

Characterizing the Mechanisms of Progression in Multiple Sclerosis

Evidence and New Hypotheses for Future Directions

E. M. Frohman, MD, PhD; M. Filippi, MD; O. Stuve, MD, PhD; S. G. Waxman, MD, PhD; J. Corboy, MD; J. T. Phillips, MD, PhD; C. Lucchinetti, MD; J. Wilken, PhD; N. Karandikar, MD, PhD; B. Hemmer, MD; N. Monson, PhD; J. De Keyser, MD; H. Hartung, MD, PhD; L. Steinman, MD; J. R. Oksenberg, PhD; B. A. C. Cree, MD, PhD; S. Hauser, MD; M. K. Rache, MD

Major advancements have been achieved in our ability to diagnose multiple sclerosis (MS) and to commence treatment intervention with agents that can favorably affect the disease course. Although MS exacerbations and the emergence of disability constitute the more conspicuous aspects of the disease process, evidence has confirmed that most of the disease occurs on a constitutive and occult basis. Disease-modifying therapies appear to be modest in the magnitude of their treatment effects, particularly in the progressive stage of the disease. Therapeutic strategies currently used for MS primarily target the inflammatory cascade. Several potential mechanisms appear to be involved in the progression of MS. Characterizing these mechanisms will result in a better understanding of the various forms of the disorder and how to effectively treat its clinical manifestations. It is our objective within this 2-part series on progression in MS to offer both evidence-based observations and hypothesis-driven expert perspectives on what constitutes the cause of progression in MS. We have chosen areas of inquiry that appear to have been most productive in helping us to better conceptualize the landscape of what MS looks like pathologically, immunologically, neuroscientifically, radiographically, and genetically. We have attempted to advance hypotheses focused on a deeper understanding of what contributes to the progression of this illness and to illustrate new technical capabilities that are catalyzing novel research initiatives targeted at achieving a more complete understanding of progression in MS.

Arch Neurol. 2005;62:1345-1356

THE PATHOLOGIC MECHANISM OF PROGRESSION IN MULTIPLE SCLEROSIS

Relapse and Progression

Relapses and progression of disability are the 2 basic clinical phenomena of multiple sclerosis (MS). Relapses are considered to be the clinical expression of acute inflammatory demyelination in the central nervous system (CNS), whereas progression is considered to reflect chronic demyelination, gliosis, and axonal loss. Early in the disease, remission of symptoms is likely due to resolution of inflammation, channel redistribution, and re-

myelination; however, following recurrent attacks, axonal damage is more likely to occur, and axonal loss accumulates. Hence, the balance between injury and repair likely determines the progression of MS.

Recent reports^{1,2} have emphasized the importance of axonal degeneration in contributing to permanent neurologic deficits in patients with MS. The extent of axonal loss is highly variable, with axonal density within plaques ranging from 20% to 80% of that in the periplaque white matter.

Hypothesis, Evidence, Future Directions

We hypothesize that the accumulation of axonal destruction underlies clinical pro-

Author Affiliations are listed at the end of this article.

gression in patients with MS. Support for this contention derives from our understanding that inflammation correlates with the extent of axonal transection in active MS lesions. However, the magnitude of axonal loss in chronic lesions suggests that mechanisms other than inflammatory demyelination may contribute to axonal damage at later stages of the disease.^{1,2} Axons in MS lesions may be destroyed in 2 different ways.³ During acute demyelination, axons are likely damaged owing to inflammatory mediators, such as proteases, cytokines, and free radicals. An association between the number of CD8 T cells and the extent of axon damage has been reported.² A CD8 major histocompatibility complex (MHC) class I-mediated pathway of axon destruction has been suggested from experimental studies.⁴ Inducible nitric oxide may also mediate axon damage.⁵ This acute phase of massive axonal injury, however, lasts only for a few days to weeks. In contrast, axonal degeneration continues in silent inactive plaques.³ Although merely a few axons are destroyed at a given time point, the accumulation of their destruction can contribute to progression of disability. Chronically demyelinated axons may degenerate owing to the lack of trophic support from myelin and oligodendrocytes. Mice that lack certain myelin proteins (myelin-associated glycoprotein [MAG] and photolipid protein [PLP]) demonstrate late-onset axonal disease, and there is evidence of an increased incidence of wallerian degeneration in MAG-deficient mice.⁶

Axonal injury and loss of MS lesions have major clinical consequences for the patient. Clinical deficits induced by inflammation and demyelination are principally reversible, whereas functional loss due to axonal degeneration appears to be permanent. Although the CNS has a large reserve capacity, irreversible structural damage accumulates in MS brains. Although substantial damage can be sustained, permanent clinical deficits appear to coincide with a time when the functional reserve capacity is exhausted. Therefore, the need to develop axon-protective therapy for MS will be crucial to our attempt to slow disease progression (see part 27).

THE IMMUNOLOGIC FEATURES OF PROGRESSION IN MS

Role of T Cells in Progression

The clinical characteristics that separate secondary progressive MS from relapsing-remitting disease are better defined than the immunologic differences. It has been suggested that priming of myelin-reactive T cells occurs as part of the disease process in MS.^{8,9} Primed T cells reactive to myelin antigens may develop a phenotype, making them more resistant to regulatory processes such as programmed cell death. One might expect that these T-cell clones would retain effector functions such as interferon γ secretion but be resistant to the regulatory effects of various therapeutic interventions. It is possible that this resistance to programmed cell death could be an explanation of why interventions such as anti-CD4 therapy were ineffective in patients with MS.¹⁰ Studies¹¹ on the immune response in patients who are receiving

anti-CD4 therapy have suggested that naive cells, rather than differentiated T_H1-like cells, are eliminated.

