

Demyelination and Early Remyelination in Experimental Allergic Encephalomyelitis Passively Transferred With Myelin Basic Protein-Sensitized Lymphocytes in the Lewis Rat

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Abstract

Histological studies were performed on Lewis rats with experimental allergic encephalomyelitis (EAE) passively transferred by myelin basic protein (MBP)-sensitized syngeneic spleen cells in order to determine the relationship between demyelination and neurological signs. Neither inflammation nor demyelination was present on the day prior to the onset of neurological signs but both were present in the spinal roots and spinal cord on the day of onset of tail weakness (4 days after passive transfer). Demyelination and the neurological signs both increased over the next 48 h. There was evidence that the caudal roots were more severely affected than the rostral roots. The peripheral nerves were spared. Demyelination in the spinal cord was concentrated in the dorsal root entry and ventral root exit zones. The initial stages of repair of demyelinated spinal root fibres by Schwann cells were observed on the earliest day that clinical recovery commenced (day 7). At this time some demyelinated fibres were closely associated with debris-free Schwann cells, and occasional fibres were completely invested by 1-2 layers of Schwann cell cytoplasm. Remyelination (compact myelin lamellae formation) by Schwann cells was first observed in the spinal roots on day 9. By the time of complete clinical recovery (day 11) the majority of affected spinal root fibres had thin new myelin sheaths. Repair of central nervous system myelin by oligodendrocytes was slower than peripheral nervous system myelin repair. Investment of demyelinated spinal cord axons by oligodendrocytes was observed on day 9, and remyelination by these cells was seen on day 10. We conclude that the neurological signs of passively induced MBP-EAE can be accounted for by demyelination of the lumbar, sacral and coccygeal spinal roots and spinal cord root entry and exit zones, and that the subsequent clinical recovery can be explained by investment and remyelination of demyelinated peripheral and central nervous system fibres by Schwann cells and oligodendrocytes respectively.

Introduction

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the nervous system and is widely studied as an animal model of multiple sclerosis (MS), a human central nervous system (CNS) demyelinating disease of unknown aetiology. EAE may be actively induced by inoculation with whole CNS tissue, purified myelin basic protein (MBP) or proteolipid protein (PLP), and adjuvants, or passively induced by the transfer of lymphocytes from animals actively inoculated with one of these antigenic preparations. Passive EAE was originally

induced by the direct transfer of lymph node cells from animals sensitized to whole spinal cord (Paterson, 1960). More recently, techniques have been described for the *in vitro* augmentation of donor lymphocyte activity (Panitch and McFarlin, 1977; Richert et al., 1979), and these have ultimately led to the development of MBP-specific T cell lines and clones which are capable of transferring disease in low doses (Ben-Nun et al., 1981; Ben-Nun and Cohen, 1982; Zamvil et al., 1985).

EAE, either actively or passively induced, may have an acute or a chronic relapsing course, but each form produces the same neurological signs, namely tail paralysis and limb ataxia, weakness and paralysis. Acute EAE is a monophasic disease like the human disease, acute disseminated encephalomyelitis, while chronic relapsing EAE has a relapsing remitting course like typical MS. In chronic relapsing EAE there are large plaques of CNS demyelination as in MS. However, in acute EAE such plaques are rare or do not occur. In fact, because of reports of the absence of demyelination in some animals with neurological signs of EAE it has been suggested that the neurological signs of acute EAE and of the first episode of chronic relapsing EAE are due not to demyelination (Hoffman et al., 1973; Lassmann and Wisniewski, 1979; Panitch and Ciccone, 1981; Raine et al., 1981; Simmons et al., 1981, 1982; Kerlero de Rosbo et al., 1985), but to other factors such as oedema (Paterson, 1976; Simmons et al., 1982; Kerlero de Rosbo et al., 1985; Sedgwick et al., 1987) or an impairment of monoaminergic neurotransmission (Carnegie, 1971; White, 1984). It has also been suggested that neurological recovery from acute EAE is too rapid to be accounted for by remyelination, and therefore that demyelination cannot be the cause of the neurological signs (Panitch and Ciccone, 1981; Simmons et al., 1981). However, these studies have either failed to use sensitive histological techniques to detect demyelination or failed to examine thoroughly the whole nervous system, particularly the lumbar, sacral and coccygeal spinal cord and the peripheral nervous system (PNS), which is known to be involved in EAE. We have recently shown that demyelination in the PNS and the CNS is an important cause of the neurological signs in actively induced acute EAE by demonstrating demyelination and nerve conduction abnormalities in the dorsal root ganglia of rabbits (Pender and Sears, 1982, 1984, 1985) and rats with whole spinal cord-induced EAE (Pender and Sears, 1986), at the ventral root exit zones of the spinal cord in rats with whole spinal cord-induced EAE (Pender, 1986a, 1988a) and in the dorsal roots, ventral roots and ventral root exit zones in rats with MBP-induced EAE (Pender, 1986b, 1987, 1988b, c). Furthermore, the rapid neurological recovery in rats with whole spinal cord- or MBP-induced acute EAE is associated with the restoration of nerve conduction in the PNS and CNS at the time of investment and remyelination of demyelinated fibres by Schwann cells and oligodendrocytes respectively (Pender, 1989).

In view of our findings that demyelination is an important cause of the neurological signs in actively induced acute EAE, we hypothesized that

demyelination could also account for the neurological signs of passively induced acute EAE. The present study was undertaken to test this hypothesis. Because of the highly predictable and early onset of neurological signs 4 days after passive transfer, we were able to make clinicopathological correlations immediately before and immediately after the onset of neurological signs, as well as during and immediately after clinical recovery.

Materials and methods

Animals

Lewis rats (JC strain) were bred by the Animal Breeding Establishment of the John Curtin School of Medical Research. Rats, 12-15 weeks old, were used as both donors and recipients of spleen cells.

Sensitization of donor animals

MBP was prepared from guinea-pig spinal cord as described by Deibler et al. (1972). MBP in 0.9% saline was emulsified in an equal volume of incomplete Freund's adjuvant containing 4 mg/ml of added *Mycobacterium butyricum*. Each rat received 0.1ml of emulsion in a footpad of each hindfoot. The total dose of MBP was 50 µg/rat.

Induction of passive EAE

Cells for passive transfer were generated according to the method of Panitch and McFarlin (1977). Single-cell suspensions were prepared from the spleens of donor rats sensitized 10-12 days previously with MBP as described above. Cells were cultured at a concentration of 2×10^6 cells/ml in RPMI 1640 with added 5% fetal calf serum, 5×10^{-5} M 2-mercapto-ethanol, 200 mM L-glutamine, penicillin and streptomycin. Concanavalin A was added at 2 µg/ml, and 50 ml cultures were incubated at 37°C in an atmosphere of CO₂ (10%), O₂ (7%) and N₂. Cells were harvested after 72 h and washed with Hanks' balanced salt solution. 5×10^7 viable cells were injected into a lateral tail vein of each recipient male rat.

Controls

For control purposes, spleens were removed from normal donors and the spleen cells were cultured with concanavalin A as above for 72 h. 5×10^7 viable cells were injected into a lateral tail vein of each of four male rats.

