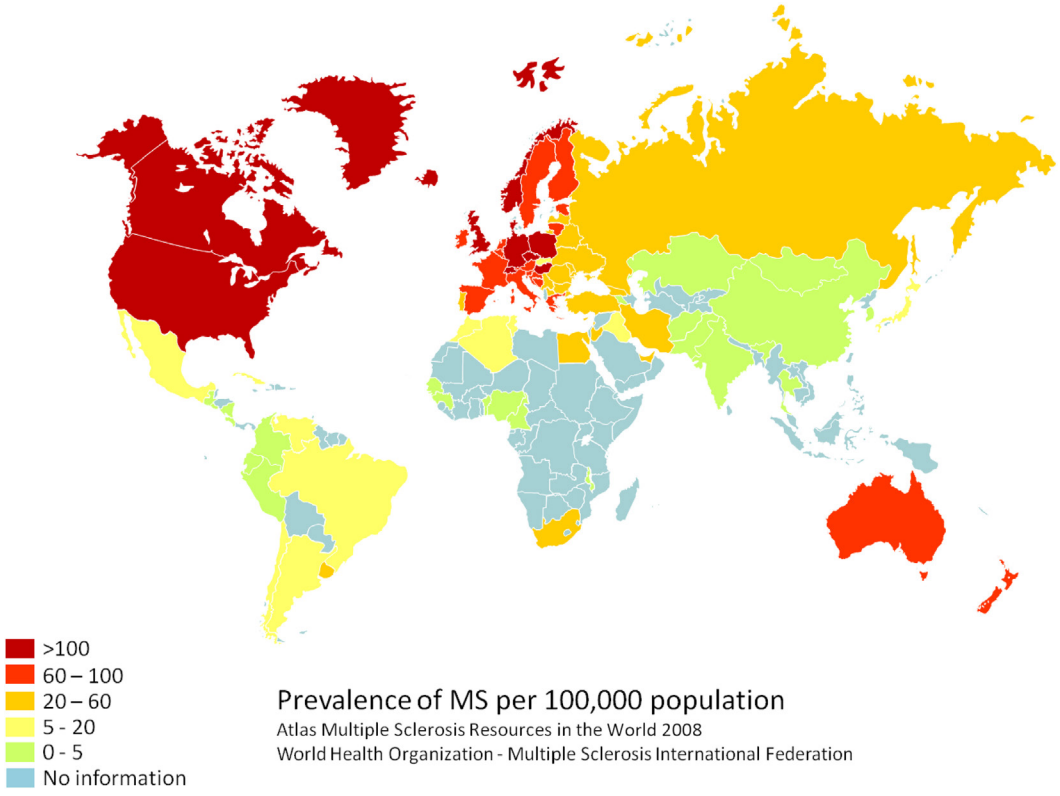

FIGURE 30.2 **Prevalence of multiple sclerosis (MS) per 100,000 population in 2008.** *Source: Data from World Health Organization; 2008. Atlas Multiple Sclerosis Resources in the World 2008.*[14] *Figure produced using Servier Medical Art.*

individuals display a higher risk of developing MS.[16] Studies of twins have shown an excess of monozygotic concordance compared with dizygotic concordance. Adoption studies and comparison of half-siblings and full-siblings reveal that genetic sharing is essential in familial aggregation of the disease, although discordance between most monozygotic twins also suggests a critical role of environmental factors in disease onset.[16]

The involvement of genes of the MHC region on chromosome 6p21 in disease susceptibility is one of the most consistent results of genetic association.[16] In particular, it is clear that specific haplotypes of the human leukocyte antigen (HLA)-DRB1*1501, -DQB1*0602, and -DQB1*0603 of MHC class II are strongly associated with higher susceptibility to MS. Higher frequencies of HLA class II allele DRB15 have been found in patients showing early-onset disease.[16] This increased susceptibility may be due to the *HLA-DRB1* and/or *HLA-DQB1* genes themselves, related to the function of these molecules in the immune response: antigen presentation or binding, T-cell recognition and selection of peptides, and intracellular signaling pathways. HLA-DR and -DQ molecules have been suggested to have binding characteristics that promote presentation of specific sets of self peptides, for example myelin peptides.[7] MS-associated HLA-DR and -DQ molecules could play a role in the determination of T-cell repertoire.[7] Although there is less information about genetic risk associated with HLA class I alleles, additional susceptibility alleles in class I loci, independent from class II alleles, have been identified. HLA-A*0301 increases the genetic risk of developing MS independently of DRB1*15, DQB1*06, and HLA-A*0201 decreases general susceptibility. HLA class I molecules may participate in the disease via the same mechanisms as HLA class II molecules, or via their interaction with cytotoxic $CD8^+$ T cells and natural killer (NK) cells.[17]

Polymorphisms of the T-cell receptor (TCR) have also been suggested to be associated with autoimmunity and with susceptibility to MS, with no significant evidence.[16] An allele of apolipoprotein E_4 ($ApoE_4$), which is a factor in lipid metabolism and has a significant role in remodeling and repair of nerve tissue, may be linked to faster progression of MS.[7,18] Cytotoxic T-lymphocyte antigen (CTLA)-4 is thought to be a candidate susceptibility gene for many autoimmune diseases, such as Graves disease, type 1 diabetes, and MS. CC-chemokine receptor 5 polymorphism has been shown to influence MS pathogenesis. Other candidates whose functions are related to immune response have been proposed, including interleukin-1 (IL-1) receptor gene, immunoglobulin loci, and tumor necrosis factor (TNF)-α and β alleles, but without conclusive evidence.[7]

Technological advances, including great progress in complete genome sequencing, have enabled the analysis of thousands of single-nucleotide polymorphisms (SNPs) in thousands of individuals, and the subsequent identification of linkage to different genomic regions.[17] Genome-wide association studies (GWAS) are based on the screening of statistical differences between cases and controls for the most frequent alleles.[17] GWAS confirmed the strong association of the *HLA-DRB1* risk locus on chromosome 6p21.3, but susceptibility genes outside the HLA region also came out with a genome-wide significance ($p < 10^{-8}$). These included *IL2Rα* (encoding interleukin receptor-2α) on chromosome 10p15 and *IL7R* (encoding interleukin receptor-7) on chromosome 5p13, both involved in cell survival, apoptosis, and immune response; and *CD58* (lymphocyte function-associated antigen-3) on chromosome 1p13, participating in cell-to-cell adhesion and immune response.[18]

The International Multiple Sclerosis Genetics Consortium (IMSGC) in collaboration with the Wellcome Trust Case Control Consortium (WTCCC2) has performed a large-scale GWAS, based on a dataset of 9772 MS cases versus 17,376 non-affected individuals.[19] This study highlighted more than 50 non-MHC regions containing SNPs with strong evidence of association. Among the identified MS risk loci, 29 have never been reported previously (Table 30.2). Some of the candidate genes identified by the study encode molecules involved in the immune response (*IL2Rα*, *IL7R*, *TNFRSF1A*, *TNFSF14*) or in costimulatory and signal transduction (*CD58*, *CD86*, *STAT3*, *TAGAP*). Another candidate gene, *CYP27B1*, is related to vitamin D metabolism, which may influence the autoimmune reaction in MS. The mechanisms involving the molecules coded by susceptibility genes *IL2Rα* and *VCAM-1* are targeted by therapies for MS.[19] Another GWAS identified three SNPs that have never been reported with MS, located at the *EOMES*, *MLANA*, and *THADA* loci, which show strong evidence of association ($p < 10^{-9}$).[20] Some additional SNPs display suggestive evidence of association with MS, and are known to be associated with autoimmune disorders such as Crohn disease, psoriasis, and rheumatoid arthritis.[19,20]

The role of SNP *TNFRSF1A*, encoding tumor necrosis factor receptor-1 (TNFR1), has been investigated through functional studies. The MS risk allele leads to expression of a soluble form of TNFR1, thus blocking TNF binding and subsequent nuclear factor-κB (NF-κB) signaling and TNFR1-mediated apoptosis. The MS-associated TNFR1 variant may act like TNF-blocking drugs, which have been suggested to promote or exacerbate the disease course.[21]

The list of MS susceptibility-associated genes is far from being complete and requires proper interpretation and further functional studies.

Environmental Factors

Based on the geographic prevalence of the disease decreasing with exposure to sunlight, some studies have suggested that solar radiation may be protective in MS.

TABLE 30.2 Non-Major Histocompatibility Complex Single-Nucleotide Polymorphisms (SNPs) with Genome-Wide Significant Association with Multiple Sclerosis

Chromosome	SNP	*p* Value	Risk Allele	Alleles	Gene of Interest	Biological Function
1	rs11581062	2.50×10^{-10}	G	A/G	*VCAM-1*[a]	Cell adhesion
1	rs1335532	2.00×10^{-9}	A	A/G	*CD58*	Cell-to-cell adhesion, immune response
3	rs11129295	1.20×10^{-9}	T	C/T	*EOMES* [a]	Developmental processes
3	rs9282641	1.00×10^{-11}	G	A/G	*CD86*	Cell activation and survival, immune response
5	rs6897932	2.60×10^{-6}	C	C/T	*IL7R*	Cell survival, immune response
6	rs1738074	6.80×10^{-15}	C	C/T	*TAGAP* [a]	Cell activation, immune response
9	rs2150702	3.28×10^{-8}	G	A/G	*MLANA* [a]	Melanosome biogenesis
10	rs3118470	2.00×10^{-9}	C	C/T	*IL2RA*	Apoptosis, immune response
11	rs630923	2.80×10^{-7}	C	A/C	*CXCR5*	Cell migration, immune response
12	rs1800693	1.80×10^{-10}	C	C/T	*TNFRSF1A*	Activation of NF-κB signaling, apoptosis, regulation of inflammation
12	rs12368653	2.00×10^{-7}	A	A/G	*CYP27B1*	Vitamin D metabolism
17	rs9891119	4.60×10^{-7}	C	A/C	*STAT3*	Cell differentiation, immune response
17	rs8070463a	9.55×10^{-8}	T	T/C	*TBX21*	Developmental processes, control of cytokine expression
19	rs1077667	9.40×10^{-14}	C	C/T	*TNFSF14* [a]	Costimulatory factor, cell proliferation, apoptosis

