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The Neurobiology of Multiple Sclerosis: Genes, Inflammation, and Neurodegeneration

Review

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The autoimmune model of multiple sclerosis (MS) pathogenesis provided for many years a useful but incomplete conceptual framework for understanding the complex array of factors that lead to the loss of immune homeostasis, myelin and axonal injury, and progressive neurological symptoms. The availability of novel tools in molecular neurogenetics and increasingly sophisticated neuroimaging technologies, together with the revitalization of MS neuropathology, has created a new paradigm for the multidisciplinary study of this disease. This is reflected by the growing resolution of the MS genomic map, discovery of delicate inflammatory networks that are perturbed in MS, identification of mediators of demyelination, and recognition that cumulative axonal loss and neuronal injury are the histological correlates of neurological disability. Together, these advances have set the stage for the development of therapeutic approaches designed to target the demyelinating and neurodegenerative components of the disease and promote repair.

Introduction

Multiple sclerosis (MS), the most common cause of chronic neurologic disability beginning in early to middle adult life, has in recent years emerged as a subject of considerable interest to the neuroscience community. The global burden of MS has a number of truly remarkable characteristics, including an increasing incidence over the past century, an influence of latitude on risk, and increased risk in both females and white populations, especially those of northern European ancestry. Tracing the historical roots of MS has proven to be difficult, given the lack of knowledge of clinical-anatomic localization in neurology prior to the late 19th century. Notable is the paucity of convincing MS-like illnesses in the historical record prior to this time. Two reports from the late 13th century described women afflicted with chronic, multifocal, and partially remitting neurologic illnesses that conceivably might have been MS; these were the cases of a woman named Halla contained in the Icelandic Saga of St. Thorklar (Poser, 1994) and of a Dutch woman named Lidwina van Schiedam (Medaer, 1979). These uncertain cases excepted, MS makes its first clear appearance in 1822 in the diaries of Augustus D'Este, the illegitimate grandson of King George III (Firth, 1948).

Although it is not known whether MS is indeed a "new" disease, studies of the prevalence of MS conducted in multiple regions of the world suggest that the incidence increased steadily during the 20th century. It is possible that some of this increase is an artifact of improved case ascertainment, but most investigators believe that it is, at least in part, real, suggesting the presence of new environmental triggers. For many years multiple sclerosis was considered an immune-mediated disorder, primarily of interest to immunologists. However, the situation changed in the mid-1990s with the recognition that a neurodegenerative process, unresponsive to immunosuppression, was responsible for progressive neurological impairment. This new information, largely derived from results of clinical trials and reassessment of the neuropathology of MS, has brought myelin biology to the forefront of MS research. It emphasizes the need to understand, in the context of this disease, the axonal changes that follow demyelination, axon-myelin interactions essential to normal neuronal function and survival, and oligodendrocyte differentiation and remyelination.

This review builds on both well-established and emerging concepts of pathogenesis and their relationship to therapy, and the focus is on three core themes underlying the disease process: genes, inflammation, and neurodegeneration. No brief review of MS can fully address the breadth of issues related to this complex and multifactorial disease, and for more detail a number of outstanding sources exist (see for example Waxman, 2005 and Compston et al., 2006). The current challenges for the MS research community are to integrate large volumes of data from multiple sources to develop improved models of pathogenesis, and to translate these into effective therapies for the two million affected individuals worldwide.

Clinical Features

Symptoms of MS result from interruption of myelinated tracts in the central nervous system (CNS); the peripheral nervous system is spared. Initial symptoms are commonly one or more of the following: weakness or diminished dexterity in one or more limbs, a sensory disturbance, monocular visual loss (optic neuritis), double vision (diplopia), gait instability, and ataxia. Onset may be abrupt or insidious, and early symptoms may be severe or seem so trivial that a patient may not seek medical attention for months or years. As the disease worsens, bladder dysfunction, fatigue, and heat sensitivity occurs in most patients. Ancillary symptoms include Lhermitte's symptom (an electric shock-like sensation down the spine and into the limbs evoked by neck flexion), hemifacial weakness or pain, vertigo, and brief tonic spasms and other paroxysmal symptoms (thought to represent discharges originating along demyelinated axons). Cognitive deficits are common, especially in advanced cases, and include memory loss, impaired attention, problem-solving difficulties, slowed information processing, and difficulties in shifting between cognitive tasks. Depression is experienced by ~60% of patients during the course of the illness, and suicide is 7.5-fold more common than in age-matched controls.

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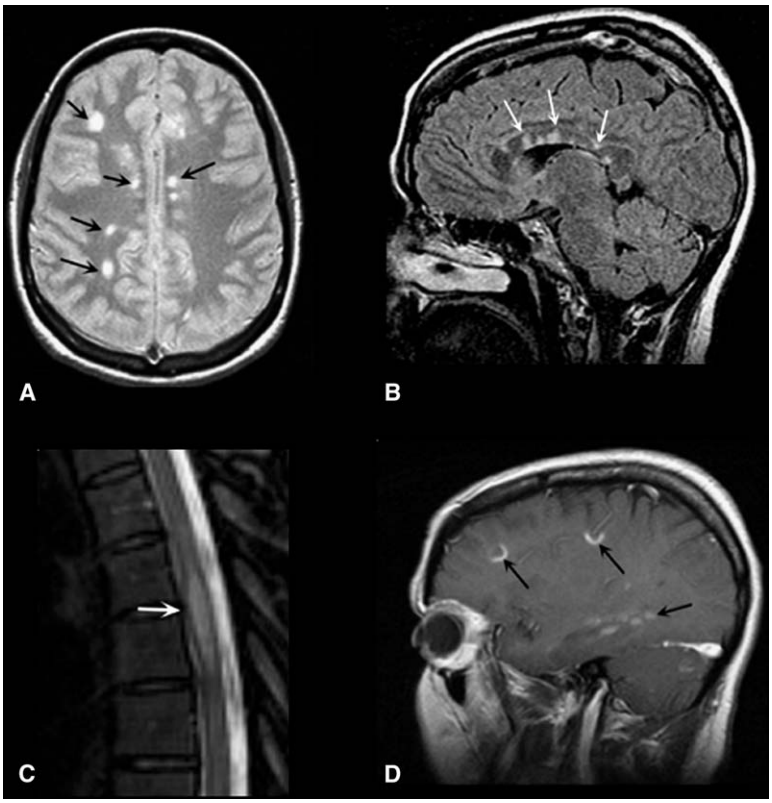

Figure 1. MRI Images in MS

(A) Axial first-echo image from T2-weighted sequence demonstrates multiple bright signal abnormalities in white matter, typical for MS.

(B) Sagittal T2-weighted fluid attenuated inversion recovery (FLAIR) image in which the high signal of CSF has been suppressed. CSF appears dark, while areas of brain edema or demyelination appear high in signal as shown here in the corpus callosum (arrows). Lesions in the anterior corpus callosum are frequent in MS and rare in vascular disease.

(C) Sagittal T2-weighted fast spin echo image of the thoracic spine demonstrates a fusiform high-signal-intensity lesion in the mid thoracic spinal cord.

(D) Sagittal T1-weighted image obtained after the intravenous administration of gadolinium DTPA reveals focal areas of blood-brain barrier disruption, identified as high-signal-intensity regions (arrows).

The diagnosis of MS has been revolutionized by magnetic resonance imaging (MRI) technology, which reveals multiple, asymmetrically located white matter lesions distributed throughout the white matter of the CNS, with a predilection for the corpus callosum and deep periventricular regions (Figure 1). New lesions are heralded by breakdown of the blood-brain barrier associated with perivenous inflammation and detected by extrusion of the heavy metal gadolinium across the blood-brain barrier. Spinal cord lesions are frequently present and can be detected with high sensitivity using high-field MRI. Ancillary tests, used primarily in uncertain or problematic cases, consist of cerebrospinal fluid (CSF) studies revealing low levels of inflammation with mononuclear cells (generally <50 cells/mm^3) and raised levels of immunoglobulin, including antibodies with restricted clonotypes (oligoclonal bands). Evoked potentials in the visual, auditory, or sensory pathways may also be helpful in identifying additional, silent lesions (Hauser and Goodin, 2005).

