

CHAPTER 34

Association between cell-mediated demyelination and astrocyte stimulation

Marion E. Smith and Marc A. Sommer

Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305, and VA Medical Center, Palo Alto, CA 94304, U.S.A.

Introduction

Myelin, the multilayered membrane surrounding the axon, is an active participant in nervous system function. It is closely integrated with the metabolic viability of the neuronal cell body and axon, and when the neuron dies or the axon is cut, the myelin sheath disintegrates. Conversely, nerve conduction slows drastically when the myelin is disrupted. In primary demyelinating disease, the myelin sheath is the target of attack, leaving the axon relatively unscathed. Primary demyelinating diseases in the human nervous system include multiple sclerosis, acute disseminated encephalomyelitis, and acute leukoencephalopathy.

In the laboratory a primary demyelinating disease can be induced in various experimental animals to serve as a model system for the human diseases. This demyelinating condition known as experimental allergic encephalomyelitis (EAE) has many features in common with multiple sclerosis. These include the invasion of lymphocytes and macrophages into the CNS parenchyma, destruction of myelin by phagocytic cells with sparing of axons, and oligoclonal IgG bands in the CSF. Since EAE is induced by immunization of laboratory animals with white matter or purified myelin, and myelin is specifically attacked, this disease has the characteristics of an autoimmune disease. The first protein to be identified as a sensitizing antigen was myelin basic protein (MBP). Purified MBP alone, used as the im-

munizing agent, induces EAE (reviewed by Brostoff, 1984). More recently, however, the structural protein of CNS myelin, proteolipid protein (PLP), has also been shown to cause EAE with somewhat different characteristics (Williams et al., 1982).

Galactocerebroside, a myelin lipid, has also been implicated as modifying the immune response to myelin basic protein to enhance demyelination (Raine et al., 1981).

Many investigators have shown conclusively that EAE is initiated by T cells. EAE can be passively transferred to a naive animal by lymph node cells from an immunized animal (Patterson, 1960), or by specific T cell lines sensitized to myelin basic protein (Ben Nun and Lando, 1983). EAE, on the other hand, cannot be transferred with serum. Nevertheless, there is much evidence that B cells and macrophages entering the CNS may contribute to the pathogenesis of the disease, particularly the destruction of myelin. A role for antibody in the disease process has been proposed by a number of investigators, and work described here lends further support to this idea. The influence of antibody-mediated demyelination may also affect other cells in the CNS.

Another characteristic of multiple sclerosis is the severe gliosis which accompanies the demyelinating process, and persists in old lesions as a scar. Astrocytes begin to hypertrophy with the onset of the active MS lesion, and the fibers rapidly fill the space between the demyelinated axons (Raine,

1984). Similarly, with the onset of EAE the astrocytes very early show increased staining to the astrocyte marker protein, the glial fibrillary acidic protein (GFAP) (Smith et al., 1983). A number of theories have been proposed for the mechanism of astrocyte stimulation, one of which invokes the effects of certain cytokines secreted by the activated cells from the immune system in cell-mediated demyelination. Evidence for stimulation of astrocytes by cytokines will be discussed further.

In recent years, investigators working on demyelination mechanisms in animal models have uncovered a network of interrelations between neural and immune cells. These findings have been applied to multiple sclerosis to greatly expand our understanding of this human disease.

Agents of demyelination

It has become evident that the actual agent of myelin destruction is the phagocytic cell which inserts its processes between the lamellae of the myelin sheath, peels them off, and ingests the membranous vesicles. This process was first described by Lampert and Carpenter (1965) in rats with EAE. Further studies of EAE by Lampert revealed two distinct demyelinating processes. In addition to myelin stripping and phagocytosis, lysis of the myelin lamellae was also observed in the vicinity of the mononuclear cell as shown by separation of the lamellae at the major dense line, which then form vesicular structures (Lampert, 1967). Myelin debris was identified within the mononuclear cells, thus classifying them as macrophages. Similarly, in multiple sclerosis as well as in other demyelinating diseases, the macrophage initiates the attack on the myelin and ingests the lamellae, probably by receptor-mediated endocytosis (Prineas, 1985). Vesiculation of the myelin has also been observed in MS, but the relation to vesicular degeneration and post-mortem autolysis is not clear (Prineas, 1985).