NF-κB: nuclear factor-κB.
[a]*Novel susceptibility genes identified by Sawcer* et al.[19]
Source: Gourraud et al.,[17] *Oksenberg* et al.,[18] *Sawcer* et al.,[19] *and Patsopoulos* et al.[20]

A first mechanism is based on the immunosuppressive effect of solar ultraviolet radiation: sunlight exposure may increase suppressor T-cell activity and decrease helper and autoreactive T-cell activity.[7,18] A second mechanism that has been proposed involves vitamin D biosynthesis, which is responsive to sunlight exposure. The prevalence of MS is low where vitamin D is abundant, as in sunny regions. It has been suggested that the hormone 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], which is the most effective metabolite of vitamin D, may have a protective effect on genetically susceptible individuals. Receptors for this molecule are found in particular on activated T lymphocytes, and have been shown to be overrepresented in Japanese patients with MS. Strong evidence based on EAE models suggests that 1,25-$(OH)_2D_3$ is a natural inhibitor of the autoimmune mechanisms underlying MS.[11]

Behavioral and lifestyle influences are also thought to enhance the risk of MS. For example, smoking has been proposed to be associated with MS emergence in a large population of individuals living in Norway.[22] The risk

of developing MS among smokers has been shown to be twice as high as among non-smokers, which may in part be explained by tobacco's toxic effects on the immune system and CNS.[22]

Epidemiological studies have highlighted a possible influence of parasitic infections on the MS disease course. MS patients infected with helminths, eukaryotic parasitic worms living and feeding in the host organism (e.g. in the digestive tract), show significantly fewer relapses, decreased activity on MRI, and a generally reduced disease severity compared with uninfected patients. There is evidence of a direct immunosuppressive function of helminthic infections in the inflammatory-driven response in MS.[23] Parasitic infection may be associated with the secretion of suppressive cytokines such as IL-10 and transforming growth factor-β (TGF-β) by regulatory T cells, and with the inhibition of T-cell proliferation and production of proinflammatory cytokines such as interferon-γ (IFN-γ) and IL-12. The reduction in parasitic infections may thus account for the increased risk of developing MS observed in developed countries.[23]

Infectious Factors

Several infectious agents have been proposed as a possible cause of MS, such as Epstein–Barr virus (EBV), rubella virus, measles virus, retroviruses, herpes virus, and zoster virus,[6,7] but such associations are unconfirmed. The most consistent evidence of involvement of a potential infectious etiology for MS is based on epidemiological studies.

EBV is a ubiquitous B-lymphotropic herpes virus that is responsible for infectious mononucleosis; it generally differs from other members of the herpes virus family because it may affect and transform antibody-producing B cells and plasma cells in the periphery as well as in the brain.[24] Epidemiological studies have reported a near-complete presence of anti-EBV antibodies in MS patients compared with healthy controls, who express a lower seropositivity.[7] Higher humoral responses to EBV has been found in the CSF of MS patients and antigen-specific immunoblotting has demonstrated that EBV proteins, EBV nuclear antigen-1 (EBNA-1) and the early protein BRRF2 may be targets for oligoclonal immunoglobulin G (IgG) bands in the CSF of MS patients.[24] Increased levels of EBV-specific $CD4^+$ and $CD8^+$ T-cell responses have been reported in the CSF and blood of MS patients,[24] supporting the hypothesis of a specific immune activation against the virus of viral proteins. Ectopic lymphoid structures in non-lymphoid tissues (e.g. the CNS) targeted by chronic inflammatory processes are thought to play a role in maintaining immune responses against persistent antigens.[25] The question of whether accumulation of EBV-infected cells in the CNS is a primary event in MS or a consequence of another disease mechanism is still unanswered.

Varicella zoster virus (VZV) DNA has been detected in CSF and peripheral blood mononuclear cells taken during MS relapse from patients with RRMS, which suggests an association between VZV infection and MS.[7]

Human endogenous retroviruses (HERVs) may play a role in autoimmune diseases and more particularly MS. HERV sequences have been isolated from cell culture of MS patients. Increased antibody reactivity to specific HERV epitopes is found in MS serum and CSF, and cell-mediated immune responses have also been reported. Although HERV-encoded proteins can have neuropathogenic effects, the causal role of HERVs in MS pathogenesis remains to be determined.[26]

Human herpes simplex virus-6 (HHV-6) is a β-herpes virus, which is T-lymphotropic and may also infect many different host cells such as monocytes. It has been proposed that RRMS patients experienced HHV-6 active infection, supporting the idea of a potential association between the viral active replication and relapse episodes. The link between SPMS and the potential role of HHV-6 is less well described, but it is thought that HHV-6 does not actively contribute to SPMS, whereas it may act as a trigger for MS attacks in patients with RRMS.[27]

Chlamydia pneumoniae is a bacterial pathogen of the respiratory tract that is responsible for community-acquired pneumonia. In spite of controversial results regarding its presence in the CSF of MS patients, *C. pneumoniae* is thought to be neurotropic and has been associated with CNS infections, since it has been shown to infect the CNS vasculature, initiating neurodegenerative disease.[7] Although MS patients are more likely to display *C. pneumoniae* in their CSF, evidence for the role of this bacterium in the etiology of MS is incomplete.

Other Factors

There is evidence supporting the influence of sex hormones in disease activity in both MS and EAE. Some studies have shown that pregnancy may be associated with a lower relapse rate in MS, with an increase in disease activity in the postpartum period. MRI studies support the idea of a correlation between estradiol and progesterone levels and gadolinium-enhancing lesions in women affected by MS; women with high levels of estradiol and low levels of progesterone have significantly higher levels of disease activity than women with low levels of both hormones. Experimental studies have suggested a protective effect of testosterone in EAE mice, and others have shown a decrease in disease severity correlated to high levels of estriol in EAE models, either after administration or during pregnancy. Sex hormones may influence MS onset and activity by

affecting immune reactivity, including cytokine secretion, although the exact mechanism is not known.[11]

A positive strong correlation between dietary fat and MS prevalence has been found in some ecological studies. Linoleic acid, a polyunsaturated essential fatty acid, may significantly reduce the progression and severity of MS disability. Different mechanisms have been proposed to explain this influence on the etiology of MS. Myelin is composed of 75–80% lipids, of which polyunsaturated fatty acids are the major component. Polyunsaturated fatty acids may participate in membrane stability, susceptibility of the myelin sheath to demyelinating agents, and immune function.[11]

Investigating the environmental and non-genetic mechanisms underlying MS pathogenesis is difficult because of the large sample sizes required, and genetic causes may or may not be involved. However, new treatments for MS could be proposed based on such studies.

IMMUNE PATHOGENESIS OF MULTIPLE SCLEROSIS

Experimental animal models reinforce the concept of an autoimmune reaction in the CNS. It has been suggested that the immune system recognizes CNS myelin as foreign and is subsequently activated to attack it.[7] One hypothesis for this initiation is the possible activation of autoreactive cells by cross-reactivity between self antigens and foreign agents, a phenomenon known as molecular mimicry.

Molecular mimicry occurs when peptides of self antigens and infectious agents share sequences or have structural similarities, leading T and B cells to react with CNS antigens. Myelin basic protein (MBP), the major constituent of the myelin sheath of oligodendrocytes, is one autoantigen that shares similar MHC-II binding and TCR motifs with viruses such as herpes simplex virus (HSV).[7] When recognizing self antigens, cross-reactivity of self reactive T cells with foreign antigens may trigger activation of these cells, leading to crossing of the BBB and tissue damage upon recognition of antigens in the brain[28] (Fig. 30.3).

A second hypothesis for infectious induction of MS is bystander activation of autoreactive immune T cells, which occurs non-specifically during infections. Viral infections cause the activation of antigen-presenting cells (APCs) such as dendritic cells, which then trigger preprimed autoreactive T cells to initiate the autoimmune response.

Another mechanism of bystander activation involves inflammatory cytokines, superantigens, and toll-like receptor (TLR) activation, which subsequently activate autoreactive T cells in a TCR-independent manner. Virus-specific T cells can also initiate bystander activation depending on specific TCR recognition. Activated virus-specific T cells migrate to the infected CNS, where they kill infected cells presenting viral epitopes. This results in secretion of inflammatory cytokines including nitric oxide by dying cells, such as $CD8^+$ T cells and macrophages, leading to the destruction of self tissue and release of autoantigens. In the infectious context presentation of autoantigens may then activate autoreactive T cells.[7]

Pathophysiology

MS is considered an autoimmune disease, occurring when Th1 cells recognize components of the myelin sheath.[5,7] The pathological hallmark of MS is the demyelinated plaque, which consists of a defined hypocellular area characterized by loss of myelin, relative preservation of axons, and formation of an astrocytic scar. Lesions are found in optic nerves, periventricular white matter, brainstem, cerebellum, and spinal cord white matter, and they often surround medium-sized blood vessels.[1] Demyelination can also involve the gray matter, associated with meningeal inflammation. Acute highly inflamed white matter lesions are also characterized by axonal transection, probably due to a lack of myelin protection and oligodendrocyte support.[6] MRI diagnosis and CSF findings influence the classification of clinical syndromes associated with MS. High-field MRI highlights several distinct areas for MS lesions: infratentorial, callosal, juxtacortical, periventricular, and other white matter areas.[29] Variations in clinical disability during disease progression may be explained by structural differences among lesions, as assessed by the water diffusion coefficient and the degree of lesion hypointensities on T1-weighted MRI.[5]

Four pathological MS patterns have been identified based on the extent of myelin loss, localization and extension of plaques, oligodendrocyte destruction, and relative contribution of different immune cells.[30] Pattern I is dominated by infiltration of T cells and macrophages and may involve proinflammatory molecules such as TNF-α and IFN-γ; pattern II displays antibody and complement deposition and involves myelin oligodendrocyte glycoprotein (MOG)- and MBP-specific antibodies; pattern III is characterized by distal oligodendrogliopathy-associated demyelination, and may be induced by focal cerebral ischemia or toxic virus; and pattern IV results from a metabolic defect leading to non-apoptotic degeneration of primary oligodendroglia and occurs primarily in PPMS. All these patterns start from T-cell mediated inflammation and result in active demyelination and subsequent acute axonal injury. Lack of trophic support by oligodendrocytes then leads to chronic axonal injury in inactive demyelinated plaques.[30]

