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Neurogenesis and Neuroprotection in the CNS — Fundamental Elements in the Effect of Glatiramer Acetate on Treatment of Autoimmune Neurological Disorders

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Abstract Multiple sclerosis (MS) is no longer considered to be simply an autoimmune disease. In addition to inflammation and demyelination, axonal injury and neuronal loss underlie the accumulation of disability and the disease progression. Specific treatment strategies should thus aim to act within the central nervous system (CNS) by interfering with both neuroinflammation and neurodegeneration. Specific treatment strategies to autoimmune neurological disorders should aim to act within the CNS by interfering with both neuroinflammation and neurodegeneration. The cumulative effect of Glatiramer acetate (GA; Copaxone®, Copolymer 1), an approved drug for the treatment of MS, reviewed herewith, draws a direct linkage between anti-inflammatory immunomodulation, neuroprotection, neurogenesis, and therapeutic activity in the CNS. GA treatment augmented the three processes characteristic of neurogenesis, namely, neuronal progenitor cell proliferation, migration, and differentiation. The newborn neurons manifested massive migration through exciting and dormant migratory pathways, into injury sites in brain regions, which do not normally undergo neurogenesis, and differentiated to mature neuronal phenotype, thus, counteracting the neurodegenerative course of disease. The plausible mechanism underlying this multifactorial effect is the induction of GA-reactive T cells in the periphery and their infiltration into the CNS, where they release immunomodulatory cytokines and neurotrophic factors in the injury site.

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Introduction

Neurodegenerative diseases are characterized by significant axonal and neuronal pathology. A case in point is multiple sclerosis (MS), which was historically considered to be a genuine autoimmune demyelinating disease, but is now recognized as a complex disease involving not only disturbances to the peripheral immune system [1], but also damage to oligodendrocytes, neurons, and axons [2–4]. Axonal and neuronal degeneration, initiated at disease onset and revealed when compensatory central nervous system (CNS) resources are exhausted, are major determinants of the irreversible neuronal disability. The interactions between the immune cells, neurons, and glia in the CNS are highly complex, and their understanding is necessary for the development of more rational treatment strategies [5]. Of particular interest is the potential to modulate these interactions in a specific manner, so as to prevent disease activity and arrest its progress. Furthermore, efforts at present are directed toward modalities that may also lead to neuroprotection and neurogeneration and thus alleviate existing disease impairment.

Autoimmune diseases are traditionally viewed as an outcome of malfunctioning of the immune system in which the immune cells react against the body's own proteins or organs [6]. In MS, and in its animal model, experimental autoimmune encephalomyelitis (EAE), the induction of immune cells of the T-helper 1 type (Th1), which secrete

pro-inflammatory cytokines against myelin components in the CNS, initiates a detrimental cascade of events and results in the formation of the sclerotic plaques [1]. Intervention with immunomodulatory agents may bring about the induction of regulatory mechanisms leading not only to a reduction of the inflammatory autoimmune response, but also to a neuroprotective effect, which counteracts the neurodegenerative process. Such a modulatory process within the CNS is central to the therapeutic control of MS [5]. An even more desirable strategy for approaching CNS diseases, including MS, would be the induction of regenerative processes leading to neurogenesis. Although historically the general belief has been that the adult CNS of mammals has very limited regenerative capacity [7], it was demonstrated already four decades ago that new functional neurons were constantly generated from neural stem cells in some restricted areas of the mammalian brain throughout life [8]. Moreover, a large body of evidence has emerged in recent studies for the existence, in many regions of the adult CNS, of stem cells with the potential to give rise to new neurons that reside [9–11]. These findings raise the possibility that endogenous neural stem cells could be mobilized, spontaneously or by the application of therapeutic strategies, to replace dying neurons in neurodegenerative diseases.

The currently available treatments for MS are directed mainly toward the alleviation of the autoimmune response. These include immunosuppressive agents such as Immuran or Azathioprine, which are applied in severe cases [12], but more commonly agents acting as immunomodulators are employed. The most widely used immunomodulatory agents are several recombinant versions of interferon beta (IFNβ), namely, the IFNβ−1b Betaferon [13], the IFNβ-1a Avonex [14] and Rebif [15], and the drug glatiramer acetate (GA, a synthetic copolymer known also as Copolymer 1 and Copaxone®), which was developed in our laboratory [16].

In this review article, we summarize our current understanding of the mechanism by which GA induces its therapeutic effect. We demonstrate that in addition to its anti-inflammatory immunomodulatory effect on the autoimmune process, GA augments neurotrophin (NT) expression, thus leading to neuroprotection and lessening of the neuronal/axonal damage. Furthermore, this peripheral immunomodulatory treatment enhances the naturally occurring neurogenesis and leads to the expansion of newly formed neuroprogenitor cells and their differentiation into mature neurons.

In Vivo Manifestations of GA

GA is a standardized, randomized mixture of synthetic polypeptides consisting 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 [17]. GA has long been known to have both a suppressive and a protective effect in EAE induced in different species: guinea pigs, rabbits, various mouse strains, and two kinds of monkeys [16–18]. In addition, GA ameliorates EAE induced by several encephalitogenic antigens, such as myelin basic antigen (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) or peptide thereof. Treatment with GA decreases the incidence and the severity of the disease and postpones its onset when administered by different routes and schedules. Daily injections of GA for 8–10 days drastically reduced the clinical manifestation, almost to complete disappearance, when started either immediately after disease induction (prevention), or when the disease was fully developed (suppression), and even in the late chronic phase of the disease (delayed suppression). The therapeutic effect of GA in MS was extensively reviewed in several articles [19, 20], which summarize various clinical trials; these indicated that GA slows the progression of disability and reduces both relapse rate and magnetic resonance imaging-defined disease activity in MS patients.