The phagocytic cell

A breakdown of the blood-brain barrier has been shown to accompany the infiltration of cells in the

guinea pig and rat with EAE (Cutler et al., 1967; Hirano et al., 1970; Juhler et al., 1984). Electron microscopy of EAE lesions have shown peripheral blood macrophages as well as T and B cells invading the CNS parenchyma through the microvessel walls (Raine, 1985; D'Amelio et al., 1990). Identification of the cells composing the infiltrate has been made with the use of monoclonal antibodies, and the principal invaders into the spinal cord of the Lewis rat with EAE have been shown to be T helper cells and Ia-positive cells, including macrophages, B cells, or activated helper cells (Hickey et al., 1983). Evidence is accumulating that other antigen-presenting cells, the microglia, may be the resident macrophages of the CNS. Although microglia are located throughout the CNS parenchyma, they are also found surrounding blood vessels as a component of the perivascular glia limitans (Lassmann et al., 1991). Like macrophages in general, they phagocytize latex beads, show non-specific esterase activity, and express Fc and type 3 complement receptors (Frei et al., 1987). Microglia have been shown to proliferate and phagocytize neuronal debris at the site of injury in the presence of an intact blood-brain barrier (Streit and Kreutzberg, 1988). The perivascular microglia are bone marrow-derived, stain positively for Ia, and appear to function as antigen-presenting cells (Hickey and Kimura, 1988). Peripheral blood macrophages and microglia stain similarly to the same cell markers (e.g., *Mac-1*) (reviewed in Dickson et al., 1991), thus it is difficult to assess their relative participation in EAE perivascular infiltrates and in myelin phagocytosis. Although both the peripheral blood macrophages and microglia have been implicated as agents of myelin destruction, few studies have reported of their capacity and propensity to phagocytize myelin.

In the acute EAE lesions or active MS plaques "foamy macrophages" appear at the edge of the active lesion and react positively with stains for neutral lipids such as cholesterol ester and triglyceride. These lipids are believed to arise from esterification of cholesterol and hydrolysis of phospholipids derived from myelin. Cholesterol ester, a lipid not present in the normal nervous system, can be

detected by lipid analysis of MS plaques (Yu et al., 1982), and in brain and spinal cord of EAE rats (Maggio et al., 1972). During active demyelination, macrophages contain recognizable myelin debris. This laboratory has been actively engaged in examining in detail the conditions by which macrophages and microglia can be induced to phagocytize myelin, and the mechanism of selectivity of the myelin attack which leaves the axon relatively undamaged.

The role of antibody in cell-mediated demyelination

Antibody-mediated demyelination in vitro

When rat peritoneal macrophages elicited with thioglycollate were cultured with purified myelin radiolabeled in the lipids, the myelin quickly associated with the macrophages, but most of the myelin appeared to be localized on the outside of the cell membrane, giving the macrophages a ragged appearance. Very little radioactive cholesterol ester was formed. If the myelin was pre-incubated with

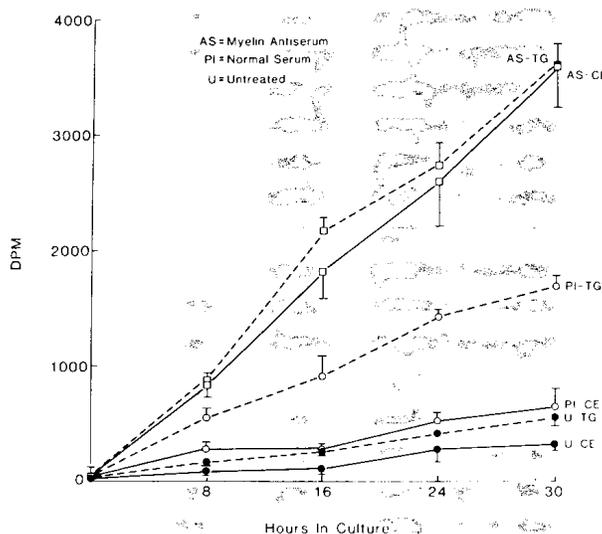


Fig. 1. Time course of synthesis of radioactive cholesterol ester (CE) and triglyceride (TG) in cultured macrophages incubated with [14 C]-labeled myelin pre-treated with antimyelin serum (AS), pre-immune serum (PI), or untreated (U). Points represent the average of three separate experiments \pm S.E.M. (From Trotter et al., 1986.)