Hypothesis, Evidence, and Future Directions

One could hypothesize that a more inflammatory profile of myelin-reactive T cells correlates with disease progression. Increased CD40 ligand expression on T cells from patients with progressive MS has resulted in increased interleukin (IL) 12 production.¹² Increased responses to myelin peptides showed a correlation with disability.¹³ A correlation between tumor necrosis factor α (TNF- α)-producing CD4 T cells and changes in T2 lesion load has also been reported.¹⁴

Both CD8 and CD4 T cells contribute to the cellular infiltrate of demyelinating lesions in patients who have MS with evidence of CD8 T-cell enrichment and clonal expansion.¹⁵ Some of these brain-infiltrating CD8 T cells have persisted in the cerebrospinal fluid or blood for longer than 5 years, suggesting that they may play a role in disease progression.¹⁶ The CNS-reactive CD8 T-cell responses have been demonstrated in these patients.¹⁷ Recent technical advancements in flow cytometric assays allow evaluation of antigen-specific CD4 and CD8 T-cell proliferative responses in patients with MS. These studies demonstrate that CNS-reactive T cells are not restricted to the CD4 T-cell subset. In fact, autoreactive HLA antigen class I-restricted CD8 T-cell responses are widely prevalent in MS.¹⁸ A higher prevalence of autoreactive CD8 T-cell responses was noted in patients with relapsing-remitting MS compared with other MS subtypes.¹⁸

The CNS-specific T-cell responses are found in patients with MS and healthy subjects. However, the functional attributes of these cells are distinct in the 2 groups, in which CNS-targeted T cells from patients are thought to be more differentiated compared with those from healthy subjects, suggesting that the cells may have experienced self-antigen *in vivo*.^{8,19} When CD4 and CD8 T cells were specifically evaluated for their functional profiles, differences were noted in both subsets of cells.¹⁸ Although autoreactive CD4 T cells appeared to exhibit a more T helper (T_H)1-type profile, autoreactive CD8 T cells showed a mixed functional profile in which higher interferon γ and chemokine receptor 3 expression was accompanied by higher IL-10 expression in patients with MS.

Similar to regulatory subpopulations of CD4 T cells, CD8 T cells have also been implicated as regulatory cells in both experimental autoimmune encephalomyelitis (EAE) and MS.²⁰⁻²² It is possible that the progression of disease in MS depends on a relative lack of regulatory T-cell function. In keeping with this hypothesis, a deficient CD8 T-cell response to glatiramer acetate (Copaxone, Teva Neuroscience, Kansas City, Mo) was found in patients with MS but not in healthy subjects.²⁰ Although patients with MS have widespread, CNS-specific CD8 T-cell responses, glatiramer acetate therapy can restore glatiramer acetate-specific CD8 responses to the levels found in healthy subjects.²⁰ A complex pathogenic and regulatory balance may exist within the CD8 T-cell subset. These findings strengthen the need for defining the role of CD8 T cells in disease progression.

Role of B Cells in Progression

The concept that autoantigens can drive B-cell clonal expansion and generate an autoantibody pool that contributes to autoimmunity has been demonstrated in other autoimmune diseases.²³ However, it has been observed that if the B-cell–monocyte ratio is high, progression of MS is more likely to occur.²⁴ In the EAE model, a role of B cells in the recovery from inflammatory demyelination has also been hypothesized.²⁵

Hypothesis, Evidence, and Future Directions

We hypothesize at least 3 different roles that a B cell might play in the progression of MS. The first is through B-cell clonal expansion. If new antigens have been exposed through ongoing myelin damage, B cells that recognize these newly exposed antigens in the CNS may undergo clonal expansion and differentiation, resulting in a larger pool of antigen-presenting cells participating in the immune response. Second, if the patient has recently had an infection that generated a B-cell response in which the resultant antibody not only recognized the viral or bacterial components but also was cross-reactive with some antigen in the CNS, it is possible that such infections may lead to breakthrough disease by indirectly generating antibodies that are cross-reactive to self-antigens in the CNS. This concept is known as molecular mimicry and occurs in some infectious states, such as human T-cell lymphotropic virus type 1.²⁶ A third possibility relates to the process of immunoglobulin class switching that occurs in B cells. All B cells initially produce IgM, and at a certain point, they are induced to produce IgG instead. In several autoimmune states, the IgG component of the immunoglobulins contains the bulk of autoreactivity rather than the IgM component.²⁷ This switch of self-reactive B cells from IgM to IgG producers could contribute to disease progression.

Each of the mechanisms by which B cells can contribute to progression in MS can potentially be prevented. For example, receptor editing is a phenomenon by which B cells that recognize self-antigens attempt to neutralize their autoreactive potential by replacing the light chain they are currently expressing with a newly rearranged one.²⁸ It has been demonstrated that receptor editing occurs in B cells in the cerebrospinal fluid of patients with MS.²⁹ Receptor editing should discourage further damage to the CNS tissue by autoantibody deposition. However, receptor editing could fail to prevent autoreactivity and instead generate a new antibody with greater self-antigen reactivity or reactivity to more than 1 self-antigen, which could lead to disease progression. In addition, one might be able to prevent B-cell–mediated pathogenesis by depleting the B cells altogether. This approach has led to suppression of autoimmunity in other diseases with known B-cell involvement.^{27,30}

Determinant (Epitope) Spreading

The terms *determinant spreading* and *repertoire broadening* describe the same phenomenon. The concept of epitope spreading emerged in the early 1990s to describe 2

phenomena: (1) diversity at the level of the T-cell receptor V gene (variable) use and (2) cellular and humeral immune response diversification from a single to numerous antigenic determinants.³¹ Epitope spreading is not necessarily a pathogenic immune response but may instead be required for the effective clearing of various infectious agents.³²

Hypothesis, Evidence, and Future Directions

Epitope spreading may contribute to the occurrence of disease exacerbations in patients who have MS with a relapsing-remitting phenotype. One group recently showed epitope spreading to overlapping PLP peptides in patients with a clinically isolated demyelinating syndrome.³³ The same authors reported that *HLA-DP*–restricted epitopes may be recognized by initiating or early-driving clones at disease onset.³⁴ We hypothesize, as these processes occur, that specific therapeutic interventions would be less effective and disease progression could occur.

