Update on multiple sclerosis, its diagnosis and treatments

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Abstract
Multiple sclerosis (MS) is a central nervous system disorder, characterized by mononuclear cell inflammation, demyelination and often with extensive axonal injury. It was first described neuropathologically in the late 1800s. MS has an interesting geographical epidemiology, with a higher rate at latitudes further from the equator in both directions. Women outnumber males by about 2:1; this ratio has been increasing in recent years. Genome wide association studies have thus far identified over 50 genetic susceptibility loci, and these are rapidly expanding. Several environmental risk factors have been identified, including low serum vitamin D levels, exposure to Epstein-Barr virus and cigarette smoking. MS displays a heterogeneous disease course; most patients with the disease begin with a relapsing-remitting course, but often eventually develop steady disability progression. A small percentage of MS patients have a progressive course without clinical relapses. Several treatments are now available to decrease relapse rate and slow the accumulation of disability in patients with relapsing MS, but there is currently no effective treatment to slow the progressive forms of MS.

Keywords: autoimmunity; cerebrospinal fluid; magnetic resonance imaging; multiple sclerosis; oligoclonal bands.

Introduction
Multiple sclerosis (MS) is a disease that is confined to the central nervous system (CNS) and is characterized by inflammation, demyelination and axonal loss. Both genetics and environmental factors are implicated in MS development, but the cause of the disease is still unknown. It is generally believed to be mediated by an autoimmune process, but this is unproven. MS is typically characterized by recurrent attacks of neurological disability. Worldwide, there are an estimated 2.5 million cases of MS, of which about 70% are women. Interestingly, the ratio of females to males with MS has been increasing (1). Age of onset is typically in the third or fourth decade, but this also appears to be changing, with increasing recognition of MS onset during childhood. MS incidence varies by race, being the highest among persons of northern European ancestry. MS is rare among Asian populations and in the black population of Africa. However, MS is not uncommon in African Americans, with a risk of about half that of the Caucasian American population (2).

Sir Augustus Frederick d’Este (1794–1848), grandson of George III, is considered the earliest recorded person for whom a definite diagnosis of multiple sclerosis can be made (3). His 22 years of illness were recorded in his diaries, which began in 1822, when he was 28 years old, with the last entry in 1846, when he was 52. He died in 1848 at age 54, but his diaries were not discovered until a century later.

During the course of his disease he developed symptoms and problems very typical of MS, including leg weakness, arm clumsiness, tremors, spasms, numbness, dizziness, bladder and sexual disturbances. Four years prior to his death, he began to use a wheelchair and became bed-bound by the time of his death.

The first clinical-pathologic description of MS was in 1868 by Jean-Martin Charcot, who reported the presence of perivascular inflammatory infiltrates in the CNS of patients with intermittent episodes of neurologic dysfunction (4).

Genetics of MS
The study of twins is a classic means of distinguishing between genetic and environmental factors as determinants of susceptibility to disease. In a population-based study of MS, monozygotic (“identical”) twins showed a 28% concordance rate for MS, compared to 4% for non-identical twin pairs (the same as the 5% concordance rate for non-twin siblings) (5). The increase in concordance rate for genetically identical twins demonstrates a major genetic component in the susceptibility to MS (6). However, as the majority of identical twins do not both have MS when one does, the data suggest a role for environmental factors, which may include epigenetic factors (7).

Approximately 15%–20% of MS subjects have at least one relative with MS, possible MS, or optic neuritis. Genetics appears to be the most critical factor in families with several cases of MS, rather than shared environment (8). Studies of
Environmental influences on risk

Epidemiologic studies support an environmental component in triggering MS. That identical twins are usually discordant for MS (approx. 30% concordance rate) supports factors other than genetics as triggers for MS. Current thought is that MS develops in genetically susceptible subjects with a critical environmental contribution. A strong relationship exists between geographic latitude and the risk of developing MS, both below and above the equator. Prevalence in tropical areas is only about 1/10th of that in cooler latitudes. However, recent evidence indicates the latitudinal gradient may be decreasing (1, 2, 11, 12).

Microbial infections (virus or bacteria) are the main candidates as environmental triggers. A variety of infectious agents have been proposed to be related to MS development. Through the decades, evidence in favor of various infectious agents as triggers for MS has been reported, only to be later discounted. Distemper, measles, herpes simplex, hepatitis B and Epstein-Barr viruses and Chlamydia pneumonia, are among several of the infectious organisms that have been suggested to be causative in MS (13). At present, evidence points most strongly at an Epstein-Barr virus (EBV) association with MS.

