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Immunomodulation by the Copolymer Glatiramer Acetate

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Glatiramer acetate (GA; Copaxone®, also known as Copolymer 1 or Cop-1), a copolymer of amino acids, is very effective in the suppression of experimental autoimmune encephalitis (EAE), the animal model for multiple sclerosis (MS), in various species including primates. The immunological cross-reaction between the myelin basic protein and GA serves as the basis for the suppressive activity of GA in EAE, by the induction of antigen-specific suppressor cells. The mode of action of GA is by initial strong promiscuous binding to major histocompatibility complex class II molecules and competition with MBP and other myelin proteins for such binding and presentation to T cells. Suppressor T cells induced by GA are of the Th2 type, migrate to the brain and lead to *in situ* bystander suppression. Clinical trials with GA, both phase II and phase III, were performed in relapsing–remitting MS (RRMS) patients, and demonstrated efficacy in reducing the relapse rate, decreasing MRI-assessed disease activity and burden and slowing progression of disability. GA is generally well tolerated and is not associated with influenza-like symptoms and formation of neutralizing antibodies seen with β-interferons. It exerts its suppressive effect primarily by immunomodulation, and has recently shown ameliorating effect in a few additional autoimmune disorders as well as in graft rejection. At present GA is considered a valuable first-line treatment option for patients with RRMS.

Keywords: **Copaxone®; glatiramer acetate; multiple sclerosis; relapsing–remitting MS; rheumatoid arthritis; transplantation; ulcerative colitis**

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INTRODUCTION

Glatiramer acetate (GA), known also as Copolymer 1 (Cop 1) and by its brand name Copaxone®, is a synthetic copolymer of amino acids, developed in our laboratory (Arnon, 1996; Teitelbaum *et al.*, 1999a). It is an approved drug for the treatment of multiple sclerosis, and exerts its activity by immunomodulation.

Multiple sclerosis (MS) is a chronic, inflammatory disease of the CNS, usually diagnosed in young adults, and is characterized by localized myelin destruction and axonal damage or loss (Halpike, 1983; Lucchinetti *et al.*, 2001). Although the aetiology and pathogenesis of MS remain largely unknown, there are indications that the disease is of autoimmune nature. Data obtained both from MS patients and from the animal model, experimental autoimmune encephalomyelitis (EAE), point to the involvement of T-cell-mediated immune response towards several myelin antigens, including myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) (Ben-Nun *et al.*, 1996; Martin *et al.*, 1992; Steinman *et al.*, 1995). However, in addition to the involvement of activated lymphocytes, myelin specific antibodies may be also implicated in the pathogenesis of MS (Warren *et al.*, 1994). In view of the autoimmune nature of MS, the drugs recommended for its treatment, particularly for the most common type of the disease, the relapsing–remitting MS, were designed for reduction of the autoimmune responses. Limited success and low tolerability of the general immunosuppressents led to the introduction of general immunomodulators, namely several forms of recombinant interferon β, as well as the more specific immunomodulator GA.

GA is composed of the amino acids L-alanine, L-lysine, L-glutamic acid and L-tyrosine, in a molar ratio of 4.2:3.4:1.4:1.0 (Teitelbaum *et al.*, 1971). It was designed to simulate the MBP, one of the major myelin auto antigens involved in the induction of EAE. Indeed, GA was shown to suppress EAE very efficiently in several species, including primates (Arnon, 1996; Teitelbaum *et al.*, 1974). Moreover, GA is cross-reactive with MBP, and its suppressive effect may be explained in terms of this cross-reactivity. However, the suppressive effect of GA, which stems from its immunomodulatory capacity, is not limited to EAE and MS, and can be demonstrated in the case of several other autoimmune disorders, such as uveoretinitis (Zhang *et al.*, 2000).

The mechanism of activity of GA involves three modes of action, at various levels of the immune response (Arnon *et al.*, 1996). The prerequisite step is the binding of GA to

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Abbreviations used: EAE, experimental allergic encephalomyelitis; EDSS, extended disability status scale; GA, glatiramer acetate; MBP, myelin basic protein; MHC, major histocompatibility complex; MRI, magnetic resonance imaging; MS, multiple sclerosis; TCL, T-cell line; TCR, T-cell receptor.

various MHC class II molecules. Subsequently, three processes may occur: competition for binding of the cross-reactive antigen MBP or other myelin proteins to the MHC; TCR antagonism, namely competition between the complexes of MHC with MBP peptides or with GA, at the TCR level; and induction of specific T suppressor cells which can cross the blood–brain barrier, accumulate in the brain (Aharoni *et al.*, 2000), as well as induce bystander suppression (Aharoni *et al.*, 1998). This suppressive immunomodulatory activity is the rationale for the broader effect of GA, in other diseases that relate to immunological disorders.

In this review article we will describe the studies in experimental animals which demonstrate the efficacy of GA, mainly in EAE, but also in other experimental autoimmune diseases, as well as neurodegenerative disorders. Moreover, we will show that tissue rejection, both graft vs host response (GVH) and host *vs* Graft (HVG) reactions, are effectively ameliorated by GA. We will show that in all these cases the mechanism of activity can be traced to the induction of Th2 suppressor cells and to a Th1 to Th2 shift, in accordance with the immunomodulatory activity of GA.