Cytokine Effects on Progression

According to the T_H paradigm, activated CD4 lymphocytes are categorized into T_{H1} or T_{H2} cells according to their cytokine phenotype.³⁵ Although it is now recognized that activated helper CD8 T cells can also be categorized into T cytotoxic 1 (T_c1) and T_c2 subsets (analogous to CD4 T_{H1} and T_{H2} cells, respectively),³⁶ herein we will primarily focus on the role of CD4 T_h subsets in progression of MS.

The adaptive immune system induces T cells to change from a naive phenotype to either effector cells or memory cells. The T_{H1}/T_{H2} phenotype reflects the functional capabilities following T-cell activation.³⁵ In the human immune system, T_{H1} cells secrete interferon γ , TNF- β , and IL-2, whereas T_{H2} cells produce IL-4, IL-5, IL-6, and IL-13.³⁷ The role of T_H phenotypes (eg, T_{H1p} , T_{H0})³⁸ in human diseases has yet to be clearly defined. However, cytokines associated with the T_{H1} response, such as IL-12 and TNF- α , appear to correlate with disease progression.^{12,39}

Hypothesis, Evidence, and Future Directions

We hypothesize that T helper cells of a T_{H1} cytokine phenotype contribute to disease progression in MS, whereas self-antigen–specific T_{H2} cells prevent CNS autoimmune disease. Intracellular cytokine staining confirmed that peripheral blood mononuclear cells from patients with progressive MS express more IL-12 on activation than those from healthy controls.⁴⁰ Another T_{H1} cytokine, interferon γ , is a potent inducer of surface MHC class II expression on a variety of antigen-presenting cells.⁴¹ The clinical significance of this cytokine in MS pathogenesis was demonstrated when it was shown that the systemic administration of interferon γ caused exacerbations in patients with relapsing-remitting MS.⁴²

Numerous approved and experimental MS pharmacotherapies have been shown to promote a shift or deviation to a T_{H2} cytokine profile.^{43,44} Unfortunately, administration of a myelin basic protein peptide (amino acids 83-99) designed as an altered peptide ligand to induce a

T_H2 cytokine profile in MS was followed by disease exacerbations in several patients.⁴⁵ However, another study using this altered peptide ligand did not show disease worsening, but some patients experienced adverse allergic responses.⁴⁶

The view that T_H1 cytokines are proinflammatory and T_H2 cytokines anti-inflammatory may be oversimplified. Although T_H1 cytokines promote the activation of antigen-presenting cells and the clearance of intracellular pathogens, T_H2 cytokines support antibody class switching in mice, promote the elimination of blood-borne pathogens, and may contribute to autoimmune disease in humans.³⁵ Finally, cytokines such as IL-17 and IL-23, which are gaining a more prominent role in EAE pathogenesis, will need to be studied for their role in MS disease progression.⁴⁷

Lymphocyte Trafficking and Progression

Once T cells are activated, these lymphocytes travel through blood vessels in the brain and spinal cord and are captured by molecules on the blood vessel wall that bind to counterreceptors on the activated lymphocyte. After they are firmly bound to the cerebrovascular endothelium, these cells can then elaborate matrix metalloproteinases capable of digesting collagen type IV and fibronectin, which facilitates transmigration.⁴⁸

Hypothesis, Evidence, and Future Directions

Each step in the process of transendothelial trafficking represents a potential checkpoint. For example, the capture and binding of lymphocytes to the blood vessel wall can be blocked by drugs that interfere with the adhesion molecules on the lymphocyte and endothelial wall, including monoclonal antibodies to α 4-integrin.⁴⁹ The process of transmigration can be stopped by statins that block the addition of lipid moieties on certain molecules in the membrane that are critical for maintaining the shape of the T cell. Statins block the prenylation of ras homology protein molecules on the cell surface that are linked to the migration of lymphocytes into the brain.⁵⁰

Immunologists hypothesized in the early 1990s that lymphocytes used specific addresses to home to targets like the CNS.⁴⁹ By identifying the address used to send lymphocytes to a particular organ, it was argued that blocking the particular molecule with an "address-like signature" would thus abolish pathologic homing but would leave lymphocytes free to move elsewhere. One type of molecule, termed α 4-integrin, on T lymphocytes allows T cells to recognize vascular cellular adhesion molecule 1 in the brain endothelium.⁴⁹ The α 4-integrin that recognizes vascular cellular adhesion molecule 1 is an essential step required for the capture of lymphocytes. Vascular cellular adhesion molecule 1 usually is not expressed at high levels in blood vessels in the brain, although in animal models and in MS its expression is increased. Administration of α 4-integrin antibodies in EAE reversed the paralytic disease and blocked encephalitogenic T-cell clones from entering the brain.⁴⁹

Antibody to α 4-integrin was successful in phase 2 MS clinical trials, in which it reduced the relapse rate by 50% and diminished the number of new gadolinium-

enhancing lesions in a 6-month clinical trial by nearly 90%.⁵¹ The year 1 results from 2 phase 3 (class I) studies on the use of natalizumab in relapsing MS have been reported. The first (AFFIRM) compared natalizumab with placebo, whereas the second (SENTINEL) compared weekly intramuscular interferon beta-1a (Avonex, Biogen Idec, Cambridge, Mass) and placebo with intramuscular interferon beta-1a and natalizumab. Both investigations show highly statistically significant beneficial effects of natalizumab on clinical (relapses) and radiographic measures of disease activity. The Food and Drug Administration approved natalizumab on November 23, 2004, for the treatment of relapsing forms of MS, yet it was later withdrawn because of the appearance of progressive multifocal leukoencephalopathy in 2 patients in the SENTINEL trial (Food and Drug Administration Web site: <http://www.fda.gov/cder/drug/advisory/natalizumab.htm>).

