Mechanisms of action of interferons and glatiramer acetate in multiple sclerosis

Suhayl Dhib-Jalbut, MD

Article abstract—MS is an immunologically mediated disease, as determined by observation of the response to immunotherapy and the existence of an animal model, experimental autoimmune encephalitis. Interferon (IFN) β-1b, IFN β-1a, and glatiramer acetate, the therapies used for relapsing or remitting MS, have mechanisms of action that address the immunologic pathophysiology of MS. The IFNs bind to cell surface-specific receptors, initiating a cascade of signaling pathways that end with the secretion of antiviral, antiproliferative, and immunomodulatory gene products. Glatiramer acetate, a synthetic molecule, inhibits the activation of myelin basic protein-reactive T cells and induces a T-cell repertoire characterized by anti-inflammatory effects. Although the two classes of drugs have some overlapping mechanisms of action, the IFNs rapidly block blood–brain barrier leakage and gadolinium (Gd) enhancement within 2 weeks, whereas glatiramer acetate produces less rapid resolution of Gd-enhanced MRI activity. IFN β has no direct effects in the CNS, but glatiramer acetate-specific T cells are believed to have access to the CNS, where they can exert anti-inflammatory and possibly neuroprotective effects.

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Multiple sclerosis is a challenging disease in terms of our understanding of the etiology and underlying pathophysiology and in the design of effective therapies. Several factors, such as exposure to certain viruses, are proposed to be involved in the etiology of MS. In addition, genes that encode HLA or T-cell receptor phenotypes may be important predisposing factors.[1]

Regardless of etiology, MS appears to be immunologically mediated, as demonstrated by the observation that MS responds to immunotherapy and by the existence of an animal model, experimental autoimmune encephalomyelitis (EAE). The prevailing hypothesis is that autoreactive T cells of the $CD4^+$ T helper (Th)1 population orchestrate the pathogenetic process in MS.[2] These cells recognize antigen(s) presented by macrophages or dendritic cells and are consequently activated to secrete proinflammatory cytokines: interleukin (IL)-1, interferon (IFN)γ, and tumor necrosis factor (TNF). These allow the upregulation of adhesion molecules and their ligands on the blood–brain barrier (BBB) endothelial cells and lymphocytes, respectively. Autoreactive T cells can then adhere to the BBB endothelium and secrete metalloproteinases. This leads to the digestion of the BBB matrix membrane, allowing activated T cells to invade the CNS. This phase of the process is believed to correlate with the appearance of gadolinium (Gd)-enhancing lesions on MRI.

Amplification of immunoreactivity takes place in the CNS, where T cells are further activated by antigen(s) presented on microglia, resulting in the secretion of proinflammatory cytokines and chemokines that attract and retain inflammatory cells in the CNS. Effector mechanisms that mediate demyelination include the ability of activated macrophages to strip myelin and to secrete myelinotoxic substances such as TNFα, nitric oxide (NO), and free radicals. Additional mechanisms may include complement-dependent antibody-mediated damage and a direct attack on oligodendrocytes by CD8+ cytotoxic T cells.

The extensive inflammation and the chronicity of the process may result in damage to axons, a marker of irreversible disability. Recovery is believed to be mediated by Th2 helper cells, which secrete anti-inflammatory cytokines, such as IL4, IL10, and transforming growth factor (TGF)β, that can deactivate macrophages.

Recent evidence suggests that MS may be a heterogeneous disease with various pathologic subtypes.[3] Therefore, it follows that increased understanding of the pathophysiologic processes of MS should enhance the design of more effective therapies.

Over the past decade MS patients have benefited enormously from therapeutic research efforts. In 1990 there were no drugs to treat MS, but today there are four FDA-approved treatments: IFN β-1a (Avonex), IFN β-1b (Betaseron), glatiramer acetate (Copaxone), and mitoxantrone (Novantrone). An-

From the Department of Neurology, University of Maryland School of Medicine, and the Baltimore VA Medical Center, Baltimore, MD.

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Address correspondence and reprint requests to: Dr. Suhayl Dhib-Jalbut, Department of Neurology, University of Maryland Hospital, 22 S. Greene St., Rm. N4W46, Baltimore, MD 21201.

