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François Blanchette
Oliver Neuhaus

Glatiramer Acetate

Evidence for a dual mechanism of action

François Blanchette (✉)
Scientific Affairs Director (Europe)
Teva Pharma S.A.
Immeuble Palatin 1
1 cours du Triangle
92936 Paris La Defense Cedex, France
Tel.: +33 (0) 155917936
Fax: +33 (0) 155917970
Mobile: +33 (0) 625692711
E-Mail:
francois.blanchette@tevafrance.com

Oliver Neuhaus
Dept. of Neurology
Kreiskrankenhaus Sigmaringen
Hohenzollernstrasse 40
72488 Sigmaringen, Germany

Abstract Glatiramer acetate is a disease-modifying drug approved for the treatment of relapsing-remitting multiple sclerosis. Since its discovery almost four decades ago, and in particular since the observation of its beneficial clinical effects in the late 1980s and early 1990s, numerous data have been generated and contribute pieces of a puzzle to help explain the mechanism of action of glatiramer acetate. Two major themes have emerged, namely (i) the induction of glatiramer acetate-reactive TH2 immunoregulatory cells, and (ii) the stimulation of neurotrophin secretion in the central nervous system that may promote neuronal repair.

Key words Glatiramer acetate · multiple sclerosis · inflammation · neuroprotection · T-cell

Introduction

Glatiramer acetate (GA; Copaxone® or Copolymer-1) was first synthesised over thirty years ago as a research tool to explore the biology of experimental autoimmune encephalomyelitis (EAE) by a group of research scientists from the Weizmann Institute in Israel, notably Ruth Arnon, Michael Sela and Dvora Teitelbaum. This early animal work led to the subsequent development of GA as a disease-modifying treatment for relapsing remitting multiple sclerosis, for which indication it was approved in 1996 [6]. Over the last decade, GA has established itself, together with the β-interferons, as the reference first-line treatment for this form of multiple sclerosis. In parallel, significant progress has been made in our understanding of the mechanism of action of this drug, implicating both modulation of the immune system and neuroprotection. This review will discuss the latest research in this field and attempt to bring together the different pieces of the puzzle into a unified hypothesis of the mechanism of action of GA.

What is glatiramer acetate?

Glatiramer acetate was originally designed as a synthetic analogue of myelin basic protein (MBP) in the late 1960's. It is a standardised mixture of polypeptides randomly polymerised from four constituent L-amino acids, namely L-glutamate, L-lysine, L-alanine and L-tyrosine, in a defined molar ratio of 0.14:0.34:0.43:0.09, the same as that found in the amino acid sequence of MBP. The size of these polypeptides ranges from 4.7 to 11 KDa.

The rationale behind the design of GA was to create a synthetic molecular mimic of MBP which could be used to explore the biology of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. At the time, EAE was generally induced by inoculation of rodents with crude myelin preparations from the spinal cord of guinea-pigs or other animals. Purified individual myelin components such as MBP were not readily available for use at this time, and the primary structure of such proteins had not been elucidated. It was therefore decided to initiate a programme of synthesis of MBP analogues with the same overall amino acid composition as MBP, that could be used to induce

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EAE in a more reproducible fashion than was possible with crude myelin preparations.

The team from the Weizmann Institute noted to their surprise that, paradoxically, one of the MBP analogues synthesised, that would later be known as GA, did not in fact induce EAE, but instead prevented the development of EAE in response to crude myelin preparations [45].

This was the first milestone in a large series of subsequent research exploring the protective effects of GA in EAE in animals [6], followed by the first pilot studies indicating a beneficial effect in human multiple sclerosis [9]. These findings were confirmed in a large randomised clinical trial, published in 1995 [22], which opened the door to regulatory approval for GA in relapsing remitting multiple sclerosis the following year.

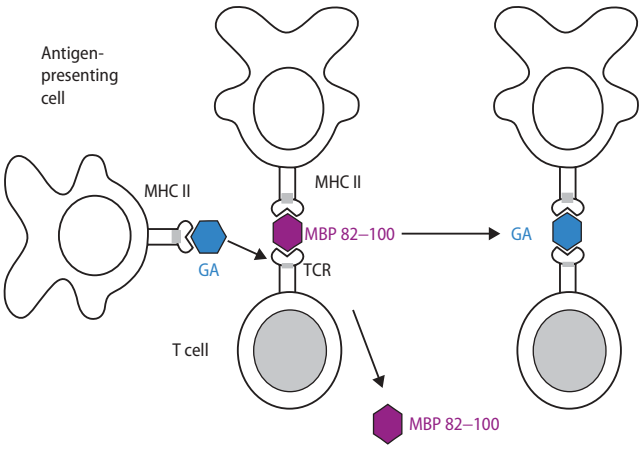


Fig. 1 Antagonism between glatiramer acetate (GA) and the myelin basic protein epitope MBP 82–100 for the T cell receptor. MHC II: type II major histocompatibility proteins; TCR: T cell receptor

Glatiramer acetate, MBP and T cell receptors

The first studies of the mechanism of action of GA focussed on potential competition between GA and MBP-derived antigens for different immune cell-mediated events [20, 44]. However, it should be noted that these observations were made using in vitro test systems and their relevance to the mode of action of the drug in vivo is not entirely clear, since MBP is normally found within the central nervous system whereas GA peptides do not normally cross the blood-brain barrier.

