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The neuropathology of chronic relapsing experimental allergic encephalomyelitis induced in the Lewis rat by inoculation with whole spinal cord and treatment with cyclosporin A*

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Summary. Chronic relapsing experimental allergic encephalomyelitis was induced in Lewis rats by inoculation with guinea-pig spinal cord and complete Freund's adjuvant followed by treatment with low-dose cyclosporin A. In most animals, tail and limb weakness developed in a relapsing remitting pattern but in some these signs were persistent or progressive from onset. Histological studies during the early stages of clinically active disease (< 25 days after inoculation) revealed inflammation and primary demyelination in the central nervous system (CNS), particularly the spinal cord, and in the peripheral nervous system (PNS), specifically the ventral and dorsal roots and dorsal root ganglia. Animals studied in the later stages of clinically active disease (> 28 days after inoculation) had extensive spinal cord demyelination but minimal PNS demyelination. In these animals, large plaques of demyelination with gliosis and prominent plasma cells occurred particularly in the thoracic spinal cord, and lesions of different ages were present within the spinal cord. CNS and PNS remyelination by oligodendrocytes and Schwann cells, respectively, was present in all animals studied later than 18 days after inoculation (the time of the first remission, if it occurred). In both early and late clinically active disease electron microscopy revealed macrophages invading and destroying CNS myelin sheaths. Active demyelination was sometimes found in regions of CNS remyelination, suggesting that remyelinated fibres were being attacked. Axonal degeneration occurred in the spinal cord. During clinical remission there was CNS and PNS remyelination and much less inflammation; however, active demyelination still occurred to a limited degree.

Key words: Experimental allergic encephalomyelitis – Demyelination – Cyclosporin A – Lewis rat – Multiple sclerosis

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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 have either an acute or chronic relapsing course. Chronic relapsing EAE more closely resembles MS, both in terms of the clinical course and the neuropathology. It is readily induced in certain strains of guinea-pigs and mice, and the neuropathology has been well studied in these species [1, 3, 8–10, 30, 37]. It has been more difficult to induce chronic relapsing EAE in the Lewis rat, although this animal is highly susceptible to acute EAE and widely studied for this purpose. The neuropathology of chronic EAE in the Sprague Dawley rat has been well described [10] but there have been limited studies on the neuropathology of chronic relapsing EAE in the Lewis rat [4, 18, 21].

Recently Polman et al. [28] have induced chronic relapsing EAE in the Lewis rat by inoculation with guinea-pig spinal cord and treatment with low-dose cyclosporin A (CsA). The present study was undertaken to investigate the neuropathology in this model. A brief preliminary report of this work has been published [26].

Materials and methods

Animals

Female Lewis rats (JC strain) bred by the Central Animal Breeding House of the University of Queensland were used. The rats were kept in cages of five and were fed rat and mouse cubes and water *ad libitum*.

Preparation of inoculum

Each batch of inoculum was prepared by homogenizing a mixture of 1 g guinea-pig spinal cord, 1 ml 0.9% saline, 1 ml complete Freund's adjuvant (Difco) and 10 mg *Mycobacterium tuberculosis* H37RA (Difco).

Induction of chronic relapsing EAE

Under ketamine/xylazine anaesthesia rats, 7–10 weeks old, were inoculated by the intradermal injection of 0.05 ml inoculum into the

medial footpad of the right hindfoot. Commencing on the day of inoculation the rats were given subcutaneous injections of CsA (Sandoz; 4 mg/kg) on alternate days until 22 days post-inoculation (DPI) inclusive.

Clinical assessment

The rats were examined daily from 7 DPI. Tail, hindlimb and forelimb weakness were each graded on a scale of 0 (no weakness) to 4 (complete paralysis) as previously described [23].

Controls

Three types of control were used. First, six rats were inoculated as above but not given CsA. Second, three rats were given CsA as above but were not inoculated. Third, one rat was inoculated as above except that the inoculum did not contain spinal cord, and it was then treated with CsA according to the above protocol. All controls were female rats 7 – 10 weeks old at the time of inoculation/initiation of CsA treatment.

Histological studies

Under ketamine/xylazine anaesthesia rats were perfused through the left ventricle with 0.9% saline followed by 2.5% glutaraldehyde/2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3 – 7.4). The brain, optic nerves, spinal cord, dorsal and ventral roots, dorsal root ganglia, spinal nerves and sciatic nerves were removed and immersed in fixative. The tissues were postfixed with 2% osmium tetroxide in phosphate buffer and embedded in HistoResin (LKB, Bromma, Sweden) or postfixed with 1% osmium tetroxide (Dalton's solution) and embedded in Epox 812 (Ernest F. Fullam, Schenectady, NY). Some of the brain specimens were embedded in HistoResin without osmication. HistoResin sections (1 μm) were stained with cresyl fast violet [19, 22]. Epox 812 sections (1 μm) were stained with toluidine blue for light microscopy, and ultrathin sections were double-stained with uranyl acetate and lead citrate and examined with an Hitachi-300 or Philips T400 electron microscope.

