PERIPHERAL NERVE DEMYELINATION INDUCED BY INTRANEURAL INJECTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS SERUM

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ABSTRACT

Intraneural injection of sera from rabbits with experimental allergic encephalomyelitis, induced by sensitization with bovine brain white matter in complete Freund's adjuvant, produced focal primary demyelinating lesions in rat sciatic nerves. Demyelinating activity was removed by prior incubation of antisera with central (CNS) and peripheral nervous system (PNS) myelin but not with liver or kidney, and was heat-labile and complement-dependent. Recipient animals developed a sensorimotor disturbance of their toes and ankles on the side injected with antiserum. Twenty minutes after antiserum injection, Schwann cells showed focal cytoplasmic outpouching and their external mesaxons opened. Between 1 and 8 hours after injection vacuolation, splitting and vesiculation of myelin became increasingly prominent at Schmidt-Lanterman clefts and paranodal regions, with concomitant degenerative changes in Schwann cell cytoplasm. Polymorphonuclear cell infiltration and endoneurial edema were apparent at this time. Substantial demyelination occurred before the appearance of phagocytic cells. Between 8 hours and 3 days many nerve fibers were surrounded and attacked by invading macrophages. Axons became demyelinated progressively over several internodes by macrophage phagocytosis. Early signs of remyelination were observed by 5 days. These findings suggest that antibodies directed against antigens common to both CNS and PNS myelin can produce in vivo peripheral nerve demyelination.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is a prototype autoimmune disease of the central nervous system (CNS) often considered a model of human demyelinating disorders, such as acute disseminated encephalomyelitis and possibly multiple sclerosis (1, 38, 41). EAE can be produced by immunization of animals either with CNS tissue homogenates or with myelin basic protein, usually in complete Freund's adjuvant (CFA) (1, 2, 38, 41).

EAE can be transferred from sensitized donors to normal animals with lymph node cells (4, 29, 30, 37, 55). T-cell depleted animals lose their capacity to develop EAE (13, 17, 36). Indices of cell mediated immunity correlate well with the clinical onset of the disease (1, 2, 38). Thus the T-cell system, which

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is important in delayed-type responses, seems to be essential for the production of EAE. The observation of penetration of mononuclear cell processes into myelin lamellae, peeling off (20, 22, 23) or exerting a lytic effect upon myelin (11, 12), has been used as further evidence for the role of a cellular response in EAE (38, 41).

On the other hand evidence has been presented suggesting that antibody may play a part in the production of demyelination in white matter-induced EAE (WM-EAE). Serum from animals with WM-EAE demyelinates organotypic CNS cultures (5, 52, 54). In vitro demyelinating activity in rabbit WM-EAE serum is complement-dependent and present in the γ2-globulin (7S) component (3), and the demyelinating factor(s) in EAE guinea pig serum is IgG2 immunoglobulin (27). Previous attempts to transfer EAE to normal animals with antiserum using systemic administration have been unsuccessful (reviewed in reference 9). A disturbance of the blood-brain barrier in EAE animals has been well documented (15, 19, 23, 28, 34) and the intact barrier in normal recipient animals may have prevented the entrance of serum proteins into the CNS, possibly accounting for the inability to transfer demyelination. Injection of antiserum directly into CNS tissues also has failed to induce EAE lesions (38, 51), but intracerebral or intraspinal cord injections of serum produced considerable artifacts which hampered interpretation (51). Intraventricular injection of EAE serum failed to produce demyelination in retinal tissue, whereas injection of activated lymphocyte supernatants plus anti-spinal cord antibodies induced focal primary demyelination (8). The only report claiming passive transfer of EAE with antiserum alone describes replication of a delayed hypersensitivity reaction three days after intraventricular injection of serum, but this study did not present convincing morphological evidence of demyelination (16).

We recently documented that intraneural injection of serum from rabbits with experimental allergic neuritis (EAN), induced by sensitization with whole peripheral nerve homogenates (WN-EAN), produces a focal demyelinating lesion in the recipient animal's peripheral nerve (48, 50). Since root and nerve lesions are often found in EAE animals (44, 45, 56, 58) we attempted to produce demyelinating lesions in normal rats by intraneural injection of WM-EAE serum alone (51). The present work reports the observation that WM-EAE serum does demyelinate in vivo peripheral nerve, and describes the morphological features and chronological evolution of the resulting demyelinating lesion.

