ABSTRACT

Six disease-modifying therapies are approved for the treatment of multiple sclerosis: glatiramer acetate, 2 formulations of interferon (IFN) β-1a (one administered by subcutaneous injection and one administered by intramuscular injection), IFNβ-1b, natalizumab, and mitoxantrone. These agents are thought to act primarily by suppressing central nervous system (CNS) inflammation, which may also produce secondary neuroprotective effects. Inflammatory CNS demyelination requires a sequence of tightly controlled immune processes, including antigen presentation to T cells, additional cell-cell contacts between antigen-presenting cells and T cells that are required for full T-cell activation, the migration of T cells through the endothelial lining, and the release of several pro-inflammatory or anti-inflammatory chemical mediators. IFNβ and glatiramer acetate (GA) interrupt several of these processes, resulting in decreased CNS infiltration of lymphocytes and inflammation. GA may also produce neuroprotective effects by modulating the release of neurotrophic factors, chemical mediators that are produced by several cell populations that promote neuronal development and survival. Natalizumab blocks specific cell-surface receptor molecules that are required for lymphocytes to migrate from the circulation to the CNS, producing long-lasting suppression of the infiltration of CD4+ and CD8+ T lymphocytes. Natalizumab may produce a rebound increase in the number of T2 lesions on magnetic resonance imaging after discontinuation. Mitoxantrone is an antineoplastic agent that interferes with DNA replication in rapidly dividing cells, resulting in lymphocyte depletion. It is not known to produce neuroprotective effects.

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ALL of the disease-modifying therapies that are currently approved for the treatment of multiple sclerosis (MS) act primarily by suppressing central nervous system (CNS) inflammation. However, recent evidence suggests that these agents may also possess secondary neuroprotective properties. This paper reviews the immunomodulatory and neuroprotective mechanisms by which current therapies improve outcomes in patients with MS.

MS TREATMENT OPTIONS: THE ROLE OF CNS INFLAMMATION

Six medications are currently approved by the US Food and Drug Administration for the treatment of MS. First-line treatments include glatiramer acetate (GA) and 3 formulations of interferon (IFN) β (IFNβ-1a for intramuscular injection once weekly; IFNβ-1a for subcutaneous injection 3 times weekly; and IFNβ-
Mitoxantrone and natalizumab are generally reserved for patients who cannot tolerate or do not respond adequately to GA or IFNβ. Although mitoxantrone and natalizumab are similar in efficacy to the first-line agents, they are associated with potentially serious safety concerns. Natalizumab has been associated with an increased risk of progressive multifocal leukoencephalopathy, an opportunistic CNS infection that is potentially fatal.1-4 Mitoxantrone is associated with an increased risk of cardiotoxicity.5

Nearly all of the approved or investigational therapies for MS are based on a model of MS pathogenesis in which an important early step is the interaction between antigen-presenting cells (APCs; especially dendritic cells, but also monocytes or B cells) and T lymphocytes in secondary lymphoid organs (Figure 1).6,7 This interaction results in the activation and clonal expansion of T cells. The activating antigen might be a foreign (eg, viral) peptide or a CNS autoantigen that has escaped the brain as the result of some type of insult. T cells that enter the CNS but do not encounter the specific antigen to which they have been sensitized rapidly exit the CNS. If a T cell encounters an antigen that is identical or very similar to the antigen that it has been sensitized to detect, it becomes reactivated and begins to express a range of inflammatory mediators, cytokines, and free radicals that damage the axon and the surrounding myelin sheath. Activated T cells may also activate B cells, which differentiate into plasma cells. Plasma cells are capable of secreting antibodies, some of which bind to myelin antigens.

The activation of T cells by APCs requires 2 distinct cell-cell signaling pathways.8 The first signal (sometimes referred to as “signal 1”) occurs when an APC presents antigen, complexed with major histocompatibility complex (MHC), to T cells. MHC possesses an antigen-binding cleft that holds a peptide antigen that is typically 8 to 11 amino acids in length.9 Binding of the MHC-antigen complex to specific cell-surface receptor molecules on T cells provides the first signal for T-cell activation. The second signal (sometimes referred to as “signal 2,” or the T-cell costimulatory signal) requires the interaction between several cell-surface costimulatory molecules on APCs and corresponding receptor molecules on T cells. For example, one costimulatory signal pathway involves the interaction of a specific costimulatory molecule known as B7 on APCs with its receptor molecule (CD28) on T cells. T-cell costimulation occurs both during the initial activation of T cells in the lymph nodes and when T cells are reactivated by APCs in the CNS. T cells that do not receive the appropriate costimulatory signal do not initiate inflammatory responses and may undergo apoptotic cell death.10,11