Clinical assessment and management of recipient animals

The recipient animals were examined daily from the 2nd day after passive transfer, as previously described (Pender, 1986b). Tail

weakness was assessed by holding the animal by the base of the tail and observing tail movement. It was graded as follows: 0 = no weakness; 1 = weakness of distal tail only, the distal tail failing to curl around the examiner's finger; 2 = weakness of the whole tail but with the proximal tail still being able to be erected vertically against gravity; 3 = severe weakness with only a flicker of tail movement; 4 = complete flaccid paralysis of the tail. Hindlimb weakness was graded thus: 0 = no weakness; 1 = slight dragging of the toes of the hindfoot; 2 = severe dragging of the hindfoot but not of the rest of the hindlimb; 3 = severe dragging of the whole hindlimb; 4 = total paralysis of the hindlimb. If the hindlimb involvement was asymmetrical, the mean grade was used. The forelimbs were assessed in a similar way to the hindlimbs. Nociception was assessed by determining if vocalization occurred in response to noxious mechanical stimulation of the tail or of the digits of the hindlimbs. The mechanical stimulus consisted of firm pressure between the thumbnail applied to the side of the tail being tested and the index finger pad applied to the opposite side. Histological studies were carried out on two controls (5 and 10 days after passive transfer) and on 11 rats with EAE (3, 4, 4, 5, 6, 7, 8, 9, 10, 11 and 11 days after passive transfer).

Histological studies

Under ether anaesthesia the rats were perfused through the left ventricle with 0.9% saline followed by 150 ml of 2.5% glutaraldehyde/2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3-7.4). The brain, spinal cord, dorsal and ventral roots, dorsal root ganglia, spinal nerves and sciatic and tail nerves were removed and immersed in fixative. Most blocks of tissue were post-fixed with 2% osmium tetroxide, dehydrated in ascending ethanols, embedded in HistoResin (LKB, Bromma, Sweden), sectioned (1 μ m) and stained with cresyl fast violet, as previously described (Pender, 1985). Some blocks were post-fixed with 2% osmium tetroxide, embedded in Epox 812 (Ernest F. Fullam, Schenectady, NY, U.S.A.), sectioned (1 μ m) and stained with toluidine blue. In the EAE rats examined 3-6 days after passive transfer the lumbar, sacral and coccygeal dorsal and ventral roots were embedded individually in order to compare the involvement of different segments. A total of 105 root sections were assessed (see Table 1). However, in the EAE rats examined 7-11 days after passive transfer and in the control rats, all of the cauda equina were embedded together. The sections were examined with a Zeiss Axiophot microscope. The extent of demyelination in each region was graded as

follows by estimating the proportion of myelinated fibres demyelinated in a cross-section examined with a 40 x /0.75 Plan-Neofluar objective: 0 = no demyelinated fibres; 1 = < 1%; 2 = 1-10%; 3 = 10-30%; 4 = 30-50%; 5 = > 50% demyelinated fibres. For ultrastructural assessment, ultrathin Epox 812 sections were double-stained with uranyl acetate and lead citrate and examined in an Hitachi-300 or Philips T400 electron microscope.

Results

Clinical findings

Distal tail weakness commenced 4 days after passive transfer. Over the next 24h the weakness ascended, with resultant complete flaccid paralysis of the tail. The ascending tail weakness was accompanied by an ascending impairment of tail nociception as previously described in Lewis rats with actively induced MBP-EAE (Pender, 1986b). Hindlimb weakness commenced 5-6 days after passive transfer and was usually maximal on days 6-7. Forelimb weakness occurred occasionally. Recovery commenced at 7-8 days. Tail nociception and hindlimb motor function had returned to normal by day 9 and tail motor function usually by day 11. The typical clinical course is illustrated in Fig. 1. None of the four rats receiving spleen cells from normal donors developed neurological signs.

Histological findings in controls

No significant histological abnormalities were detected in the rats examined 5 and 10 days after the transfer of spleen cells from normal donors.

Histological findings in rats receiving MBP-sensitized spleen cells

The histological and clinical findings in the rats receiving MBP-sensitized spleen cells are summarized in Tables 1 and 2.

Day 3. The rat studied on day 3, before the onset of neurological signs, showed no evidence of inflammation or demyelination in the CNS or PNS.

Day 4. The two rats studied on day 4 showed grade 2-3 tail weakness and absent nociception over the distal third of the tail and no hindlimb weakness. The majority of random 1 μ m transverse sections through individual sacral and coccygeal dorsal and ventral roots showed grade 1-2 primary demyelination (Fig. 2). However, the mean grade of demyelination was 0.8-1.0 (see Table 1), emphasizing that only a small proportion of the fibres at any one cross-sectional level (1 μ m thick) were affected. Some of these demyelinated fibres

were completely demyelinated but the majority were in the process of being demyelinated by mononuclear cells that had invaded the myelin sheath. The process affected both large and small diameter fibres.

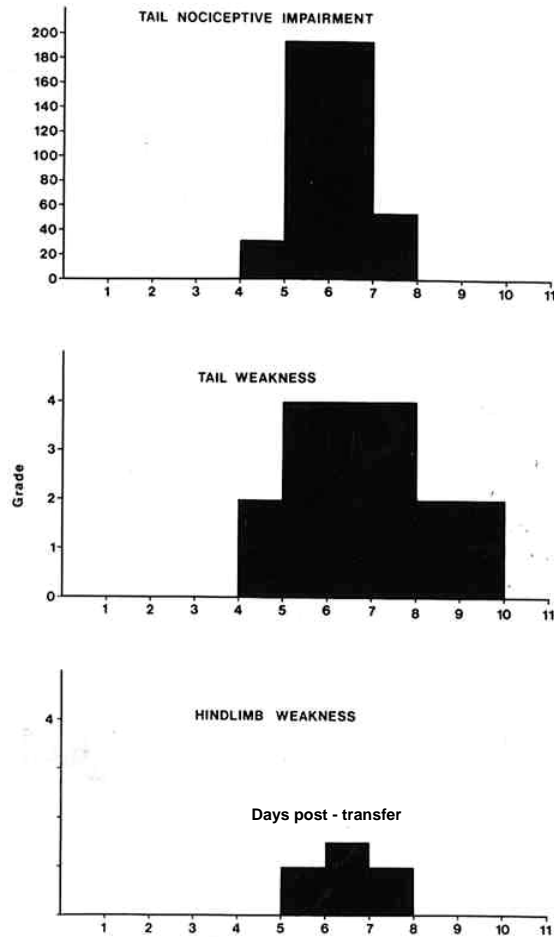


Fig. 1. Profiles of the neurological signs in a Lewis rat with EAE passively transferred by MBP-sensitized lymphocytes, showing the development of and recovery from: impairment in tail nociception (upper panel); tail weakness (middle panel); and hindlimb weakness (lower panel). See Materials and Methods for grading of tail and hindlimb weakness. As the nociceptive impairment commenced distally, 'length of tail affected' refers to length from the tail tip.

Demyelination was found in sections taken from proximal and distal segments of roots; however, the sections through roots lying adjacent to the spinal cord showed less demyelination than sections through the more distal parts. The roots were infiltrated with mononuclear and polymorphonuclear leukocytes and extravasated erythrocytes. Oedema was also present. Similar inflammatory and demyelinating lesions were present in the lumbar dorsal and ventral roots. The lumbar, sacral and coccygeal dorsal root ganglia showed minimal demyelination but there was considerable inflammation in the connective tissue surrounding the ganglia and adjacent spinal nerve. The spinal nerves themselves showed minimal

involvement. Transverse sections through the lumbar, sacral and coccygeal spinal cord showed grade 1-4 demyelination in the CNS (oligodendrocyte-myelinated) parts of the dorsal root entry and ventral root exit zones. In some sections there appeared to be a cone of demyelinated CNS axons projecting into the roots (Fig. 3). There was grade 1 demyelination in the remainder of these sections of the spinal cord and this was restricted to the subpial region except for rare demyelinated fibres associated with perivascular infiltrates in the deep white matter. There was meningeal, subpial and deep perivascular infiltration predominantly with mononuclear leukocytes but also with polymorphonuclear leukocytes, pyknotic cells and extravasated erythrocytes. There was also some white matter oedema. Occasional fibres in the white matter showed dilatation of the periaxonal space within the myelin sheath as described by Brosnan et al. (1988). Similar inflammatory changes were present in the spinal cord grey matter. Sections through the brain revealed meningeal, subpial and deep perivascular infiltration in the brainstem, particularly the medulla. The cerebellum was spared and there was minimal infiltration of the cerebral parenchyma.