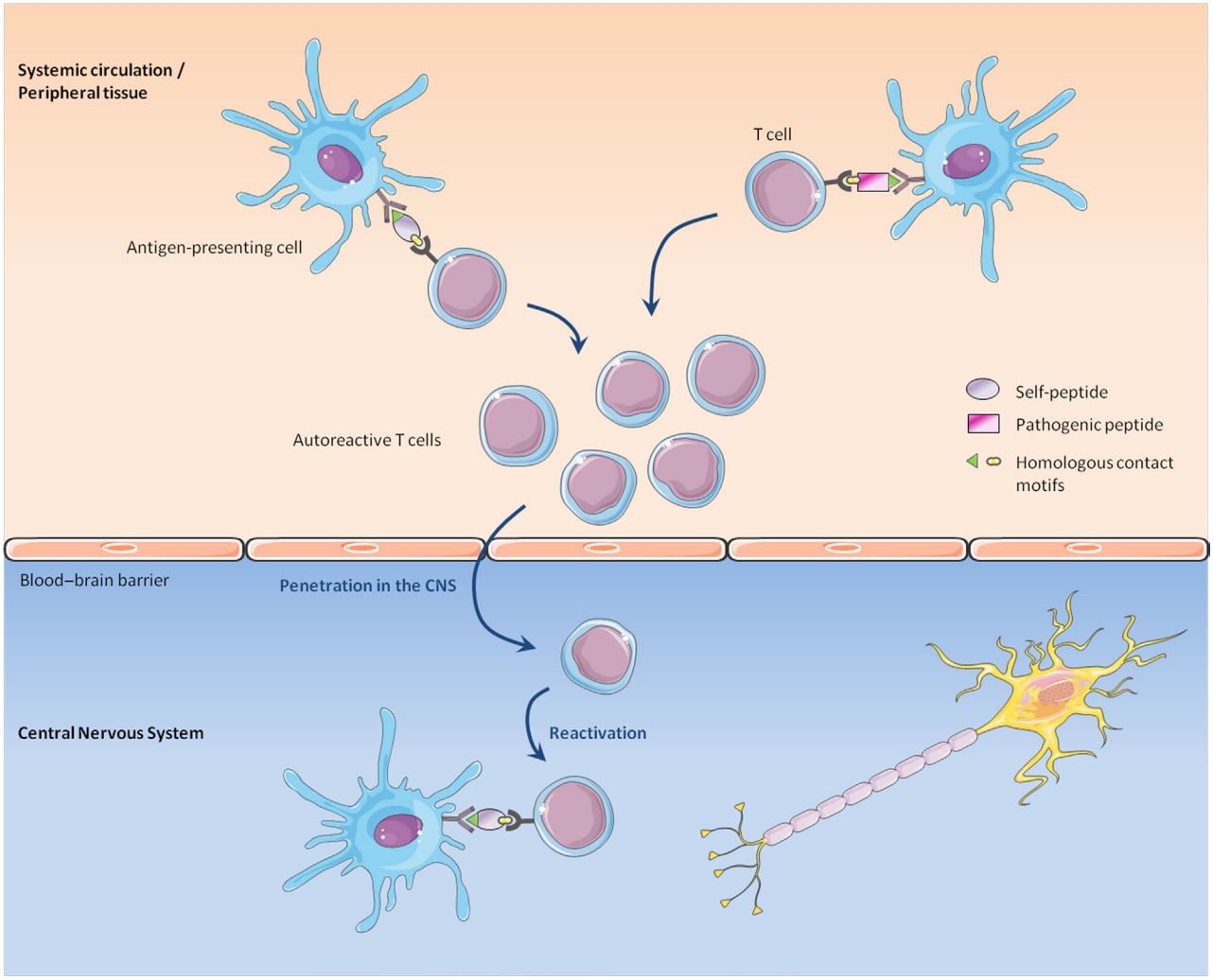

FIGURE 30.3 **Possible mechanism of cross-reactivity between self antigens (myelin basic protein, proteolipid protein, myelin oligodendrocyte glycoprotein) and infectious agents (Epstein–Barr virus, herpes simplex virus).** *Figure produced using Servier Medical Art.*

Autoimmunity-Related Events

During the initial state of the inflammatory response, activated T cells with encephalitogenic potential are recruited from the periphery to the CNS. Upon activation, T cells express integrins on the cell surface, which enable them to bind to specialized capillary endothelial cells of the BBB. Then, they migrate across the disrupted BBB through the endothelium and the endothelial basal lamina into the CNS parenchyma.[6] Following activation and recruitment of leukocytes, endothelial cells reorganize their membrane in multiple cup-shaped microdomains enriched with cellular adhesion molecules such as intracellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These molecules surround the migrating T cells and allow their passage across the endothelium through individual cells.[26] MRI clinical observations of acute and chronic active MS lesions reveal the breakdown of the BBB, which allows the infiltration of lymphocytes and leukocytes into the CNS. The release of proinflammatory cytokines such as IFN-γ and TNF-α activates cerebral endothelial cells and modulates the BBB phenotype by induction of several inflammatory genes. They affect BBB integrity through several mechanisms including inhibition of junctional protein expression. Activated T cells that migrate across the BBB express matrix metalloproteinases, which can disrupt the subendothelial basal lamina[31] (Fig. 30.4). This process is followed by a second amplification of the immune response within the CNS, as pathogenic T cells are reactivated by fragments of myelin antigens and other factors are involved in myelin degradation and damage to oligodendrocytes. Numerous non-specific, blood-derived and CNS inflammatory cells release myelin-toxic substances (e.g. lymphotoxin, nitric oxide, and perforins).[3] Activated macrophages and microglial cells secrete proinflammatory cytokines such as TNF-α and IFN-γ, toxic reactive oxygen species (ROS), or proteolytic and lipolytic enzymes. Cytotoxic activity of $CD8^+$ T cells may also participate in damaging the

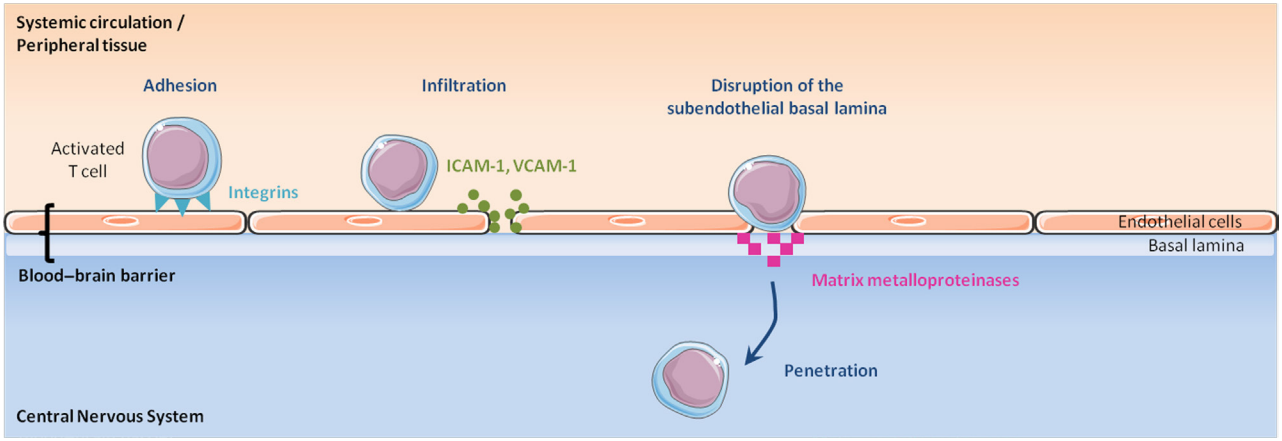

FIGURE 30.4 Blood–brain barrier-related events leading to disruption of subendothelial basal lamina and migration of lymphocytes into the CNS. ICAM: intracellular cell adhesion molecule; VCAM: vascular cell adhesion molecule. *Figure produced using Servier Medical Art.*

axons that have undergone demyelination.[3] Intrathecal synthesis of immunoglobulins is increased in MS patients. Once inflammation has started, myelin-specific B cells as well as antibodies infiltrate the disrupted BBB, enter the CNS, and release antibodies that contribute to myelin destruction through different mechanisms. These include complement-mediated opsonization, which facilitates phagocytosis by macrophages, complement-mediated cytolysis, or stimulation of antibody-dependent cell-mediated cytotoxicity by binding to NK cells.[6]

Spontaneous remyelination occurs in the context of inflammation-driven demyelination, via the recruitment and action of OPCs, which may express developmental genes, usually expressed during early myelogenesis, to re-ensheath demyelinated areas with newly generated myelin. Studies have highlighted the reactivation of oligodendrocyte transcription factor-1 (Olig-1) during remyelination in MS patients, which initiates the process of myelin regeneration. Other molecules including chemokine CXC and CC receptors are responsible for the recruitment of OPCs in the damaged areas.[5] Human and experimental models of CNS demyelination have reported that spontaneous remyelination occurs in response to myelin injury, and that modulation of the immune response may promote the remyelination process in patients with acute or early MS.[8] However, spontaneous remyelination fails to compensate for demyelination, resulting in chronic axonal injury and permanent neurological disability. Despite a large number of surviving OPCs in many MS lesions, failure of myelin repair may be due to an inadequate or insufficient recruitment of OPCs, or to a failure of recruited OPCs to differentiate into remyelinating oligodendrocytes.[8] Depletion of OPCs in some patients may be explained by the effect of MOG on OPCs: patients displaying MOG-directed antibodies may be deficient in OPCs.[8] Repeated remyelination and demyelination may also lead to progressive loss of function and depletion of OPCs.[8] Demyelinated axons may be unresponsive to remyelination because of intrinsic axolemmal alterations, for example expression of inhibitory cell surface molecules such as polysialylated neural cell adhesion molecule (PSA-NCAM), neurofilament fragmentation, or energy failure[6] (Fig. 30.5).

