

Chapter 17

White Matter Damage in Multiple Sclerosis

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17.1 Introduction

Multiple sclerosis (MS) is the leading cause of nontraumatic neurological disability in young adults in the United States and Europe. MS is an inflammatory demyelinating disease of the central nervous system (CNS) and generally considered a predominantly autoimmune disease, mediated by an autoreactive and aberrant T cell attack against CNS elements, particularly myelin. In line with this notion, immunomodulatory and anti-inflammatory therapies prove to be effective, especially early in the disease phase. However, the disease often progresses relentlessly in later disease stages without much evidence for acute inflammation and no obvious effect of anti-inflammatory therapies (Stadelmann et al. 2011).

MS initially presents as a relapsing-remitting disease in most patients, with immunological attacks (relapses) which may last from days to weeks with a posterior recovery phase. However, the antigenic stimuli that initiate or perpetuate this abnormal immune reactivity are still a matter of intense research and debate. Focal lesions showing perivascular inflammation, infiltration of immune cells, axonal degeneration, oligodendroglial death, and demyelination characterize this chronic and degenerative disease (Prineas et al. 2002). MS lesions can arise anywhere in the CNS but, they show a predilection for the optic nerve, spinal cord, brain stem, and periventricular areas. Furthermore, brain tissue immediately adjacent to the subarachnoid space, i.e. subpial gray matter, is especially vulnerable to demyelination (Bo et al. 2003), a fact that has only recently been appreciated. Mostly, within a patient, lesions of similar age

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resemble each other with respect to the extent and pattern of inflammation and remyelination (Lucchinetti et al. 1999, 2000; Patrikios et al. 2006).

Demyelination results in slower conduction or complete failure of transmission, leading to symptoms or neurological deficits associated with damage to the CNS, especially white matter tracks. Some of the most common findings are optic neuritis and visual impairment, weakness, sensory disturbances, lack of motor coordination, ataxia, nystagmus, and cognitive dysfunction. However, the clinical presentation of MS varies greatly and correlates well with the multiplicity of lesions and their distribution at various anatomical sites within the brain and spinal cord.

MS affects women more often than it does men. Its incidence is approximately 3.6 cases among women and 2 cases among men per 100,000 individuals per year. Its prevalence varies geographically: higher MS incidence is classically associated with countries with cold climates and at high latitudes (averaging 90–100 cases per 100,000 individuals), although recent studies report that this gradient has become attenuated after 1980, apparently due to increased incidence in lower latitudes (Alonso and Hernan 2008). Other factors than genetic determinants could also be influencing this observed change, such as infections or vitamin D and sun exposure, which are correlated with MS incidence (Giovannoni and Ebers 2007). Other environmental conditions, such as smoking and reproductive factors, are thought to be involved in the female-to-men incidence ratio for MS.

17.2 Characteristics of MS

17.2.1 *Clinical Observations and Diagnostic Criteria for MS*

Although MS starts with one attack, the profile of the disease is the multiplicity of relapses which underlies CNS dysfunction. Visual impairment to different degrees, orbital pain and frontal headaches are often the presenting symptoms, and disease diagnosis is based on clinical criteria supported by visualization of CNS white matter lesions by magnetic resonance imaging (MRI) following established standards, recompiled in the McDonald Criteria (Polman et al. 2011). MS lesions are typically disseminated in time and space. Dissemination in time involves more than one relapse, whereas dissemination in space implies involvement of more than one area of the CNS. The multiplicity of MS plaques and their location at various anatomic sites may account for the great variability of clinical symptoms and signs. Diagnostic complementary evidence to MRI scans includes analytical examination of cerebrospinal fluid (CSF), and evaluation of visual evoked potentials (VEP). CSF analysis can provide evidence of the immune and inflammatory nature of lesions, being the presence of different oligoclonal IgG bands and an elevated IgG index indicative of a CSF abnormality, whereas VEP typical of MS are delayed in time but with well-preserved wave form.

17.2.2 *Types of MS*

MS patients present several common patterns of symptoms, and each pattern is associated with variable intensities of inflammatory response. According symptoms and the course of disease, MS patients are classified into different subtypes. The most common form, affecting 85–90 % of newly diagnosed patients, is relapsing-remitting multiple sclerosis (RRMS), which is characterized by discrete clinical “attacks” or “relapses” followed by subsequent improvement. RRMS is the most typical presentation in younger patients with a mean age of onset of around 30 years. It is characterized by relapses of neurological dysfunction that last weeks to months and affect various locations of the brain, optic nerves and/or spinal cord. Multifocal areas of abnormality are found on magnetic resonance scanning, typically (but not exclusively) in the white matter. The MRI appearance of some lesions exhibit enhancement after intravenous administration of gadolinium, indicating breakdown of the blood–brain barrier as a result of active inflammation (reviewed in Stys et al. 2012). Within 10 years of disease onset, approximately 50 % of patients with RRMS will develop a slow, insidiously progressive, neurological deterioration and CNS atrophy (usually progressive gait impairment), with or without clinical attacks superimposed. This is termed secondary progressive multiple sclerosis (SPMS), and 90 % of RRMS patients advance into this stage within 30 years of disease onset. A minority of patients (approximately 10–15 %) have primary progressive multiple sclerosis (PPMS), which is characterized by a progressive course from onset, with occasional plateaus and absence of clinically evident relapses. In addition, PPMS presents less conspicuous inflammation on MRI. Due to its difference from RRMS, and particularly because of the absence of any relapse, it has been suggested that PPMS may represent a different disease (Thompson et al. 1997). However, although the initial courses of RRMS and PPMS are very different, the progressive phases of each proceed at remarkably similar rates (Scalfari et al. 2010) and, intriguingly, the conversion from RRMS to progressive MS tends to occur in a well-defined age window of 35–50 years, which is the same as the typical age of disease onset in patients with PPMS (Leray et al. 2010). Finally, approximately 5 % of the MS patients develop progressive relapsing multiple sclerosis (PRMS), which is characterized by worsening from onset with clear, acute relapses (with or without recovery), and with periods between relapses with continuing progression of deterioration (Lublin and Reingold 1996; Rovaris et al. 2006).