THE NEUROSCIENCE OF PROGRESSION IN MS

Role of Astrocytes in Progression

In new MS lesions, astrocytes proliferate and become hypertrophic. In chronic lesions, astroglial scarring can form an obstacle that prevents repair. A primary dysfunction of astrocytes in MS might be involved in lesion formation and progression of disability. A loss of astrocytic β ₂-adrenergic receptors in MS might explain many of the pathologic changes of the disease and play a role in both inflammation-mediated injury and progressive neurodegeneration.⁵² Activation of β ₂-adrenergic receptors by norepinephrine increases intracellular levels of cyclic adenosine monophosphate (cAMP), which controls many astrocytic functions.

Hypothesis, Evidence, and Future Directions

We hypothesize that lack of β ₂-adrenergic receptors may allow astrocytes to express adhesion, MHC class II, and B7 costimulatory molecules and to act as antigen-presenting cells that can initiate inflammatory reactions.⁵³ During inflammation, lymphocytes, microglia, and macrophages release excessive amounts of glutamate. The lack of β ₂-adrenergic receptors on astrocytes may impair glutamate uptake and contribute to excitotoxic damage of oligodendrocytes through overactivation of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors. The receptor defect may also facilitate the release from astrocytes of proinflammatory cytokines such as TNF- α ,⁵⁴ which is involved in myelin and oligodendrocyte destruction, and result in the expression of nitric oxide synthase.⁵²

Evidence is mounting that axons and oligodendrocytes use astrocyte-derived lactate as an energy source. Lactate is generated from astrocytic glycogenolysis that is stimulated by norepinephrine via β ₂-adrenergic receptor activation. The lack of astrocytic β ₂-adrenergic receptors in MS might prevent an adequate lactate supply to axons, especially in situations of increased neuronal activity.⁵⁵ Intracellular cAMP in astrocytes also stimulates the production of various trophic factors, including neuregulin, nerve growth factor, and brain-derived growth factor.⁵⁶ Neu-

