The Macrophage as the Demyelinating Agent: a Role for Antimyelin Antibodies

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THE MECHANISM OF MYELIN DESTRUCTION

Multiple sclerosis (MS) is characterized by the focal destruction of myelin in the central nervous system. It is generally believed that macrophage-like cells actively phagocytize the myelin by surrounding the nerve fiber, peeling away the outer myelin lamellae, and ingesting the myelin droplets (1). Macrophages filled with lipid particles are seen in the vicinity of the lesions. These "foamy" macrophages stain positively with stains for neutral lipids such as Oil Red O, and multilamellar bodies can be seen within the cell body by electron microscopy. The bulk of the neutral lipids contained within the macrophages are composed of cholesterol ester and triglycerides, lipids not usually present as components of the membranous nervous system. Cholesterol, a major lipid of the myelin sheath is esterified to form cholesterol ester, and the fatty acids of the cholesterol ester and the triglycerides are formed by hydrolysis of myelin phospholipid. These metabolic conversions of myelin lipids presumably take place within the macrophage cell body after phagocytosis of the myelin membrane.

Origin of Phagocytes

The question whether the phagocytes which attack the myelin sheath are derived from blood monocytes infiltrating the nervous system at the time of the demyelinating event, or are the resident microglia which become activated, is at present unanswered, but it is likely that phagocytes of both origins may participate in the myelin attack. When microglia are activated, it is difficult or even impossible to distinguish them from macrophages. Microglia, like blood macrophages, are believed to be derived
from monocytic blood cells (2), and show the ability to present antigen (3). Microglia contain the surface antigen MAC-1, the receptor for acetylated LDL, and stain for nonspecific esterase (4). Some minor differences between the two cells in culture have been noted; the microglia lack peroxidase and the ruffled edges of peritoneal macrophages, but retain the ability to proliferate (4).

A ROLE FOR ANTIBODY IN DEMYELINATION

Multiple Sclerosis

At the present time multiple sclerosis is thought to involve an immunological element, although the event that initiates the process is unknown. Evidence of immunological processes in MS includes the invasion of perivenular lymphocytes and monocytes into the CNS, the similarities between MS and experimental allergic encephalomyelitis (EAE), particularly the chronic relapsing form, and the presence of IgG in the cerebrospinal fluid (CSF) (5).

The presence of large amounts of immunoglobulin in the CSF is an invariable feature of multiple sclerosis, such that it is used as a diagnostic tool. The immunoglobulin appears to be synthesized within the nervous system, probably by B-cells or plasma cells migrating into the CNS in the course of the immunological episode. A number of investigators have detected CSF antibodies against brain or myelin constituents (e.g., Carson et al. and Ichikawa et al. detected antibody to myelin basic protein (MBP) (6,7), Kasai et al. to glycolipids (8), and Moller et al. to myelin-associated glycoprotein (9)). On the other hand, Chou et al. (10) could not confirm the presence of antibody to MBP, and Rostami et al. (11) did not find antibody to galactocerebroside in CSF of MS patients. At this time there is no agreement as to whether there is myelin antibody specificity in the CSF of MS patients.

IgG has been detected within and on the surface of macrophages in areas of demyelinating lesions of MS brain. The macrophage immunoglobulin often displayed an uneven distribution similar in appearance to capping. Prineas and Graham (12) interpreted these observations as an indication that Fc receptors linked the macrophage to IgG directed against an antigen in the myelin sheath. The myelin ingested into the phagocytic cell was attached to coated pits, a sign of receptor mediated phagocytosis (13).

Experimental Allergic Encephalomyelitis

The immunological aspects of this autoimmune disease, often used as an animal model for multiple sclerosis, is on a much firmer basis. EAE has been clearly shown to be a T-cell mediated disease (14,15). Transfer of T-cells from a line specifically sensitized to MBP will cause EAE in normal mice (16). Humoral agents were not considered to be involved in the disease because EAE could not be transferred with serum.

