PRIMARY DEMYELINATION AS A NONSPECIFIC CONSEQUENCE OF A CELL-MEDIATED IMMUNE REACTION*

BY HENRYK M. WISNIEWSKI and BARRY R. BLOOM

(From the Departments of Pathology, Microbiology and Immunology, and Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461)

Two types of demyelination are generally recognized and result from myelin damage by exogenous or endogenous agents. Primary demyelination is characterized by segmental myelin damage without primary changes in the axon. In secondary demyelination both the axoplasm and the myelin undergo degeneration as in ischemic necrosis or axon death consequent upon dystrophic processes or in Wallerian degeneration. In these conditions, myelin degeneration is a secondary phenomenon, a sequela to death of the axon. In contrast, in primary demyelination, myelin damage is the principal, and often only, lesion.

In considering the great number of pathological conditions in which primary demyelination, i.e. segmental myelin damage without death of the axon, is observed, two general patterns emerge. In some cases, myelin damage occurs in the absence of inflammatory cells, for example in lesions produced by diphtheria toxin, lead poisoning, and metabolic disorders (diabetes). Of greater interest, perhaps, are those pathological conditions in which inflammation is the consistent feature associated with the demyelination. These include the human demyelinating diseases such as acute disseminated encephalomyelitis, poly-ganglio-radiculo-neuritis, and possibly multiple sclerosis, and the experimental situations of experimental allergic encephalomyelitis (EAE), experimental allergic neuritis and distemper encephalomyelitis (1). While some of these situations clearly involve viruses as primary etiologic agents, all are associated with inflammatory type demyelination. However, in a viral demyelinating disease such as progressive multifocal leukoencephalopathy histiocytic cells appear at the advanced stage of myelin destruction and are presumed to remove already damaged myelin (2). In contrast, in virus-induced demyelinating encephalitis caused by canine distemper (3), and perhaps in subacute sclerosing panencepha-
litis (SSPE) in man (4), hematogenous cells of the type seen in EAE appear at the very early stages of nerve tissue damage. The mechanism of myelin damage in all of these situations remains unclear. In some, it is possible that segmental demyelination may be the result of the death of myelinated cells caused by the infecting organism, e.g. the distemper virus. A second hypothesis holds that whatever the initial exogenous trigger, inflammatory demyelination represents destruction of autologous myelin by an autoimmune process.

A further possibility worthy of consideration is that damage to myelin could occur as a nonspecific consequence of a specific cell-mediated immune reaction occurring to an antigen in the vicinity of a myelinated nerve. In this situation both primary (segmental) and secondary demyelination could occur as a consequence of immunological reactions to a variety of different agents, perhaps to viral antigens or mycobacterial antigens in neurotuberculosis and leprosy. It is the purpose of this investigation to ascertain whether myelin can be damaged as a nonspecific consequence of a specific delayed-type hypersensitivity reaction directed at a nonnervous tissue antigen, in this case, tubercle bacillus or soluble products thereof.

Materials and Methods

Both human and animal material were studied. The human specimens were cranial nerves and samples of pons and medulla taken from two cases of tuberculous meningitis.