**Interferon β**

All of the IFNβ formulations used for MS therapy bind to IFNβ receptors on T cells and APCs.11 Engagement of IFNβ receptors initiates several intracellular events that interfere with the process of antigen presentation, including prevention of the upregulation of MHC, costimulatory molecules, and T-cell receptor molecules.11 IFNβ does not extensively penetrate the CNS at the doses typically used to treat MS, and therefore probably acts primarily on antigen presentation in the lymph nodes.

Activated T cells in circulation also require chemotactrant molecules (chemokines) to migrate from the vasculature into sites of inflammation. Chemokines are expressed by several cell types, including endothelial cells, astrocytes, and microglia.12 Two chemokines—monocyte chemotactric protein-1 and interleukin (IL)-
are especially important in T-cell infiltration to sites of inflammation.\textsuperscript{13} Several studies have demonstrated that IFNβ downregulates the expression of these chemokines or blocks their activity.\textsuperscript{11} Another critical step in the migration of T cells to sites of inflammation is the adhesion of T cells to the vascular endothelium. T-cell adhesion requires the interaction of specific cell-surface adhesion molecules on T cells (eg, very late antigen-4 [VLA-4]) and receptor molecules on endothelial cells (eg, intercellular adhesion molecule-1). IFNβ downregulates VLA-4, resulting in reduced T-cell adherence to the endothelial membrane.\textsuperscript{11}

Finally, the migration of T cells from the vasculature to the parenchyma requires the release of proteolytic enzymes by T cells (eg, matrix metalloproteinase-9 [MMP-9]) that help to digest the endothelial lining and the extracellular matrix. IFNβ is a potent inhibitor of MMP-9,\textsuperscript{14-17} and therefore decreases the ability of T cells to migrate from the circulation to the parenchyma.

There is no evidence that IFNβ produces a direct neuroprotective effect. However, the diverse actions of IFNβ on T-cell activation, chemoattraction, lymphocyte adhesion, and transendothelial migration may indirectly promote neuroprotection in patients with MS by suppressing CNS inflammation. This hypothesis is supported by studies in which IFNβ has been shown to significantly reduce brain atrophy in patients with MS. For example, Rudick et al examined the effects of IFN therapy on brain atrophy in 140 patients with relapsing-remitting MS (RRMS).\textsuperscript{18} Atrophy was common among patients with MS treated with placebo: approximately 20% of patients exhibited a loss of more than 2% of brain parenchymal volume from baseline after 2 years (Figure 2).\textsuperscript{18} Treatment with weekly intramuscular IFNβ-1a significantly reduced atrophy after the second year of treatment, but not the first year (Figure 3).\textsuperscript{18}

**Glatiramer Acetate**

Glatiramer acetate is a random synthetic polymer of 4 amino acids (L-alanine, L-glutamic acid, L-lysine, and L-tyrosine) in a molar ratio of 4.6:1.5:3.6:1.9. GA reduces CNS inflammation that is mediated by CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells, and may also produce indirect neuroprotective effects in patients with MS.

CD4\textsuperscript{+} T cells were long believed to be the dominant T-cell subset in the pathogenesis of MS. When activated by APCs bearing an appropriate antigen complexed with MHC, CD4\textsuperscript{+} T cells differentiate into either helper T cell (T\textsubscript{H}1) 1 cells (which release several pro-inflammatory cytokines, including IFNγ, tumor necrosis factor-α and IL-2), or T\textsubscript{H}2 cells (which release anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, and transforming growth factor-β).\textsuperscript{5,19} GA structurally resembles myelin-derived antigen, but promotes the maturation of anti-inflammatory T\textsubscript{H}2 cells that migrate to the CNS and suppress inflamm-
tion. GA treatment also increases the number of circulating CD8+ T cells, a response that has been correlated with the clinical efficacy of GA. The precise mechanism by which CD8+ cells suppress CNS inflammation is not known, but may involve killing of autoreactive CD4+ cells. Finally, GA also has been shown to modify the development of APCs in animal models of MS by promoting the development of anti-inflammatory monocytes.