The prognosis of MS varies widely. The factors responsible for this variability are unclear and it therefore remains difficult to estimate prognosis in individual patients at the time of diagnosis. The outcome of an attack depends on the severity and extent of axonal injury, and this largely determines the degree of recovery or the persistence of neurological deficits. Usually, after inflammation has resolved and myelin debris has been removed, the conduction of the neural impulses is reestablished in the denuded nerve fibers. Although the resolution of inflammation allows a remission with full or partial recovery of neurologic deficits, the appearance of

new plaques, which may develop at any time throughout the course of the disease, would cause a relapse. Disability becomes permanent when the structural continuity of the nerve fibers is disrupted and wallerian degeneration develops (reviewed in Prineas et al. 2002).

Clinical indicators of a relatively good prognosis are female gender, younger age of onset, optic neuritis, sensory attacks, complete recovery from attacks, few attacks, and long inter-attack interval. Relatively poor prognostic factors include male gender, predominant cerebellar and motor involvement, incomplete resolution of attacks, progressive course from onset, frequent early attacks, and short inter-attack interval (Keegan and Noseworthy 2002).

In general, the average duration of the disease is 25–30 years. One-third of patients have a benign course, remain fully functional, and show little disability for 15 years after disease onset. In contrast, malignant MS is defined by a rapid, progressive course, leading to significant neurological deficits and even death (Lublin and Reingold 1996; Prineas et al. 2002).

17.2.3 Etiology of MS

MS is a complex and multifactorial disease which cannot be associated to a single genetic or environmental factor and, at least, interactions between a potential genetic susceptibility and environmental factors are implicated in the origin of MS (Zamvil and Steinman 2003). It is widely accepted that the etiology of this illness has autoimmune and inflammatory grounds, and that a derailment of the immune system leads to cell and antibody-mediated attacks on myelin.

17.2.3.1 Autoimmunity

The immune system plays an integral role in the initiation and progression of MS (Hemmer et al. 2002; Keegan and Noseworthy 2002). Thus, MS can be seen as a disease in which genetically susceptible individuals, upon encountering an environmental stimulus such as an infection, generate an autoimmune attack against CNS myelin based on molecular mimicry between infectious and myelin antigens.

Autoimmune-mediated reactions against myelin, which are based on the inappropriate immunological attack towards self-antigens, constitute the most widely accepted hypothesis for MS etiology, although the initial trigger of autoimmunity is still unknown. This autoimmune hypothesis is supported by similarities between the MS pathology and experimental allergic encephalomyelitis (EAE), the dominant MS animal model, which is induced by immunization with brain and spinal cord myelin extracts. In this model, myelin antigen-specific CD4⁺ T cells can induce CNS inflammation, demyelination, and neurodegeneration, resulting in the loss of motor functions or paralysis (Brown and Sawchenko 2007).

17.2.3.2 Genetic Susceptibility

Familiar occurrence is recognized and the disease has been reported in monozygotic and dizygotic twins; however, the genes that contribute to MS susceptibility are difficult to identify because they exert a relatively modest effect on disease risk. In support of the genetic contribution, certain gene variants in the class II major histocompatibility complexes (MHCs) occur more frequently among MS patients than among the general population (Prineas et al. 2002). Thus, two large genetic studies have revealed an association of MS in a genetic region of the European and North American Caucasian population (Lincoln et al. 2005; Sawcer et al. 2005). Risk alleles are located in human leucocyte antigen (HLA) class II region. Specifically, HLA-DRB1 and HLA-DQB1 alleles (DR15 haplotype) are linked to MS susceptibility, with possible interactions between them and closing neighboring variants. This link was confirmed in the largest genome-wide association study conducted to date, which also revealed two other immune-related targets including the receptors for the interleukins IL-2 and IL-7 (Hafler et al. 2007; Lundmark et al. 2007). Some evidence indicate that the complexity of class II loci points to epistatic interactions at this locus, with interactions between susceptibility and resistance alleles (Giovannoni and Ebers 2007).

17.2.3.3 Environmental Factors

Increasing rate of MS in countries with cold climate and a high latitude, together with the higher incidence in women, suggest that early life events have an influence in MS development. Although, individuals who migrate from a cold climate to a warm climate after 15 years of age retain the higher risk associated with their native locality, high levels of vitamin D decrease the risk for MS. This suggests an immunomodulatory role of the vitamin D on T cells (Giovannoni and Ebers 2007). In addition, smoking before the onset of MS has a significant, although moderate, risk factor the subsequent development of MS.

17.2.3.4 Transmissible Agents

Aside from the genetic and environmental component, the role of transmissible agents as a cause of MS is a relatively popular hypothesis. It is possible that exposure to an unidentified infectious agent may occur during the early years of life. Several pathogens have been postulated to trigger the immune reaction: measles virus, Epstein-Barr virus (EBV), human herpes virus 6, retroviruses, and *Chlamidia pneumoniae*. Among them, data associating EBV infection with MS remains strong. Thus, people with symptomatic EBV infection are at increased risk of developing MS, similar to people with high titers of anti-EBV antibodies. There are also observations supporting EBV-induced molecular mimicry as an underlying mechanism in MS autoimmunity. This association may be causative, or it may simply be a phenomenon required for disease onset (Giovannoni and Ebers 2007).

17.3 Pathophysiology of MS

MS is a chronic, degenerative disease of the CNS which pathological hallmarks are inflammation, demyelination, neurodegeneration, oligodendroglial death, and axonal degeneration; these alterations occur either focally or diffusely throughout the white and gray matter in the brain and spinal cord.

Pathophysiological models of MS should reproduce the generation of acute demyelinating lesions and their evolution into chronic sclerotic plaques, as well as an unpredictable clinical course that is characterized initially by recurrent relapses and later by steady progression. Although MS is regarded as a white matter disease, and the principal features of the disease are demyelination, perivascular inflammation, and the presence of plaques within the white matter, the incidence of demyelination and oligodendrocyte or neuron/axon injury are also prominent and widespread in gray matter structures, such as the cerebral cortex, thalamus, and basal ganglia (Lassmann 2007; Stadelmann et al. 2008). Thus, a particularly high prevalence of plaques in the cerebral cortex has been observed in progressive stages of the disease, and constitutes a significant proportion of the overall pathology of the brains of MS patients.