Day 5. The rat studied on day 5 showed grade 4 tail weakness, absent nociception along the whole length of the tail, and grade 1 hindlimb weakness. The majority of random transverse sections through individual lumbar, sacral and coccygeal dorsal and ventral roots showed grade 1-2 demyelination, with a mean grade of 1 (see Table 1). The findings were similar to those in the roots of day 4 rats. There were more fibres in the process of being demyelinated than there were completely demyelinated fibres. The dorsal root ganglia showed inflammation and slight demyelination; inflammation was particularly prominent in the connective tissue around the ganglia. There was minimal involvement of the spinal nerves. The sciatic and tail nerves were normal. There was grade 1-4 demyelination in the CNS parts of the dorsal root entry and ventral root exit zones of the lumbar, sacral and coccygeal spinal cord. Grade 1 demyelination was present in the remainder of these sections of the spinal cord but this was restricted to the subpial region and immediate vicinity of some penetrating blood vessels. There was some oedema of the white matter and there were occasional fibres showing dilatation of the periaxonal space within the myelin sheath. Inflammation was also present in the grey matter of the spinal cord. Sections through the brain showed prominent oedema and inflammation in the dorsomedial medulla bilaterally. Other regions of the brainstem showed perivascular infiltration. There was minimal infiltration of the cerebellum and cerebrum.

TABLE 1

NEUROLOGICAL SIGNS AND QUANTITATIVE ASSESSMENT OF
DEMYELINATION AFTER TRANSFER OF MBP-SENSITIZED LYMPHOCYTES

Days after transfer	Tail weakness (grade)	Tail nociceptive impairment (length of tail affected (mm))	Hindlimb weakness (grade)	Demyelination of spinal roots (mean grade ^a)	Demyelination of CNS parts of spinal cord root entry and exit zones (mean grade ^a)	Relative proportions of partially ^c and completely demyelinated fibres in roots
3	0	0	0	0 <i>n</i> ^b = 10	0 <i>n</i> ^b = 5	-
4	2	66	0	1.0 <i>n</i> = 15	1.9 <i>n</i> = 4	Partial >complete
4	3	61	0	0.8 <i>n</i> = 41	2.1 <i>n</i> = 6	Partial >complete
5	4	195	1	1.0 <i>n</i> = 21	2.4 <i>n</i> = 6	Partial >complete
6	4	195	2	1.8 <i>n</i> = 18	2.9 <i>n</i> = 7	Complete >partial

^a Mean grade of demyelination (see materials and Methods) in transverse sections through individual dorsal and ventral roots or through the spinal cord.

^b Total number of sections.

^c Fibres in the process of being demyelinated

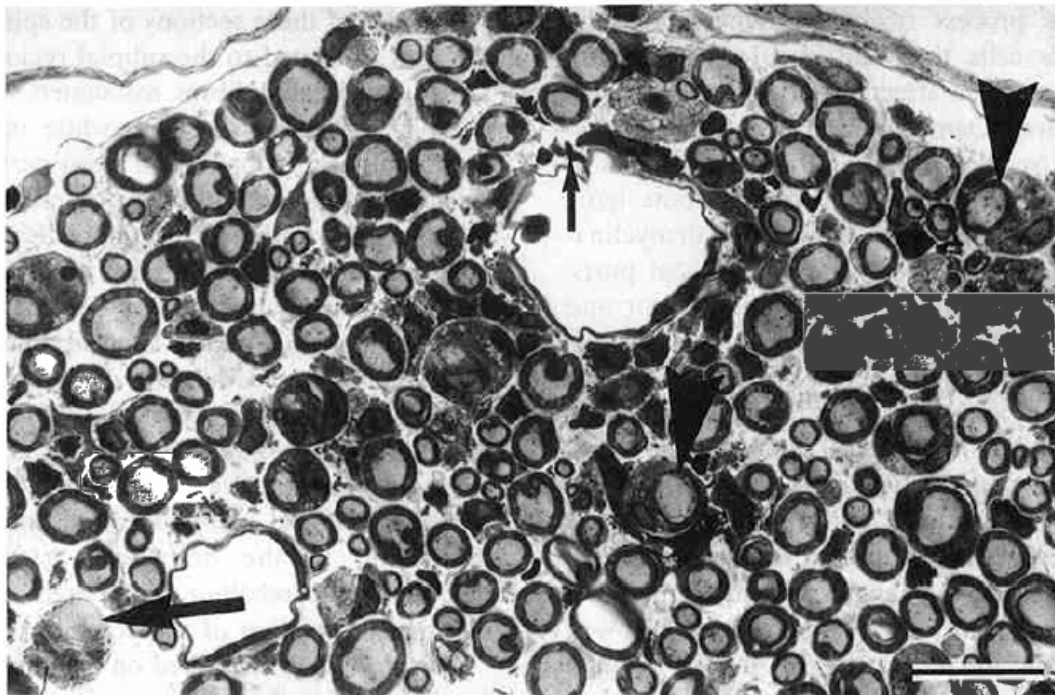


Fig. 2. Transverse section through an S4 ventral root of a rat 4 days after the passive transfer of MBP-sensitized lymphocytes. Several fibres are in the process of being demyelinated (arrowheads). One completely demyelinated fibre (large arrow) and extravasated erythrocytes (small arrow) can also be seen. HistoResin section stained with cresyl fast violet. Bar = 25 μ m.

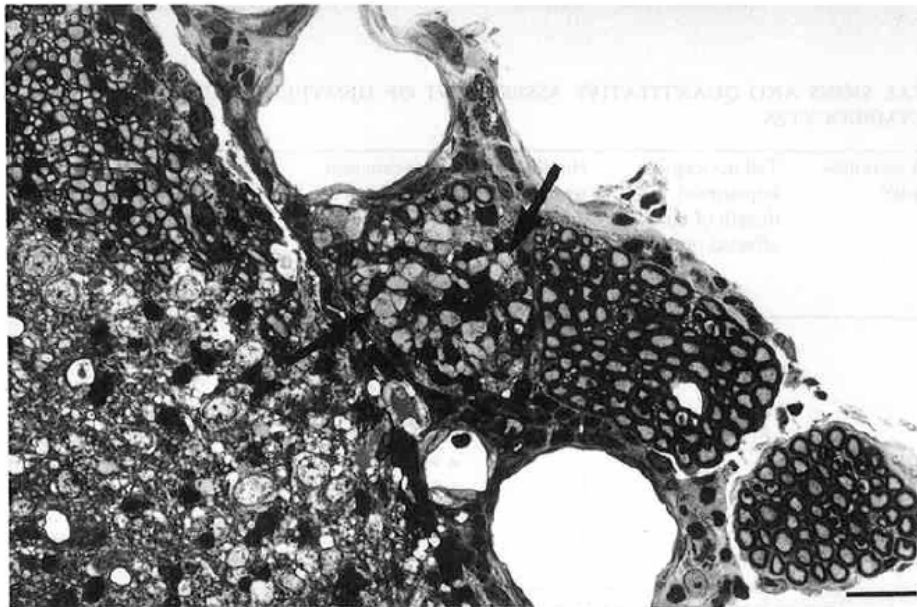


Fig. 3. Transverse section through the dorsal root entry zone of the coccygeal spinal cord of a rat 4 days after the passive transfer of MBP-sensitized lymphocytes. There is a cone of demyelinated axons (arrows) extending into the dorsal root. HistoResin section stained with cresyl fast violet. Bar = 25 µm.