For example, GA can bind with high affinity to major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APC) which recognise MBP antigens. This prevents MBP antigens from binding to these MHC proteins themselves and thus from stimulating these lymphocyte populations. In particular, GA has been shown to bind to the MLA-DR class of MHC proteins which are specifically expressed in high amounts in autoimmune diseases such as multiple sclerosis [16, 17].

It has also been demonstrated that GA can compete specifically with MBP-derived antigens, in particular the immunodominant 82–100 epitope of MBP, for the T cell receptor [4]. The docking of APCs to T cells mediated by binding of MHC II molecules presenting MBP to the T cell receptor can thus be displaced and prevented by APCs presenting GA, which bind preferentially to the T cell receptor (Fig. 1). Unlike the binding of MBP 82–100 to the T cell receptor, which results in functional activation of the T cell, binding of GA to the T cell receptor does not activate the T cell, and thus behaves as a T cell receptor antagonist with respect to MBP.

Also relevant is the concept of GA as an altered peptide ligand [20]. Although the primary structure of GA structurally resembles that of epitopes on MBP, it is not identical and is unlikely to share the same three-dimensional structure. This means that the concerted conformational change of ligand and receptor that underlies activation of APCs or T cells by MBP will not take place when the receptors bind GA rather than MBP, the binding of GA being unproductive. The consequence of this is that GA inhibits immune cell activation and inflammation in response to myelin antigens.

Glatiramer acetate and T cell phenotype

More recently, evidence has accrued that exposure to GA can alter the properties of GA-reactive T cells. Given the extreme heterogeneity of individual peptide sequences in GA preparations, it is not surprising that certain of these peptide epitopes will be recognised by T cell receptors on circulating peripheral white blood cells in most individuals, as part of the memory T-cell pool in the intrinsic immune system repertoire, even if these individuals have never been exposed to GA itself. This means that most, if not all, of us have some GA-reactive T cells circulating in our bloodstream at any time. These cells can then be stimulated to proliferate and secrete cytokines when exposed to GA. In 1990, Pette et al. published a split-well cloning method to select and propagate clonal lines of T cells specific for a given antigen isolated from peripheral blood using cycles of stimulation and dilution in vitro [38].

Using such a method, it was possible to determine the profile of cytokine expression from GA-specific T cells stimulated with GA and APCs [39]. Some GA-specific T cell lines expressed essentially pro-inflammatory cytokines such as interleukin-2, interleukin-5 and interferon-γ, and are thus classified as TH1 cells. Other cell lines expressed predominantly anti-inflammatory cytokines such as interleukin-4, interleukin-10 and TGF-β, and correspond to TH2 cells. In addition, TH0 T-cells, which express both types of cytokines, are also present

as precursor T cells which can differentiate into either TH1 or TH2 cells when activated.

An important action of GA is to induce a shift in the phenotype of GA-reactive T cells from a TH1 to an TH2 pattern of cytokine release. In a survey of the cytokine profile of over 700 GA-reactive T cell lines [37], we found that, whereas most T cell lines from healthy donors or from untreated multiple sclerosis patients show a TH1 pattern of cytokine release, those from patients treated with GA predominantly behave like TH2 cells (Fig. 2). Similar observations have been reported in a subgroup of patients included in the US pivotal trial who had been treated with GA for six years, suggesting that this phenotypic shift is stable over time [11].

Based on these findings, we can propose a model of the mechanism of action of GA [36] (Fig. 3), whereby a latent population of GA-reactive T cells in the circulation proliferate in response to administration of GA and differentiate into a TH2 anti-inflammatory phenotype. With repeated treatment, these cells become chronically activated and can cross the blood-brain barrier into sites of inflammatory damage to the nervous system. Once inside the nervous system, they can no longer be activated by GA itself, which does not enter the brain, but are re-activated by local CNS antigens, perhaps myelin degradation products presenting MBP epitopes, with which they are cross-reactive. Local release of anti-inflammatory cytokines from the GA-reactive T cells upon stimulation can attenuate inflammation caused by infiltrating pro-inflammatory T-cells and microglia by a bystander suppression effect [5, 36].

A further piece has been added to this puzzle by a series of experiments indicating that the ability of GA to promote a shift in T cell phenotype involves APCs, in particular, dendritic cells [49]. It was demonstrated that, in the absence of APCs, naïve T cells are unresponsive to GA. However, GA will directly act on the dendritic cell to inhibit the production of inflammatory mediators from these cells. These mediators include IL-12p70, which is an important polarising factor for T cells, inducing them to differentiate towards a TH1 phenotype in the presence of non-specific stimuli such as LPS or CD40 ligands. If naïve T cells are incubated with dendritic cells that have been previously exposed to GA, or with culture medium from such cells, then they are no longer able to differentiate to a TH1 phenotype in the presence of non-specific stimuli, presumably because IL-12p70 secretion from the dendritic cell has been blocked by GA.

Multiple sclerosis, neurotrophic factors and glatiramer acetate

Apart from its immunomodulatory activity at the level of T cells, the second major component of the mechanism of action of GA involves a neuroprotective effect on damaged axons and neuronal cell bodies in the central nervous system mediated by modulation of the expression of certain neurotrophic factors.