The generally accepted pathophysiological model for MS is centered on an immune-mediated attack against CNS myelin antigens, in which several components of the immune system generate an inflammatory response that damages myelin and axons, leading to the formation of acute plaques (Frohman et al. 2006; Fontoura and Garren 2010). However, although MS is considered an autoimmune CNS inflammatory disease, it is widely accepted that neurodegeneration is the predominant pathophysiological substrate of disability. Indeed, there is a strong correlation between inflammation and neurodegeneration (Zipp and Atkas 2006; Franciotta et al. 2008; Frischer et al. 2009; Lee et al. 2011); however, debate remains regarding whether inflammation is a primary or secondary process during both the onset and the development of MS (Trapp and Nave 2008; Craner and Fugger 2011).

17.3.1 *Inflammation in MS*

Inflammatory events in MS could be due to an autoimmune reaction against myelin antigens, which is more profound in actively demyelinating lesions and involves both cellular and humoral immunity with the participation of macrophages, T lymphocytes, and B cells. In the early stages of the disease process, T cells are primed in the periphery by antigen-presenting dendritic cells, and activated CD4⁺ T lymphocytes cross the blood–brain barrier and react with myelin and/or oligodendroglia antigens (Fig. 17.1). There is also evidence for humoral autoantibodies produced by local B cells binding myelin and other CNS components, as well as expression of immune-associated molecules, such as major histocompatibility antigens, adhesion molecules or pro-inflammatory cytokines, interleukin 12 (IL-12), and tumor necrosis factor- α (TNF- α). All of these factors contribute to the generation of a diffuse

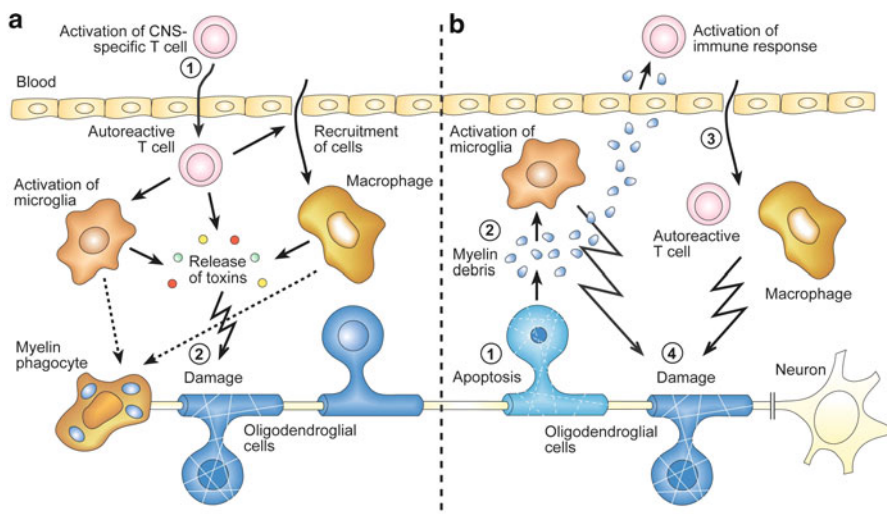


Fig. 17.1 Alternative views of the mechanisms of lesion formation in MS. **(a)** Activated T cells migrate into the CNS (1) and initiate inflammatory events including recruitment of blood macrophages, activation of local microglia, and the release of toxins. This leads to myelin destruction, oligodendrocyte death, and clearance of damaged tissue by phagocytes (2). **(b)** Viruses, glutamate, and other agents can cause extensive oligodendrocyte apoptosis in tissue foci (1). As a consequence, large amounts of myelin debris are generated (2) overwhelming the physiological mechanisms of elimination of apoptotic leftovers and thus triggering inflammation. Subsequently, T cells and macrophages invade the CNS (3) and initiate a stereotyped autoimmune attack of myelin (4) as described in **(a)**. Reproduced from Matute and Pérez-Cerdá (2005)

inflammatory process, with infiltrates mainly composed by lymphocytes and macrophages and with additional activation of the resident microglia.

In general, the inflammation process occurs during all stages of MS, although it changes as the disease progresses. In acute MS, infiltrating macrophages and activated T cells are predominant in focal demyelinated lesions. In addition, in chronic MS there is a diffuse inflammatory/degenerative process supported by microglia and macrophages that may be more or less independent of current focal inflammation (Kerschensteiner et al. 2009; Edan and Leray 2010). In this chronic phase, particularly oligodendrocytes, myelin, and axons degenerate in the CNS, causing a wide variety of symptoms that often progress to physical and cognitive disabilities.

17.3.2 Neurodegeneration in MS

Although MS has been mainly considered an immune disease, there is an evolving concept regarding MS as a primary neurodegenerative disease with secondary inflammatory demyelination. Thus, early axonal damage can result from a direct interaction with immune cells, there is a positive correlation between axonal transection and the

degree of inflammation in white matter MS lesions undergoing demyelination (Zipp and Atkas 2006; Trapp and Nave 2008; Nikić et al. 2011). In addition, recent studies support the concept that neurodegeneration is an independent process in MS, which may explain why current disease-modifying therapies predominantly targeting immunomodulatory mechanisms have a reduced efficacy against the development of permanent physical disability in the later stages of MS (Craner and Fugger 2011).

In the vast majority of MS patients, disease develops into a progressive stage in which neurological deterioration continues in the absence of relapses (Dutta and Trapp 2011). Alternatively, others are diagnosed with PPMS, in which neurological dysfunction occurs without relapses from disease onset (Dutta and Trapp 2011). In all instances, imaging, neuropathological studies, and animal studies of MS show that markers for neurodegeneration (primarily axonal damage and atrophy) appear during the progressive phase of the illness and correlate with neurological disability. Although the mechanisms of axonal degeneration are uncertain, inflammation and demyelination appear to be major risk factors (Ferguson et al. 1997; Trapp et al. 1998; reviewed in Trapp and Nave 2008). With this in mind, numerous studies have focused on axonal protection as a major therapeutic goal in MS, both promoting remyelination and analyzing different aspects of spontaneous remyelination and axonal recovery (e.g., Patrikios et al. 2006; Nikić et al. 2011).