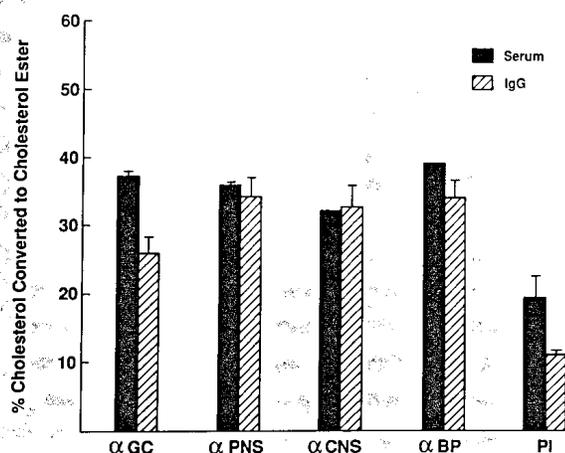


Fig. 2. Percent of total myelin cholesterol converted to cholesterol ester by cultured macrophages incubated for 30 h with myelin opsonized with antisera or IgG to galactocerebroside (GC), PNS myelin, CNS myelin, myelin basic protein (BP) or pre-immune serum (PI). Each value represents the average of 1-3 separate experiments. Bars represent \pm S.E.M.

antiserum to purified CNS myelin raised in rabbit, then incubated with cultured macrophages, the association of myelin with the macrophages was greatly increased, the cells were round and smooth with vesicular structures inside, and much more radioactive cholesterol ester and triglyceride was formed. Pre-immune serum enhanced the production of cholesterol ester only slightly, although some triglyceride was formed (Fig. 1). Surprisingly, antibody to PNS myelin also stimulated cholesterol ester production (Trotter et al., 1986). Since the antiserum contained antibody to many myelin proteins, as well as galactocerebroside, a number of antibodies to myelin constituents were tested for their ability to augment phagocytosis and cholesterol esterification. Antibodies tested included those to galactocerebroside, MBP, proteolipid protein, GM1 ganglioside, and the PNS myelin constituents P_0 and P_2 proteins. Of these, only antibodies to galactocerebroside and MBP showed stimulatory properties (Fig. 2). The amount of cholesterol ester and triglyceride produced when these antibodies were pre-incubated with myelin, then administered to macrophages was nearly as high as with antibody

to whole CNS or PNS myelin. Heating the serum before pre-incubation with myelin did not destroy the activity of the serum, and in most cases purified IgG from the antisera retained most of the stimulating activity (Fig. 2). Myelin purified from both the CNS and PNS were phagocytized by similar mechanisms, that is, the cell association and metabolism of both kinds of myelin was augmented to an equal extent by antibody to CNS myelin, PNS myelin, galactocerebroside, or MBP (Smith et al., 1990). Goldenberg et al. (1989) have also reported increased opsonic activation in rabbit anti-myelin antiserum, in the IgG prepared from this serum, in anti-MBP serum, and in anti-galactocerebroside serum, but not in anti-myelin-associated glycoprotein serum or serum from rabbits injected with Freund's adjuvant alone (Goldenberg et al., 1989).

These results are consistent with a receptor-mediated mechanism for myelin phagocytosis with IgG serving as a ligand between the myelin and the Fc receptor of the macrophage, as suggested by Prineas' group for multiple sclerosis and EAE (Prineas and Graham, 1981; Epstein et al. 1983). Complement appears not to be involved in the phagocytic mechanism, although it may play a role in damaging the myelin for more efficient phagocytosis (Cammer et al., 1986). Therefore, the Fc receptors, but not the C₃ complement receptors are instrumental in ingesting myelin. Epstein et al. (1983) have reported the attachment of myelin lamellae to coated pits on the macrophage surface, a strong evidence for ligand-mediated phagocytosis.

Serum antibody in EAE

After immunization of Lewis rats with purified myelin, the course of development of EAE follows a predictable pattern. At about 10 days post-immunization the animal begins to lose weight, by 12 days it is paralyzed in the hind legs, but by 16 days it has partially recovered and is walking. Serum drawn from the rat at 7 days post-immunization showed no opsonizing activity for myelin phagocytosis, but the activity rose thereafter, at about the time the animals show early signs of EAE (9–10 days), and continued to rise until 27–28 days after immuniza-

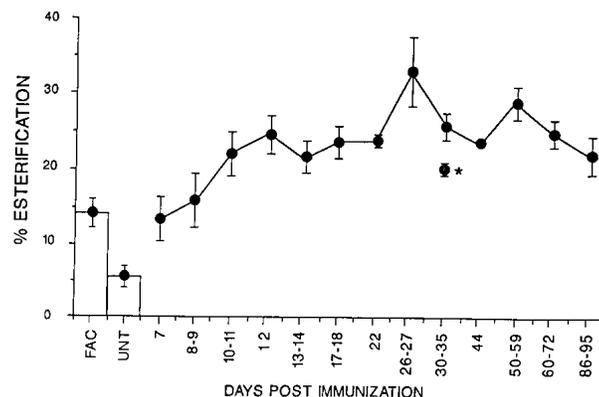


Fig. 3. Percent of cholesterol esterified in macrophage cultures incubated with [¹⁴C]-labeled myelin opsonized with serum from rats at different times after immunization with purified myelin. Incubation time was 30 h. Point marked with asterisk represents a culture containing myelin pre-opsonized with serum showing very little antibody. Each point represents experiments from 3–6 different serum pools. FAC, Freund's adjuvant control serum; UNT, untreated myelin. Bars represent ± S.E.M. (From Sadler et al., 1991.)