TABLE 2

TEMPORAL PROFILES OF CLINICAL SEVERITY AND OF MYELIN DAMAGE AND REPAIR IN RATS RECEIVING MBP-SENSITIZED LYMPHOCYTES

Days after transfer	Clinical severity ^a	PNS myelin (spinal roots)	CNS myelin (spinal cord, predominantly root entry and exit zones)
3	0	No demyelination	No demyelination
4	2	Demyelination	Demyelination
4	3	Demyelination	Demyelination
5	5	Demyelination	Demyelination
6	6	Demyelination	Demyelination
7	6	Demyelination	Demyelination
		Investment of some demyelinated fibres by Schwann cells	
8	2	Demyelination	Demyelination
		Investment of most demyelinated fibres by Schwann cells	
9	2	Demyelination	Demyelination
		Investment of most demyelinated fibres by Schwann cells	Investment of some demyelinated fibres by oligodendrocytes
		Remyelination of occasional fibres with up to 2 myelin lamellae	
10	1	Remyelination of the majority of affected fibres with up to 5 myelin lamellae	Demyelination
			Investment of most demyelinated fibres by oligodendrocytes
11	1	Remyelination of the majority of affected fibres with up to 11 myelin lamellae	Demyelination
			Investment of most demyelinated fibres by oligodendrocytes
			Remyelination of some fibres with up to 3 myelin lamellae
11	0	Remyelination of the majority of affected fibres with up to 8 myelin lamellae	Demyelination
			Investment of most demyelinated fibres by oligodendrocytes
			Remyelination of some fibres with up to 3 myelin lamellae

^a Sum of grades of tail and hindlimb weakness.

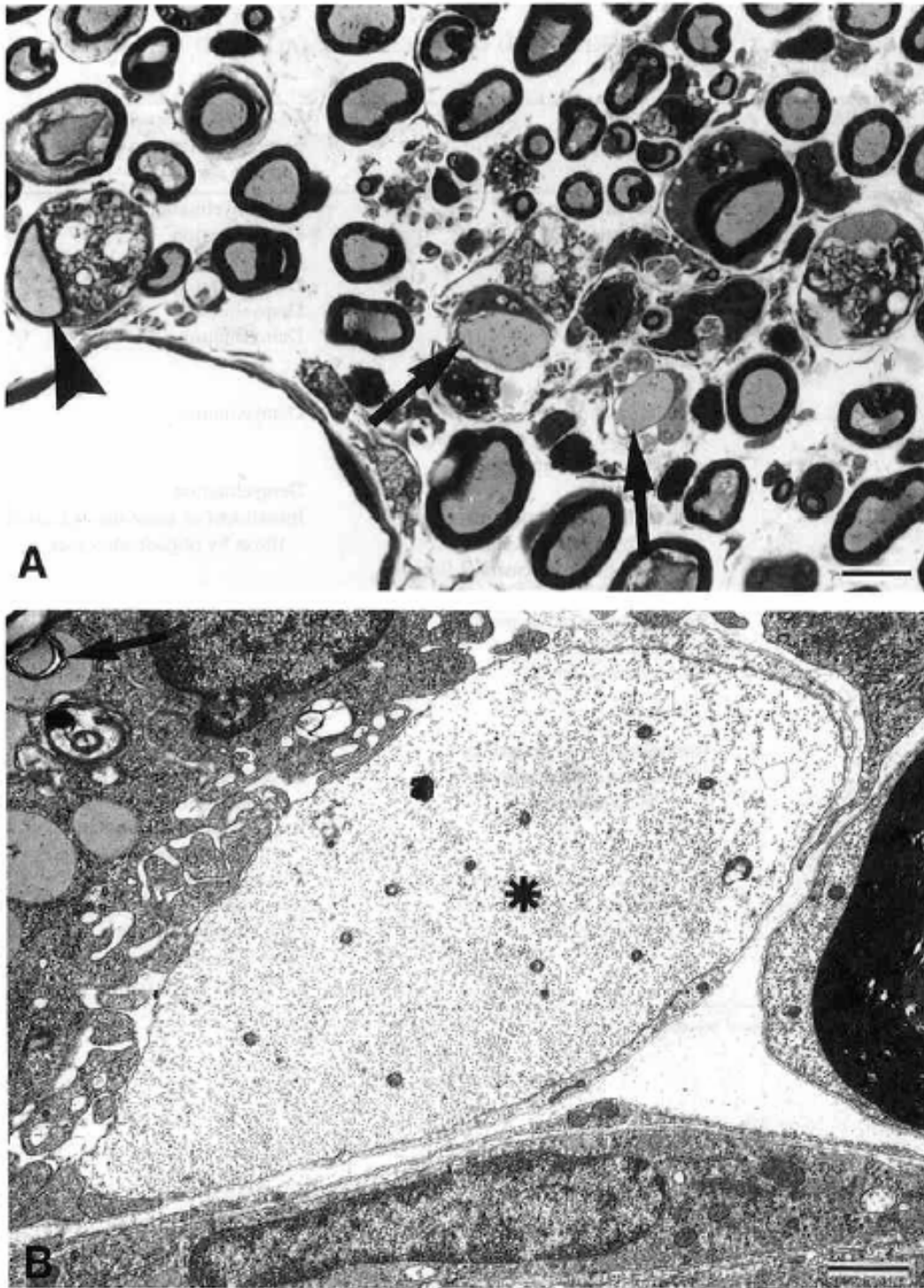


Fig. 4. A: Transverse section through an L6 ventral root of a rat 6 days after the passive transfer of MBP-sensitized lymphocytes. A fibre in the process of being demyelinated (arrowhead) and completely demyelinated fibres (arrows) can be seen. HistoResin section stained with cresyl fast violet. Bar = 10 μ m. B: Electron micrograph of a first coccygeal ventral root of the same rat. There is a completely demyelinated axon (asterisk) associated with a macrophage containing myelin debris (arrow). Bar = 1 μ m.

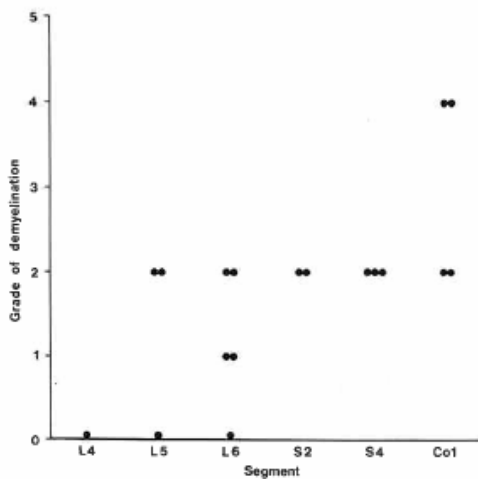


Fig. 5. Scatter diagram showing the grades of demyelination in transverse sections through individual lumbar (L), sacral (S) and coccygeal (Co) roots (dorsal or ventral) in a rat with hindlimb weakness, tail paralysis and absent tail nociception, 6 days after the transfer of MBP-sensitized lymphocytes.

Day 6. The rat studied on day 6 showed grade 4 tail weakness, absent nociception along the whole length of the tail, and grade 2 hindlimb weakness.