Peripheral Immunomodulation

The mechanism by which GA induces its beneficial effect in animals and in patients was extensively investigated over the years in several laboratories [16, 18]. These studies demonstrated that GA exerts its therapeutic activity by immunomodulating various levels of the immune response, which differ in their degree of specificity. The initial step is the binding of GA to major histocompatibility complex (MHC) molecules. GA undergoes a very rapid, high level, and efficient binding to various MHC class II molecules on murine and human antigen presenting cells (APCs) and even displaces peptides from the MHC binding site [21]. This competition for binding to the MHC can consequently lead to inhibition of various pathological effector functions. Recently, several groups have observed that GA treatment leads to generalized alterations of various types of APCs. Specifically, GA changes the properties of dendritic cells and monocytes, so that they stimulate Th2-like responses [22]. The modulation on the level of the innate immune system is the least specific step and can be beneficial for the modulation of detrimental immune responses to various antigens including in transplanted grafts [23]. However, in addition to the MHC blocking, in the case of the MS immunodominant encephalitogenic epitope of MBP-peptide 82-100, GA has been shown to act by T cell receptor antagonism as an altered peptide ligand, in a strictly antigen-specific manner [24]. This interference is already indicative of the specificity of this agent in the therapy for MS/EAE.

The above activities, however, do not necessarily play an essential role in the modulation of MS and EAE *in vivo*

because it is unlikely that GA can reach to the CNS in sufficient amounts to compete efficiently with the relevant myelin antigens *in situ*. It is therefore likely that, in CNS diseases, additional immunomodulatory mechanisms/factors that can access the blood–brain barrier (BBB), mediate the therapeutic activity of GA. We have previously demonstrated that GA-treated animals (either by subcutaneous injections or by oral administration) develop GA-specific T cells in the peripheral immune system. These cells can adoptively transfer protection against EAE [25]. Furthermore, T cell lines and hybridomas could be isolated from spleens of animals rendered unresponsive to EAE by GA [26]. Both cell types act as regulatory suppressor cells, as they inhibit both the *in vitro* response of MBP specific effector cells and the *in vivo* manifestation of EAE induced by different CNS antigens.

The GA-induced cells were characterized as Th2/3 cells secreting large amounts of anti-inflammatory cytokines such as interleukin (IL)-4, IL-10 and transforming growth factor (TGF)-β, but not Th1 cytokines, in response to both GA and MBP [27]. Other myelin antigens such as PLP and MOG were incapable of activating the GA-induced cells to secrete Th2/3 cytokines. Yet, EAE induced by PLP or MOG can be suppressed by GA and by GA-induced cells, probably by "bystander mechanisms" [28]. Furthermore, a shift from a Th1-biased cytokine profile toward a Th2-biased profile has also been observed in GA-treated MS patients [29–31], indicating that such GA-specific cells are indeed involved in its therapeutic effect. In the above studies, the presence of GA-induced Th2 regulatory cells was demonstrated only in the periphery, namely, in the spleens and lymph nodes of experimental animals, or in the peripheral blood mononuclear cells in humans. More recent studies show that such regulatory T cells function also in the organ in which the pathological processes of EAE and MS occur, as described in the following.

In Situ Immunomodulation in the CNS

The existence in the CNS of GA-specific T cells, induced in the periphery either by injection or by oral treatment with GA, was demonstrated by their actual isolation from brains of actively sensitized GA-treated mice, and by the localization of GA-specific cells in the brain after their passive transfer to the periphery. Thus, a specific ex vivo reactivity to GA, manifested by cell proliferation and by Th2 cytokine secretion, was observed in whole lymphocyte population obtained from brains of EAE-induced mice treated by GA parenterally [32] or orally [33]. Moreover, highly reactive GA-specific T cell lines that secrete *in vitro* IL-4, IL-5, IL-10, and TGF-β in response to GA and cross-react with MBP at the level of Th2 cytokine secretion were obtained from both brains and spinal cords of GA-treated mice. In contrast, no reactivity to the control antigen lysozyme could be obtained in lymphocytes isolated from the CNS of mice injected with lysozyme. Furthermore, an *in situ* immunomodulatory effect, induced by GA treatment in the brains of EAE-induced mice, was manifested by a decrease in the level of the inflammatory cytokine IFN-γ and by the secretion of the anti-inflammatory cytokine IL-10, in response to the encephalitogen MBP.

The ability of the GA-specific T cells from the periphery to cross the BBB and accumulate in the CNS was confirmed by the adoptive transfer of fluorescently labeled GA-specific cells (intraperitoneally) and their subsequent detection in the brain. Indeed, the GA-specific cells were present in the brain 7 and even 10 days after their injection to the periphery [32, 33], whereas lysozyme-specific cells (serving as control) were absent in the CNS in those times. Hence, GA-specific cells induced either actively by immunization with GA, or passively by adoptive transfer, penetrate and accumulate in the CNS. There is currently a consensus that the brain is not an immune-privileged site and that activated T cells, regardless of their specificity, cross the BBB. However, T cells specific to non-CNS antigens (as in the case of lysozyme-specific T cells) subsequently migrate back or die, whereas T cells specific to CNS antigens can be stimulated *in situ* and persist. The presence of the GA-specific cells in the brain can possibly be attributed to their cross-reactivity with MBP [27, 28]. While this cross-reactivity may not be essential for the GA-specific cells to reach the brain, it may still enable their *in situ* activation.

