Mechanism of Action of Glatiramer Acetate in Treatment of Multiple Sclerosis

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Summary: Glatiramer acetate (GA) (Copolymer-1, Copaxone, Teva, Israel, YEAK) is a polypeptide-based therapy approved for the treatment of relapsing-remitting multiple sclerosis. Most investigations have attributed the immunomodulatory effect of GAs to its capability to alter T-cell differentiation. Specifically, GA treatment is believed to promote development of Th2-polarized GA-reactive $CD4^+$ T-cells, which may dampen neighboring inflammation within the central nervous system. Recent reports indicate that the deficiency in $CD4^+CD25^+FoxP3^+$ regulatory T-cells in multiple sclerosis is restored by GA treatment. GA also exerts immunomodulatory activity on antigen presenting cells, which participate in innate immune responses. These new findings represent a plausible explanation for GA-mediated T-cell immune modulation and may provide useful insight for the development of new and more effective treatment options for multiple sclerosis.
Key Words: Multiple sclerosis, glatiramer acetate, immunomodulatory agents, mechanism of action, antigen presenting cells.

INTRODUCTION

Glatiramer acetate (GA) (Copolymer-1, Copaxone, Teva, Israel, YEAK) is a pool of synthetic peptides randomly composed of L-tyrosine (Y), L-glutamic acid (E), L-alanine (A), and L-lysine (K) with an average length of 40 to 100 residues. GA was synthesized in this manner more than 30 years ago to most closely resemble the encephalitogenic properties of myelin basic protein (MBP), one suspected auto-antigen in multiple sclerosis (MS). Surprisingly, instead of inducing experimental autoimmune encephalomyelitis (EAE), the murine model of MS, immunizations with GA protected mice from subsequent attempts to induce EAE.[1] This seminal observation was followed by various clinical trials. Whereas early open-label studies already suggested clinical benefit in the 1980s,[2,3] these findings had to be interpreted with caution as drug production was not yet standardized. In 1991, a phase III multicenter, double-blind, placebo-controlled trial with standardized GA preparation was initiated in 11 medical centers in the United States, with 251 relapsing–remitting MS patients.[4] Within two years of treatment, the relapse rate decreased approximately 30% in GA-treated patients leading to approval of GA treatment of MS in many countries worldwide in 1995. A later double-blind, placebo-controlled study demonstrated a reduction in the number of gadolinium-enhancing lesions in patients receiving GA compared to a placebo during a nine-month study period.[5] Additional data showed that GA may also have a favorable effect in preventing tissue loss at a later diseased stage.[6,7] Based on these favorable clinical and imaging data, subcutaneously administered GA is one of the most widely prescribed drugs used today for the treatment of relapsing–remitting MS.

Many investigators have attempted to address the immunologic basis for the clinical effects of GA in MS and MS models.[8,9] Although different potential mechanisms have been considered, most investigations have attributed the immunomodulatory activity of GA to alterations in T-cell antigen reactivity, focusing on its influence on the adaptive immune response. Early *in vitro* studies established that GA can bind to major histocompatibility complex (MHC) class II molecules and suggested that GA might preferentially alter presentation of myelin antigens to auto-reactive T-cells.[10,11] Studies in EAE and MS have extensively demonstrated that GA treatment

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promotes development of Th2-polarized GA-reactive $CD4^+$ T cells.[1,12–14] These Th2 cells can potentially accumulate in the CNS[15] where they might release anti-inflammatory cytokines[12,15–17] and neurotrophic factors,[18] dampening the activity of nearby auto-aggressive T cells, a process known as "bystander suppression."[15] Recent reports indicate that GA treatment also exerts immunomodulatory activity on antigen presenting cells (APCs),[19–23] a part of the innate immune system. These newer findings may provide a plausible explanation for the observed Th2 deviation under GA treatment and raise the question as to whether GA is solely an antigen-specific T cell-directed immunotherapeutic agent as currently assumed. In this article, we review the various effects of GA on the adaptive and innate immune system and describe how these two arms of the immune system interact with one another during GA therapy.