Random transverse sections through individual dorsal and ventral roots showed grade 0-2 demyelination in the lumbar segments, grade 2

demyelination in the sacral segments and grades 2 and 4 demyelination in the coccygeal segments (Figs. 4A and 5). Thus these findings indicate that the more caudal roots are more severely affected. The mean grade of demyelination in the spinal roots was 1.8 (see Table 1), which was considerably greater than that present in the animals studied on days 4 and 5. By this stage the vast majority of affected fibres had been completely demyelinated, although some fibres were still in the process of being demyelinated. Most of the completely demyelinated fibres were associated with macrophages containing myelin debris. Small and large fibres were demyelinated. The presence of primary demyelination was confirmed by electron microscopy which revealed demyelinated in-tact axons closely associated with macrophages containing myelin debris (Fig. 4B). There was slight inflammation and demyelination in the dorsal root ganglia. The spinal nerve showed some infiltrating mononuclear cells but no demyelination. The sciatic and tail nerves were normal. Transverse sections through the cervical, thoracic, lumbar, sacral and coccygeal spinal cord showed grade 2-4 demyelination in the CNS parts of the dorsal root entry and ventral root exit zones (Fig. 6). Grade 1 demyelination was present in the remainder of these sections of the spinal cord but this was restricted to the subpial region

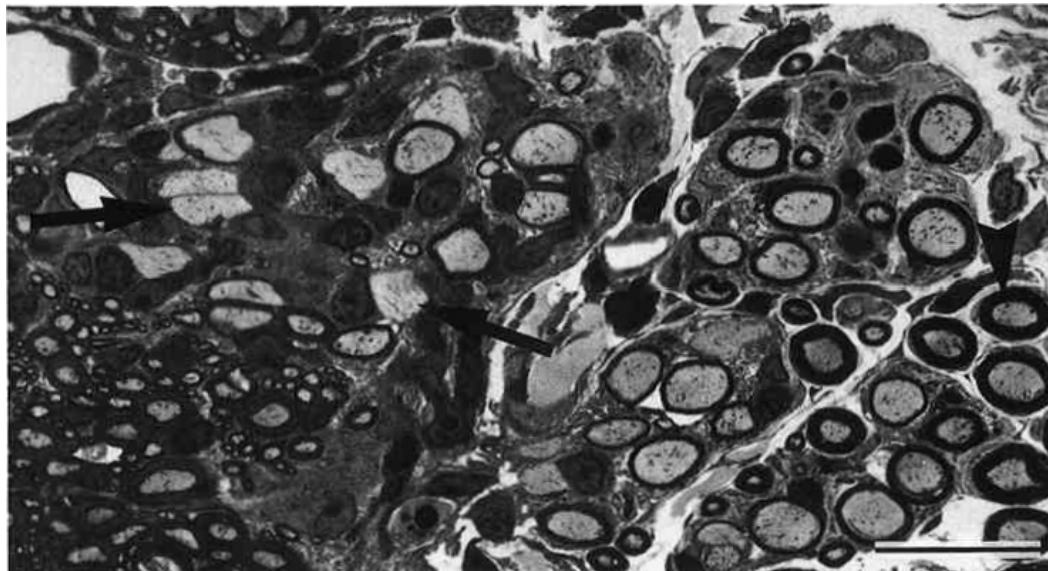


Fig. 6. Transverse section through the ventral root exit zone of the L5 spinal cord of a rat 6 days after the passive transfer of MBP-sensitized lymphocytes. Demyelinated axons (arrows) can be seen in the CNS part of the ventral root exit zone. Normal PNS myelin can be seen in the ventral roots (arrowhead). HistoResin section stained with cresyl fast violet. Bar = 25 µm.

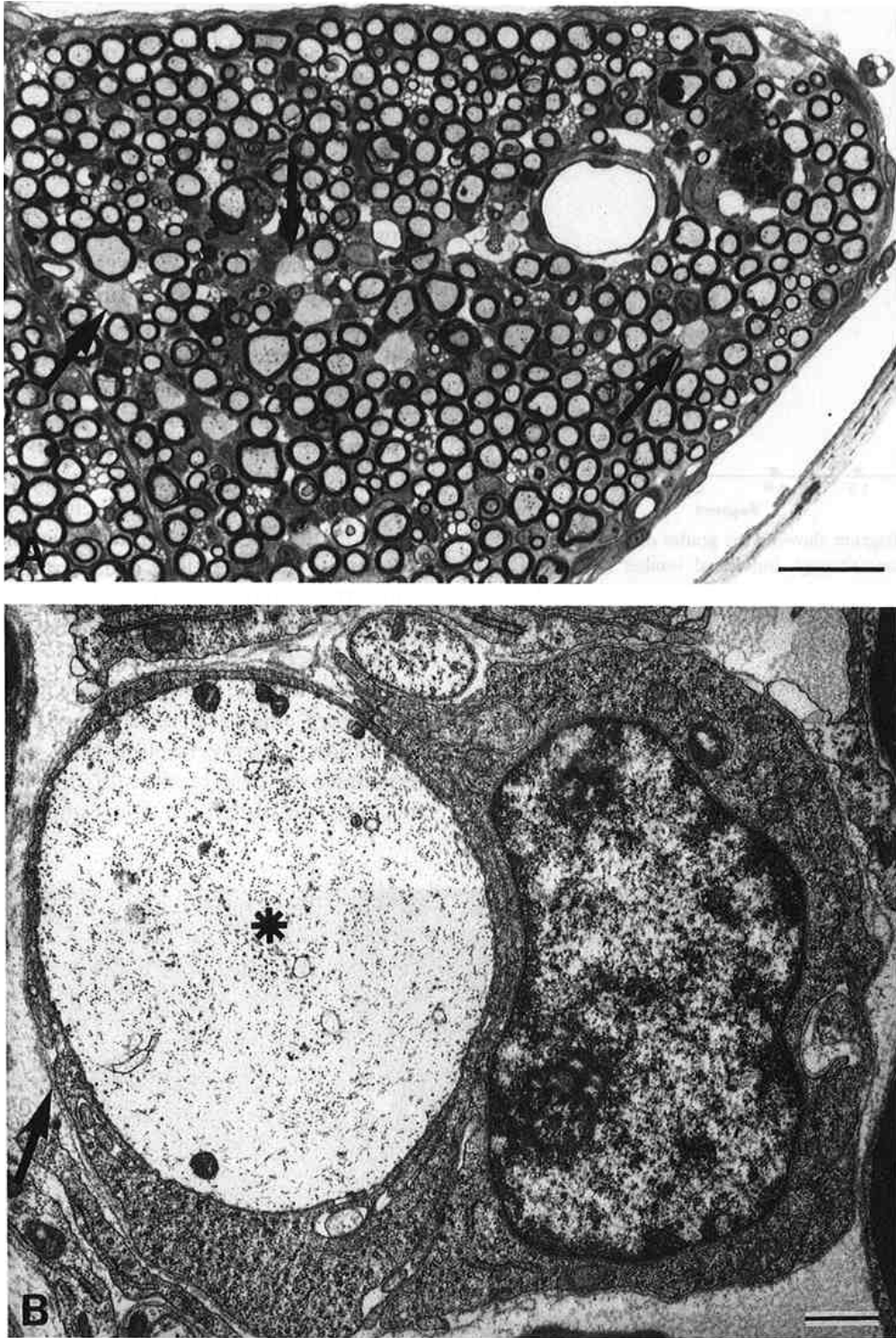


Fig. 7. Light and electron microscopic appearance of the cauda equina of a rat 7 days after the passive transfer of MBP-sensitized lymphocytes. A: Many completely demyelinated axons can be seen (arrows). Epox 812 section stained with toluidine blue. Bar = 25 μm . B: Electron micrograph showing investment of a demyelinated axon (asterisk) by a Schwann cell within a basal lamina (arrow). Bar = 1 μm .