MATERIALS AND METHODS

Preparation of antiserum

To produce WM-EAE sera 11 male New Zealand albino rabbits weighing 2.3 to 2.7 kg were immunized with a total of 0.8 ml of a mixture containing 0.2 g homogenized fresh bovine brain white matter, 0.2 ml saline and 0.4 ml CFA (Difco). The inoculum was given intradermally or subcutaneously at 4 sites on the back. Eight of 11 immunized rabbits developed clinical signs of EAE of various degrees between 13 and 26 days after sensitization. Serum was obtained from
these animals 12 and 21 days after sensitization, within 1 day of the clinical onset of EAE, and 8 or 10 days after onset.

Control sera were obtained from 3 rabbits inoculated with 0.2 g homogenized fresh bovine liver, CFA and saline, and 3 rabbits inoculated with 10 mg bovine serum albumin (BSA) (Sigma), CFA and saline. Control rabbits were bled 2 to 3 weeks after sensitization. All sera were stored in multiple sterile single-use vials at $-70^\circ$C.

**Evaluation of antiserum.**

Sera were evaluated for in vivo peripheral nerve demyelinating activity 2 days after intraneural injection into rat sciatic nerve using our previously described semi-quantitative methods (50). We tested two WM-EAE sera with the highest in vivo demyelinating activity to examine the organ specificity, heat lability, and complement dependency of peripheral nerve demyelinating activity according to the methods already described (50).

**Sequential study.**

To study the chronology of the morphological changes produced in rat sciatic nerve after WM-EAE serum injection, we selected the WM-EAE serum with the most potent demyelinating activity by morphological criteria (50). This serum was obtained from a rabbit 14 days after immunization, on the day of the clinical onset of EAE. Twenty $\mu$l of WM-EAE serum was injected into the subperineural portion of one sciatic nerve of 24 male Wistar rats (250–300 gm) (48). Control serum was injected into the contralateral nerve. Injected rats were observed for signs of leg or foot weakness. At least two animals were sacrificed at each of the following post-injection times: 20 minutes, 1 hour, 3 hours, 5 hours, 8 hours, 15 hours, 24 hours, 2 days, 3 days, and 5 days. After pentobarbital anesthesia rats were fixed by intra-aortic perfusion with 3.6% glutaraldehyde. The sciatic nerves were excised and dissected into four 1.5 mm long portions above, at, and below the site of needle insertion. The tissues were then postfixed with 1% OsO$_4$, dehydrated in a series of ethyl alcohols, cleared in propylene-oxide and embedded in epoxy (48). One percent toluidine blue-stained sections ($1 \mu$m thick) were studied by light microscopy. Ultrathin sections were double stained with uranyl acetate and lead citrate and studied with an electron microscope.

**RESULTS**

**Evaluation of demyelinating activity of WM-EAE serum.**

Sera from 7 of 11 rabbits immunized with bovine cerebral white matter produced focal demyelinating lesions in rat peripheral nerves. Of these 7 donor rabbits 5 had developed clinical EAE and 2 appeared normal. Three of the 4 donor rabbits whose sera did not show in vivo peripheral nerve demyelinating activity developed clinical EAE and 1 remained normal. The most potent demyelinating activity was present in sera obtained between 14 and 26 days after sensitization. However, there was no apparent correlation between demyelinating activity and the clinical severity of the EAE. Control sera did not induce measurable demyelination in recipient nerves.

Incubation of the two most potent WM-EAE sera with either lyophilized purified bovine CNS or PNS myelin removed demyelinating activity, while activity of these two sera persisted after incubation with bovine liver or kidney. WM-EAE sera heated at $56^\circ$C for 30 minutes did not produce demyelinating lesions in nerves of rats decomplemented by prior injections of cobra venom factor.
Clinical observation.