As with multiple sclerosis, myelin destruction in EAE is macrophage mediated. Lampert (17) has described stripping of the myelin lamellae on whole myelin segments
MYELIN PHAGOCYTOSIS

Figure 1. Cell associated radiolabel after different incubation times of macrophage monolayers with $^{14}$C-labeled myelin. Each point represents total cell bound dpm/cell bound + supernatant dpm. AS, Antiserum-treated myelin; U, untreated; PI, preimmune serum.

by invading mononuclear cells. The myelin fragments are then ingested into the cell body and transformed into amorphous globoid lipid bodies. The myelin lamellae have been observed attached to coated pits on the macrophage surface, as with MS. Epstein et al. (18) have suggested that the targeted activity of macrophages which bind to an antibody-myelin complex through their Fc receptors may explain the specificity of myelin destruction, leaving the axon intact.

ANTIBODY EFFECTS ON MYELIN DESTRUCTION

Experimental Findings

The results of a number of experiments from this laboratory have supported a role for antibody in cell mediated demyelination. These experiments utilize cultures of thioglycollate elicited peritoneal macrophages from the adult rat. After the macrophages are harvested and the cultures established, they are transferred to a defined medium and incubated with purified myelin, the lipids of which contain a $^{14}$C label.
When untreated myelin was presented to the cultures much of the myelin quickly became cell associated, but if the myelin was pretreated with antiserum to purified CNS myelin raised in rabbits, much more myelin became cell associated. After 30 hours about 45% of the total myelin radioactive counts were cell associated compared to 23% with untreated myelin and 20% with preimmune serum treated myelin (Fig. 1).

Examination of the macrophage cultures after incubation with antiserum treated myelin showed many more myelin spheres within the macrophage cell bodies than with preimmune serum treated myelin or untreated myelin. The latter appeared to adhere to the outside surface of the macrophage without being ingested, giving the cells a ragged appearance (19).

Analysis of lipids in the cultures after 30 hours incubation revealed that with antibody treated myelin much of the radioactivity was found in cholesterol ester and triglyceride, while very much less was present in cultures incubated with preimmune treated or untreated myelin (Fig. 2). About four times as much radioactivity in
Figure 3. Accumulation of $^{14}$C-labeled cholesterol ester (CE) and triglyceride (TG) after 30 hours culture of macrophage monolayers with radioactive myelin treated with various antisera. a Myelin, antiserum to purified CNS or PNS myelin; a GC, antiserum to GC; a BP, antiserum to MBP, a MAG, antiserum to MAG, PI, preimmune serum, U, untreated myelin. Reprinted by permission from the editors of the Journal of Neurochemistry (19).

cholesterol ester and about twice as much in triglyceride was present after 30 hours compared to that in cultures with preimmune serum treated myelin. It was interesting that although untreated myelin associated with the macrophages to a large extent (Fig. 1), there was very little conversion of cholesterol to cholesterol ester. When the radioactive cholesterol ester formed from the antibody treated myelin was hydrolyzed, both the cholesterol portion and the fatty acid of the cholesterol ester were found to be radioactive, with about 66% of the total counts in the cholesterol and 34% of the counts in the fatty acid. Thus, both the cholesterol and fatty acid of the cholesterol ester were derived from the myelin, rather than from serum (19).

Specificity of the Antibody

ImmunobLOTS of the anti-CNS myelin serum revealed the presence of antibody to all four MBPs, the proteolipid protein (PLP), the cyclic nucleotide phosphohydrolase (CNpase), and a high molecular weight protein, probably the myelin-associated glycoprotein (MAG). Antibody to the glycolipid, galactocerebroside (GC), was also present (19). Specific antibodies to myelin constituents were tested as myelin op-
sonizing agents to determine which of these might be the active factor(s). Antibodies active in promoting uptake of myelin and cholesterol ester formation included antiserum to MBP and to GC (Fig. 3), while anti-MAG and anti-PLP (not shown) did not appear to be active. Antibody to whole peripheral nerve myelin, which also contains GC and MBP, was fully as active as that to CNS myelin in promoting the metabolism of central myelin. Heating the serum to 56° for 30 minutes to destroy complement did not alter the opsonizing activity; therefore complement does not appear to participate in the opsonization.