Effects on the adaptive immune system

As with conventional peptide antigens, GA can bind to MHC class II molecules on the surface of APCs.[10,24] In association with MHC class II molecules, GA is recognized by T cells via their antigen-specific T-cell receptor. Early *in vitro* studies indicated that GA may compete with myelin antigens for the binding to MHC class II. Specifically, it was observed that GA binding to MHC class II could inhibit the activation of T cell lines specific for MBP.[25] However, a later study demonstrated that the stereoisomer of GA, D-GA, which contains solely D-amino acids, could effectively bind to MHC class II,[26] but failed to suppress EAE.[27] These findings indicate that GA may not primarily act as an MHC class II antagonist.

It is well established that in most MS patients, GA treatment induces a population of $CD4^+$ GA-reactive Th2 cells,[12–14,28] which is associated with clinical benefit.[29] It seems very unlikely that sufficient amounts of GA can reach the CNS to locally activate GA-reactive T cells. It is believed that GA-reactive Th2 cells are generated in the periphery, accumulate (along with pathogenic non-GA-specific elements) in the CNS of patients with MS and release anti-inflammatory cytokines in a process termed "bystander suppression" (see FIG. 1). Many studies in EAE and MS have generated the concept that GA-reactive Th2 cells may be reactivated within the CNS through cross-recognition of myelin antigen.[12,16] This assumption was supported by two observations. First, GA-reactive Th2 cells could be identified in the CNS of GA-treated mice protected from EAE.[15] Second, in some but not all studies,[30] several GA-specific Th2 cell lines generated from MS patients or mice could cross react with MBP at the level of cytokine secretion.[12,15–17,31]

$CD4^+CD25^+$ regulatory T cells (Treg) are an important subclass of regulatory cells that engage in the maintenance of immunologic tolerance by actively suppressing self-reactive lymphocytes.[32,33] Forkhead transcription factor Foxp3 is the key transcription factor in the physiological development of Treg.[34] Its genetic defect results in impaired function of Treg, which is associated with in an autoimmune and inflammatory syndrome in humans as well as in mice.[35] Similarly, the experimental deletion of Treg in mice causes various spontaneous organ-specific autoimmune diseases.[36] Viglietta et al.[37] reported that in patients with MS, similar to other autoimmune conditions,[38] effector function and frequency of Treg is significantly decreased in the peripheral blood. Several studies provided evidence for a role and mechanism of action of GA in the induction of $CD4^+CD25^+$ Treg. *In vitro* exposure to GA resulted in an elevated production of interleukin-10 (IL)-10 by Treg.[39] In another study, GA promoted the conversion of $CD4^+CD25^-$ to $CD4^+CD25^+$ Treg through the activation of Foxp3.[40] GA treatment led to a significant increase in Foxp3 expression in $CD4^+$ T cells in MS patients whose Foxp3 expression was reduced at baseline. GA-reactive $CD4^+CD25^+$ T-cell lines generated from GA-treated MS patients expressed high levels of Foxp3 that correlated with increased T-cell regulation.[40] Thus, besides the well-known preferential Th2 differentiation of T cells, GA appears to normalize frequency and function of Treg in MS, which represents an additional immunomodulatory effect of GA.

More recently, it was reported that GA treatment also induces a population of $CD8^+$ GA-reactive T cells. In untreated MS patients, GA-reactive $CD8^+$ T-cell responses were found to be significantly lower compared with healthy individuals. Treatment with GA restored these $CD8^+$ responses[41] and enhanced release of IFN-γ by these cells,[42] which appears to be associated with a positive clinical response.[42] Although the *in vivo* function of these cells is still not entirely understood, a recent report indicated that GA-reactive CD8+ T cells may suppress pro-inflammatory effector T-cell function in a manner similar to $CD4^+CD25^+$ Treg.[43,44]