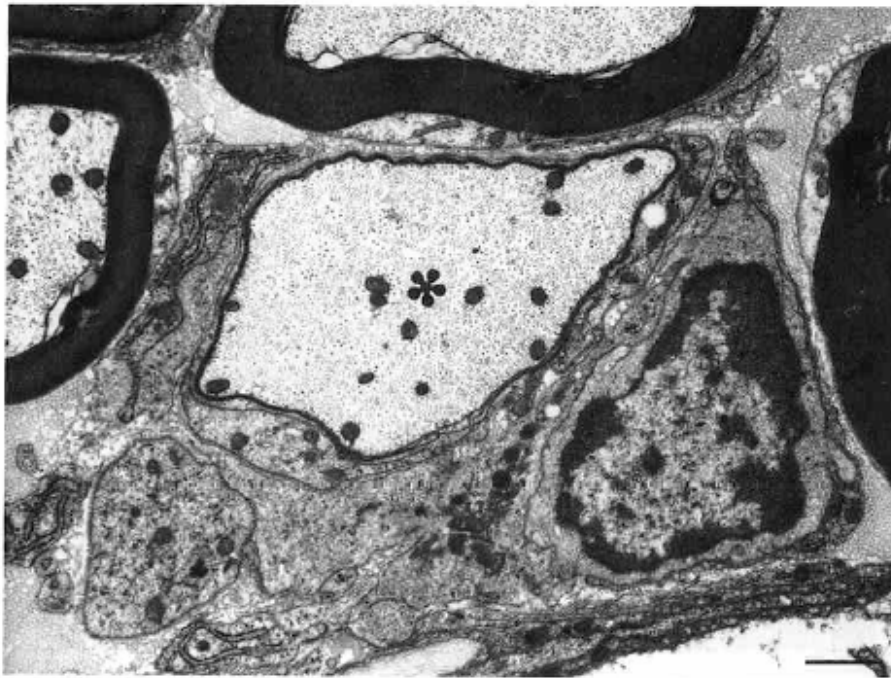


Fig. 8. Electron micrograph of the cauda equina of a rat 9 days after the passive transfer of MBP-sensitized lymphocytes. There is an axon (asterisk) in the process of being remyelinated. Bar = 1 μ m.

Inflammatory infiltrates were present throughout the white matter and were also found in the grey matter. Fibres showing dilatation of the periaxonal space within the myelin sheath were rarely found. Sections through the brain revealed perivascular infiltrates and some oedema in the brainstem and perivascular infiltrates at the junction between the optic chiasm and base of the cerebral hemispheres but there was minimal inflammation in the rest of the cerebrum and in the cerebellum. No demyelination was detected within the brain.

Day 7. The rat examined at this stage showed grade 4 tail weakness and grade 2 hindlimb weakness. Clinical recovery had not commenced in this rat but had commenced in some other rats at this stage (see Fig. 1). Transverse sections through the cauda equina revealed grade 1-2 demyelination (Fig. 7A). The vast majority of affected fibres were completely demyelinated, although there were occasional fibres in the process of being demyelinated. Some of the completely demyelinated fibres were associated with myelin debris-laden macrophages but the majority were not. Some completely demyelinated fibres were closely associated with debris-free Schwann cells. Electron microscopy confirmed this association with Schwann cells and revealed that occasionally such fibres were completely invested by 1-2 layers of Schwann cell cytoplasm within a basal lamina (Fig. 7B).

Grade 3 demyelination was present in the CNS parts of the root entry/exit zones but there was minimal demyelination (grade 1) in other parts of the spinal cord.

Day 8. This rat had shown clinical recovery from grade 4 to grade 2 tail weakness and had recovered from grade 1 hindlimb weakness to normal hindlimb function. The light and electron microscopic histological findings were similar to those in the rat examined on day 7 except that increased proportions of demyelinated cauda equina fibres were closely associated with debris-free Schwann cells and were being invested by these cells.

Day 9. This rat had recovered from grade 4 to grade 2 tail weakness and from grade 1 hindlimb weakness to normal hindlimb function. There was grade 2 demyelination in the cauda equina. The majority of demyelinated fibres were now closely associated with debris-free Schwann cells. There was only a mild inflammatory infiltrate. Electron microscopy revealed that the majority of demyelinated PNS fibres were being invested by Schwann cells and that some were being remyelinated (with 1-2 lamellae of compact myelin) (Fig. 8; Table 2). Grade 3-5 demyelination was present in the CNS parts of the spinal cord root entry/exit zones but there was only grade 1 demyelination in other

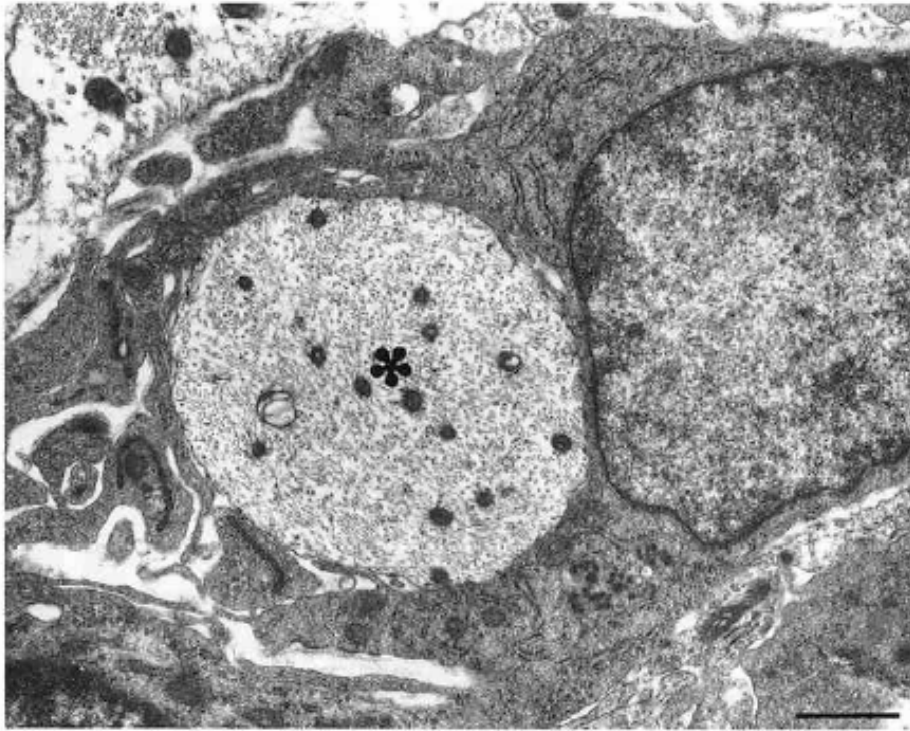


Fig. 9. Electron micrograph of the sacral spinal cord of a rat 9 days after the passive transfer of MBP-sensitized lymphocytes. A demyelinated axon (asterisk) is being invested by an oligodendrocyte. Bar - 1 μ m.

