THE MECHANISM OF ACTION OF TREATMENTS FOR MULTIPLE SCLEROSIS

Michael K. Racke, MD
The Ohio State University
Columbus, OH

Olaf Stüve, MD, PhD
University of Texas Southwestern Medical Center
Dallas, TX

The therapeutic landscape for multiple sclerosis (MS) is rapidly changing. There are now many FDA approved disease modifying therapies for MS including: IFN-β-1a (Avonex, Rebif), IFN-β-1b (Betaseron, Extavia), pegylated interferon-β -1a (Plegridy), glatiramer acetate (Copaxone, Glatopa), mitoxantrone (Novantrone), natalizumab (Tysabri), fingolimod (Gilenya), teriflunomide (Aubagio), dimethyl fumarate (Tecfidera), daclizumab (Zinbryta) and alemtuzumab (Lemtrada). This lecture will highlight the proposed mechanisms of these therapeutic agents for the treatment of multiple sclerosis. It will also touch on the mechanism of several promising therapies nearing FDA approval including the monoclonal antibody therapies daclizumab and ocrelizumab and those with similar mechanisms in trial (ofatumumab) and autologous hematopoietic stem cell transplantation.

Introduction:

The therapeutic landscape for multiple sclerosis (MS) is rapidly changing. The first disease modifying therapy, interferon-β-1b (IFN-β-1b) was introduced in 1993. There has been significant global experience with the first-line injectable therapies (IFN-β-1a, IFN-β-1b and GA) and their side effect profiles have been well characterized. The relative safety and efficacy of these therapies has provided prescribers with a level of comfort in recommending them to patients with MS. Injectable therapies can be uncomfortable and inconvenient for patients. The new and emerging agents provide alternative modes of administration including oral agents and intravenous infusions along with improved efficacy; however, these therapies are not without risks.

I. Monoclonal Antibodies:

Alemtuzumab:

Alemtuzumab is a humanized monoclonal antibody which targets CD52, an epitope expressed on T and B lymphocytes, natural killer cells and most monocytes, but it is not expressed on hematopoietic precursors. (1,2) Treatment with alemtuzumab results in rapid depletion of CD52 containing cells by antibody dependent cellular toxicity. (3) Following treatment, reconstitution of these cell populations is staggered with return to baseline for monocytes and B cells at 3 months, CD8+ T cells around 30 months and CD4+ T cells at approximately 61 months (4).

Daclizumab:

Daclizumab is a humanized monoclonal antibody against the α subunit, CD25, of the IL-2 receptor on T cells, B cells, macrophages and natural killer cells (5). Interleukin-2 plays a key role in T cell activation and proliferation. Cluster of differentiation-25 (CD-25) blockade selectively inhibits activated T cells which play an important role in the pathogenesis of auto-immune disease and therefore, this drug is of interest in the treatment of MS. In addition, daclizumab has been shown to increase the quantity of $CD56^{bright}$ natural killer (NK) cells (a regulatory subset of NK cells) which down regulate adaptive T cell responses. (6) Administration of 1mg/kg of daclizumab every 4 weeks results in blockade of 95% of CD25 on T cells.

Ocrelizumab:

Ocrelizumab is a humanized, recombinant monoclonal antibody against CD20 on B cells. (7) It has been shown to enhance antibody dependent cell mediated cytotoxicity and leads to a reduction in complement dependent cytotoxicity similar to rituximab (8). Clinical trials were recently completed studying ocrelizumab in both relapsing remitting and primary progressive multiple sclerosis. Positive results of these studies have been presented and were recently published (41, 42).

Ofatumumab:
Ofatumumab is a type I, humanized monoclonal (IgG1κ) antibody against a novel epitope of CD20 on B lymphocytes. It is believed to mediate B cell lysis by complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity (9, 10). It targets a CD20 epitope which is distinct from that targeted by rituximab, by binding both small and large extracellular loops of the CD20 surface antigen (11, 12).

Natalizumab:
Natalizumab is a recombinant humanized monoclonal antibody which antagonizes the $\alpha_4\beta_1$ integrin (also known as very late activation antigen or VLA-4) expressed on the surface of activated lymphocytes and monocytes. It is a selective adhesion molecule inhibitor which binds specifically to the α_4 subunit (13). This prevents activated leukocytes from adhering to and migrating across the endothelial blood brain barrier yielding its therapeutic effect in MS.

II. Oral Agents:
Fingolimod:
Fingolimod was the first oral agent FDA approved for use in RRMS. Its novel mechanism of action, efficacy, and oral route of administration made it an attractive alternative to previously used parenteral therapies. Unlike other drugs used in MS and other autoimmune diseases, fingolimod modulates the immune response, creating a relative leukopenia that is reversible with discontinuation of the drug. This, theoretically, spares the body's ability to fight infection, although some infections have shown an increased incidence. Fingolimod is a sphingosine-1-phosphate (S1P) receptor modulator. S1P is a phospholipid that is primarily produced in the plasma by erythrocytes, but is ubiquitous in the body (14). One of the roles of S1P is chemoattraction and motility, particularly with regard to lymphocytes. The active form of fingolimod, binds non-specifically to 4 of the 5 S1P receptor types. The S1P1 receptor is highly expressed on unactivated lymphocytes. Binding of fingolimod to the S1P1 receptor causes abnormal phosphorylation, resulting in internalization and degradation of the receptor. The decrease in S1P1 receptor expression on plasma lymphocytes results in their sequestration in the lymph nodes, thus preventing lymphocyte activation and subsequent transit to sites of inflammation.