tion, at the time when the animal is well on his way to complete recovery. Some cholesterol is esterified from macrophages incubated with myelin opsonized with serum from animals immunized with Freund's adjuvant alone or from those up to 7 days after immunization with myelin. Cholesterol ester production begins to increase from serum taken from animals at about 10 days post-immunization (Fig. 3) (Sadler et al., 1991). After reaching a peak at 27–28 days, the opsonizing activity of the serum decreases somewhat, but remains relatively high up to 95 days post-immunization, the last point tested. Assays of antibody titer of MBP and proteolipid protein by ELISA, as well as electroblots of myelin proteins immunostained with serum from rats at different times after immunization generally showed that the antibody levels followed the pattern of the opsonizing activity (Fig. 4) (Sadler et al., 1991). Although MBP and proteolipid protein showed up well on the immunoblots, no anti-galactocerebroside could be detected in any of the serum samples. This antibody appears not to be made in the Lewis rat, possibly accounting for the relatively small amount of demyelination seen in this species.

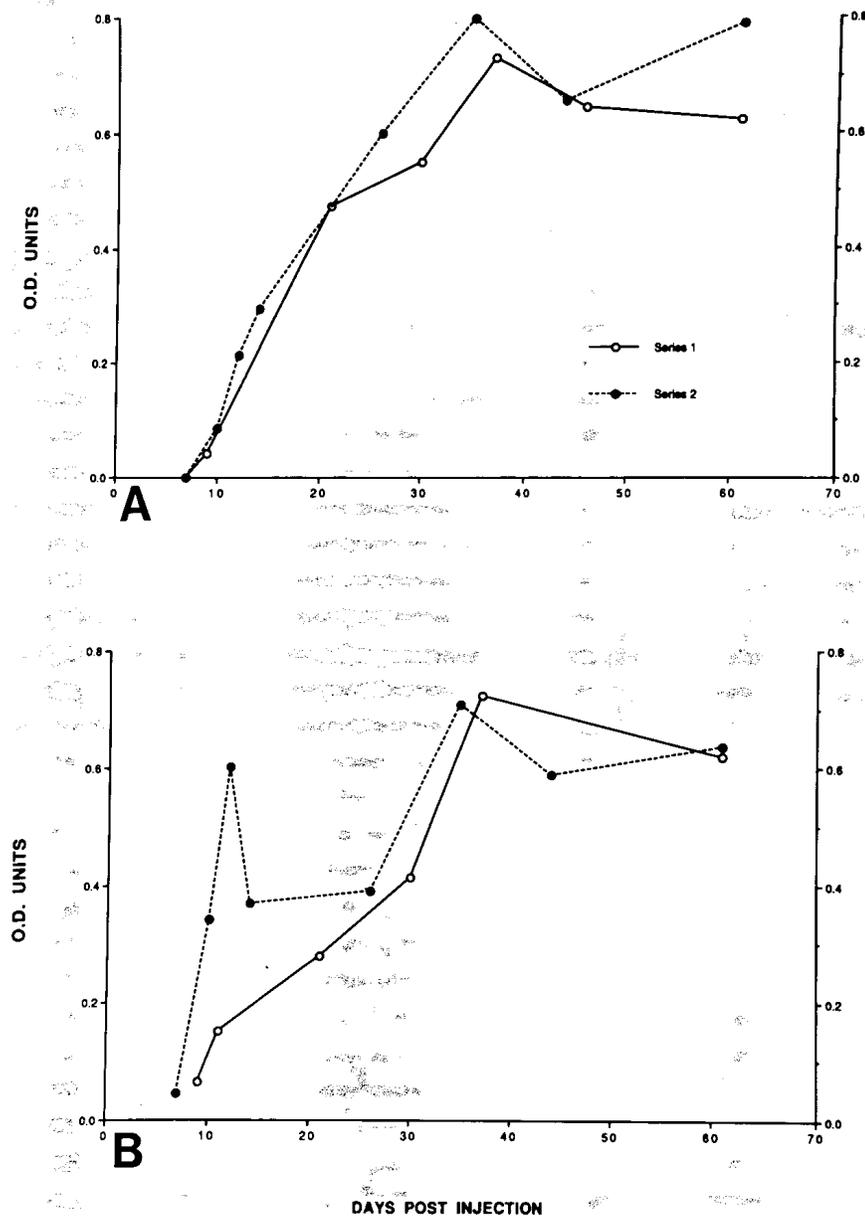


Fig. 4. Relative myelin basic protein (A) and proteolipid protein (B) titers from two series of sera from EAE rats at different times after immunization with purified myelin. Each serum sample is a pool from two identically treated rats. Microtiter plates were coated with myelin basic protein or proteolipid protein and test sera were suitably diluted using alkaline phosphatase for visualization. (From Sadler et al., 1991.)

If antibody affects myelin destruction, one may ask why the animal recovers quickly when the antibody persists over a period of several months. The blood-brain barrier is believed to return to normal by 21 days after immunization in the Lewis rat, and

after this time serum antibody can no longer gain access to the CNS. We have found, however, that demyelinating lesions are active long after the animal has recovered. B cells may be trapped in the CNS with the recovery of the blood-brain barrier,