The animal material consisted of guinea pigs sensitized with 1.5 mg killed tubercle bacilli in Freund’s complete adjuvant injected into the foot pads and nuchal muscles as described previously (5). The animals were used 4-8 wk after sensitization and were challenged with one of four sets of antigens: purified protein derivative (PPD) (125 μg/0.1 ml); Old Tuberculin (1:200); sonicated H₃Ra (100 μg/0.1 ml), and living avirulent tubercle bacilli, BCG or R,Rv (10⁶-10⁷ viable organisms/0.1 ml). Under general anesthesia (nembutal) the skull was exposed and 2 mm posterior to the coronal and 2 mm lateral to the sagittal suture a burr hole was made. In the same animals the atlanto-occipitalis membrane and both sciatic nerves were exposed and 0.1 ml of the antigen to be tested was injected into the lateral ventricle; 0.2 ml into the cisterna cerebellomedullaris; 0.01 ml into the left peroneal and tibial branches of the sciatic nerve. The right sciatic nerve served as a control and was injected with 0.01 ml of saline solution. All injections were carried out with a 0.01 ml of microsyringe (Hamilton Co., Inc., Whittier, Calif.) with a fine (no. 30) gauge needle which, for sciatic nerve injection, was bent to an angle of 40° to facilitate entry. Injections into the lateral ventricle were performed using stereotaxic apparatus. Antigen was injected by hand into the cisterna cerebellomedullaris and peroneal and tibial branches of the sciatic nerve. In five guinea pigs, laminectomy at L-5 or L-6 level of the spinal cord was performed and 100 μl of PPD (125 μg) was injected bilaterally into the posterior tracts and subarachnoid space. Each of the antigens used in the study was also injected into nonsensitized, control guinea pigs at the same time. At 1, 2, 3, 4, and 10 days after challenge, animals were anesthetized, heparinized, and killed by perfusion with 5% glutaraldehyde in Sorenson phosphate buffer. Control animals were also sacrificed at the same time intervals. After perfusion, samples of tissue for light and electron microscopic studies were taken from the following areas: peroneal and tibial branches of the sciatic nerves, intracranial portion of II, III, V, VI, and VII cranial nerves, C-1, L-5, L-6, and S-1 spinal cord with anterior and posterior roots, and samples of brain tissue close to the lateral and IV ventricle and the base of the brain, together with the leptomeninges. The tissue was postfixed with 2% Dalton’s chrome osmium solution, dehydrated in graded concentrations of ethanol, immersed in propylene oxide, and embedded in epon. 1-μm sections were cut and stained with toluidine blue. Thin sections of selected blocks and areas were cut, stained with uranyl acetate and lead citrate, and examined in a Siemens electron microscope (Siemens Corp., Medical Industrial Div., Iselin, N.J.). The formalin-fixed human material (III, IV, V, VI, VII cranial
DEMYELINATION AND A CELL-MEDIATED IMMUNE REACTION

nerves C-1 roots, and samples of tissue from the base of the brain) were embedded in paraffin for routine histologic examination (Hematoxylin & Eosin, Bodian, and modification of Speilmeyer methods), and in plastic for electron microscopic studies according to the methods described above.

Results

Injection of antigen into the subarachnoid space or ventricles of delayed-hypersensitive guinea pigs invariably produced lesions exhibiting primary demyelination. Tuberculin-sensitized guinea pigs challenged either with Old Tuberculin, PPD, live or dead tubercle bacilli displayed similar morphological changes. In the majority of instances the presence of inflammatory cells was limited to the surface of the ependyma and the subarachnoid space. On occasion, however, cells aggregated in the Virchow-Robin space and around the entry zone of the cranial nerves, and penetrated the brain parenchyma. In such areas, demyelinated axons, axons undergoing demyelination, and myelin-laden macrophages were observed. By and large the extent of demyelination was proportional to the degree of inflammation. Axons showing Wallerian-like degeneration (secondary demyelination) were also present particularly in foci with extensive inflammation. Peroneal and tibial branches of the sciatic nerves injected with tuberculin or tubercle bacilli displayed extensive accumulation of inflammatory cells in the interfascicular connective tissue. Although cells penetrated the perineurium only rarely, once cells were found among the nerve fibers, demyelination was a common picture. Control animals revealed only minimal cellular reactions along the injection tracts, consisting of occasional polymorphonuclear leukocytes and a few mononuclear cells.

