Enhanced Myelination in Autoimmunity and in Normal Development Induced by Glatiramer Acetate

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Multiple sclerosis (MS) is a complex multifaceted disease in which inflammatory autoimmune attack in the central nervous system (CNS) leads to the destruction of the myelin sheath and the formation of demyelinated lesions in the white matter [1]. Diffused molecular and cellular changes in normal-appearing white matter and cortical demyelination have also been recognized as a component of MS pathology. This widespread demyelination results in impaired nerve conductivity and neuroaxonal damage. Thus, the essential challenge for MS therapy is not only to ameliorate the inflammatory aspect of the disease, but to promote neuroprotective repair mechanisms beyond their limited spontaneous occurrence, in particular remyelination.

Glatiramer acetate (GA, Copaxone®, Israel) is an amino acid copolymer, an approved drug widely used as a first-line treatment for MS [2]. Cumulative evidences indicate that GA affects various levels of the innate and the adaptive immune response, inducing deviation from pro-inflammatory to anti-inflammatory pathways [3]. This includes competition for the binding of antigen-presenting cells, driving dendritic cells, monocytes, and B cells towards anti-inflammatory responses, induction of Th2/3 and T-regulatory cells, and down-regulation of both Th1 and Th17 cells. The immune cells induced by GA reach into the CNS and secrete in situ anti-inflammatory cytokines, thus alleviating the pathological inflammatory processes [4]. In addition to its immunomodulatory activity, GA treatment induces elevation in the CNS levels of several neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), suggesting neuroprotective repair consequences [5]. To examine whether GA can indeed affect the repair and remyelination we applied two systems: the animal model of MS, experimental autoimmune encephalomyelitis (EAE), and postnatal myelination in the developing CNS of newborn mice.

**Remyelination in EAE**

The effect of GA treatment on remyelination was investigated in relapsing-remitting EAE induced by myelin proteolipid protein (PLP) peptide in which widespread demyelination is the main pathological manifestation, and in chronic EAE induced by myelin oligodendrocyte glycoprotein (MOG) peptide in which degeneration processes prevail. We observed reduced myelin damage as detected by scanning electron microscopy (SEM) and immunohistochemistry in EAE-inflicted mice treated with a late therapeutic GA regimen (initiated when there was already extensive myelin damage), suggesting the induction of actual repair processes [6]. This was further confirmed by applying transmission electron microscopy (TEM), which facilitates the visualization of newly myelinated axons [7]. Indeed, quantitative spinal cord TEM analysis of remyelination compared to demyelination revealed a significant increase in the relative remyelination, by seven- and threefold over untreated EAE mice, when GA treatment was applied during the first or second disease exacerbation, respectively [Figure 1A].

This pattern was also evident on MRI using magnetization transfer ratio (MTR), which focuses on macromolecules, indicating myelin loss. Overall assessment of the whole brain by histogram analysis as well as detection of specific affected areas by voxel-based analysis revealed restoration of the MTR values to the normal level following GA treatment [8].

The effect of GA in this system is attributed to increased proliferation and survival of oligodendrocyte progenitor cells (OPCs) and their recruitment into injury sites [Figure 1B], thus enhancing myelin repair in situ. Furthermore, GA treatment induces a morphological transformation of OPCs from the earlier bipolar stage to the more mature multiprocessed form, suggesting an effect on the differentiation along the oligodendroglial maturation cascade towards myelin-producing cells [6].

**Postnatal Myelination in the Developing CNS**

It was subsequently questioned whether the remyelination demonstrated in EAE-induced mice is solely due to the anti-inflammatory activity of GA in the inflamed CNS. Addressing this issue we recently investigated whether GA can affect postnatal myelogenensis in the developing nervous system under...
Figure 1. The effect of GA treatment on myelination in EAE [A,B] and on postnatal myelinogenesis [C,D]

[A] TEM micrograph of a remyelination zone in the spinal cord of a GA-treated EAE-induced mouse, depicting oligodendrocyte cell surrounded by newly remyelinated axons, and quantitative assessment of the remyelination to demyelination ratio of three mice per treatment group at each time point.