Neurotrophic factors are proteins which regulate growth and differentiation, and also programmed cell death, in developing and mature neurones, and other

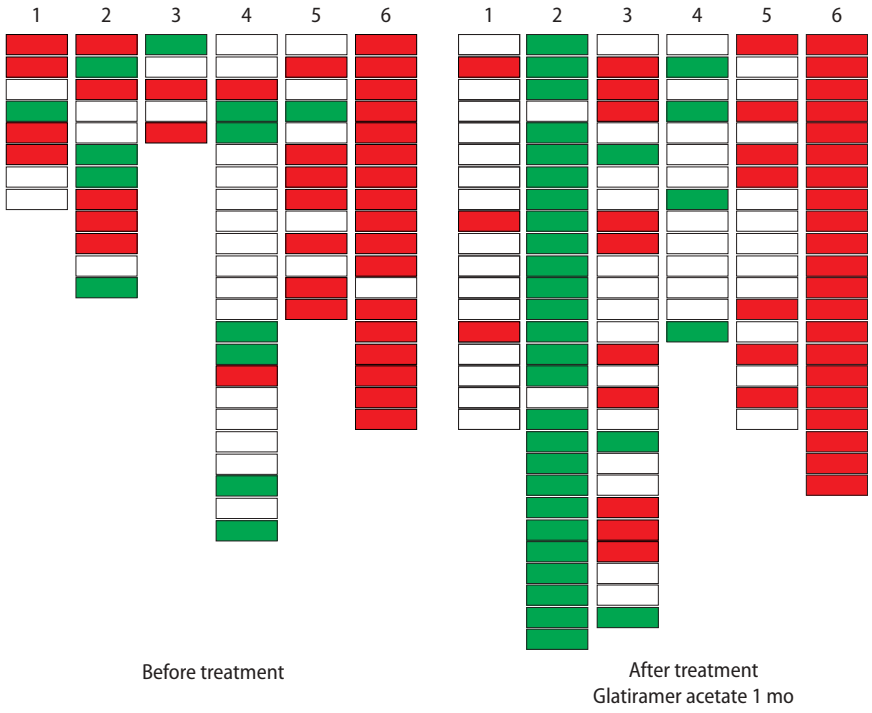


Fig. 2 Phenotype of GA-sensitive T-cell lines from MS patients before treatment (top) and one month after (bottom) treatment with GA. The blocks represent individual T-cell clones of T_H1 (■), T_H0 (□) and T_H2 (■) phenotypes isolated from six patients, each represented by a single column 1-6. Data are adapted from Neuhaus et al. [37], with permission from National Academy of Science, USA, Copyright 2000

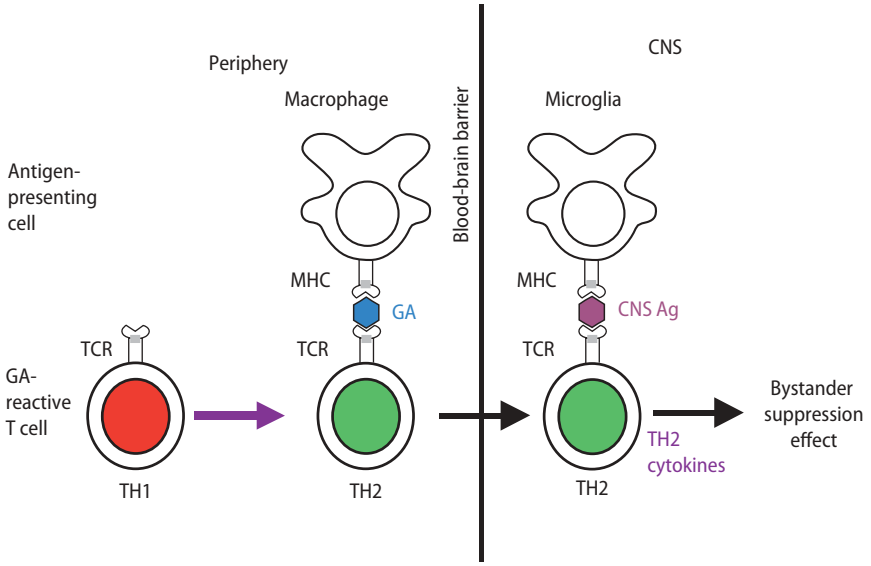


Fig. 3 Schematic representation of the proposed mechanism of action of the immunomodulatory effects of glatiramer acetate (GA) in multiple sclerosis. CNS AG: CNS antigen; MHC: major histocompatibility proteins; TCR: T cell receptor. Reproduced from Neuhaus et al. [36], with permission

neural crest derived cells such as oligodendrocytes. Several families of neurotrophic factor have been identified, of which the best characterised are the neurotrophin family, of which nerve growth factor was the first described, the neurotrophic cytokines, such as ciliary neurotrophic factor (CNTF) and the GFL family, exemplified by glial-derived neurotrophic factor (GDNF). Each family consists of several members which share some structural homology and a common signalling pathway on target cells. Each different neurotrophic factor acts on a specific population of target neurones in a specific context during neuronal differentiation, maturation or repair.