There is also evidence suggesting a link between autoimmunity, clinical phenotype, and neurodegeneration in MS. MS patients develop antibodies to oligodendrocytes, myelin, and other neuronal antigens that cause neurodegeneration and contribute to the pathogenesis of the disease (Franciotta et al. 2008; Lee et al. 2011).

These data strongly suggests that the molecular mechanisms responsible for axonal degeneration may differ between early and late stages of MS, and that there is an unclear link between the mechanisms of neurodegeneration and focal/diffuse inflammation and their relative contribution to the clinical deficits observed in different phases of the disease.

17.3.3 Oligodendrocyte Damage and Demyelination

The depletion of oligodendrocytes is a recognized feature of MS lesions, becoming more apparent as the disease evolves (Raine 1994). In compliance with this idea, Fas expression is elevated in oligodendrocytes in chronic active and chronic silent MS lesions. Fas is a cell surface receptor that transduces cell death signals, and its upregulation in oligodendrocytes suggests that Fas-mediated signaling might contribute to immune-mediated oligodendrocyte injury and subsequent demyelination in MS (D'Souza et al. 1996). In turn, treatment of oligodendrocytes with antibodies against myelin-oligodendrocyte glycoprotein (MOG) leads to an increase in Ca^{2+} influx and activation of the MAPK/Akt pathways, a signaling cascade relevant to the initial steps of MOG-mediated demyelination (Marta et al. 2005).

A number of experimental studies have demonstrated a strong positive correlation between oligodendrocyte susceptibility to injury and the extent of CNS

inflammation in EAE. In a knockout mouse system, absence of oligodendrocyte protective factors increases oligodendrocyte susceptibility to injury and augments the inflammatory reaction and the severity of symptoms (Butzkueven et al. 2002; Balabanov et al. 2007). In contrast, mice lacking proapoptotic genes or overexpressing antiapoptotic molecules, specifically in oligodendrocytes, display resistance to EAE and inflammatory demyelination (Hisahara et al. 2000; Hövelmeyer et al. 2005). A recent study has described that the oligodendrocytic overexpression of the dominant-negative form of interferon regulatory factor-1 (IRF-1), a severity factor for both MS and EAE, results in significant protection against EAE with a reduction of inflammatory demyelination and with oligodendrocyte and axonal preservation (Ren et al. 2011). These data suggest that oligodendrocytes are actively involved both in the regulation of EAE and in the network of neuroimmune responses. Therefore, exploring oligodendrocyte-related pathogenic mechanisms in addition to conventional immune-based mechanisms may have important therapeutic implications in MS.

On the other hand, early MS lesions have a prephagocytic nature and display primary oligodendrocyte injury in the absence of microglial activation and adaptive T cell or B cell responses (Barnett and Prineas 2004; reviewed in Matute and Pérez-Cerdá 2005; Fig. 17.1). The trigger for that selective and apparently primary oligodendrocyte damage is unknown; however, it may include viruses, glutamate, ATP, and other agents known to be oligotoxic (reviewed in Matute 2011; Matute and Cavaliere 2011). These data correlate with previous pathological characterizations of MS lesions, such that some plaques are highly suggestive of a primary oligodendrocyte dystrophy rather than an autoimmunity process (Lucchinetti et al. 2000). More recent studies on autopsy material from patients in early, active stages of MS show little evidence of T cell or B cell infiltration in areas of brisk demyelination and oligodendrocyte loss; they only show macrophage infiltration and microglial activation, which is evidence of an innate immune response that is triggered to clear debris (Henderson et al. 2009).

Likewise, lack of adaptive immune response occurs in multiple system atrophy, a degenerative disorder where the main target of the disease process is the oligodendrocyte, which shows prominent secondary myelin degeneration and a reactive microgliosis (Wenning et al. 2008). In addition, inducing primary death of oligodendrocytes per se does not engender an autoimmune reaction, despite causing robust demyelination, even in the case when they induced concomitant strong stimulation of the immune system (Locatelli et al. 2012).

These findings suggest that MS lesions may initiate with oligodendrocyte death of unknown origin in the absence of inflammatory activity, and that the heterogeneity observed in the neuropathology of the lesions within and among patients may be a reflection of the time point at which a given lesion is observed. Therefore, it is possible that primary oligodendrocyte injury leads to microglial activation, with the adaptive T cell and B cell response appearing as a secondary event. In this context, the innate immune system may play a much more fundamental role than previously thought in MS lesion pathogenesis, and therapies aimed at this side of the immune response will prove effective, including in progressive stages of the disease (Fontoura and Garren 2010).

17.4 Mechanisms Involved in White Matter Damage in MS

17.4.1 Microglial Activation

In addition to changes to oligodendrocytes and neurons, microglia are also relevant to MS pathophysiology (He and Sun 2007) and these glial cells play important roles in both the destructive and restorative phases of MS. Specifically, reactive microglia may be both deleterious or protective in MS pathogenesis (Muzzio et al. 2007; Sanders and De Keyser 2007).

MS is associated with the activation of immune cells, including macrophages, peripheral blood mononuclear cells and microglia. Microglial cells originate from monocyte/macrophage precursors and are regarded as the major immunocompetent cell type of the nervous system, constituting approximately 10 % of all cells in the brain. The immune response of the brain is spatially segregated from the peripheral immune response by the blood–brain barrier and, together with astrocytes and infiltrating peripheral immune cells, is predominantly executed by microglia. Thus, microglial cells, as brain-resident immune cells, are a sensor of pathological signals in the CNS and play a major role in host defence and tissue repair in the brain. They are rapidly activated and respond with morphological changes, transforming the resting ramified microglia into an amoeboid form with phagocytic activity, proliferation, and the production of a wide array of inflammatory mediators (Kreutzberg 1996; Cuadros and Navascués 1998). Past studies have shown that exposure to different factors, such as lipopolysaccharide, interferon- γ , or β -amyloid, leads to microglial activation and induces the production of various pro-inflammatory mediators that are potentially neurotoxic (Meda et al. 1995; Zielasek and Hartung 1996). These mediators include nitric oxide, prostaglandin E2, pro-inflammatory cytokines (e.g., TNF- α , IL-1 β , and IL-6), and reactive oxygen species (reviewed in Bi et al. 2011).