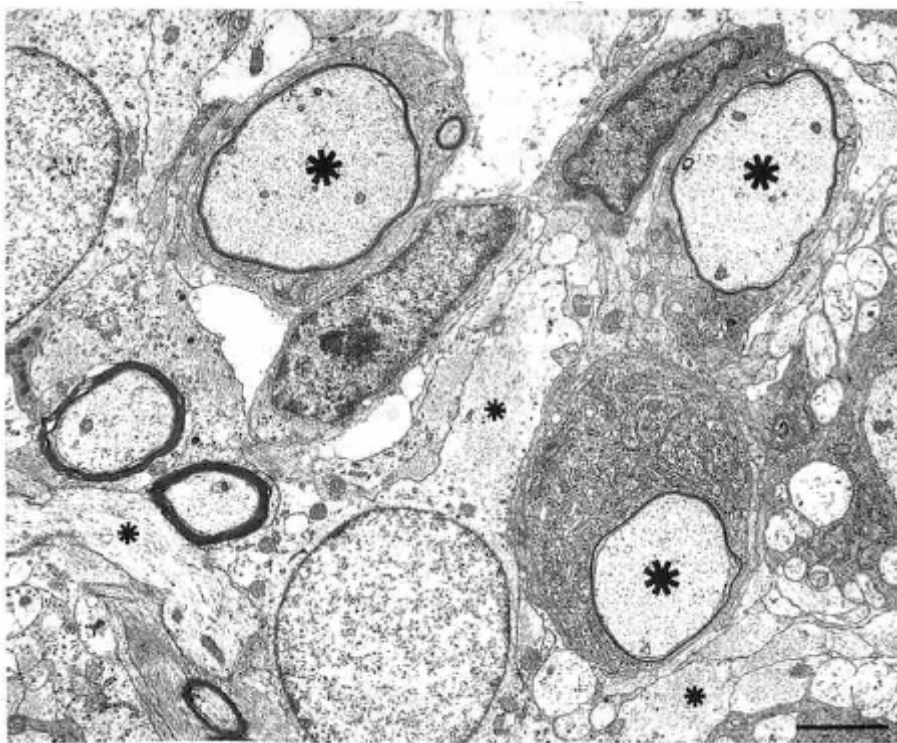


Fig. 10. Electron micrograph of the dorsal root entry zone of the sacral spinal cord of a rat 10 days after the passive transfer of MBP-sensitized lymphocytes. There are three axons (large asterisks) being remyelinated by oligodendrocytes. Astrocytic processes containing intermediate filaments (small asterisks) can also be seen. Bar = 2 μ m.

regions of the spinal cord. There was mild inflammatory cell infiltration of the spinal cord. Electron microscopy of the spinal cord showed demyelinated intact axons some of which were invested by debris-free oligodendrocyte processes (Fig. 9).

Day 10. The rat examined at this stage had recovered from grade 4 to grade 1 tail weakness and from grade 1 hindlimb weakness to normal hindlimb function. Light microscopy revealed that the majority of affected cauda equina fibres were being remyelinated by Schwann cells. Most of the other affected cauda equina fibres were closely associated with Schwann cells. There was mild inflammatory cell infiltration of the cauda equina. Electron microscopy of the cauda equina revealed remyelination with up to five myelin lamellae. Light microscopic examination of the spinal cord revealed findings similar to those in the rat studied on day 9. Electron microscopy of the spinal cord showed that the majority of demyelinated axons were being invested by oligodendrocyte processes and that occasional fibres were being remyelinated by oligodendrocytes producing 1-2 myelin lamellae (Fig. 10). Repair of CNS myelin appeared to be more advanced in the central tissue projecting into the roots than in other regions.

Day 11. One of these rats had made a full clinical recovery from grade 4 tail weakness to normal tail motor function (grade 1 tail weakness had been present on day 10). The other had shown recovery from grade 4 to grade 1 tail weakness, from absent to normal tail nociception and from grade 1 hindlimb weakness to normal hindlimb function. The light microscopic findings in the cauda equina were similar to those in the rat examined on day 10, with the majority of affected fibres being remyelinated by Schwann cells (Fig. 11A). Electron microscopy revealed remyelination with up to 11 myelin lamellae (Fig. 11B; Table 2). There was a slight inflammatory infiltrate in the cauda equina. As with the rats studied on days 9 and 10 there were occasional fibres in the process of being demyelinated and some fibres undergoing axonal degeneration. Axonal degeneration in the cauda equina was prominent in the rat that had residual tail weakness when examined on day 11. Light microscopic examination of the spinal cord showed features similar to those in the rats studied on days 9 and 10. Electron microscopy revealed that now the majority of demyelinated spinal cord axons were invested by oligodendrocyte processes which often formed more than one layer, and that some fibres were being remyelinated by oligodendrocytes producing up to three myelin lamellae (Table 2).

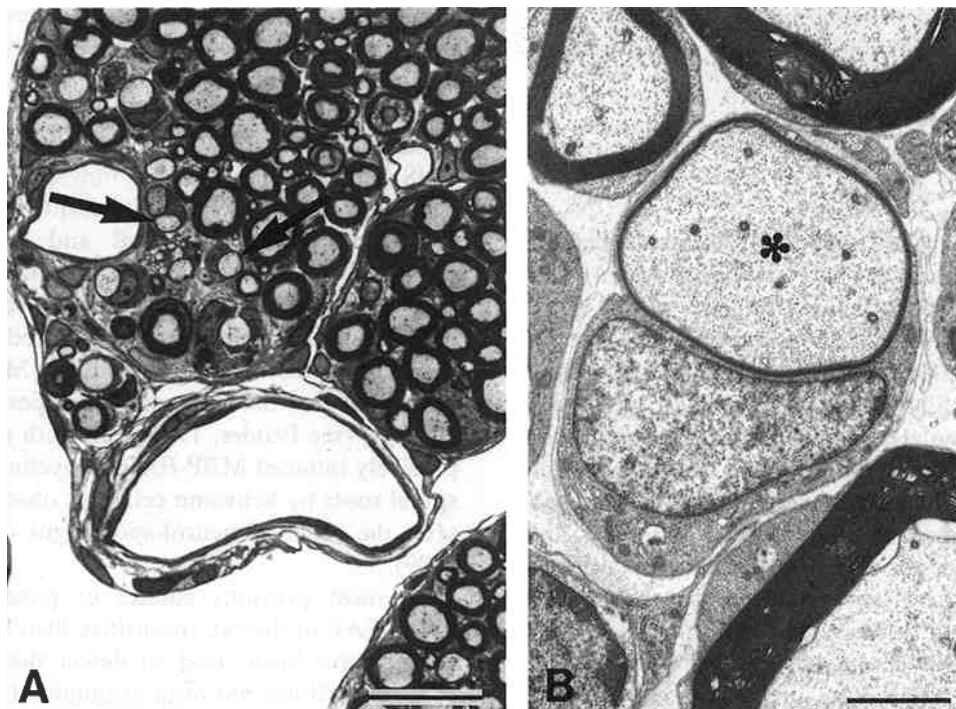


Fig. 11. Light and electron microscopic appearance of the cauda equina of a rat 11 days after the passive transfer of MBP-sensitized lymphocytes. A: There are several axons (arrows) in the process of being remyelinated. HistoResin section stained with cresyl fast violet. Bar = 25 μ m. B: Electron micrograph showing an axon (asterisk) in the process of being remyelinated by a Schwann cell. Bar = 2 μ m.

