

# Immunopathology of multiple sclerosis

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**Abstract**—Multiple sclerosis (MS) is an immune-mediated disease of the CNS that is characterized by inflammation, demyelination, and axon loss. The inflammation in MS appears to be caused by an overactive pro-inflammatory T<sub>H</sub>1 profile in T cells. Demyelination can result as a consequence of direct damage to myelin by inflammatory cells or indirectly because of the environment produced by inflammation. Axon loss occurs in MS lesions starting early in the disease. Treatments now approved by the FDA are most effective in the inflammatory phase of the disease. Because axon loss is irreversible and is responsible for long-term disability, therapies are also needed that enhance remyelination or are neuroprotective.

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Multiple sclerosis (MS) is an immune-mediated, chronic inflammatory disease of the CNS. The prevalence of MS is estimated to be 400,000 persons in the United States and 2 million people worldwide.<sup>1</sup> The natural history of MS in most patients is characterized by a progressive neurologic deterioration.<sup>2</sup> In addition to sustained physical disability, 45% to 65% of patients with MS experience cognitive dysfunction that is usually irreversible.<sup>3</sup> Other common co-morbidities are fatigue and depression, with the lifetime prevalence of the latter estimated to be between 37% and 54%.<sup>4</sup>

Patients with MS exhibit different patterns of disease activity. The majority of patients (approximately 85%) initially present with relapsing–remitting MS (RRMS), which is characterized by relapses with full recovery or with residual deficits.<sup>2</sup> At least 50% of patients with RRMS develop secondary progressive MS (SPMS), which is characterized by disease progression with or without occasional relapses. Approximately 10% of MS patients exhibit primary progressive MS (PPMS), which is progressive from the onset with occasional temporary improvements. The least common form of MS, progressive relapsing MS (PRMS), is progressive from the onset with acute relapses.

In considering the pathogenesis of MS, two different approaches are common. One point of view considers the disease to consist of two distinct phases, an inflammatory and a neurodegenerative phase.<sup>5</sup> According to another point of view, the disease process has three components: 1) an initial phase of inflammation, which leads to 2) demyelination, and this ultimately results in 3) axon loss. The loss of axons is considered to be the ultimate cause of the permanent clinical disability that occurs during the

disease. In this review of the immunopathology of MS, these three components of the disease will be considered separately.

**Inflammation.** MS is predominantly a T cell-mediated inflammatory disorder, with activation and entry into the CNS of T cells specific for myelin antigens (e.g., myelin basic protein, proteolipid protein, myelin oligodendrocyte glycoprotein, or myelin-associated glycoprotein).<sup>6</sup> In the CNS, naive CD4 T cells differentiate into T<sub>H</sub>1 and T<sub>H</sub>2 cells, which produce different cytokines and have different effector mechanisms (figure 1). T<sub>H</sub>1 cells produce pro-inflammatory cytokines, such as interleukin-2 (IL-2), tumor necrosis factor (TNF), and interferon (IFN)- $\gamma$ . T<sub>H</sub>1 cytokines activate antigen-presenting cells (APCs), promote T<sub>H</sub>1 differentiation, and inhibit T<sub>H</sub>2 differentiation. In contrast, T<sub>H</sub>2 cells produce anti-inflammatory cytokines, such as IL-4, IL-5, IL-6, IL-10, and IL-13. These cytokines regulate humoral immunity, downregulate local inflammation, promote T<sub>H</sub>2 differentiation, and inhibit T<sub>H</sub>1 differentiation. The inflammation seen in MS appears to be largely due to a misguided and overactive T<sub>H</sub>1 response.

A model of the pathologic mechanisms that occur in MS is shown in figure 2. Pre-existing autoreactive T cells are activated outside the CNS by foreign microbes, self proteins, or microbial superantigens. The activated T cells cross the blood–brain barrier through a multistep process. First, activated T cells that express integrins can bind to adhesion molecules on the surface of the endothelium. Then the T cells must pass through a barrier of extracellular matrix (ECM) in a step that involves matrix metallo-

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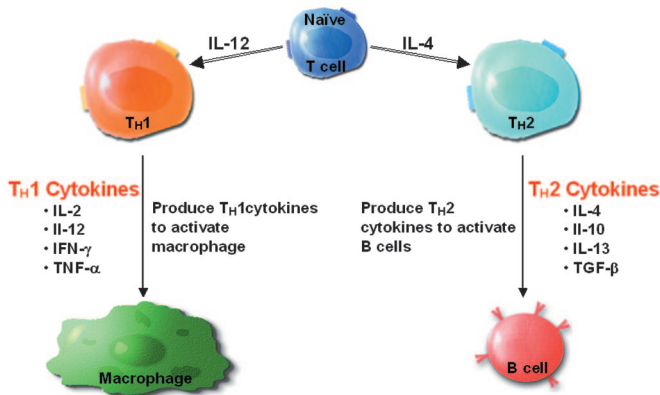