Figure 4. Time course of accumulation of cell associated radiolabel (uptake) and production of radioactivity in cholesterol ester in macrophage cultures incubated with 14C-labeled myelin treated with serum from Lewis rats with acute EAE induced by sensitization with purified CNS myelin, or from Freund’s adjuvant-injected controls (FAC), or with untreated myelin. From Smith ME, DeJong LJ. Neurochem Res 1987;12:167–172. Reprinted with permission from the editor.
Antibodies in EAE

Studies with rat macrophages incubated with myelin pretreated with serum from Lewis rats with EAE induced by sensitization with purified CNS myelin produced analogous results (Fig. 4). Purified myelin preincubated with EAE serum became macrophage associated to a much larger extent than untreated myelin or myelin preincubated with serum from rats injected with Freund’s adjuvant alone (FAC). Untreated myelin was taken up by macrophages much more than FAC serum treated myelin, but very little cholesterol ester was produced. Macrophages ingesting myelin treated with anti-CNS myelin serum produced more than twice as much cholesterol ester as from FAC serum treated myelin, and more than four times that from untreated myelin.

Figure 5. Accumulation of radiolabeled cholesterol ester in macrophage cultures incubated with $^{14}$C-labeled myelin pretreated with serum from Lewis rats at increasing time after immunization with purified myelin in Freund’s complete adjuvant. Percent cholesterol esterified is measured by dpm cholesterol ester/dpm cholesterol ester + cholesterol.
Figure 6. Immunoblot of separated CNS myelin protein showing the appearance of antibodies to MBP (MBP) and (PLP) in serum of Lewis rats immunized with purified myelin in Freund’s complete adjuvant. Lane 1, Freund’s adjuvant control serum; lanes 2–9, serum from animals days 7–14 after immunization.

The amount of stimulation of myelin uptake and cholesterol ester production from anti-CNS myelin serum increased with the time after immunization of the animals. Thus the myelin treated with serum taken from rats on day 7 after myelin injection produced only small amounts of cholesterol ester, then the production gradually increased so that the myelin treated with serum obtained 18 days after immunization yielded 34% esterification of the myelin cholesterol (Fig. 5).

This increase generally paralleled the amount of antibody present in the serum. At 7 days little or no antibody was visible on an immunoblot, but on day 8, antibody to MBP began to appear and gradually increased up to 14 days (Fig. 6). Further increases in antibody titer were seen up until about 30 days, then the antibody
Figure 7. Production of radioactivity in cholesterol ester (CE) and triglyceride (TG) by macrophages incubated with myelin pretreated with serum from rats with acute EAE as a result of immunization with purified myelin (EAE-My), of MBP immunization (EAE-BP), or serum from Freund's adjuvant-injected controls (FAC). Reprinted with permission from the editor of Neurochem Res.

decreased somewhat, but remained high up to 92 days after immunization, the last time point tested. These experiments indicate that the level of antibody correlates well with the stimulation of myelin uptake and the amount of cholesterol ester produced in our in vitro model of this cell mediated demyelinating disease.
Although the antibody specificity in anti-CNS myelin serum appeared to be mainly toward MBP, antibody to PLP was the appearance of MBP on immobilized PLP. Anti-PL antibody also gradually increased throughout the time period tested. Antibody to GC was absent in the rat that was tested. Therefore, the cholesterol ester production may be due to stimulation by the antibody to MBP. Although we previously showed that rabbit antibody to PLP did not stimulate activity of the anti-serum, it is the same as that produced with whole myelin (Fig. 7).