Although early microscopic studies reported demyelination in the cerebral cortex of affected brains (Brownell and Hughes, 1962), MS is generally perceived as a white matter disease. There is, however, an increasing interest in the involvement of gray matter in MS, and some observers believe that cortical plaques are important contributors to motor, sensory, and cognitive disability in MS (Kidd et al., 1999; Peterson et al., 2001). Cortical demyelination appears to be very rare in acute or early relapsing MS and reduction of cortical thickness is seen primarily in patients with long-standing disease (Bozzali et al., 2002), suggesting that cortical pathology follows the white matter disease (Kutzelnigg et al., 2005). Microglia activation and diffuse axonal injury are typically associated with active lesions in the cortex, but the classical perivascular lymphocytic infiltration characteristic of the white matter plaques is absent (Bo et al., 2003; Peterson et al., 2001; Kutzelnigg et al., 2005). Overall, there is an emerging consensus that cortical lesions and atrophy are major contributors to disease burden in patients with MS. The development of novel animal models displaying demyelinating lesions in the cortex (Merkler et al., 2006) will be of great value to fully assess the role of cortical pathology in MS.

The Basis of Neurological Disability

Although MS can vary from a benign illness to a rapidly evolving and incapacitating disease, most patients with MS ultimately experience progressive disability. Approximately 85% of all MS patients manifest initially as recurrent attacks of neurologic dysfunction (relapses); these occur 1–2 times annually and, especially in the early phases, are typically followed by gradual improvement (remissions) over several months. Women are affected with relapsing-remitting MS twice as frequently as men. Approximately 15% of patients begin with a purely progressive course, termed primary progressive MS; interestingly, these patients tend to be older than relapsing-onset patients and are male as often as female.

MS relapses are associated with the development of new, focal, and usually permanent MRI lesions. These areas of altered signal intensity, once established, reflect fluid shifts due to a combination of demyelination and gliosis. Serial studies in relapsing MS indicate that approximately six out of seven newly formed MRI

lesions are clinically silent. Furthermore, even symptomatic lesions do not in most cases produce permanent disability, as remissions are the rule, especially early in the disease course, and the correlation between the total lesion load detectible by MRI and concurrent disability is weak (Goodin, 2006). Nevertheless, the development of a large number and volume of lesions during the initial years of MS is strongly associated with a greater risk of disability occurring years later (Brex et al., 2002; Rudick et al., 2006). There is a widely circulated aphorism in the field that a relapse in 2006 may produce disability in 2016. This thinking has led to the widespread application of anti-inflammatory disease-modifying therapies for MS, largely in the hope that reduction in the accumulation of focal lesions not only decreases relapses but might also lessen late neurodegeneration and ultimate neurologic disability.

Disability in MS is not due primarily to the effects of relapses, but results from chronic progressive worsening, usually manifested as progressive spastic weakness of the limbs. For patients with relapsing-onset MS, the risk of transitioning from a relapsing to a progressive course is relatively linear and approximately 2.5% annually. Fifteen years after diagnosis, fewer than 20% of patients with MS have no functional limitation, 50% to 60% require assistance when ambulating, 70% are limited or unable to perform major activities of daily living, and 75% are not employed. In a recently published natural history study, females reached disability endpoints at an older age than males (Confavreux and Vukusic, 2006a). However, the most important clinical factor influencing progression to disability was age at clinical onset: the younger the onset, the younger the age achieving the disability milestones. In contrast, no other variables, including relapsing or progressive course, substantially affected the time to reach severe disability (Confavreux et al., 2003; Confavreux and Vukusic, 2006b). Furthermore, times from clinical onset to a Kurtzke Expanded Disability Status Scale (EDSS) score of 6 (walking with unilateral aid) and 7 (wheelchair bound) were primarily influenced by the time the patient reached a score of 4 (limited walking but without aid). In contrast, no other variables had a measurable influence on the time elapsed from a score of 4 to a score of 6 or 7, or from a score of 6 to a score of 7. These observations suggest that once a certain pathologic threshold is reached, most patients with MS progress along a common irreversible neurodegenerative pathway.

Despite important advances in therapeutics for MS, none of the currently available disease-modifying drugs have yet been shown to significantly alter the long-term natural history of the disease (Feldmann and Steinman, 2005; Hohlfeld and Wekerle, 2004; Noseworthy, 2003). Further, the partial, negligible, or deleterious effects that some approaches have yielded in the clinic, despite being successful at the bench, reflect the complex molecular interactions operating in MS and the limitations of current working hypotheses as faithful models of disease pathogenesis. These models support the occurrence of two overlapping and connected arms, inflammatory and neurodegenerative (Figure 2). Any satisfactory understanding of the biology of MS must explain the temporal relationship between these two phases of MS (Figure 3).

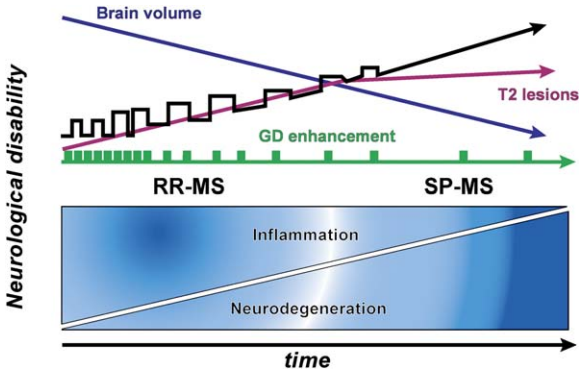

Figure 2. Natural History of MS

Four clinical patterns are recognized by international consensus. Approximately 85% of patients experience relapsing-remitting MS (RR-MS), characterized by the abrupt start of symptoms and acute episodes of worsening (exacerbations or relapses) with complete or partial recovery. Between these episodes, patients may be clinically stable, may experience gradual progression of disability, or may undergo a combination of both. Approximately 50% of patients with RR-MS convert to secondary progressive MS (SP-MS) within 10 years of disease onset. The secondary progressive phase is characterized by gradual progression of disability with or without superimposed relapses. In contrast, patients with primary progressive MS (PP-MS, approximately 10% of patients with MS) experience gradual progression of disability from onset without superimposed relapses. Patients with progressive relapsing MS experience gradual progression of disability from disease onset, later accompanied by one or more relapses; this clinical pattern affects ~5% of patients. An important conceptual development in the understanding of MS pathogenesis has been the compartmentalization of the mechanistic process into two distinct but overlapping and connected phases, inflammatory and neurodegenerative. Axonal loss begins most likely at disease onset and accumulates. Conversion of relapsing-remitting to secondary progressive occurs once axon loss surpasses the capacity of the CNS to compensate for loss of function.

Genetic Susceptibility

MS clusters with the so-called complex genetic diseases, a group of common disorders characterized by modest disease risk heritability and multifaceted gene-environment interactions. The genetic component in MS is primarily suggested by familial aggregation of cases and the high incidence in some ethnic populations (particularly those of northern European origin) compared with others (African and Asian groups), irrespective of geographic location. High frequency rates are found in Scandinavia, Iceland, the British Isles, and North America (about 1–2 in 1,000). Lower frequencies are found among southern Europeans. The disease is uncommon among Samis, Turkmen, Uzbeks, Kazakhs, Kyrgyzis, native Siberians, North and South Amerindians, Chinese, Japanese, African blacks, and New Zealand Maori (Rosati, 2001). According to some observers, this characteristic geographical distribution implicates a pathogen that is not ubiquitously distributed (see below). However, this prevalence pattern can also be explained, at least in part, by the geographical clustering of northern Europeans and their descendants (Sotgiu et al., 2003).