Figure 1. The differentiation of naive CD4 T cells into  $T_H1$  and  $T_H2$  cells. From Yong 2002,<sup>27</sup> with permission.

proteases (MMPs), which are enzymes that play a role in both the degradation of ECM and the proteolysis of myelin components in MS. The T cells are locally re-activated when they recognize their antigen on the surface of local APCs. The activated T cells secrete cytokines that stimulate microglial cells and astrocytes, recruit additional inflammatory cells, and induce antibody production by plasma cells. Anti-myelin antibodies, activated macrophages or microglial cells, complement, and tumor necrosis factor alpha (TNF- $\alpha$ ) are believed to cooperate in producing demyelination. In the neurodegenerative phase of the disease, excessive amounts of glutamate are released by lymphocytes, microglia, and macrophages. The glutamate activates various glutamate receptors (AMPA and kainate receptors), and the influx of calcium through ion channels associated with different glutamate receptors may cause necrotic damage to oligodendrocytes and axons. Different steps of the putative pathologic cascade may eventually be targeted therapeutically for optimal treatment of MS.

**Demyelination.** Demyelination in MS can result from direct damage to myelin by inflammatory cells or indirectly from the environment created by inflammation. Some remyelination does occur in MS lesions, but the myelin is usually thinner than before, with shorter internodes.<sup>7</sup> The following factors are postulated to inhibit remyelination in MS:<sup>8</sup> 1) the loss of oligodendrocytes and oligodendrocyte precursors as a consequence of attack by immune cells; 2) inhibitory signals produced by inflammation; 3) the obstruction of oligodendrocytes by astrocytic scarring; and 4) the reduced receptiveness of injured axons to remyelination.

The acute neurologic dysfunction that occurs during a relapse is considered to be due to depolarization-induced conduction block at demyelinated regions of axons.<sup>9</sup> Recovery is hypothesized to be due to an increase in the number of Na<sup>+</sup> channels along the demyelinated parts of the axon, partially restoring axon conduction.<sup>10</sup> Current research in animal models of MS suggests that dysregulated ion channel expression (e.g., Ca<sup>2+</sup> or Na<sup>+</sup> channels) can cause

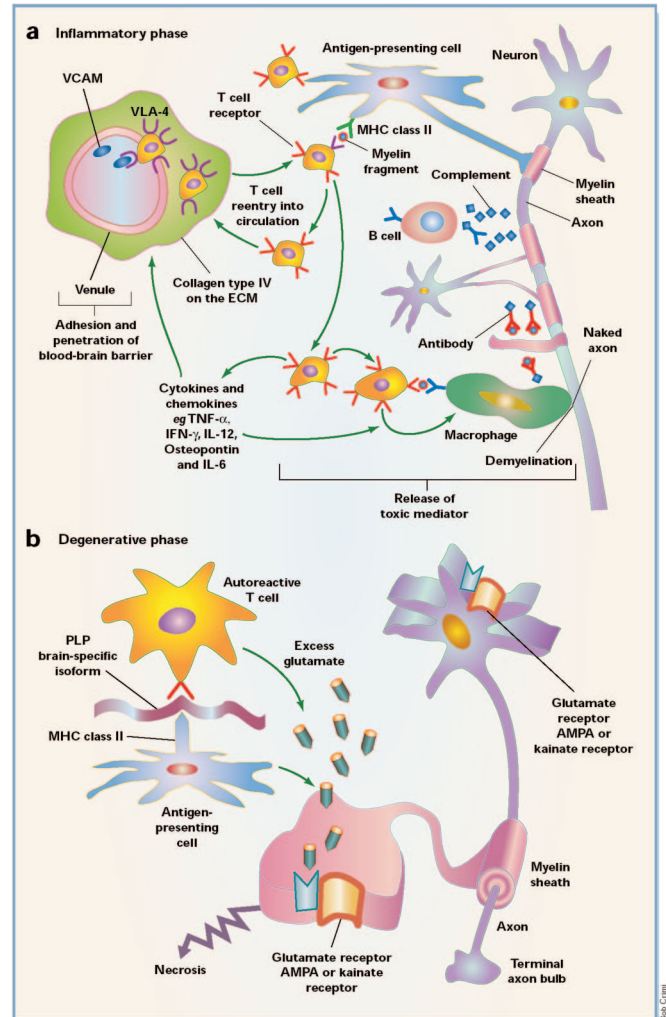


Figure 2. The two stages of the progression of MS. (a) Autoimmune attack. (b) Neurodegeneration. ECM = extracellular matrix. From Steinman 2001, with permission.<sup>5</sup>

neuron injury or disrupt neuron signaling in MS. Both of these processes could lead to neurologic dysfunction.<sup>11</sup>