There is evidence that the constant activation and release of pro-inflammatory factors promotes the development of neurodegenerative diseases and therefore, microglial activation plays an important role in the pathophysiology of these diseases (Bi et al. 2011). Therefore, inhibition of pro-inflammatory mediators in microglia attenuates the severity of Alzheimer's disease, Parkinson's disease, trauma, multiple sclerosis, and cerebral ischemia (Koning et al. 2007; Krause and Müller 2010; Qian et al. 2010).

In RRMS, as well as in progressive MS, active tissue injury is associated with microglial activation (Prineas et al. 2001). Activated microglia and microglial nodules are invariably seen in the normal-appearing white matter (NAWM) of patients with progressive MS. In addition, microglial activation is also seen in many other neuroinflammatory or neurodegenerative diseases in the absence of pathological changes resembling those in MS, such as selective primary demyelination (Czeh et al. 2011). Thus, microglial activation might contribute to neurodegeneration in MS, but additional mechanisms are required to trigger patterns of tissue damage that are specific to MS. Oxidative burst by activated microglia seems to have a major role in the induction of demyelination and progressive axonal injury in MS

lesions (Lassmann et al. 2012). Furthermore, microglia might also have neuroprotective functions depending on the types and triggers of activation, and could stimulate remyelination via removal of damaged tissue and secretion of neurotrophic molecules (Czeh et al. 2011).

17.4.2 Reactive Astrocytosis

Reactive astrocytes contribute to the glial scar that fills the demyelinated plaque in MS (Holley et al. 2003), however its role in disease progression is controversial. Astrocytes can support migration, proliferation and differentiation of oligodendrocyte progenitors, and at the same time they can also promote inflammation and damage to oligodendrocytes and axons (Williams et al. 2007).

Astrocytes can be targeted by the immune reaction, as in neuromyelitis optica (NMO), a disease closely resembling MS. NMO is characterized by demyelinating lesions of the optic nerve and spinal cord that are typically more destructive than MS lesions. This disease is characterized by antibodies against an antigen of astrocytic foot processes, aquaporin 4 (AQP4) (Lennon et al. 2005; Roemer et al. 2007). Recent data indicate that anti-AQP4 antibodies from NMO patients are able to induce selective astrocyte death in vivo and, at higher dosage, demyelination and axonal damage (Bradl et al. 2009; Bennett et al. 2009; Sharma et al. 2010).

This circumstance raises the question of whether the target structure in MS is necessarily the myelin sheath or oligodendrocyte. To date, no anti-AQP4 antibodies or astrocyte depletion have been observed in MS; however, the search for the antigen is still ongoing (Vyshkina and Kalman 2008; Stadelmann et al. 2011).

17.4.3 Axonal Damage and Loss

Degeneration of demyelinated CNS axons is increasingly recognized as a common accompaniment to inflammatory demyelination. Axons may be damaged by the same underlying primary degenerative processes that affect the myelinating unit, or they might undergo secondary degeneration by virtue of demyelination.

A number of studies highlight the emerging role of disturbed axonal ion homeostasis in the process of neurodegeneration. Internodal axons express glutamate receptors (Stirling and Stys 2010), and it is possible that these could be chronically overactivated, under pathological conditions, leading to primary axonal pathology. There is solid evidence that glutamate levels are increased in the human brain (Srinivasan et al. 2005) as a consequence of altered glutamate homeostasis (Vallejo-Illarramendi et al. 2006) and thus, trigger excitotoxic destruction of oligodendrocytes and myelin as well as of axons (Matute et al 2001; Domercq et al. 2005). AMPA and kainate axonal receptor activation can induce a small amount of Ca^{2+}

entry, which in turn releases further Ca^{2+} from the axoplasmic reticulum by opening intracellular calcium channels, known as ryanodine, or through of phospholipase C activation as well as L-type Ca^{2+} channels opening (Ouardouz et al. 2009a, b). The functional significance of these signaling mechanisms by glutamate receptors in axons is unknown but they may serve to amplify axonal Ca^{2+} signals which seem to be weak because of the limited quantity of cation available in the narrow space. In turn, high local concentrations of Ca^{2+} generated by these receptors may result in focal swellings and irreversible axonal transactions (Ouardouz et al. 2009b; Stirling and Stys 2010).

In addition, aberrant expression of Na^+ channels, acid-sensing Na^+ channels, glutamate receptors and voltage-gated Ca^{2+} channels has been detected in dystrophic or demyelinated axons. Alterations in the expression and/or activity of these ion channels could directly or indirectly lead to intra-axonal Ca^{2+} accumulation and concomitant axonal degeneration. As a consequence, such ion channels could be potential targets for neuroprotective pharmacological therapies in patients with MS (Lassmann et al. 2012).

Alternatively, axonal damage in MS might be a secondary phenomenon. For example, it could be damaged by the release of glutamate, reactive oxygen or nitric oxide species, and cytotoxic cytokines from immune cells in the vicinity of inflammatory plaques (Siffrin et al. 2010; Matute 2011) or could also occur through non-inflammatory mechanisms that cause disruptions of the close physical and biochemical relationship between axons and their myelin sheaths (Stys et al. 2012). The loss of myelin greatly enhances the propensity of axons for transport disturbance (Trapp and Stys 2009). Thereby, energy production may be compromised owing to mitochondrial disruption (Mahad et al. 2009) and $\text{Na}^+\text{-K}^+\text{-ATPase}$ -mediated ion transport may be reduced in many demyelinated axons in the MS brain (Young et al. 2008), which could bias such an axon towards a state of virtual hypoxia. The resulting mismatch between energy supply and demand could culminate in degeneration (Stys 2004; Trapp and Stys 2009).