Evidence of risk heritability in the form of familial recurrence has long been known. The degree of familial aggregation can be determined by estimating the ratio of the prevalence in siblings versus the population

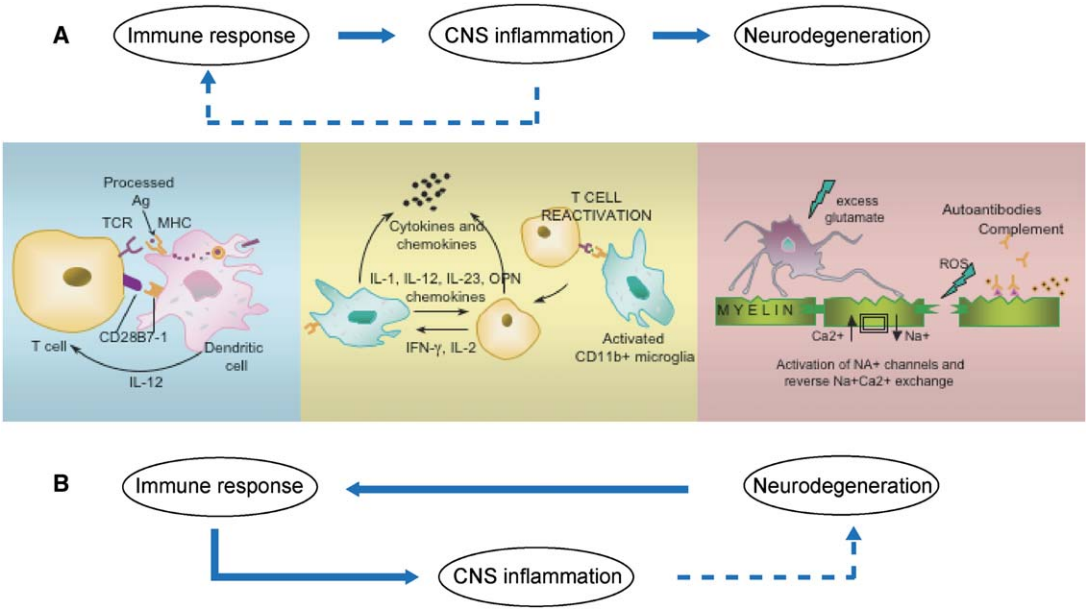

Figure 3. Models of Disease Pathogenesis in MS

The traditional neuropathological view of MS (A) highlights CNS injury as a consequence of an autoimmune response. An alternative hypothesis (B) proposes that activation of autoimmune cells occurs as a consequence of toxic insults to CNS cells. Infections, for example, may be asymptomatic but cause cytophatic effects to target cells in the course of an antiviral response. The prolonged release of neural antigens may then induce inflammatory responses.

prevalence of the disease (λ_s). For MS, the λ_s ranges between 20 (0.02/0.001) and 40 (0.04/0.001). Half-sibling (Sadovnick et al., 1996), adoptee (Ebers et al., 1995), spouse (Ebers et al., 2000), and risk assessment studies performed in Canada appear to confirm that genetic, and not environmental, factors are primarily responsible for the familial clustering of cases. However, an intriguing association with month of birth was observed in the Canadian familial cases, reflecting perhaps an interaction between genes and an environmental factor operating during gestation or shortly after birth (Willer et al., 2005). Concordant sibs tend to share age of symptom onset rather than year of onset, supporting a genetic effect on the familial recurrence. Concordance in families for early and late clinical features has been observed as well, suggesting that in addition to susceptibility, genes may influence disease severity or other aspects of the clinical phenotype (Barcellos et al., 2002; Brassat et al., 1999; Kantarci et al., 2002). Twin studies from different populations consistently indicate pairwise concordance (20%–30% in identical twin pairs compared to 2%–5% in like-sex fraternal twin pairs), providing additional evidence for a genetic etiology in MS. Overall, neither the recurrence familial rate nor the twin concordance supports the presence of a Mendelian trait. Modeling the available data predicts that the MS-prone genotype results from multiple interacting polymorphic genes, each exerting a small or at most a moderate effect to the overall risk. Their incomplete penetrance and moderate individual effect probably reflects epistatic interactions and postgenomic events; these may include genes that rearrange somatically to encode a vast variety of immune receptors, posttranscriptional regulatory mechanisms, and incorporation of retroviral sequences. An additional layer of difficulty is encountered when genetic heterogeneity is considered, whereby specific genes or alleles influence susceptibility and pathogenesis in some affected individuals, but not in others.

The *HLA-DRB1* gene on chromosome 6p21 is the strongest genetic factor identified as influencing MS susceptibility (Figure 4). The association of MS with *HLA* genes, specifically the *DRB1*1501* allele, has been a consistent finding across nearly all populations (Oksenberg and Barcellos, 2005). Recent studies suggest the possibility that complex *trans HLA-DRB1* allelic interactions may determine the balance between susceptibility and resistance (Barcellos et al., 2003, 2006; Dyment et al., 2005). For example, there is a *DRB1*15* dose effect on susceptibility, and the *DRB1*15/08* and *DRB1*15/14* genotypes are high risk and protective, respectively. The exact mechanism(s) by which the *DRB1* gene influences susceptibility to MS remain undefined, but are likely related to the physiological function of HLA molecules in immune responses, including antigen binding and presentation, and T cell repertoire determination by negative selection of high-avidity autoreactive T cells within the embryonic thymic microenvironment (Stratmann et al., 2003; Wucherpfennig, 2005). Although a recent high-density single nucletoide polymorphism (SNP) study covering the MHC region assigns the entire association signal to the *HLA* class II region (Lincoln et al., 2005), the debate concerning the role of non-HLA class II genes mapping to this region continues, with some data suggesting that additional disease genes lie within the central class III and/or telomeric to the class I *HLA* regions.

The identification of the true predisposing gene or genes within the *HLA* region has been held back by the extensive linkage disequilibrium (LD) across the