In 2004, a Canadian group studying pediatric MS (which is rare) reported the finding of a significantly higher rate of having had EBV in 30 children with MS, compared to age- and gender-matched control children (14). In the MS group, 83% compared to 42% of the control children had evidence of EBV in the past. A number of separate epidemiologic studies have shown a statistically higher rate of EBV in adults with MS (almost 100%) compared to adults without MS (90%–95% positive). Risk is higher in those with higher titers to EBV (15). Risk of developing MS is statistically greater in persons with a clinical history of mononucleosis (16). Clearly, these association data do not prove causation. To be certain that any infectious agent is involved in the pathogenesis of MS, controlled randomized trials demonstrating that vaccinations or specific therapies targeting a particular infectious agent lead to an altered incidence or course of MS will be required. It should be noted that, despite intense scrutiny, no infectious organisms have been identified in MS-affected CNS tissues, and there is no evidence to support communicability of MS (17).

Two main mechanisms have been proposed to explain how infectious agents could induce MS despite lack of evidence for a continuing infection: 1) molecular mimicry, involving the activation of autoreactive T and B cells by cross-reactivity between self-antigen and foreign agents; and 2) bystander activation, meaning that autoreactive cells are activated because of non-specific inflammatory events that occur during infection. Another possibility is that a combination of these two mechanisms could induce MS after an infection (18).

Molecular mimicry in MS pathogenesis is a candidate for linking the epidemiologic and genetic findings (19). Lang and colleagues demonstrated that a T cell receptor (TCR) from an MS patient recognized both a DRBI*1501-restricted myelin basic protein (MBP) peptide and a DRB5*0101-restricted EBV peptide (20). The crystal structure of the DRB5*0101-EBV peptide complex had structural similarities to the DRBI*1501-MBP peptide complex at the surface presented for TCR recognition. DRB1*1501 and DRB5*0101 are in strong linkage disequilibrium with one another.

Interestingly, some infections may be protective in MS. The “hygiene hypothesis” suggests that many autoimmune diseases may result from the advent of societal sanitation in the developed world and the concomitant lack of exposure to certain infections, particularly helminths (21). Persistent parasite infections have also been associated with amelioration of established MS (22). Non-infectious environmental factors, such as low vitamin D levels may also be important risk factors.

A prospective study identified decreased risk of MS with increasing serum vitamin D levels prior to MS clinical development. Vitamin D is not found naturally in many foods, and is mainly produced endogenously via ultraviolet light exposure to skin. Differences in vitamin D levels might help to explain the latitudinal prevalence differences noted in MS (23).

Interestingly, an association between vitamin D and genetic expression of the MS-associated HLA-DRBI*1501 allele has been identified (24). Vitamin D acts via vitamin D receptors, which translocate to the nucleus where they have effects on gene expression via vitamin D receptor elements (VDRE). The major histocompatibility complex (MHC) class II allele HLA-DRBI*1501 has a conserved VDRE in its proximal promoter region, which leads to increased expression. This VDRE is not found in other MHC class II genes. Investigators have speculated that increased HLA-DRBI*15 expression during T cell development in the thymus may play a role in the deletion of autoimmune T cells.

Smoking cigarettes appears to confer a mildly increased risk for MS development. In a study that compared women who had never smoked to those that had, women who smoked had an MS incidence rate of 1.6 times that of those that had never smoked (95% confidence interval: 1.2, 2.1). The MS risk for those with past smoking history was 1.2 times that of
never smokers after adjustment for age, latitude and ancestry. Relative rate increased with increasing cumulative smoking history (25). The association of smoking with increased MS risk has been confirmed in a recent meta-analysis (26). Whether smoking is also associated with increased severity of established MS, or progression from relapsing-remitting to progressive MS is less certain, although several studies have suggested this (26).

Obesity during young adulthood is associated with increased risk of developing MS (27). Although at first this association may seem improbable, there are a number of primarily pro-inflammatory factors associated with obesity (28).

**New neuropathology findings in MS**

Historically, there are certain pathological changes that serve to define MS. First, the lesions (“plaques”) are discrete, circumscribed lesions. Second, the lesions are located mainly around blood vessels in a perivenular distribution (so called “Dawson’s fingers” or perivascular inflammatory “cuffs”). Third is that there may be extensive myelin loss with relatively less destruction of nerve fibers, or “axons” and little to no destruction of nerve cells. Although plaques may occur anywhere in the CNS, they tend to occur more frequently in some areas, such as the corpus callosum, the optic nerves and the cervical region of the spinal cord. Cells of the immune system, macrophages, T cells, plasma cells, and B cells, are all present in the inflammatory component of most lesions. Inflammation correlates with the occurrence of new (gadolinium-enhancing) lesions observed by magnetic resonance imaging (MRI) (29).