Once the presence of the GA-specific Th2 cells in the CNS was confirmed, their ability to actually function as suppressor cells in the diseased organ and secrete anti-inflammatory cytokines *in situ* had to be verified. Using APCs isolated from the brain, it was found that the Th2 secretion in response to both GA and MBP could be supported by CNS and by peripherally originated APCs [33]. The reactivity of the GA-induced T cells in the CNS was studied using a double labeling approach in which pre-labeled specific T cells are adoptively transferred and their expression in the brain subsequently detected immunohistologically, thus allowing the tracing of the cytokines secreted by a specific subset of T cells on the level of the whole CNS tissue [34]. As demonstrated in Fig. 1, the GA-specific cells in the brain manifested intense expression of the two potent regulatory anti-inflammatory cytokines IL-10 and TGF-β, but showed no trace of the detrimental inflammatory cytokine IFN-γ. Of special interest is the finding that IL-10 and TGF-β were expressed not only by the GA cells but also by CNS resident cells in their vicinity such as astrocytes. In contrast, the overall expression of IFN-γ was drastically reduced [34]. These results indicate that the GA-specific T cells induce a bystander immunomodulatory effect on the CNS resident cells themselves. IL-10 is a potent regulatory cytokine in autoimmunity that inhibits Th1 cells and macrophage

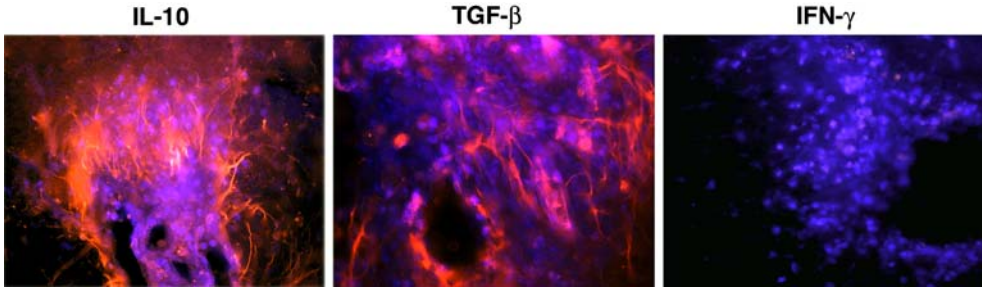

Fig. 1 Immunohistochemical analysis of cytokines expression by GA-specific cells in the brain. Activated Hoechst-labeled, GA-specific cells were injected into the peritoneum of EAE induced mice. After 7 days, the mice were perfused, and brain sections were stained immunocytochemically for IL-10, TGF-β, and INF-γ. Merged images of sections depicted GA specific T cells (*blue*) and their immunohistological cytokine expression (*red*), manifesting extensive expression of IL-10 and TGF-β but not a trace of INF-γ. Note that IL-10 and TGF-β are expressed not only by the GA labeled cells but also by unlabeled cells within their vicinity. These surrounding bystander cells have elongated astrocyte-like morphology, and their astrocyte nature was further corroborated, indicating bystander effect of the GA-cells on the CNS resident cells

activation, in addition to its effector multifunctional therapeutic reactivity [35]; TGFβ is known for its ability to suppress cytotoxic T cell response and to reduce tumor necrosis factor α and IFN-γ and other factors that contribute to myelin damage [36]. Hence, the *in situ* ability of GA-specific infiltrating cells to express and induce the expression of these potent modulating cytokines in the CNS cells may indeed contribute to GA therapeutic activity by restraining the inflammatory pathological process. Nevertheless, the effect of GA is not restricted to anti-inflammation, but involves more specific CNS-related effects.

Neuroprotection and Augmentation of Neurotrophic Factors in the Brain

In addition to the secretion of Th2/3 cytokines, GA-specific T cells produce the potent NT brain-derived neurotrophic factor (BDNF), as demonstrated for T cell lines from peripheral mouse [37] and human [38, 39] origin. The *in vitro* production of BDNF by GA-induced cells—by whole lymphocyte populations and by T cell lines, originating from both the CNS and the periphery—was shown at the protein and the messenger RNA (mRNA) levels [40]. Furthermore, GA-specific T cells demonstrated extensive BDNF staining in brains of EAE mice that had been adoptively transferred with GA cells, in contrast to the low (background) expression in the corresponding brain region of EAE-untreated mice [34]. Members of the NT family such as BDNF, NT-3, and NT-4 are important regulators of neuronal function and survival [41, 42]. Besides their well-established role in neuronal development, process growth, and regulation of synaptic plasticity, they have the capacity to protect neurons against various pathological insults. NTs also affect the regeneration of mature oligodendrocytes and oligodendroglial precursors and consequently improve myelin repair [43]. BDNF, in particular, was shown to rescue degenerating neurons, promote axonal outgrowth, remyelination, and regeneration [42, 44]. Hence, modulation of NTs, especially BDNF, in the CNS is of major therapeutic consequence. Therefore, the accumulation of GA-specific cells that express BDNF in the brain *in situ* is highly significant.

BDNF was elevated not only in brains of adoptively transferred mice but also in brains of mice that were injected daily (subcutaneously) with GA as such [40], similar to the practice used in the treatment of MS patients. Hence, in mice with untreated MOG peptide-induced EAE, there was a reduction in BDNF expression (compared to naive mice) in various brain regions, e.g., the cortex, the striatum, and the accumbens nucleus. In contrast, in EAE mice treated with GA, starting at various stages of the disease, BDNF expression, as demonstrated on the protein level by immunohistochemistry and on the mRNA level by *in situ* hybridization, was significantly higher and similar to its level in normal mice (Fig. 2). It should be noted that in the early phase of disease, there was a slight elevation of BDNF in certain brain regions such as the cortex, compared to naive mice, reflecting naturally occurring self-repair mechanisms [45]. Yet, as the disease progresses to chronic EAE, the level of BDNF declined drastically, much below that of the naive control, indicative of the impairment caused by the disease. The chronic disease phase in EAE/MS is regarded as the stage in which exhausted self-compensating neuroprotection fails, and extensive neurodegeneration takes place. Hence, the restoration of BDNF to the normal level, even when GA treatment started in this late stage, is of particular significance. BDNF is not the only neurotrophic factor affected by GA treatment; a similar phenomenon was found for two additional neurotrophic factors—NT3 and NT4 [40]. Reduced levels of BDNF in the serum and the cerebral spinal fluid of MS patients, and its reversal by GA treatment, have been reported recently [46], indicating that this effect of GA is relevant to human therapy as well.