A few hours after the intraneural injection of WM-EAE serum with PNS demyelinating activity, the foot muscles of most recipient rats were weak. Extent of the defect ranged from impaired toe abduction lasting several hours to paralysis of toe and ankle movements and loss of response to pin prick stimuli developing after 5 hours and persisting for seven days. Most animals showed signs of improvement after 8 days and appeared normal by 14 to 16 days. No clinical abnormalities were observed on the side injected with control serum.

Sequence of morphological events following intraneural injection of WM-EAE serum.

Macroscopic examination of WM-EAE serum-injected nerves revealed mild swelling of the injected portion of the nerve, approximately 0.7 cm in length. Dilatation and congestion of epineurial blood vessels was present infrequently. On cross section the surface of the nerves was slightly translucent. Rarely single pinpoint-shaped hemorrhages were found at the epineurial injection site of both WM-EAE and control serum injected nerves.

By light microscopy, both WM-EAE and control serum-injected nerves had certain changes in common. A few myelin sheaths were crushed along the needle tract. This was usually associated with acute axonal swelling with aggregation or absence of axoplasmic organelles. Mild endoneurial edema (Fig. 1), infiltration of a few polymorphonuclear cells, destruction of mast cells and swelling of endothelial cells of the microvasculature were occasionally present in the endoneurium. Mild epineurial inflammation without perineurial involvement was found in most injected nerves. Ultrastructurally, dilatation of endoplasmic reticulum (ER) and swelling of mitochondria were occasionally present in Schwann cell cytoplasm. We did not find demyelinated fibers in nerves injected with anti-BSA serum, but anti-liver serum-injected nerves had a few demyelinated axons.

Focal demyelinating lesions extended about 2 cm along nerves injected with WM-EAE serum (Fig. 2). The lesions usually involved more than half of the fascicular cross section area. The lesions remote from the injection site tended to be located in the subperineurial region. Although signs of Wallerian degeneration were sometimes noted distal to the injection site, only demyelinating changes were found proximal to it.

Twenty minutes after the intraneural injection of WM-EAE serum, the earliest time point studied, myelin sheaths and axons appeared normal. A minimal degree of endoneurial edema and infiltration by a few polymorphonuclear cells were noted. A striking abnormality was observed in the configuration of Schwann cells. Some Schwann cells had irregular focal outfoldings or mushroom-shaped outpouchings of cytoplasm (Fig. 3). The cytoplasmic organelles of these Schwann cells were generally normal although at times there was dilatation of smooth ER. We did not find discontinuities of the Schwann cell
Fig. 1. Cross section of a rat sciatic nerve proximal to the site of control serum (anti-BSA) injection. There is a mild endoneurial edema in the center of this fascicle two days after injection. X 64

Fig. 2. Sciatic nerve proximal to the site of WM-EAE serum injection. At low magnification two days after injection the demyelinating lesion is characterized by endoneurial edema, a perineurial inflammatory infiltrate (left) and increased endoneurial cellularity. X 60 (A higher magnification view is shown in Fig. 8).

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Fig. 3. Twenty minutes after WM-EAE serum injection, a mushroom-shaped outpouching of Schwann cell cytoplasm is seen. Organelles in Schwann cell cytoplasm appear almost normal except for mild dilatation of smooth ER. × 14,600
surface membrane. The external mesaxons of most myelinated fibers were opened slightly (Fig. 4). Schwann cell processes surrounding unmyelinated fibers were somewhat retracted. In the most extensive lesions, nuclei of some Schwann cells had an increased chromatin rim.