It has been known for decades that there are plaques in white matter tracts traversing gray matter structures, such as the thalamus. More recently it has been recognized that many MS plaques are located in cortical gray matter. These plaques are typically smaller and less inflammatory than white matter lesions and are not readily seen by MRI imaging. Some evidence exists that remyelination is more extensive in gray matter than white matter lesions (30).

In addition to demyelination, axons are frequently lost in MS, including early in the disease process. Demyelination and axon injury are thought by some to result from the toxic molecules produced by inflammatory cells, such as glutamate, nitric oxide, peroxynitrite TNF-α and reactive oxygen species (31). Other studies implicate antibodies, directed against myelin (32). There may be direct stripping of myelin from axons by activated macrophages. These mechanisms are not mutually exclusive. Axon loss is felt by many to be the pathologic correlate of disability. Antibodies to axon components have been associated with progressive disease, but proof of causation is lacking (33).

**Immunopathology of MS**

Evidence suggests that CD4+ autoreactive T cells are a central factor in MS pathogenesis. Increased numbers of activated CD4+ T cells are found in MS cerebrospinal fluid (CSF), as well as increased levels of cytokines, such as tumor necrosis factor (TNF) and interleukin (IL)-2. MS has been thought to be mediated by CD4+ T cells with a Th1 helper (Th1) phenotype, characterized by the production of interferon (IFN)-γ and IL-2. Another T cell subset characterized by the production of IL-17, named Th17 cells, appears to be important in MS pathogenesis, at least in some individuals with MS. Th17 cells represent a lineage distinct from the Th1 and Th2 phenotypes (34, 35). The presence of IL-17 in MS lesions (36) has been demonstrated. Elevated serum IL-17 concentrations may identify a subset of relapsing-remitting MS patients with a primarily Th17-mediated disease that is not responsive to therapy with IFN-β (37). The cytokines IL-6, transforming growth factor (TGF)-β, IL-21, and IL-23 are important for the differentiation and/or activation and expansion of Th17 cells (38).

Th1 or Th17 cells are independently capable of inducing the animal model for MS, experimental autoimmune encephalomyelitis (EAE) (39). In the EAE model, a first wave of Th17 cells enters through the choroid plexus, which then instigate a secondary wave of Th1 cells into the CNS via venules (40). Based on this model, Th17 and Th1 cells could both play critical, but distinct roles in MS pathogenesis.

Despite the focus on and evidence of the importance of CD4+ T cells in MS, the potential involvement of CD8+ T cells cannot be dismissed. CD8+ T cells are present in inflammatory MS lesions (41). Also, examination of MS lesions had shown clonal expansion of CD8+ T cells based on T cell receptor analysis (42). One hypothesis would be that MS lesion formation is initiated by Th1 and Th17 cells, while amplification and damage are mediated by CD8+ T cells (43).

Humoral immune abnormalities in the cerebrospinal fluid (CSF) are typically observed in MS, implicating roles for B cells and plasma cells in its pathogenesis. CSF alterations in MS include increased IgG (IgM and IgA) with an “oligoclonal” pattern on electrophoresis. These findings, though found in >90% of MS patients, are not specific and can be seen in other CNS inflammatory disorders. Elevated intrathecal Ig has been associated with poorer MS prognosis (44, 45). In particular, anti-lipid IgM is associated with enhanced early disease activity and worse prognosis (46). The advent of rituximab, a monoclonal antibody therapy that specifically lysed B cells, confirmed the key role of B cells and their products in MS. Rituximab targets CD20, a cell surface molecule confined to B cells, leading to depletion of circulating B cell. Treatment of relapsing MS with rituximab led to rapid and profound reduction in gadolinium enhancing lesions on brain MRI in three separate studies in relapsing MS subjects (47). Depletion of B cells in the blood was accompanied by depletion of B cells in CSF but, notably, reduction of enhancing brain lesions was not accompanied by reduction in CSF immunoglobulins or oligoclonal bands (48). Whether the critical role of B cells occurs in the periphery, in the CNS, or in both locations has not yet been determined.