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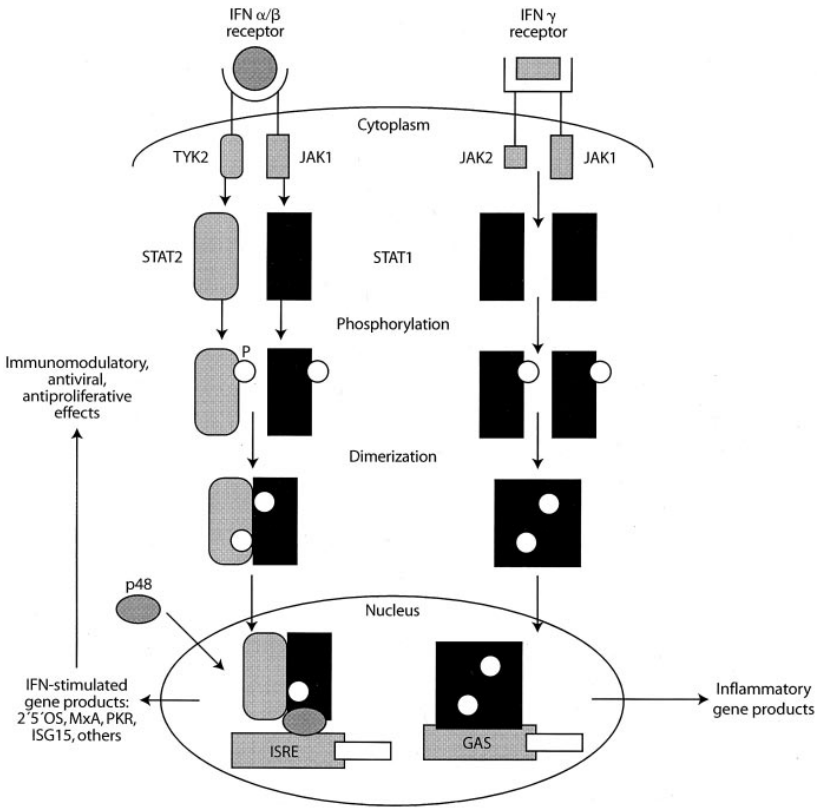

Figure 1. Type I and type II interferons (IFNα, -β, and -γ) bind to species-specific cell surface receptors. Binding induces a cascade of signaling pathways that eventually lead to the secretion of IFN-stimulated gene products such as MxA protein and PKR. These gene products have immunomodulatory, antiviral, and antiproliferative actions that account for the usefulness of IFNs in the treatment of cancer, viral infections, and MS.

other formulation of IFN β-1a, Rebif, is available in many countries and is expected to become available in the United States. IFN β-1a and IFN β-1b are type I IFNs indicated for relapsing–remitting (RR) MS. Glatiramer acetate is also approved for RRMS, and mitoxantrone has been approved for worsening RRMS and for progressive relapsing and secondary progressive MS. This article reviews the current state of knowledge about the mechanisms of action of the drugs approved for RRMS, the IFNs and glatiramer acetate.

Interferons. IFNs are proteins secreted by cells in response to invading organisms. In general, they have antiviral and anti-inflammatory effects, and they modulate the immune system. The type I IFNs include IFN α, and IFN β. These are primarily produced by fibroblasts and have strong anti-inflammatory properties. Type II IFN includes IFNγ, which is produced primarily by cells of the immune system. This review focuses on the type I IFNs, which are used clinically in the treatment of MS.

The commercially available IFNs include IFN β-1b and IFN β-1a. The difference between the two IFNs is that IFN β-1a is glycosylated, whereas IFN β-1b is not. In addition, IFN β-1b contains one amino acid substitution from the natural molecule. Their biologic effects are probably quite similar, although there are purported differences in terms of antigenicity and other properties.[4,5] Moreover, differences in dosage regimens and route of administration may produce different responses.[6-11]

All IFNs bind to cell surface species-specific receptors. Binding induces a cascade of signaling pathways, the end result of which is secretion or production of a number of proteins called IFN-stimulated gene products. These gene products are antiviral, antiproliferative, and immunomodulatory (figure 1). Experimental evidence in animal models and in humans indicates that IFN β has several potential mechanisms of action in MS (table 1).[12,13]

T-cell activation. T-cell activation occurs as a result of T-cell receptor recognition of processed antigen in the context of HLA class II molecules expressed on antigen-presenting cells. The formation of this trimolecular complex alone is insufficient for T-cell activation. A second signal delivered by co-stimulatory molecular interaction, such as B7/CD28 or CD40/CD40L, is required for T-cell activation. In

Table 1 *IFNβ mechanisms of action in MS*

Reduction in T-cell activation
Inhibition of IFNγ effects
Induction of immune deviation
Inhibition of blood–brain barrier leakage
CNS effects (?)
Antiviral effect (?)