One way to reduce long-term disability for patients with MS would be to promote remyelination of axons.<sup>7</sup> This could be achieved by enhancing endogenous remyelination or by limiting damage to oligodendrocytes. A more complex method of promoting remyelination is to transplant exogenous myelin-forming cells (e.g., oligodendrocyte precursor cells, Schwann cells, olfactory ensheathing cells, neural stem cells, or xenografts). One difficulty with this latter approach is the need to implant the cells within the appropriate regions of the CNS where demyelination has occurred. Despite the difficulties associated with this approach, pilot studies have begun to explore implantation of autologous Schwann cells into MS lesions. A phase I study in five MS patients was started in 2001 at Yale University.<sup>7</sup> The site of implantation was to be biopsied after 6 months to determine the extent of remyelination. However, after three of planned five patients were

treated, this trial was terminated in 2002 because of lack of evidence that the approach led to significant myelin formation.<sup>12</sup> The optimal transplant strategy would allow precursor cells to be administered regionally, with a chemoattractor homing mechanism causing implantation preferentially into demyelinated areas.

**Axon loss.** In the past, the MS lesion was generally considered to be an area of demyelination with preservation of axons. However, it has been observed that destruction of axons does occur, with axon loss being reported in otherwise normal-appearing white matter.<sup>13</sup> Axon damage and transection occur in both acute and chronic inflammatory plaques as a consequence of demyelination, and have been observed in active and chronic lesions from patients with clinical disease durations ranging from 2 weeks to 27 years.<sup>14</sup> Although the initial postmortem studies in patients with MS led to the conclusion that axon loss occurred late in the progression of MS,<sup>15</sup> a recent study reported that axon damage was most extensive during the first year after onset of MS, with fewer acutely injured axons observed in the lesions of patients with disease durations greater than 10 years.<sup>16</sup>

Axon transection is irreversible and is most abundant in areas of inflammation. Later axon damage may result from changes in the microenvironment in the CNS or from sublethal injury during acute inflammatory events, resulting in decreased long-term survival. Therefore, the following mechanisms have been proposed for demyelination-induced axon loss: 1) a direct effect through the loss of trophic influences from oligodendrocytes; 2) as a consequence of sustained demyelination-induced conduction block; and 3) indirectly through the increased vulnerability of the exposed axon to noxious agents.<sup>17</sup> The progressive disability that occurs in MS is believed to result from cumulative axon damage and degeneration, along with neuron loss due to Wallerian degeneration. Drop-out of damaged axons over time may result in premature stress and damage to surviving axons within the same pathways, thereby producing further loss and functional impairment.

Brain white matter contains, by volume, 46% axons, 24% myelin, 17% glia, and 13% blood vessels and blood and tissue fluid.<sup>15</sup> Therefore, a CNS correlate of axon loss is brain atrophy, which may be caused either by tissue loss within the lesion or by Wallerian degeneration in related fiber pathways.<sup>15</sup> At all stages of the disease, an increased rate of atrophy occurs in the brain and spinal cord of patients with MS (0.6–1% per year reduction in brain volume in patients versus 0.1–0.3% per year for healthy controls).<sup>15</sup> Atrophy is most evident in progressive forms of MS and may be a predictor for the conversion from RRMS to SPMS because, once a threshold of axon transection is exceeded, MS patients may enter an irreversible secondary progressive stage. Inflammatory or demyelinating lesions are followed by progressive atrophy for months or

years, suggesting that there is a potential time period after acute inflammation during which appropriate neuroprotective therapy might be beneficial in preventing long-term disability.<sup>15</sup>

Recent MRI studies examining brain atrophy in patients with MS have yielded new information about the pathologic processes that occur during disease progression. One study reported that regional brain atrophy evolves differently in MS patients according to their clinical phenotypes, with ventricular enlargement predominant in patients with RRMS and cortical atrophy more common in patients with progressive forms of the disease.<sup>18</sup> In another study, conducted in patients recruited within 3 months of the onset of a clinically isolated syndrome (CIS) suggestive of MS and followed for 3 years, early development of MS was associated with progressive gray-matter atrophy but not white-matter atrophy.<sup>19</sup> Gray-matter atrophy might identify patients who are most in need of early treatment with DMAs. A postmortem study conducted in 55 MS patients and 32 matched controls reported a significant decrease in the volume and axon density in the corticospinal and posterior sensory tracts compared with controls.<sup>20</sup> Furthermore, the fibers lost in MS appeared to be preferentially small fibers with diameters  $<3 \mu\text{m}^2$ . Therefore, axon loss in MS is widespread and selectively affects the smaller fibers.