That myelin-reactive T cells can be recovered from the blood of healthy subjects without MS suggests that immunoregulatory mechanisms exist (49). Spontaneous remissions
in relapsing-remitting MS (RRMS) also indicate an intrinsic immunoregulatory control. A subset of T cells, called regulatory T cells, are able to inhibit EAE severity and enhance its resolution (50). The mechanisms through which regulatory T cells function are not completely known, but the production of the anti-inflammatory cytokines IL-10 and TGF-β has been implicated (51). A defect in the function of regulatory T cells in MS patients has been reported (39). Growing evidence indicates the existence of a subset of regulatory B cells that can suppress immune-mediated disease progression. Their suppressive activity appears largely related to IL-10 secretion (52). Interestingly, persistent helminth infections have been associated with enhancement of these regulatory networks (22, 53).

Intrinsic CNS glial cells are also implicated in MS immunopathogenesis. Microglia are considered the resident macrophages of the CNS and are quickly activated by injury or pathogens (54). Microglia provide functions similar to other tissue macrophages, including phagocytosis, antigen presentation and production of cytokines, eicosanoids, complement components, excitatory amino acids (glutamate), proteinases, oxidative radicals and nitric oxide (55). Currently, there are no unique histochemical markers that distinguish intrinsic microglia from macrophages which have invaded the brain during inflammation (56). Based on the pathology of active lesions, macrophages/microglia actively participate in myelin breakdown during MS; phagocytosis of myelin proteins in the lesions by these cells is a reliable indicator of ongoing demyelinating activity.

In addition, activated microglia and macrophages express molecules critical for antigen presentation to T cells, including MHC II and B7-1 and B7-2 molecules. Activated microglia produce a wide range of factors, such as chemokines, prostaglandins, nitric oxide, and the cytokines IL-1β and TNFα. Many of these microglial products are pro-inflammatory, but some may also have neuroprotective roles (55).

“Inside-out” vs. “outside-in”?

Despite decades of work, the manner by which MS lesions are initiated is not understood. One concept is that an initial and progressive neuronal, glial and/or myelin disorder leads to a secondary inflammatory reaction, which exacerbates injury and provokes a vicious cycle. The “inside-out” mechanism of MS pathogenesis has been termed, which implies a fundamental abnormality behind the blood-CNS-barrier with subsequent recruitment of inflammatory cells from the peripheral blood. An early loss of oligodendrocytes without concomitant lymphocyte or macrophage invasion was described in some acute MS lesions (57). At present, there is no clear way to distinguish this from the “outside in” hypothesis of MS pathogenesis. That genetic risk factors identified thus far are primarily related to the immune system would appear to better support the “outside in” concept (10).

Diagnosis of MS

Several sets of diagnostic criteria have been put forth over recent decades. With the advent of MRI, the inclusion of typical imaging findings into the diagnosis was inevitable and needed. Thus, the McDonald criteria for MS diagnosis were developed and were subsequently refined and revised (58). Accumulating data has allowed yet greater refinements. The 2010 McDonald criteria allow dissemination in time to be met by a single MRI, in the event of the concurrent presence of a non-symptomatic gadolinium enhancing lesion and non-enhancing lesions typical of demyelination (59). With the minimal MRI criteria now needed for diagnosis, CSF findings typical of MS (elevated IgG, elevated IgG index, presence of oligoclonal bands) may be critically important in cases that have atypical features.

Clinical subcategories of MS

The course of MS is variable. Based on disease course, a panel of experts on MS developed a categorization scheme with four groups: relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), progressive relapsing and primary progressive MS (PPMS) (60). A subgroup of patients with RRMS have “benign” MS in which patients remain functionally unimpaired after 10–15 years of disease. The most common initial is relapsing-remitting MS (RRMS). The extent of recovery from a relapse or “attack” varies a great deal between patients and from one attack to the next. One half or more of patients with RRMS go on to develop SPMS, in which the patient has progressing deficits in the absence of clear-cut attacks. However, clear-cut attacks may be superimposed upon a progressive course in SPMS patients. Primary progressive MS patients have a steadily progressive course without clinical relapses. Interestingly, the gender ratio in PPMS is about 50% male, 50% female rather than the female predominance of RRMS and SPMS. The clinical course of MS is related to the age of the patients at onset, with an increased percentage of patients with progressive disease at later ages of onset.

Clinically isolated syndromes

Clinically isolated syndrome (CIS) is the first clinical demyelinating event in a person with a high risk of developing MS. Such individuals have several silent lesions on brain or spinal cord MRI, or other risk factors, such as CSF typical of MS. Initiation of a β-interferon after the first event, before the diagnosis of MS can be made, has been shown to delay the development of definite MS in three large studies (61). Early treatment appears to delay short-term disability progression, but it is not yet clear if early treatment alters long-term progression.

