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Topical Review

Is the Myelin Membrane Abnormal in Multiple Sclerosis?

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Introduction

Myelin, a multilamellar membrane surrounding nerve fibers of both the central and peripheral nervous systems, is derived from the plasma membrane of the oligodendrocyte (CNS) and Schwann cell (PNS). The chemical composition of myelin differs markedly from that of its progenitor membrane. In addition, the chemical composition of CNS myelin differs from that of PNS myelin. This review will be concerned only with CNS myelin.

Myelin lipid, which accounts for approximately 75% of the dry weight (20% for most cell membranes), consists of cholesterol, phospholipids and galactolipids in a ratio of 4 : 3.5 : 2. Myelin has less protein than plasma membranes (25% as compared to 75%) and has fewer types. There are two major myelin proteins, the extrinsic or basic protein (MBP) and the intrinsic or Folch-Lees proteolipid apoprotein. Lipophilin has been purified from human proteolipid fraction and constitutes about 50% of this fraction. Although the lipids of myelin are considered to exist predominantly in a bilayer, the arrangement of the proteins has not been fully elucidated. Evidence to date suggests that the chemical architecture of the bilayer will be similar to that of most plasma membranes.

In man, when destruction and loss of myelin occurs but the nerve fiber remains intact, a "demyelinating disease" termed multiple sclerosis (MS), is acquired. Since the disease was first described, a number of etiologic theories has been proposed, the most important of which are (a) an infectious or environmental agent, or (b) an abnormal immune mechanism is responsible for disease induction. A large body of knowledge has been accumulated, but both cause and mechanism of demyelination remain unknown.

Pathologically, myelin is destroyed, axons are preserved, gliosis occurs and "plaques" are formed. These lesions are scattered throughout the CNS white matter although a prediliction for discrete sites exists. Oligodendrocytes are also lost at sites of myelin destruction, leading to speculation that this cell may be the primary disease site. There is no evidence to support this hypothesis, but investigations, to establish whether an oligodendrocyte cell surface antigen(s) against which an autoimmune response could be directed, are underway in a number of laboratories.

Since MS is a disease of myelin, much of current research is based on the theory that a primary biochemical abnormality of myelin may exist. To date, no unequivocable evidence for an abnormality in the chemical composition of MS myelin has been detected. Advances in chemical technology will permit a more complete elucidation of the protein and lipid moieties of normal and diseased myelin (which may explain some of the structural abnormalities to be described later). Studies on the physical structure of myelin suggest that MS myelin has an altered structure.

This review will deal with the hypothesis that MS is the result of a membrane defect and the data obtained by biochemical and biophysical techniques that compare the structure of myelin from normal and diseased tissue will be discussed.

Biochemical Studies

The Chemical Composition of Normal Myelin

Myelin is generally isolated from normal human central nervous system white matter by sucrose

Table.

Lipid[a]	Myelin[a]
Cholesterol	27.7
Cerebroside	22.7
Sulfatide	3.8
Ethanolamine phosphatides	15.6
Lecithin	11.2
Sphingomyelin	7.9
Phosphatidylserine	4.8
Phosphatidylinositol	0.6
Plasmalogens (ethanolamine phosphatides)	12.3
Gangliosides[b]	600.0[a]

[a] % total lipid weight.
[b] μg sialic acid/g myelin.

density gradient ultracentrifugation methods [4, 52, 60]. Myelin prepared in this manner is free of nerve axons, neurons, and other cells of the white matter. Only the noninvolved areas of white matter (free of plaques) were used to prepare myelin from patients who died with multiple sclerosis.

The Table lists the lipid composition of normal human brain myelin [59]. Cholesterol, cerebrosides, ethanolamine phosphatides, and lecithin are the major lipids. Gangliosides are associated with myelin but account for less than 1% of the total lipid; GM_1 and GM_4 represent 75% of the total ganglioside content [89]. The fatty acid residues of myelin lipids vary considerably.

The major myelin proteins of normal human brain (the basic protein and lipophilin) have been isolated and purified [40, 52, 56]. Polyacrylamide gel electrophoresis of myelin has established that there are several minor protein components as well [30, 58]. High molecular weight proteins are present, one of which is called Wolfgram protein [81]. Also, there is at least one glycoprotein [32, 64] and some small molecular weight proteins, none of which have been characterized.