The plausible candidates for mediating this effect are the Th2/3 GA-specific T cells induced in the periphery by GA

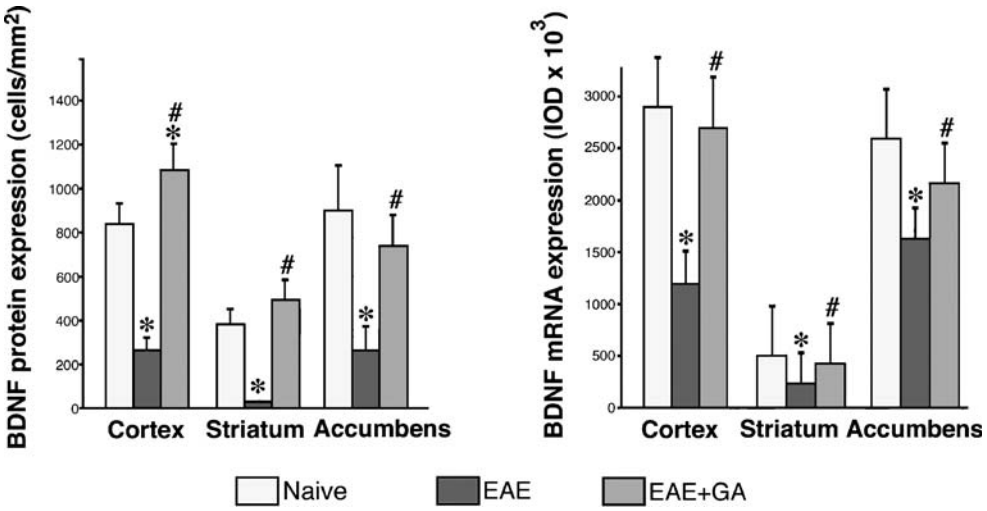

Fig. 2 BDNF expression in brains of EAE-induced mice at the chronic disease phase—60 days after induction—and the effect of GA treatment. Immunohistochemical analysis of BDNF protein level (*left*) and *in situ* hybridization of BDNF mRNA (*right*). BDNF in EAE untreated mice declined below that of the naive control, whereas GA treatment (10 daily injections 2 mg/mouse, starting at day 45) restores it to the normal level. Quantitative analysis of protein expression was performed by counting positively stained cells and of mRNA by measuring integrated optical density (*IOD*). *Asterisk* significant effect over naive control, *number sign* significant effect over EAE-untreated mice

treatment [27, 28] and shown to produce BDNF [37–40], penetrate the CNS [32, 33], and express BDNF *in situ* [34]. Yet, most of the NT-positive cells were CNS resident cells—neurons and astrocytes [40]. It has been demonstrated that diverse CNS cell types, glial and neurons, produce NTs [41, 42], which are stored in vesicles and undergo regulated release [44]. Hence, similarly to its bystander effect on the expression of the anti-inflammatory cytokines IL-10 and TGF-β [34], GA augments NTs expression by the CNS resident cells themselves. It was claimed that anti-immunomodulatory treatments might distract the beneficial neuroprotective consequence of inflammation—neurotrophic factor release from the infiltrating immune cells. Yet, in the GA-treated mice, BDNF positive astrocytes co-expressed IL-10 but not IFN-γ, contradicting the association between NTs production and inflammatory activity in EAE/MS [45, 47].

The elevated immunoreactivity due to treatment with GA could result from either the augmentation of NTs synthesis and the expansion of new producing cells, or higher uptake and redistribution of NTs, originally released from neighboring cells. The fact that elevated expression was found also on the level of mRNA using *in situ* hybridization is supportive of a genuine enhanced NTs synthesis. Because NT receptors such as the full-length BDNF receptor, tyrosine kinase receptor B, have been found in neurons in the vicinity of MS plaques and in reactive astrocytes in MS lesions [47], it is possible that their elevated production is actually of functional relevance and counteracts the neurodegenerative disease course.

These studies indicate that peripheral immunomodulatory treatment can restore impaired NT expression in the CNS. The therapeutic manifestation of this effect is the actual protection/preservation of the CNS population and reduction of the axonal/neuronal damage [48, 49]. Hence, histopathological analysis of brains from MOG peptide-induced EAE (untreated) mice revealed multiple neuronal malformations manifested in axonal transection, sparse processes, and fiber deterioration. Furthermore, multiple widespread lesions were observed in various brain regions, indicative of considerable neuronal and axonal loss. An additional deformation in cell morphology in EAE mice was enlargement and swelling of the neuronal cell body accompanied by margination of the nucleus. In contrast, in brains of EAE mice treated with GA, considerably less damage was detected, revealing fewer deteriorating fibers, reduced amount of lesions with smaller magnitude and lower number of marginized cell nuclei. The beneficial effect of GA was manifested even when treatment started long after the appearance of disease—in the chronic disease phase, thus indicating actual repair mechanisms. Furthermore, a thin layer of fibers was frequently observed over the lesions in the GA-treated animals, suggesting surviving filaments or axonal sprouting and regeneration. These effects may point to an ongoing process of neurogenesis.

Neurogenesis

According to several reports, new functional neurons are constantly generated from neuronal stem cells throughout life [50], and stem cells with potential to give rise to new neurons reside in many different regions of the mammalian brain [51, 52]. Thus, neurogenesis occurs and persists in the adult brain, where it may contribute to repair and recovery after injury. Indeed, in the neuroproliferative brain areas—the subventricular zone (SVZ) of the lateral ventricle and the hippo-

campus subgranular zone (SGZ)—multi-potent cells manifest increased proliferation and migration in pathological situations. Moreover, progenitor cells from the SVZ that migrate through the rostral migratory stream (RMS) to the olfactory bulb can be triggered to differentiate into astrocytes and neurons [53]. Although brain insults such as cerebral ischemia [54], apoptosis [55], or autoimmune inflammatory demyelination [53] were shown to enhance self-neurogenesis, its therapeutic significance is limited, as it fails to regenerate functional neurons that compensate for the pathological damage. Therapeutic strategies are therefore contemplated to promote neurogeneration processes [56].