Besides activation and alteration of T cells, GA treatment also induces a humoral response to itself in most patients, which peaks approximately 3 months after treatment initiation.[45] Just as individuals who are naive to GA treatment sometimes have pre-existing (naive) GA reactive T cells,[28] some untreated MS patients reveal an unprimed humoral response against GA, mainly of an IgM, IgG1, and IgG2 isotype.[46] GA-treated MS patients also produce IgG1 and IgG2 anti-GA antibodies, but in contrast to unexposed individuals, GA-treated MS patients frequently develop high titers of IgG4 antibodies against GA.[46] Preferential secretion of IgG4 antibodies might occur secondary to the induction of GA-reactive Th2 cells, as isotype switching to IgG4 is regulated by the Th2 cytokine IL-4. To date, it is considered controversial whether antibodies against GA are of clinical relevance. In general, IgG4 antibodies have strong neu-

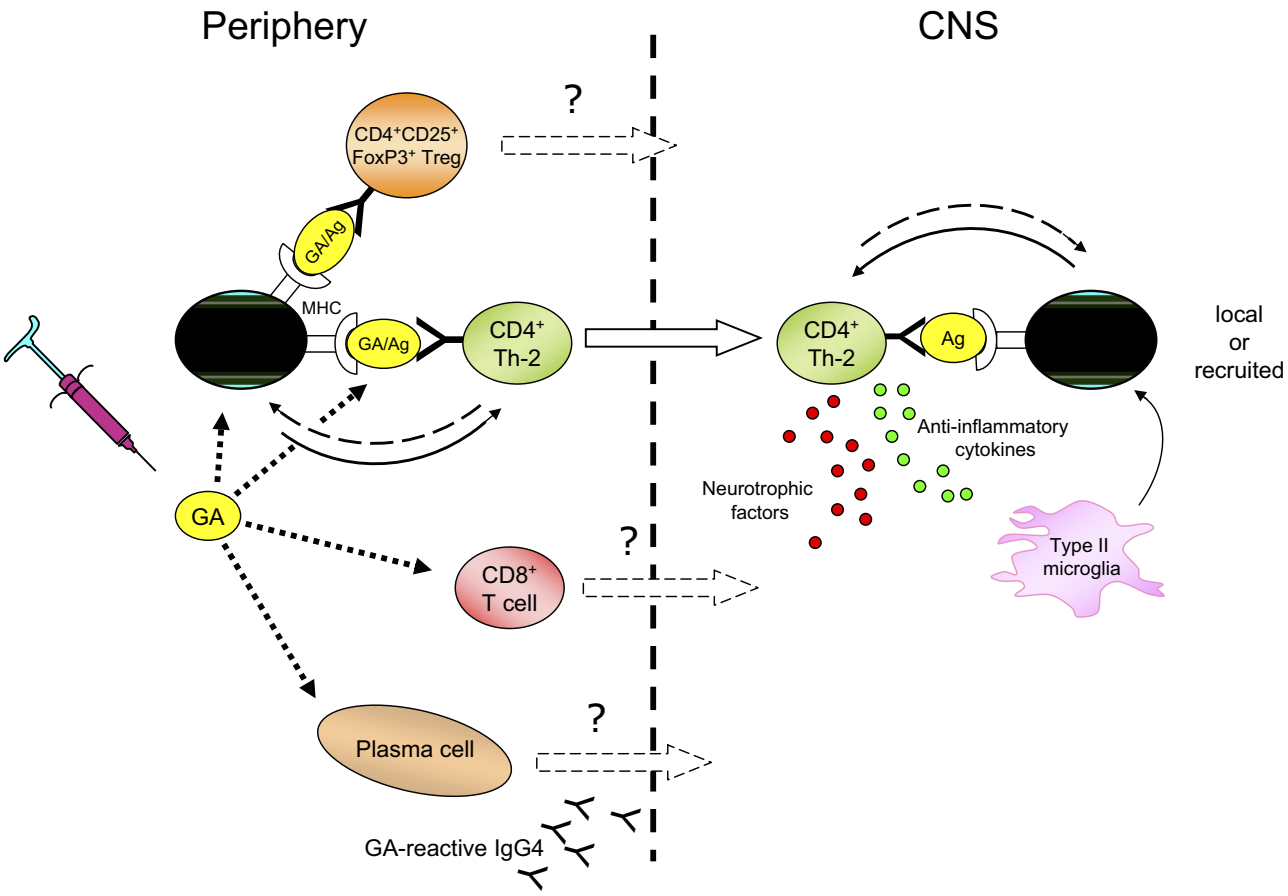

FIG. 1. Cross-talk between type II antigen presenting cells (APCs) and regulatory T-cell populations in glatiramer acetate (GA)-mediated immune modulation. GA treatment exerts effects on APC and T cells that result in the induction of a specific population of Th2 cells and $CD4^+CD25^+FoxP3^+$ regulatory T cells (Treg) in the periphery. Type II APC and Th2 cells may facilitate the development of each in a positive feedback mechanism, as type-2 monocytes tend to induce Th2 cells, and Th2 cell-derived anti-inflammatory cytokines may promote development of type II APC. GA-reactive Th2 cells are believed to cross the blood-brain barrier and to be locally reactivated within the CNS through cross recognition of myelin antigen. In response, these cells may secrete anti-inflammatory cytokines and neurothrophic factors dampening neighboring inflammation ("bystander suppression"). Another feedback loop between APC and T cells may develop within the CNS, as Th2-cytokines might promote type II differentiation of resident APC, such as microglia. GA treatment is also associated with induction of GA-reactive $CD8^+$ T cells, although their *in vivo* function remains to be determined. Finally, consistent with the Th2 shift, GA-reactive plasma cells secrete anti-GA antibodies (≺), preferentially of an IgG4 isotype. Whether these antibodies enter the CNS or may neutralize some of the immunomodulatory effects of GA is not yet known. (GA-Ag = glatiramer acetate antigen; ? = Presumed transmigration of immune cells across the blood-brain barrier.)