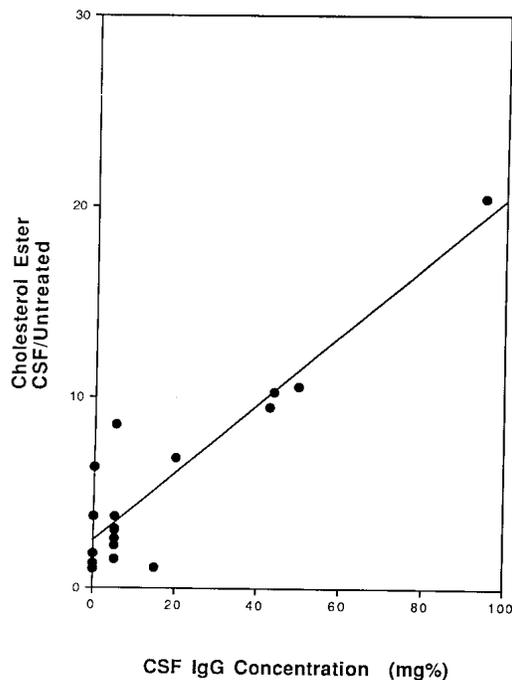


Fig. 5. Correlation between opsonization activity and IgG concentration of cerebrospinal fluid (CSF) from rabbits immunized with myelin. Abscissa represents percent of cholesterol esterified by macrophages, incubated with myelin opsonized with CSF/percent CE with untreated myelin.

and may continue to secrete antimyelin antibodies, leading to further demyelination.

CSF antibody in EAE

That antibody is actually present in the CNS was conclusively shown in rabbits with EAE (Sommer et al., 1992). EAE was induced in rabbits by immunization with purified myelin, and CSF samples were taken from animals with acute symptoms of EAE just before death. CSF from those immunized rabbits which did not show clinical symptoms was collected at 37–42 days after immunization. The CSF was used for total immunoglobulin measurement, immunoblots for analysis of specific antibodies, and opsonization of myelin for measurement of phagocytosis and cholesterol production by macrophages. With one exception, measureable amounts of IgG were found only in those animals showing clinical symptoms. There was a direct cor-

relation between CSF IgG content, and the ability of the CSF to augment phagocytosis and cholesterol ester production (Fig. 5). Blots of myelin proteins using EAE CSF at a dilution of 1:50 for immunostaining revealed antibody to myelin basic protein, proteolipid protein, other unknown high molecular weight proteins, and, in most cases, galactocerebroside (Sommer et al., 1992).

This work shows that antibody to myelin constituents is present within the nervous system in EAE, and that a direct correlation between the IgG content and the opsonizing effect on phagocytosis of myelin *in vitro* can be demonstrated. This is evidence that humoral factors are involved in macrophage-mediated demyelination in EAE. The amount of specificity of IgG in the CSF undoubtedly reflects the degree of acute immunological and destructive activity proceeding in the CNS.