In the context of potential neurotrophic effects of GA, the neurotrophic factor that has received the most attention is brain-derived neurotrophic factor (BDNF). BDNF is a dimeric polypeptide belonging to the neurotrophin family that acts on a rather broad population of target neurones by regulating the expression of genes involved in neurite outgrowth, synaptogenesis and apoptosis through activation of a cell surface receptor tyrosine kinase, trkB. Although BDNF was originally thought to be produced exclusively by neurones or astrocytes, it has since been shown that certain immune cells, notably T cells, B cells and monocytes also express BDNF, which can be secreted when these cells are stimulated [8, 10, 24]. This observation has led to the development of the concept of neuroprotective immunity, whereby release of BDNF from activated immune cells penetrating the nervous system following tissue insult may contribute to protecting neurones from damage and promoting neuronal repair [25].

With respect to multiple sclerosis, BDNF immunoreactivity has been demonstrated in inflammatory infiltrates in the brain of patients post mortem [24]. Subsequently, it was shown that actively demyelinating lesions contain large numbers of monocytes and T cells which express BDNF, and that the BDNF receptor trkB is upregulated in neurones in these lesions [43].

Several lines of evidence, of different strengths of clinical relevance, now exist indicating that GA may be able to promote neuroprotection in multiple sclerosis by facilitating the release of neurotrophic factors at lesion sites (Fig. 4).

Glatiramer acetate and neuroprotection: in vitro studies

Given that an important cellular target of GA, namely T cells, can produce and secrete BDNF, it was important to find out whether BDNF production from GA-reactive T cells could be modulated by GA. This was indeed the case, and two independent laboratories demon-

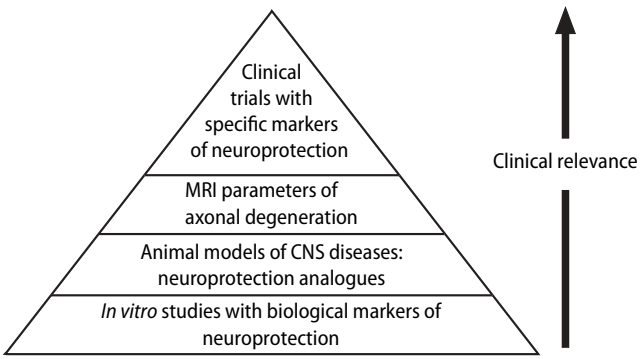


Fig. 4 Lines of evidence for a neuroprotective effect of glatiramer acetate in multiple sclerosis

strated increased expression of BDNF upon stimulation with GA in GA-reactive T cell lines obtained from either healthy volunteers or multiple sclerosis patients treated with GA [12, 52]. This increased expression could be observed both at the mRNA and protein levels and was accompanied by increased secretion of BDNF into the extracellular milieu (Fig. 5). Importantly, a stimulation of BDNF secretion was observed irrespective of the cytokine phenotype of the T cell line, being equally robust in pro-inflammatory TH1 cell lines as in anti-inflammatory TH2 cell lines.

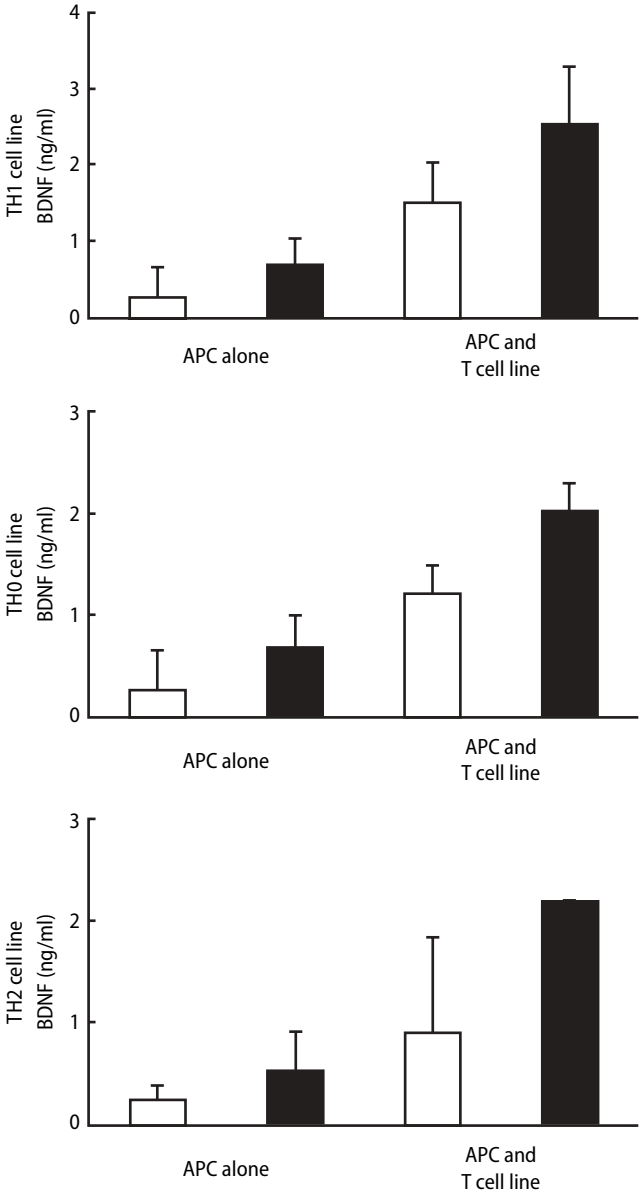


Fig. 5 Secretion of brain-derived neurotrophic factor (BDNF) by glatiramer acetate-reactive T cell lines isolated from peripheral blood. T cell lines were cultivated in the presence of antigen-presenting cells (APCs) and with (filled columns) or without (open columns) glatiramer acetate. Reproduced from Ziemssen et al. [52], with permission