During the first 5 hours after WM-EAE serum injection, polymorphonuclear cell infiltration and subperineurial and endoneurial edema became prominent. By light microscopy the number of nerve fibers with split myelin sheaths gradually increased over this period. In longitudinal sections examined with the electron microscope these abnormalities were identified as intralamellar splitting and vacuolation of myelin in the early phase with increasing vesiculation thereafter. These changes seemed to begin at paranodal areas and Schmidt-Lanterman (SL) clefts (Fig. 5) and usually accompanied “smudging” of myelin lamellae of various degrees. In the most severe lesions Schwann cell cytoplasmic organelles were increasingly swollen and their cytoplasm appeared diffusely homogenous and rarefied by 5 hours. In cross sections of those nerves with severe Schwann cell changes, vesicular dissolution of myelin lamellae was often present at the outer and inner surfaces of the myelin sheath. A lesser degree of myelin vesiculation was sometimes seen at the periphery of the lesion in fibers without evident Schwann cell damage. Loosening of the myelin sheath was first apparent at the outermost lamellae. In these fibers it appeared that intraperiod lines of myelin lamellae became fragmented, split, and melted away. Major dense lines seemed to persist longer and split to give a railway-like pattern of myelin dissolution (Fig. 6). Loosening of lamellae, usually associated with severe Schwann cell changes, was less frequently observed than myelin vesiculation. Only a few mononuclear cells could be found in the endoneurium by 5 hours and changes in myelin and Schwann cells were not associated with any infiltrating cells. At times axons of demyelinating fibers were mildly abnormal with dilatation of smooth ER and dwindling of axon size, as evidenced by the increased number of axonal organelles and axolemmal folds. However, most nerve fibers had normal appearing axoplasm and axolemma. Nerves injected with control antisera did not have similar abnormalities of myelin sheaths or Schwann cells.

Eight hours after the injection of WM-EAE serum, the myelin sheaths of most nerve fibers within the demyelinating lesion underwent extensive vesiculovacular disruption, sometimes extending to one-third of the internodal length (Fig. 7). With the light microscope vesiculovacular disruption of myelin looked like a gray halo surrounding the axons. Demyelinating fibers were not associated with the invading processes of phagocytic mononuclear cells, a cell type which could be found scattered in the endoneurium only after extensive search.

Between 15 hours and 3 days after WM-EAE serum injection the endoneurial space was infiltrated with large mononuclear cells and macrophages (Fig. 8), and endoneurial edema became pronounced. The large mononuclear cells had a diameter of 15 μm to 50 μm, were rich in polysomes and rough ER, and had well developed Golgi complexes. Their cytoplasm was more electron dense
FIG. 4. Myelinated fiber-associated Schwann cell, 20 minutes after WM-EAE serum injection. The external mesaxon is slightly opened (arrow). × 21,000

FIG. 5. Longitudinal section of a myelinated nerve five hours after WM-EAE serum injection. Schmidt-Lantermann clefts are dilated (arrows) with vesiculovacuolar dissolution of myelin sheaths. Axonal diameter narrows abruptly. No phagocytic mononuclear cells were found in proximity to this lesion. × 6,200
Fig. 6. Five hours after WM-EAE serum injection, the outer myelin lamellae of a large myelinated fiber appear loosened and damaged. A railway-like pattern of myelin dissolution is also seen (arrow). × 27,600

Fig. 7. Cross section of a nerve fiber eight hours after WM-EAE serum injection shows extensive myelin vesiculation and vacuolation over half the axonal circumference. Axon size is reduced as judged by the degree of myelin thickness. × 12,700
Fig. 8. Two days after WM-EAE serum injection, higher magnification view of Fig. 2 shows demyelinated axons surrounded by a gray halo (arrowhead), and mononuclear cells (double arrowheads) scattered throughout the endoneurium. Nerve fibers are now associated with phagocytic cells (arrow). × 430

Fig. 9. Longitudinal section of myelinated fibers 2 days after WM-EAE serum injection. Paranodal demyelination and internodal segmental demyelination occur in association with phagocytic cells. Mononuclear cells (arrow) and polymorphonuclear cells (arrowhead) infiltrate the endoneurium. × 390

Fig. 10. Longitudinal section 3 days after WM-EAE serum injection. There is paranodal demyelination of the uppermost fiber and segmental demyelination associated with phagocytic cells of the second fiber. × 910
than that of Schwann cells and their nuclei had a thin rim of chromatin and large nucleoli. These cells appeared to be dense around the endoneurial microvasculature. Myelin sheaths of most nerve fibers in the lesions of this age were associated with phagocytic mononuclear cells and very rarely with polymorphonuclear cells. In longitudinal sections macrophages were seen to invade the myelin sheath at various points along the same internode. Paranodal demyelination and severe internodal myelin destruction were prominent in the same internodes (Figs. 9, 10, 11). Ultramicroscopically, macrophage processes were at times present in association with split and detached lateral loops of myelin sheaths. Schwann cell cytoplasm around these demyelinated fibers seemed to have degenerated and disappeared. At the center of the lesions unmyelinated nerve fibers also lost their Schwann cell envelopes, leaving denuded axons within the basal lamina (Fig. 12). In these areas fibrinous deposits and extravasation of red cells were sometimes present around endoneurial vessels.