On the other hand, neuronal antigens have recently been identified as targets of the immune reaction. Specific immune reactions against neurofilament, beta-synuclein, contactin-2/TAG-1, and neurofascin lead to CNS inflammation (reviewed in Stadelmann et al. 2011). Importantly, anti-neurofascin antibodies have been identified in a proportion of MS patients and these antibodies have been shown to aggravate axonal damage and clinical disease in the EAE model (Mathey et al. 2007).

17.4.4 Oligodendroglial Injury

17.4.4.1 Excitotoxicity and Calcium Dysregulation

Primary and/or secondary alterations in glutamate signaling cause excitotoxicity that contribute to MS pathology. Thus, numerous studies carried out in cellular and animal models of MS as well as in postmortem brain and in patients indicate that

excitotoxicity mediated by Ca^{2+} -permeable glutamate receptors contributes to oligodendrocyte death, demyelination, and tissue damage in MS.

Glutamate dyshomeostasis results from primary and/or secondary inflammation as a consequence of the autoimmune attack to the CNS and/or resulting from ongoing cell damage within the brain and spinal cord. Thus, activated microglia releases cytokines and free radicals that diminish glutamate uptake. This in turn elevates the extracellular levels of this transmitter, resulting in overactivation of Ca^{2+} -permeable glutamate receptors which lead to oligodendrocyte excitotoxicity (Sánchez-Gómez et al. 2003; Domercq et al. 2007). Moreover, activated microglia increases their expression of the glutamate-cystine exchanger which contributes further to raising the levels of glutamate and its toxicity (Domercq et al. 2007). Other mechanisms accounting for glutamate dyshomeostasis include genetic variability in the promoter of the major glutamate transporter, EAAT2, which results in lower transporter expression (Pampliega et al. 2008). Finally, an additional component of the genetic background linking MS and deregulation of glutamate signaling and Ca^{2+} -dyshomeostasis may lie in a polymorphism in the Ca^{2+} -permeable AMPA receptor subunit GluR3, an abundantly expressed subunit in oligodendrocytes, which is associated with a subgroup of patients responding to interferon beta therapy in MS (Comabella et al. 2009).

In addition to glutamate, ATP signaling can trigger oligodendrocyte excitotoxicity via activation of Ca^{2+} -permeable P2X7 purinergic receptors expressed by these cells (Matute et al. 2007). Importantly, sustained activation of P2X7 receptors *in vivo* causes lesions which are reminiscent of the major features of MS plaques, and treatment with P2X7 antagonists of chronic EAE reduces demyelination and ameliorates the associated neurological symptoms. These results are in line with data in P2X7 null mice showing that this deficiency suppresses the development of EAE (Sharp et al. 2008), and at odds with earlier observations indicating that the lack of P2X7 receptors aggravates EAE (Chen and Brosnan 2006). These apparent discrepancies may be caused by the different strains of P2X7 KO mice used, and can be explained by compensatory mechanisms developed in the genetically modified animals during development and maturation. In this regard, pharmacological blockade of P2X7 receptors with selective antagonists after disease onset is probably a more relevant approach to study the importance of these receptors to MS than the use of P2X7 null mice.

In addition, P2X7 RNA and protein levels are elevated in normal appearing axon tracts in MS patients, suggesting that signaling through P2X7 receptors in oligodendroglia is enhanced in this disease which may render this cell type more vulnerable to ATP dysregulation (Matute et al. 2007). The increased expression of P2X7 receptors in axon tracts before lesions are formed indicates that this feature may constitute a risk factor associated with newly forming lesions in MS; this receptor subunit may thus prove to be a diagnostic and/or prognostic clinical biomarker for MS. On the other hand, blockade of P2X7 receptors protects oligodendrocyte from dying; this property has therapeutic potential for halting the progression of tissue damage in MS (Matute and Cavaliere 2011).

17.4.4.2 Mitochondrial Damage and Oxidative Stress

Current attention in the MS research is focused on the role of mitochondrial injury in demyelination and neurodegeneration (Trapp and Stys 2009; Witte et al. 2010). Mitochondrial injury induced by oxidative stress might underlie the pathological features of MS lesions, such as oligodendrocyte apoptosis, demyelination, destruction of thin-caliber axons, and lack of remyelination (Lassmann et al. 2012).

Evidence for mitochondrial damage in MS lesions was originally identified from biochemical analyses of impaired activity of mitochondrial enzymes in chronic active lesions in MS (Lu et al. 2000). Subsequent detailed immunohistochemical investigations of respiratory chain proteins revealed profound mitochondrial injury, possibly reflecting increased oxidative damage in areas of initial tissue injury within active MS lesions (Mahad et al. 2008). In general, the data indicate that active tissue damage in MS and key features of the pathology of MS lesions can be explained as a result of mitochondrial injury and oxidative stress.

In oligodendrocytes, mitochondrial injury results in the generation of oxygen free radicals and release of apoptotic factors leading activation of caspase-dependent death pathways (Sánchez-Gómez et al. 2003). In addition, dysfunction mitochondrial triggers release of apoptosis-inducing factor, its translocation into the nuclei, and activation of poly-ADP-ribose polymerase (PARP), a mechanism demonstrated *in vivo* in the experimental model of oligodendrocyte destruction and demyelination induced by cuprizone intoxication (Veto et al. 2010). Furthermore, *in vitro* oligodendrocyte progenitor cells are more resistant to mitochondrial injury than are mature oligodendrocytes, but are impaired in their ability to differentiate and form myelin sheaths (Ziabreva et al. 2010). This observation could explain the failure of remyelination in chronic MS plaques, despite the presence of oligodendrocyte progenitor cells (Chang et al. 2002).