Myelin basic protein (A_1 or encephalitogenic protein) is a single polypeptide chain with a mol wt 18,400 of known sequence [16, 33]. Eighteen percent of the amino acids are basic (lys, arg) at neutral pH. It contains a single tryptophan residue and no cysteines. Several post translational modifications have been demonstrated and are presumed to be responsible for the microheterogeneity observed in all MBP preparations. These modifications include the phosphorylation of serine and threonine, deamidation of glutamine and asparagine, loss of C-terminal arginine and the presence of methionine sulfoxide [20, 26, 27, 55]. The significance of this microheterogeneity is not known, although it may be implicated in the stabilization of the multilamellar structure of myelin, which will be described later.

Lipophilin has been assigned a mol wt of about 28,000 by analytical ultracentrifugation [40]. It's sequence has not been fully elucidated; however, most of the sequence of the bovine proteolipid apoprotein is known and a mol wt of 30,000 has been determined [73]. This protein is a single polypeptide chain containing 65% nonpolar amino acids, 10 cysteines, and 4 tryptophans. Lipophilin has 2–3 mol/100 mol covalently bound fatty acids which in part accounts for its strong hydrophobic character [40].

The Chemical Composition in Multiple Sclerosis

Myelin Lipids

There have been extensive lipid analyses performed on the plaques, areas immediately adjacent to plaques, normal-appearing white matter, and myelin isolated from intracranial white matter of deceased MS patients.

In the MS plaque, there is a decrease in total lipid [22, 25, 53] largely a result of myelin loss. Specifically, cholesterol is decreased and a concomitant increase in cholesterol esters is found [65, 71, 78]. Phospholipids (predominantly phosphatidylethanolamine) are decreased [22, 25, 43, 88]. Also, a reduction in the cerebroside-sulfatide ratio has been reported [22, 29, 53]. Although the ganglioside content appears to be significantly decreased, the alteration in the distribution of gangliosides was most striking and one ganglioside (G_{M4}) was found to be totally absent [90]. The composition of the lipid in areas adjacent to plaques is similar to plaque material, but the changes vary proportionally to the age of the lesion.

Lipid analyses performed on MS cerebral white matter and cord tissue show differences similar to that of plaque material when compared to normal tissue (non-MS). The total lipid content is decreased; cholesterol esters are present but cholesterol levels appear to be normal; phospholipids (acidic) are significantly decreased as are cerebrosides [2, 22, 25, 42, 57, 77, 79, 91]. A significant reduction in the sulfatide content of MS white matter [2, 79] but an increase in sulfatides in MS cord tissue similar to plaque material [91] has been reported. The ganglioside concentration in MS white matter [90] and in cord tissue appears to be essentially normal, but differences in ganglioside distribution similar to plaque tissue were observed [91].

Reports on the lipid composition of MS myelin (excluding plaque and periplaque material) com-

pared to normal myelin have been contradictory. The cholesterol content is similar in both, and no cholesterol esters have been detected [37, 45]. A decrease in a number of phospholipids has been reported [21, 80]. However, normal levels [36, 74] have been reported by some investigators. Compared to normal myelin, the cerebroside/sulfatide content of MS myelin has been reported to be increased, decreased, or normal [21, 23, 36, 74, 80, 82]. Several studies have reported no difference in fatty acid content and degree of unsaturation between normal and MS tissue [24, 36, 74], whereas other investigators have reported changes [6, 21, 47, 78, 80]. These apparently conflicting results may reflect variations in the extent, duration, or severity of the disease or may suggest a multifactorial etiology giving rise to a number of diseases loosely called MS at the present time.

Myelin Proteins

There is a general agreement among most investigators that the total protein content of MS-plaques, -periplaque tissue, -cerebral white matter and -cord tissue is decreased when compared to normal tissue on a wet weight basis. This decrease in MS tissue is considered to be the direct result of myelin loss. However, the protein/lipid ratio of MS myelin is the same or slightly elevated compared to that found in normal human myelin [6].