When EAE was produced with MBP instead of whole myelin, the stimulating activity of the anti-serum was identical to that produced with whole myelin.
MYELIN PHAGOCYTOSIS

Antiserum Effects in Microglia

A purified culture of microglia was produced using a modification of the procedure of Glia and Baker (20). This method utilizes the procedure of McCarthy and de Vellis (21) in which dissociated cells from newborn rat brains are placed in primary culture. After culturing for some days the oligodendroglia and microglia are detached from the astrocyte bed by shaking, the microglia are allowed to adhere to a second culture dish, and the oligodendroglia washed off. The result is an almost homogeneous population of microglia. When these cells were incubated with myelin treated with antisera to MBP and to GC for 30 hours it was observed that almost every cell took up myelin. As with peritoneal macrophages, the antisera to MBP and GC stimulated the appearance of cholesterol ester (Fig. 8), indicating that intrinsic brain macrophages are able to participate in myelin uptake and metabolism, as has been shown with peritoneal macrophages. Microglia have been shown to contain Fe receptors, and the mechanism is probably similar to that postulated by Epstein et al. (18).

Demyelination in vivo and in vitro

The possibility that antibody to GC is involved in demyelination has been considered by other investigators. Raine et al. (22) showed that guinea pigs immunized with MBP developed the severe clinical symptoms and CNS inflammatory response of EAE, but showed little demyelination, while those immunized with whole CNS white matter or MBP + GC developed the identical disease as well as demyelinating lesions. Anti-GC sera applied to organotypic cultures cause demyelination (23), and experimental neuritis can appear after long-term immunization with GC (24). Antibody to GC appears to be a more likely candidate for a humoral role in demyelination, since GC is found on the outside of the myelin membrane, and is therefore more accessible to the antibody. MBP, on the other hand, is believed to be located on the cytoplasmic face, and therefore might not be exposed to antibody. It is interesting that the Lewis rat is notable for its failure to develop extensive demyelination in EAE, and this may be related to our finding that it does not produce antibody to GC.

Although only inflammatory lesions were observed during the acute clinical stages of EAE in Lewis rats, during the recovery stage severe demyelinating lesions were observed in some of the spinal cords, and active phagocytosis of myelin was seen as late as 42 days after immunization with myelin. Although anti-GC antibody was not present, the long exposure to high amounts of MBP and possibly PLP may have promoted demyelination.

MACROPHAGES AND PROTEOLYSIS

The Macrophage as a Secretory Cell

In addition to its phagocytic function the macrophage is a major secretory cell, and a number of its secreted products can be destructive agents. A partial list of
Table 1. Injurious Factors Secreted by Macrophages.

<table>
<thead>
<tr>
<th>Category</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteases</td>
<td>Plasminogen Activator</td>
</tr>
<tr>
<td></td>
<td>Collagenase</td>
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<tr>
<td></td>
<td>Elastase</td>
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<tr>
<td></td>
<td>Acid Protease</td>
</tr>
<tr>
<td>Phospholipases</td>
<td>Complement Components</td>
</tr>
<tr>
<td>Reactive Metabolites of Oxygen</td>
<td>Superoxide</td>
</tr>
<tr>
<td></td>
<td>Peroxide</td>
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<tr>
<td></td>
<td>OH Radical</td>
</tr>
<tr>
<td></td>
<td>Singlet Oxygen</td>
</tr>
<tr>
<td>Arachidonate Metabolites</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td></td>
<td>Leukotrienes</td>
</tr>
<tr>
<td></td>
<td>OH Eicosatetraenoic acids</td>
</tr>
</tbody>
</table>

Products secreted by macrophages includes several proteases, phospholipases, complement components, reactive metabolites of oxygen, and arachidonic acid metabolites (Table 1). A more comprehensive list has been compiled by Nathan et al., (25). Secreted products which might be injurious to myelin include proteases, phospholipase, and oxygen metabolites, while complement, which has been shown to be activated by myelin, may have a potentiating role (26). In some kinds of demyelinating conditions myelin appears to be disrupted extracellularly. The regular membranous lamellae split at the major dense line, then form vesicles prior to their dissolution. Such a mechanism has been observed in EAE and experimental allergic neuritis (EAN) (17,27,28). Myelin vesiculation is also a prominent feature of certain kinds of myelin disruption which are not cell mediated, such as is found in spinal cord trauma (29).

Proteolytic Enzymes

Vesiculation of the myelin membrane may be a result of proteases and phospholipases secreted by macrophages. The extreme vesiculation seen in spinal cord injury has been attributed to an influx of calcium, thereby activating phospholipase and proteases (30). Calcium-activated protease has been shown to be present in the myelin membrane (31).