[B] Left panel: progenitor oligodendrocyte expressing NG2 (red), extending multiple processes, intertwines several transected nerve fibers (green)
Right panel: mature oligodendrocytes expressing O4 (red) accumulating in spinal cord lesions of GA-treated EAE-induced mice

[C] Representative spinal cord sections and quantitative assessment of myelinated axons compared to the total number of axons on postnatal day 14. More MBP-encircled axons are depicted in GA-injected compared to PBS-injected mice

[D] Left: representative TEM micrographs depicting larger axons with thicker myelin sheath in GA-injected mice
Right: linear regression of the interaction between the treatment and axonal diameter on myelin thickness, and the average g-ratio for six mice per treatment group

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non-pathological conditions, when injected on postnatal days 7–21 [9]. Immunohistological and ultrastructural analyses revealed significant elevation in the number of myelinated axons as well as in the thickness of the myelin encircling them and the resulting g-ratios, in spinal cords of GA-injected mice compared to their phosphate-buffered saline (PBS)-injected littermates, on postnatal day 14 [Figure 1C & D]. GA induced also an increase in the axonal diameter, implying an effect on the overall development of the nervous system. It should be noted that when the myelination process was apparently completed (postnatal day 21), the extent of myelinated axons and their morphological appearance did not differ between the GA-injected and the PBS-injected mice. Thus, GA accelerates myelin development without inducing an excessive or aberrant myelination.

A prominent elevation in the amount of OPCs and their proliferation (detected by BrdU incorporation) as well as in mature oligodendrocytes indicated that similar to the findings in EAE, the effect of GA in postnatal myelination is linked to differentiation along the oligodendroglial maturation cascade. As previously found in EAE, GA-postnatal injection resulted in increased expression of BDNF and insulin-like growth factor (IGF-1), which are known to promote myelination, suggesting that the mode of action of GA can be linked to its neurotrophic effect. Notably, the GA-injected newborn mice exhibited better performance in a rotating rod test than their PBS-injected littermates, suggesting that the accelerated myelin development results in functional advantage in sensorimotor functions.

During remyelination, features of developmental myelination are recapitulated. For example, some exons of the MBP gene that are present during fetal development are expressed again in oligodendrocytes during demyelinating diseases [10]. The effect of GA on postnatal myelogenesis as well as on remyelination in EAE is therefore relevant to its therapeutic effect in MS, supporting the notion that the repair process in the CNS can be up-regulated by therapy, irrespective of the inflammatory process.

References

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Capsule
Chromatin state dynamics during blood formation
Chromatin modifications are crucial for development, yet little is known about their dynamics during differentiation. Hematopoiesis provides a well-defined model to study chromatin state dynamics; however, technical limitations impede profiling of homogeneous differentiation intermediates. Lara-Astiaso et al. developed a high sensitivity indexing-first chromatin immunoprecipitation approach to profile the dynamics of four chromatin modifications across 16 stages of hematopoietic differentiation. The authors identified 48,415 enhancer regions and characterized their dynamics. They found that lineage commitment involves de novo establishment of 17,035 lineage-specific enhancers. These enhancer repertoire expansions foreshadow transcriptional programs in differentiated cells. Combining our enhancer catalog with gene expression profiles, we elucidate the transcription factor network controlling chromatin dynamics and lineage specification in hematopoiesis. Together, these results provide a comprehensive model of chromatin dynamics during development.

“Others have seen what is and asked why. I have seen what could be and asked why not”
Pablo Picasso (1881-1973), Spanish painter, sculptor, printmaker, ceramicist, stage designer, poet and playwright. One of the greatest and most influential artists of the 20th century, he is known for co-founding the Cubist movement and collage, and for the wide variety of styles that he helped develop and explore