At the periphery of severe two to three day old demyelinating lesions, Schwann cell cytoplasm usually had a mildly dilated ER but otherwise appeared normal. Infrequently Schwann cells showed the mushroom-like changes of cytoplasm observed in the 20 minute old lesion. In some of these nerve fibers, macrophage processes seemed to penetrate into the external mesaxon, pushing terminal cytoplasmic mass away from the myelin sheath (Fig. 13). On one occasion an island of Schwann cell cytoplasm was observed in the space between the basement membrane and the outer surface of the myelin sheath. Degenerating membranous materials, possibly Schwann cell debris, were often present in this space (Fig. 14).

In nerves examined at this stage (15 hours to 3 days), an occasional Schwann cell showed signs of phagocytic activity. Axons were decreased in diameter but otherwise appeared normal. Evidence of Wallerian degeneration was occasionally observed in the distal portion of demyelinating fibers. Inflammatory changes in the perineurium were prominent and consisted of separation of perineurial cell lamellae, edema, and invasion of poly- and mononuclear cells (Fig. 2).

Between 3 and 5 days after WM-EAE serum injection almost all nerve fibers in the demyelinaive lesion were surrounded by macrophages, and many fibers showed segmental demyelination extending through several internodes. Myelin vesiculation and splitting were not major patterns of demyelination at this stage. Schwann cells with mitotic figures were occasionally found at the periphery of the lesion. On day 5 active Schwann cells rich in polysomes and rough ER occasionally surrounded completely demyelinated axons, but the initial stages of remyelination were not yet observed.

DISCUSSION

This report describes, for the first time, the observation that white matter-induced EAE serum alone produces focal demyelinaive lesions in normal recipient peripheral nerve after intraneural injection. The capacity of WM-
EAE sera to produce in vivo peripheral nerve demyelination is removed by absorption with either CNS or PNS myelin but not with liver or kidney. The sera heated at 56°C for 30 minutes do not produce demyelinating lesions in nerves of rats decomplemented by cobra venom factor. Thus, the in vivo
Fig. 12. Three days after WM-EAE serum injection, unmyelinated nerve fibers have lost their Schwann cell envelopes. The basal lamina persists. × 25,800

Fig. 13. Three days after injection. Process of a possible mononuclear cell (arrow) penetrates into the outer mesaxon, pushing the terminal Schwann cell cytoplasmic mass away from its myelin sheath. ER in Schwann cell cytoplasm is dilated. × 17,600

Peripheral nerve demyelinating factor(s) in WM-EAE serum appears to be complement-dependent antibody.

The presence of demyelinating antibodies in serum from animals with EAE was first reported by Bornstein and Appel in 1961 (5). They demonstrated that
serum from whole spinal cord-induced EAE animals produces demyelination in myelinated organotypic cerebellum cultures. This in vitro demyelinating activity was attributed to complement-dependent antibodies which react specifically with myelin sheaths and possibly oligodendrocytes in cultured CNS tissue (3, 27). Despite the consistent reproducibility and the elaborate studies of this immune response (5, 6, 7, 41, 42), the role of humoral factor(s) or antibody in the pathogenesis of EAE has been long in question (2, 53, 54). This is in part because previously demyelinating antibody could be detected only by using CNS cultures (38).

The degree of demyelinating activity of WM-EAE sera observed in peripheral nerves in vivo correlates with that seen in vitro in mouse dorsal root ganglion cultures (Saida T. et al, unpublished observation). This relationship between in vivo and in vitro demyelinating activity is similar to that reported for EAN serum from rabbits sensitized with whole peripheral nerve (50). It is not clear whether the demyelinating WM-EAE serum factor demonstrated in this study is similar to the anti-spinal cord antibodies reported by Brosnan et al. (8), which produced retinal demyelination only with supplementation of products of activated lymphocytes, because their methods differed from ours.