Glatiramer acetate and neuroprotection: animal studies

Although such studies indicate that T cells can secrete BDNF in response to GA in vitro, it is important to demonstrate that this also occurs where the released BDNF can make a difference, that is at the site of tissue damage within the nervous system. This is obviously difficult to observe in patients with multiple sclerosis, although GA-reactive TH2 cells that produce BDNF have recently been isolated from the cerebrospinal fluid of patients with multiple sclerosis treated with GA [21, 53]. On the other hand, this issue can be addressed in animal studies.

In the EAE mouse model of multiple sclerosis, BDNF secretion has been evaluated in the nervous system after adoptive transfer of GA-reactive T cells [3]. These cells were isolated from a donor mouse treated with GA, labelled with a fluorescent tag and inoculated into a recipient mouse in which EAE was induced with spinal cord homogenate. One week later, abundant fluorescent cells could be identified in many areas of the nervous system where they had migrated by perivascular infiltration. These cells expressed the anti-inflammatory cytokines interleukin-10 and TGF-β characteristic of TH2 cell lineages but not interferon-γ, a pro-inflammatory cytokine present in TH1 cells. Intense BDNF immunostaining was associated with these cells (Fig. 6). In contrast, in control EAE mice which had not been inoculated with GA-reactive T cells, cerebral BDNF staining was modest. The same group went on to show that direct treatment of EAE mice with GA produced a sustained augmentation of the levels of BDNF in the brain [1], principally associated with infiltrating T cells.

The consequence of BDNF release from GA-reactive T cells infiltrating the nervous system may be enhanced neuroprotection. In certain rodent EAE models, significant axonal disruption, reminiscent of that seen in human multiple sclerosis, is observed in the chronic phase of the pathology [30]. Axonal damage can be quantified in these models by measuring immunostaining for amyloid precursor protein (APP) and non phosphorylated neurofilament (SMI-32), which are markers of truncated axons. In EAE mice treated with GA, the extent of axonal damage has been shown to be attenuated markedly compared to untreated mice, with a 85 % reduction in SMI-32 staining and a 63 % reduction in APP staining [19].

In the latter experiment, it could be argued that the reduction in axonal damage is a consequence of attenuated inflammation in the nervous system rather than a direct effect of GA. This issue has been addressed in another animal model of immune attack on the nervous system, myelin oligodendrocyte glycoprotein (MOG)-induced optic neuritis in the rat [31]. In this model, inflammatory damage to the optic nerve precedes apoptotic death of the retinal ganglion cells whose axons