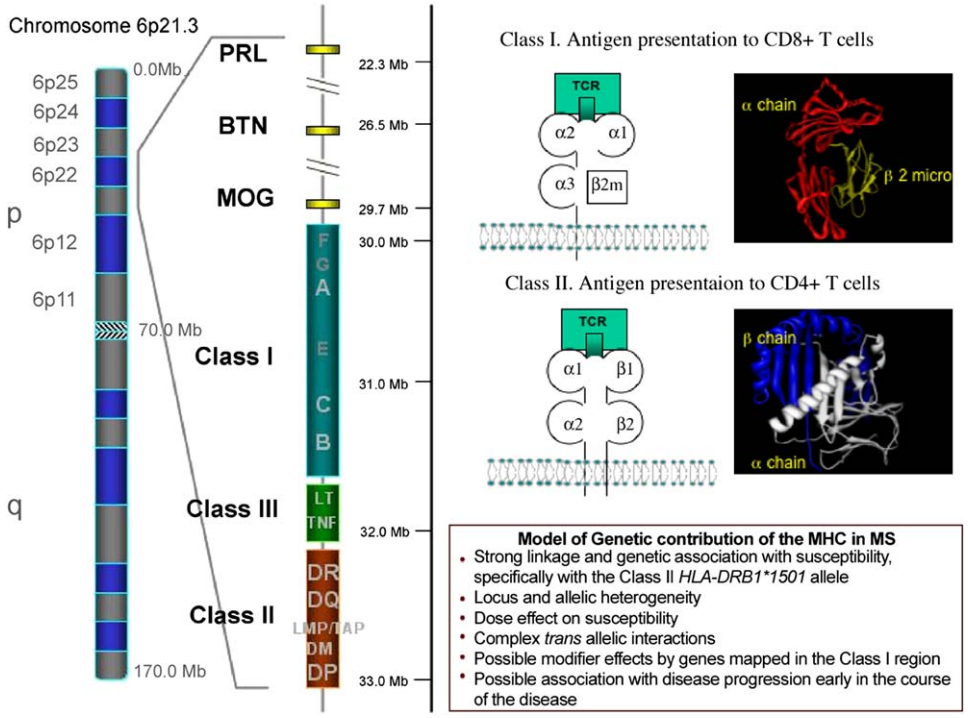

Figure 4. The 6p21-23 Chromosomal Region and MS

The full sequence of the MHC region was completed and reported in 1999. From 224 identified loci, 128 are predicted to be expressed and about 40% are predicted to have immune response functions. The diagram shows the relative positions of class I and II loci involved in antigen presentation. Other genes mapped in the MHC region include complement proteins, genes for the steroid 21-hydroxylase, tumor necrosis factor, and heat-shock proteins, collectively known as class III. The locus contains at least one gene (*HLA-DRB1*) influencing susceptibility to multiple sclerosis.

locus (Miretti et al., 2005). LD refers to the presence of alleles at neighboring loci segregating together at the population level more frequently than would be expected according to the genetic distance that separates them. LD generates long-range correlations among allelic variations in the human genome, the so-called "haplotype-blocks." The maintenance of these blocks is most likely due to the nonuniform distribution of recombination, which tends to occur at "hot spots" demarcating one block from the next. The extended haplotypes are the result of recent population history and, if common in a population, may indicate recent positive selection events. Available data show that LD extends over greater physical distances in the *MHC* region than elsewhere in the genome as a result of reduced recombination rate in the region, 0.44 cM/Mb, compared with a genome-wide average of 1.2 cM/Mb. Because LD patterns differ between populations, a powerful approach to resolving this complex genetic obstacle will be to scrutinize and compare a large number of MS haplotypes in well-characterized datasets from distinct populations. In the case of MS, this can be accomplished by comparing high-risk groups of northern European descent versus low-risk nonwhite populations. African Americans are at a lower risk for MS when compared with northern Europeans and white Americans (Kurtzke et al., 1979; Wallin et al., 2004), but tend to have a more aggressive disease course (Cree et al., 2004). In a recent study of *HLA-DRB1* and *-DQB1* alleles and haplotypes in an African-American MS cohort, a selective association with *HLA-DRB1*15* was revealed, indicating a primary role for the *DRB1* gene in MS independent of *DQB1*0602* (Oksenberg et al., 2004). It is then likely that *HLA-DRB1* constitutes the centromeric boundary of the class II *DR-DQ* association in MS. African-American patients also exhibited a high degree of allelic heterogenity as disease association at the *DRB1* locus was found for *DRB1*1501, DRB1*1503*, and *DRB1*0301* alleles. The haplotypic features of the *DRB1*1501-DQB1*X* (non 0602) and *DRB1*1503*-positive chromosomes indicated an older African origin for the HLA-associated MS susceptibility gene(s), predating the divergence of human ethnic groups, rather than being solely due to genetic admixture with people of European descent. *HLA-DRB1*1501* has a relative low frequency in Africa. Positive selection for this allele appears to have occurred in Europeans, but not in Africans, and although the factors which drove this selection, presumably some infectious pathogen, are unknown, one possible consequence was a heightened susceptibility to MS in Europe, a disorder almost nonexistent in Africa.

As indicated before, compared to European Americans, African Americans are at low risk for MS, supporting the presence of genetic risk factors that occur at higher frequency in Europeans. Because the sections of the genome in African Americans inherited from their European or African ancestors have only had an average of six generations of recombination, extended LD is present in these segments, and non-MHC disease genes are potentially amenable to identification through admixture mapping using relatively low numbers of ancestry-informative genetic markers (Patterson et al.,

2004; Seldin et al., 2004). Admixture mapping is based on the observation that, on average, 80% of the ancestry of African Americans is West African and about 20% is European, and works by searching through the genome for sections with an unusually high proportion of European or African ancestry compared with the average. African Americans affected with MS will inherit a higher-than-average proportion of African or European ancestry, depending on which population has a higher risk for disease at the genetic level. We have already had successes with admixture mapping and just recently identified a locus on chromosome 1 where there is a significantly high proportion of European ancestry in MS chromosomes compared with the genome-wide average and controls; followup should identify the exact gene responsible for the admixture signal associated with MS (Reich et al., 2005).

Unequivocal genetic linkage and association was repeatedly demonstrated in the HLA region. Using the elegant formulation of Risch (1990), it is possible to use the values of *HLA* allele sharing by descent in sibships and estimate the proportion of λs that is explained by the HLA-DRB1 locus. Using data from 98 multicase MS families, we estimated that the *HLA* region accounts for 17%–60% of the genetic susceptibility in MS (Haines et al., 1998). Yet even at the upper bound of this estimate, much of the inheritance of MS remains unexplained. The lack of an obvious and homogeneous mode of transmission has slowed progress by preventing the full exploitation of classical genetic epidemiologic techniques for gene discovery. Nevertheless, an approach that dominated MS genetics until recently involved first determining the chromosomal region of the genetic effect by linkage analysis, which has been extremely productive for mapping genes responsible for monogenic diseases. The establishment of genetic linkage requires the collection of family pedigrees with more than one affected member to track the inheritance of discrete chromosomal segments that deviate from independent segregation and cosegregate with the disease. The potential of linkage mapping for gene identification in complex diseases was highlighted in studies of type 2 diabetes, Crohn's disease, and schizophrenia (Stefansson et al., 2002). To assess the full power of the linkage approach and upgrade the MS genetic map, one of the largest linkage screens ever performed in an autoimmune disease was recently completed by an international consortium (Sawcer et al., 2005). Unequivocal linkage was demonstrated in the *MHC* region (as expected) and suggestive linkage was identified on broad regions of chromosomes 17, 19, and 5; fine mapping is in progress. However, the statistical scrutiny of this map indicates that the next generation of studies attempting to identify the genes influencing the development of this disease will need to rely on association methods and large DNA collections. Genome-wide association studies harbor great potential for complex disorders, but a number of very important challenges, including how to interpret results obtained from large numbers of statistical tests, and how to detect biologically meaningful interactions between polymorphisms that confer disease risk, will need to be overcome. Progress in developing affordable high-throughput genotyping technology and a better understanding of the complex functional structure embedded in the human genome suggest that the tools may finally be at hand to achieve the elusive goal of whole-genome association studies. A powerful trio-based study using 500,000 SNP arrays is currently in progress by this consortium, and results are expected by early fall. This screen will provide a more definitive MS genetic map, but all candidate loci will require stringent independent replication. Furthermore, interpretation of genomic data must take into account that variants of interest may result from segmental duplications (Fredman et al., 2004), inversions (Stefansson et al., 2005), loss of imprinting (Mummert et al., 2005), paralogous or other regions with polymorphic genomic imbalances (Iafrate et al., 2004), or genes resistant to X-inactivation (Carrel and Willard, 2005). It is also important to consider that preferential allelic expression provides an additional source of variability (Lo et al., 2003b; Yan et al., 2002), and copy number polymorphisms (CNPs) contribute substantially to normal human genomic variation for numerous genes involved in neurological function, regulation of cell growth, and regulation of metabolism (Sebat et al., 2004).