Our recent observations suggest that anti-galactocerebroside antibody may be a common demyelinating factor in WM-EAE and WN-EAN sera. Both WM-EAE and WN-EAN sera demyelinate organotypic cultures of CNS (52) and peripheral nervous system (PNS) (Saida T. et al., unpublished observa-
tion), and also peripheral nerves in vivo (48, 50, 51). Anti-galactocerebroside antibody titers are elevated in both WM-EAE and WN-EAN sera (52). Further, antisera to galactocerebroside produced demyelination in cultured CNS and PNS tissue (52). The detection of antibody to galactocerebroside in EAE and EAN produced by immunization with white matter or peripheral nerve is not surprising, since galactocerebroside is a glycolipid hapten (18, 32, 46) and is one of the major constituents of both CNS and PNS myelin (33). Although WM-EAE sera with CNS and PNS demyelinative activity always had elevated antibody titers to galactocerebroside, some high titer sera with CNS demyelinating activity failed to demyelinate PNS both in vivo (51) and in culture (52). This suggests the possible existence of a protective factor in some WM-EAE sera preventing PNS demyelination by galactocerebroside antibody.

Strong evidence in support of the pathogenic importance of galactocerebroside is our recent observation that 13 out of 31 rabbits hyperimmunized with galactocerebroside developed EAN (49). Further, peripheral nerve demyelinating activity of those galactocerebroside-induced EAN animals could be transferred passively by direct injection of anti-galactocerebroside antisera into sciatic nerves of normal rabbits or rats (47, 49). Therefore, it is possible that antibody to galactocerebroside is at least in part responsible for the production of demyelination in the PNS and perhaps also in the CNS of whole white matter-induced EAE animals. On the other hand, antibody to CNS-basic protein produced neither in vivo PNS demyelination (51) nor demyelination in CNS cultures (54; Saida T. et al., unpublished observation), despite the well known ability of CNS myelin basic protein to produce EAE.

It is widely accepted that circulating antibody alone is not primarily involved in the induction of EAE (1, 2, 4, 9, 13, 17, 28, 36, 38). However, the production of clinical paralysis and demyelination by intraneural injection of WM-EAE serum raises the possibility that circulating factor(s) participate at some stage in the immunopathogenesis of demyelination in WM-EAE, at least in some species. The transferred lesions in PNS may have relevance to the pathogenesis of WM-EAE lesions, since WM-EAE animals often develop demyelinative lesions in peripheral nerve roots as well as CNS lesions (44, 45, 56, 58). A lack of blood-nerve barrier has been shown at the dorsal root ganglion and nerve root, and less frequently at distal peripheral nerve, in normal animals of some species (19, 36, 37). In addition animals with EAE are reported to have a disturbed blood-brain barrier (15, 19, 23, 28, 34). This could provide an access route for circulating demyelinating antibodies. Alternatively antibodies produced locally by infiltrating lymphocytes and plasma cells could contribute to antibody-mediated demyelination.

The demyelinative lesions in WM-EAE and those occurring after intraneural injection of WM-EAE serum, while not identical, share many pathological features. These similarities provide support for the possible role of antibodies in the production of demyelinative lesions of WM-EAE.

After intraneural injection of WM-EAE serum, in vivo demyelination evolves rapidly in the injected fascicle. Within one hour the cytoplasm of Schwann
cells at the center of the lesion becomes swollen or distorted. Between one and 8 hours after injection lamellar splitting, vacuolation and vesiculation of myelin rapidly progress at SL incisures and paranodal areas, without mononuclear cell participation. Schwann cell cytoplasm around these fibers becomes rarefied. Between 15 hours and 3 days after injection macrophages invade myelin sheaths, stripping off myelin lamellae with phagocytosis of myelin debris. By 5 days most axons are completely demyelinated over several internodes and some bare axons are wrapped by activated Schwann cell cytoplasm. At the periphery of the demyelinating lesion Schwann cell destruction is inconspicuous and macrophages coexist with surviving Schwann cell processes. Polymorphonuclear cell infiltration and endoneurial edema is maximum over the first few days and subsides thereafter. The morphological patterns of demyelination induced by intraneural injection of WM-EAE serum are identical in character and evolution to those produced in a similar fashion in rat peripheral nerve by WN-EAN serum (48, 50) and by anti-galactocerebroside serum (47).

