# GLATIRAMER ACETATE: MECHANISMS OF ACTION IN MULTIPLE SCLEROSIS

Tjalf Ziemssen and Wiebke Schrempf

Multiple Sclerosis Center Dresden, Neurological University Clinic
Dresden University of Technology, Dresden 01307, Germany

I. Introduction
II. Pathology and Immunology
III. MS as Neurodegenerative Disease
IV. The Janus Face of CNS-Directed Autoimmune Inflammation
V. Neurotrophic Factors Are Released by Different Immune Cells
VI. Glatiramer Acetate: Historical Remarks
VII. GA: Overview of Clinical Studies
VIII. GA: Imaging Studies
IX. GA: Animal Models
X. GA in MS: Mechanisms of Action
A. Effects of GA by Binding to MHC Class II Molecules
B. Effects of GA on the APC Level
XI. Effects of GA on the B-Cell Level
XII. Effects of GA on the T-Cell Level
A. Th1–Th2 Shift
B. GA as Altered Peptide Ligand
C. Secretion of Neurotrophic Factors
D. Induction of GA-Specific CD8+ Suppressor T Cells
E. Induction of Regulatory CD4+CD25+ T Cells
XIII. Conclusions
References

Glatiramer acetate (GA), formerly known as copolymer 1, is a mixture of synthetic polypeptides composed of four amino acids resembling the myelin basic protein (MSP). GA has been shown to be highly effective in preventing and suppressing experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis (MS). Therefore, it was tested in several clinical studies and so approved for the immunomodulatory treatment of relapsing-type MS.

In contrast to other immunomodulatory MS therapies, GA has a distinct mechanism of action: GA demonstrates an initial strong promiscuous binding to major histocompatibility complex molecules and consequent competition with various (myelin) antigens for their presentation to T cells. In addition, antigen-based therapy generating a GA-specific immune response seems to be the prerequisite for GA

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therapy. GA treatment induces an *in vivo* change of the frequency, cytokine secretion pattern and the effector function of GA-specific CD4+ and CD8+ T cells, probably by affecting the properties of antigen-presenting cells such as monocytes and dendritic cells. As demonstrated extensively in animal experiments, GA-specific, mostly, T helper 2 cells migrate to the brain and lead to *in situ* bystander suppression of the inflammatory process in the brain. Furthermore, GA-specific cells in the brain express neurotrophic factors like the brain-derived neurotrophic factor (BDNF) in addition to anti-inflammatory T helper 2-like cytokines. This might help tip the balance in favor of more beneficial influences because there is a complex interplay between detrimental and beneficial factors and mediators in the inflammatory milieu of MS lesions.

## I. Introduction

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS). It is believed to be a multifocal immune-mediated disorder in which the myelin sheath or the oligodendrocyte is targeted by the immune system in genetically susceptible people. There is a considerable heterogeneity in terms of clinical, radiological, and pathological changes, mirrored by a high intrapatient and interpatient variability in the clinical course and its manifestations. The disease affects approximately 0.1% of the population in temperate climates. It is a disease of young people with a lifelong and often disabling course. MS is manifested in physical symptoms (relapses and disability progression), CNS inflammation, brain atrophy and cognitive dysfunction.

About 85% of the patients begin with a relapsing-remitting disease, where neurological symptoms and signs develop over several days, plateau, and then usually improve over days to weeks. Approximately two-thirds of patients with relapsing-remitting MS (RRMS) undergo a conversion to a secondary progressive disease course (SPMS), where relapse frequency lessens over time and progressive neurological dysfunction emerges. The remaining 15% of patients begin the disease course with a gradually progressive neurological dysfunction, typically a slowly worsening myelopathy [primary progressive MS (PPMS)].

## II. Pathology and Immunology

The pathological hallmark of MS is the demyelinating plaque which consists of infiltrating lymphocytes and macrophages, damage to the blood–brain barrier, and loss of myelin.

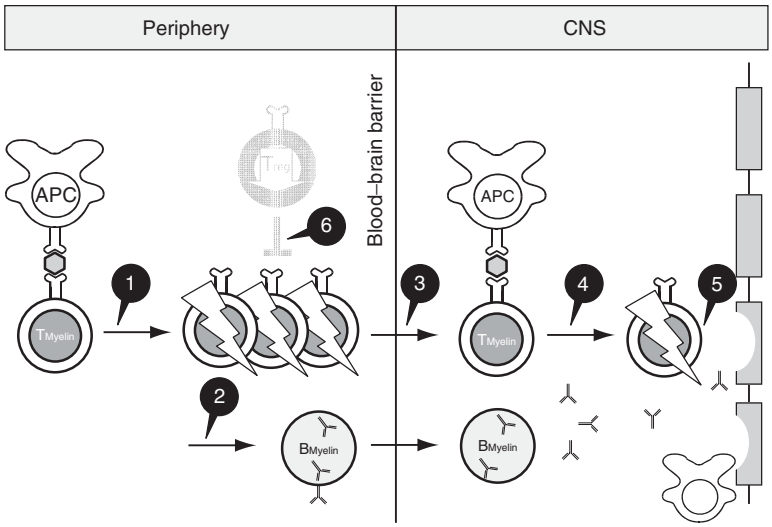


FIG. 1. Hypothetical pathophysiological cascade in MS. Details (inclusive numbers) in the text.

Oligodendrocytes synthesize and maintain the axonal myelin sheath of up to 40 neighboring nerve axons in the CNS. Compact myelin consists of a condensed membrane, spiraled around axons to form the insulating segmented sheath needed for saltatory axonal conduction; voltage-gated sodium channels cluster at the unmyelinated nodes of Ranvier between myelin segments, from where the action potential is propagated and spreads down the myelinated nerve segment to trigger another action potential at the next node.