We will also describe the clinical trials and investigations that led to the approval of GA as a drug, as well as the follow-up studies in patients and evaluation of their clinical and immunological parameters. These are highly valuable for the assessment of the risk–benefit relationship in the use of GA for the treatment of multiple sclerosis.

Finally, in view of the information accumulated on the mode of action of GA in various models of autoimmune and other immunological disorders, both in laboratory animals and patients, the mechanism by which it exerts its effect will be discussed.

STUDIES IN EXPERIMENTAL ANIMAL MODELS

GA was designed to simulate the MBP, one of the major myelin-derived autoantigens that induces experimental autoimmune encephalomyelitis—an experimental animal model of MS, and which has been implicated in the pathogenesis of MS (Teitelbaum *et al.*, 1974). GA was demonstrated to suppress EAE induced by MBP in a variety of species: guinea pigs, rabbits, mice and two species of monkeys—rhesus monkeys and baboons (Fig. 1). In contrast to rodents, where GA inhibits the onset of the disease, in primates it was used in treatment of the ongoing disease. A remarkable degree of suppression of EAE by GA was demonstrated in all species studied, even though different encephalitogenic determinants of MBP are involved in disease induction in the different species. Furthermore, GA was effective in suppressing the chronic relapsing EAE, a disease which shows a closer resemblance to MS, which can be induced by either spinal cord homogenate or encephalitogenic peptides derived from proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) (Arnon, 1996). Thus, the suppressive effect of GA in EAE is a general phenomenon and is not restricted to a particular species, disease type or the encephalitogen used for EAE induction. More recent studies have demonstrated that, in addition to the parenteral route of administration used in all the studies described so far, oral administration of GA is also effective in suppressing EAE in rats, mice and in primates (Teitelbaum *et al.*, 1999b). Furthermore, oral GA was more effective than oral MBP in suppressing the disease (Vollmer, 1998; Weiner, 1999).

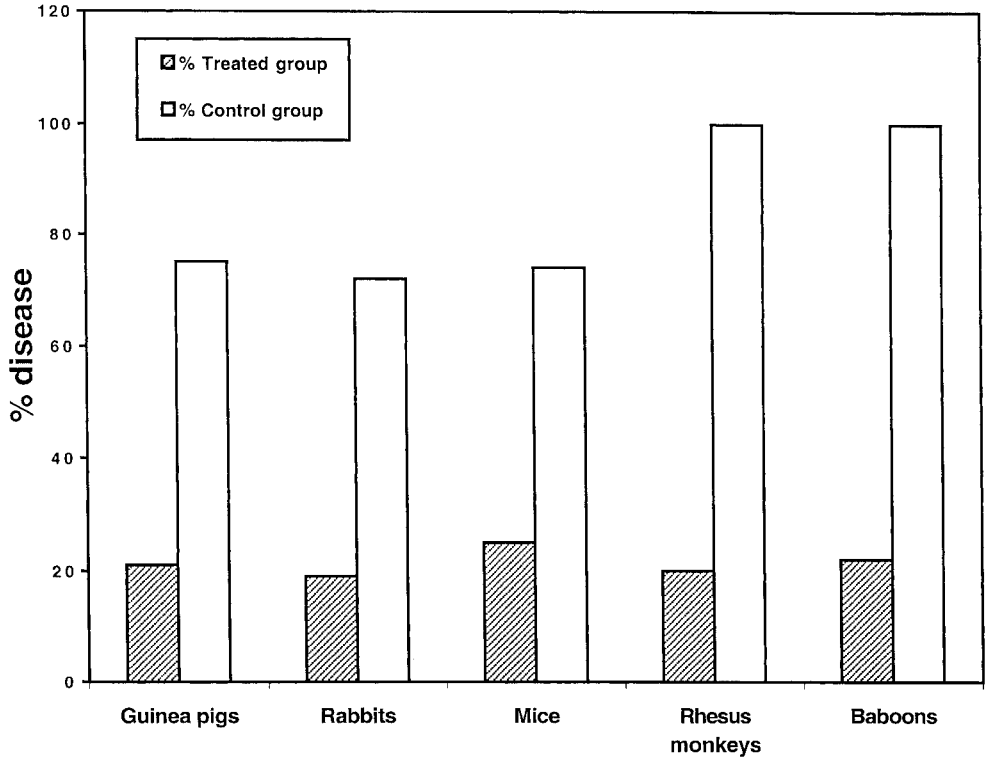

Figure 1. Suppression EAE by GA in various species. Incidence of disease in GA-treated animals as compared to untreated controls.

The suppressive effect of GA in EAE is a specific one, since GA lacked any suppressive effect on the immune response in several systems—humoral and cellular immune responses to a variety of antigens and vaccination against various induced infections (Teitelbaum *et al.*, 1996a). GA treatment also did not suppress other experimental autoimmune diseases, including myasthenia gravis, thyroiditis, diabetes and systemic lupus erythematosus (Brosh *et al.*, 1997). However, as will be described below, GA has recently been reported to inhibit another autoimmune disorder, namely experimental uveoretinitis (Zhang *et al.*, 2000), a disease interrelated with MBP and EAE. Unpublished results from our laboratory show that GA may be also effective in the case of experimental colitis.