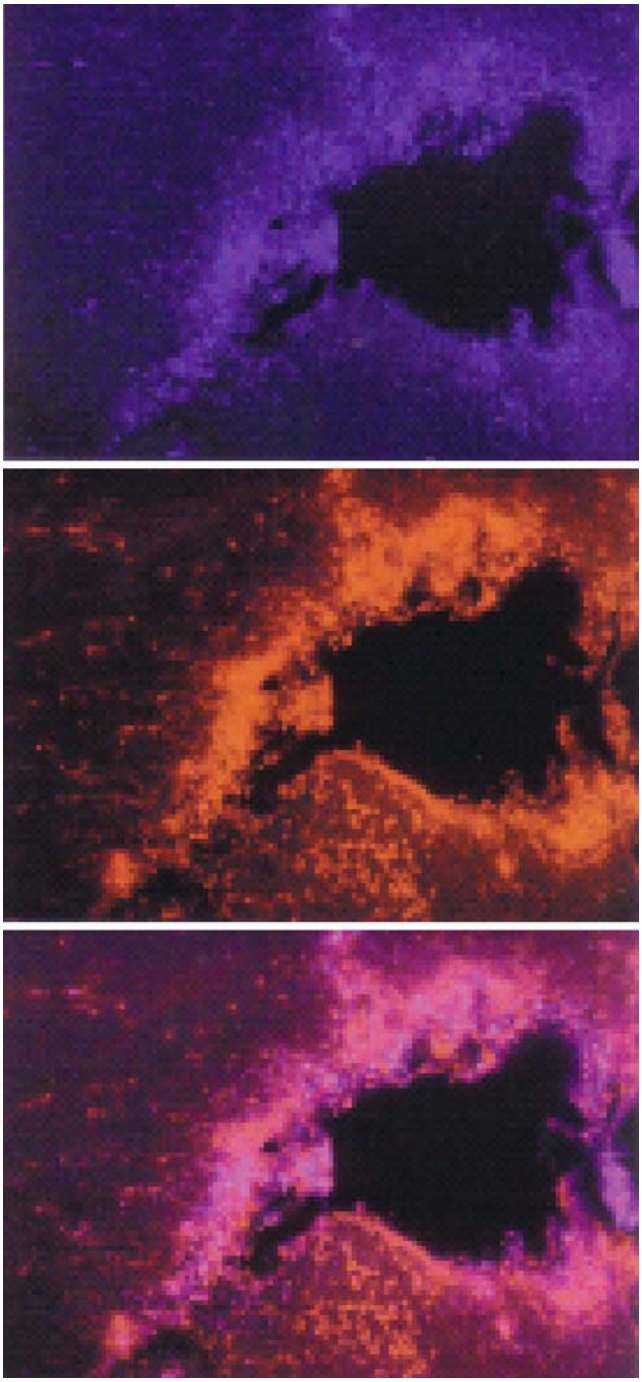


Fig. 6 Brain-derived neurotrophic factor (BDNF) in glatiramer acetate (GA)-reactive T cell lines in the periventricular space in the brains of mice with experimental autoimmune encephalomyelitis. Top: fluorescent Hoescht blue staining of GA)-reactive T cell lines; centre: immunocytochemical staining of BDNF in the same brain section; bottom: overlap of the two images. Reproduced from Aharoni et al. [3], with permission from National Academy of Sciences, USA. Copyright 2003

lie therein, and the two processes can thus be temporally distinguished. If the rats are treated preventively with GA for two weeks prior to inoculation with MOG, both inflammatory demyelination of the optic nerve and apoptosis of the retinal ganglion cells can be prevented. However, if GA is given at the same time as MOG inoculation, only the late degeneration of the retinal ganglion cells and not the earlier inflammatory attack on myelin can be prevented. These findings strongly suggest that the neuroprotective effects of GA in this model are direct, and not secondary to inflammation.

Evidence for repair-promoting effects of GA has also come from an electron micrographic study of myelin integrity in spinal cord in the MOG-EAE mouse model [2]. This showed that not only could GA prevent demyelination of axons running in the spinal cord if given prior to MOG inoculation, but it could also promote remyelination of damaged axons when GA was administered 21–34 days after the first appearance of inflammatory pathology and clinical signs.

Glatiramer acetate and neuroprotection: MRI studies

Magnetic resonance imagery (MRI) can provide information in patients on many different aspects of the disease process in multiple sclerosis. The different conventional and experimental MRI paradigms available for use in multiple sclerosis, the disease processes on which they provide information, and some of the studies performed with immunomodulatory treatments are described in detail elsewhere in this volume by Zivadinov. A summary of the principal MRI findings obtained for GA is provided in Table 1. Several of the effects of GA that have been observed are consistent with a neuroprotective action in these patients.

One of the more robust effects of glatiramer acetate is to slow the rate of accrual of chronic T1 hypointense lesions or 'black holes'. These represent an end-state of 'burnt-out' inflammatory lesions in which significant axonal loss and tissue destruction has taken place [47, 48]. The number of 'black holes' visible on MRI can be considered a global marker of irreversible axonal loss [48] and is well correlated with the rate of accumulation of irreversible disability [7, 46, 47]. A reduction in the number of 'black holes' may reflect neuroprotective or remyelinating processes within lesions.

The European/Canadian Glatiramer Acetate Study [13] was a randomised, double-blind, placebo-controlled clinical trial which evaluated a number of MRI outcome measures in patients with relapsing remitting multiple sclerosis treated with GA. The rate of formation of 'black holes' was compared in the two treatment groups [14]. This required serial MRI imaging to distinguish new hypointense T1 lesions that extinguished over time from those that persisted. When extinction occurs, this usually happens between five and twelve months after the initial appearance of the lesion. In the European/

Table 1 Magnetic resonance imaging (MRI measures) and effects of glatiramer acetate (GA). * Except in one study, where no significant effect was observed

MRI Measure	Disease process	Disease measure	Disability correlation	Effect of GA	Reference
Gadolinium-enhancing T1 lesions	Acute inflammatory activity	Global	Weak	Reduced number of new lesions	[13, 50]
T2 lesions	Oedema with moderate demyelination, gliosis and axonal loss	Global	Weak	Reduced lesion load	[13, 50]
'Black holes' (T1 hypointense lesions)	Significant demyelination with severe tissue damage, gliosis and axonal loss	Global	Strong	Decreased rate of formation	[14]
Magnetic resonance spectroscopy (MRS)	Axonal and neuronal injury, irreversible axonal loss and reduced cerebral volume	Focal	Strong	Decreased rate of decline	[26–29, 34]
Magnetisation transfer ratio (MTR)	Oedema, demyelination, axonal loss, severe pathological destruction	Focal	Strong	Reduced signal	[26, 40]
Brain atrophy measures	Tissue loss (axons, neurons, myelin)	Focal	Strong	Decreased rate of atrophy*	[18, 41, 42, 51]

Canadian study, the rate of formation of 'black holes' started to decline in the GA treatment group from the sixth month onward, whilst it remained constant in the placebo group. During the seventh and eighth month, the rate of formation was significantly lower in the GA treatment group (Fig. 7), corresponding to a 50 % reduction in the rate of conversion during the last month. Out of the 275 new lesions which were identified as T1 hypointense lesions at Month 1 and were followed up for eight months, 44 lesions in the placebo group and 21 lesions in the GA-treated groups remained hypointense and could be classified as permanent 'black holes' after nine months of treatment.