Fingolimod likely has other effects that may be responsible for its efficacy. The S1P1 and S1P3 receptors are expressed on astrocytes. *In vitro* studies of active and chronic MS lesions show an increase in S1P1 and S1P3 expression. In the experimental autoimmune encephalitis (EAE) model, injections of S1P into mice resulted in astrogliosis. Thus, a decrease in the expression of S1P may be protective to astrocytes in the CNS (14). In addition, treatment of cultured human astrocytes with fingolimod resulted in decreased production of pro-inflammatory cytokines.

Teriflunomide:
Teriflunomide is the active metabolite of leflunomide, a drug FDA-approved for the treatment of rheumatoid arthritis (15). Leflunomide is converted to teriflunomide by various cytochrome P450 isoenzymes (16). Though leflunomide is an efficacious treatment for rheumatoid arthritis, it has potentially serious side effects such as interstitial lung disease and hepatotoxicity. Toxicity is thought to be related to ineffective enzymatic conversion of leflunomide to teriflunomide. Due to risks with leflunomide, the active metabolite, teriflunomide was developed as a therapy for multiple sclerosis.

Teriflunomide's primary mechanism of action is inhibition of dihydro-orotate dehydrogenase (DHODH) (15). Dihydro-orotate dehydrogenase is necessary for the de novo synthesis of pyrimidines and, thus, DNA replication in rapidly proliferating cells such as lymphocytes. Because teriflunomide inhibits lymphocyte proliferation by interfering with DNA replication, its effect is cytostatic rather than cytotoxic. Other mechanisms of action for teriflunomide have been proposed based upon studies in the murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). These other mechanisms include decreased production of interferon gamma, decreased T cell chemotaxis, and increased secretion of the anti-inflammatory cytokine, interleukin-10 (17).
Dimethyl fumarate:
Fumaric acid esters have been used off-label for treatment of psoriasis in Europe for some time. A formulation of the FAEs dimethylfumarate and monoethylfumarate with the trade name, Fumaderm, was approved for treatment of psoriasis in 1994 in Germany. One of these FAEs, dimethylfumarate, was approved as a therapy for MS. Dimethylfumarate (DMF) is almost completely hydrolyzed in the small intestine to its active metabolite,

monomethylfumarate (MMF) (18). Absorption of MMF is decreased by concurrent ingestion of food, though it remains highly bioavailable (19). Its metabolism does not require the cytochrome p450 system, thus, few drug interactions would be expected. Monomethyl fumarate has a half-life of approximately 12 hours and the excretion of metabolites is primarily via respiration.

The mechanism of DMF *in vivo* is not fully understood. Its effects *in vitro* appear similar to those of other FAEs. Fumaric acid esters have been shown to shift the cellular cytokine profile from the pro-inflammatory Th1 state to the less inflammatory Th2 state. In humans and mice, this shift is in part due to stimulation of type 2 dendritic cell differentiation. Type 2 dendritic cells subsequently produce the anti-inflammatory cytokines, IL-10 and IL-4 (20). The shift from the Th1 to the Th2 state may also result in the apoptosis of activated T cells and decrease the expression of adhesion molecules, ICAM-1 and VCAM, protecting the CNS from influx of activated lymphocytes. It has also been shown to decrease the antigen-presenting ability of monocytes and macrophages (21).

III. First line agents

Glatiramer acetate

Glatiramer acetate (previously known as Copolymer 1 or Cop-1) is a random polymer of glutamic acid, lysine, alanine, and tyrosine, that is of considerable interest for its ability to inhibit EAE and reduce the frequency of relapses in RRMS. It was originally designed at a time when T cell recognition of protein antigens was poorly understood, so the thought was that random polymers with amino acid composition similar to known encephalitogens may be able to cause disease. Interestingly, while Cop-1 was never able to cause disease, animals that received this agent were subsequently resistant to developing EAE. Several mechanisms have been proposed to explain these findings.

Our own studies suggested that Cop-1 could inhibit the *in vitro* response of several antigen-specific murine T cell hybridomas and impair human MBP-specific T cell lines from lysing targets in the context of three HLA-DR types associated with MS by competition with nominal antigen for MHC binding (22). This was at a time when it had only recently been determined that T cells needed to recognize peptide antigens in the context of MHC molecules.