In our own studies, we intended to elucidate the effect of EAE induction on neurogenesis and the resulting differentiation toward the neural lineage. An additional goal was to investigate whether peripheral immunomodulatory treatment by GA injections, at different stages of the disease, can augment the neurogenesis process. To that end, the studies were performed on MOG peptide-induced EAE [48], in both C57BL/6 mice and in yellow fluorescent protein (YFP 2.2) transgenic mice, which selectively express YFP on their neuronal population [57].

The combination of two detection markers allowed the evaluation of the number of new neurons—the overall expression of the immature marker double-cortin (DCX) and enumeration of the cells emerging during the concurrent bromodeoxyuridine (BrdU)/GA injection [48]. Those BrdU/DCX dual-stained cells were the cells that actually differentiated into the neuronal lineage after the GA administration. Employing this approach, it was demonstrated that, indeed, EAE induction as such triggered increased neuroprogenitor proliferation in the neuroproliferative zones (SVZ and SGZ) of the brain after disease appearance, but this effect was of short duration and subsequently declined to levels below that of the naive control mice. This indicates that the impairment inflicted by the disease cannot be compensated just by the self-neurogenesis. In contrast, GA treatment, applied at various phases of this chronic EAE model, augmented the neuronal proliferation in both SVG and SGZ to a higher level than that observed in the EAE mice, and this effect persisted for a prolonged duration. Of special significance is the neuroproliferative consequence of GA treatment when initiated in the chronic phase of the disease because this phase in EAE/MS is regarded as the stage in which exhausted self-compensating neurogenesis fails and extensive neurodegeneration overcomes [58, 59].

Neuroprogenitors originating in the SVZ were mobilized into the route in which they normally migrate in adults, namely, the RMS. This mobilization was somewhat increased in EAE mice, but GA treatment augmented it even further. The therapeutic relevance of this effect is implied by the enhanced neuronal migration found in EAE mice, which exhibited slight, short-term disease and spontaneous recovery. Still, in the GA-treated mice, the neuroprogenitor migration was not confined to the RMS and was observed in other brain regions, e.g., the lateral cortical stream—the neuronal migratory route naturally found in the embryonic forebrain [60]. Furthermore, neuronal progenitors diverged from the classic neuroproliferative zones and the migratory streams and spread to adjacent atypical brain regions that do not normally undergo neurogenesis such as the striatum, nucleus accumbens, and cortex.

At early time points after GA and BrdU injection (1 to 10 days after their last injection), $BrdU^+$ neuroprogenitors expressed the immature neuronal marker DCX, characteristic of migrating and differentiating neurons [60, 61], and displayed migratory morphology [62]. It has been doubted whether progenitor cells retain their ability to proliferate after leaving the neuroproliferative zones [63]. But in EAE mice treated by GA, we did find small clusters of BrdU/DCX co-expressing cells in the striatum and in the nucleus accumbens, suggesting local divisions. Furthermore, staining with phosphorylated histone (an endogenous marker of cells in M phase) indicated that some DCX^+ cells in these regions had indeed divided just before perfusion, suggesting *in situ* proliferation outside the classic neuroproliferative zones. At a later time point (1 month after completion of GA treatment), DCX^+ cells with branching processes and $BrdU^+$ cells expressing the mature neuronal marker NeuN and displaying mature morphology were found (Fig. 3a). Thus, the three processes comprising neurogenesis: neuronal proliferation, migration, and differentiation were increased after GA treatment.

The most striking and significant findings were that the newly generated neurons were attracted or recruited to regions of damage manifesting massive migration through existing and dormant migration pathways, into injury sites in brain regions, which do not normally undergo neurogenesis (Fig. 3b). Clusters of newly formed cells co-expressing the immature or the mature neuronal markers (BrdU/DCX and BrdU/NeuN) were situated in areas with deteriorating fibers and neuronal loss, around the margins and inside lesions in various brain regions such as the striatum, cortex, and nucleolus accumbens (Fig. 3c). BrdU/NeuN dual-stained cells were not found in corresponding regions of naive mice, confirming that neurogenesis does not normally occur in the adult rodent cortex. But, directed migration of new neurons toward injury sites had already been demonstrated after cerebral ischemia [54]. Our own results demonstrated also that in untreated EAE mice, a few DCX positive cells can be identified. Still, although lesions in EAE mice treated with GA were less extensive, the number of progenitors migrating into them was drastically higher. Moreover, in lesions occupied by the DCX-stained cells, fibers extended into lesions, suggesting induction of axonal regeneration or sprouting, by the neuroprogenitor (Fig. 3c). These newly formed neurons could constitute a pool for the replacement