Role of the Environment

Epidemiological, clusters or outbreaks, and migration studies have been widely used to illustrate potential environmental influences on MS. Although the interpretation of most of these studies has been difficult, in part due to the small number of study participants in the individual reports, the results have been influential and do suggest a role for environmental factors in MS, and in some cases, they suggest the existence of critical time periods for exposure to putative environmental disease agents. A large number of environmental exposures have been investigated. Those include viral and bacterial infections, nutritional and dietary factors, well water consumption, exposure to animals, minerals, trauma due to accident or surgery, pollution, solar radiation, temperature, rainfall, humidity, chemical agents, metals, organic solvents, and various occupational hazards. Common viruses are among the most frequently studied and biologically plausible putative infectious agents related to MS pathogenesis, and many have been proposed at one time or another to be the causative MS agent. Prominent candidates have included measles, rubella, mumps, and the herpes viruses, including Epstein Barr virus (EBV), herpes simplex virus (HSV) 1 and 2, varicella zoster virus, and HHV6. Strong evidence for a role of EBV in particular, a ubiquitous herpesvirus with a worldwide distribution, has been indicated by epidemiologic (Ascherio et al., 2001) and laboratory studies (Cepok et al., 2005; Levin et al., 2005). A higher risk of infectious mononucleosis (associated with relatively late EBV infection) and higher antibody titers to the latency-associated antigen EBNA1 are associated with MS, and conversely, individuals never infected by EBV are at low MS risk. No compelling mechanistic explanation to account for the EBV-MS relationship is known.

Attempts to isolate the causative environmental trigger(s) in MS have been largely unproductive and have failed to provide breakthrough insights into mechanisms of disease susceptibility and pathogenesis. This may be due to heterogeneity operating also at the level of

causative factors. Whether the genotype dictates different forms of the same disease in response to a common causative agent or other trigger or whether the genotype reflects different diseases with completely separate environmental causes is not known. The expectation that any single agent would have enough specificity and universality to account for all cases of this disease seems unlikely. However, it would be premature to dismiss the possibility that a previously undetected agent could be responsible for MS, acting either as a trigger for an autoimmune response, or even as a chronic active CNS infection directly responsible for symptoms.

Triggering and Persistence of Inflammation

Early in the 1930s, Rivers and others defined the laboratory autoimmune disease experimental allergic (or autoimmune) encephalomyelitis (EAE) (Rivers and Schwenker, 1935). EAE can be induced in a variety of animal species, including nonhuman primates, by immunization with myelin proteins or their peptide derivatives. When studied in genetically susceptible animals, immunization induces brain inflammation accompanied by varied signs of neurologic disease. EAE and MS share common clinical, histologic, immunologic, and genetic features; hence EAE is widely considered to be a relevant model for the human disease (Steinman and Zamvil, 2006). Demonstration in the early 1960s that EAE could be adoptively transferred by myelin-sensitized T cells inaugurated the era of T cell immunology in MS research, an approach that in many respects dominates the field to this day. Although the inflammatory changes that occur in MS may ultimately be shown to be secondary rather than primary (inflammation and selective destruction of brain elements may also occur in nonautoimmune conditions, including genetic disorders such as adrenoleukodystrophy or chronic virus infections such as HTLV-1), numerous studies in blood, cerebrospinal fluid, and brain tissues of individuals with MS have exposed cellular and humoral immune responses against CNS antigens that are not as prominent in non-MS controls (Hafler et al., 2005). These studies provide the rationale for a disease model driven by the loss of immune homeostasis and uncontrolled immune responses against structural CNS components.

During the initial state of the inflammatory response, lymphocytes with encephalitogenic potential are activated in the periphery and home to the CNS, become attached to receptors on endothelial cells, and then proceed to pass across the blood-brain barrier (BBB), through the endothelium and the subendothelial basal lamina into the interstitial matrix. Remarkably, the presence of immunocompetent cells with autoimmune potential appears to be an embedded characteristic of the (healthy) immune system in vertebrates (Genain et al., 1994; Hohlfeld et al., 2000). These cells may provide important inflammatory signals necessary for wound healing, angiogenesis, neuroprotection, and other maintenance functions. Furthermore, CNS-specific T cells can provide neurotrophic factors such as BDNF and contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood (Ziv et al., 2006). The transition from physiological to pathological autoimmunity involves at least two factors: (1) the loss of immune homeostasis, normally maintained through the induction of anergy or apoptosis, receptor downregulation, editing, and anti-idiotypic/cellular regulatory networks, and (2) the engagement and activation of lymphocytes by adjuvant signals including, conceivably, recurrent exposures to exogenous pathogens. This could occur via nonspecific polyclonal activation of T and B cells by bacterial or viral antigens, or, alternatively, as a consequence of structural homology between a self-protein and a protein in the pathogen, a process commonly referred as molecular mimicry (Steinman et al., 2002). It is notable, for example, that components of the myelin sheath share amino acid homologies with proteins of measles, influenza, herpes, papilloma, and other viruses. These pathogens acquired sufficient homology to engage myelin-specific T cells and drive a misguided response (Lang et al., 2002). Amino acid identity may not even be required for cross-reactivity to occur between the autoantigen and the mimic, as long as they share chemical properties at critical residues that allow anchoring to antigen-presenting molecules and interaction with the T cell antigen receptor.

Once activated, T cells express surface molecules called integrins, which mediate binding to the specialized capillary endothelial cells of the BBB. One such integrin, VLA-4, binds the vascular cell adhesion molecule (VCAM) expressed on capillary endothelial cells following induction by TNF-α and IFN-γ during an inflammatory response. Building on successful modulation of demyelination in animal models with anti $\alpha4\beta1$ integrin antibodies (Yednock et al., 1992), clinical trials with Natalizumab, a humanized monoclonal antibody directed against the $\alpha4$ integrin of the adhesion molecule VLA-4, were conducted, and very encouraging results were reported in both MS (O'Connor et al., 2004) and Crohn's disease (Ghosh et al., 2003). Unfortunately, this modality of therapy resulted in an unexpected compromise of CNS immune surveillance mechanisms in a small number of individuals who were concomitantly treated with immunomodulatory or immunosuppressive therapies, resulting in progressive multifocal leukoencephalopathy (PML) due to JC virus infection (Sheridan, 2005). At least one affected patient was on Natalizumab monotherapy at the time PML developed. Ransohoff (2005) proposed that Natalizumab may activate bone marrow B cells, which appear to serve as natural reservoir for PML virus in the healthy adult and promote viral replication and passage of the virus to the CNS.

As the activated T cells migrate across the BBB to reach the CNS parenchyma, they express gelatinases (matrix metalloproteinases, MMP) responsible for lysis of the dense subendothelial basal lamina. The clinical relevance of metalloproteinases is underscored by the observation that some members of this family of molecules are present in the cerebrospinal fluid of patients with MS, but not in normal controls (Leppert et al., 1998). TNF-α-converting enzyme (TACE, ADAM 17), a member of the ADAM (a disintegrin and metalloproteinase) family of enzymes, releases TNF-α from its cell membrane-bound precursor by proteolytic cleavage. In a longitudinal study of 11 relapsing-remitting MS patients, TACE mRNA expression in peripheral blood mononuclear cells showed a significant correlation with the number of lesions (Seifert et al., 2002). Thus, metalloproteinases may act not only as mediators of

cell traffic across the BBB, but may also increase the inflammatory reaction through TNF processing. Furthermore, a direct neurotoxic effect for metalloproteinases has been proposed as well; microinjection of activated MMPs into the cortical white matter of experimental animals results in axonal injury, even in the absence of local inflammation (Newman et al., 2001). Beta interferon, currently the most frequently employed treatment for MS, works in part by reducing expression of MMP by activated T lymphocytes and interfering with their passage across the BBB (Leppert et al., 1996; Stuve et al., 1996).