The specific effect of GA in EAE may be explicable in terms of immunological specificity. Indeed, marked cross-reactivity was demonstrated between GA and MBP, both at the cellular and the humoral levels of the immune response. Thus, using monoclonal antibodies, we could demonstrate clearly that several monoclonal anti-MBP antibodies reacted with GA and vice versa (Teitelbaum *et al.*, 1991). At the cellular level, cross-reaction was observed both *in vitro* and *in vivo* (Webb *et al.*, 1973). Of interest is the very good correlation between the extent of immunological cross-reactivity and the suppressive effect on EAE of various synthetic copolymers, and particularly interesting is the observation that a polymer resembling GA in all parameters, except that it is built from D-amino acids rather than L-amino acids, does not cross react with MBP and has no EAE-suppressing activity whatsoever (Webb *et al.*, 1976).

Studies in experimental animal models conducted during the last decade have focused both on the immunological properties of GA, thus contributing to the understanding of its mode of action, and on extending its suppressive effect to models other than EAE. These include the following.

Promiscuous binding to MHC class II molecules (*in vitro*)

It was demonstrated that GA exhibits a very rapid, high and efficient binding to many different MHC class II haplotypes on living murine and human antigen presenting cells (Fridkis-Hareli *et al.*, 1994). Furthermore, it can bind in the polymeric form, and hence processing of GA is not required prior to its binding to MHC molecule (Fridkis-Hareli *et al.*, 1995). More recently, GA was also shown to interact with purified HLA-DR molecules, DR1, DR2 and DR4, with high affinity (Fridkis-Hareli and Strominger, 1998). Furthermore, the fraction of GA that was eluted from the different DR molecules had a similar amino acid composition to that of intact GA, indicating that the same types of determinants are involved in the binding to different MHC class II molecules. As a result of its high and efficient binding to MHC class II molecules, GA is capable of competing for binding with MBP and other myelin-associated proteins, such as PLP and MOG. Moreover, GA can efficiently displace MBP-, PLP- and MOG-derived peptides from the MHC binding site, whereas it could not be displaced once bound to the MHC by these antigens (Teitelbaum *et al.*, 1996b, 1999a).

Inhibition of T cell responses by GA (*in vitro*/*ex vivo*)

It has been demonstrated that GA can competitively inhibit the immune response to MBP of diverse MBP-specific murine and human T cell lines and clones, which have different MHC restrictions and respond to different epitopes of MBP (Racke *et al.*, 1992; Teitelbaum *et al.*, 1988, 1992). GA also inhibited the response of T cell lines reactive with PLP and MOG peptides (Teitelbaum *et al.*, 1988). The results suggest that the observed inhibition was due to competition between GA and nominal antigen for the MHC peptide binding site. This mechanism may be less specific, and indeed GA was shown to also inhibit *in vitro* some other immune responses such as the response of murine T cell hybridoma specific to porcine insulin (Racke *et al.*, 1992) and type II collagen-reactive T cell clones (Fridkis-Hareli *et al.*, 1998). Nonetheless, the response to a variety of other antigens such as PPD, lysozyme and ovalbumin was not affected by GA (Teitelbaum *et al.*, 1999a). In addition to the relatively non-specific MHC-blocking, GA was shown to inhibit the response to the immunodominant epitope of MBP, peptide 82–100, in a strictly antigen-specific manner by acting as T cell receptor (TCR) antagonist. The TCR antagonistic activity could not be demonstrated for MBP 1–11 and PLP 139–151, yet it was shown for all tested MBP 82–100-specific T cell lines/clones derived from mice and MS patients (Aharoni *et al.*, 1999).

Induction of antigen specific T-suppressor (Ts) cells

In vivo studies have demonstrated that GA-treated animals (either by subcutaneous injections or by oral administration) develop GA-specific Ts cells in the peripheral immune system. These cells can adoptively transfer protection against EAE (Lando *et al.*, 1979; Vollmer, 1998). Furthermore, Ts cell hybridomas and lines could be isolated from spleen cells of mice and rats rendered unresponsive to EAE by GA. Both cell types produced *in vitro* inhibition of MBP specific effector lines and inhibited *in vivo* EAE induced by different CNS antigens (Aharoni *et al.*, 1993). These Ts cells were characterized as Th2/3 type cells secreting anti-inflammatory cytokines such as IL-4, IL-10 and TGFβ but not Th1 cytokines, in response to both GA and MBP. Other myelin antigens such as PLP, MOG and $\alpha\beta$ crystalline could not activate the GA-induced Ts cells to secrete Th2 cytokines. Yet the disease induced by PLP and MOG can be suppressed by 'bystander suppression' mechanism (Aharoni *et al.*, 1996, 1998; Vollmer, 1998). More recently, it has been demonstrated that these GA-specific Th2 suppressor T cells accumulate in the brain. They can secrete the modulating cytokines *in situ* (Aharoni *et al.*, 2000, 2002), in response to the myelin proteins, which may explain the therapeutic effect of GA.

Potential of GA for the suppression of autoimmune disorders other than EAE

The capacity of GA to induce specific Th2 suppressor T cells, in addition to its recognition by various MHC class II

molecules, prompted several studies exploring the possibility that it might be effective in suppressing other autoimmune disorders. Thus, the findings that GA contains binding motifs of the rheumatoid arthritis-associated HLA-DR-1 (DRBR*0101) or HLA-DR4 (DRB1*0401) molecules (Fridkis-Hareli *et al.*, 1999) and can also inhibit type-II collagen-reactive T cell clones, suggest that it might demonstrate suppressive activity in animal models for rheumatoid arthritis (Fridkis-Hareli *et al.*, 1998). GA has also demonstrated inhibitory suppressive activity in experimental autoimmune uveoretinitis (Zhang *et al.*, 2000), as well as in an animal model of colitis, as shown recently in our laboratory (Aharoni *et al.*, unpublished results). In these studies, colitis was induced in mice by TNBS, and oral treatment with GA reduced significantly the various pathological manifestations of the disease.

