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Distinct pathological patterns in relapsing–remitting and chronic models of experimental autoimmune enchephalomyelitis and the neuroprotective effect of glatiramer acetate

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ABSTRACT

The respective roles of inflammatory and neurodegenerative processes in the pathology of multiple sclerosis (MS) and in its animal model experimental autoimmune encephalomyelitis (EAE) are controversial. Novel treatment strategies aim to operate within the CNS to induce neuroprotection and repair processes in addition to their anti-inflammatory properties. In this study we analyzed and compared the *in situ* pathological manifestations of EAE utilizing two different models, namely the relapsing–remitting PLP-induced and the chronic MOG-induced diseases. To characterize pathological changes, both transmission electron microscopy (TEM) and immunohistochemistry were employed. The effect of the approved MS drug glatiramer acetate (GA, Copaxone) on myelin damage/repair and on motor neuron loss/preservation was studied in both EAE models. Ultrastructural spinal cord analysis revealed multiple white matter damage foci, with different patterns in the two EAE models. Thus, the relapsing–remitting model was characterized mainly by widespread myelin damage and by remyelinating fibers, whereas in the chronic model axonal degeneration was more prevalent. Loss of lower motor neurons was manifested only in mice with chronic MOG-induced disease. In the GA-treated mice, smaller lesions, increased axonal density and higher prevalence of normal appearing axons were observed, as well as decreased demyelination and degeneration. Furthermore, quantitative analysis of the relative remyelination versus demyelination, provides for the first time evidence of significant augmentation of remyelination after GA treatment. The loss of motor neurons in GA-treated mice was also reduced in comparison to that of EAE untreated mice. These effects were obtained even when GA treatment was applied in a therapeutic schedule, namely after the appearance of clinical symptoms. Hence, the remyelination and neuronal preservation induced by GA are in support of the neuroprotective consequences of this treatment.

1. Introduction

In multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), the immune system provokes the detrimental process via autoimmune inflammatory mechanisms, leading to disseminated demyelination, which is the primary morphological hallmark of these diseases [1–4]. However, MS is currently recognized as a complex multifaceted disease with central role for axonal and neuronal pathology [5–8]. Permanent clinical disability is revealed when a threshold of neuronal loss is exceeded, and CNS compensatory resources are exhausted. Yet, increasing evidence indicates that axonal and neuronal injury begins at early disease stages, both in the white and in the gray matter areas, supporting degenerative disease course [5–9]. The relative roles of the inflammatory and the degenerative processes in MS pathology and the extent of neuronal loss, particularly in early disease phases, are highly controversial. Differences may be attributed to the heterogeneity of this disease and its variable patterns of CNS pathology [1,2]. In this respect, the use of different EAE models, obtained by immunizing susceptible strains with various myelin antigens, such as oligodendrocyte glycoprotein (MOG) or proteolipid protein (PLP), which mirror different aspects of the human pathology, may lead to deeper understanding [10,11].

Various aspects of axonal pathology have been demonstrated in EAE, such as axonal transaction, fragmentation, swelling, vacuolization, Wallerian-like degeneration, changes in neurofilament

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phosphorylation and in sodium channel distribution as well as transport deficits [8,11,12]. In addition, somatic neuronal pathology particularly of lower motor neurons (MNs) has also been implicated in EAE, although contradictory information on its extent and kinetics has been indicated. For example, in the MOG-induced model some previous studies did not detect MNs loss [13] or gray matter involvement [14], whereas recently significant loss of lower MNs has been reported [15]. Evidence for MNs loss has been found in other EAE models i.e. in the chronic phase of Biozzi mice [16] and the acute stage of Lewis rats [17] induced by spinal cord homogenate, as well as in a passive model induced by adoptive transfer of PLP specific T-cells [15]. Since axonal and neuronal injuries are central factors in the determination of neurological disability, a detailed *in situ* analysis of CNS damage along the disease course in different EAE models is important.

Subsequent to the pathological MS/EAE processes, intrinsic protective routes are stimulated, probably in an attempt to repair the damage and restore tissue integrity [18]. Recruitment of oligodendrocyte precursor cells into the lesions and remyelination of the demyelinated axons are key processes [19,20]. In addition, it has been demonstrated that neuronal progenitor cells migrate from neuroproliferative zones to injury sites, with a potential for neurogenesis [21]. However, in spite of the repair capabilities of the adult brain, remyelination and restoration in MS/EAE are only partial and are manifested mainly during the early disease phase. Current treatments for MS have shown efficacy in ameliorating the immune inflammatory process [22], but their capability to stimulate neuroprotective and repair processes within the CNS has not been proven. It is therefore paramount to evaluate MS treatments according to their neuroprotective consequence, namely prevention of demyelination and axonal/neuronal damage, as well as augmentation of remyelination and repair.