After traversing the BBB, pathogenic T cells are reactivated by fragments of myelin antigens. Recent data suggest that this is a two-step process (Platten and Steinman, 2005). Primed CD4+ T cells are first engaged by CD11c-expressing antigen presenting cells (APCs) in the perivascular space before moving into the parenchyma. Reactivation induces additional release of proinflammatory cytokines that stimulate CD11b microglia, further open the BBB, and stimulate chemotaxis, resulting in additional waves of inflammatory cell recruitment and leakage of antibody and other plasma proteins into the nervous system. Both CD11c and CD11b are members of the integrin family expressed on neutrophils, monocytes, NK cells, and activated T and B cells. The activated C11b+ cells also contribute to the inflammatory milieu by secreting T cell-activating factors such as IL-12, IL-23, and toxic mediators such as nitric oxide (NO) and oxygen radicals. Pathogenic T cells may not be capable of producing or inducing tissue injury in the absence of the secondary leukocyte recruitment. For example, in EAE mediated by adoptive transfer of myelin-reactive encephalitogenic T lymphocytes, these cells are among the first to infiltrate the CNS, but constitute only a minor component of the total infiltrate in the full-blown lesion. Despite being very effective in preventing EAE, anti-CD4 antibody therapy has not been successfully translated to humans (Lindsey et al., 1994). CAMPATH-1H, which targets the CD52 antigen present on lymphocytes and monocytes and causes prolonged T cell depletion, showed substantial reduction in disability at 6 months in relapsing, but not secondary progressive, MS patients, perhaps due to the suppression of ongoing inflammation in these patients with active inflammatory disease (Coles et al., 2006). The dramatic appearance of thyroid autoimmunity in up to one-third of treated patients may be related to the relaxation of T cell-mediated homeostatic mechanisms induced by the anti-CD52 therapy (Goodnow et al., 2005).

B cell activation and antibody responses appear to be necessary for the full development of demyelination, both in humans and in experimentally induced diseases in animals. In most MS patients, an elevated level of immunoglobulins synthesized intrathecally can be detected in the cerebrospinal fluid. Although the specificity of these antibodies is mostly unknown, anti-MBP specificities have been detected. Myelin-specific autoantibodies have been detected bound to the vesiculated myelin fragments, at least in some patients (Genain et al., 1999; O'Connor et al., 2005). Recent data suggests that antibodies specific for aquaporin-4 water channel, a component of the dystroglycan protein complex located in astrocytic foot processes at the blood-brain barrier, appear to be a biomarker of neuromyelitis optica, an demyelinating disease that selectively affects optic nerves and spinal cord and is considered an inflammatory condition that phenotypically resembles a severe variant of MS (Lennon et al., 2005). Myelin-specific infiltrating B cells have been detected in the MS brain, and in the CSF and brain parenchyma of affected individuals there is an elevated frequency of clonally expanded B cells with properties of postgerminal center memory or antibody-forming lymphocytes (Baranzini et al., 1999; Colombo et al., 2000; Corcione et al., 2004; Owens et al., 1998; Smith-Jensen et al., 2000; Zhang et al., 2005). Limited histological data suggests that B cell differentiation, affinity maturation, and antibody secretion occur primarily in the Virchow-Robin and meningeal spaces in MS, which may assume secondary lymph node-like characteristics. Antibodies may participate in myelin and axonal destruction through different mechanisms such as opsonization, which facilitates phagocytosis by macrophages; complement fixation; or stimulation of antibody-dependent cell-mediated cytotoxicity (ADCC) by binding to natural killer cells. The systemic administration of B cell-depleting antibodies, such as the humanized anti-CD20 monoclonal antibody Rituximab, is currently under evaluation in MS (Cree et al., 2005; Cross and Stark, 2005), and positive results from a phase 2 trial of Rituximab in relapsing-remitting MS were recently reported. Rituximab causes transient depletion of CD20+ pre-B and mature B cells, but not stem or plasma cells. B cell depletion affects antibody production, as well as B cell-mediated antigen presentation and activation of T cells and macrophages. Antagonism of B cell-activating factor of the tumor necrosis factor family (BAFF; also known as BLyS—B lymphocyte stimulator), an important survival factor for peripheral B cells, may be an alternative or an adjunct to Rituximab therapy to enhance autoimmune B cell sensitivity to intrinsic tolerance mechanisms (Goodnow et al., 2005). BAFF is primarily produced by radioresistant lymphoid stromal cells, but a recent report suggests that it is also produced in the brain by astrocytes and is upregulated in MS lesions (Krumbholz et al., 2005). An anti-BAFF antibody-based therapy is currently being tested in systemic lupus erythematosus.

Modulating the Immune Response

Interferon β (IFNβ) is a member of the type I interferon family and, as a recombinant reagent, is the most frequently prescribed therapeutic to control exacerbations in relapsing-remitting MS. IFNβ has pleiotropic effects, including antagonism of IFN-γ-mediated MHC upregulation on antigen-presenting cells, alteration of the profile of cytokine expression, modulation of apoptotic pathways, and blockade of migration across endothelia. Overall, IFNβ has been shown to decrease clinical relapses, reduce brain MRI activity, and possibly slow progression of disability. However, the effect of this treatment is partial, and a substantial proportion of patients are not responders. Therapy has been associated with a number of adverse reactions, including flu-like symptoms, transient laboratory abnormalities, menstrual disorders, increased spasticity, and dermal reaction. Furthermore, the significance of long-term effects and

impact on disease progression has not been determined. Baranzini and collaborators applied predictive modeling tools to a longitudinal gene expression dataset generated from MS patients treated with IFNβ (Baranzini et al., 2005). The analysis identified sets of gene triplets whose expression, when tested before the initiation of therapy, can predict favorable response with up to 87% accuracy. It is noteworthy that the genes in the top-scoring triplet were *Caspase-2*, *Caspase-10*, and *FLIP*, three apoptosis-related molecules. Despite the relatively high predictive accuracy of these models, the functional link between genes and therapeutic effects of this drug is still unclear.

Glatiramer Acetate, a polymer molecular mimic of a region of myelin basic protein, is another popular FDA-approved drug for MS. It affects the cytokine expression pattern, but it also may saturate MHC molecules, preventing efficient presentation of autoantigens by monocytes and dendritic cells, and may induce active T cell suppression against MBP (Farina et al., 2005). A new generation of therapeutic synthetic peptides developed based upon a better understanding of the molecular structure of HLA molecules may be available for clinical trials in the near future (Stern et al., 2005). Glucocorticoids are also potent inhibitors of antigen presentation function. The chemotherapeutic drug cyclophosphamide is lympholytic and stimulates production of anti-inflammatory Th2 cytokines. Additional experimental therapies focus on interference with antigen presentation to encephalitogenic T cells (altered peptide ligands, intravenous antigens), induction of a Th2 response, T cell depletion (anti-CD52 or anti TCRVβ), blockade of adhesion molecules, administration of anti-inflammatory cytokines (IL-10, TGF-β), or neutralization of proinflammatory cytokines (type IV phosphodiesterase inhibitors, nerve growth factor, TNFR p55 Ig fusion protein, anti TNF-αIgG1). Anti-TNF biologicals (monoclonal antibodies and soluble receptor) have been extensively and successfully used in rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, and psoriasis. However, treatment of MS using lenercept, a TNFRp55-FC construct, appears to have increased the frequency of relapses. N-(3,4,-Dimethoxycinnamoyl) anthranilic acid (3,4-DAA), an orally active synthetic derivative of the amino acid tryptophan metabolite anthranilic acid, suppressed proliferating of myelin specific T cells, inhibited production of proinflammatory cytokines, and reversed EAE paralysis; tryptophan metabolites may represent a novel class of drugs to control inflammation in MS (Platten et al., 2005).