In order to phenotype the cells responding to GA, we used a flow cytometric approach where the T cells responding to GA by proliferation or by other activation markers would allow one to characterize those cells. Using this novel flow cytometric approach, we were able to demonstrate that both CD4+ and CD8+ T cells responded to GA. Interestingly, while both healthy individuals and MS patients made a robust CD4+ T cell response to GA, only CD8+ T cells from healthy individuals made a significant response to GA (23). In addition, similar to what had been observed by others, over time the CD4+cells response to GA declined, but there was a differential response to GA with a subsequent expansion of CD8+ T cells in MS patients on GA therapy. When examining the ability of CD8+ T cells from MS patients to exhibit this regulatory function directly ex vivo, the regulatory function was impaired, but showed significant improvement in the MS patients after several months of therapy on GA. Thus, GA appears to be able to correct a deficit in a regulatory CD8 T cell response in MS patients that can be observed in healthy individuals (24).

Interferon-beta

It is now recognized that IFNβ has a number of immunoregulatory properties that interfere with cell migration, cell-cell adhesion, cell activation, and antigen presentation (25). The migration of inflammatory cells into the CNS is mediated by adhesion molecules on various cell types (26). Expression of very late activation antigen (VLA)-4, an integrin on the surface of leukocytes that binds to vascular cell adhesion molecule (VCAM)-1 on endothelial cells and other cell types, was shown to be downregulated by IFNβ (27, 28). In this regard, IFNβ increases levels of VCAM-1 in the sera of MS patients (29). Increased levels of soluble VCAM-1 correlated with a decrease in MRI lesions, suggesting that IFNβ decreases VCAM-1 expression on CNS endothelial linings and T-cell trafficking into the CNS (30).

Perhaps the most important mechanism, by which IFNβ benefits MS patients, is through effects on leukocyte trafficking through extracellular matrix (ECM) barriers (31). Following their passage across the endothelial barriers, leukocytes still have to transverse the basement membrane (basal lamina) of brain venules to access the brain parenchyma. Matrix metalloproteinases (MMPs) are proteolytic enzymes that are considered the physiologic mediators of cell migration through ECM. In 1996, two studies showed that the migration of activated T lymphocytes across a barrier of fibronectin, or matrigel (a synthetic basement membrane), was mediated by

MMP-9, and that IFNβ treatment reduced the production of MMP-9 by activated T cells, as well as their migration *in vitro* (32, 33). Subsequently, it was shown that IFNβ reduces the transmigration of T cells, even in the presence of a strong chemokine gradient, by inhibiting the chemokine-induced upregulation of MMP-9 (34). Another study assessed the migratory capacity of T cells derived from IFNβ-1b treated MS patients, untreated MS patients, and healthy controls (35). Lymphocytes derived from patients treated for less than 2 years with IFNβ migrated at a low rate that was indistinguishable from that of cells isolated from healthy donors. However, long-term treatment with IFNβ (>3.5 years) was associated with a reversion of the migration to a high level that did not differ statistically from that of cells isolated from untreated MS patients. The biological significance of these observations was underscored by another investigation that compared monthly serum levels of MMP-9 and its antagonist, tissue inhibitor of MMP-type 1 (TIMP-1), in patients with RR-MS to healthy controls (36). Serum MMP-9 levels were significantly elevated in RR-MS patients compared to healthy controls, and high MMP-9 and low TIMP-1 levels preceded appearance of new Gd-enhancing lesions.

Although controversial, some data suggests that IFNβ promotes Fas (CD95) mediated programmed cell death (apoptosis) of T cells (37-39). It remains to be evaluated further if IFNβ therapy exerts a regulatory effect on peripheral T cells through an apoptosis-promoting mechanism. Finally, it is important that interferons were originally described as proteins produced by immune competent cells to increases the resistance of surrounding tissue against viral infections. Their use in MS was suggested because of indirect evidence implying viral infections in this disease (40). However, there has been no data presented to date supporting the concept that viral inhibition underlies IFNβ effects on MS in any way.

IV. Stem cell transplantation

Stem cell transplantation has been used almost as long as the interferons to treat multiple sclerosis. Initially, patients with secondary progressive MS were treated with various stem cell regimens, but good treatment outcomes were not obtained. Some felt that even for stem cell transplantation to be effective, it had to be initiated earlier in the disease course. The HALT-MS clinical trial was initiated in patients with relapsing-remitting (RR) MS with active CNS inflammation relatively early in the course of their disease. We hypothesized that high dose immunosuppressive therapy (HDIT) and autologous hematopoietic cell transplantation (HCT) would remove disease-causing cells and induce a re-set of the immune system, and thereby control disease. Patients were selected based on significant loss of neurological function (Expanded Disability Status Scale (EDSS) 3.0-5.5) and failure of MS disease modifying therapy (DMT) to control disease activity. At 5 years after HDIT / HCT and, importantly, with no post-transplant immunosuppressive therapy administered, event-free survival (EFS) was 69%, defined as absence of progression, relapse activity, or new MRI lesions (43).

Conclusion:

The therapeutic pipeline for MS is enriched with novel agents that have the potential to provide improved efficacy and ease of administration for patients. Selection of an appropriate disease modifying therapy, however, will become increasingly more complex as more therapies become available. The risk to benefit ratio of a given therapy must be carefully considered in each individual patient, in addition to the order in which potential future therapies are offered.

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