In a very recent publication it was reported that GA is effective also in an animal model of ALS-vaccination where GA delayed the onset of the disease and increased the life span nearly 25%, compared with control mice (Angelov *et al.*, 2003).

Still in connection with multiple sclerosis, it was recently demonstrated that GA blocks the production of the IL-1 β-induced RANTES, as well as the levels of RANTES mRNA, in human astroglial cells (Li and Bever, 2001). These findings and the involvement of the nuclear factor κB in the process are significant, since the RANTES polypeptide has strong chemoattractant activity for T lymphocytes and monocytes/macrophages that are implicated in the pathogenesis of MS.

Inhibition by GA of manifestations of graft rejection

The pathological process of immune rejection is mediated by T cells that recognize alloantigens presented on self MHC molecules as non-self. In view of the strong capacity of GA to bind promiscuously to MHC class II, it was evaluated as a potential inhibitor of graft rejection. During the B10D2–BALB/c model of graft vs host disease (GVHD), which is similar to the MHC-matched bone marrow transplantation in human, GA demonstrated significant inhibition of the GVHD and improved survival of the mice (Schlegel *et al.*, 1996). Treatment with GA completely abolished cytotoxic activity towards host targets, prevented the Th1 cytokine secretion (IL-2 and IFNγ) and induced beneficial TH2 anti-inflammatory response (Aharoni *et al.*, 1997). More recently, it was shown that GA can also inhibit the manifestation of host vs graft (HVG) rejection (Aharoni *et al.*, 2001). Thus, it prolonged skin graft survival, and inhibited the functional deterioration of thyroid grafts in various strain combination of mice, across minor and major histocompatibility barriers. GA was similar in its activity to the potent immunosuppressive drug FK506 and more effective than cyclosproin A (Fig. 2). Here again, GA inhibited Th1 response and induced Th2 cytokines.

T cell immunity to GA induces neuroprotection

Recent studies have revealed an additional pan of GA activity which might be relevant also to MS. It was demonstrated that, similarly to MBP, active immunization with GA as well as adoptive transfer of T cells reactive to GA can inhibit the progression of secondary degeneration after crush injury of the rat optic nerve (Kipnis *et al.*, 2000). Furthermore, vaccination with GA protected neurons against glutamate cytotoxicity, while immunity to MBP and MOG which provides effective neuroprotection after axonal injury, did not protect the neurons from toxicity caused by glutamate (Schori *et al.*, 2001). It is of interest that, in these experiments, immunization with GA protected retinal ganglion cells from death induced by ocular hypertension in rats (Schori *et al.*, 2001). It was also demonstrated that activated GA-specific T cells secrete significant amounts of brain-derived neurotrophic factor (BDNF), a neurotrophin that plays a major role in neuronal survival. It was thus recently suggested that GA be used as a therapeutic vaccine for neurodegenerative diseases (Kipnis and Schwartz, 2002). It was further proposed that the protective effect of GA vaccination is obtained through a well-controlled inflammatory reaction, and that the activity of GA in driving this reaction derives from its ability to serve in a dual action, including the ability to activate spectrum of self-reactive T cells (Kipnis and Schwartz, 2002).

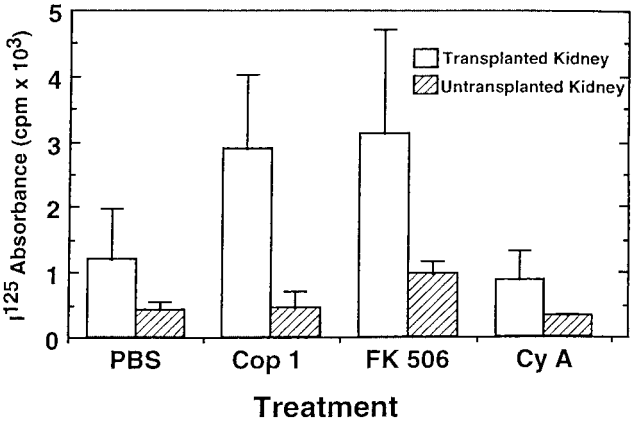

Figure 2. Effect of GA treatment on thyroid rejection in the B10D2-BalB/c mode. Thyroid glands from B10D2 donors were transplanted in the kidney's capsules of BALB/c mice. Recipient mice were treated daily with: PBS i.p. from day −6; 6000 μg/day of GA (ip+sc) from day −6; 1 μg/day of CsA i.p. from day −6; and 300 μg/day of FK506 i.p. from day −1 before transplantation. One week after transplantation, mice were injected i.p. with μc I^{125}, and the radioactivity of each kidney was measured 20 h later. For each treatment, the mean I^{125} absorbance of the recipient kidneys (solid bars) and the mean I^{125} absorbance of the untransplanted kidneys (striped bars) is demonstrated. Groups of four to 10 mice were used for each point (from Aharoni *et al.*, 2001).