tralizing activity, although they do not bind to fragment crystallizable (Fc) receptors or activate the complement system. However, serum from GA-treated patients containing antibodies against GA did not inhibit the ability of GA to stimulate GA-reactive T cells, indicating that *in vitro* anti-GA Ig had no neutralizing effect.[45] Interestingly, Brenner et al.[47] reported that relapse-free patients displayed higher titers against GA than patients with an active disease course under GA treatment, indicating a beneficial rather than effect-neutralizing role of antibodies against GA. In fact, in an animal model of CNS demyelinating disease, GA-specific antibodies were shown to promote myelin repair,[48] an effect which might contribute to the proposed neuroprotective properties of GA in MS.

Effects on the innate immune system

Although past investigations of GA primarily focused on its effects on the adaptive immune system, especially on T cells, emerging evidence supports the concept that GA may also act on APCs. The interaction between APCs and T cells is fundamental for any adaptive T-cell immune response. Several groups have reported that in vitro GA treatment leads to a broad antigen-nonspecific alteration of APC function.[19-21,49–52] Possibly the first report regarding the effect of GA on the innate immune system was derived from an *in vitro* study in which GA altered the activation of a human monocytic cell line.[49] Specifically, GA inhibited the induction of HLA proteins as well as the release of tumor necrosis factor (TNF) and cathepsin B by THP-1 cells. *In vitro* GA treatment was

also shown to alter the activation status of freshly isolated human monocytes.[19,20] Weber et al.[19] reported that GA inhibited lipopolysaccharide (LPS)-mediated expression of APC activation markers, including CD150/SLAM, CD25, and CD69. Furthermore, GA-treated monocytes released significantly lower levels of TNF- and IL-12, two inflammatory Th1-polarizing cytokines. Another study demonstrated that GA treatment not only reduced the release of inflammatory cytokines, but also enhanced production of Th2 polarizing IL-10 by monocytes.[20] A similar cytokine shift was observed in microglial cells, an APC population that is believed to have a key role in the reactivation of T cells within the CNS. *In vitro*-generated human dendritic cells also released less TNF[21] and IL-12[50] on *in vitro* GA exposure. Most notably, GA-treatment of dendritic cells promoted Th2 differentiation of naive T cells without affecting APC capability for inducing T-cell proliferation.[21]