A more recent study used a combined magnetic resonance spectroscopy (MRS) and magnetisation transfer ratio (MTR) approach to address irreversible tissue damage [26]. The MRS signal, measured as the N-acetylaspartate/creatinine ratio, is thought to be a marker of axonal and neuronal integrity, whereas the MTR signal is considered a marker of the integrity of the myelin sheath. The study compared fourteen patients treated with high-dose β-interferon to fifteen treated with GA. Changes in MRS and MTR signals after one year of treatment were compared after one year of treatment. The MRS signal in a volume of interest centred on the corpus callosum increased by 7.8 % ($p = 0.03$) in the GA treatment group whilst no significant change (+1.2 %) was observed in the β-interferon group. Concerning the MTR signal measured over the whole brain, this increased in both treatment groups (+2.78 % in the GA group and +1.94 % in the β-interferon group), but the change from baseline was only significant in the GA treatment group ($p = 0.01$). Again, these results suggest that treatment with GA can have a neuroprotective effect in patients with multiple sclerosis, which may have a relevant clinical impact on the rate of progression of disability.

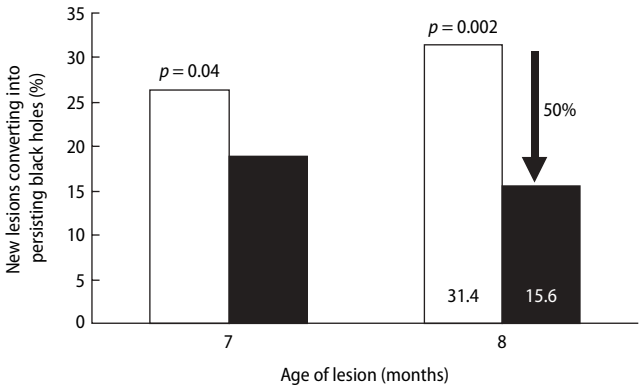


Fig. 7 Number of new persistent T1 hypointense lesions ('black holes') observed in the seventh and eighth treatment month in the European/Canadian Glatiramer Acetate Study. Open columns: placebo group; filled columns: glatiramer acetate treatment group. Adapted from Filippi et al. [14]

Glatiramer acetate and neuroprotection: clinical data

With respect to data on GA and neuroprotection, the highest level of clinical relevance, and the most important one, relate to data from clinical trials incorporating specific markers of neuroprotection into their design. At the moment, there are no such definitive data with GA. Nonetheless, prospective analyses of the data from the United States pivotal trial of GA in relapsing remitting multiple sclerosis and its long-term extension have provided important clues that treatment with GA can effectively slow down progression of disability [15, 22, 23], which would be the expected clinical correlate of a neuroprotective effect. Survival analysis of the three-year outcome data from this trial (Fig. 8) revealed that there was a highly significant reduction in the percentage of patients whose disability worsened by more than 1.5 EDSS steps in the GA treatment group (21.6 % of patients) compared to the placebo group (41.6 %; $p = 0.001$).

Other important data from the pivotal trial and its open-label long-term extension showed that a high pro-

portion of patients remained stable or improved in terms of EDSS disability scores. For the original GA treatment group, this proportion was 81.6 % at the end of the double-blind, placebo-controlled period [15, 22, 23] and 64.4 % for those patients continuously treated with GA for an average of ten years [15, 22, 23]. An interesting aspect of these data was that patients who were originally randomised to placebo never caught up the gap in disability progression once they were switched to active GA treatment, which provides a hint that early treatment may be important in preventing disability.

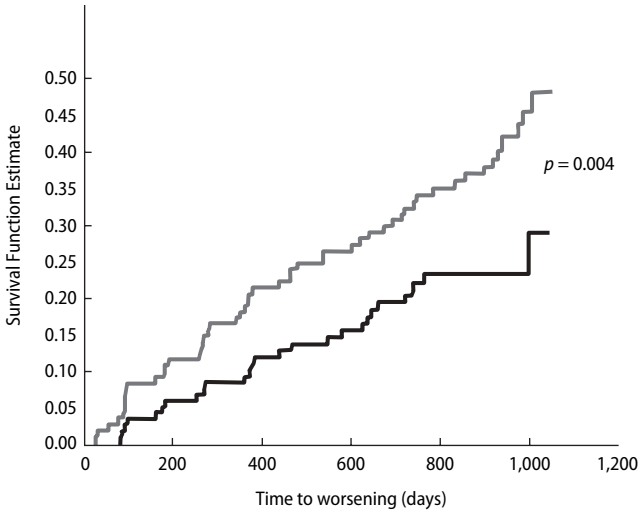


Fig. 8 Survival analysis of patients reaching a threshold of deterioration by 1.5 points in the US pivotal trial of glatiramer acetate. Grey: placebo group; black: glatiramer acetate treatment group. Adapted from Johnson et al. [23], with permission

Early treatment is the object of a current randomised, placebo-controlled, prospective clinical trial of GA in subjects presenting with a clinically isolated syndrome (CIS), the PreCISe study (cited in [33]). After randomisation to GA or placebo, patients are followed for 36 months or until conversion to clinically definite multiple sclerosis occurs, after which all patients have the opportunity to continue GA treatment in an open-label extension until five years from randomisation. The PreCISe study includes a number of ancillary trials that address prospectively the issue of neuroprotection using a variety of MRI parameters as well as clinical variables. The double-blind phase of this study will end in late 2008, which means that the first, preliminary results may be expected in early 2009. Given that GA has shown efficacy in more severe and advanced types of disease (RRMS)