Other approaches that have also proven effective in blocking EAE, such as the use of statins, which inhibit LFA-1, modulate MHC expression, and induce immune-deviation, and antihistamines, which engage H1 receptors found in MS brain, may provide a new therapeutic strategy in MS for previously approved drugs. A preliminary trial with statins has shown some degree of efficacy (Vollmer et al., 2004). Similarly agonists for the peroxisome proliferator-activated receptor-α and angiotensin blockers may prove to be effective in MS by inducing a cytokine shift from the proinflammatory to the anti-inflammatory type (Feldmann and Steinman, 2005). Finally, it is possible to reverse ongoing paralysis in the EAE model by using vectors encoding regulatory cytokines (or inflammatory cytokine inhibitors) or by tolerizing the immune system via injection of naked DNA encoding myelin antigens together with DNA encoding the cytokine IL-4. DNA vaccination has been taken into the clinic for infectious disease and cancer, and trials are now being organized to apply this approach to autoimmune diseases, including MS (Feldmann and Steinman, 2005).

A key but unresolved question is whether a single mechanism of tissue damage is operative in MS, or whether fundamentally distinct pathologies are present in different cases. Heterogeneity has been observed in terms of whether the inflammatory infiltrate is associated with the deposition of antibody and activation of complement, and whether the target of the pathology is the myelin sheath or the oligodendrocyte (Lucchinetti et al., 2004). A recent study of very early MS lesions describes extensive apoptosis of oligodendrocytes as the earliest structural change observed (Barnett and Prineas, 2004). These changes, associated with microglial activation but without other evident inflammatory infiltrates, resembled ischemic changes. One critically important question is whether this heterogeneity reflects fundamentally distinct mechanistic processes responsible for axonal damage, which may require overlapping restorative strategies.

Neurodegeneration

The traditional neuropathological view of MS highlights myelin loss as the key event leading to impaired propagation of action potentials across the exposed region of the axon and ensuing neurological deficits. However, the early literature on MS already described substantial axonal damage in active lesions (for an historical review see Kornek and Lassmann, 1999). Contemporary high-resolution histopathological studies reveal abundant transected and dystrophic axons in sites of active inflammation and demyelination, and confirm that partial or total axonal transection begins early in the disease process (Bitsch et al., 2000; Ferguson et al., 1997; Trapp et al., 1998). Axonal damage appears to take place in every newly formed lesion, and the cumulative axonal loss is considered now to be the reason for progressive and irreversible neurological disability in MS (Neumann, 2003). Furthermore, reduced axonal density is also observed in inactive and remyelinating lesions, cortical tissue, and the normal-appearing white matter in the brain and spinal cord. Pathological studies indicate that as many as 70% of axons are lost from lateral corticospinal tracts in patients with advanced paraparesis (Bjartmar et al., 2000). Advanced MRI techniques increasingly provide a clinically useful and quantitative window into the dynamic processes that result in progressive axonal and neuronal loss over time. Examples include accelerated rates of whole brain atrophy (Miller et al., 2002); gradual worsening of focal (T1) lesions over time (Bagnato et al., 2003); reductions in the predominantly neuronal/axonal marker n-acetyl aspartate (NAA) as assessed by spectroscopy (Gadea et al., 2004); disruption of individual white matter tracts, measured by diffusion tensor imaging (Pagani et al., 2005); and evidence of plasticity and altered functional connectivity by functional MRI (Cader et al., 2006). Taken together, these in vivo methods lend further support to the concept that axonal

and neuronal loss is responsible for the persistent neurological dysfunction that occurs in patients with MS (Filippi and Rocca, 2005).

Knowledge of the mechanisms that trigger axonal injury is far from complete and it is unclear whether demyelination is a prerequisite for axonal injury in MS. Mice null for the glial cyclic nucleotide phosphodiesterase (*Cnp1*) gene developed axonal swellings and neurodegeneration throughout the brain, leading to hydrocephalus and premature death, but the ultrastructure, periodicity, and physical stability of myelin are apparently not altered (Lappe-Siefke et al., 2003). On the other hand, mice deficient for myelin associatge glycoprotein (MAG) show late-onset axonal disease preceded by paranodal axon atrophy with reduced neurofilament spacing, suggesting that an underlying disruption of myelin can lead to a delayed and progressive axonal loss (Li et al., 1994). Late-onset degeneration and disability also occurs in proteolipid protein (PLP) null mice (Griffiths et al., 1998). This also may be the case in human MS (Garbern et al., 2002). Demyelination results in reduced support for the axons as well as redistribution of ion channels, destabilization of axonal membrane potentials, reduced excitability, and conduction block. Axons can initially adapt and restore conduction, explaining remissions, but eventually distal and retrograde degeneration occurs. Therefore, the early promotion of remyelination and preservation of oligodendrocytes remains an important therapeutic goal in MS. Another source of functional CNS plasticity is the potential for axonal remodeling independent of myelin integrity. In a modified EAE model, spinal cord inflammation resulted in extensive interneuron sprouting and connectivity associated with neurological recovery (Kerschensteiner et al., 2004).

In apparent contrast to the model of primary oligodendrocyte disease, some evidence suggests that axonal damage is mediated directly by resident and invading inflammatory cells and their toxic soluble products, in particular microglia, macrophages, and CD8+ T lymphocytes. Indeed, neurons and axons express class I MHC molecules, leaving them vulnerable to cytotoxic T cells (Hoftberger et al., 2004). Axon-specific antibodies and complement may also mediate axonal injury (Zhang et al., 2005). A recent study in EAE provided convincing evidence that axonal damage is associated with tau phosphorylation and aggregation (Schneider et al., 2004); the tau pathology was dependent on inflammation and could be partially prevented by early prednisolone treatment.

Liu et al. (2006) add to the growing body of evidence in support of CNS-immune system cross-talk by demonstrating a role for neurons in controlling the function of inflammatory T cells within their immediate microenvironment. Neuron-T cell contact interaction results in the local differentiation of T cells with a CD15+TGF-β1+CTLA-4+Foxp3+ phenotype that suppresses proliferation of encephalitogenic CD4+ T cells and progression of EAE. Transforming growth factor-β (TGF-β) is a critical differentiation factor for the generation of these regulatory T(reg) cells, through upregulation of Smad3 and IL-9. Remarkably, the T(reg) cells appear to be converted from already-committed and activated encephalitogenic T cells in the CNS environment. Interleukin-6 (IL-6) completely inhibits the generation of Foxp3(+) T(reg) cells induced by TGF-β in peripheral tissues, but together, IL-6 and TGF-β induce the differentiation of pathogenic IL-17-producing cells from naive T cells. T-helper-17 is a recently described CD4+ lineage distinct from classical Th1/Th2 cells implicated in a growing list of autoimmune diseases. TGF-β acts to upregulate the IL-23 receptor, providing a positive autocrine signal for expansion of Th17 cells (Bettelli et al., 2006; Mangan et al., 2006). The overall cytokine milieu leads to the development of mutually exclusive effector or regulatory inflammatory pathways, which may be key to understanding how inflammation and infection is linked to the development of CNS autoimmunity and neurodegeneration.

Resident microglia activated in the neuroinflammatory process are likely to cause CNS tissue injury through the release of mediators such as NO and oxygen radicals, vasoactive amines, complement, proteases, cytokines, and eicosanoids. Chronic "inactive" (e.g., noninflammatory) MS lesions, and even normal-appearing white matter in MS cases, are also characterized by activated microglia and raised concentrations of NO (Lassmann, 2003). Excessive amounts of NO have been linked to neurological symptoms as a result of direct injury to oligodendrocytes and axons (Acar et al., 2003; Smith, 2005). For example, demyelinated axons exposed in vitro to NO experience significant conduction block, whereas myelinated axons were affected only at higher concentrations (Redford et al., 1997). Possible mechanisms include the direct nitration of Na channels and inhibition of mitochondrial respiration, leading to reduced ATP production. Aminoguanidine, a selective inhibitor of NO, prevents the clinical development of EAE and reduces inflammation and demyelination (Cross et al., 1994). On the other hand, mice genetically deficient for NOS2A were shown to be highly susceptible to EAE induced by immunization with myelin oligodendrocyte glycoprotein (MOG) (Sahrbacher et al., 1998), consistent with the presence of complex and systemic regulatory networks in autoimmune demyelination.