The Major Immune Players

Role of T-Lymphocyte Mediated Immunity

Autoreactive T cells may recognize myelin-related epitopes. T cells that are reactive to self antigens are normally deleted in the thymus by the mechanism of central tolerance, resulting in an immune repertoire that recognizes exogenous pathogens. In MS defects in self tolerance may lead to production of antigen-specific T cells with TCR alterations that are able to recognize self proteins and produce autoimmune reactions.[7] During inflammatory events, autoreactive T cells release cytokines that participate in migration and recruitment of cells to specific targets, initiating the inflammatory response.[6] Myelin-protein specific T cells that are retrieved from the peripheral circulation in healthy adults as well as in MS patients are controlled by peripheral tolerance that involves costimulatory signaling, transcriptional and epigenetic mechanisms, and regulatory T (Treg) cells.[7] Although not completely understood, dysfunction of Treg cells is thought to participate in MS pathogenesis.[32] Through a direct action on Treg cells, tolerogenic or immunogenic dendritic cells maintain immune reactions and thus contribute to the homeostasis of CNS immunity. Altered or dysfunctional dendritic cells have been described in MS patients.[32] Autoreactive T cells undergoing clonal expansion and clonotypes with unique TCR repertoires have been detected in MS lesions. $CD4^+$ T cells are found predominantly in the perivascular infiltrates and in the parenchyma, while $CD8^+$ T cells are identified in brain tissue as well as in

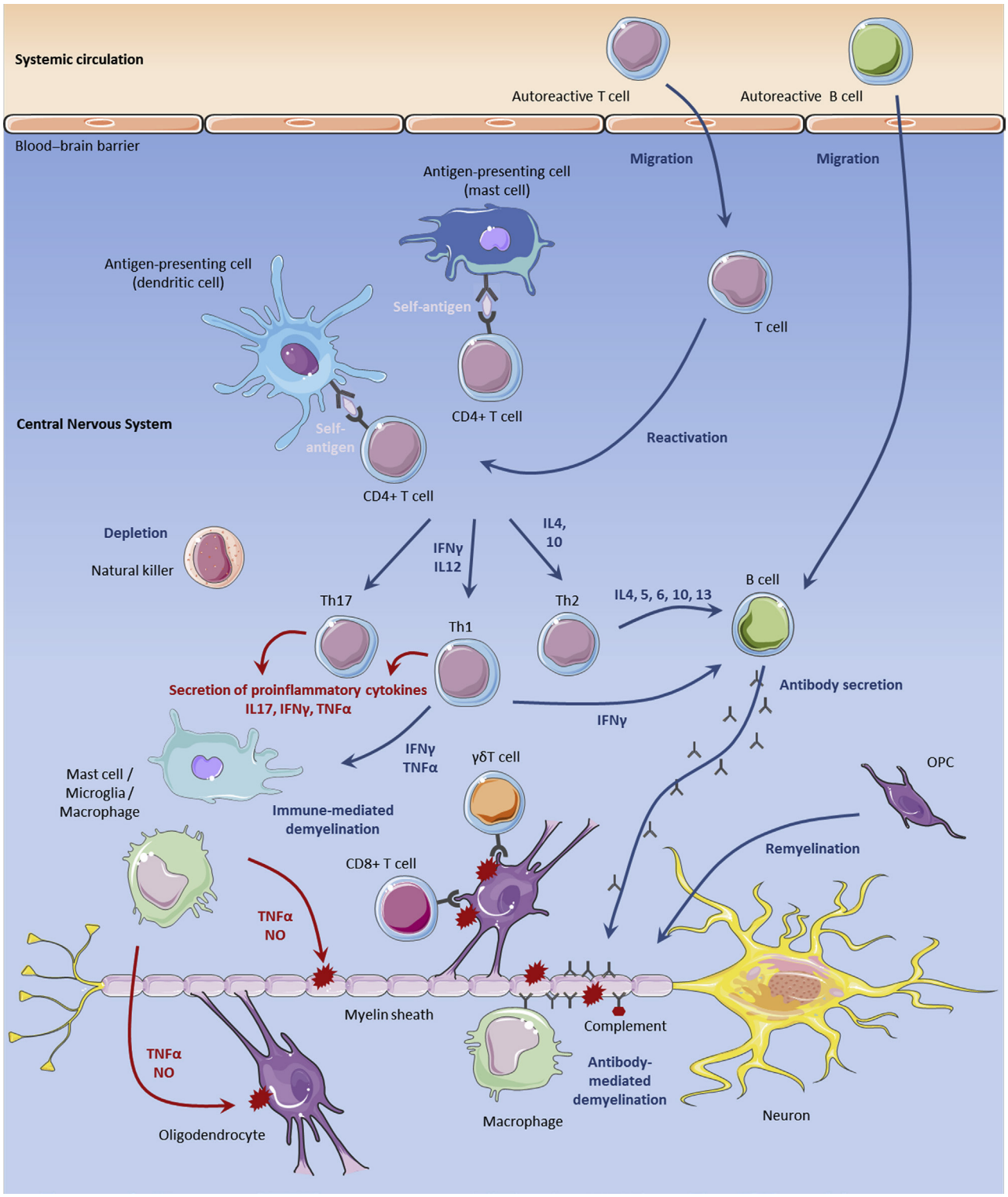

FIGURE 30.5 **Inflammation-driven demyelination: possible inflammatory events leading to demyelination and remyelination in the CNS.** IFN: interferon; IL: interleukin; TNF: tumor necrosis factor; NO: nitric oxide; Th: T-helper cell; OPC: oligodendrocyte progenitor cell. *Figure produced using Servier Medical Art.*

peripheral blood.[7] These cells may represent a pool of memory T cells, which could be recruited during acute attacks, but this mechanism remains unclear.

Current evidence exists that MS primarily involves $CD4^+$ autoreactive T cells as a central element for the autoimmune hypothesis of MS. $CD4^+$ T cells have been identified as CNS- and CSF-infiltrating cells in MS patients. In the EAE model, the injection of myelin protein components or spinal cord homogenate into animals induces a $CD4^+$ T-cell driven autoimmune disease course that is similar in many respects to that observed in MS.[7] Some HLA class II molecules are thought to increase the genetic risk of developing MS. Their role as antigen-presenting molecules to pathogenic $CD4^+$ T cells also supports the importance of $CD4^+$ T cells in the immunopathogenesis of MS. The frequency of high-avidity myelin-specific $CD4^+$ T cells that respond to low antigen concentrations to myelin proteins has been shown to be significantly higher in MS patients than in healthy controls, and they express mostly a proinflammatory phenotype.[7]

T-helper (Th) cells are $CD4^+$ effector cells that are differentiated in response to exposure to specific interleukins. Th cells may express different phenotypes based on their cytokine secretion profile and provide immune responses to mediate protection against pathogens.[33] When T cells are polarized to Th1 (proinflammatory phenotype), inflammation is promoted. Th1 cells secrete IFN-γ in large quantity, and also TNF-α, lymphotoxin-α (LT-α), and IL-2. IFN-γ produced by Th1 mediates protection against intracellular pathogens by activating macrophages. The number of mononuclear cells that secrete IFN-γ is increased in blood and CSF of MS patients, and IFN-γ is present in MS lesions.[34] In EAE, antibodies against LT-α and TNF-α have been shown to modulate the disease course, supporting the role of Th1 cells in EAE.[34]

Chemokines and their receptors play a central role in the inflammatory reaction. Among the different chemokine receptors, CXCR3 and CCR5 show preferential expression on Th1 cells. Chemokines induce leukocyte adhesion to the endothelium, and play an important role in the infiltration of inflammatory T cells through the BBB into the CNS.[7] Deregulated Th1 response to myelin components leads to an autoimmune reaction and brain tissue inflammation in EAE.

The damaging role of proinflammatory cytokines in MS is controversial. IFN-γ delivered intrathecally to EAE mice may increase the expression of the anti-inflammatory molecule TNFR1 on CNS-infiltrated lymphocytes. The subsequent binding of TNF-α on TNFR1 induces apoptosis of these cells. Mice treated with IFN-γ show a significant decrease in EAE inflammatory features (e.g. number of inflammatory infiltrates) and a general inhibition of the disease course.[35] These data challenge the exclusive detrimental function of proinflammatory cytokines such as IFN-γ in EAE and in MS, and the exclusive use of anti-inflammatory therapies in MS.

The Th2 phenotype is characterized by secretion of anti-inflammatory cytokines including IL-4, IL-5, IL-6, IL-10, and IL-13. Th2 cells induce B-cell proliferation and stimulate antibody production.[30] IL-4 and IL-13 provide positive feedback for Th2 cell differentiation, and IL-4 may inhibit the development of Th1 and Th17 proinflammatory phenotypes. Th2 responses can downregulate autoimmune disease. MS is associated with a parallel upregulation of proinflammatory Th1 cytokines and an immune response downregulation of anti-inflammatory Th2 cytokines such as IL-10 and TGF-β, a factor produced by activated T lymphocytes that has suppressive effects on both T- and B-cell related immunity.[34]

A distinct $CD4^+$ T-cell subpopulation has been identified as (IL-17-producing) Th17 cells. Proinflammatory cytokine IL-17 and regulated cytokine IL-23 are increased in CNS lesions and in peripheral blood mononuclear cells of MS patients. Increased peripheral blood Th17 cells may be associated with MS severity.[36] IL-17 receptors are seen in acute and chronic MS plaques.[33] In EAE models, induced disease is more severe after transfer of IL-17-producing $CD4^+$ T cells precultured with IL-23 than after transfer of Th1 cells, supporting the hypothesis of a critical role for IL-23 in EAE.[5] The Th17 population and IL-23-induced secretion of IL-17 are emerging as critical mediators of chronic autoimmune diseases. Both Th1 and Th17 cell populations may promote inflammation in MS, acting in parallel through different mechanisms of action[36]; however, the impact of Th17 cytokines remains unclear. Overexpression of T-cell specific IL-17A in EAE mice does not exacerbate the pathological course of the disease, and deficiency in IL-17A and IL-17F does not show a clear beneficial impact on the development of EAE. This suggests that Th17 cells may exert their pathogenicity through other factors or mechanisms of action. IL-17A produced by Th17 cells may disrupt the BBB by inducing the production of ROS, which are harmful to brain endothelium permeability (downregulation and reorganization of tight junction molecules), leading to breakdown of the BBB and infiltration of immune cells into the CNS. Conversely, IL-17A-deficient mice show lower levels of ROS production and BBB disruption.[36] These findings suggest a role for IL-17 in maintaining BBB integrity, but not in disease development.