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the absence of a second signal, T cells become anergic. Activated T cells can proliferate and differentiate into effector T cells, including Th and cytotoxic T cells. Th cells can be divided into two phenotypes. Th1 cells secrete inflammatory cytokines, which lead to macrophage activation and, in the case of MS, mediate destruction of myelin. Th2 cells secrete anti-inflammatory cytokines, inhibit the inflammatory effects of Th1 cells, and activate B cells to produce antibodies.[14]

Evidence from our work and that of others indicates that IFN β can interfere with T-cell activation in several ways.[12] First, IFN β counteracts many of the proinflammatory effects of IFN γ. This occurs primarily because of competition for shared signaling and transcription factors induced by these cytokines (figure 1). For example, IFN γ enhances HLA class II molecules, and this may be the mechanism by which IFN γ worsens MS.[15] IFN β inhibits the upregulation of HLA class II, which is believed to interfere with antigen processing and presentation, and consequently with T-cell activation.[16] Second, IFN β may have an effect on co-stimulatory molecule interaction, including B7/CD28[17] and CD40:CD40L.[18] By interfering with these two groups of molecules, IFN β could inhibit T-cell activation, including the activation of myelin-reactive T cells.[16]

Immune deviation. Several studies[12,13] have shown that IFNβ can tilt the balance in favor of an anti-inflammatory response either by inhibiting Th1 or by promoting Th2 cytokine production. For example, IFN β enhances peripheral blood mononuclear cell secretion of IL10[19] and inhibits IL12,[20] a key proinflammatory cytokine. It is unclear whether changes in these cytokines correlate with response to therapy. Immune deviation as a therapeutic mechanism for IFN β in MS is controversial, in view of recent findings indicating that IFN β can upregulate a number of proinflammatory gene products in human peripheral blood mononuclear cells.[21] This appears to indicate that a proinflammatory response to IFN β may in some way be beneficial, or that the net balance is in favor of an anti-inflammatory response, or that its mechanism of action may be entirely unrelated to cytokine changes.

Blood–brain barrier effects. MRI indicates that IFN β has a prominent effect on the BBB. In NIH studies,[22] almost 90% of MS patients treated with IFN showed a rapid and robust decrease in the number of Gd-enhancing lesions on MRI. Although this may be the dominant mechanism of action of IFNβ in MS, the decrease in enhancing lesions does not necessarily correlate with the clinical response to IFN β in the long run.

IFN β probably affects the BBB by two mechanisms: by interfering with T-cell adhesion to the endothelium, although the evidence for this is not strong,[23] and by inhibiting the ability of T cells to get into the brain. Some MS patients treated with IFN β demonstrated a rise in serum soluble vascular cell adhesion molecule (sVCAM), which correlated with a reduction in the number of MRI Gd-enhancing lesions.[24] sVCAM may act as a decoy by binding VLA-4 on T cells, thus inhibiting their attachment to the endothelium of the BBB.

IFN β may also interfere with T-cell/endothelial-cell adhesion by inhibiting HLA class II expression on endothelial cells, which can also function as ligands for T cells.[25] T cells secrete proteases and gelatinases, one of which is matrix metalloproteinase 9 (MMP9). MMP9 digests the matrix membrane and allows T cells to enter the brain. That MMP9 may be involved in MS pathogenesis is suggested by evidence of elevated levels of MMP9 in the spinal fluid of MS patients and its correlation with the number of enhancing lesions on MRI.[26,27]

IFN β inhibits MMP9 production by activated T cells,[28,29] which may explain the dramatic effect of IFN β in inhibiting the opening of the BBB. However, the possibility that MMP9 may be a marker of injury rather than a therapeutic target for IFN β should be kept in mind.[30]

Despite speculation that IFN β may have an effect in the CNS because of in vitro evidence of an effect on glial cells in humans[16,31] and the demonstration of accessibility to the CNS in healthy mice,[32] there is no clinical evidence that IFN β enters the brain in humans.