Phagocytosis of myelin by microglia

Active microglia can be prepared for culture from primary cultures of newborn rat brains. In the process of disrupting the brains and preparing primary cultures by the procedure of McCarthy and DeVellis (1980), many neural cells are broken. Microglia,

TABLE I

Effect of GM-CSF on esterification of myelin cholesterol by cultured microglia

Myelin treatment	Percent cholesterol esterified	
	Control	+ GM-CSF
BP antiserum	32.2 ± 3.3	36.9 ± 3.9
BP IgG	27.5 ± 4.8	28.5 ± 8.6
FAC serum	20.0 ± 2.3	22.6 ± 7.0
FAC IgG	23.7 ± 3.3	27.1 ± 5.9
Untreated	11.5 ± 3.2	29.0 ± 3.9

Myelin containing lipids labeled with [¹⁴C] was pre-incubated with myelin basic protein antiserum or its IgG, or with serum or IgG from complete Freund's adjuvant-injected rabbits, or left untreated. The treated microglia were incubated with 300 U/ml GM-CSF for 20 h prior to addition of myelin. The myelin-antibody mixture or untreated myelin was incubated with microglia for 30 h.

which are plentiful in the CNS at that time, are actively engaged in cleaning up the cell debris during the early time in culture. After incubating the primary cultures for a few days, the flasks are gently rocked, and the supernatant with the cell debris are poured off. The debris is centrifuged, gently resuspended, and replated. After an hour in culture, the non-adherent cells are washed off, and a relatively pure microglial culture remains behind. These cells show non-specific esterase activity, and stain positively to *Mac-1* and other antibodies specific for macrophages.

Microglia prepared by this procedure show somewhat different properties from thioglycollate-elicited macrophages. Whereas macrophages survive well in the defined medium N2 (Bottenstein and Sato, 1979), microglia, after 24 h, lose their adherent properties, become very small, and float away into the medium. If granulocyte-macrophage stimulating factor (GM-CSF) is present in the defined medium, the cells remain adherent, and often proliferate. GM-CSF is known to be a potent growth factor for microglia (Giulian and Ingeman, 1988; Suzumura et al., 1990).

If antibody-treated myelin is added to the microglial culture 24 h or less after culture in defined medium, they remain adherent, actively phagocytize myelin, and produce large amounts of cholesterol ester. With untreated myelin, however, the adherent properties are lost, and although some myelin is phagocytized, very few microglia remain on the plates after 30 h of culture. If the microglia are pre-treated with GM-CSF overnight, then incubated with myelin, all cultures, both those fed either antibody-treated myelin or untreated myelin, remain adherent. Although generally the GM-CSF did not enhance cholesterol esterification when antibody-treated myelin was administered to the cultures, much more untreated myelin was phagocytized (Table I). In many experiments, as much cholesterol ester was produced from untreated myelin as from that complexed with antibody. Peritoneal macrophages did not show a marked increase in phagocytosis of untreated myelin with GM-CSF treatment.

We have observed that when microglial cultures contain astrocytes, and these cultures are allowed to incubate for several days, most of the microglia float away except for those adhering to the astrocytes. It appears that the astrocytes offer a sustaining factor or an attractive surface that promotes microglial survival. There is some evidence that this factor(s) may be one or more of the colony-stimulating factors. Hao et al. (1990) have found a factor in astrocyte cultures identified as CSF-1 that stimulates the proliferation of mouse brain macrophages. They were able to show CSF-1 mRNA in astrocytes on the CSF-1 receptor mRNA in the macrophage-like cells. No GM-CSF mRNA, however, was found. Others, using the polymerase chain reaction, found that GM-CSF mRNA was present in unstimulated astrocyte cultures, and that GM-CSF and G-CSF could be detected after stimulation with LPS or murine cytomegalovirus infection (Wesselingh et al., 1990). It appears, therefore that microglia, with proper stimulation, can phagocytize myelin and produce neutral lipid in the absence of antibody, while peripheral macrophages do not show this independence.

Other effects of phagocytic cells

A wide variety of factors, many of them injurious to the surrounding tissues are known to be secreted by the macrophage. A list of these factors includes various proteases, phospholipases, complement components, free radicals, and arachidonic acid metabolites, including prostaglandins and thromboxanes (Cohn, 1978; Wightman et al., 1981; Bonney et al., 1985). Secretion of certain of these factors is modulated by external cytokines (Gordon, 1986). Active investigation is now in progress as to whether microglia also secrete some or all of these injurious substances. It has been shown that activated microglia produce free radicals such as superoxide anion (Colton and Gilbert, 1987), and it is likely that these cells with suitable stimulation will be found to secrete some, if not all of the substances secreted by macrophages. A number of cytokines are also secreted by macrophages; both macrophages and microglia when activated produce interleukin 1