Two independent studies investigated how GA affects monocytes in MS patients. In both studies, monocytes were freshly isolated from GA-treated patients without any additional *in vitro* exposure to GA. Compared to untreated MS patients and healthy subjects, monocytes from GA-treated MS patients expressed significantly lower levels of the activation marker CD150/SLAM and released less TNF on stimulation with low concentration of LPS.[19] In the second study, Kim and colleagues[20] reported that the basal and induced release of IL-10 was significantly enhanced in monocytes from GA-treated patients, whereas the production of IL-12 was reduced, defining an anti-inflammatory "type II" monocyte phenotype. These studies clearly indicate a systemic effect of GA treatment on monocytes that may promote Th2 differentiation of T cells *in vivo*. Theoretically, these findings raise the possibility that GA treatment may compromise innate immune responses in GA-treated MS patients. However, GA-treatment does not appear to be predisposed to infections. In this regard, one *in vitro* finding might be of relevance (i.e., GA only inhibited activation of monocytes that were challenged with suboptimal concentrations of toll-like receptor ligands, such as LPS).[19] Higher concentrations of LPS could override the inhibitory effect of GA, which could explain why the capability of monocytes to efficiently clear infections is not diminished in GA-treated MS patients. Future longitudinal studies are necessary to define whether initiation of GA treatment truly leads to a reduction of APC reactivity in the individual MS patient. This type of study will also allow correlation between altered APC reactivity and a drug-related benefit to determine the extent of the clinical relevance of these GA-mediated effects on the APCs.

Cross-talk between type II APC and regulatory T-cell populations

It has become established that the phenotype of APC influences differentiation of T cells and that reciprocally differentiated T cells modify APC function. In this regard, monocytes cultured with Th2 supernatants developed a phenotype similar to GA-treated monocytes. This finding indicates that GA-reactive Th2 cells can exert a positive feedback on the development of type II monocytes. In fact, type II monocyte development may even occur secondary to the induction of GA-reactive Th2 cells. However, other evidence suggests the opposite scenario (i.e., that APCs may be the primary target of GA and that GA-induced type II APCs mediate T-cell deviation). First, in vitro, GA exerted a direct effect on various APC populations resembling its effect in vivo, in the absence of T cells.[19,20] These GA-treated APCs were capable of promoting development of Th2 cells when co-cultured with naive (untreated) Th0 cells in the absence of GA.[21] Second, *in vivo* GA treatment exerted a systemic effect on monocytes and possibly on monocyte-derived APCs. However, the frequency of GA reactive Th2 cells in the peripheral blood of GA-treated MS patients is only approximately 1 in 20,000, raising the question as to whether Th2 cytokines derived from these cells could be sufficient to mediate type II APC development. Most strikingly, studies in genetically altered mice indicate that *in vivo* GA treatment can induce type II monocytes in the absence of T cells.[53] Further studies are necessary to determine the pathway by which GA treatment may alter APC and T-cell function in MS patients.