Antiviral effects. MS may be caused by a virus in a subset of patients, on the basis of circumstantial evidence.[33,34] The clinical courses of human herpesvirus (HHV) infections and MS have some similarities, such as chronicity, dormancy, reactivation in the case of herpes and relapses in the case of MS, and vulnerability to stress and hormonal imbalance. HHV6 in particular may be involved in the pathogenesis of MS in a subset of patients.[35] It was recently reported that treatment with valaciclovir can decrease the number of MRI-enhancing lesions in a subset of MS patients with active disease.[36] Therefore, if a viral infection is a cause of MS in some patients, IFN β may have an additional therapeutic effect in this group through its antiviral properties.

Glatiramer acetate. Glatiramer acetate (copolymer-I) is a synthetic molecule composed of four amino acids: glutamine, lysine, alanine, and tyrosine. These four amino acids are represented in myelin basic protein (MBP), which is a suspect antigen involved in the induction of autoimmunity in MS.[37] The polypeptide was originally produced in an attempt to mimic MBP and to induce EAE.[38] Instead of inducing disease, the copolymer prevented the induction of EAE in animals.[39] This finding triggered clinical studies of the use of glatiramer acetate in MS.[40,41] Because of the randomness of the amino acid composition and the relatively short length of the peptide, glatiramer acetate has the ability to bind to HLA class II (DR) molecules, including HLA DR2. This binding property suggests several mechanisms of action, based on experimental evidence in EAE

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Table 2 *Potential mechanisms of action of glatiramer acetate in MS*

Inhibition of myelin-reactive T cells
Induction of anergy in myelin-reactive T cells
Induction of anti-inflammatory Th2 cells
Bystander suppression in the CNS
Neuroprotection

and more recently in MS patients treated with the drug (table 2; figure 2).

Inhibition of myelin-reactive T cells. Studies have demonstrated the ability of glatiramer acetate to inhibit the activation of MBP-reactive T cells.[42] Myelin-reactive Th1 clones exposed to increasing doses of glatiramer acetate manifest dose-dependent inhibition of proliferation and IFN γ production with relative antigen specificity.[43] In addition, glatiramer acetate induces anergy in MBP-reactive T cells in vitro[43] and modulates T-cell receptor recognition of the MBP-immunodominant peptide$_{82–100}$.[44] Collectively, these findings suggest that glatiramer acetate can interfere with T-cell activation.

Induction of anti-inflammatory Th2 cells. Studies both in EAE and in humans indicate that a likely in vivo mechanism of action of glatiramer acetate involves the induction of immunomodulatory Th2 cells.[42] Such glatiramer acetate-specific T cells may exert their protective action by entering the CNS compartment[45] and by the production of anti-inflammatory cytokines in response to cross-recognition of myelin antigens (bystander suppression).

Induction of glatiramer acetate-reactive Th2 cells presumably occurs because glatiramer acetate can act as an altered peptide ligand that delivers a weak signal to T cells, resulting in preferential Th2 cell activation.[46] Our recent studies (unpublished data) indicate that glatiramer acetate-reactive Th2 cells are generated as early as 2 months after treatment is initiated[47] and are sustained for up to 9 years (figure 3), despite a drop in the precursor frequency of these cells. A possible explanation for the sustained Th2 phenotype despite reduced proliferation is that glatiramer acetate results in a progressive deletion of high-affinity T cells or, alternatively, may induce a subset of nonproliferating immunoregulatory T cells with anti-inflammatory properties.