The amino acid derivative *N*-acetylaspartate (NAA) is predominantly found in neurons and is considered a marker for their density and integrity.<sup>21</sup> A recent study, conducted using conventional MRI and calculating whole-brain NAA (WBNA) concentrations with MR spectroscopy, reported that the mean WBNA concentration was significantly lower in patients at presentation with CIS compared with controls ( $p < 0.0001$ ).<sup>22</sup> This finding confirms earlier MRI studies showing that significant axon pathology occurs in patients at the earliest clinical stage of MS. Furthermore, atrophy appeared only after a delay of months following acute inflammation, again suggesting that there may be a window of opportunity for neuroprotective treatment.

**Apoptosis.** Both necrosis and apoptosis play a role in the progression of MS. When necrosis occurs in MS lesions, it produces enhancing lesions caused by the substantial breakdown of the blood–brain barrier. Apoptosis occurs pathologically in MS, in part because the loss of myelin causes neurons to be much more susceptible to binding of agents that induce apoptosis rather than necrosis. Apoptosis is probably at least partially responsible for the progression that leads to permanent disability and explains why lesion activity on MRI scans can be quiescent at a time when the disability of the patient continues to increase.

In MS lesions, apoptosis occurs in neurons, oligodendrocytes, and leukocytes. The precise role of apoptosis in disease progression is under active investigation. In one study, extensive areas of apo-

ptotic oligodendrocytes were observed in new lesions of some MS patients.<sup>23</sup> A recent report described the pathologic findings observed in immunostained tissues from 12 patients with RRMS who had died during or shortly after a relapse.<sup>24</sup> Instead of the previously described inflammatory and necrotic changes, widespread apoptosis of oligodendrocytes in the absence of a cellular immune response was observed. New symptomatic lesions showed extensive oligodendrocyte apoptosis in myelinated tissue in which T cells, macrophages, and neurons appeared normal. At the same time, no lesions exhibiting apoptosis of oligodendrocytes were found in six patients with chronic MS. This suggests that apoptosis of oligodendrocytes may represent a very early stage in the evolution of lesions that underlie acute relapses of MS. Therefore, myelin destruction in MS lesions may be caused by processes other than those mediated by macrophages.

**Patterns of pathology.** Although demyelinating lesions are associated with inflammatory infiltrates containing T cells and macrophages, four different patterns of pathology with resulting demyelination have been observed in MS lesions.<sup>23,25</sup> Type I patterns are T cell-mediated and account for 19% of lesions. They lead to a pattern of demyelination that is macrophage-mediated, either directly or by macrophage toxins. Type II lesions are both T cell- and antibody-mediated and, at 53%, are the most common pathology observed in MS lesions. This pattern results in demyelination via specific demyelinating antibodies and complement. Type III lesions occur in 26% of lesions and are related to distal oligodendroglial pathology; degenerative changes in distal processes occur that are followed by apoptosis. Type IV lesions are responsible for only 2% of lesions and result from primary oligodendrocyte damage followed by secondary demyelination. This latter pattern was observed only in a small subset of patients with PPMS. The heterogeneity of demyelination processes in patients with MS implies that different therapies may be required to provide optimal treatment.

All of the current FDA-approved treatments specifically target the immune system and are most effective in patients who are undergoing active inflammation. However, inflammatory cells may have a neuroprotective role in the repair of damaged tissue. For example, macrophages stimulate remyelination in organ culture and immune cells provide neuroprotective mediators (e.g., nerve growth factor and glial cell line-derived neurotrophic factor).<sup>26</sup> Consequently, complete blockage of all inflammatory processes within lesions may not have maximal benefit.<sup>25</sup> Other targets are also possible for the treatment of MS, such as inhibiting the ability of T cells to cross the blood-brain barrier, inhibiting demyelination, or promoting remyelination. Cytotoxic agents, such as mitoxantrone, may provide additional benefits for treating MS, and neuroprotective agents might reduce the axon loss that is responsible

for long-term disability. In the future, optimal treatment of MS may consist of combination therapy with agents that have different mechanisms of action.