In the MS-plaque and periplaque tissue, there is a decrease in both the basic protein and the proteolipid protein [29, 58, 66, 68]. Recently, this reduction in both myelin proteins has been demonstrated in MS cord tissue, as well [91]. The normal-appearing MS white matter appears to have a normal proteolipid content but a decrease in basic protein [91, 58]. There has been one report [67] of an almost total loss of basic protein in MS myelin. However, most investigators have found no significant reduction of the myelin specific proteins [30, 41, 74, 83]. Since the reported differences have been observed using polyacrylamide gel electrophoresis only, the quantitative significance of such changes must be interpreted with caution and must await isolation and characterization of the individual proteins. The gel patterns have shown other differences between normal and MS material such as increases in large molecular weight components, one of which is identified as glial fibrillary acidic protein [58]. The apperance of low molecular weight components has been observed, but is not a consistent finding in all MS samples.

The amino acid composition and sequence of the normal human basic protein is known [16, 52]. The amino acid composition of MS basic protein appears to be similar to the normal, but the entire sequence has not been elucidated. Those fragments of the MS basic protein that have been sequenced in this laboratory are similar to the normal (*unpublished data*); however, there has been one report [19] that peptide 44–49 had a 50:50 substitution of a seryl for a glycyl residue in the basic protein from one MS patient. This same substitution has been seen in other mammalian species; therefore, its occurrence in MS basic protein may not be disease related. The amino acid composition of lipophilin from normal and MS myelin appears to be similar (*unpublished data*, this laboratory); however, no sequence data on the MS protein is available. Isolation, characterization and elucidation of the primary structure of the remaining myelin proteins has yet to be determined.

In order to determine whether an actual abnormality exists in the protein composition or structure of MS myelin, a careful comparison of the composition of the myelin specific proteins from normal and diseased tissue will have to be undertaken. Since MBP displays microheterogeneity, it will be necessary to compare the corresponding microheteromers of MS basic protein with the normal counterparts.

At least 15 enzymes have been reported to be integral constituents of CNS myelin. Two enzymes, 2′, 3′-cyclic nucleotide 3′-phosphohydrolase [49] and cholesterol ester hydrolase pH 7.2 [31] are myelin specific; the remainder are found in other subcellular fractions, as well, and are involved in lipid metabolism and transport activities.

When the enzyme activity of 2′,3′-cyclic nucleotide 3′-phosphohydrolase purified from MS myelin was compared to the normal enzyme activity, it was found to be decreased by one half [45]. No significance was attached to this finding by the investigators as they could not establish whether the total enzyme concentration was decreased or whether half of the enzyme had been inactivated in some way.

Membrane Organization

The study of the membrane organization of myelin involves the study of both intrabilayer and interbilayer interactions. The intrabilayer interactions are concerned with lipid-lipid, protein-lipid, and protein-protein interactions that are found in the bilayer. These have been studied by spin label, fluorescence, and X-ray techniques. The interbilayer interactions responsible for inducing and maintaining the unique multilayer organization of myelin have been studied with X-ray diffraction techniques primarily.

Intrabilayer Interactions

The lipid moiety of the normal myelin membrane is believed to exist in the form of a bimolecular leaflet. The results of early studies using physical techniques suggested that the protein moiety formed a monolayer across the polar head groups of the lipid molecules at each surface of the bilayer [5, 70, 87]. On the basis of more recent studies, this model is no longer tenable. Using a number of different techniques, it has been established that the basic protein is located on the cytoplasmic side of the bilayer only [1, 12, 14, 44, 46, 51, 63]. The interaction of the basic protein with the polar head groups of lipid molecules is largely electrostatic [28, 61, 72], although there are domains that penetrate the bilayer and interact hydrophobically [7, 62, 85] with the lipid interior.

On the other hand, the intrinsic protein (lipophilin) appears to be imbedded in the membrane [34, 35, 44, 63, 85] and spans the bilayer with portions exposed at both surfaces [84]. In this case, the lipophilin-lipid interaction is hydrophobic, but it also interacts electrostatically with acidic lipids [9, 13, 38]. It is surrounded by 20–30 molecules of boundary lipid and induces phase separation by attracting acidic lipids to its boundary layer. These studies are summarized in a recent review [8].