CLINICAL INVESTIGATIONS

Two recent comprehensive review articles dedicated almost exclusively to this subject described in detail the various clinical trials that led to the approval of GA as a drug for the treatment of multiple sclerosis, and its evaluation (Sela and Teitelbaum, 2001; Simpson *et al.*, 2002). In the following we will relate to these clinical studies briefly and focus on additional findings that were reported more recently.

Early clinical trials

Based on the efficacy demonstrated by GA in suppressing EAE in all species including primates, both rhesus monkeys and baboons, two early clinical trials were conducted, one in Israel (Abramsky *et al.*, 1977) and the other in the USA (Bornstein *et al.*, 1982). The former, in which only four patients participated, receiving the same, relatively low dose (2–3 mg, two to three times a week for 6 months), indicated possible slight improvement in disability, and mainly no apparent adverse affect of GA. The latter, conducted in 16 patients with relapsing–remitting or chronic progressive MS, was actually a phase I trial, using increasing dosage, and led to the definition of the optimal dose, i.e. 20 mg GA daily, administered subcutaneously. While efficacy could not be evaluated in this early trial, GA treatment was well tolerated in all patients, with no toxicity noted and no adverse effects in the clinical disease recognized.

Another double-blind trial, conducted in two centers, in New York and Texas, included 106 patients suffering from chronic progressive MS (Bornstein *et al.*, 1991). The primary outcome measure of this trial was confirmed progression of disability by full-grade change in the EDSS. Out of 23 patients that fulfilled this criterion, nine were in the GA treated group and 14 in the placebo group, and thus did not manifest a statistically significant difference. Progression rates at 12 and 24 months were higher for the placebo group with a 2 year probability of 29.5% compared with 20.4% for the treated groups ($p = 0.088$). The difference in the 2 year progression of 0.5 EDSS units ($p = 0.03$) was significant.

Phase II/III trials—therapeutic efficacy

As discussed extensively by Simpson *et al.* (2002), GA has shown efficacy in treating patients with the relapsing–remitting disease (RRMS). Thus, in three randomized double-blind trials [including a 2 year pilot trial (Bornstein *et al.*, 1987), a larger US 2 year pivotal trial (Johnson *et al.*, 1995) and a 9 month European/Canadian (Comi *et al.*, 2001) studies] GA, at a dose of 20 mg once daily, administered subcutaneously in patients with RRMS, was significantly more effective than placebo for the respective primary endpoint of each trial (proportion of relapse-free patients, relapse rate and number of enhancing lesions on MRI scans).

For patients receiving GA compared with those receiving placebo in the two larger comparative studies, the mean relapse rate (covariate adjusted) at study endpoint was 29% lower in the large US trial (where relapse rate was the primary endpoint) and 33% lower in the European/Canadian study (where relapse rate was the tertiary endpoint). In the pilot trial, GA recipients had a mean relapse rate 78% lower, and they were more than twice as likely to be relapse-free, than placebo recipients. Relapse-related results in this pilot trial have not been reproduced in larger trials, possibly due to the patient populations having a shorter duration of disease and a higher baseline relapse rate than those in subsequent studies.

Glatiramer acetate decreased activity and burden of disease, as assessed by analysis of MRI scans, in patients enrolled in the European/Canadian study (Comi *et al.*, 2001) where certain MRI measures were the primary and secondary endpoints. For the primary outcome measure, patients in the GA-treated group demonstrated 29% fewer gadolinium-enhancing CNS lesions (areas of acute inflammation representing disruption of the blood–brain barrier) than patients in the placebo group. For secondary MRI outcomes, GA showed significantly greater lesion reductions (ranging from 30 to 82.6%) than placebo. Although this 9 month trial period was considered too short to demonstrate a significant reduction in the volume of hypointense T_1 lesions (representing areas of demyelination and axonal loss), further analysis of these scans has shown that, after 8 months, the proportion of new T_2 lesions evolving into these hypointense T_1 lesions ('black holes') in patients receiving GA was half that shown in patients receiving placebo (p value$<$ 0.002).

Progression to sustained disability, as measured by the Kurtzke Expanded Disability Status Scale (EDSS), was secondary endpoint in the two long-term trials. Patients with RRMS treated with glatiramer acetate in the pivotal US trial were significantly more likely to experience improved disability, and placebo recipients were more likely to experience worsening disability. The overall disability status was also significantly improved in this trial, although the change was modest. The pilot trial showed positive trends in delaying the onset or worsening of disability, although it did not have adequate statistical power to evaluate this outcome.

The beneficial effect of GA persisted far beyond the duration of the trials. Thus, the relapse-rate for an extension period (up to 35 months) of the US trial suggested a sustained benefit for patients receiving GA vs those receiving placebo (Johnson *et al.*, 1998). Furthermore, the annualized relapse rate for patients who had received GA throughout the 6 year active-treatment extension phase was 72% less than the annualized relapse rate at study entry ($p = 0.0001$; Johnson *et al.*, 2000). Patients receiving GA for 8 years (Johnson *et al.*, 2002) had an annualized relapse rate for the eighth year of 0.16 (equivalent to one relapse in 6 years) compared with a baseline annualized rate of 1.49 (based on the rate for the 2 year pretreatment period; Fig. 3).