Glatiramer acetate also may modulate the activity of neurotrophic factors (neurotrophins). These molecules are primarily expressed during CNS development, and they also may be important in the pathogenesis of MS and other neurodegenerative diseases. Neurotrophins are produced by immune cells and neurons, they provide trophic support to injured axons, and they may promote the preservation of axons. Some neurotrophins (eg, brain-derived neurotrophic factor and neurophin-3) have been shown to stimulate oligodendrocyte proliferation and remyelination. Neurotrophins also produce immunoregulatory effects that may be important in MS, including the suppression of MHC expression by microglia, downregulation of costimulatory molecules B7 and CD40, and decreased monocyte migration.

In animal models of MS, GA-specific T cells have been shown to cross the blood-brain barrier and release neurotrophic factors and anti-inflammatory cytokines.

The efficacy of GA for the treatment of primary progressive MS (PPMS), which many experts believe is primarily a disorder of neurodegeneration rather than inflammation, has recently been evaluated in the PROMISE clinical trial. Patients with PPMS were randomized to treatment with GA at a dose of 20 mg per day by subcutaneous injection or placebo for up to 3 years. The primary efficacy end point (the likelihood of disease progression) did not differ significantly between the 2 treatment groups. This may have been due to a much lower rate of progression in both groups than was anticipated when the study was designed. However, post-hoc analysis revealed that GA was associated with a significant decrease in the rate of disease progression among men, but not among women (Figure 4). This finding suggests that GA may produce neuroprotective effects in male patients with MS, and that a larger clinical trial may be required to demonstrate significant improvement in disease progression for men and women.

### Natalizumab

As previously described, infiltration of T lymphocytes into the CNS is a pivotal early step in the development of inflammation and demyelination in MS. Natalizumab, a monoclonal antibody that blocks the cell-surface adhesion molecule VLA-4, acts by preventing leukocyte migration. Natalizumab is an immunoglobulin G4 antibody that does not bind complement or induce cell killing. Natalizumab was approved for the treatment of MS on the basis of 2 phase III clinical trials. The AFFIRM (Natalizumab Safety and Efficacy in RRMS) clinical trial demonstrated that natalizumab decreased the annualized relapse rate by 68% compared with placebo and the risk of confirmed progression of disease by 42% over 2 years. Natalizumab also significantly reduced gadolinium-enhancing lesions, T2 lesions, and brain atrophy. The SENTINEL (Safety and Efficacy of Natalizumab in Combination with IFNβ-1a in Patients with RRMS) study compared the combination of natalizumab and IFNβ-1a versus IFNβ-1a alone in patients with at least 1 relapse on IFNβ therapy. Compared with IFNβ-1a alone, combination therapy was associated with a 24% reduction in the...
risk of sustained disability after 2 years, and also significantly reduced relapse rates and T2 lesions.

Recent research has examined the effects of natalizumab on immunosurveillance of the CNS in patients with MS by performing lymphocyte profiles from samples of cerebrospinal fluid (CSF) and peripheral blood.\textsuperscript{31,32} Natalizumab markedly decreased leukocyte infiltration into the CNS. An analysis of T-cell subsets revealed a decrease in CD8\(^+\) T cells of approximately 5-fold in patients treated with natalizumab compared with untreated patients, but nearly a 50-fold reduction in CD4\(^+\) T cells. Natalizumab treatment was also associated with a decrease in penetration of B cells and plasma cells into the CNS. At an additional follow-up evaluation 6 months later—long after natalizumab would be expected to have completely cleared from circulation—patients who had received natalizumab continued to exhibit significant depletion of CD4\(^+\) and CD8\(^+\) T cells in the CNS (Figure 5).\textsuperscript{31} Thus, at least some pharmacologic effect of natalizumab persists for at least 6 months after the last dose.

The optimal duration of natalizumab therapy is not well defined, and it is unclear whether the benefits of treatment persist after treatment is discontinued. In one phase II clinical trial, relapse rates were significantly lower for patients who were treated with natalizumab during 6 months of therapy, but this benefit of treatment was almost entirely lost within 6 months of treatment discontinuation.\textsuperscript{33} More recently, an analysis of magnetic resonance imaging outcomes for 21 patients from the AFFIRM and SENTINEL clinical trials demonstrated that the number of new or enlarging T2 lesions increased after natalizumab was discontinued, especially among patients with the shortest duration of natalizumab treatment.