The relationship between mitochondrial alterations and oxidative stress is complex. Mitochondrial dysfunction results in generation of free radical and conversely, oxidative stress also drives mitochondrial dysfunction by several different mechanisms. Free radicals disrupt mitochondrial enzyme function, modify mitochondrial proteins and accelerate their degradation, interfere with *de novo* synthesis of respiratory chain components, and can directly induce mitochondrial DNA damage (reviewed in Lassmann et al. 2012). In active MS lesions, the expression of enzymes involved in free radical production is markedly increased, most prominently in areas of initial tissue injury, and oxidized DNA and lipids are abundantly present (Van Horssen et al. 2011; Fischer et al. 2012). The signs of oxidative stress in MS lesions are concomitant with the upregulation of proteins involved in antioxidant defence mechanisms (van Horssen et al. 2010) and might explain the high levels of expression of molecules associated with endoplasmic reticulum stress, such as CHOP or BiP (Cunnea et al. 2011).

Oxidized DNA and lipids in apoptotic oligodendrocytes and dystrophic axons strongly supports the contribution of these alterations to demyelination and neurodegeneration (Haider et al. 2011). Moreover, it is known that iron accumulates in

the aging human brain where it is predominantly stored in oligodendrocytes and detoxified by its binding to ferritin. As shown *in vitro*, intracytoplasmic accumulation of Fe^{2+} in oligodendrocytes might in part explain the high susceptibility of these cells to degeneration under conditions of oxidative stress induced by inflammation and mitochondrial dysfunction (Zhang et al. 2005). Importantly, oligodendrocyte destruction releases accumulated Fe^{2+} into the extracellular space and, its uptake into cells within the lesions might further amplify oxidative damage and increase the susceptibility of the surrounding tissue to free-radical-driven demyelination and neurodegeneration.

17.5 Neuropathology of MS

Common histopathological hallmarks of MS is the presence of multifocal areas of inflammatory demyelination and axonal loss distributed over time and space within the brain and spinal cord white and gray matter. Although demyelination in MS is largely restricted to focal lesions, other aspects of its pathology are less confined (Prineas et al. 2002). Whether inflammatory demyelination is primary or secondary in the disease progression remains controversial (Trapp and Nave 2008). This is due to the difficulty of obtaining appropriate MS specimens to evaluate the temporal course of each individual lesion.

Classical active MS lesions are described as perivascular inflammatory infiltrates, consisting mainly of T cells and macrophages, together with myelin breakdown and degeneration of axons. These features led to a pathophysiological view in which the disease trigger is an immune dysregulation (Hu and Lucchinetti 2009; Lassmann 2011). However, recent neuropathological studies have provided evidence of primary oligodendropathy as a cause of demyelination (Barnett and Prineas 2004; Prineas and Parrat 2012). If this were the common mechanism of MS, the disorder would be regarded as a primarily degenerative disorder rather than an autoimmune disease (Nakahara et al. 2010; Stys et al. 2012).

17.5.1 Focal Lesions and Diffuse Damage in MS

Focal plaques of demyelination in white matter are the diagnostic hallmark of MS pathology at all stages of the disease. They are typically classified into four categories: classic active lesions, slowly expanding lesions, inactive lesions and remyelinated shadow plaques (Prineas et al. 2002). In addition, the so called NAWM may show some pathological changes (Allen et al. 2001), and fine evaluation of myelin in gray matter areas resulted in different types of cortical pathologies in MS brains (Kutzelnigg et al. 2005).

17.5.1.1 Classical Active Lesions

These lesions are the most abundantly found in patients with acute and relapsing disease. In contrast, they become rare during the progressive stage of the disease (Prineas et al. 2002; Lassmann 2011; Lassmann et al. 2012). Active MS lesions display activated macrophages which contain remnants of myelin sheaths taken up during the demyelinating process, reactive astrocytes forming a glial scar intermixed with variable T cell (both CD4⁺ and CD8⁺) and B cell perivascular and parenchymal infiltrates, and blood–brain barrier breakage. Axonal injury characterized by transections and swellings is also pronounced, and its extent correlates with the number of lymphocytes (Trapp and Nave 2008). In this highly inflammatory environment, macrophages can be densely packed either throughout the lesion (acute plaques) or at the periphery (chronic active plaques).

The architecture of a chronic active plaque varies with the different temporal developmental sequence of injury from the center (less recent) to the edges (more recent) where the lesions expand. Thus, lesions commonly have an onion-like shape with a layer of initial tissue injury (“prephagocytic” area characterized by activated microglia) surrounding a region of early myelin phagocytosis (early active), a zone of advanced myelin digestion (late active) and an inactive central area which often shows early but unstable remyelination while inflammation is active (Lassmann et al. 2012). Early and late active demyelination can be distinguished by the presence of minor myelin proteins (MOG and myelin-associated glycoprotein) or major myelin proteins including myelin basic protein and proteolipid protein as degradation products within macrophages (Hu and Lucchinetti 2009). Most probably, chronic active lesions recapitulate events encountered in acute lesions. However, the initial stages of MS lesion formation are not well characterized as a consequence of the limited availability of human samples with recent new lesions.

17.5.1.2 Slowly Expanding Lesions

This lesion type accounts for approximately half of the lesions in progressive MS. They probably reflect a gradual expansion of preexisting lesions in the absence of major blood–brain barrier disturbance (Prineas et al. 2002; Lassmann 2011). These lesions display at their edge a narrow zone of robustly activated microglia with prominent acute axonal injury and macrophages containing early myelin degradation products. In addition, they are surrounded by diffusely activated microglia, and their inner part shows a dense astrocytic scar, profound axonal loss, absence of myelin without signs of remyelination, and nearly complete loss of oligodendrocytes, macrophages and microglia. Little is known about the mechanisms leading to cell extinction in the core of these lesions. It is conceivable that the absence of inflammatory reaction may limit the local capacity of restoring damaged myelin (Gay 2007), since phagocytes support initiating proliferation and differentiation of oligodendrocyte progenitor cells (Zhao et al. 2005).

17.5.1.3 Inactive Lesions

Inactive lesions are the most frequent lesion type found at all stages of MS (Prineas et al. 2002). In particular, this type of lesion accounts for the great majority of plaques in patients with long-standing disease. They are hypocellular in nature, have a glial population composed largely of small fibrous astrocytes, are clearly demarcated from the surrounding NAWM, and display no demyelinating or neurodegenerative activity at the lesion border. The core of these lesions is devoid of myelin and oligodendrocytes and has no signs of remyelination. Usually axon densities are very reduced and embedded in astrocytic scar tissue; and infiltrates of T or B lymphocytes are rare. In addition, the appearance of microglial cells changes from a few ramified cells inside the lesion to a densely packed population of ramified cells immediately outside the plaque (Lassmann et al. 2012).