Bystander suppression in the CNS. Bystander suppression implies that glatiramer acetate-reactive T cells are capable of entering the CNS and recognizing cross-reactive antigen(s), probably myelin antigen(s). These T cells can then secrete anti-inflammatory cytokines and suppress inflammation. Although it is technically difficult to demonstrate glatiramer acetate-reactive T cells in the human CNS, recent EAE evidence supports this mechanism.[45] Furthermore, glatiramer acetate-reactive T cells may have a neuroprotective effect on neurons and axons.[48] In MS patients, glatiramer acetate treatment reduces the proportion of new MS lesions that evolve into "black holes,"[49] suggesting a potential neuroprotective effect. Therefore, glatiramer acetate may have a beneficial clinical effect in the long term because axonal degeneration is believed to cause irreversible damage in MS. Whether glatiramer acetate acts systemically, centrally, or both in humans is unclear. The fact that the drug inhibits the appearance of new MRI Gd-enhancing lesions[50] could suggest a significant systemic effect.

IFNβ and glatiramer acetate compared. IFNβ and glatiramer acetate have different but overlapping mechanisms of action, and both ultimately result in a decreased proinflammatory response in the periphery and the CNS (table 3). However, IFNβ rapidly blocks BBB leakage and Gd enhancement within 2 weeks, whereas glatiramer acetate activity on the BBB produces less rapid and dramatic resolution of Gd-enhanced MRI activity.

The clinically evident effects of glatiramer acetate may appear to be delayed compared with the onset of IFN effects. The time-dependent effect of treatment with glatiramer acetate was observed in the United

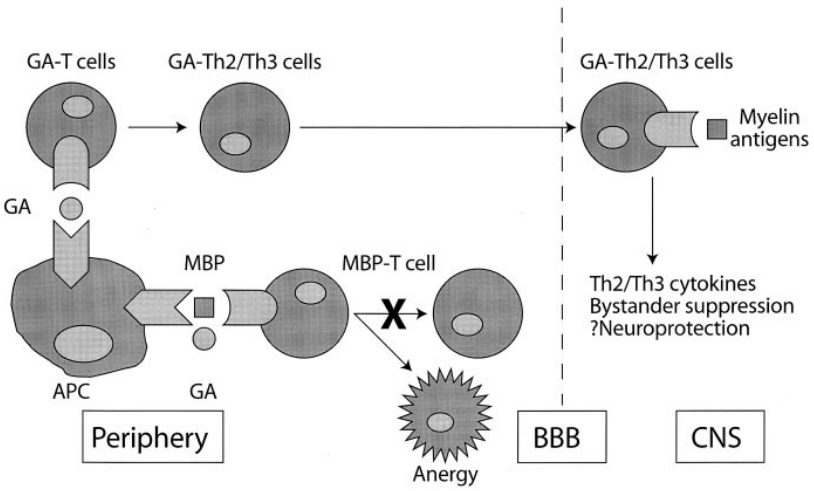

Figure 2. Mechanism of action of glatiramer acetate (GA) summarized. After SC injection, GA binds HLA class II (DR) on antigen-presenting cells in lymph nodes. As a result, GA can block the activation of myelin-reactive T cells or render these cells anergic. In addition, GA induces GA-specific Th2 cells that cross the blood–brain barrier (BBB) and produce bystander suppression as a result of cross-recognition of myelin antigens. These cells may also have a neuroprotective function.

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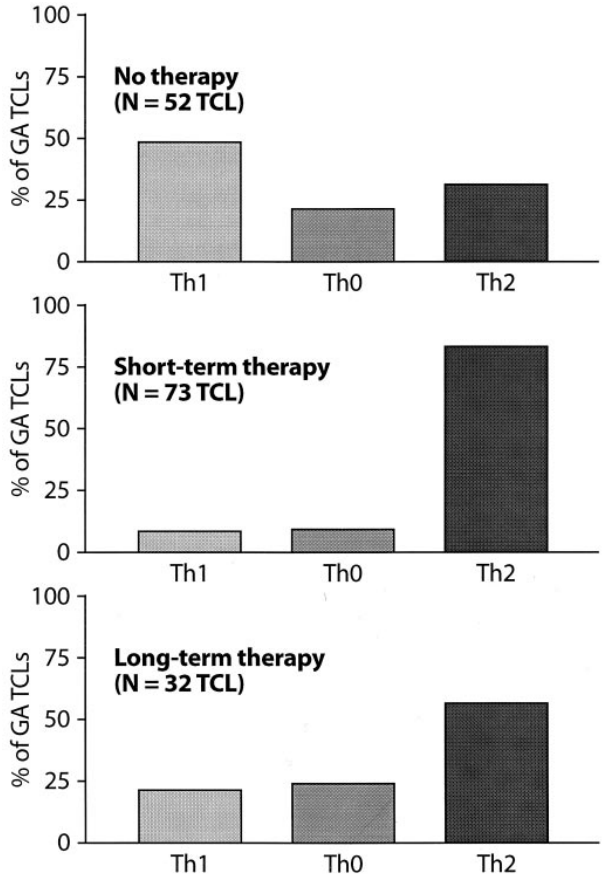