Conditioned medium from macrophage cultures has been shown to contain proteolytic enzymes which hydrolyze the basic protein in myelin (32). Plasminogen activator, as well as an enzyme inhibited by a metal chelator (EDTA), possibly elastase, appeared to be the active enzymes secreted into the medium. Although the macrophage medium was effective only on lyophilized myelin and not on freshly prepared myelin, Cammer et al. (33) were later able to show that complement potentiates the proteolytic effect so that the conditioning medium as well as plasmin is able to degrade myelin basic protein in freshly prepared myelin.
MYELIN PHAGOCYTOSIS

A number of proteolytic enzyme inhibitors, especially those of plasminogen activator, injected into EAE rats have been found to suppress the lesions and clinical symptoms of EAE (34,35). The mechanism of inhibition of EAE symptoms and lesions was assumed to be the prevention of proteolysis of myelin proteins.

On the other hand, the inhibition of plasminogen activator may have also inhibited macrophage migration into the CNS. Plasminogen activator has an important role in cell migration and matrix breakdown by the formation of plasmin, which certain cells use to break down barriers to migrate through the vessels (36). Macrophages, fibroblasts, and several types of malignant cells have especially high levels of bound plasminogen activator localized to sites on the plasma membrane. Fibronectin and other components of the extracellular matrix are directly degraded by plasmin. In experiments where Lewis rats were immunized with whole myelin and injected with a number of protease inhibitors the numbers of cells infiltrating the CNS as measured by the DNA levels were substantially decreased. Although the onset of EAE symptoms was usually accompanied by a 2- to 4-fold increase of DNA in the spinal cord, the increase of DNA in rats injected with inhibitors of plasminogen activator and other proteolytic enzymes was substantially less (37). Furthermore, the lesions appeared to be small and punctate, with very few cells migrating beyond the immediate periphery of the blood vessels. It is likely, therefore, that the plasminogen activator, elastase, and collagenase secreted by macrophages may be important factors in allowing entrance of the cells into the CNS, in addition to their proteolytic action on myelin.

Other Macrophage-secreted Products

In addition to proteolytic enzymes and complement, phospholipases and reactive metabolites of oxygen may also participate in myelin destruction. Activated macrophages were shown to contain a number of phospholipases and to secrete these into the culture medium (38). The culture medium could be shown to degrade myelin lipids, and, in some cases, myelin. Phospholipases generate polyunsaturated fatty acids, especially arachidonic acid, a precursor of a number of metabolites which produce inflammation. Furthermore, polyunsaturated fatty acids are oxidized by reactive oxygen species to form lipid peroxides, which produce cellular edema (39). Reactive oxygen species also aggregate myelin proteins concomitantly with the accumulation of lipid peroxidation products (40). The secretory products of microglia have not been systematically explored, but secretion of superoxide anions in response to certain activating agents has been demonstrated in vitro (41). It is likely that activated microglia will be shown to produce many of the factors secreted by macrophages.

CONCLUSIONS

The activated macrophage is believed to be the main agent of myelin destruction in cell mediated demyelinating diseases. It possesses the capability of destroying myelin extracellularly by secreting a number of agents shown to damage myelin,
including proteases, complement, phospholipases, and reactive oxygen species. In addition, the macrophages phagocytize myelin by receptor mediated endocytosis, as shown by the presence of coated pits. Much evidence supports the mechanism of the Fc receptor-antibody ligand for myelin phagocytosis. At this time it is not known to what extent each of these mechanisms is involved in the various demyelinating diseases, and it is possible that different factors are important for demyelination in MS and EAE. A role for the brain macrophages, the microglia, is also likely, and investigation of the properties of these cells is underway. It is probable that most of the physiological properties of macrophages will be found in microglia also, under the appropriate conditions. This area of study has just recently emerged, and the physiology and role of microglia will undoubtedly be better understood in the near future.

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REFERENCES


MUltiple Sclerosis
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EDITORS

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