Figs. 1 and 2 show the sciatic nerve with aggregates of myelin-laden macrophages next to the demyelinated nerve fibers resulting from challenge with tuberculin in tuberculin-sensitive guinea pigs. Around the vessels (Fig. 1) some hematogenous cells without myelin debris were also seen. In the affected nerves, as in the CNS, axons undergoing Wallerian-like degeneration were found. However, they were seen only in areas of the most extensive inflammation. The best defined areas of demyelination were observed in guinea pigs injected with PPD into the posterior columns and the subarachnoid space of the lumbar spinal cord. In these animals demyelinating plaques were seen outside the traumatic zone in the vicinity of the subpial vessels with perivascular cuffs of inflammatory cells. Fig. 3 illustrates the perivascular accumulation of inflammatory cells intermixed with groups of demyelinated nerve fibers. Higher magnification of this area (Fig. 4) shows naked neurons surrounded by macrophages with myelin debris. Splitting of the myelin sheaths and the presence of dark droplets within the boundaries of the nerve fibers are indicative of active demyelination (electron micrographs of such nerve show that these droplets are myelin debris in the cytoplasm of macrophages which penetrated with myelin sheath). Fig. 5 is an electron micrograph of one such area showing extensive vesicular and netlike disruption of the myelin sheath adjacent to myelin-laden macrophages. Fig. 6 demonstrates active stripping of myelin lamellae by the mononuclear cell processes. The normal locking axoplasm of the nerve fiber which is undergoing active demyelination should be noted. In areas of active demyelination, the plasma membrane of the
Fig. 1. Overall view of demyelination in sciatic nerve 72 h after challenge with killed, sonicated tubercle bacilli. V, vessels, surrounded by macrophages and mononuclear cells. Between the demyelinated axons (arrows) myelin laden macrophages are present. × 200.

Fig. 2. Macrophages with myelin debris adjacent to naked (arrows) axons. Sciatic nerve as above. × 560.

Mononuclear cells was often “covered” with the disrupted and altered myelin lamellae giving an appearance of “myelination” of the mononuclear cells (Figs. 7 and 8).

Human cranial nerves obtained from patients with neurotuberculosis (Fig. 9)
Fig. 3. Cuffs of inflammatory cells around the subpial vessels of the spinal cord, posterior tract in animals 72 h after challenge with PPD. Arrows point to the groups of demyelinated axons. × 150.

Fig. 4. Area of active demyelination from the above. Long arrows indicate nerve fibers invaded by debris-laden macrophages. Short arrows show axons with splitting of the myelin sheath. At the E/M level it was found that some splits correspond to areas where vesicular disruption of the myelin took place; others, where pockets of the invading cell cytoplasmic processes were interdigitated with the myelin lamellae. Double arrows point to denuded axons surrounded by macrophages containing myelin debris. × 560.
FIG. 5. Extensive netlike disruption of the myelin sheath around normal-looking axon (A) in area infiltrated by hematogenous cells. M, mononuclear cells with myelin debris. × 6,000.

FIG. 6. Active stripping of the myelin sheath by a macrophage. Arrow points to fingerlike process stripping the remaining few myelin lamellae. × 10,000.
Fig. 7. Mononuclear cell with myelin debris covered with altered myelin lamellae. Outer myelin lamellae of the nerve fiber in the center appeared also to "fuse" with the membranes of the macrophage. × 10,000.

Fig. 8. Higher magnification of the area where the myelin lamellae are in very close apposition to the plasma membrane of the macrophage. × 43,200.
were also extensively infiltrated by inflammatory cells. The same area, when stained for axons (Fig. 10) showed that the majority of them were well preserved. However, the stain of myelin (Figs. 11 and 12) demonstrated loss of myelin sheath and active demyelination. Electron microscopic studies of these nerves (Fig. 13) revealed that in areas infiltrated by the mononuclear cells, the myelin sheaths had undergone vesicular disruption of the type seen in the experimental

Figs. 9-13. These are derived from human autopsy cases with neurotuberculosis.

Fig. 9. Extensive cellular infiltrate in the cranial nerve. Hematoxylin and eosin stain. × 150.