Dysfunction of the suppressor function of Treg cells may be associated with MS. Treg cells can be classified into two subsets, natural and inducible Treg cells, according to the molecules expressed on their surface or their cytokine secretion profile.[32] Treg-cell mediated suppression plays a central role in the control of autoreactive T cells and the induction of peripheral tolerance. Although the frequency of $CD4^+/CD25^+$ Treg cells does

not differ from healthy controls, $CD4^+/CD25^+$ Treg cells in MS patients appear to be functionally impaired or to have deficits in their maturation or in the thymic output. EAE studies further support this hypothesis, and the adoptive transfer of $CD4^+/CD25^+$ Treg cells from naïve mice prevents or reduces the severity of EAE. Inactivation or depletion of Treg cells using anti-CD25 antibody may increase susceptibility to EAE and prevent secondary remissions in myelin SJL mice immunized with proteolipid protein (PLP).[32]

$CD8^+$ T cells have been detected in MS plaques and may have regulatory functions in the progression of the disease. Experimental studies have suggested that myelin-specific $CD8^+$ T cells induce severe CNS autoimmunity, which has similarities to MS. Prominent oligoclonal expansions of $CD8^+$ memory T cells have been found in the CSF and brain tissue of MS patients. The $CD8^+$ cytotoxic T-cell response to MBP is increased in MS patients.[7] Cytotoxic $CD8^+$ T cells may kill glial cells, leaving axons exposed; they mediate axonal transection in active MS lesions.[3,6]

Role of Humoral Immunity

The observation that immunoglobulin levels are increased in the CSF of MS patients is supporting evidence for a role of B cells and humoral immunity in MS immunopathogenesis.[7] It has also been observed that B cells, plasma cells, and myelin-specific antibodies are present in chronic and acute MS lesions with demyelination.[37] Mutant mice engineered to produce high levels of MOG-specific antibodies displayed higher EAE severity and more progressive disease, even after disease induction with myelin components other than MOG.[37] In progressive MS, organized ectopic lymphoid follicles have been observed close to inflammation-driven demyelinated MS lesions. They resemble secondary lymphoid organs in the CNS and contain proliferating B cells and follicular dendritic cells. The formation of ectopic (CNS) lymphoid tissue may play a role in maintaining immune responses against persistent antigens.[25]

Antibodies directed against proteins and lipids of the myelin sheath and against molecules in the CNS are secreted by B cells that have migrated into the CNS or from serum that has extravasated through the BBB.[13] Secretion of myelin-specific antibodies by B cells may lead to the destruction of myelin within plaques. B cells act as APCs for autoreactive T cells, and antibodies participate in the uptake and processing of antigen. B cells also provide costimulation to autoreactive T cells. Antibodies can opsonize myelin for macrophage phagocytosis and thus cause demyelination, and also target and address other cells to inflammatory sites.[37] B cells may exert a beneficial role in MS by influencing cytokine production to maintain a balance in the Th1/Th2 profile through their antigen-presenting function. Antibodies that normally recognize CNS components may also promote myelin repair and enhance remyelination, or have a regulatory function, for example by inducing T-cell anergy.[37]

Role of Innate Immune Mechanisms

Inflammation in MS may be initiated by Th1 cells that cross the BBB after activation in the periphery, recognize their target antigen, and activate non-specific effectors, for example macrophages and microglia, which are largely responsible for demyelination and axonal damage. Dendritic cells present in the CSF compartment and in MS lesions may contribute to the chronicity of MS and may play a role in PPMS.[32] TLRs present on dendritic cells may display inappropriate signaling in MS and EAE, leading to a potential inhibition of the immunosuppressive effects of Treg cells and thus contributing to the maintenance of MS.[7] Mature dendritic cells may be retained in the inflamed tissue and drive naïve T cells to a Th1 phenotype by secreting IL-12.[32]

Mast cells are increased in the CSF of MS patients, as well as in MS plaques and acute lesions. They have been suggested to act as APCs and to mediate a shift in the Th1/Th2 population.[7] NK cells have been associated with MS, based on their observed depletion in peripheral blood, plaques, and CSF of MS patients, as well as in EAE. NK could be involved in suppression of autoimmunity by producing cytokines or by inducting target lysis.[7] γδT cells have been found in chronic MS lesions and in the CSF of MS patients. These cells lyse oligodendrocytes via perforin, supporting a role in MS pathogenesis.[7]

Role of Self Antigens

The most studied candidate self antigens are the proteins that constitute the myelin sheath, such as MBP, MOG, myelin-associated glycoprotein (MAG), and PLP. MBP and PLP are the most abundant proteins that compose the myelin. PLP has been shown to induce autoreactive T- and B-cell responses in MS.[7] These proteins are expressed within the intracellular surface of myelin membranes of the sheath and are involved in maintaining the structure of compact myelin. MOG is less abundant and is specifically expressed on the surface of myelin, making it a target for demyelinating antibodies and cellular immune responses in MS. All of these self antigens can induce EAE in mice, rats, guinea pigs, and non-human primates.[7] Other myelin and non-myelin antigens targeted by $CD4^+$ T cells include 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), myelin-associated oligodendrocytic basic protein (MOBP), oligodendrocyte-specific glycoprotein (OSP), αB-crystalline, S100β protein, and myelin lipid components.[7]

Damage to myelin can be considered as an epiphenomenon following non-specific polyclonal activation

of T and B cells by bacterial or viral antigens, or due to molecular mimicry, when proteins in the pathogen share molecular features with self antigens, such as specific myelin epitopes.[6]

CLINICAL FEATURES OF MULTIPLE SCLEROSIS

Clinical Features in Different Types of Multiple Sclerosis

There is much variability in the clinical features of MS, in the onset as well as in the progression of the disease. Symptoms result from the interruption of myelinated tracts in the CNS, whereas the peripheral nervous system is not disturbed.[6] The frequency of new symptoms depends on the age of onset. If the onset is before 20 years of age, the majority of symptoms relate to optic sensitivity, while motor deficits are prevalent with onset at later ages. The most common features of MS are paralysis, sensory disturbances, lack of coordination, and visual impairment.[13]

Current models for MS disease progression support the occurrence of two overlapping and connected stages, one driven by inflammation and one by neurodegeneration, with no clear understanding of their temporal relationship. Most MS cases start with attacks of neurological disturbance, which are probably mediated by autoimmune reactions leading to inflammatory lesions and demyelination. These attacks are followed by complete or partial recovery and thought to be free of disease progression. About half of these cases turn into a progressive phase characterized by steady worsening of the disease with superimposed relapse episodes, and often characterized by an inability to walk and progressive paralysis. Occasionally, clinical disability begins with the progressive phase, in the PPMS clinical pattern. The progressive stage is likely to be driven by degeneration of both the myelin sheath and the axons, and subsequent brain and spinal cord atrophy underlie motor symptoms[13] (Table 30.3).

Clinical Symptoms

Clinical symptoms of MS often correlate with the functional localization of impaired conduction in MS lesion.[1] Lesions in the cerebrum result in hemifacial weakness, pain, and motor impairments, as well as the cognitive deficits that are common in advanced cases, including memory and attention problems and slowed information processing.[1,6] Later in the disease, patients may display psychiatric manifestations: dementia, bipolar disorders, pathological laughter and crying, and psychosis. Depression is experienced by 60% of patients.[6]

TABLE 30.3 Clinical Features of Multiple Sclerosis

Lesion Site	Clinical Symptoms
Cerebrum	Hemifacial weakness and pain
	Motor impairments
	Cognitive deficits (memory and attention deficits, slowed information processing)
	Psychiatric features (dementia, bipolar disorder, depression)
Cortex	Motor, sensory, and cognitive disability
Optic nerve	Optic neuritis (often monocular acute blurring or loss of vision)
Cerebellum	Postural and action tremor
	Limb incoordination
	Gait instability
	Ataxia
Brainstem	Diplopia (double vision)
	Vertigo
	Impaired speech and swallowing
	Paroxysmal symptoms
Spinal cord	Weakness
	Diminished dexterity
	Spasticity (paraparesis, shaking, progressive ambulatory disability, stiffness)
	Autonomous disturbances (sexual impotence, bladder dysfunction, constipation)
	Pain (Lhermitte sign)
Other	Fatigue
	Temperature sensitivity

Source: Compston and Coles[1] and Hauser and Oksenberg.[6]

Cortical demyelination is believed to follow white matter pathology and to be rare in acute or early relapsing MS. Cortical plaques lead to focal cortical deficits underlying motor, sensory, and cognitive disability in MS.[1,6] Imaging in patients with high-strength magnets, functional MRI, and positron emission tomography, as well as cortical thickness and CNS atrophy measures, may provide useful information to characterize gray matter demyelination and better correlate cortical atrophy with specific disabilities, including cognitive dysfunction, fatigue, and depression.[2]

One of the most common initial symptoms is visual impairment, including reduced visual acuity and unilateral painful loss of vision. Demyelination commonly affects the visual system from the optic nerve to the cerebrum, since approximately 40% of the brain is involved in visual function. Among the neuro-ophthalmic symptoms, optic neuritis is the most clinically isolated demyelinating

syndrome. Optic neuritis is caused by inflammation or demyelination of the optic nerve and results in acute blurring or loss of vision, usually monocular.[1]

Some common early symptoms are caused by MS lesions located in the brainstem. They include diplopia (double vision), vertigo, impaired speech and swallowing, and paroxysmal symptoms thought to originate from discharges along demyelinated axons.[1,6] Lesions in the cerebellum are responsible for postural and action tremor, and for limb incoordination, gait instability, and ataxia.[1,6]

The 2005 revision of the criteria for MS diagnosis focuses on characterizing lesions of the spinal cord and aims to simplify the diagnosis of PPMS.[38] Spinal cord lesions can be detected with high sensitivity using high-field MRI.[6] Lesions in the descending motor pathways of the spinal cord lead to weakness or diminished dexterity in the limbs, especially the legs. Spasticity, including spastic paraparesis, shaking, and difficulty walking, progressively turns into complete disability, and stiffness is also observed as the disease worsens. Lesions of motor and sensory fibers of the spinal cord and hypoactivity result in autonomic disturbances such as sexual impotence, bladder dysfunction, and constipation.[1,6] Ancillary symptoms include the Lhermitte sign, an electrical sensation running down the spine and into the limbs triggered by neck flexion, which is attributed to spontaneous discharges of demyelinated axons.[1,6] Fatigue and temperature sensitivity are other symptoms that occur in most patients[1,6] (Table 30.3).