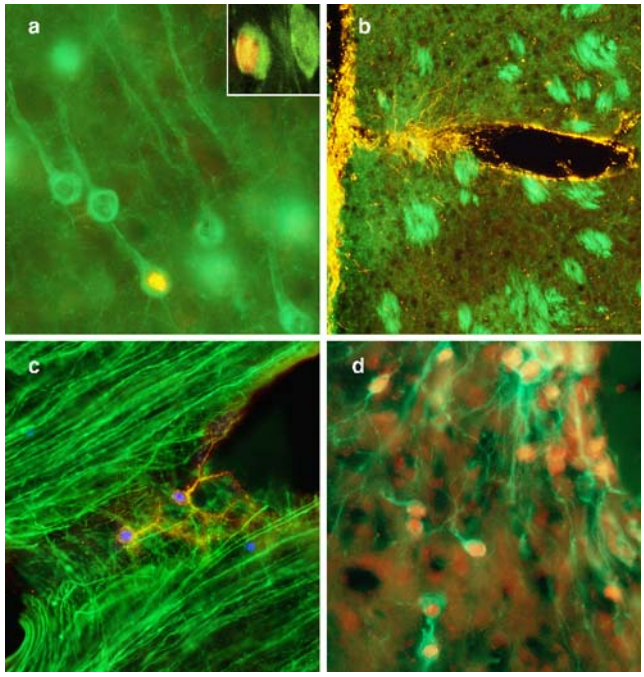

Fig. 3 Fate tracing of neuronal progenitor cells generated in the course of GA treatment in EAE mice. **a** Pyramidal neuron in the cortex (occipital, layer 6), born during the concurrent injections of the proliferation marker BrdU and GA, to an EAE-induced transgenic mouse that selectively express YFP on its neuronal population. One month after completion of GA treatment, neurons co-expressing BrdU (*yellow*) and YFP (*green*) with apical dendrites and axons are seen, indicative of mature functional neurons. *Inset*, BrdU positive cell (*yellow*) co-expressing the mature neuronal marker NeuN (*green*). **b** Migration of neuronal progenitors toward injury sites. One month after completion of GA treatment, cells expressing the immature neuronal marker DCX (*orange*) are migrating from the RMS toward a lesion in the striatum. **c** Penetration of neuronal progenitors into injury sites. Neuroprogenitors born during the concurrent injections of BrdU and GA, co-expressing BrdU (*blue*) and DCX (*orange*) are depicted inside a lesion in the frontal cortex (layer 5/6), accompanied by axonal sprouting and extension of YFP-expressing neuronal fibers (*green*) into the lesion. **d** Neuronal progenitor cells expressing DCX (*green*) in brain of GA-treated EAE mice. The cells migrated to injured sites in the region of the nucleus accumbens and manifest extensive expression of the neurotrophic factor BDNF (*red*) that supports neuroprotection and regeneration of neural elements

of dead or dysfunctional cells and/or induce a growth-promoting environment that supports neuroprotection and axonal growth. The latter activity was evidenced by BDNF expression of the new neurons (Fig. 3d). It can thus be concluded that an immunomodulatory treatment can essentially induce neurogenesis and the formation of new neurons in sites of injury, thus counteracting the neurodegenerative course of disease.

Concluding Remarks

Despite major progress in the elucidation of the mechanism of neuronal death, in the context of neurodegenerative diseases, including MS, it has not been proven hitherto that the currently available drugs provide neuroprotective effect. Actually, until now it was suggested that such effects apply only to agents such as acetylcholine inhibitors in the context of Alzheimer disease. Drugs whose main activity mechanism is explicitly centered on neuroprotection have not demonstrated their efficacy, perhaps because of a lack of appropriate methodology for their evaluaxtion. In the case of MS or its animal model, EAE, the available treatment modalities lean mainly on immunomodulation and thereby the induction of an anti-inflammatory effect.

That said, there is nonetheless real hope that a remedial effect may be attained by promoting neuroprotection and neurogenesis, based on the known self-repair mechanisms that prevail during the disease. However, as presented in this review article, the neuroprogenitor proliferation in the animal model of MS, namely, in EAE mice, is elevated after disease induction but only for a short duration, thus indicating that the self-repair mechanisms as such are not sufficient and fail to overcome the neurodegenerative process. On the other hand, the immunomodulatory drug GA, which is one of the current treatments for MS, induces neuroprotection and augmentation of the self-neurogenesis triggered by the pathological process. This results in proliferation of neuroprogenitor cells, their migration through exciting and dormant migratory pathways, into injury sites in brain regions, which do not normally undergo neurogenesis, and differentiation to mature neuronal phenotype, thus counteracting the neurodegenerative disease course. Furthermore, studies in patients demonstrated that treatment with GA reduces the formation of permanent T1W “black holes,” which have been associated with irreversible neurological disability [64]. It was also shown recently, by quantifying the resonance intensity of the neuronal marker *N*-acetylaspartate that GA treatment leads to a significant increase in axonal integrity, suggesting axonal metabolic recovery and protection from sublethal axonal injury [65]. This may explain the long-term beneficial effect of GA in patients followed up for 20 years and more [66]. These studies support the notion that GA confers neuroprotection not only in the animal model but in MS as well. Finally, cumulative evidence reported by several laboratories dealing with animal models of some neuronal trauma [67, 68] and neurodegenerative diseases such as Parkinson’s disease [69] and atrophic lateral sclerosis [70] indicate that GA may have both neuroprotective and neurogenerative effect on a broader spectrum of neurodegenerative disorders. This may pave the way for the development of additional therapeutic modalities that might provide a more satisfactory solution for the treatment of neurological disorders.