As for its safety profile, from all these clinical trials it emerges that GA is well tolerated. The most commonly reported treatment-related adverse events are localized injection-site reactions and transient post-injection systemic

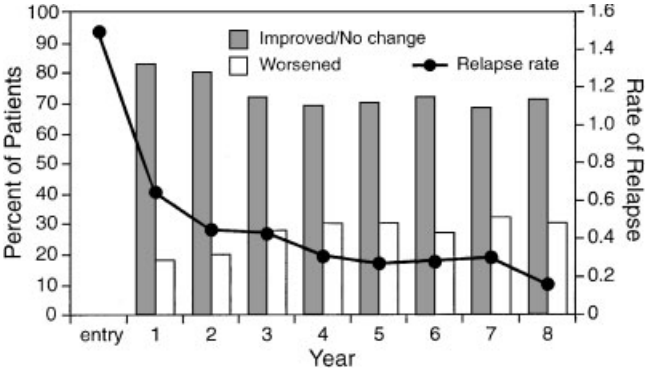

Figure 3. Results of long-term (8 years) prospective open trial of GA for relapsing-remitting MS. Yearly EDSS change by year of study. All patients received GA (from Johnson *et al.,* 2002).

reactions, manifested in facial-flushing, chest tightness, dyspnoea, palpitations, tachycardia and anxiety, which are mild and self-limiting (Johnson *et al.*, 1995, 1998). GA is not associated with the influenza-like syndrome or the formation of neutralizing antibodies that are reported in patients treated with interferon β. Based on the above they conclude that GA is a valuable first-line treatment option for MS patients (Simpson *et al.*, 2002).

A recent review discusses the risk-benefit assessment of glatiramer acetate in multiple sclerosis (Ziemssen *et al.*, 2001). The authors indicate that GA was found to slow the progression of disability and to reduce both relapse rate and MRI-defined disease activity and burden. The two major mechanisms that have been proposed to explain the effect of GA in EAE, namely the induction of GA-reactive Th2 regulatory suppressive cells and the interference with T cell activation as an altered peptide ligand, apply in MS patients as well. The most common adverse effects were mild and consisted mainly of injection-site reactions. Furthermore, antibodies to GA do not interfere with its clinical effects. Hence, they conclude that, overall, GA is very well tolerated and has excellent risk–benefit profile in RRMS patients.

MODE OF ACTIVITY OF GLATIRAMER ACETATE

Studies on the mode of action of GA have been conducted both in animal models and in humans.

Mode of action in experimental animal models

Animal studies on the mechanism of action have demonstrated that GA binds promiscuously to different MHC class II molecules. Following this interaction, three processes may occur in parallel: (1) competition for binding of MBP or other myelin proteins to the MHC class II, and thus inhibition of the induction of antigen-specific effector T cell functions; (2) TCR antagonism—competition at the level of TCR between the complex of MBP-derived peptides with class II MHC antigen and the complex of GA with class II antigen; and (3) activation of specific T suppressor cells of the Th2 type. We have recently demonstrated that, indeed, the GA-specific suppressor cells cross the blood–brain barrier and form clusters in the brain (Aharoni *et al.*, 2000), and consequently can secrete the anti-inflammatory cytokines *in situ* in the target organ and induce bystander suppression (Aharoni *et al.*, 1998). It is currently believed that this mechanism is more relevant *in vivo* than HLA competition or TCR antagonism.

Immunomodulation by GA in humans

In the last few years, since the time GA has been in clinical in use, several studies performed by different groups have been published on the immunological effects of GA in humans (Dabbert *et al.*, 2000; Duda *et al.*, 2000a,b; Miller *et al.*, 1998; Neuhaus *et al.*, 2000; Qin *et al.*, 2000). These human studies, as summarized below, are in almost complete agreement with the results reported previously in animals and lend credence to the proposed mechanism of action, particularly in relation to the immunological effect of GA.

Antibody response. Evaluation of the immunological responses to GA in MS patients revealed that all patients treated with GA developed anti-GA antibodies, whereas placebo-treated patients were negative (Brenner *et al.*, 2001; Teitelbaum *et al.*, 2003). The antibody level peaked at 3 months after initiation of treatment and reached a level of 8–20-fold above baseline. It decreased at 6 months and remained low. The anti-GA reactive antibodies were of IgG but not IgM or IgE class. During the follow-up period, both IgG1 and IgG2 isotypes exhibited kinetics similar to that of the total IgG. IgG_1 levels were 2–3-fold higher than those of IgG_2 at all time points examined. Similar patterns of antibody profile were found in three different sets of clinical trials (a total of 130 patients). The anti-GA antibodies did not interfere with the GA activity *in vitro*—they did not inhibit its binding to MHC molecules and T cell stimulation, nor did they inhibit the Th2 cytokine secretion of a human GA-specific clone. Most significantly these anti-GA antibodies are non-neutralizing and they do not interfere at all with the therapeutic effect of GA nor do they correlate with the reported side effects of GA (Brenner *et al.*, 2001). Furthermore, as shown in Table 1, the patients' sera with the highest GA antibody titre did not affect at all the capacity of GA to block the EAE symptoms (Teitelbaum *et al.*, 2003).