Measures of Disease Progression

Both the clinical definition and therapeutic trials now require quantification of physical disability in MS. The Kurtzke Expanded Disability Status Scale (EDSS) is the standard measurement of neurological impairment in MS commonly used in clinical trials. A measure of overall neurological impairment was first given by the Disability Status Scale (DSS), which ranges from 0 (normal neurological examination) to 10 (death due to MS). The DSS evolved into the EDSS by dividing each step into two and defining functional system grades at each step. The EDSS quantifies disability in eight functional systems: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, and other. It ranges from 1.0 to 4.5 for people affected with MS who are fully ambulatory, and from 5.0 to 9.5 for people with MS with impaired ambulation. The maximum grade, 10.0, represents death due to MS.[39] Although the Kurtzke EDSS is a standard method to evaluate disability, its use is limited by inconsistencies between evaluators and its insensitivity to clinical changes at a specific grade.

Cognitive dysfunction is not strongly correlated with the EDSS score, and can be rated through tests such as the Paced Auditory Serial Addition Test (PASAT) and the Symbol–Digit Modalities Test (SDT). Other clinical scales such as the Scripps Neurological Rating Scale (SNRS) and the Trojano Neurological Rating Scale are based only on the neurological examination. The Multiple Sclerosis Functional Composite (MSFC) includes quantitative functional measures of three key clinical issues of MS: leg functional ambulation, arm/hand function, and cognitive function. This measure of disability may be a consistent method by which to define the clinical status of MS patients.

Quantifying MRI techniques may be more sensitive, objective, and reliable than clinical methods in detecting and defining MS disease activity, although many MS lesions have no clinically detectable symptoms.[6]

PROGRESSIVE MULTIPLE SCLEROSIS AS AN UNMET NEED

About half of RRMS patients shift to a SPMS within 10 years of the initial diagnosis; this clinical form is characterized by a steady progression of neurological damage with or without relapses and the occasional minor remission or plateau.[9] Only about 15% of MS patients develop PPMS, with slow continuous worsening of neurological decline and no identifiable relapses or remissions.[9] PPMS displays a later (about 10 years) onset than RRMS. Available data on clinical phenotypes show differences in epidemiology of RRMS and progressive MS, raising the issue of a different pathogenic mechanism being responsible for the onset of the disease. The female to male ratio for PPMS is about 1:1, whereas it is between 2:1 and 3:1 for RRMS.[10] The genetic risk of developing MS associated with MHC-II genes is similar in RRMS and in PPMS, as well as other identified susceptibility genes.[40] Although the geographic distribution of progressive MS does not reflect the general distribution of MS, progressive MS is more frequent in relatively resistant populations (e.g. blacks and Asians).[10]

It is hypothesized that SPMS and PPMS emerge as the result of axonal and neuronal damage, consequent to accumulating multifocal inflammatory events, and leading to permanent and irreversible neurological disability. It is likely that the conversion of RRMS into SPMS occurs when the CNS fails to compensate for demyelination and subsequent axonal loss with spontaneous recovery mechanisms.

Comparison in Pathophysiology

Demyelinating lesions of white matter, identified by the prominence of macrophages containing myelin degradation products, are more frequent in RRMS than in SPMS or PPMS, whereas the inflammatory reaction is

more pronounced in SPMS than in PPMS. There is also a clearer cortical demyelination in progressive MS than in RRMS. Acute axonal damage associated with inflammation and lack of trophic support from myelin sheaths seems to be more prominent in progressive MS than in RRMS.[10] Progressive MS is characterized by increased demyelination in white and gray matter, with less inflammation, which is more diffuse, within the lesions, and with acute axonal damage.

In progressive MS, inflammation is trapped behind a closed BBB. The severity of inflammation of the meninges and the formation of lymph follicle structures are associated with the activity of cortical lesions and with activation of microglial cells. Activated T cells release diffusible factors in the parenchyma, leading to CNS damage, either directly or indirectly via microglia activation. Soluble factors may include specific demyelinating antibodies, but they have not been identified.[25] In progressive MS, neurodegeneration may result in overreaction of microglial cells which produce neurotoxic factors upon stimulation by T cells.[25]

Another feature of demyelination and neurodegeneration (e.g. loss of structure and/or function of neurons) in progressive MS is mitochondrial injury. Damaged axons found in active lesions of chronic MS display profound mitochondrial alterations, leading to energy failure. Accumulation of Na^+ is observed in the axoplasm. As axonal Na+ concentrations increase, the Na^+/Ca^{2+} exchanger exchanges axoplasmic Na^+ for extracellular Ca^{2+}, leading to a toxic excess of Ca^{2+} in the axon and finally axonal degeneration. It is unclear what initiates mitochondrial impairment in MS, but it is likely that tissue injury is a result of inflammatory processes in the CNS.[25]

Comparison Based on Brain Imaging Techniques

MRI studies reveal smaller and less frequent gadolinium-enhanced lesions, indicating areas of active inflammation, in PPMS compared with SPMS patients. Lesions observed in PPMS may be associated with the progression and severity of disability. Gadolinium-enhanced brain inflammatory lesions in PPMS are more commonly observed in early disease.[40] Multifocal T2-hyperintense lesions of PPMS frequently affect the spinal cord, most commonly in the cervical region.[10] Proton magnetic resonance spectroscopy enables the quantification of disease evolution by measuring the white matter concentration of brain *N*-acetyl aspartate (NAA), a marker of neuronal integrity.[6,10] The concentration of NAA as assessed by proton magnetic resonance spectroscopy appears to be reduced more in PPMS and SPMS than in RRMS.[40] An increase in diffusion abnormalities on diffusion tensor imaging is seen in PPMS, attesting to an accumulation of pathology in gray matter over time.[40]

Magnetization transfer ratio imaging allows quantification of cerebral myelin content, and can be used to assess demyelination and remyelination. Patients with PPMS display a reduction in magnetization transfer ratio values in gray matter, which is associated with clinical disability and cognitive impairment. These values do not differ significantly in PPMS and RRMS cases.[10]

Quantitative MRI shows that cerebral atrophy of both gray matter and white matter occurs frequently in early PPMS, to a smaller extent in SPMS, and even less in RRMS.[10] Brain atrophy is an early clinical feature of PPMS and an indication of disease progression. Spinal cord atrophy is more pronounced in PPMS and SPMS than in RRMS. Cortical lesions using double-inversion-recovery imaging are observed in PPMS patients more than in RRMS, relative to disease duration,[10] again suggesting that gray matter abnormalities are correlated with clinical disability in PPMS.

TREATMENT OF MULTIPLE SCLEROSIS

MS therapy can be divided into specific and symptomatic approaches. MS-specific treatments approved by the US Food and Drug Administration (FDA) include injectable therapies (IFN-β and glatiramer acetate), oral therapies (teriflunomide, dimethyl fumarate, and fingolimod), and infusion therapies (mitoxantrone and natalizumab). Detailed effects and mechanisms of action of long-term MS drugs are summarized in Table 30.4. These treatments mainly target the ongoing inflammation and therefore are effective only in patients with RRMS, or those with SPMS who continue to have relapses. The short aims of specific therapy in MS are to reduce clinical relapses and to decrease the accumulation of new MRI lesions, as they both are linked with poor prognosis. The long aim of specific MS therapy is to delay the evolution from relapsing to progressive MS, as progressive MS is primarily dominated by neurodegenerative processes that are mainly indifferent to the available therapy.

To this end, a two-fold strategy is adopted, which consists of short-term treatment with steroids that aims to reduce the accumulation of disease burden after a relapse, and long-term treatment with licensed drugs that aim to steady the MS process.

As such, patients who have PPMS or SPMS without superimposed relapses only benefit from symptomatic therapy. Symptomatic treatments encompass a wide range of drugs directed to treat a very wide range of symptoms that includes walking impairment, spasticity, fatigue, bladder dysfunction, pain, and depression. Among the symptomatic drugs, dalfampridine is the sole therapy approved to treat walking impairment exclusively in MS patients.

TABLE 30.4 Treatments for Multiple Sclerosis: Effects and Potential Mechanisms of Action

Treatment	Dose	Route of Administration	Frequency of Dose	FDA Approval	Target	Mechanism of Action	Effects	Stage of the Study
FIRST LINE								
IFN-β[41]								
IFN-β_{1b}	250 μg	s.c.	Every other day	1993/2009	Immune system	Modulates cytokine expression profile to an anti-inflammatory phenotype	Reduced relapse rate, disability progression, and MRI activity vs placebo	Recombinant form currently studied for control of disease course and development of brain lesions vs placebo (ClinicalTrials.gov identifier: NCT01464905)
IFN-β_{1a}	30 μg	i.m.	Once a week	1996		Inhibits T-cell activation and proliferation		
IFN-β_{1a}	22 μg	s.c.	3 times a week	2002		Blocks T-cell migration across endothelium		
	44 μg	s.c.	3 times a week					
Glatiramer acetate[42]	20 mg	s.c.	Daily	1996	Immune system	Modulates cytokine expression profile to an anti-inflammatory phenotype	Reduced relapse rate and accumulation of disability vs placebo	
	40 mg	s.c.	3 times a week (PMID: 23686821)	2014		Induces antigen-specific suppressor T cells		
						Reduces antigen presentation		
						Corrects $CD8^+$ T-cell regulatory deficit		
Teriflunomide[43]	7 mg	Oral	Daily	2012	Inhibits mitochondrial dihydroorotate dehydrogenase	Modulates T-cell responses	Reduced relapse rate, disability progression, and MRI activity vs placebo	
	14 mg							

Continued

TABLE 30.4 Treatments for Multiple Sclerosis: Effects and Potential Mechanisms of Action—cont'd