Figure 3. Percentages of glatiramer acetate-reactive T-cell lines (TCL) classified as Th1, Th0, or Th2 were compared in patients who had had short-term (1–10 months; 73 TCL) or long-term (6–9 years; 32 TCL) glatiramer acetate therapy, or who had not taken glatiramer acetate (52 TCL). Classification of Th phenotype was based on the ratio of IFNγ (a Th1 marker) to IL5 (a Th2 marker). A ratio >2 was classified as Th1, 0.5 to 2 as Th0, and <0.5 as Th2.

States pivotal trial[51] and in the European/Canadian trial,[50] and is consistent with the immunologic activity of glatiramer acetate. That is, in vivo studies have suggested that, over time, glatiramer acetate treatment induces glatiramer acetate-specific T cells to proliferate and secrete anti-inflammatory cytokines that are typical of Th2 regulatory or suppressor T cells. A proportion of the glatiramer acetate-specific T cells can be cross-stimulated by MBP and its immunodominant fragments to secrete the same regulatory cytokines. In the EAE model, these cells confer protection from clinical disease.[50] Patients treated with glatiramer acetate demonstrated a reduction in proinflammatory cytokines and an increase in anti-inflammatory cytokines.[52] Anti-inflammatory cytokines peaked during the first 6 months of treatment and then gradually decreased, whereas proinflammatory cytokine levels continued to decrease.

These immunologic observations are consistent with the delayed MRI effects. Therefore, although IFN β has the desired effect of rapidly blocking inflammation, it is less likely to have an effect in the CNS compartment once inflammation sets in because there is no evidence that IFN β can access the CNS in pharmacologically relevant concentrations. Therefore, glatiramer acetate-specific T cells may have a distinct ability to enter the CNS, downregulate inflammation at the lesion site, and perhaps contribute to neuroprotection.

Table 3 *Comparison of activities of glatiramer acetate and INFβ*

	Glatiramer acetate	IFNβ
Interference with T-cell activation	Yes	Yes
Decrease in Th1 and enhancement of Th2 cytokines	Yes	Yes
Induces Th2 cells*	Yes	No
Inhibits T-cell/BBB transmigration*	No	Yes
CNS effects	Yes	No
Neuroprotection	Yes?	No?
Antibodies	Inert	Neutralizing

* Critical differences.

Combination therapy. Few studies have addressed the possibility of combining IFN β and glatiramer acetate.[53-55] In vitro evidence[53] suggests a possible additive effect on the inhibition of myelin-reactive T cells. There is also evidence that the combination of the two drugs is safe, as can be measured clinically and by MRI.[55] On the other hand, IFN β has significant antiproliferative effects and therefore has the potential to inhibit the generation of glatiramer acetate-reactive T cells (unpublished data). In addition, work in EAE mice has suggested that the combination of the two drugs is counterproductive.[54] Furthermore, because IFN β blocks BBB leakage,[22] this could interfere with the migration of glatiramer acetate-specific T cells into the brain. If this is the case, the combination would be counterproductive.

We recently addressed these concerns in a group of five MS patients receiving a combination of IFN β-1a and glatiramer acetate treatment as part of a multicenter safety study.[55] Specifically, we addressed the question of whether IFN β interferes with the generation of glatiramer acetate-reactive Th2 cells, which are believed to underlie the mechanism of action of this drug. The data were compared with data obtained from a group of 12 MS patients receiving glatiramer acetate monotherapy. Glatiramer acetate-reactive T-cell lines from patients receiving monotherapy or combination therapy both showed Th2 bias, as reflected by increased levels of IL-5 and decreased levels of IFN γ (figure 4).[56]

These findings suggest that the combination of the two drugs is unlikely to compromise the ability of glatiramer acetate to induce a Th2 response. What is unclear is whether IFN β blocks the entry of the

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