Results

Clinical findings

The rats inoculated with spinal cord and adjuvants but not given CsA developed neurological signs 11 – 14 DPI and recovered fully by 18 – 20 DPI (Fig. 1 A). Only one of these rats had a second clinical episode, which commenced 19 DPI and had resolved by 23 DPI. No further clinical episodes occurred in this animal. The rats given CsA alone and the rat inoculated with adjuvants (without spinal cord) and given CsA did not develop neurological signs during a period of observation of 26 – 30 days after inoculation/initiation of CsA treatment.

The majority of rats inoculated with spinal cord and adjuvants and given CsA developed tail weakness commencing 11 – 16 DPI. Over the next 2 days the tail usually became completely paralyzed and hindlimb weakness developed. Most of the affected animals recovered from this episode and had minimal or no residual deficit by 18 – 22 DPI. Of those that recovered, 85% had a second episode commencing 19 – 26 DPI. The pattern, severity and temporal profile of the neurological signs in the second episode were similar to those in the first episode. Clinical recovery from the second episode was usually complete 26 – 34 DPI. Of these rats 60% had a third episode commencing 30 – 34 DPI and had recovered completely from this episode 33 – 37 DPI. One rat had a fourth episode commencing 44 DPI and resolving by 50 DPI and a fifth episode commencing 52 DPI and resolving by 57 DPI. The clinical profile of a rat with a typical relapsing course is shown in Fig. 1 B. Some of the rats (25%) recovered incompletely or not at all from the first episode and had a chronic persistent or chronic progressive clinical course, although partial remissions often punctuated the course. Forelimb weakness usually developed in these animals. Neurological signs persisted up to 48 DPI in some of these animals and then resolved.

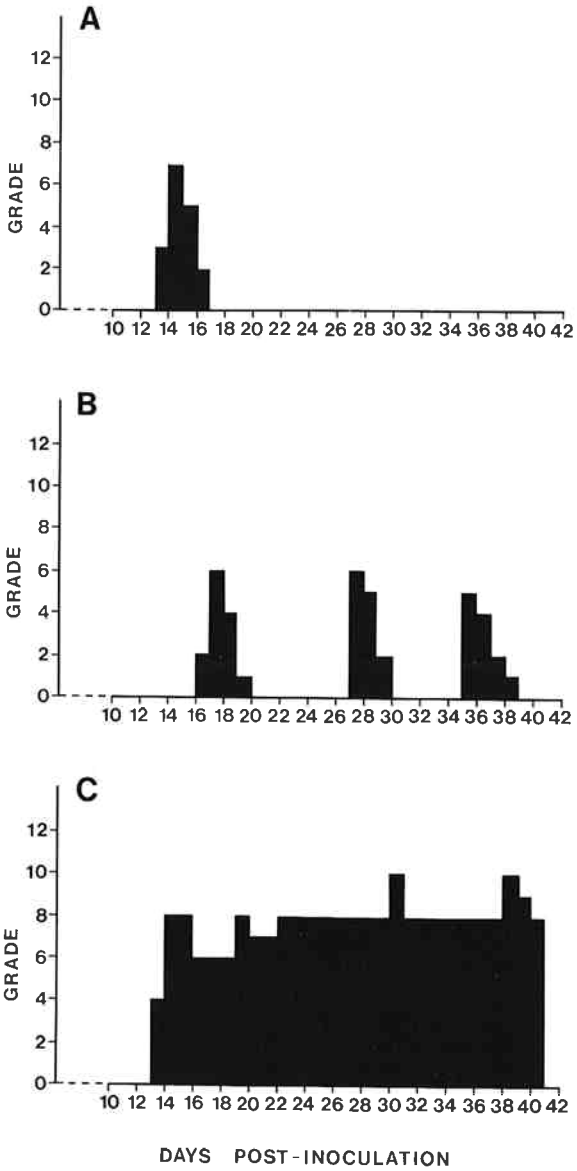

Fig. 1. Clinical profiles of Lewis rats inoculated with spinal cord and adjuvants and untreated (**A**) or treated with low-dose cyclosporin A (CsA) (**B** and **C**). The clinical course is acute and monophasic in (**A**), chronic relapsing in (**B**) and chronic persistent in (**C**)

The temporal profile of a rat with a chronic persistent clinical course that was terminated for histological study 41 DPI is shown in Fig. 1C. Some animals had clinical courses intermediate between the relapsing form illustrated in Fig. 1B and the persistent form shown in Fig. 1C.