Treatment	Dose	Route of Administration	Frequency of Dose	FDA Approval	Target	Mechanism of Action	Effects	Stage of the Study
Dimethyl fumarate[44,45]	240 mg	Oral	Twice daily	2013	Activates the antioxidant transcription factor Nrf2 pathway	Upregulates Th2 response	Reduced relapse rate and MRI activity vs glatiramer acetate	
						Reduces cell death	Reduced disability progression vs placebo	
SECOND LINE								
Natalizumab[46]	300 mg	i.v.	Every 4 weeks	2006	Targets α_4 subunit of VLA-4 receptor	Prevents migration of leukocytes across BBB into CNS	Reduced relapse rate, disability progression, and MRI activity vs placebo	
Fingolimod[47]	0.5 mg	Oral	Daily	2010	Modulates S1PR types 1, 2, 3, and 5	Prevents CCR7-positive lymphocytes, including naïve and central memory T cells, from exiting lymph nodes	Reduced relapse rate, disability progression, and MRI activity vs IFN-β_{1a}	
THIRD LINE								
Mitoxantrone[48]	12 mg/m^2 (lifetime cumulative limit 140 mg/m^2)	i.v.	Every 3 months	2000	Inhibitor of topoisomerase II	B- and T-cell suppression	Reduced relapse rate, disability progression, and MRI activity vs placebo and vs IFN-β_{1a}	
						Inhibits T-cell migration		

FDA: Food and Drug Administration; IFN: interferon; s.c.: subcutaneous; i.m.: intramuscular; i.v.: intravenous; Nfr2: nuclear factor (erythroid-derived 2)-related factor 2; VLA: very late antigen; S1PR: sphingosine-1-phosphate receptor; Th: T-helper; BBB: blood–brain barrier; CCR: chemokine receptor; MRI: magnetic resonance imaging.

Short-Term Treatments for Acute Relapse

Glucocorticoids have short-term effects on the speed of functional recovery in patients with acute attacks of MS and are widely used in the treatment of acute exacerbations in RRMS. They exert different immunomodulatory mechanisms of action, including inhibition of antigen presentation,[6] anti-inflammatory effects such as reduction of edema and arachidonic acid metabolites, decrease of proinflammatory cytokine expression,[7] and inhibition of lymphocyte proliferation.[33] Usually, they are administered intravenously at high doses during relapse periods of MS and steroid tapering is not suggested. A 2013 trial with a small number of patients reported that administration of bioequivalent doses of oral methylprednisolone is not inferior to intravenously methylprednisolone in terms of the safety and efficacy profile (measured as EDSS and MRI activity 4 weeks after MS relapse).[49] However, before any extension to clinical practice, these promising results have to be confirmed in larger clinical trials with longer follow-up.

Long-Term Treatments

The long-term relapsing MS treatment scenario is quickly growing and therapeutic decisions strictly depend on the course of MS. In general, two different strategies can be adopted.

The first is the *escalation strategy,* which is a therapeutic strategy based on a reasonable decision-making procedure in which drugs with the best risk–benefit ratio are first preferred and, if needed, drugs with increasing power or toxicity (but that are not necessarily more effective) are successively adopted.[50]

The second strategy is the *induction strategy*, which represents a more aggressive approach in which powerful immunosuppressant drugs are used right from the beginning to tackle the disease process hard and early with the aim of resetting the immune system. However, this clinical benefit is gained at the expense of the risk of clinically relevant side effects.[50] Patients who suffer from a non-aggressive form of RRMS are candidates for the escalation strategy, whereas those who suffer from an aggressive form of RRMS are more likely to be put through to an induction strategy.

In a simplified scenario, approved long-term MS therapy can be grouped into three categories based on their presumed target or mechanism of actions: (1) inhibitors of immune cell trafficking, such as natalizumab and fingolimod: by blocking very late antigen-4 (VLA-4) from binding to VCAM-1, natalizumab prevents adhesion of leukocytes to the endothelium and their subsequent transmigration into the CNS[33,51]; by targeting the receptors of sphingosine-1-phosphate (S1PR), fingolimod inhibits the egress of T cells from lymph nodes; (2) inhibitors of cell replication, such as teriflunomide and mitoxantrone: teriflunomide inhibits the mitochondrial dihydroorotate dehydrogenase, which reduces the *de novo* synthesis of pyrimidine nucleotides in proliferating cells, but does not inhibit the salvage pathway used by resting cells; mitoxantrone affects the DNA topoisomerase II, leading to an impairment of T- and B-cell proliferation[33]; (3) immunomodulators, such as dimethyl fumarate, interferons, and glatiramer acetate: dimethyl fumarate directly activates the antioxidant transcription factor nuclear factor (erythroid-derived 2)-related factor-2 (Nrf2) pathway, leading to a counteraction of oxidative stress; interferons promote antagonism of IFN-γ-mediated MHC upregulation on APCs, modulate apoptosis,[6] and inhibit T-cell activation and proliferation[33]; finally, glatiramer acetate promotes a shift in cytokine expression to an anti-inflammatory profile, induces antigen-specific suppressor T cells, and inhibits antigen presentation.[41]

All of the FDA-approved drugs to treat MS reduce annual relapse rate, stabilize or decrease the MRI lesion burden, and limit the accumulation of disability. Nevertheless, they vary significantly in magnitudes of benefits. Interferons, glatiramer acetate, and the recently approved teriflunomide are expected to be approximately 30% effective against relapses, 38% effective at slowing short-term progression, and 50% effective at reducing enhancing lesions and T2 lesions on MRI.[52] Conversely, fingolimod, natalizumab, mitoxantrone, and the recently licensed dimethyl fumarate reduce annual relapse rate by up to 68%, progression rate by up to 42%, and enhancing and Th2 lesions by up to 90% and 85%, respectively.[52–54] However, moving beyond efficacy, the first generation of MS therapies (interferons and glatiramer acetate) displays a well-defined reasonably safe profile, whereas the use of the more efficacious natalizumab and mitoxantrone is limited by safety concerns.

Natalizumab carries the risk of progressive multifocal leukoencephalopathy[55]; this risk is directly related to evidence of previous JC virus exposure, duration of natalizumab exposure, and previous use of immunosuppressants. Mitoxantrone carries the risk of leukemia (which approaches 1%) and of cardiotoxicity, limiting its use at a life cumulative dose of 140 mg/m^2.[56] The safety profile of the recently approved oral therapies (fingolimod, teriflunomide, and dimethyl fumarate) remains to be proven in a general MS population. None of these drugs has been shown to be safe during pregnancy or breastfeeding, and in women who need to continue therapy during pregnancy, only glatiramer acetate appears to be relatively safe (pregnancy category B).[57] Emerging treatments for RRMS are summarized in Table 30.5.

TABLE 30.5 Treatments for Multiple Sclerosis: Evidence from Clinical Trials

Treatment	Target/Mechanism of Action	Effects	Phase	Stage of the Study
CLINICAL TRIALS IN RRMS				
Alemtuzumab[58] (approved by EMA)	Monoclonal antibody, targets molecule CD52	Reduced relapse disability progression and MRI activity rate vs IFN-β_{1a}	III	Currently studied for long-term safety and efficacy (ClinicalTrials.gov identifier: NCT00930553)
Laquinimod	Modulates adaptive T-cell immune responses (by interfering with nuclear factor-κB)	Reduced relapse rate, disability progression, and MRI activity vs placebo	III	Currently studied for efficacy, safety, and tolerability vs IFN-β_{1a} (ClinicalTrials.gov identifier: NCT01975298)
Dalcizumab[59,60]	Monoclonal antibody, binds to CD25	Reduced relapse rate, disability progression, and MRI activity vs placebo	III	Currently studied for safety and efficacy vs IFN-β_{1a} (ClinicalTrials.gov identifier: NCT01064401)
Ocrelizumab[61]	Monoclonal antibody, binds to CD20 antigen	Reduced MRI activity vs placebo and IFN-β_{1a}	III	Currently studied for the potential reduction in disease activity vs IFN-β_{1a} (ClinicalTrials.gov identifier: NCT01247324; NCT01412333)
CLINICAL TRIALS IN SPMS				
Ocrelizumab	Monoclonal antibody, binds to CD20 antigen	NA	III	Currently studied for safety and efficacy in PPMS vs placebo (ClinicalTrials.gov identifier: NCT01194570)
Fingolimod	Modulates S1PR types 1, 2, 3, and 5	NA	III	Currently studied for safety and efficacy in PPMS vs placebo (ClinicalTrials.gov identifier: NCT00731692)
Siponimod	Modulates S1PR types 1 and 5	NA	III	Currently studied for safety and efficacy in SPMS vs placebo (ClinicalTrials.gov identifier: NCT01665144)
Mastinib	Tyrosine kinase inhibitor, primarily targets mast cells	NA	II/III	Currently studied for safety and efficacy in PPMS or relapse-free SPMS vs placebo (ClinicalTrials.gov identifier: NCT01450488)
Natalizumab	Targets the α_4 subunit of VLA-4 receptor	NA	II/III	Currently studied for safety and efficacy in PPMS and SPMS vs placebo (ClinicalTrials.gov identifier: NCT01077466; NCT01416181)

RRMS: relapsing–remitting multiple sclerosis; EMA: European Medicines Agency; MRI: magnetic resonance imaging; IFN: interferon; SPMS: secondary progressive multiple sclerosis; S1PR: sphingosine-1-phosphate receptor; VLA: very late antigen; NA: not applicable; PPMS: primary progressive multiple sclerosis;
Source: Loma and Heyman[33] and Lutterotti and Martin.[51]

Symptomatic Treatment: Aminopyridines

Potassium-blocking drugs such as aminopyridines (4-aminopyridine and 3,4-diaminopyridine) have been proposed as treatment for MS to improve nerve conduction in demyelinated axons. Potassium channels are found in high density in the internodal part of the axon covered by myelin, and they tend to decrease action potential amplitude and duration. Potassium channel blockers have the opposite effect. They can increase action potential amplitude and duration, improving nerve conduction in experimental demyelinating diseases.[62] However, their wide use in MS has always been hindered by the lack of evidence-based guidelines for aminopyridines as symptomatic treatment of MS, and by safety concerns about their side effects (e.g. seizures).[63] Dalfampridine, the sustained release version of 4-aminopyridine, overcomes safety concerns and improves walking impairment in about one-third of treated MS patients.[64,65]