Fig. 10. Similar area as above stained with Bodian technique to show that the majority of axons in the affected nerves appear normal. × 150.
DEMYELINATION AND A CELL-MEDIATED IMMUNE REACTION

FIG. 11. This photomicrograph demonstrates that in areas of cellular infiltrates the majority of the nerve fibers lose their myelin sheath (black longitudinal lines correspond to remnants of the myelin sheath). Luxol fast blue and PAS. × 300.

FIG. 12. 1 μm toluidine blue section showing partially demyelinated nerve fibers (arrows). × 560.

model. It is interesting to note the presence of some plasma cells here, since they are invariably observed in SSPE and multiple sclerosis plaques.

Discussion

When tuberculin-sensitive guinea pigs were challenged either with the tubercle bacillus or with the tuberculin protein either in the central or peripheral nervous
Fig. 13. Electronmicrograph taken from area of active demyelination. A mononuclear cell, possibly a plasma cell, is present between two almost completely demyelinated nerve fibers. Note that the remnants of the myelin sheath shows vesicular and netlike disruption similar to that seen in experimental animals. $\times 6,000$. 

355
Demyelination and a Cell-Mediated Immune Reaction

Demyelination appeared to be proportional to the intensity of the inflammatory reaction. In all cases, it was impossible to distinguish between reactions provoked by living or sonicated killed tubercle bacilli, Old Tuberculin, or tuberculin PPD. In the areas exhibiting most pronounced cellular inflammation, especially at later times after challenge, the tuberculin reaction damaged not only the myelin sheaths, but also the axons. A difference in intensity of inflammation and demyelination between nerves and roots was seen, perhaps best explained by the fact that peripheral nerves have a perineurium known to be a barrier to infection, and perhaps inflammation (6, 7). An essentially indistinguishable histopathological picture emerged from observations on the brains of two patients who died of tuberculous meningoencephalitis.

As early as 1932 Burn and Finley (8) reported that living or dead tubercle bacilli and their products, when placed in direct contact with the leptomeninges of tuberculous animals initiated a marked clinical and pathological reaction characterized by weakness, convulsions, and death with intense inflammatory exudate distributed throughout the subarachnoid space. A similar picture including perivascular damage was reported by Rich and McCordock (9) as well. A recent elegant series of histopathologic studies on human neurotuberculosis presented by Dastur and associates described myelin destruction in the spinal roots and perivascular demyelination in the white matter of the brain (10-12). Demyelination of the roots was attributed to compression (strangulation) (11) by the leptomeningeal exudate. In the present experiments, a leptomeningeal exudate was seen, but appeared to be a direct function of the cellular infiltrate in the inflammatory reaction. However, the parenchymal perivascular demyelination they postulated to be a consequence of a delayed-type hypersensitivity reaction probably occurred due either to brain antigens or to tuberculoprotein antigens in the area (12). The present studies are a direct confirmation of that view.

We believe that the demyelination after a tuberculin reaction in the brain may serve as a useful model for approaching other demyelinating diseases particularly by permitting study of the purely immunological histopathology. It has most obvious relevance to the origin of neurologic damage in CNS tuberculosis, and in the tuberculoid form of leprosy, both brought about largely by cell-mediated immune reactions. This is dramatically made clear by the excellent morphologic studies of Sunderland (7), Job (13), Nishiura (14), as well as the more classical studies of Wade (15) and Kanolkar (16) in leprosy in which a considerable degree of pathology in this form of the disease appears to be a nonspecific consequence of the delayed hypersensitivity reaction to the products of the M. leprae. In these situations as well as in the present study, as a consequence of intensive inflammatory reaction, there was often secondary demyelination resulting from both myelin and axon destruction. The mechanism of myelin damage and sometimes concomitant destruction of the axon remain unclear. Based on such experimental models as EAE or EA neuritis, a number of mechanisms have been proposed (17-20): (a) myelin damage by either circulating or locally produced antibodies; (b) direct destruction of myelin by sensitized lymphoid cells; and (c) myelin damage either by mediators produced by sensitized lymphocytes or
secondarily by activated macrophages influenced by lymphocytes. In the present model, the histologic picture suggests that the last of these mechanisms is likely to be most significant. The sequence of events observed was as follows: perivascular accumulation of hematogenous cells, infiltration of surrounding parenchyma by cells from the perivascular cuffs, vesicular disruption and stripping of the myelin lamellae by the hematogenous cells, and phagocytosis of the myelin sheaths. The morphogenesis is thus similar to that seen in EAE (17), and similar as well to lesions studied in distemper (3) and SSPE (4) encephalitis, and suggests that some components of demyelination even in the viral-induced conditions could result from a similar mechanism (1). This model suggests that if the exogenous source of antigen were removed, by appropriate treatment or immunological means, the degree of neurologic damage would be limited, and in the case of primary demyelination, could be reversible. This point stands in contradistinction to demyelination which might occur as a result of an autoimmune process, in which even if the initiating antigen were removed, the autoimmune process would likely be progressive.