T cell response to GA in naive MS patients. Several studies have demonstrated the presence of GA reactive T cells in peripheral blood mononuclear cells (PBMC) of both untreated MS patients and normal individuals (Brenner *et al.*, 2001; Brosnan *et al.*, 1985; Duda *et al.*, 2000b; Farina *et al.*, 2001). The proliferative response to GA in naive MS and normal individuals could be inhibited by anti DR but not anti-DQ antibodies (Brenner *et al.*, 2001; Duda *et al.*, 2000a,b). Another study claims that class I-restricted T cells are also involved in this reactivity (Ragheb and Lisak, 2000). These results indicate that the proliferation induced by GA is mediated by the TCR and is MHC-restricted. Thus, there is compelling evidence that GA is recognized as a conventional antigen and not as a mitogen or superantigen.

T cell response to GA in treated MS patients—Th1 to Th2 shift. The proliferative responses to GA, MBP and PPD were followed up for 2 years in 86 patients participating in the phase III open-label study in Israel (Brenner *et al.*, 2001). Following an initial, slight increase, the response to GA was markedly and gradually reduced as a function of time in trial. The proliferative response to MBP which was low at baseline, showed also a trend toward reduction with time, of borderline significance. On the other hand, the response to the non-relevant antigen—PPD, which was high at baseline—did not change during the trial. Recent results from several groups (Duda *et al.*, 2000a; Farina *et al.*, 2001; Qin *et al.*, 2000; Ragheb and Lisak, 2000) confirm these observations. The decline in the proliferative response to GA may reflect an antigen-induced cell death due to the repetitive stimulations, anergy, or a shift to a Th2 type of response.

Table 1. Effect of MS Sera from GA-treated patients on EAE blocking activity of GA

Serum	Disease inoculum	Serum added to inoculum		Serum injected i.p.	
		EAE incidence	Blocking (0%)	EAE incidence	Blocking (0%)
—	MSCH (Control)	9/10	—	4/5	—
	MSCH+GA	0/10	100	0/5	100
NMS	MSCH (Control)	9/10	—	4/5	—
	MSCH+GA	0/10	100	0/5	100
MS-1	MSCH+GA	0/10	100	0/5	100
MS-2	MSCH+GA	0/10	100	0/5	100
MS-3	MSCH+GA	0/10	100	0/5	100
MS-4	MSCH+GA	0/10	100	0/5	N.T
MS-5	MSCH+GA	0/10	100	0/5	100
MS-6	MSCH+GA	0/10	100	0/5	N.T

EAE was induced in (SJL/J × BALB/c) F_1 mice by injecting 5 mg MSCH in CFA.
For EAE blocking GA (250 μg) was added to the MSCH inoculum. Tested sera were either added to the disease inoculum or injected intraperitoneally (i.p.).
N.T., not tested.

Different lines of evidence suggest that GA treatment induces a shift from Th1 to Th2 response: (a) such a shift is indicated by the pattern of the anti GA antibody isotypes, namely, higher IgG_1 than IgG_2 (Brenner *et al.*, 2001); (b) treatment of MS patients with glatiramer acetate led to an elevation of TGFβ, IL-10 and suppression of TNFα mRNA from PBMC (Miller *et al.*, 1998); (c) recent observations from several groups on short-term and long-term GA specific T cell lines (TCL) demonstrate that TCL from untreated MS patients and healthy controls are predominantly of the Th1 type secreting IFNγ and TNFα; on the other hand, TCL derived from GA-treated patients are predominantly Th2 cells secreting IL-4, IL-5 and IL-13 (Dabbert *et al.*, 2000; Qin *et al.*, 2000); (d) using an automated ELISPOT assay it was demonstrated that there is increase of GA reactive T cells producing IL-4 or IFNγ. The elevated IFNγ response was partially mediated by CD8+ T cells after stimulation with very high concentrations of GA (Farina *et al.*, 2001). These findings led to the development of an immunological assay for assessing the efficacy of GA in multiple sclerosis patients. A recent pilot study revealed that out of 15 patients who responded clinically to GA, 13 (86%) showed increase of T cells secreting IL-4 and IFNγ, whereas only two (22%) out of nine clinical non-responders met these immunological criteria (Farina *et al.*, 2002). (e) The above elevated IFNα response was partially mediated by CD8+ T cells after stimulation with very high concentration of GA (Farina *et al.*, 2001). This was recently corroborated in a study demonstrating that, whereas GA-induced CD4+ T cell responses are comparable in healthy individuals and MS patients, CD8+ T cells are significantly lower in untreated MS patients. Treatment with GA resulted in up-regulation of these CD8+ responses with restoration to levels observed in healthy individuals (Karandikar *et al.*, 2002). Both CD4+ and CD8+ GA-specific responses are HLA-restricted.

Cross-reactivity between GA and MBP. Cross reactivity between GA and MBP, which may explain its suppressive activity, was demonstrated in animals both at the humoral level, mainly by monoclonal antibodies (Teitelbaum *et al.*, 1991), and at the cellular level, using both *in vivo* (delayed hypersensitivity) and *in vitro* (lymphocyte transformation) assays (Webb *et al.*, 1973). Most of the Th2/3 TCL isolated from treated rodents were shown, however, to cross-react with MBP only at the level of Th2/3 cytokine secretion but not by Th1 cytokine secretion nor by proliferation (Aharoni *et al.*, 1996, 1998; Teitelbaum *et al.*, 1999b). Similarly, most studies on human TCL specific to GA demonstrated that MBP did not induce cross proliferation (Burns *et al.*, 1986). In contrast to proliferation there is clear evidence that GA and MBP may cross-stimulate human T cells at the level of cytokine secretion (Neuhaus *et al.*, 2000; Qin *et al.*, 2000). In addition to cross-stimulation with MBP that was demonstrated in hundreds of cell lines, two GA-specific T cell lines could be stimulated to produce IFN-γ with another myelin antigen—MOG (Neuhaus *et al.*, 2000). Interestingly, it was also demonstrated that, with increasing duration of treatment, the surviving GA-reactive T cells become more 'degenerate' and respond to an increasing number of components from a combinatorial peptide library (Duda *et al.*, 2000b). However, this response still led to the secretion of Th2 cytokines.