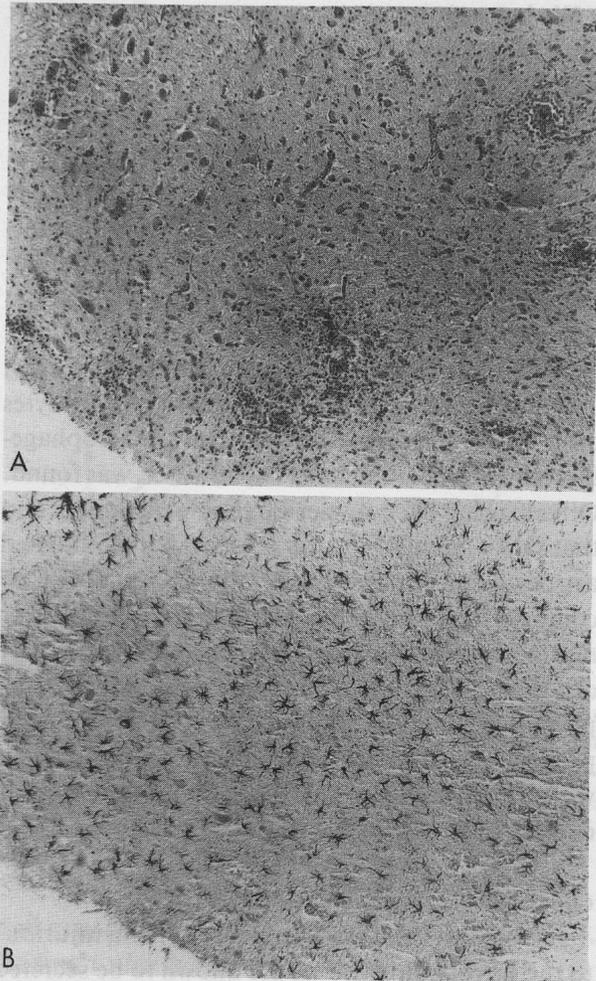


Fig. 6. EAE spinal cord with acute EAE. *A*. Cross section of spinal cord stained with H and E ($\times 90$). Numerous focal EAE lesions are visible. *B*. Adjacent section immunostained for glial fibrillary acidic protein. Activated astrocytes are evenly distributed, and not especially associated with the sites of lesions. (From Smith et al., 1983.)

(Guilian et al., 1986), and tumor necrosis factor (Sawada et al., 1989). These and other cytokines, including IL-6 may interact with neural cells when phagocytic cells enter the nervous system.

Astrocyte stimulation in demyelinating disease

As mentioned earlier, the multiple sclerosis plaque is largely composed of filamentous protein derived from hypertrophied astrocytes. As the axons are

stripped of their myelin, they become embedded in a gliotic matrix of which the principal protein is the glial fibrillary acidic protein (GFAP). This protein was first isolated from multiple sclerosis plaques (Eng et al., 1971), and it has become a widely-used marker for astrocytes and astrocytic gliosis. The antibody is used extensively in pathology to detect by immunocytochemical techniques the presence of gliosis.

GFAP increase in EAE

An increase in immunostaining for GFAP in spinal cord of Lewis rats immunized with myelin was seen about the time that clinical symptoms became evident. Early in the course of the disease (10–12 days post-immunization) fibrous processes in white matter became heavily stained; then, later, hypertrophied astrocyte cell bodies and their processes became evident in grey matter (Smith et al., 1983). The increased staining was general throughout the tissues, and did not correspond to the site of the lesion (Fig. 6), suggesting the influence of a freely-diffusible factor. Although an increase was evident in incorporation of radioactive amino acids into the GFAP, as well as into all of the neural filaments, the amount of GFAP in the spinal cord did not seem to increase in the acute disease in the rat. Later, in the course of the disease, after about 18 days post-immunization, Aquino et al. (1988) showed a gradual and steady rise of GFAP to a level about twice that of the control, remaining high through 65 days post-immunization. The mRNA for GFAP in astrocytes also increased with the rise in GFAP, although the time course was somewhat different. An increase in GFAP mRNA was seen as early as 10 days post-immunization, reached levels 6–8 times greater than control values at 11–14 days, then slowly declined. At 65 days post-immunization the message was still four times the control level (Aquino et al., 1990).

In chronic relapsing EAE in the SJL/J mouse large gliotic scars which immunostained heavily with GFAP antibody were observed after several relapses. Over the course of 6 months the amount of GFAP in the CNS (brain and spinal cord combined)

more than doubled in relation to the 70 kDA neurofilament protein (Smith and Eng, 1987). This model, therefore, appeared to resemble human MS in relation to the development of an intense glial scar during a series of demyelinating episodes. Investigation of the relationships of the clinical relapses to the GFAP gene expression is currently underway.