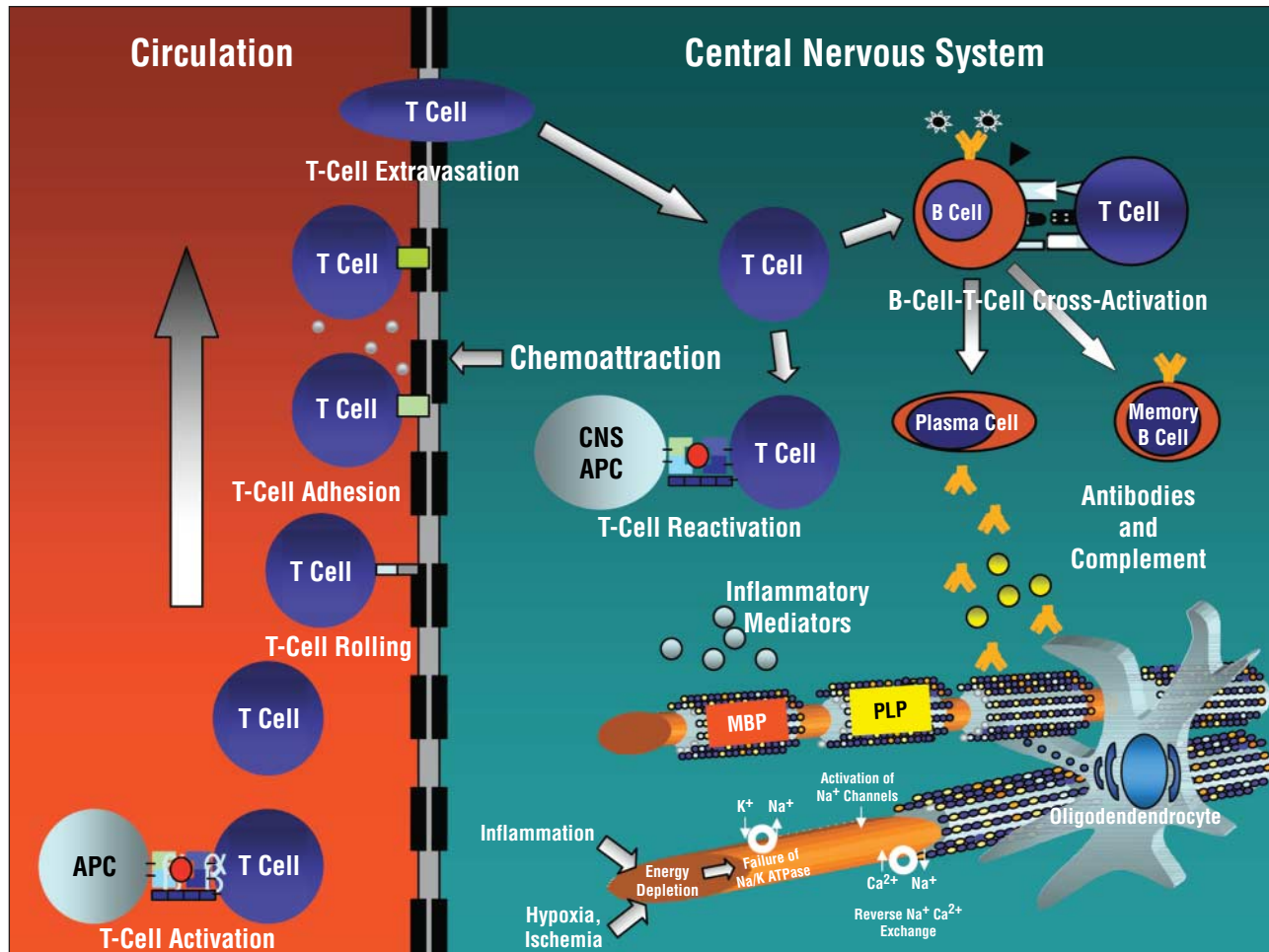


Figure. Proposed mechanisms that appear to be germane to trafficking, inflammation, demyelination, and ultimately axonal dysfunction and loss. According to this model, any of several triggers, including nitric oxide produced as a result of inflammation, can lead to axonal energy rundown with subsequent activation of persistent sodium channels owing to depolarization. Sodium influx through these channels drives reverse Na^+ and Ca^{2+} exchange, thus importing high levels of Ca^{2+} into the axon, where it can activate injurious enzymes and liberate free radicals that damage axons. CNS indicates central nervous system; APC, antigen-presenting cells; MBP, myelin basic protein; PLP, phospholipid protein; and ATPase, adenosine triphosphatase.

regulin, which is a survival factor for oligodendrocytes, has been studied in astrocytes of patients with MS and was found to be reduced in active and chronic lesions.⁵⁷

Potential Role of Sodium Channels in Progressive MS

Although axonal injury is frequent in early stages of MS and contributes to the acquisition of nonremitting deficits, evidence suggests that excitotoxicity is not a principal trigger.⁵⁸ It has been firmly established that calcium-mediated injury can lead to persistent axonal dysfunction and axonal degeneration within CNS white matter.⁵⁹ Biophysical evidence indicates, as shown in the **Figure**, that following a spectrum of insults, reverse sodium-calcium exchange, triggered by sodium influx via voltage-gated sodium channels, can produce injurious sustained calcium influx.⁶⁰ Pharmacologic block of the sodium-calcium exchanger and of sodium channels is protective, preventing axonal degeneration in response to a spectrum of experimental models of axonal injury.⁶⁰

On the basis of pathologic examination of MS tissue, it has been suggested that hypoxialike tissue injury is a com-

ponent of MS lesions.⁶¹ Nitric oxide is present at increased concentrations within MS lesions and interferes with mitochondrial function. This observation suggests a role for energy failure in producing axonal injury in MS. Electrically active axons were found to be particularly sensitive to the damaging effects of nitric oxide.⁵ It has been shown that sodium channel blockers prevent nitric oxide-induced injury of CNS axons, suggesting that inflammatory events (which are associated with nitric oxide production) in MS can also trigger a component of the tissue injury cascade in MS (Figure).⁶²

Irrespective of the trigger, operation of this axon-damaging cascade in MS requires colocalization of sodium channels and the sodium-calcium exchanger in close proximity along axons that are destined to degenerate. This link was shown to be related to the coexpression of the voltage-gated sodium channel, together with the sodium-calcium exchanger, in injured CNS axons (but not in uninjured axons) in both EAE and MS.⁶³

The *in vitro* studies that demonstrated this axon-damaging cascade have recently been extended by an *in vivo* study in which the sodium channel blocker phenytoin was shown to have a neuroprotective effect in pro-

gressive EAE.⁶⁴ In that study, phenytoin decreased the degree of axonal degeneration, maintained action potential conduction, and substantially improved clinical outcome.⁶⁴

Hypothesis, Evidence, and Future Directions

On the basis of these findings, it has been hypothesized that sodium channel blockade may have a neuroprotective effect, preserving axonal integrity and function and thereby preventing nonremitting deficits in MS.⁶⁵ The putative neuroprotective mechanism of action of these drugs (which appear to target molecules located within neurons) makes them ideal candidates for adjunctive therapy (see part 27).

CNS Repair and Regeneration

Progression in MS is likely the result of impaired axonal regeneration following immune-mediated injury. Understanding the mechanisms of how axonal regeneration is inhibited in the CNS has important clinical implications for MS. Three inhibitor proteins, neurite outgrowth inhibitor (Nogo), MAG, and oligodendrocyte myelin glycoprotein, inhibit CNS neural regeneration through the Nogo receptor and associated p75 neurotrophin receptor and leucine-rich repeat and immunoglobulin domain-containing Nogo receptor-interacting (LINGO) protein. In mice that underwent thoracic cord hemisection, those that were Nogo-A deficient demonstrated increased regrowth of axons after the traumatic injury.⁶⁶ Both MAG and oligodendrocyte myelin glycoprotein also appear to inhibit axon regeneration through binding of the Nogo receptor complex.⁶⁷ Once the Nogo receptor is activated, transmission of the signal is mediated by protein kinase C and cAMP. Interestingly, elevated levels of cAMP attenuate the ability of MAG to inhibit axon regeneration *in vitro*.⁶⁸

Hypothesis, Evidence, and Future Directions

On the basis of these studies, we hypothesize that cAMP levels might affect axonal regeneration following spinal cord injury. In mice in which the levels of cAMP were increased, axonal regeneration was significantly improved. Cytokines such as IL-6 are also increased in response to increased levels of cAMP. In addition, IL-6 appears to be effective in attenuating MAG inhibition of axon regeneration. Perhaps drugs such as phosphodiesterase inhibitors will be targeted to regulate cAMP levels and reduce MAG inhibition of axon degeneration in immune-mediated CNS injury. Increasing neurite outgrowth, reducing glial scar formation, and increasing functional recovery might influence disease progression in MS.

THE NEURORADIOLOGIC FEATURES OF PROGRESSION IN MS

Neuroradiologic Detection of Tissue Injury in MS Progression

Conventional magnetic resonance imaging (MRI) is widely used for diagnosing and monitoring MS; however, the

correlation between conventional MRI and clinical findings of MS is still limited.⁶⁹ Among the reasons for these radiologic and clinical discrepancies, a major role has been attributed to the low pathologic specificity of the abnormalities seen on conventional MRI studies and the inability of conventional MRI to quantify the extent of the damage of the normal-appearing tissue. In addition, lesion location itself is another variable that plays a significant role in determining the level of disability.