Glatiramer acetate (GA, Copaxone®), an approved drug for MS treatment, is effective in the prevention and suppression of EAE induced by various encephalitogens in several species [23]. The therapeutic activity of GA has been attributed to its immunomodulatory effect at different levels of the immune response. In the periphery GA binds promiscuously to major histocompatibility complex molecules (MHC), acting both as an MHC blocker [24] and a T-cell receptor antagonist [25], leading to inhibition of pathological effector functions. GA has also been shown to modulate the properties of dendritic cells and monocytes, so that they preferentially stimulate T-helper (Th)-2 like responses [26,27]. Indeed, it has been demonstrated in several EAE models, as well as in MS patients, that GA is a potent inducer of Th2/3 cells that secrete high levels of anti-inflammatory cytokines [28,29]. It was also demonstrated in EAE models that these cells can cross the blood brain barrier (BBB), accumulate in the CNS [30], and express *in situ* interleukin 10 (IL-10) and transforming growth factor-β (TGF-β) [31]. Recent studies demonstrated a drastic reduction in the level of Th-17 cells in the CNS of EAE mice following GA treatment, with concomitant elevation of T-regulatory cells [32,33].

During recent years, cumulative results indicate that in addition to its immunomodulatory activity, GA induces neuroprotective consequences. For example, GA treatment of MOG-induced EAE mice restored the impaired levels of brain derived neurotrophic factor (BDNF), as well as of two other neurotrophic factors – NT3 and NT4 [34]. The relevance to human therapy is supported by the reduced levels of BDNF in the serum and cerebral spinal fluid of untreated MS patients, and its reversal by GA therapy [35]. Moreover, in mice with MOG-induced EAE, GA treatment augmented neurogenesis of neuronal progenitor cells that migrated into injury sites and differentiated to mature neurons [36]. The neuroprotective effect of GA was manifested also on the primary target of the EAE/MS pathology – the myelin. Using scanning electron microscopy, we recently demonstrated reduced myelin damage in EAE mice in which treatment was initiated in the chronic disease phase, when demyelination was already manifested, suggesting active remyelination [37]. However, validation of the effect of GA on myelin formation has not yet been shown.

In the present study we analyzed *in situ* spinal cord (SC) white matter pathology, namely axonal demyelination and degeneration, in two widely used MS models: the PLP-induced relapsing–remitting EAE and the chronic EAE form induced by MOG, at various time points during disease progression. Using transmission electron microscopy (TEM) that facilitates the visualization of newly formed myelin, we verified the spontaneous remyelination stimulated in response to myelin destruction, as well as the proactive process following treatment with GA. We further studied the involvement of neuronal somatic pathology during the disease course, by quantifying the extent of motor neuron loss in these two models. We report herewith on distinct pathological patterns characteristic to each EAE model, and on a beneficial effect of GA treatment in elevating the extent of remyelination and in reducing axonal damage as well as neuronal loss.

2. Materials and methods

2.1. Mice

C57BL/6 and (SJL/JxBALB/c)F1 mice were purchased from Harlan (Jerusalem, Israel). YFP 2.2 mice selectively express YFP in their nervous system [38], and were kindly provided by Joshua R. Sanes (Washington University, St. Louis, MO). Female mice, 8–12 weeks of age, were used and kept under specific pathogen free (SPF) environment. All experiments were approved by the Institutional Animal Care and Use Committee of the Weizmann Institute.

2.2. Induction and evaluation of EAE

Relapsing–remitting EAE was induced in (SJL/JxBALB/c)F1 mice by the peptide encompassing amino acids 139–151 of proteolipid protein (PLP) synthesized by Genscript (Piscataway, NJ). Chronic EAE was induced in C57BL/6 mice by injecting a peptide consisting of amino acids 35–55 of myelin oligodendrocyte glycoprotein (MOG), synthesis by Genscript. Mice were injected subcutaneously at the flank, with 200 μl emulsion containing 200–300 μg of the encephalitogenic peptide in incomplete Freund's adjuvant enriched with 3 mg/ml heat-inactivated Mycobacterium tuberculosis (Sigma, St. Louis, MO). Pertussis toxin (Sigma), 200–250 μg/mouse was injected intravenously immediately after the encephalitogenic injection and 48 h later. Mice were examined daily. EAE was scored as follows: 0-no disease, 1-limp tail, 2-hind-limb paralysis, 3-paralysis of all limbs, 4-moribund condition, and 5-death.

2.3. Glatiramer acetate (GA, copaxone, copolymer 1)

GA consists of acetate salts of synthetic polypeptides containing four amino acids L-alanine, L-glutamate, L-lysine, and L-tyrosine [23]. GA from batch 242905809, with an average molecular weight of 7700 kDa, obtained from Teva Pharmaceutical Industries (Petah Tiqva, Israel) was used throughout the study. GA treatment was administered by consecutive 7–10 daily subcutaneous injections (2 mg/mouse, in Phosphate Buffered Saline, PBS) either as a prevention treatment starting one day following disease induction, or as a suppression treatment beginning after the appearance of clinical manifestations. Layouts of the GA treatment schedules in characteristic experiments are demonstrated in Table 1 and Fig. 1.