The hallmark CNS lesion in EAE is perivenuous cuffing of mononuclear cells, macrophages, and occasional plasma cells (12, 22). Concomitantly, areas of demyelination appear in close proximity to areas of inflammation. Ultrastructurally, myelin sheaths are directly attacked by invading mononuclear cell processes (1, 20, 23, 25). These processes seem to be specifically directed toward normal-appearing myelin. At times myelin vesiculation and lamellar changes are found in close proximity to the inflammatory reaction without the direct invasion of mononuclear cells (22). Similar direct attacks on myelin sheaths by invading mononuclear cells has been found in the PNS of EAE animals (58). Early in the development of CNS lesions of EAE, oligodendroglia undergo degenerative changes (11, 20, 43). Schwann cells in the PNS of EAE animals have been reported to be normal (58), but they are normal in the PNS of chronic EAE (40, 44). Virtually every kind of myelin change has been described in EAE. These include myelin vesiculation, splitting, intramyelinic swelling, vacuolation, smudging, changes in lamellar periodicity and opening of major or minor dense lines (22, 41).

An important difference between EAE lesion and WM-EAE serum-mediated PNS demyelinating lesion is that intraneural injection of WM-EAE serum does not produce perivenular cuffings of small lymphocytes. Perivascular lymphocytic cuffing is a characteristic early pathological finding in EAE (22, 41) and has been considered a sign of delayed or tuberculin-type hypersensitivity to encephalitogenic myelin basic protein (38). However, perivenular infiltration of large mononuclear cells and macrophages as found in EAE is conspicuous 15 hours after intraneural injection. Also present in nerves after WM-EAE serum injection are polymorphonuclear cell infiltration and fibrinous exudates, perhaps introduced by biologically active products of complement proteins. Intense polymorphonuclear cell infiltration and extravasation of fibrin have been described in WM-EAE produced in rhesus monkeys (12) and the CNS of “hyperacute” WM-EAE animals (22). Myelin basic protein also can induce EAE lesions accompanied by polymorphonuclear cell infiltration
and fibrinous exudates (25), even though anti-basic protein antibodies neither produce in vitro CNS demyelination (54; Saida T et al., unpublished observation) nor in vivo PNS demyelination (51).

Pronounced Schwann cell abnormalities are a feature of antiserum-mediated in vivo demyelination but not of the PNS of "ordinary" EAE (58). The Schwann cell injury is followed by myelin dissolution and subsequent phagocytic cell invasion, all occurring within 15 hours of WM-EAE serum injection. This sequence of events is analogous to that described after diphtheria toxin injection (59), x-irradiation (31), and some toxic neuropathies (24, 26). In those situations, except for x-irradiation, the temporal appearance of Schwann cell damage, myelin disintegration and macrophage invasion is prolonged over days and months. Acute inflammatory responses such as polymorphonuclear cell infiltration or fibrinous exudates are not seen in these conditions but were found in our material. Damage to Schwann cells did not occur in control serum-injected nerves or in nerves injected with WM-EAE serum after absorption with PNS or CNS myelin or after inactivation by heating at 56°C for 30 minutes. Thus the Schwann cell changes produced by WM-EAE serum seem to be antibody-dependent complement-mediated and not due to toxic, ischemic or some other artifactual effect. It is conceivable that Schwann cell degeneration induced by our strongly demyelinating WM-EAE serum is an exaggeration of a phenomenon which takes place insidiously in the PNS of WM-EAE animals. Schwann cell membranes could share common antigenic sites with myelin which react with demyelinating antibodies. Analogous early degeneration of oligodendroglia is described in EAE animals (11, 20, 43) and after an application of WM-EAE serum in CNS cultures (5, 41, 42).