Assuming that APCs are the primary target through which GA mediates T-cell immune deviation, one would anticipate that Th2 deviation and/or induction of Treg should not be restricted to GA-reactive T cells. A recent study by Allie et al.[54] investigated the phenotype of T-cell lines specific for GA, MBP, or tetanus toxoid generated from MS patients before and after GA treatment. T-cell differentiation was assessed by the ratio between IFN-γ and IL-5 release. In this longitudinal study, *in vivo* GA treatment biased differentiation of all T cell lines toward a Th2 phenotype, indicating that Th2 differentiation occurred independent of T-cell antigen specificity.[54] However, another study did not describe an antigen-independent Th2 deviation of established T-cell responses on GA treatment, and supported the concept that Th2 deviation may primarily occur in GA-reactive T cells.[42] Although apparently conflicting, both findings might be valid. First, a cross-sectional study comparing untreated patients to GA-treated patients may be less sensitive to detect minor changes in T-cell differentiation compared to a longitudinal study investigating the same patients before and after treatment. Second, it is plausible that an APC-driven Th2 deviation may be pronounced in

GA-reactive T cells, as every APC that presents GA should have been in contact with GA and undergone type II differentiation prior to T-cell activation. The concept of an antigen-nonspecific effect of GA is further supported by the fact that GA treatment has been shown to be clinically beneficial in other models of autoimmune or inflammatory conditions, such as arthritis, uveoretinitis,[55] inflammatory bowel disease,[56] and graft rejection.[57]

Although T cells might not be the primary target of GA, they are most likely the effector cells of GA-mediated immune modulation. Deficiencies in regulatory T cells have been associated with MS pathogenesis[38] and GA-mediated restoration of T-cell regulation correlates with clinical benefit.[40] In EAE, adoptive transfer of GA-reactive T cells alone can inhibit EAE induction by various encephalitogens,[16,31,58] similar to GA treatment itself, and GA-reactive T cells accumulate in the CNS of protected animals. Thus, whereas GA may mediate a primary effect on APC independent of T cells, the type II APC-induced regulatory T cells may be the effector cells of GA-mediated immune modulation.

Possible neurotrophic effects

Some experimental data indicate that GA may have direct neuroprotective properties. *In vitro*, GA-reactive T cells can produce neurotrophic factors such as brain-derived neurotrophic factor (BDNF).[17,18] BDNF is an important factor for differentiation and survival of neurons and is required for maintenance of various glial cell functions.[59] Although the ability to produce BDNF is unlikely to be restricted to GA-reactive T cells, it may relate to the activation status of immune cells (e.g., T cells).[60] In this regard, the continuous activation by daily GA application may promote BDNF production of GA-reactive T cells *in vivo*. As activation of immune cells also facilitates their transmigration across the blood-brain barrier,[61] accumulation of BDNF-producing GA-reactive T cells within the CNS of patients with MS may occur, in proportion to the population frequency of these cells. This concept is supported by findings derived from EAE studies. Adoptively transferred GA-reactive T cells are detected within the CNS of mice with EAE[15] and produce BDNF *in situ*.[62] These putative neurotrophic effects of GA may not be restricted to CNS autoimmune disease. In an optic-nerve injury model, GA-specific T cells prevented the secondary degeneration of axons and similarly accumulated at the site of injury producing neurotrophic factors.[63] In an animal model of glaucoma, GA reduced loss of retinal ganglion cells without affecting intraocular pressure.[64] GA administration protected motor neurons from acute and chronic degeneration[65] and adoptive transfer of GA-reactive T cells enhanced survival of dopaminergic neurons in a mouse model of Parkinson's disease.[66,67] Thus, GA may exert neurotrophic and/or protective properties in addition to immunomodulatory effects. Their relevance in human neurodegenerative diseases, including MS, remains to be determined.

CONCLUSIONS

Although GA is one of the most widely prescribed drugs for treatment of relapsing–remitting MS, its mechanism of action is still not entirely understood. GA treatment induces a preferential Th2 deviation of T cells and promotes restoration of frequency and function of Treg in MS. Recent reports demonstrated that GA also exerts immunomodulatory effects on APCs, such as monocytes. These new findings may provide a plausible explanation for GA-mediated T-cell immune modulation. Whereas it remains to be determined whether APCs, T cells, or both are the primary pharmacological target for GA, immune modulation of APC and T cells appears to engage a positive feedback mechanism. These novel observations should contribute to a better understanding of the mechanism of action of GA and may provide useful insight for the development of new and more efficient agents.

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