The early increase in immunochemical staining early in disease was unexplained in view of the failure to demonstrate an increase in GFAP amounts until much later. In the acute disease in the Lewis rat there was evidence that some astrocytic cell division was taking place. [³H]Thymidine uptake into astrocytes could be demonstrated by radioautographic visualization in the spinal cord of rats with

EAE, while none were seen in the Freund's adjuvant-injected control (Smith et al., 1987) (Fig. 7). On the other hand, fewer labeled astrocytes could be found in the chronic relapsing EAE mouse where the disease is much milder, and develops over a much longer time course. It was concluded that very little of the increase in staining of the GFAP early in EAE, and the subsequent increase in amount of GFAP could be due to astrocyte division.

It was observed by electron microscopy that very early in the disease the glial filaments which are normally arranged in tight bundles disperse as the processes become swollen with watery cytoplasm (Eng et al., 1989). We have suggested that the early increase in GFAP immunostaining is due, at least in



Fig. 7. Astrocytes immunostained for glial fibrillary acidic protein showing uptake of [³H]thymidine (arrows) by radioautography. Smaller labeled cells are lymphocytes ($\times 700$).

part, to the edema which causes dissociation of the filaments, thereby exposing more antigenic sites to the antibodies.

Cytokines and astrocyte stimulation

Of the cytokines invoked as stimulating agents for astrocytes, interleukin-1 (IL-1) has received the most attention. Giulian and Lachman (1985) showed IL-1 to be a potent mitogen for astrocytes in culture, but not for oligodendroglia. IL-1-like activity was detected in the brain 10 days after stab wound injury, suggesting that IL-1 released by inflammatory cells may promote the formation of astroglial scars (Giulian and Lachman, 1985). IL-1 injected into the cerebral cortex of adult rats promoted astrogliosis and new blood vessel growth, and Giulian's group suggested that amoeboid microglia were the likely source of IL-1 (Giulian et al., 1986, 1988). Tumor necrosis factor (TNF) has also been invoked as a cytokine-promoting astrocyte proliferation. Microglial cells when treated with interferon- γ and endotoxin develop tumor cytotoxicity and produce TNF- α (Frei et al., 1987). Selmaj et al. (1990) showed that in serum-free medium TNF, as well as IL-6 and lymphotoxin were mitogenic for astrocytes, while IL-1 was not active under these conditions. More recently, however, these investigators found that TNF, although inducing proliferation of astrocytes, down-regulated GFAP mRNA (Selmaj et al., 1991). Unfortunately, measurement of the GFAP message in IL-1 stimulated astrocytes has not yet been reported.

At the present time it appears as if in cell-mediated demyelination a factor, perhaps a colony-stimulating factor, is secreted by the astrocyte which stimulates the phagocytic cell, either the invading peripheral macrophage or the microglial cell. These cells may be further stimulated by phagocytosis of the myelin-antibody complex, and may, in turn, produce a factor, perhaps IL-1, which promotes hypertrophy and some hyperplasia of the astrocytes (Fig. 8). Such a mutually dependent unit is probably not self-sustaining, but the interaction may be initiated and/or modulated by other factors both from immune cells such as lymphocytes, and from other

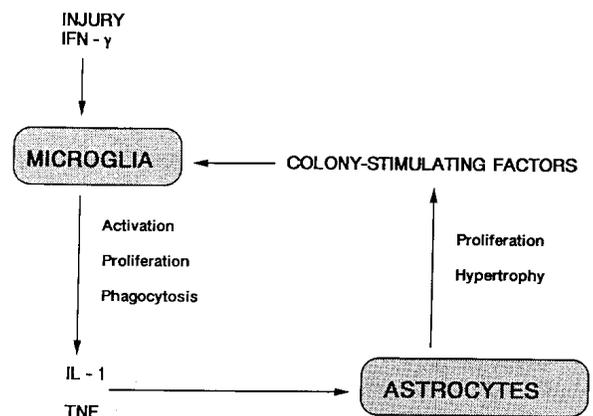


Fig. 8. Diagram of possible interactions between microglia and astrocytes in CNS injury or cell-mediated demyelination with astrocyte proliferation and formation of the gliotic plaque.

neural cells. At this time this sequence of events is mostly speculative, but recent results in our laboratory have shown the respiratory burst of macrophages phagocytizing myelin to be greatly enhanced by the presence of antibody-complexed myelin (Nguyen and Smith, unpublished observations). It is possible that secretion of other factors, including cytokines, may also be stimulated by antibody-mediated events. The promotion of astrogliosis by cytokines could be also a general mechanism for the astrocyte response in many kinds of CNS injury where antibody is not present. The microglia as resident macrophages of the nervous system have the potential to be activated by internal mechanisms, which could then start a sequence of events leading to the astrocyte response.

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