Autologous Hematopoietic Stem Cell Transplantation

The transplantation of hematopoietic stem cells (HSCs) is a widely used therapy for patients with hematopoietic malignancies and solid tumors. Autologous HSC transplantation has also been successfully used to target autoimmune responses in many autoimmune diseases, including MS.[66]

A graft containing HSCs is obtained from the patient's bone marrow or mobilized from the marrow into the circulation. Non-myeloablative chemotherapy and immune-depleting biological agents are administered before the transplantation to destroy the autodestructive immune system. The immune system is regenerated from the infused autologous HSCs.[66] Following HSC transplantation in patients with severe progressive MS, improvement or stabilization of the neurological condition has been observed over a follow-up of almost 4 years, with a decrease or stabilization of EDSS and no clear correlation with toxicity of the conditioning regimen.[66] HSC transplantation is more likely to be effective for MS patients with active CNS inflammation and poor disability, by controlling the inflammatory response and reducing clinical relapses. In addition, it may slow or stop further neurological decline, with an improvement in disability over the years.[66]

Autologous HSC transplantation performed in MS patients since the late 1990s has proven to be a successful treatment; it is based on replacement of the immune system rather than suppression. However, HSC transplantation does not seem to be effective in all patients; in particular, SPMS patients experience poor outcomes compared with patients with aggressive highly active MS, but this therapy remains promising.[66] Phase I and II studies have shown encouraging results. Phase III randomized controlled trials in MS, and in autoimmune diseases such as rheumatoid arthritis, are currently being carried out in Europe and North America.[66]

Future Challenges for Treatment of Progressive Multiple Sclerosis

Current Prospects

At the time of writing, no treatments have been approved for both PPMS and SPMS without superimposed relapses. Moreover, the last phase III clinical trials with interferons, glatiramer acetate, and rituximab in progressive MS had negative results.[67] However, several new compounds are currently undergoing clinical development for progressive MS therapy, including immunomodulatory as well as non-selective and selective immunosuppressive drugs. Emerging therapies for progressive MS are summarized in Table 30.5.

Neuroprotective Strategies

Neuroprotective, restorative, and repair-promoting treatment strategies are slowly emerging. New therapeutic strategies have evolved that specifically target the neurodegenerative aspect of MS (e.g. anti-LINGO-1, riluzole ion channel blockers, statins). However, although some of these molecules displayed neuroprotective effects in animal models of MS, very few have been tested in clinical MS. Currently, the antibodies anti-LINGO-1 and riluzole are the most promising treatments for progressive MS.

LINGO-1 is a protein expressed on oligodendrocytes and neurons, which regulates OPC differentiation and remyelination. LINGO-1 is upregulated in MS plaques, and in animal models of MS LINGO-1 antagonism improves remyelination.[68] A phase II clinical trial with anti-LINGO-1 antibody (BIIB033) is ongoing in subjects with RRMS when used concurrently with interferons (ClinicalTrials.gov identifier: NCT01864148).

Riluzole (Rilutek®) inhibits glutamatergic activity by blocking the release of glutamate and aspartate and by altering the function of glutamate receptors and sodium channels. Riluzole decreases disease severity in EAE.[69] A phase II clinical trial on 15 patients with PPMS showed that riluzole seems to reduce the rate of cervical cord atrophy and the development of hypointense T1 brain lesions on MRI, although its effect on decreasing brain atrophy is poor.[70]

Stem and Progenitor-Cell Based Therapies

New therapeutic strategies for the progressive stage of MS are based on promoting reconstitution of functional myelin. Oligodendrocyte-mediated remyelination depends on the transcription factor Olig-1, which could be a target for MS therapy. Remyelination is also enhanced by cytokines such as IL-1β, and is affected by a chronic inflammatory or highly gliotic environment.[6] Growth factors have been shown to expand OPCs.[6] Therapies aimed at axonal protection based on Na^+ channel inhibitors may control the Na^+ influx in the axon and limit the entry of extracellular Ca^{2+}. Two sodium channel blockers, phenytoin (Dilantin®) and flecainide (Tambocor®), have neuroprotective effects in EAE models,[6] and may be a therapy for progressive MS.

Chronic demyelination and remyelination failure may be due to concurrent loss of OPCs and oligodendrocytes, following a decrease in recruitment of OPCs. OPC repopulation in areas of demyelination appears to be one potential therapeutic strategy to promote remyelination. OPCs have been used to promote remyelination in rodent models of focal CNS acute demyelination.[6] Human OPCs engrafted in a myelin-deficient mouse restore axonal myelination, with extensive myelin production and compaction at 12 weeks post-transplantation.[6] Yet, the environment inhibits or fails to promote differentiation or regeneration. In a context of demyelination, this could be overcome by a therapy based on engineered precursor cells, which may deliver therapeutic molecules to damaged areas to confer protection or to stimulate remyelination.[6]

The idea that stem cells could be a therapy in chronic inflammation-mediated demyelinating and neurodegenerative diseases is based on neurogenesis occurring at an adult age within specific areas of the brain. Stem cells,

of either embryonic or adult origin, retain the ability to renew themselves for long periods and to differentiate into a wide range of specialized cell types. These functional properties may overcome the limitations of disease-modifying therapies in neurological disorders. In MS, stem-cell based therapeutic strategies focus on two issues that may have a neuroprotective effect on demyelinated areas: immunomodulation and remyelination. Transplantation of mesenchymal stem cells (MSCs) and neural stem/precursor cells (NPCs) shows protective effects in EAE, which are likely to be based on immune regulation and promotion of repair in the CNS.[71]

Intravenously infused MSCs have a beneficial effect on the severity and progression of EAE. MSCs can be isolated from some connective adult tissues such as bone marrow. They participate in the regulation of the immune system, for example by inducing T-cell anergy, altering B-cell proliferation, or migrating to inflammatory regions under specific conditions to attenuate the inflammatory response. MSCs may also promote repair in damaged tissues by secreting stimulating factors. Clinical trials are being carried out in patients with MS to assess the therapeutic relevance and safety of MSC injection.[71,72] These include a double-blind, randomized, cross-over phase I/II study that is evaluating the safety and the efficacy of the intravenous administration of autologous MSCs to patients with active MS resistant to currently available therapies [MEsenchymal StEm Cells for Multiple Sclerosis (MESEMS) trial; Clinicaltrials.gov identifier: NCT01854957].

In EAE, transplanting NPCs protects animals from paralysis.[71] The mechanism remains unclear, but it seems that NPCs exert neuroprotective function by a mechanism other than cell replacement. They improve remyelination, rescue degenerated axons, and modulate the immune response.[71] NPCs display immunomodulatory properties in the injured CNS. In both acute and chronic EAE models intracerebroventricularly injected NPCs migrate to inflamed areas and regulate inflammation, leading to a decrease in demyelination and axonal damage. A higher frequency of T-cell apoptosis has been observed upon transplantation of NPCs in EAE. Systemically injected NPCs can modulate peripheral immunomodulation in lymph nodes and attenuate severity in EAE.[73] NPCs directly injected in damaged areas of the brain also exert therapeutic effects by rescuing degenerated axons and improving the remyelination process in demyelinated areas. Intravascularly or intrathecally injected NPCs show only limited potentiation for remyelination, but they can improve the survival and regeneration of endogenous neural cells, including glial and neural progenitors. NPCs may also express and deliver neurotrophins and growth factors, which play a role in the neuroprotective effect. Clinical trials are being developed to investigate the safety and effects of NPC transplantation.[71,73]

Transplanted NPCs remain in the niche of endogenous neural stem cells in an undifferentiated state, or migrate out of the niche in a defined differentiated phenotype, depending on the inflammatory conditions. Yet the exact mechanisms underlying the NPC response to the microenvironment are unknown. The concept of therapeutic plasticity aims to describe the various therapeutic actions of stem cell transplants *in vivo*, particularly the ability to adapt functions to the specific microenvironment. Some functions of transplanted NPCs, including immune regulation, are based on intercellular communication. NPCs may communicate with host cells (e.g. cells of the immune system) via secretion of cytokines, chemokines, growth factors, and other mediator molecules, cell-to-cell interactions, or secretion of extracellular membrane vesicles.[73]

FUTURE DIRECTIONS

One of the major challenges in MS is to understand the mechanisms underlying the pathogenesis of the disease. MS is a complex disease that affects young adults who are genetically susceptible and are then exposed to a precipitating environmental factor or infectious agent. MS lesions are likely to originate from an autoimmune disorder, which involves various cell types and modulating molecules of the immune system. Autoimmunity may be the consequence of molecular mimicry, that is, the activation and recruitment of autoreactive immune cells due to structural homology between a self antigen and an exogenous pathogenic protein. Similarities in the disease observed in both human MS and EAE provide strong evidence for this hypothesis.[7] Susceptibility genes have been identified and genome-wide analysis is now available. Improving techniques of genomic investigation may lead to a better understanding of genetic associations with the disease.[20]

Many aspects remain unclear, including functional relations between the myelin sheath and the axon. White matter demyelinated lesions of the CNS in MS patients start with destruction of the myelin sheath of neurons by inflammatory reactions. The progressive stage of the disease is then dominated by axonal damage and neurodegeneration of chronically demyelinated neurons. However, it is still uncertain whether inflammation in MS is primary or secondary.[2] Less is known about MS cortical lesions, but it seems that inflammation does not occur in damaged areas in the gray matter. Brain imaging has suggested that the pathogenesis of MS includes a distinct and more global cortical pathology. In addition, as anti-inflammatory therapies appear to have no

effect on the progressive stage, the question is raised whether inflammation initiates tissue damage in progressive MS.

Another major question is whether PPMS is the same disease as RRMS. The underlying mechanisms in both clinical patterns remain unknown. However, differences in disease course, epidemiology, and clinical pathology assessed by MRI suggest different forms of pathogenesis in RRMS and PPMS.[10]

Elucidating the cause of MS may provide key information in understanding the chronology of events leading to demyelination and axonal degeneration. Together with a consistent animal model, this could be helpful in further determining relevant targets for MS treatments. Therapeutic strategies aim at modulating the immune response, protecting the CNS, potentiating remyelination, and improving nerve conduction. Future prospects in MS therapy are based on stem cells; their systemic transplantation has been shown to protect the CNS from degeneration driven by chronic inflammation in EAE, through immunomodulatory or neuroprotective mechanisms.[47]

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