Hypothesis, Evidence, and Future Directions

We hypothesize that application of modern magnetic resonance-based techniques such as magnetization transfer MRI, diffusion tensor MRI, and magnetic resonance spectroscopy to the assessment of patients with MS has significantly changed the notion of MS as a demyelinating disease.⁷⁰ First, axonal damage, which may be represented by either axonal loss or dysfunction, has been recognized as one of the main contributors to clinical worsening over time. This pathologic process is an early phenomenon in the course of MS; it has been detected even in patients at presentation with clinically isolated syndromes suggestive of MS.⁷¹ Second, widespread abnormalities, which go undetected when using conventional MRI, have been demonstrated in the normal-appearing white matter of patients with MS. Such abnormalities are more pronounced in patients with secondary and primary progressive MS⁷² and tend to worsen over time. Third, it has been shown that the gray matter is not spared by the disease process⁷³ and likely contributes to some of the symptoms of the disease, such as cognitive impairment, mood disorders, and fatigue. Finally, the application of these magnetic resonance techniques is improving our ability to obtain precise estimates of the composition and severity of damaged structures, such as the optic nerves and the spinal cord.

In the case of axonal and neuronal damage, the factors that have traditionally been viewed as potentially able to limit the clinical impact of MS (ie, resolution of inflammation, remyelination, and redistribution of voltage-gated sodium-channels in persistently demyelinated axons) are all likely to have a limited role. Functional MRI studies have demonstrated cortical changes in patients with different disease courses. The relationship found between these changes and magnetic resonance measures of brain and cord damage suggests that brain plasticity might play a major adaptive role in limiting the functional consequences of MS-related widespread tissue damage.⁷⁴

THE GENETICS OF PROGRESSION IN MS

Analysis of Gene Expression in MS

The development of multiplex analysis of transcripts from MS tissues has advanced our understanding of the disease.^{75,76} These approaches to decipher the messenger RNA transcripts found at the site of MS lesions have revealed several targets for therapy, as well as increasing our awareness of the complexity of the disease. Large-scale transcriptional analysis of MS brain tissue has revealed that

possible targets for therapy include elements of cholesterol metabolism, such as key enzymes involved in cholesterol synthesis, histamine receptors, and various cytokines including TNF, IL-15, IL-17, and osteopontin.^{75,76} Large-scale robotic sequencing of messenger RNA from complementary DNA libraries derived from MS brain plaques and gene microarray analysis of transcripts from MS lesions of various types^{75,76} have been performed by several groups.

A potential role for osteopontin (also known as Eta-1) in the progression of MS was identified.⁷⁵ In the present study, more than 11 000 clones were sequenced from libraries prepared from brain plaques in patients with MS and controls. Elucidated were 423 genes, including 26 novel genes that were present only in MS plaques and absent in control material. Transcripts for α B-crystallin, an inducible heat shock protein localized in the myelin sheath and targeted by T cells in MS, were the most abundant transcripts unique to MS plaques. The next 5 most abundant transcripts included those for prostaglandin D synthase, prostatic binding protein, ribosomal protein L17, and osteopontin.

Hypothesis, Evidence, and Future Directions

We hypothesize that mining of the data sets will reveal new targets in MS.^{75,76} Osteopontin is a secreted molecule that is critical in the polarization of cytokines toward a T_H1 bias, driving increases in interferon γ and IL-12, which may be necessary in the pathogenesis of MS.⁷⁷ Levels of osteopontin are elevated in the plasma during relapses of MS and may serve as an important surrogate marker for disease activity.⁷⁸ Levels of novel cytokines, including IL-15 and IL-17, were shown to be elevated in MS lesions and may be targets for therapy.⁷⁶ Elevated levels of transcripts for immunoglobulin and Fc receptor make therapy directed to removal of B cells an attractive possibility in MS.

Transcriptional profiling of MS tissue revealed many changes in genes involved in lipid and cholesterol metabolism.⁷⁶ Expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase was down-regulated in MS tissue, as were the expression levels of other genes encoding critical pathways in lipid metabolism. Recently, promising results in clinical studies have ignited interest in the potential application of the cholesterol-lowering HMG-CoA reductase inhibitors statins in MS therapy⁷⁹ (see part 27).

Genetic Modifiers of Progression

Although little is known about the underlying cause of disease variability, concordance for early and late clinical features has been observed in families with multiple cases of MS, suggesting that in addition to susceptibility, genes influence disease progression and other aspects of the clinical phenotype.⁸⁰⁻⁸²

Hypothesis, Evidence, and Future Directions

We hypothesize that disease progression in MS is influenced by genetic and nongenetic factors. The genomic

determinants of MS heterogeneity are most likely single-nucleotide polymorphisms. It is also important to recognize that the aggregate contribution of germline genetic variants to the disease course of a given patient with MS may be modest. This is highlighted by observations that the clinical expression of MS may be very different even between monozygotic twin siblings who both have the disease. It is therefore likely that several postgermline events influence the clinical expression of MS.

Earlier studies⁸⁰⁻⁸³ have reported intrafamilial concordance for disease course, disease severity, and age at onset. The clinical course and severity of MS may also differ between ethnic groups. This phenotypic aggregation is due to genetic sharing. In EAE, it appears that MHC genes primarily influence penetrance, whereas other loci modulate specific phenotypes, such as topographic location of lesions in the brain or spinal cord, demyelination, and severity of inflammation.⁸⁴ Similar interplay of genetic factors may apply to human disease.