NO also mediates the excitatory effect of glutamate, and the excess of glutamate released by microglia and macrophages, accompanied by a decrease in glutamate intake and metabolism, activates α-amino-3-hydroxy-5-methil-4-isoxazolp-propionic acid (AMPA), which is highly toxic to oligodendroglial cells and neurons. Blockade of AMPA-responsive glutamate receptors with AMPA antagonists ameliorates neurological sequelae in EAE, increases oligodendrocyte survival, and reduces dephosphorylation of neurofilament H, an indicator of axonal damage. Drugs affecting microglial function, such as hydroxymethyl-glutaryl coenzyme A (HMG-coA) reductase inhibitors, or agonists of peroxisome proliferator-activated receptor (PPAR) α, such as gemfibrozil, ameliorate EAE and are now being tested in MS (Platten and Steinman, 2005). Another source of glutamate release is the astrocyte, through a Ca-regulated exocytosis-like process (Bezzi et al., 2004). Interestingly, glutamate release appears to follow the activation of the CXCR4 by the chemokine stromal cell-derived factor 1 (SDF-1) and is followed by rapid release of TNF-α by the microglia, which can lead to neuronal death by apoptosis (Bezzi et al., 2001).

Recent work shows NMDA receptor (NMDAR) expression and function in oligodendrocytes (Salter and Fern, 2005; Karadottir et al., 2005). Expression was detected in several regions of the brain and at various developmental stages, but appears limited to the oligodendrocyte processes, explaining why previous studies that have focused on the somata failed to detect these receptors. This represents an unexpected and novel finding since glutamate was thought to activate exclusively the AMPA and kainate receptors in oligodendrocytes. Under pathological conditions, NMDAR stimulation may provoke oligodendrocyte injury. Using an in vitro model mimicking ischemia, Salter and Fern (2005) demonstrate that glutamate activation of the NMDAR and the subsequent intracellular Ca^{2+} overload cause the loss of the oligodendrocyte processes. Moreover, the selective NMDAR antagonist MK801 was able to confer protection in this model. Karadottir et al. (2005) simulated ischemia in brain slices by energy deprivation, causing the development of an inward current in the oligodendrocytes, partly evoked by the NMDAR. Altogether, the data shows Ca^{2+} dependence in the NMDAR-mediated oligodendrocyte injury, and indicates that Ca^{2+} influx can affect the cytoskeletal element within the processes, which will determine their stabilization or retraction. The unusual subunit composition of these receptors, which include primarily the NR1, NR2C, and NR3A subunits, suggest that novel selective NMDA receptor antagonists can be developed devoid of the side effects characteristic of the existing ones.

Once the molecular mechanism of axonal damage is triggered, ion fluxes, mitochondrial dysfunction, and activation of proteases culminate in degradation of cytoskeletal proteins and axonal disintegration (Dutta et al., 2006). The early influx of Na^+ and Ca^{2+} ions into the axoplasm as a result of channel exposure or upregulation is highly excitotoxic and leads to interrupted axonal transport and accumulation of proteins, such as amyloid precursor protein, N-type voltage-gated Ca^{2+} channels, nonphosphorylated neurofilament proteins, and metabotropic glutamate receptors (Peterson et al., 2005). Limited data suggest that neuronal cell death by apoptosis could also have a significant contribution to neurodegeneration in MS (Cid et al., 2002).

Promoting Remyelination and Repair

A characteristic of many MS lesions is the presence of large numbers of NG2-positive oligodendrocyte precursor cells (OPC) as well as PLP-positive preoligodendrocytes that survive the acute inflammatory onslaught (Chang et al., 2000). These arrested oligodendrocytes extend processes to the vicinity of surviving axons, but fail to remyelinate. The development of practical strategies to promote reconstitution of functional myelin from this locally available precursor pool represents an obvious strategy for restorative therapy (for a recent review, see Dubois-Dalcq et al., 2005). Following injury, oligodendrocyte-mediated remyelination is dependent on the transcription factor Olig-1, which represents an excellent target for therapy (Arnett et al., 2004). Inflammatory signals derived from macrophages (Kotter et al., 2005) and lymphocytes (Bieber et al., 2003; Foote and Blakemore, 2005) also influence the capacity for remyelination. Remyelination is also enhanced by cytokines such as IL-1β (Vela et al., 2002), either directly or indirectly via stimulation of IGF-1 by astrocytes, macrophages, or monocytes (Mason et al., 2001). Growth factors, such as platelet-derived growth factor (PDGF) or FGF-2, have been shown to expand OPCs (Frost et al., 2003; Woodruff et al., 2004). Negative effects on remyelination can also result from the cytokine milieu present in a chronic inflammatory or highly gliotic environment (Diemel et al., 2004; Foote and Blakemore, 2005), highlighting the complexity of the interactions. Furthermore, it is possible that the demyelinated axon is unreceptive to remyelination due to intrinsic axolemmal changes, possibly including expression of inhibitory cell surface molecules such as polysialylated-neural cell adhesion molecule (Charles et al., 2000), changes in membrane caliber, neurofilament fragmentation, or energy failure (Dutta et al., 2006).

Human OPC engrafted in a myelin-deficient (shiverer) mouse were able to restore myelination (Windrem et al., 2004), raising hope that a similar strategy might be useful in MS. Schwann cells represent another potential source of cell replacement therapy (Bachelin et al., 2005). In EAE, systemically injected neurospheres protected animals from paralysis; however, these beneficial effects appeared to be mediated by induction of immunoregulatory networks that reduced tissue damage, and not primarily by promotion of remyelination (Pluchino et al., 2005). The failure of adequate remyelination in MS appears to result from an inhospitable environment within the plaque or lack of sufficient signals for extensive myelination (Setzu et al., 2006), rather than a lack of myelin precursor cells. Therefore, it seems unlikely that therapy with nonengineered precursor cells will provide, on its own, a sufficient stimulus for remyelination. More likely, engineered cells could be used to deliver therapeutic molecules to areas of tissue damage.

The concept that progressive ("slow-burning") axonal loss occurring over time in demyelinated lesions is responsible for late chronic disability is supported by histologic studies (Bjartmar et al., 2000; Kornek et al., 2000) and by serial MRI studies revealing accelerated atrophy over time (Losseff et al., 1996). As summarized above, high concentrations of NO may contribute to mitochondrial dysfunction and raised intracellular Na^+ and Ca^{2+} (Smith et al., 2001). Consequent to demyelination, the coexpression of Na^+ channels with the Na^+/Ca^{2+} exchanger along the length of the naked axon could further contribute to Na^+ influx and raised intracellular Ca^{2+} (Craner et al., 2004). Axonal protection strategies using Na^+ channel inhibitors, either phenytoin (Lo et al., 2003a) or flecainide (Bechtold et al., 2004), have shown potential as neuroprotective agents in preclinical EAE models, and human trials are underway with phenytoin and lamotrigine.

With the aid of high-powered laboratory technologies, we are now in a position to define the full array of genes, molecules, and pathways operating in MS. This information will likely provide a reliable conceptual model of pathogenesis and lead to novel curative strategies. This goal can only be achieved if sufficient knowledge exists to distinguish disease variants, reliably classify therapeutic outcomes, and capture key individual genetic, medical, and molecular profiling variables.

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