References

1. Hellings N, Raus J, Stinissen P (2002) Insights into the immunopathogenesis of multiple sclerosis. Immunol Res 25:27–51
2. Kornek B, Lassmann H (1999) Axonal pathology in multiple sclerosis: a historical note. Brain Pathol 9:651–656
3. Bitsch A, Schuchardt J, Bunkowski S (2000) Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. Brain 123:1174–1183
4. Bjartmar C, Trapp BD (2001) Axonal and neuronal degeneration in multiple sclerosis: mechanisms and functional consequences. Curr Opin Neurol 14:271–278
5. Ziemssen T (2005) Modulating processes within the central nervous system is central to therapeutic control of multiple sclerosis. J Neurol 252(Vl)38–45
6. Feltkamp TEW (1999) The mystery of autoimmune diseases. In: The decade of autoimmunity. Elsevier, pp 1–5
7. Ramony Cajal S (1928) Degeneration and regeneration of the nervous system. Hafner, New York
8. Altman J, Das GD (1965) Autoradiographic and histological evidence of post-natal hippocampal neurogenesis in rats. J Comp Neurol 124:19–35
9. Gage FH (2000) Mammalian neural stem cell. Science 287:1433–1438
10. Kempermann G, Gage FH (2000) Neurogenesis in the adult hippocampus. Novartis Found Symp 231:220–235
11. Alvarez-Buylla A, Garcia-Verdugo JM (2002) Neurogenesis in adult subventricular zone. J Neurosci 22:629–634
12. Yudkin PL, Ellison GW, Ghezzi A Goodkin D, Hughe RA, McPherson K, Mertin J, Milanese C (1991) Overview of azathioprime treatment in multiple sclerosis. Lancet 338:1051–1055
13. Johnson KP, Knobler RL, Greenstein JL et al (1990) Recombinant beta interferon treatment of relapsing-remitting multiple sclerosis pilot study results. Neurology 40(Suppl 1):261
14. Jacobs LD, Cookfair DL, Rudick RA et al (1996) Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaboration Group (MSCRG). Ann Neurol 39:285–294
15. PRIM (Prevention of relapses and disability by interferon beta-1a subsequently in multiple sclerosis) study group (1998) Randomized, double blind, placebo controlled study of interferon beta-1a in relapsing-remitting multiple sclerosis: chemical results. Lancet 352:1498–1504
16. Arnon R (1966) The development of Cop 1 (Copaxone®), an innovative drug for the treatment of multiple sclerosis: personal reflections. Immunol Lett 50:1–15
17. Teitelbaum D, Meshorer M, Hirshfeld T, Sela M, Arnon R (1971) Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. Eur J Immunol 1:242–248
18. Arnon R, Sela M (2003) Immunomodulation by the copolymer glatiramer acetate. J Mol Recognit 16:412–421
19. Sela M, Teitelbaum D (2001) Glatiramer acetate in the treatment of multiple sclerosis. Expert Opin Pharmacother 2:1149–1165
20. Wolinsky JS (2006) The use of glatiramer acetate in the treatment of multiple sclerosis. Adv Neurol 98:273–292
21. Fridkis-Hareli M, Teitelbaum D, Gurevich E, Pecht I, Brautbar C, Kwon OJ, Brenner T, Arnon R, Sela M (1994) Direct binding of myelin basic protein and synthetic copolymer 1 class II major histocompatibility complex molecules on living antigen presenting cells-specificity and promiscuity. Proc Natl Acad Sci U S A 91:4872–4876
22. Farina C, Weber MS, Meinl E, Wekerle H, Hohlfeld R (2005) Glatiramer acetate in multiple sclerosis: update on potential mechanisms of action. Neurology 4:567–575
23. Arnon R, Aharoni R (2004) Mechanism of action of glatiramer acetate in multiple sclerosis and its potential for the development of new applications. Proc Natl Acad Sci U S A 101(Suppl 2): 14593–14598
24. Aharoni R, Teitelbaum D, Arnon R, Sela M (1999) Copolymer 1 acts against the immunodominant epitope 82-100 of myelin basic protein by T cell receptor antagonism in addition to major histocompatibility complex blocking. Proc Natl Acad Sci U S A 96:634–639
25. Lando Z, Teitelbaum D, Arnon R (1979) Effect of cyclophosphamide on suppressor cell activity in mice unresponsive to EAE. J Immunol 132:2156–2160
26. Aharoni R, Teitelbaum D, Arnon R (1993) T-suppressor hybridomas and IL-2 dependent lines induced by copolymer 1 or by spinal cord homogenate downregulate experimental allergic encephalomyelitis. Eur J Immunol 23:17–25
27. Aharoni R, Teitelbaum D, Sela M, Arnon R (1997) Copolymer 1 induces T cells of the T helper type 2 that cross react with myelin basic protein and suppress experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 94:10821–10826
28. Aharoni R, Teitelbaum D, Sela M, Arnon R (1998) Bystander suppression of experimental autoimmune encephalomyelitis by T cell lines and clones of the Th2 type induced by Copolymer 1. J Neuroimmunol 91:135–146
29. Miller A, Shapiro S, Gershtein R, Kinarty A, Rawashdeh H, Honigman S, Lahat N (1998) Treatment of multiple sclerosis with copolymer 1 (Copaxone©) implicating mechanisms of Th1 to Th2/3 immune deviation. J Neuroimmunol 92:113–121
30. Neuhaus O, Farina C, Yassouridis A, Wiendl H, Then Bergh F, Dose T, Wekerle H, Hohlfeld R (2000) Multiple sclerosis comparison of copolymer-1 reactive T cell lines from treated and untreated subjects reveals cytokine shift from T helper 1 to T helper 2 cells. Proc Natl Acad Sci U S A 97:7452–7457
31. Duda PW, Schmied MC, Cook S, Kriegler JI, Hafler DA (2000b) Glatiramer acetate (Copaxone©) induces degenerate, TH2-polarized immune response in patients with multiple sclerosis. J Clin Invest 105:967–976
32. Aharoni R, Teitelbaum D, Leitner O, Meshorer A, Sela, M, Arnon R (2000) Specific Th2 cells accumulate in the central nervous system of mice protected against EE by copolymer. Proc Natl Acad Sci U S A 97:11472–11477
33. Aharoni R, Meshorer A, Sela M, Arnon R (2002) Oral treatment of mice with copolymer 1 (glatiramer acetate) results in the accumulation of specific TH2 cells in the central nervous system. J Neuroimmunol 126:58–68
34. Aharoni R, Kayhan B, Eilam R, Sela M, Arnon R (2003) Glatiramer acetate specific T-cells in the brain express TH2/3 cytokines and brain-derived neurotrophic factor *in situ*. Proc Natl Acad Sci U S A 100(24):14157–14162
35. Bettelli E, Nicholson LB, Kuchroo VK (2003) IL-10, a key effector regulatory cytokine in experimental autoimmune encephalomyelitis. J Autoimmun 4:265–267
36. Morris MM, Dyson H, Baker D, Harbige LS, Fazakerley JK, Amor S (1997) Characterization of the cellular and cytokine response in the central nervous system following Semliki Forest virus infection. Neuroimmunology 74:185–197
37. Kipnis J, Yoles E, Porat Z, Cohen A, Mor F, Sela M, Cohen IR, Swhwartz M (2000) T cell immunity to copolymer 1 confers neuroprotection on the damaged optic nerve: possible therapy for optic neuropathies. Proc Natl Acad Sci U S A 97:7446–7451
38. Ziemssen T, Kumpfel T, Kinkert WEF, Neuhaus O, Hohlfeld R (2002) Glatiramer acetate-specific T-helper 1- and 2-type cell lines produce BDNF: implications for multiple sclerosis therapy. Brain-derived neurotrophic factor. Brain 125:2381–2391
39. Chen M, Valenzuela RM, Dhib-Jalbut S (2003) Glatiramer acetate-reactive T cells produce brain derived neurotrophic factor. J Neurol Sci 215:37–44
40. Aharoni R, Eylam R, Domev H, Labunsky G, Sela M, Arnon R (2005) The immunomodulator glatiramer acetate augments the