To assess the state of genotype-phenotype research in MS, we have identified from the literature a set of gene polymorphisms that have been significantly associated with phenotypic end points (**Table**). The list omits many reports and probably includes a few type I errors due to small sample sizes. In addition, series are retrospective, some phenotypic end points are questionable or not validated, and the confounding effects of drug treatment and/or stratification generally have not been considered. The effect of HLA genotypes (ie, both alleles at the HLA locus) on clinical phenotypes is particularly instructive.¹³⁹ In a mildly affected group of patients with MS, *HLA-DRB1**1501 homozygotes were significantly less frequent compared with patients classified as having nonmild MS. When a more stringent definition of mild MS was applied in which disease duration of at least 15 years was imposed, no *HLA-DRB1**1501 homozygotes were present in this subgroup. Furthermore, *HLA-DRB1**1501 homozygotes were observed more frequently among patients with a severe disease course in contrast to patients classified as having nonsevere disease. The observed dose effect could conceivably result from a perturbation in the balance of T_H1 and T_H2 cytokines influenced by other genes in the HLA region, such as *TNF*. For example, *HLA-DRB1**1501 haplotypes are associated with a *TNF* promoter polymorphism that modulates levels of expression of this proinflammatory T_H1 cytokine.¹⁴⁰ The observation of an HLA genotypic effect on disease outcome is also consistent with a model of protection mediated by *HLA-DRB1**1501-negative haplotypes.

THE FUTURE

The past few years have seen real progress in the development of laboratory and analytical approaches to study complex genetic disorders on a genome-wide scale and in defining the pathologic basis of demyelination. There is widespread enthusiasm that the deconstruction of the MS-prone genotype may lead to novel diagnostics and, more important, better therapeutic options for our patients. Unexpected overlap between genomic variations

Table. Examples of Gene Variants That Have Been Associated With Multiple Sclerosis Phenotype

Gene or Locus	Chromosomal Location	Allele	Associate Phenotype	Source
<i>GSTM1</i>	1p13.3	<i>Ile105</i>	Severe disability	Mann et al ⁸⁵
<i>IL-1ra/IL-1β</i>	2q14.2	allele 2 of <i>IL-1β</i> allele 3 of <i>IL-1ra</i> <i>IL-1ra</i> intron 4 VNTR	High protein expression and favorable prognosis High protein expression and favorable prognosis	Schrijver et al, ⁸⁶ Kantarci et al ⁸⁷ Sciaccia et al, ⁸⁸ Feakes et al ⁸⁹
<i>CCR5</i>	3p21-24	Δ32–base pair deletion	Age at onset was approximately 3 y later in patients carrying the deletion Progression to disability was delayed in homozygotes and heterozygotes for the deletion Lower risk of recurrent disease activity Trend toward reduced frequency in PPMS Trend toward smaller lesion burden	Barcellos et al ⁹⁰ Kantor et al ⁹¹ Sellebjerg et al ⁹² Haase et al ⁹³ Schreiber et al ⁹⁴
<i>OPN</i>	4q21-q25	1284A→C 9583 G→A Rs 4754, Rs 1126616, Rs 4660, Rs 1126772, Rs 1126859, Rs 9138, Rs 1126880, Rs 1126893	Patients with the wild-type 1284A genotype are less likely to have mild disease course and were at increased risk for a secondary-progressive clinical type Patients with the 9583 G/G genotype showed later disease onset No association with disease severity	Caillier et al ⁹⁵ Niino et al ⁹⁶ Hensiek et al ⁹⁷
<i>IL-4</i> <i>HLA</i>	5q31.1 6p21.3	<i>VNTR B1</i> <i>DRB1*1501</i>	Late onset, late onset in homozygotes <i>HLA</i> haplotypes were reported to be associated with an earlier age at disease onset, sex dimorphism, and severe, relapsing-remitting, and mild MS courses <i>HLA</i> haplotypes were reported to have no influence on disease course No <i>DRB1</i> association in some Asian populations who have a restricted disease, termed <i>neuromyelitis optica</i> , in which optic nerve and/or spinal cord involvement predominates A high prevalence of <i>DR2</i> was observed in patients with acute unilateral optic neuritis; its presence was associated with increased odds for developing definite MS; the association was most apparent among patients with signal abnormalities on the baseline brain MRI A number of small studies failed to show any association between PPMS and <i>DR2</i> , although a larger study from Northern Ireland appeared to show the association; others suggested an association between PPMS and the <i>HLA-DR4</i> haplotype, although a post hoc analysis is consistent with an effect decreasing the risk of relapsing-remitting MS in <i>HLA-DR4+</i> individuals rather than increasing the risk of PPMS	Vandenbroeck et al, ⁹⁸ Kantarci et al ⁹⁹ Engell et al, ¹⁰⁰ Madigand et al, ¹⁰¹ Duquette et al, ¹⁰² de la Concha et al, ¹⁰³ Celius et al, ¹⁰⁴ Masterman et al, ¹⁰⁵ Hensiek et al ¹⁰⁶ Poser et al ¹⁰⁷ Runmarker et al, ¹⁰⁸ Weinschenker et al, ¹⁰⁹ McDonnell et al, ¹¹⁰ Barcellos et al, ⁸² Villoslada et al ¹¹¹ Kira et al ¹¹² Hauser et al ¹¹³ Olerup et al, ¹¹⁴ de la Concha et al, ¹⁰³ McDonnell et al, ¹¹⁰ Weinschenker et al, ¹⁰⁹ Kantarci et al ¹¹⁵
<i>CD24</i>	6q21	ORF A→V	50% of <i>CD24 VN</i> patients with an expanded disability status scale score of 6.0 reached the milestone in 5 y, whereas the <i>CD24 AV</i> and <i>CD24 A/A</i> patients did so in 16 y and 13 y, respectively	Zhou et al ¹¹⁶

(continued)

associated with MS and other medical disorders might be uncovered, such as a role for *APOE4* as a modifier in both Alzheimer disease and MS. The development of reliable and predictive genomic profiles will not be trivial because of experimental constraints and practical economical and ethical considerations.