Mitoxantrone

Mitoxantrone is an antineoplastic agent that is also used to treat patients with MS who do not respond adequately to or cannot tolerate other treatments.\textsuperscript{35} Mitoxantrone kills rapidly dividing cells by interfering with DNA replication, resulting in the depletion of leukocytes.\textsuperscript{36} In clinical trials of patients with secondary progressive MS or very active RRMS, mitoxantrone has been shown to significantly reduce relapses and slow the progression of disability.\textsuperscript{37,38} It is not known to produce neuroprotective effects.

**Procedures**

**Figure 5. Natalizumab Decreases the Number of Lymphocytes in CSF**

At study entry, the number of (A) WBCs, (B) CD4\(^+\) T cells, (C) CD8\(^+\) T cells, (D) CD19\(^+\) B cells, and (E) CD138\(^+\) plasma cells in CSF was compared between patients with other neurologic diseases (OND), patients with multiple sclerosis who were not treated with natalizumab (MS), and patients with multiple sclerosis treated with natalizumab [(MS (Nat)]. A mild CSF pleocytosis was observed in MS patients who had not received natalizumab therapy (A), compared with the OND patient cohort (A). Compared with MS patients not treated with natalizumab, treatment with natalizumab significantly reduced the numbers of T lymphocytes, CD4\(^+\) T cells, CD8\(^+\) T cells, CD19\(^+\) B cells, and CD138\(^+\) plasma cells in the CSF (A–E). Six months after cessation of therapy, the CSF cellular phenotype of 14 natalizumab-treated MS patients was reanalyzed [(MS (Nat)] 6 Mo). The number of CSF WBCs (A), CD4\(^+\) T cells (B), CD8\(^+\) T cells (C), CD19\(^+\) B cells (D), and CD138\(^+\) plasma cells (E) remained significantly lower than those in MS patients not treated with natalizumab. During the 6-month interval, 1 patient had a clinical relapse. This patient (indicated by a triangle and an arrow) had the highest WBC, CD4\(^+\), and CD8\(^+\) T-cell counts of the cohort (A–C). 

CSF = cerebrospinal fluid; WBC = white blood cell.

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CONCLUSIONS

Currently approved disease-modifying therapies for MS act primarily by suppressing CNS inflammation. Lymphocyte activation and migration to the CNS are carefully regulated by numerous signaling molecules and their receptors, many of which are modulated by IFNβ and GA. IFNβ and GA may produce secondary neuroprotective effects by preventing the infiltration of autoreactive T cells into the CNS. GA may also modulate the activity of neurotrophic factors that are important in neuronal survival. Natalizumab, which is used for second-line therapy of MS, may produce long-lasting suppression of leukocyte migration to the CNS for several months after the drug has cleared from circulation.

QUESTION AND ANSWER SESSION

Q: You showed that CD4 and CD8 cells were barely detectable in CSF 6 months after natalizumab treatment. But in the rebound paper that you described, relapses were seen after 3 months. There appears to be a disconnect between cellular and clinical events.

Dr. Stüber: We were also surprised when we looked at the 6-month data to see that there was no change. We subsequently looked at other cell types, which I did not show. In the original paper we looked at effector cells—T cells and B cells. But we realized that APCs may also be affected by blocking VLA-4. Probably the most effective APCs in the brain are from the peripheral circulation, such as dendritic cells and B cells, which may be impaired in their ability to enter the CNS. If these are excluded from the brain, reactivation of T cells cannot occur. This effect may mediate the prolonged effect of natalizumab.

We also examined radiographic and clinical outcomes 14 months after the cessation of therapy in our patients, and we did not see any rebound phenomenon. I think there is one difference between our patients and those from the rebound studies is that in those studies, patients were not treated with other immunoregulatory drugs. Most of our patients were either already on IFNβ or were started on IFNβ or GA immediately after cessation of natalizumab therapy.

I think that we also have to consider the biologic half-life. In the case of natalizumab, administration of a single dose may block VLA-4, but it probably does not affect the cellular expression of VLA-4. But chronically blocking VLA-4 over a period of years may cause the cells to reduce their expression of VLA-4, so that when that blockade is removed, it may take awhile for the cell to again establish VLA-4 expression.

Dr. Greenberg: There is also evidence of a similar effect with rituximab. We have had some patients whose B cells have not come back 1 year after dosing with rituximab. The rituximab itself has been gone for months, but the cells have not recovered yet. I think that the monoclonal antibodies may produce long-term effects that we do not really understand yet.

REFERENCES