Proposed mechanism of action of Copolymer 1-specific immunodulation

As emerges from the cumulative experimental results, GA affects MS at various levels of the immune response involved, which differ in their degree of specificity. Its binding to the MHC class II molecules, which is the least specific step, is a prerequisite for its effect by any mechanism. Following this interaction, three mechanisms were clearly shown to be effective:

(1) GA can compete for binding to MHC class II with several myelin associated antigens, e.g. MBP, PLP, MOG and α,β-crystallin, resulting in inhibition of antigen specific T-cell effector functions (i.e. proliferation, interleukin secretion and cytotoxicity). This mechanism is by its nature antigen-non-specific, as MHC

blockage may also lead to interference with other immune responses, depending on the strength of TCR-MHC/peptide engagement, and consequently to immunomodulatory effect of GA in other autoimmune disorders (Weiner, 1999), or the prevention of graft rejection (Aharoni *et al.*, 1997, 2001).

(2) TCR antagonism–competition at the level of TCR between the complex of MBP-derived peptides with class II MHC antigen, and the complex of GA with class II antigen. This is a specific mechanism since it involves interaction with a specific TCR. By engaging the specific TCR, GA can also act as altered peptide ligand and induce anergy of the pathogenic T cells. Interestingly, it has been demonstrated that an important mechanism by which TCR antagonists are active *in vivo* is by the induction of Th2 regulatory T cells which mediate bystander suppression of EAE (Aharoni *et al.*, 1998; Nicholson *et al.*, 1997). An interesting new angle is the recent report that GA treatment in MS patients is associated with significant modulation of the susceptibility of their T helper cells to apoptosis (Atlas *et al.*, 2001).

(3) GA binding to the relevant MHC leads to the activation of T regulatory/suppressor cells, which are activated by shared suppressive determinants between MBP and GA, to secrete Th2 suppressive cytokines. Indeed, such suppressor cells were shown to migrate and form clusters in the brain (Aharoni *et al.*, 2000). This mechanism is a specific one and results from the cross-reactivity between GA and MBP. However, due to bystander suppression, other myelin encephalitogens (e.g. PLP and MOG) are also inhibited due to their *in vivo* colocalization with MBP.

Furthermore, it was recently demonstrated that both GA-specific Th1 and Th2 cell lines produce the brain derived neurotrophic factor (BDNF) (Ziemssen *et al.*, 2002). As the signal transduction receptor for BDNF is expressed in MS lesions, it is likely that the BDNF secreted by the GA-reactive Th2 and Th1 has neurotrophic effects in the MS target tissue. This may, therefore, be an additional aspect in the mechanism of action of GA. It is currently believed that induction of T-suppressor cells is more relevant *in vivo* than HLA competition or anergy induction, thus emphasizing that GA exerts its biological activity by effective immunomodulation.

CONCLUDING REMARKS

This review article summarizes the data available on the therapeutic activity and immunomodulatory properties of glatiramer acetate. Known by its trade name Copaxone®, GA is the only non-interferon novel drug for the treatment of multiple sclerosis. It is a synthetic random polymer of amino acids and has a specific effect on the autoimmune process involved in both EAE and MS. The results of clinical trials with GA indicate that it is an effective low-risk specific drug for the treatment of relapsing–remitting MS, capable of slowing the progression of disability and reducing the relapse rate.

While polymers are often used in pharmacopeia, e.g. as wrapping devices, or as tools allowing for slow release of drugs, GA is the first polymeric drug which is responsible for the successful treatment of a disease. It is worth mentioning again that GA is effective against multiple sclerosis, probably because of its chemical and immunological resemblance to myelin basic protein. Indeed, it can be considered (Hohlfeld and Windl, 2001) the prototype of an autoantigen-directed, autoantigen-derived selective agent. This illustrates the concept of specificity in treating autoimmune diseases, similarly to vaccines against infectious diseases, where nobody expects to have one vaccine against all diseases.

Its mode of action includes a prerequisite stage of binding to the MHC molecules, thus competing with the binding of the myelin proteins that induce the neurological damage. The macromolecular nature of GA, combined with its microheterogeneity, could actually contribute to its effectiveness by leading to its binding to MHC class II of many genetic backgrounds. However, the major role in the mechanism of activity of GA is played by the suppressor T cells that it induces, with concomitant shift of Th1 to Th2 response, thus placing it as a most effective immunomodulator for treatment of MS and possibly other autoimmune disorders, or even graft rejection.

As illustrated in this review, the number of publications describing studies on GA, whether experimental or clinical, *in vitro* and *in vivo*, grew exponentially in recent years, and it is to be hoped that in the not too distant future we shall understand even better both the mechanism of action of this drug, and—most importantly—we shall be able to evaluate the long-term impact and health improvement of the MS patient.

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