Identification of genomic variants that predispose patients to a discrete phenotype might also reveal novel disease-associated biochemical pathways and new therapeutic targets. The demonstration of even a modest genetic effect of a known gene on the course of MS could represent a major therapeutic opportunity.

Table. Examples of Gene Variants That Have Been Associated With Multiple Sclerosis Phenotype (cont)

Gene or Locus	Chromosomal Location	Allele	Associate Phenotype	Source
<i>ESR1</i>	6q21.5	<i>PvuII</i> and <i>XbaI</i> RFLP	The <i>P</i> allele-positive patients had a significantly higher progression of disability and a worse-ranked MS severity score; the study also suggests an interaction between the <i>ESR1</i> genotype and <i>DR2</i> in women with MS	Kikuchi et al ¹¹⁷
		<i>PvuII</i> RFLP	The study suggests an interaction between the <i>ESR1</i> genotype and <i>DR2</i> in women with MS	Mattila et al ¹¹⁸
<i>CD59</i>	10q24.1	-670A or exon 7 74C	Sex dimorphism	Kantarci et al ¹¹⁹
<i>CNTF</i>	11q12	Exon 2/-6, G→A null mutation	Patients with the <i>CNTF</i> -/- genotype had significantly earlier onset (17 y vs 27 y) with predominant motor symptoms	Giess et al ¹²⁰
			No correlation with age at onset, course, or severity	Hoffmann and Hardt, ¹²¹ Hoffmann et al ¹²²
<i>CRYAB</i>	11q22.3-23.1	-650C	Noninflammatory, neurodegenerative phenotype characterized by rapid PPMS course	van Veen et al ¹²³
<i>MEFV</i>	16p13.3	694M→V	Rapid progression to disability in non-Ashkenazi Jewish patients	Shinar et al ¹²⁴
<i>APOE</i>	19q13.2	<i>APOE4</i>	Increased severity, rate of progression, or disease brain activity	Evangelou et al ¹²⁵ Fazekas et al, ¹²⁶ Hogg et al, ¹²⁷ Chapman et al, ¹²⁸ Fazekas et al, ¹²⁹ Enzinger et al ¹³⁰
		<i>APOE2</i>	No effect	Ferri et al, ¹³¹ Weatherby et al, ¹³² Masterman et al, ¹³³ Schreiber et al, ⁹⁴ Savettieri et al ¹³⁴
<i>TGFB1</i>	19q13	-509/C and codon 10/+869T	Decreased severity and progression to chronic progressive disease	Ballerini et al ¹³⁵ Schmidt et al, ¹³⁶ Kantarci et al ¹³⁷
			Decreased severity defined as an EDSS score of <3 after 10 y of symptoms	Green et al ¹³⁸

Abbreviations: A/V, alanine/valine; EDSS, Expanded Disability Status Scale; M, methionine; MRI, magnetic resonance imaging; MS, multiple sclerosis; PPMS, primary progressive multiple sclerosis; RFLP, restriction fragment length polymorphism; Rs, reference SNP; SNP, single nucleotide polymorphism; VNRT, variable number of tandem repeats; V/V, valine/valine.

Accepted for Publication: June 10, 2005.

Author Affiliations: Departments of Neurology (Drs Frohman, Karandikar, Monson, and Racke), Ophthalmology (Dr Frohman), and Pathology (Dr Karandikar) and Center for Immunology (Dr Racke), University of Texas Southwestern Medical Center at Dallas; Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Milan, Italy (Dr Filippi); Department of Neurology, Heinrich Heine University, Dusseldorf, Germany (Drs Stuve, Hemmer, and Hartung); Department of Neurology, Yale University, New Haven, Conn (Dr Waxman); Department of Neurology, University of Colorado, Boulder (Dr Corboy); Texas Neurology, Dallas (Dr Phillips); Department of Neurology, Mayo Clinic, Rochester, Minn (Dr Lucchinetti); Division of Neuropsychology, Department of Veterans Affairs, Washington, DC (Dr Wilken); Department of Neurology, University of Groningen, Groningen, the Netherlands (Dr DeKeyser); Department of Neurology, Stanford University, Stanford, Calif (Dr Steinman); and Department of Neurology, University of California, San Francisco (Drs Oksenberg, Cree, Hauser, and Racke).

Correspondence: Elliot M. Frohman, MD, PhD, Department of Neurology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, TX 75235 (elliott.frohman@utsouthwestern.edu).

Author Contributions: Study concept and design: Frohman,

Filippi, Stuve, Waxman, Corboy, Phillips, Lucchinetti, Wilken, Karandikar, DeKeyser, Hartung, Steinman, Oksenberg, Hauser, and Racke. *Acquisition of data:* Frohman, Waxman, DeKeyser, and Hauser. *Analysis and interpretation of data:* Frohman, Phillips, Wilken, Hemmer, Monson, DeKeyser, Hartung, Cree, and Hauser. *Drafting of the manuscript:* Frohman, Filippi, Stuve, Waxman, Phillips, Wilken, Karandikar, Hemmer, Monson, DeKeyser, Steinman, Oksenberg, Hauser, and Racke. *Critical revision of the manuscript for important intellectual content:* Frohman, Stuve, Corboy, Phillips, Lucchinetti, DeKeyser, Hartung, Oksenberg, Cree, Hauser, and Racke. *Statistical analysis:* Wilken, Cree, and Hauser. *Obtained funding:* Waxman, DeKeyser, and Steinman. *Administrative, technical, and material support:* Frohman, Filippi, Stuve, Waxman, Hemmer, and Hartung. *Study supervision:* Frohman, Stuve, Corboy, Phillips, and Hartung.

Funding/Support: This study was supported by the Once Upon a Time Foundation, Fort Worth, Tex (Dr Frohman) and the National Institutes of Health, Bethesda, Md (Dr Racke). Dr Cree is a Sylvia Lawry Fellow of the National Multiple Sclerosis Society.

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