expression of neurotrophic factors in brains of experimental autoimmune encephalomyelitis mice. Proc Natl Acad Sci U S A 102(52):19045–19050
41. Lessman V, Gottmann K, Malcangio M (2003) Neurotrophin secretion: current facts and future prospect. Prog Neurobiol 69:341–374
42. Riley CP, Cope TC, Buck CR (2004) CNS neurotrophins are biologically active and expressed by multiple cell types. J Mol Histol 35:771–783
43. Althau HH (2004) Remyelination in multiple sclerosis: a new role for neurotrophins? Prog Brain Res 146:415–432
44. Murer MG, Yan O, Raisman-Vozari R (2001) Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol 63:7–124
45. Caggiula M, Batocchi AP, Frisullo G, Angelucci F, Patanella AK, Sancricca C, Nociti V, Tonali PA, Mirabella M (2005) Neurotrophic factors and clinical recovery in relapsing-remitting multiple sclerosis. Scand J Immunol 62:176–182
46. Azoulay D, Vachapova V, Shihman B, Miler A, Karni A (2005) Lower brain-derived neurotrophic factor in serum of relapsing remitting MS: reversal by glatiramer acetate. J Neuroimmunol 167:215–218
47. Stadelmann C, Kerscensteiner M, Misgeld T, Bruck W, Hohlfeld R, Lassmann H (2002) BDNF and gp145 trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? Brain 125:75–85
48. Aharoni R, Arnon R, Eilam R (2005) Neurogenesis and neuroprotection induced by peripheral immunomodulatory treatment of experimental autoimmune encephalomyelitis. J Neurosci 25 (36):8228–8217
49. Gilgum-Sherki Y, Panet H, Holdengreber V, Mosberg-Galili R, Offen D (2003) Axonal damage is reduced following glatiramer acetate treatment in C57/bl mice with chronic-induced experimental autoimmune encephalomyelitis. Neurosci Res 47:201–207
50. Luc DC, Song H, Colamarino SA, Ming G-L, Gage FH (2004) Neurogenesis in the adult brain: new strategies for central nervous system diseases. Annu Rev Pharmacol Toxicol 44:399–421
51. Van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in adult hippocampus. Nature 415:1000–1034
52. Picard-Riera N, Nait-Oumesmar B, Baron-Van Evercooren A (2004) Endogenous adult neural stem cells: limits and potential to repair the injured central nervous system. J Neurosci Res 76:223–231
53. Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, Evercooren AB (2002) Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. Proc Natl Acad Sci U S A 99:13211–13216
54. Jin K, Sun Y, Xie L, Peel A, Mao XO, Batteur S, Greenberg DA (2003) Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. Mol Cell Neurosci 24:171–189
55. Magavi SS, Leavitt BR, Macklis JD (2000) Induction of neurogenesis in the neocortex of adult mice. Nature 405:951–955
56. Chitnis T, Imitola J, Khoury SJ (2005) Therapeutic strategies to prevent neurodegeneration and promote regeneration in multiple sclerosis. Current Drug Targets Immune Endocrine and Metabolic Disorders 5:11–26
57. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR (2000) Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron 28:41–51
58. Bjartmar C, Wujek JR, Trapp BD (2003) Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. J Neurol Sci 15:165–171
59. Hobom M, Storch MK, Weissert R, Maier K, Radhakrishnan A, Kramer B, Bahr M, Diem R (2004) Mechanisms and time course of neuronal degeneration experimental autoimmune encephalomyelitis. Brain Pathol 14:148–157
60. Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK, Berwald-Netter Y, Denoulet P, Chelly J (1999) Doublecortin is a developmentally regulated microtubule-associated protein expressed in migrating and differentiating neurons. Neuron 23:247–256
61. Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 467:1–10
62. O'Rourke NA, Sullivan DP, Kaznowsky CE, Jacobs AA, McConnell SK (1995) Tangential migration of neurons in the developing cerebral cortex. Development 121:2165–2176
63. Gould E, Gross CG (2002) Neurogenesis in adult mammals: some progress and problems. J Neurosci 22(3):619–623
64. Filippi M, Rovaris M, Rocca MA, the European/Canadian Glatiramer Acetate Study Group (2001) Glatiramer Acetate reduces the proportion of new MS lesions evolving into "black holes." Neurology 57:731–733
65. Khan O, Shen Y, Caon C, Bao F, Ching W, Reznar M, Buccheister A, Hu J, Tselis A, Lisak R (2005) Axonal metabolic recovery and potential neuroprotective effect of glatiramer acetate in relapsing-remitting multiple sclerosis. Mult Scler 11:646–651
66. Ford CC, Johnson KP, Lisak RP, Panitch HS, Shifroni G, Wolinsky JS, the Copaxone Study Group (2006) Aprospective open-labled study of glatiramer acetate: over a decade of continuous use in multiple sclerosos patients. Mult Scler 12:309–320
67. Kipnis J, Yoles E, Cohen A et al (2000) T-cell immunity to copolymer 1 confers neuroprotection on the damaged optic nerve: possible therapy for optic neuropathies. Proc Natl Acad Sci U S A 97:7446–7451
68. Schori H, Kipnis J, Yoles E et al (2001) Vaccination for protection of retinalganglion cells against death from glutamate cytotoxicity and ocular hypertention: implication for glaucoma. Proc Natl Acad Sci U S A 98:3398–3403
69. Benner EJ, Mosley RI, Destache CJ et al (2004) Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. Proc Natl Acad Sci U S A 101:9435–9440
70. Angelov DN, Waibel S, Guntinas-Lichius O et al (2004) Therapeutic vaccine for acute and chronic motor neuron diseases: implications for amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 101:15823–15828