Histological findings

Controls

Two rats inoculated with spinal cord but not given CsA were perfused 34 DPI while in late first and second remission, respectively. The findings resembled those in rats during late remission after inoculation with spinal cord and CsA treatment (PNS and CNS remyelination with minimal ongoing inflammation and demyelination). No abnormality was found in the CNS or PNS of one rat inoculated with adjuvants alone and treated with CsA (30 DPI).

First episode (13 and 13 DPI). Inflammation and demyelination were present in the CNS and the PNS.

CNS. In the CNS the spinal cord was the site of predilection. Within the spinal cord there was meningeal, subpial and perivascular infiltration with mononuclear cells. Primary demyelination was present in the regions of subpial and perivascular mononuclear infiltration (Fig. 2a), and was prominent in the dorsal root entry and ventral root exit zones. Myelin debris was present within macrophages (Fig. 2a). Electron microscopy of the spinal cord confirmed the presence of primary demyelination and revealed invasion of myelin sheaths by macrophages (Fig. 2b). The cerebellar white matter and brainstem, particularly the medulla, were also affected. There was minimal cerebral and mild optic nerve involvement.

PNS. In the PNS the spinal roots and dorsal root ganglia were the main sites of involvement. In these regions there was perivascular mononuclear infiltration and primary demyelination (Fig. 2c, d). There were numerous macrophages containing myelin debris. There was minimal involvement of the sciatic and spinal nerves.

First remission (20 DPI). Remyelination and demyelination were present in the CNS and PNS. The distribution of lesions was similar to that in the animals studied during the first episode.

CNS. In the CNS there was remyelination by oligodendrocytes (Fig. 3a); however, many fibres were still demyelinated. Overall the degree of CNS inflammatory cell infiltration was much less severe than during the first episode but there were some areas of prominent inflammation and demyelination (Fig. 3c). Some axonal degeneration was also present in the spinal cord.

PNS. In the PNS there were fibres being remyelinated by Schwann cells (Fig. 3b), fibres that were still demyelinated and mild inflammation.

Second episode (19 and 23 DPI). There was demyelination, remyelination and prominent inflammation in the CNS and PNS.

CNS. In the CNS the spinal cord was the region most involved. There was demyelination and remyelination by oligodendrocytes. The degree of inflammatory cell infiltration was much more severe than during the first remission and similar to that seen during the first episode. Myelin debris was present within macrophages. Oedema and axonal degeneration were present. In some regions, plaques of demyelination were present and there was gliosis (Fig. 4a). There was mild involvement of the brain and minimal involvement of the optic nerves.

PNS. In the PNS the spinal roots and dorsal root ganglia were the main sites of involvement. There was demyelination, remyelination by Schwann cells, moderate inflammatory cell infiltration, oedema and axonal degeneration (Fig. 4b). There was mild involvement of the spinal nerves with demyelination, inflammatory cell infiltration and oedema. There was minimal or no involvement of the sciatic and trigeminal nerves.

Third episode (33 DPI). There was prominent demyelination, remyelination and inflammation in the CNS and prominent remyelination with minimal demyelination and inflammation in the PNS.

CNS. In the CNS the spinal cord was the predominant site of involvement. The dorsal root entry and ventral root exit zones were severely affected, but there was also prominent demyelination in other regions of the spinal cord, sometimes with plaque formation, particularly at the thoracic level. Axonal degeneration was also present. The cerebrum, brainstem and cerebellum were also affected, particularly the cerebellar white matter. These was minimal optic nerve involvement.

PNS. In the PNS there was prominent remyelination in the spinal roots and dorsal root ganglia but there were only occasional demyelinated fibres and slight inflammation. There was minimal involvement of the peripheral nerves themselves.

Chronic persistent EAE (29, 33 and 41 DPI). There was marked CNS demyelination with plaque formation, gliosis, prominent remyelination, moderate to severe inflammation and widespread axonal degeneration. In the PNS there was prominent remyelination and slight inflammation and demyelination.

CNS. The spinal cord was the main site of involvement. All levels of the spinal cord were affected but the thoracic spinal cord was the region showing most extensive disease. Lesions of different ages were clearly present. There were large plaques of demyelination (Fig. 5a, b) with