Radiologically isolated syndromes

With the advent of increasingly sensitive imaging capabilities, new entities have developed. Radiologically isolated syndrome (RIS) is a new designation, based upon the detection of abnormalities on CNS imaging that are typical for MS, but in the absence of clinical symptoms and signs (62). No
reliable prognostic indicators have been found to differentiate those RIS cases that will eventually develop clinically definite MS, although the presence of asymptomatic lesions in the cervical cord increases the risk of future clinical MS. Whether immunomodulatory therapy of those with RIS will ultimately be beneficial has not yet been determined.

### Treatment of MS

Front-line treatments for relapsing forms of MS are primarily the β-interferons and glatiramer acetate (Table 1). Among the many actions of β-interferons are the inhibition of expression of class II MHC molecules, which are critical to T cell activation, and inhibition of adhesion molecule expression that is critical to infiltration of the blood-brain barrier by inflammatory cells. Evidence suggests that treatment with β-interferons delays transition from RRMS to SPMS and that the sooner RRMS patients commence treatment with β-interferon, the better they do in the long-term (63, 64).

Glatiramer acetate (GA) is a synthetic, random polypeptide. The mechanism by which it reduces MS relapses is poorly understood, although there is evidence to suggest that it binds to MHC II molecules and may compete with myelin peptides for binding. Other data indicate that GA induces a shift toward type II monocytes, which secrete the anti-inflammatory cytokines IL-10 and TGF-β and lead to expansion of regulatory T cells that suppress CD4 T cells (65).

Mitoxantrone can be used for worsening MS, both RR and SPMS. Its several serious risks, including leukemia and cardiomyopathy, have limited its use (66).

Newer approved agents include natalizumab, a monoclonal antibody directed against α4 integrins, that reduces relapse rate by 60%–70% in RRMS (67). However, progressive multifocal leukoencephalopathy (PML) has occurred in over 150 patients treated with natalizumab. Risk of PML is higher in patients with antibodies to the causative JC virus; assays for anti-JC virus antibodies may allow more informed treatment decisions.

Fingolimod is an oral agent that modulates expression of sphingosine-1 phosphate receptors on lymphocytes (68), resulting in retention of T and B lymphocytes in lymph nodes. Because lymphocytes are not injured or deleted, immunity is retained and the cells can exit intact from lymph nodes when the drug is stopped.

There are no proven therapies to slow progression in patients with the progressive clinical subtypes, SPMS and PPMS. Because of this, uncovering the cause and treatment of the relentless progression characterizing these subtypes has increasingly become a focus of research.

### Future outlook

Over the coming 5–10 years, several new medications are likely to be approved. New anti-inflammatory and possibly neuroprotective treatments are being studied. Promising agents include humanized monoclonal antibodies directed against immune cell surface markers and oral immunomodulators.

### Conclusions

Multiple sclerosis is an inflammatory, demyelinating disease with relative axonal sparing. Signs and symptoms of MS vary widely among patients and may include a variety of neurological signs and symptoms. Neurological dysfunction in MS is thought to be the result of impaired conduction along partially or completely demyelinated segments of nerve fibers; however, it may also be related to inflammation and to axonal injury. Advances in brain imaging, immunology and molecular biology have greatly increased our understanding of MS. Several therapies are currently available for the treatment of relapsing-remitting MS, but no single treatment has demonstrated dramatic efficacy in the treatment of this disease.

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References


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Kevin A. Cross is a student at Vanderbilt University in Nashville, Tennessee, where he is majoring in history and German. During his university years, he worked each summer doing medical research, ranging from animal studies utilizing the animal model for MS, experimental autoimmune encephalomyelitis, to advanced imaging studies to quantifyate MRI in MS. He plans to attend medical school in the future.

Laura Piccio obtained her MD and PhD degrees from the University of Milan, Italy where she trained as a neurologist. In 2005 she received a post-doctoral fellowship from the National Multiple Sclerosis Society (NMSS) under the mentorship of Dr. Anne Cross at Washington University. In 2008, she joined the faculty of the Neuroimmunology Section of the Department of Neurology at Washington University. She was awarded the prestigious Harry Weaver Neuroscience Award from the NMSS in 2010. At present, her research interests are focused on the role of innate immunity in MS, and the interplay between metabolic and immune pathways in MS and its animal model.