Membrane Impermeable Labeling Reagents

The localization of the basic protein and lipophilin in normal and MS myelin has been studied by the use of the chemical labeling reagent 4,4′-diisothiocyano-2,2′-ditritiostilbene disulphonic acid (^{3}H-DIDS) [86]. From the results of this study, the authors concluded that the protein arrangement in chronic MS myelin was similar to that of the normal myelin membrane, but in myelin isolated from acute MS the arrangement differed. In the latter case, the BP was more accessible to the reagent as a result of a disruption in the membrane. This finding was confirmed by electron micrographs of the acute MS myelin, which demonstrated that almost all lamellar structure was lost and vesiculation had occurred [86]. This study implied that grossly normal-appearing MS white matter may have a different molecular architecture than that from normal myelin.

Spin Label and Fluorescent Probes

Electron spin resonance (ESR) measures the "fluidity" of localized regions of a membrane by incorporating probes that will occupy different positions within the membrane. Myelin has been shown [8, 75] to be less fluid than plasma membranes (e.g., erythrocytes, hepatocytes, and synaptosomes). The greatest difference between these membranes was observed with the fatty acid spin label, 12-doxyl stearate (nitroxide group at C-12). However, no difference in the fluidity between MS and normal myelin was detected [48], using the 12-doxyl stearate probe.

A second group of probes, fluorophores, measure fluidity by fluorescence polarization. The probe 1,6-diphenyl 1,3,5-hexatriene (DPH), which partitions into the hydrophobic portions of the membrane, was incorporated into red blood cell ghosts from patients with Duchenne muscular dystrophy (DMD) and carriers of DMD. In both cases, the fluidity was found to be decreased when compared to normal erythrocyte ghosts [50]. However, a decrease in fluidity was not observed by other investigators [15, 69] using spin labels. This lack of concordance in measurements of fluidity by the two methods was observed between normal and transformed cells in culture [39]. Using DPH and *trans*-parinaric acid as fluorescent probes, no difference in fluidity of normal and MS myelin was detected [6], which is in agreement with the spin-label studies.

Interbilayer Interactions

Model Systems

When basic protein isolated from normal human myelin was incorporated into phosphatidylglycerol (PG) vesicles, it was shown to promote the organization of the phospholipid bilayers into a multilamellar structure similar to native myelin by liquid X-ray diffraction [11]. The basic protein isolated from MS myelin was less effective in inducing this multilayered pattern [10]. In fact, even at very high concentrations, the MS-MBP was not able to induce the almost crystal-like structure induced by the normal MBP. The results suggested that the normal basic protein was responsible, in part, for the stabilization of the multilamellar structure observed in myelin, and the MS protein was less effective. Although biochemical studies have failed to establish a difference in the basic protein from MS and normal myelin to date, the liquid X-ray diffraction data would imply that a difference exists. The difficulty in demonstrating a difference may be related in part to the MBP microheterogeneity. A complete examination of the role of the various charge isomers on the formation and stability of the multilayer pattern is underway in our laboratory.

The Physical Structure of Normal and Diseased Myelin

To explore further our hypothesis that a membrane defect exists in MS, myelin from normal and diseased white matter was subjected to low angle X-ray diffraction. The patterns obtained from both normal and MS myelin showed identical spacings of about 178 Å [76], confirming that intact myelin was being studied. In the presence of long-chain fatty alcohols (*n*-decanol, *n*-dodecanol and *n*-tetradecanol) the X-ray patterns for seven normal myelin samples were unaltered. On the other hand, the X-ray pattern for all eight MS samples differed in the presence of the alcohols. Additional reflections, as well as a decrease in the width at ½ height of the third-order reflection, were observed. These changes are thought to result from the fact that MS myelin contains domains of poorly organized structure not present in normal myelin and, therefore, the alcohol can penetrate into these domains and form regular structures. This proposal was further supported by the finding that a greater number of poorly structured domains were present in the more acute form of MS than the chronic form [76]. These findings are in agreement with the DIDS-labeling studies and electron micrographs in which acute MS myelin showed loss of lamellar structure and an increase in vesiculation [86].

A recent study [18] in which wide angle X-ray diffraction was used to determine the gel phase and liquid crystalline phase lipid supports the conclusion from the previous X-ray results with fatty alcohols. Lipid in the gel phase is represented by a Bragg spacing of 4.15 Å, liquid crystalline lipid by a Bragg spacing of 4.6 Å in the X-ray diffraction pattern.