17.5.1.4 Remyelinated Shadow Plaques

This is a term traditionally used in MS for any extensive area of partial reduction in myelin density (Prineas et al. 2002). While many shadow plaques show stable remyelination and inflammation is reduced practically to control levels (Patrikios et al. 2006), others become the site of fresh activity, with new lesions forming within or overlapping previously remyelinated tissue (Prineas et al. 2002).

17.5.1.5 Normal-Appearing White Matter

Although demyelination in MS is largely restricted to focal lesions, other aspects of pathology are less confined. Thus, NAWM has been defined pathologically as macroscopically normal white matter at least 1 cm away from a plaque edge with histological abnormalities. They include astrogliosis, microglial activation, vascular hyalinization, blood–brain barrier disturbances, mild inflammation with little T cell infiltration, reduced myelin density, remyelination, axonal loss and damage, as well as microplaque formation (Allen et al. 2001; Kutzelnigg et al. 2005; Hu and Lucchinetti 2009). Interestingly, diffuse NAWM microglial activation and axonal injury are most prominent in patients with PPMS or SPMS. Moreover, the extent of NAWM pathology correlates with the extent of cortical lesions but not with focal white matter load. Indeed, if microglial activation represents early pathogenic signs in preactive lesions, microglia may play a more relevant role in the development of lesions than inflammatory cell infiltrates (Gay 2007).

17.5.1.6 Gray Matter Lesions

With the introduction of new and sensitive methods for staining myelin, it is apparent that gray matter lesions, and particularly cortical demyelination, is extensively

and significantly involved in MS pathology (Kutzelnigg et al. 2005; Trapp and Nave 2008; Hu and Lucchinetti 2009; Dutta and Trapp 2011; Lassmann 2011). Compared to white matter lesions, cortical lesions contain less blood–brain barrier breakdown, few lymphocytic and macrophage infiltration, and more efficient myelin repair, being ramified microglia the dominant population. Despite limited inflammation, demyelination is present and neuronal and axonal pathology (neuritic transection, neuronal apoptosis, synaptic loss) are prominent features of cortical lesions, questioning the basic premises of MS pathogenesis. Typically cortical lesions appear as contiguously leukocortical areas of demyelination (classified as type I lesions) or strips or bands of subpial demyelination (type III lesions) that can not be considered as focal lesions. In addition but less frequently, small perivascular areas of demyelination can be seen (type II lesions). While type I and II lesions are found at all stages of MS, including acute and relapsing MS, type III lesions are mainly seen in patients with progressive disease. In addition, cortical demyelination correlates in part with diffuse white matter but not with focal white matter lesions.

17.6 Neuroimaging of MS

Although pathological assessment is the gold standard to identify MS lesions, there are intrinsic limitations owing to the very limited availability of biopsy tissue and additionally, tissue evaluation only provides one snapshot in time, not allowing observation of the evolution of pathological changes over time. Because of that, MRI and related techniques with higher specificity are promising for a better understanding of the pathophysiology of the MS “in vivo” (Polman et al. 2011).

On conventional T2-weighted MRI images, MS lesions appear as nonspecific focal areas of signal increase and therefore, may resemble by themselves and in absence of clinical information, many other types of pathology. In MS, hyperintense signals result from confluent perivenular lesions. In contrast, T1-weighted images show less sensitivity but more specificity to MS pathology and probably T1 hypointense signals are related to edema, demyelination, axonal loss and gliosis (Filippi et al. 2012). The sensitivity of lesion detection in T1-weighted imaging can be increased selectively with the injection of gadolinium-diethylenetriaminepentacetate (Gd-DTPA), a quelated form of Gd. Normally, Gd-DTPA in serum does not cross the blood–brain barrier, so T1-weighted images obtained after its administration allow the MS enhancing lesions that have a blood–brain barrier breakdown associated with ongoing inflammation to be identified (Kermode et al. 1990). The use of Gd-DTPA is a very sensitive and reproducible way to assess MS activity in focal white matter lesions. In fact, using conventional MRI techniques is possible to distinguish between active, acute or chronic, and inactive lesions. However, an underestimation of the degree of disease activity must be taken in mind because a relatively large proportion of MS lesions have very short-lived enhancement (Cotton et al. 2003). Importantly, enhancement is probably absent in slowly expanding lesions (Hochmeister et al. 2006).

Conventional MRI approaches are basically unable to detect tissue damage in the so-called NAWM in MS characterized by diffuse microglial activation and axonal injury. Some recent techniques that are far away of being integrated into routine clinical practice at present, have shown initial correlation between dysfunction and imaging that still needs future research and validation (Moll et al. 2011; Filippi et al. 2012; Rocca et al. 2012). These include magnetization transfer (MT) imaging and its quantitative index MT ratio, diffusion tensor MRI tractography and proton magnetic resonance spectroscopy.

17.7 Conclusions

MS is a complex and heterogeneous disease with an ill defined etiology. Although it is thought that damage in MS occurs primarily in CNS myelin and in oligodendrocytes, injury to axons, and ultimately to neurons, determines disease progression. In addition, autoimmunity and neuroinflammation are crucial to fluctuating neurological deficits in RRMS and in accumulating CNS injury.

MS has been traditionally considered a white matter disease in which lesions in plaques are the major pathological hallmark. However, closer inspection of the pathology has recently revealed that neurons and synapses are also deeply altered, and that profound diffuse damage is present in normally-appearing white matter. Moreover, gray matter lesions are also present often in MS.

A key unanswered question to MS etiology is whether the disease initiates by cytodeneration or by a primary autoimmune attack. This point is critical to drug development, since most strategies to find new treatments have relied on the use of EAE as an auto-immunity animal model of disease. As a consequence, most therapeutic agents of clinical application are anti-inflammatory in nature. Therefore, alternative disease models reproducing the neurodegenerative processes underlying MS are needed to advance in its understanding and treatment.

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