Discussion

We have demonstrated that primary demyelination is present in the sacrococcygeal spinal roots and spinal cord on the day of onset of tail weakness but not on the previous day. At this stage the majority of affected fibres were in the process of being demyelinated while a smaller proportion had been completely demyelinated. Demyelination was also present in the lumbar spinal roots and spinal cord. Over the next 48 h the neurological signs progressed to involve the hindlimbs, and demyelination was found to be more extensive in the lumbar, sacral and coccygeal spinal roots and spinal cord. In addition the majority of affected fibres were now completely demyelinated while a smaller proportion were in the process of being demyelinated. In the spinal cord the demyelination was concentrated in the CNS (oligodendrocyte-myelinated) parts of the dorsal root entry and ventral root exit zones. There was minimal demyelination in other regions of the spinal cord and this was essentially restricted to the subpial regions. There was minimal, or no, demyelination in the dorsal root ganglia, spinal nerves and peripheral nerves. Thus a demyelinating polyradiculitis is an important component of the pathology of passively induced MBP-EAE as it is in actively induced MBP-EAE (Pender, 1988c). Of particular interest was our observation of early repair of demyelinated PNS and CNS fibres by Schwann cells and oligodendrocytes respectively. Occasional PNS fibres were being invested by Schwann cells 7 days after passive transfer. During the period of clinical recovery (days 7-11) this repair progressed both in terms of the proportion of demyelinated PNS and CNS fibres undergoing repair and in terms of the proportion in more advanced stages of repair (summarized in Table 2). By day 9 many PNS fibres were invested by Schwann cells and some fibres were surrounded by newly formed myelin lamellae. By the time of complete clinical recovery (day 11) most affected fibres in the spinal roots were being remyelinated. Repair of demyelinated CNS fibres by oligodendrocytes was slower than PNS myelin repair. Oligodendrocytes were investing demyelinated spinal cord axons by day 9 and remyelinating these axons on day 10. The early repair of the PNS and CNS by Schwann cells and oligodendrocytes, respectively, is similar to that recently observed in actively induced MBP-EAE and whole-spinalcord-EAE (Pender, 1989). It is notable that the time course of the evolution and resolution of neurological signs in passively induced MBP-EAE is similar to that in actively induced MBP-EAE, if one allows for the different latent periods (4 and 10 days) (see Pender, 1986b). In both actively and passively induced MBP-EAE, remyelination of the spinal roots by Schwann cells was observed 5 days after the onset of neurological signs (see Pender, 1989).

In most previous studies of passively transferred EAE in the rat, insensitive histological techniques have been used to detect demyelination, and the PNS has not been examined. Lassmann et al. (1988) demonstrated limited CNS demyelination in Lewis rats with EAE passively induced by an MBP-specific T cell line but they did not find PNS lesions. However, the only part of the PNS illustrated in their paper is a spinal root immediately adjacent to the spinal cord. Our studies revealed that this part of the spinal root tends to be spared and that one needs to examine roots at a distance from the spinal cord. Lassmann et al. (1988) did not state whether they examined these parts of the spinal roots. Furthermore, because the demyelination in the roots tends to affect fibres in a randomly scattered fashion rather than in plaques, a high resolution microscope objective is needed to detect individual demyelinated fibres. Heininger et al. (1989) have found histological and electrophysiological evidence of demyelination in the spinal cords and spinal roots of Lewis rats with EAE passively transferred by an MBP-specific T cell line. Heininger et al. indicate that the demyelination may be paranodal in distribution. Because paranodal demyelination is restricted to short lengths of individual fibres, it could easily be missed or underestimated in transverse sections and would be adequately assessed only by longitudinal sections. Vandenberg et al. (1986) have re-reported persistent neurological signs and an attenuation of MBP immunoreactivity in the spinal cords of Lewis rats after repeated intraperitoneal injections of MBP-specific T cell lines; however, this immunocytochemical technique does not distinguish between primary demyelination and demyelination secondary to axonal degeneration. Lassmann et al. (1988) and Linington et al. (1988) have augmented the severity of CNS primary demyelination and the severity of neurological signs in Lewis rats with passively transferred MBP-EAE by intravenously injecting a monoclonal antibody against a myelin/ oligodendrocyte glycoprotein.

The involvement of the PNS when rats are inoculated with purified CNS MBP is explained by the fact that the P₁ protein from the PNS is identical to CNS MBP (Brostoff and Eylar, 1972; Greenfield et al., 1973). The preferential involvement of the spinal roots with sparing of the peripheral nerves may be due to the reduced blood-nerve barrier in the roots (Olsson, 1968) and the almost 3-fold higher concentration of P₁ in the spinal root than in the peripheral nerve in the rat (Greenfield et al., 1973). In mice, CNS demyelination has been described in EAE

passively transferred by MBP-specific lymph node cells, T cell lines and T cell clones (Raine et al., 1984; Zamvil et al., 1985; Tabira and Sakai, 1987) and in EAE passively transferred by proteolipid-protein-specific T cell lines (Sato et al., 1987). The reported sparing of the PNS in mice with passively transferred MBP-EAE (Raine et al., 1984; Tabira and Sakai, 1987) could be due to interspecies differences in the blood-nerve barrier, Ia antigen expression, or MBP concentration in the spinal roots or to differences in MBP epitopes in intact CNS and PNS myelin in the mouse. Sparing of the PNS in proteolipid-protein-induced EAE is expected because of the apparent absence of proteolipid protein in the PNS (Finean et al., 1957; Folch et al., 1958).

Our findings indicate that demyelination can account for the neurological signs in passively transferred MBP-EAE in the rat. Demyelination was present on the day of onset of neurological signs and progressed over the next 2 days as did the neurological signs. The clinical progression is likely to be due not only to demyelination of a greater number of fibres but also to progression of the demyelinating process in individual internodes. It is unclear at what stage of the immune attack on individual myelin internodes conduction block occurs, but it is possible that the early stages of invasion of the sheath by mononuclear cells may not necessarily result in conduction block. Demyelination of the lumbar and sacrococcygeal ventral roots and spinal cord ventral root exit zones explains the hindlimb and tail weakness. Demyelination-induced nerve conduction block in the small-diameter myelinated (A δ) fibres in the sacrococcygeal dorsal roots and spinal cord dorsal root entry zones is likely to be the cause of the impairment of tail nociception, as suggested for actively induced MBP-EAE (Pender, 1986b). Demyelination does not need to occur in large plaques in order to produce neurological signs; the functional effects of multiple demyelinating lesions scattered along the length of a spinal root is cumulative, provided that different fibres are affected in each lesion. The ascending nature of the progression of neurological signs is at least partly accounted for by the caudally increasing length of the roots (see Simmons et al., 1982). In a study on male albino rats, Waibl (1973) showed that the lengths of the spinal roots increase progressively from the first thoracic root (2 mm) to the third coccygeal root (59 mm). Thus the probability of many lesions in an entire root increases progressively in a caudal direction. Therefore the probability of a high proportion of fibres undergoing demyelination-induced conduction block increases caudally. In addition, our study suggests that the

caudal roots may be more severely affected than the rostral roots at any one level; this would also contribute to the ascending progression of neurological signs.

As inflammation was present as well as demyelination, one could argue that components of the inflammatory response per se, such as oedema, are responsible for the neurological signs. However, in contrast to the clearly established functional consequences of demyelination, there is no proven effect of oedema on the function of the nervous system, apart from the effects of raised tissue pressure within a confined space. Further evidence that demyelination rather than inflammation per se is the cause of the neurological deficit in acute EAE comes from the observation that nerve conduction is preserved in unmyelinated axons of inflamed and demyelinated dorsal root ganglia while it is severely impaired in demyelinated fibres (Pender and Sears, 1984). Our present finding that the time course of myelin repair parallels that of clinical recovery strongly supports the hypothesis that demyelination accounts for the neurological signs of passively induced EAE, as in actively induced EAE (Pender, 1989). There is evidence from studies on non-inflammatory models of PNS demyelination that nerve conduction may be restored in demyelinated fibres in the early stages of repair before the formation of compact myelin lamellae (Bostock and Sears, 1978; Smith and Hall, 1980; Smith et al., 1982). In the lysophosphatidyl choline model, restoration of conduction occurred when demyelinated fibres became closely associated with debris-free Schwann cells (Smith and Hall, 1980; Smith et al., 1982). Thus in EAE it is possible that function may be restored in demyelinated PNS fibres that have been invested by Schwann cells, a stage first observed at the onset of clinical recovery. It is also possible that investment of demyelinated CNS fibres by oligodendrocytes, as observed on day 9, may be sufficient to restore conduction in these fibres. The present study suggests that the initial stages of clinical recovery from passively transferred MBP-EAE in the rat are due to PNS myelin repair while the later stages are due to both CNS and PNS myelin repair.

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