Structural insight into the role of myelin basic protein in multiple sclerosis

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In the article by Musse *et al.* in this issue of PNAS (1), the authors present unique and compelling physico-chemical data that demonstrate the structural instability of myelin in multiple sclerosis (MS). Such alteration in myelin stability, reported as secondary to changes in myelin basic protein (MBP) structure, may be causative in nature and/or contribute to the early course of demyelination in MS. A combination of physico-chemical and theoretical approaches enabled the authors to arrive at their conclusions and demonstrated that myelin structural information can shed light on mechanistic dysfunction in MS.

The primary findings are that an altered charge isomer of MBP (*rm*C8) that is associated with disease severity in MS (2) has less membrane depth penetration and shorter α-helix structure, making the immunodominant epitope of this protein more exposed to the cytosolic space and readily digested by proteases. This change in MBP conformation would free the epitope for T cell recognition and suggests a mechanism of action potentially initiated by alterations in myelin structure. This hypothesis is supported by a recent study describing antibody enzymes (abzymes) that catalyze MBP in a site-specific degradation (3). The C8 MBP isoform is also more abundant in immature myelin, and results of this study support the hypothesis that myelin structure in MS is developmentally immature (4), contributing to altered myelin stability and possibly the initiation of the disease.

MBP and MS

MBP is abundant in myelin, and the structures of the genes encoding MBP were among the first to be determined in the nervous system. Campagnoni and Campagnoni (5) provide an excellent review of the data demonstrating the heterogeneity of MBP and current understanding of the genetics of their formation. They review the data demonstrating that multiple isoforms of MBP are produced through the translation of separate mRNAs, resulting in a heterogeneous population of MBP structures. The recombinant C8 isoform (*rm*C8) used in this study represents the least cationic isoform of MBP and is correlated with the most severe cases of MS. There are numerous reports on the isoforms of MBP, but this study is the first to examine the effects of MBP deimination in its native membrane state.

Considerable data suggest that MBP plays a key role in the pathology of MS, although its mechanism of action has remained unclear. Antigenically related MBP was isolated from the cerebrospinal fluid of patients with MS (6). Induction of experimental allergic encephalomyelitis (EAE) with MBP produced a monophasic inflammatory disease process in guinea pigs with minimal demyelination, whereas the addition of galactocerebrosides or the use of whole myelin produced EAE with demyelination, suggesting that MBP plays a role in demyelination when in synergy with myelin lipids (7). Subsequently it was demonstrated that a single transfer of MBP-sensitized T cells from animals with EAE produced a relapsing disease process in mice with both inflammation and demyelination, similar to what is found in MS (8). This discovery drew attention to MBP isoforms as candidate autoantigens. The authors build on prior reports of MBP isoforms in MS by providing key structural data of the immunodominant epitope of *rm*C8 MBP in its native membrane form and demonstrate that it has less membrane depth penetration and shorter α-helix structure. These results take our understanding of the role of MBP in MS to a new level of structural detail and support mechanistic understanding of demyelination in MS.

Myelin from multiple sclerosis normal-appearing white matter may generate self-antigens.

MBP is localized to the cytosolic side of myelin and is therefore inaccessible to circulating immune cells. It was hypothesized that the reaction between MBP and T cells may occur at some site other than myelin membrane (7). It was recently proposed that decreased myelin compaction caused by aberrant post-translational modification of MBP may initiate a process that makes myelin susceptible to degradation (9). In support of this hypothesis, Musse *et al.* (1) show that reduction in the net positive charge of MBP not only results in hindrance to compact myelin assembly but also renders the most immunodominant epitope of MBP to be highly surface-exposed and readily digestible by myelin-associated proteases. The antigenic peptides released could initiate or sustain the immune response.

Results of a complete genomic screen for MS suggest that a multifactorial etiology is likely (10). The synergistic interaction of membrane proteins and lipids has been studied for years with a variety of techniques for elucidation of membrane structure, dynamics, and the clustering of membrane components into rafts. MBP prefers to colocalize with the lipid phosphatidylserine, most likely because of electrostatic interactions. Reports using physico-chemical approaches to investigate myelin structure indicated that both MBP and lipid components contribute to myelin stability in a synergistic manner (11, 12), and thus one cannot rule out the role of lipids in MS and demyelination.

MS Normal-Appearing White Matter

The current study models changes in MBP from MS normal-appearing white matter. For >50 years there have been various reports of biochemical alterations in MS normal-appearing white matter. Allen and McKeown (13) were the first to correlate changes in MS normal-appearing white matter with histology. There have been literally thousands of imaging studies of varying kinds that detected white matter abnormalities in regions that appeared normal on standard MRI. Although the spatial resolution of MRI is greater than the diameter of cells, these results suggest that changes in MS myelin and white matter occur early, before evidence of demyelination, and that an inherent defect in myelin structure possibly exists (14, 15). Results of the study by Musse

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et al. (1) support the hypothesis that myelin from MS normal-appearing white matter has biochemical alterations that may generate self-antigens known to sensitize T cells in MS.

Potential Applications and Future Studies

Copolymer 1 (Copaxone) is a treatment currently used as an immune modulator in MS (16, 17). It is of benefit to approximately one-third of the MS patients who take it. Its mechanism of action remains unknown, although it is believed to interfere with the antigenic fragment of MBP. Modified amino acid copolymers were reported to suppress the effects of the antigenic fragment of MBP through different effects on T cells in humanized mice (18). In light of the current study (1), structural evaluation of myelin may contribute to the development of more effective agents that suppress the immune response in MS.

The results of this study are highly significant for understanding what initiates and/or sustains the immune response in MS. The results interpret prior studies with a mechanism of action. The results indicate that myelin disintegration may be initiated in the cytosolic space, as opposed to the external extracellular surface, although the cause and course of demyelination remain unknown and myelin degradation could be occurring at both surfaces of myelin (19). And, finally, the results support the hypothesis that myelin structure in MS is similar to immature myelin, resulting in altered myelin stability. The principal author stated in his recent review of MBP (20) ". . . it remains impossible to put all of these investigations into a global picture without a detailed, structural context." The current paper provides key structural data to begin the assembly of such a global picture of changes to myelin in MS. The technical approaches of this study can be applied, not only to the study of myelin, but to other biomembranes and macromolecular tissue structures, highlighting the contributions of physical chemistry, structural biology, and bioengineering to the understanding of disease process.

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