It has not yet been shown in experimental systems that a specific immune response to an exogenous agent can initiate a secondary autoimmune EAE-like encephalitis. Clearly in neurologic diseases associated with breakdown of nervous parenchyma, detection of lymphocytes reactive to encephalitogenic protein suggests that possibility. It is well known that in the absence of Freund's adjuvant, for example, it is extremely difficult to induce EAE, e.g. Rivers et al. (21, 22) had to give 60–120 injections of 5–10 ml brain homogenate to produce disease. However, the possibility that sensitization to brain antigens may occur as a result of persistent inflammatory reactions in nervous tissue is currently under study.

**Summary**

Primary demyelination occurs in a variety of human and experimental diseases known to be associated with the presence of inflammatory cells. However, the mechanism of demyelination remains unclear. The possibility that myelin can be damaged as a nonspecific consequence of a specific delayed type of hypersensitivity reaction directed at nonnervous tissue antigens is currently under study.

Guinea pigs were sensitized to tuberculin with Freund's complete adjuvant, and were challenged in the central and peripheral nervous system either with live or killed sonicated tubercle bacilli, Old Tuberculin, or tuberculin purified protein derivative (PPD). Local inflammatory reactions were invariably produced and primary demyelination was a constant feature of the lesions. The morphological picture was rather similar to that observed in human neurotuberculosis and early tuberculoid leprosy, and in experimental allergic encephalomyelitis and distemper encephalitis in animals. The infiltrates consisted predominantly of mononuclear cells with some polymorphonuclear cells as well. Vesicular disruption of the myelin sheath in the immediate vicinity of the inflammatory cells and stripping of the myelin lamellae by the histiocytes without axonal damage were the leading features of the lesion. The results indicate that cell-mediated immune reactions to a variety of nonbrain antigens could be responsible for a
component of the demyelination seen in some inflammatory demyelinating conditions, and suggest that this system may serve as a useful model for studying the immunopathology of demyelinating disease.

We thank Dr. Robert D. Terry for his support and constructive criticism. The authors appreciate the expert technical, photographic, and secretarial assistance of Mrs. Carol Fitzgerald, Mr. Larry Gonzales, Mr. Sidney Gravney, Mrs. Judith Gaffney, Mrs. Lenore Grollman, and Mrs. Roselyne Schwartz. We express our appreciation to Doctors Marius Valsamis and Mauro Dal Canto for providing us with the human autopsy material.

Received for publication 9 September 1974.

References

1. Wisniewski, H. M. 1972. Patterns of myelin damage resulting from inflammatory and toxin-induced lesions and their relationship to multiple sclerosis. In Multiple sclerosis, UCLA Medical Forum. Wolfgram and Andrews, editors. Academic Press Inc., New York. 53.

2. Zu Rhein, S. M. 1969. Association of Papova-virions with a human demyelinating diseases (Progressive multifocal leukoencephalopathy). Prog. Med. Virol. 11:185.