In MS, the composition of the inflammatory infiltrate varies depending on the stage of demyelinating activity. Early symptoms of MS are widely believed to result from this inflammatory demyelination which leads to slowing or blockade of axonal conduction. The regression of symptoms has been attributed to the resolution of inflammatory edema and to partial remyelination.

Current concepts assume that MS occurs as a consequence of immune tolerance breakdown in genetically susceptible individuals (Hafler, 2004). The major contributing factors thus include genetics, environment, and immune dysregulation. T helper (Th) cells are considered to play a pivotal role in the whole self-reactive immune response of the CNS, primarily characterized by inflammatory demyelination. Putting experimental data from human studies and animal experiments together, the following pathophysiological cascade seems quite attractive which is shown and numbered in Fig. 1:

1. Autoreactive T cells, known to exist in any individual, are activated in the periphery, probably by molecular mimicry (i.e., recognition of epitopes that are common to autoantigens and microbial structures as exogenous triggers) or by self-antigens.
2. T cells may trigger B-cell activation and antibody formation, the latter potentially exerting detrimental effector functions at the myelin sheath (Ziemssen and Ziemssen, 2005).
3. T-cell activation enables transmigration through the blood–brain barrier to the sites of inflammation, a cascade of events influenced by adhesion molecules, chemotactic factors and migration promoters.
4. Recognizing their antigens presented by microglia, the local antigen-presenting cells (APCs), the autoreactive T cells are reactivated.
5. T cells of both CD4+ and CD8+ cytotoxic phenotypes release proinflammatory cytokines. An inflammatory cascade is initiated followed by the further recruitment of inflammatory cells like monocytes.
6. Underlying immunoregulatory defects, such as decrease of regulatory T cells in the circulation of patients with MS, allow the further pathological activation of autoreactive T cells.

## III. MS as Neurodegenerative Disease

Although MS seems to be primarily an inflammatory autoimmune disease, it has become evident that axonal loss plays an important role in the pathogenesis of disability in patients with MS. While axonal pathology was elegantly and precisely described in classic MS neuropathologic studies more than a century ago (Charcot, 1877), it has reemerged as a major focus of research (Trapp *et al.*, 1998). The important question to be addressed is not whether there is axonal loss in MS but when and to what extent does the axonal loss occur. The timing and degree of axonal loss is of importance not only in its relationship to the etiology of the disease but may be central to the appearance of clinical symptoms and the progressive deterioration associated with the disease. The fact that axonal loss is irreversible has important implications for when and which therapeutic intervention should be used.

It is likely that various mechanisms contribute to axonal damage during different stages of disease. In active lesions, the extent of axonal transection correlates with inflammatory activity while even there seems to be an inflammation-independent axonal loss (Perry and Anthony, 1999). Hence, axonal loss may be caused by inflammatory products of activated immune and glial cells, including proteolytic enzymes, cytokines, oxidative products, and free radicals, although the precise molecular mechanisms of axonal damage are poorly understood. In addition, the magnitude of axonal loss in chronic MS lesions without pronounced inflammatory

infiltrates suggests that mechanisms other than inflammatory demyelination contribute to the degeneration of axons. Several conditions interfere with attempts of axonal regrowth after lesions develop. These include the lack of neurotrophic factors that support growth, the presence of a glial scar (depending on the site of lesion) or the presence of inhibitory molecules that impede axonal growth. Evidence shows that axon degeneration following injury has similarities with the cellular mechanisms underlying programmed cell death (Perry and Anthony, 1999).

The biochemical events underlying the inflammatory and neurodegenerative phases of the disease are not known in detail and may be quite different (Steinman, 2001). Inflammation involves activation of T and B cells in the periphery, crossing the blood–brain barrier and homing to the lesion site. In the lesion, T cells are reactivated by myelin antigens, release cytokines that attract macrophages and activate microglia which start to destroy the myelin sheath. Anti-myelin antibodies bind complement, attract macrophages, and stimulate opsonization of myelin. Demyelination leads to reversible and to some extent irreversible impairment of functionality of the axon whose conduction properties are deteriorated, thus accounting for the clinical symptoms associated with relapses.

Neurodegeneration is likely to be a complex process (Chitnis *et al.*, 2005; Grigoriadis *et al.*, 2004), especially when it takes place in inflammatory disorders like MS. To a significant extent, axonal loss seems to be a major consequence of demyelination and inflammation, for example, by binding of CD8+ T cells to exposed axons and secretion of toxic factors. However, other mechanisms not directly related to demyelination and inflammation are also likely to be important. An example is excitotoxicity: glutamic acid can bind to excitatory amino acid receptors on the cell bodies, dendrites, or axon terminals of neurons and initiate a process of neuronal cell death (Steinman, 2001).

Theoretically, there are several possible relationships between inflammation and neurodegeneration in MS. Three exclusive hypotheses can be envisaged: (a) that neurodegeneration is entirely secondary to inflammation, (b) that inflammation is entirely secondary to neurodegeneration, or (c) that inflammation and neurodegeneration are entirely independent. On the other hand, nonexclusive hypotheses stating that neurodegeneration is partially dependent and partially independent of inflammation or vice versa can also be put forward, and these seem intuitively more likely (Ziemssen, 2005).

There are a number of clinical arguments in favor of some independence between the inflammatory and neurodegenerative processes. For example, in a clinical trial of alemtuzumab (Campath-1H), a monoclonal antibody directed against CD52 which leads to T-cell depletion, in secondary progressive MS (SPMS), a gradual extinction of exacerbations and lesion activity visible on magnetic resonance imaging (MRI) was demonstrated (Coles *et al.*, 1999). However, disability continued to progress in about half the patients in whom progressive brain atrophy and axonal degeneration could be observed using MRI and magnetic resonance

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