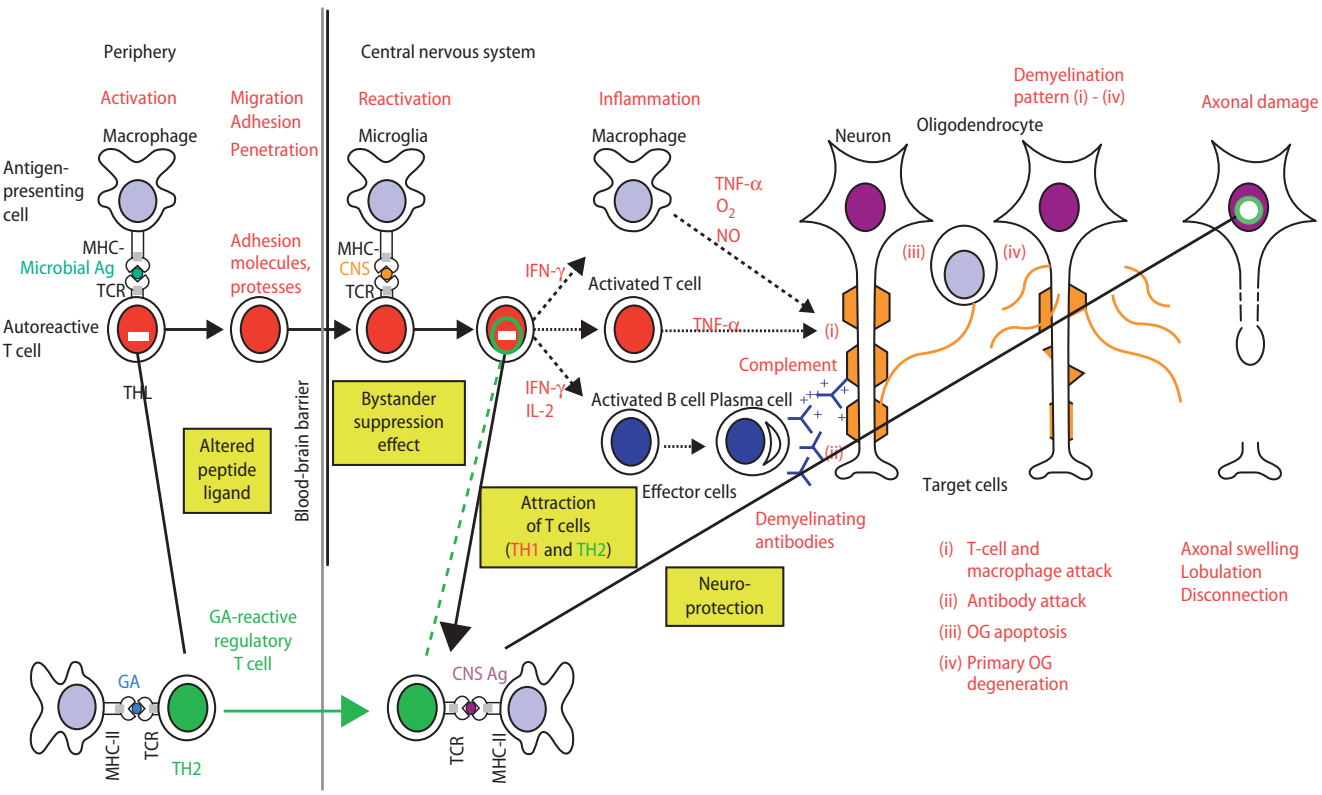


Fig. 9 Mechanism of action of glatiramer acetate in multiple sclerosis. The different mechanisms identified to date are indicated in the yellow boxes. Red: pro-inflammatory T cells; pale blue: macrophages; green: glatiramer acetate-reactive T cells: dark blue: B cells; purple: neurones; orange: oligodendrocytes. Reprinted from Neuhaus et al. [35] with permission from Elsevier. Copyright 2007

[13, 22, 32], it is reasonable to expect similar efficacy in a less severe disease type such as CIS.

Conclusions

Research into the mechanism of action of GA has been extremely productive over the last decade and provided many fascinating insights into how this agent may inhibit disease activity and slow progression in relapsing remitting multiple sclerosis. The different pieces of the puzzle can be aligned along two principal themes, immunomodulation and neuroprotection (Fig. 9). The immunomodulatory components involve effects on APCs, antagonism of the effects of myelin-related antigens on immune cells, phenotypic switch of T cells, secretion of anti-inflammatory cytokines and bystander suppression in the nervous system. The neuroprotective components involve effects on neurotrophin secretion, axonal protection and myelin repair. Information from MRI studies and clinical trials have helped demonstrate the pertinence of these different actions for the management of patients with multiple sclerosis. Of course, many pieces of the puzzle are still missing, and the questions which are still open will hopefully be answered in the next months or years.

Disclosure François Blanchette is employed by Teva. Oliver Neuhaus received honoraria for speaking from Teva, Bayer-Schering, Merck-Serono.

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