3. Wisniewski, H. M., C. S. Raine, and W. J. Kay. 1972. Observations on viral demyelinating encephalomyelitis canine distemper. Lab. Invest. 26:589.

4. Herndon, R. M., and L. J. Rubinstein. 1968. Light and electron microscopy observations on the development of viral particles in the inclusion of Dawson's encephalitis (subacute sclerosing panencephalitis). Neurology. 18:8.

5. Bloom, B. R., and B. Bennett. 1971. In, In vitro Methods in Cell Mediated Immunity. B. R. Bloom and P. R. Glade, editors. Academic Press, Inc., New York. 235.

6. Kristensson, K., and Y. Olsson. 1973. Diffusion pathways and retrograde axonal transport of protein tracers in peripheral nerves. Progress in Neurology. Vol. 1, part 2. Kerkut and J. W. Phillips, editors. Pergamon Press, Inc., New York. 85.

7. Sunderland, S. 1973. The internal anatomy of nerve trunks in relation to the neural lesions of leprosy. Observations on pathology, symptomatology and treatment. Brain. 96:866.

8. Burn, C. G., and K. H. Finley. 1932. The role of hypersensitivity in the production of experimental meningitis. J. Exp. Med. 56:203.

9. Rich, A., and H. A. McCordock. 1933. The pathogenesis of tuberculous meningitis. Johns Hopkins Med. J. 52:5.

10. Dastur, D. K., and P. M. Udani. 1966. The pathology and pathogenesis of tuberculous encephalopathy. Acta Neuropathol. 6:311.

11. Dastur, D. K., and N. H. Wadia. 1969. Spinal meningitides with radiculomyelopathy. Part 2. Pathology and pathogenesis. J. Neurol. Sci. 8:261.

12. Dastur, D. K., and V. S. Lalitha. 1973. The many facets of neurotuberculosis: an epitome of neuropathology. In Progress in Neuropathology. Vol. II. H. M. Zimmerman, editor. Grune & Stratton, Inc., New York. 351.

13. Job, C. K. 1970. Mycobacterium leprae in nerve lesions in lepromatous leprosy. Arch. Pathol. 89:195.

14. Nishiura, M. 1960. The electron microscopic basis of the pathology of leprosy. Int. J. Lepr. 28:357.

15. Wade, H. W. 1934. Tuberculoid changes in leprosy. III. The pathology of a nerve abscess. Int. J. Lepr. 2:293.

16. Khanolkar, V. R. 1964. Pathology of leprosy. In Leprosy in Theory and Practice. R. G.
Cochrane and T. F. Davey, editors. The Williams and Wilkins Company, Baltimore, Md. 2nd edition. 125.

17. Bornstein, M. B. and Iwanami, H. 1971. Experimental allergic encephalomyelitis. Demyelination activity of serum and sensitized lymph node cells on cultured nerve tissues. *J. Neuropath. Exp. Neurol.* 30:240.

18. Wisniewski, H., J. Prineas, and C. S. Raine. 1969. An ultrastructural study of experimental allergic encephalomyelitis in the peripheral nervous system. *Lab. Invest.* 21:105.

19. Arnason, B. G. W., G. F. Winkler, and N. M. Hadler. 1969. Cell-mediated demyelination of peripheral nerve in tissue culture. *Lab. Invest.* 21:1.

20. Johnson, A. B., H. M. Wisniewski, C. S. Raine, E. H. Eylar, and R. D. Terry. 1971. Specific binding of peroxidase-labeled myelin basic protein in allergic encephalomyelitis. *Proc. Natl. Acad. Sci. U.S.A.* 68:2694.

21. Rivers, T. M., D. M. Sprunt, and G. P. Berry. 1933. Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J. Exp. Med.* 58:39.

22. Rivers, T. M., and F. F. Schwentker. 1935. Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys. *J. Exp. Med.* 61:889.