At 26°C the X-ray pattern for both the normal and MS myelin were identical; that is, both membranes showed gel and liquid crystalline phase lipid. At 50°C the normal myelin X-ray pattern was identical to that observed at 26°C. However, no gel phase lipid (4.15 Å Bragg spacing) was present in the MS myelin, indicating a more fluid or disordered membrane structure [18]. To extend these findings the phase transition temperature (*Tm*), which reflects changes in mobility of the fatty acyl chain of lipids, was measured. For normal myelin the *Tm* was 62.6 ± 2.0°C and 42.0 ± 2.0°C for MS myelin, that is a difference of about 20°C. In order to determine whether this difference in *Tm* was due only to the lipid moiety of myelin, a total lipid extract was prepared. The *Tm* value obtained for the MS total lipid extract was not significantly different from that of MS myelin, whereas the *Tm* for total lipid extract from normal myelin decreased by 13°C. The results suggest that the protein moiety of normal myelin plays an important role in the stability and organization of the membrane and this role appears to be diminished in MS myelin. Since the lipid phase transition of the total lipid extract was decreased by 13°C in the normal material, a role for the lipid components is also indicated.

Developmental Studies

Myelin was isolated from the CNS white matter of 18 deceased individuals whose age at death ranged from 2 months to 78 years. Wide angle X-ray diffraction pattern and the lipid phase transition temperature was obtained for each myelin sample (*17*).

The wide angle X-ray diffraction pattern indicated that myelin structure undergoes changes with development. No gel phase lipid (Bragg spacing 4.15 Å) was discernible in myelin isolated from 2–3 month old infants. By 5 years of age this lipid was detected but was most pronounced after the age of 8 years. After the age of 50 years the 4.15 Å Bragg spacing became less intense. These results indicated that as myelin develops it undergoes a structural change from a disordered to ordered state and maintains this ordered state until individuals begin to age.

The *Tm* value was found to increase gradually from 13 to 40°C in the first 8 years of life (phase I). By the age of 20 years the *Tm* value rose sharply to 62°C, remained at this value until the age of 50 (phase II), and then decreased to 50–55°C by 75–78 years (phase III). The decrease in phase III was correlated with an increase in the concentrations of malonaldehyde and conjugated dienes formed by lipid peroxidation [17].

In the case of MS myelin, the *Tm* value was 42°C, a value obtained by the normal developing human by 5–8 years of age. This suggests that an inability to complete phase II of myelin maturation may be responsible for the low *Tm* found in MS myelin.

Concluding Remarks

The studies presented in this review of available data obtained on noninvolved areas of white matter support the view that a generalized membrane defect may be responsible for the degeneration observed in MS.

An important advance has been made by focussing on developmental changes in myelin. Although it may be premature, the fact that myelin from the central nervous system of children aged 5–8 years

shows similarities in physical structure to myelin obtained from MS patients, even though most of the components (lipids and proteins) are present in adult proportions, emphasizes the need to study myelin development in the human. An understanding of how the compact multilayered structure recognized as adult myelin is formed during growth and development may provide important clues concerning the structure of myelin in disease.

The data already available provide a possible explanation for epidemiological data, documented over many years. Epidemiological data obtained from the studies of the incidence of MS in migrant populations suggest that MS is acquired early in life and a latent period exists before the clinical onset of the disease. Briefly, if an individual moves from a high-incidence area to a low-incidence area before the age of 12–15 years, he or she will develop MS with the frequency found in the new environment, whereas if the individual moves after the age of 15 years, he or she carries the incidence of MS from the place of origin to the new environment [3, 54]. The wide angle X-ray diffraction data of myelin during development showed that mature myelin was not formed until the age of 17–20 years. If myelin is not fully mature when an individual moves from a high- to a low-incidence area or vice versa, the myelin will be susceptible to whatever causes myelin breakdown in the new environment. Alternatively, after the adult myelin is formed it becomes less susceptible to attacking etiological agents, so that the incidence of MS is that of the environment of origin. Although this is largely speculative, it does point to a correlation between physical structure of myelin and degeneration.

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