**Conclusions.** The pathologic conditions that occur in MS include inflammation, demyelination, and axon loss. MS is predominantly a T cell-mediated inflammatory disorder with overproduction of pro-inflammatory cytokines. Demyelination can result from either direct damage to neuronal myelin by inflammatory cells or indirectly because of the extracellular environment. This demyelination can cause neurons to be more susceptible to apoptosis. Although demyelination may produce the relapses that occur during the disease, long-term disability is due primarily to irreversible axon loss and cell death. Current FDA-approved treatments for MS target the inflammatory phase of the disease. Therefore, new therapies are needed that enhance remyelination or are neuroprotective.

## References

1. National Multiple Sclerosis Society—About research [online]. Available at: <http://www.nationalmssociety.org/Research-FactSheet.asp>. Accessed June 24, 2004.
2. Kieseier BC, Hartung HP. Current disease-modifying therapies in multiple sclerosis. *Semin Neurol* 2003;23:133–146.
3. Bagert B, Camplair P, Bourdette D. Cognitive dysfunction in multiple sclerosis: natural history, pathophysiology and management. *CNS Drugs* 2002;16:445–455.
4. Minden SL, Schiffer RB. Affective disorders in multiple sclerosis. Review and recommendations for clinical research. *Arch Neurol* 1990;47:98–104.
5. Steinman L. Multiple sclerosis: a two-stage disease. *Nature Immunol* 2001;2:762–764.
6. Martino G, Furlan R, Brambilla E, et al. Cytokines and immunity in multiple sclerosis: the dual signal hypothesis. *J Neuroimmunol* 2000;109:3–9.
7. Stangel M, Hartung HP. Remyelinating strategies for the treatment of multiple sclerosis. *Prog Neurobiol* 2002;68:361–376.
8. Hohlfeld R. Myelin failure in multiple sclerosis: breaking the spell of Notch. *Nature Med* 2002;8:1075–1076.
9. Kornek B, Lassmann H. Neuropathology of multiple sclerosis—new concepts. *Brain Res Bull* 2003;61:321–326.
10. Waxman SG. Sodium channels as molecular targets in multiple sclerosis. *J Rehab Res Dev* 2002;39:233–242.
11. Waxman SG. Ion channels and neuronal dysfunction in multiple sclerosis. *Arch Neurol* 2002;59:1377–1380.
12. The Myelin Project progress report [online]. Available at: <http://www.myelin.org/12082003.htm>. Accessed Mar 12, 2004.
13. Neumann H. Molecular mechanisms of axonal damage in inflammatory central nervous system diseases. *Curr Opin Neurol* 2003;16:267–273.
14. Trapp BD, Peterson J, Ransohoff RM, et al. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998;338:278–285.
15. Miller DH, Barkhof F, Frank JA, Parker GJ, Thompson AJ. Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance. *Brain* 2002;125:1676–1695.
16. Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Bruck W. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain* 2002;125:2202–2212.
17. Halfpenny C, Benn T, Scolding N. Cell transplantation, myelin repair, and multiple sclerosis. *Lancet Neurol* 2002;1:31–40.
18. Pagani E, Rocca MA, Gallo A, et al. Regional brain atrophy evolves differently in MS patients according to their clinical phenotypes. *Neurology* 2004;62(suppl 5):A287–288. Abstract.
19. Dalton CM, Chard DT, Davies GR, et al. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. *Brain* 2004;127:1101–1107.
20. DeLuca GC, Ebers GC, Esiri MM. Axonal loss in multiple sclerosis: a pathological survey of the corticospinal and sensory tracts. *Brain* 2004;127:1009–1018.
21. Gonen O, Catalaa I, Babb JS, et al. Total brain N-acetylaspartate: a new measure of disease load in MS. *Neurology* 2000;54:15–19.
22. Paolillo A, Piattella MC, Pantano P, et al. The relationship between inflammation and atrophy in clinically isolated syndromes suggestive of multiple sclerosis. A monthly MRI study after triple-dose gadolinium-DTPA. *J Neurol* 2004;251:432–439.

23. Lucchinetti C, Bruck W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000;47:707-717.
24. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann Neurol* 2004;55:458-468.
25. Lassmann H, Bruck W, Lucchinetti C. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. *Trends Mol Med* 2001;7:115-121.
26. Kieseier BC, Hartung HP. Multiple paradigm shifts in multiple sclerosis. *Curr Opin Neurol* 2003;16:247-252.
27. Yong VW. Differential mechanisms of action of interferon-beta and glatiramer acetate in MS. *Neurology* 2002;59:802-808.