We occasionally observed a form of demyelination similar to that described in EAE (58). At the periphery of demyelinating lesions induced by WM-EAE serum injection, where we did not find morphologically altered Schwann cells, processes of mononuclear cells invaded directly into nerve fibers penetrating normal-appearing myelin sheaths and displacing portions of the Schwann cell cytoplasm. The absorption of injected antiserum by nerve parenchyma or the dilution of antiserum with interstitial tissue fluid at the periphery of the lesion might account for an apparent lack of damage to Schwann cells or myelin sheaths. Sufficient antibody could be present to sensitize the myelin of these normal-appearing fibers, resulting in an attack by phagocytic mononuclear cells.

Myelin vesiculation and intramyelinic swelling in the absence of mononuclear phagocytes occurs between 3 and 8 hours after WM-EAE serum injection. Similar abnormalities are often observed in the active PNS and CNS lesions of chronic EAE animals (43, 44). In the CNS of acute EAE Lewis rats, vesicular transformation of myelin is also found before the association of mononuclear cells (20). Although myelin abnormalities such as splitting, vesiculation, or loosening induced by WM-EAE serum injection first appear following Schwann cell changes, they could result at least partly from antibody-dependent complement-mediated damage to myelin, since the demyelinating activity of WM-
EAE sera is absorbed by myelin. The occasional presence of myelin vesiculation without apparent Schwann cell damage, seen at the periphery of WM-EAE serum-mediated lesions may be an indication of such a mechanism.

Fifteen hours after WM-EAE serum injection, when monocytes and macrophages are numerous in the endoneurium, loosening of myelin lamellae and myelin vesiculation is often observed in the vicinity of the invading cells. These findings resemble the "lytic" changes described in the CNS of EAE animals (10, 20, 22) where mononuclear cells are presumed to release enzymes leading to myelinolysis. A pattern of loosened myelin lamellae induced by WM-EAE serum in vivo is similar to that described as "even separation" of myelin lamellae in the CNS of EAE animals (20, 21, 22, 39).

The pathological features of antiserum-mediated in vivo PNS demyelination resemble in many ways those observed in serum-induced demyelination in CNS and PNS cultures. Demyelinative changes in CNS cultures following application of WM-EAE serum also evolve rapidly (5, 42). Degeneration of myelin-forming cells (oligodendroglia) is frequently observed in cultures, starting prior to the obvious destruction of myelin lamellae. The characteristic findings of in vitro myelin destruction are large intramyelinic swellings and melting or smudging of myelin sheaths. We often observed similar intralamellar splitting with myelin vacuolation and smudging of sheaths early after WM-EAE serum injection. Myelin vesiculation, which we saw frequently in this study, is described in the CNS and PNS of EAE animals (10) but is found less frequently in CNS cultures after WM-EAE serum application (52). Contact of phagocytic mononuclear cells with demyelinating nerve fibers is seldom observed in CNS cultures, where phagocytosis of myelin is mainly accomplished by astroglial processes (42, 52). The limited numbers of macrophages available probably explains the less frequent occurrence of macrophage-associated phagocytosis of myelin in CNS cultures.

Hirano et al. studied serum-induced demyelination in PNS cultures using sera from Guillain-Barre syndrome patients (14). One of the earliest changes observed in PNS cultures was opening of the external mesaxon of Schwann cells. Loosening, vesiculation, splitting and vacuolation of myelin lamellae where also found, starting at paranodal loops and SL clefts. Phagocytosis of myelin, possibly by macrophages, was only rarely found.

A low grade inflammatory reaction within the endoneurium and a few demyelinated nerve fibers seen in control serum-injected nerves might have resulted from effects of needle insertion and reactions to heterologous serum and/or its acute phase reactants. These problems were discussed elsewhere (48). The nature of minimal demyelination produced by anti-liver serum could not be characterized further because its degree was such that the activity was lost non-specifically during adsorption or heating procedures.

Our results suggest that antibodies directed against antigens common to both CNS and PNS myelin can produce in vivo peripheral nerve demyelination. The demyelinating lesions produced by intraneural injection of WM-EAE serum share many pathological features with the PNS and CNS lesions of
WM-EAE animals. It is possible that the serum demyelinating factor(s) demonstrated in this study could play a role in the pathogenesis of PNS demyelination of rabbits with WM-EAE, if not in the primary induction of the disease.

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