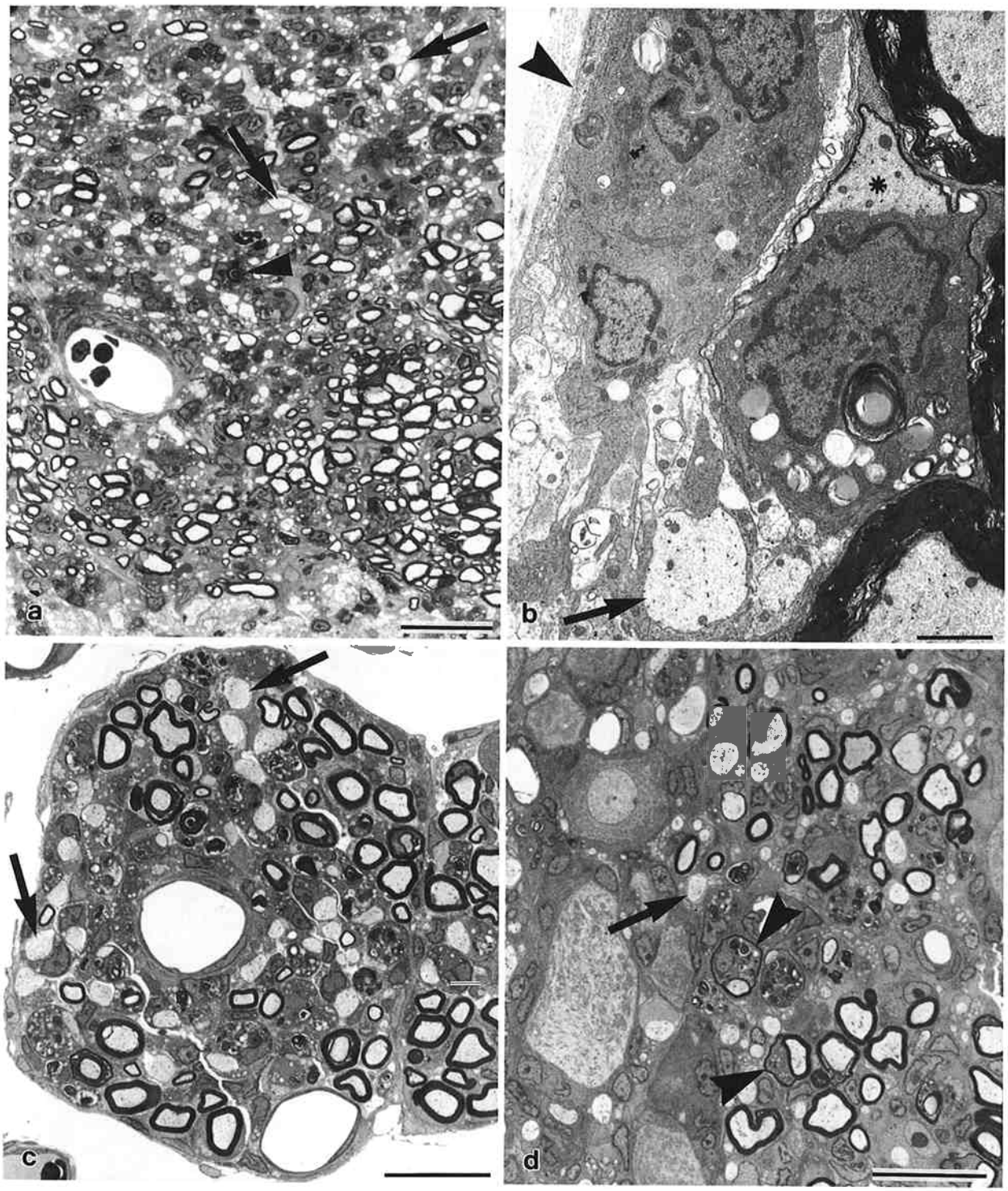

Fig. 2a–d. First episode of experimental allergic encephalomyelitis (EAE) [13 days post-inoculation (DPI)]. These sections and all subsequent ones are from rats inoculated with spinal cord and treated with CsA. **a** Transverse epoxy section through T6 spinal cord showing demyelinated axons (*arrows*), mononuclear infiltration and myelin debris (*arrowhead*). Toluidine blue. **b** Electron micrograph of L5 spinal cord showing a fibre (*asterisk*) being invaded by a macrophage. A completely demyelinated axon (*arrow*) can also be seen. The glia limitans is indicated by an *arrowhead*. **c, d** Epoxy sections through the cauda equina (**c**) and a sacral dorsal root ganglion (**d**) showing demyelinated axons (*arrows*), mononuclear infiltration and myelin debris. Some myelin sheaths are being invaded by macrophages (*arrowheads*). Toluidine blue. *Bars* **a** = 25 μm; **b** = 2 μm; **c, d** = 25 μm

gliosis and prominent remyelination by oligodendrocytes. In some regions the remyelination by oligodendrocytes was sufficiently extensive to form shadow plaques (Fig. 5c). Within the demyelinated lesions there were many macrophages laden with undigested myelin debris (Fig. 5b). Myelin debris was not found within astrocytes or oligodendrocytes although rounded membrane-bound lipid droplets were often found in astrocytes (Fig. 6d).

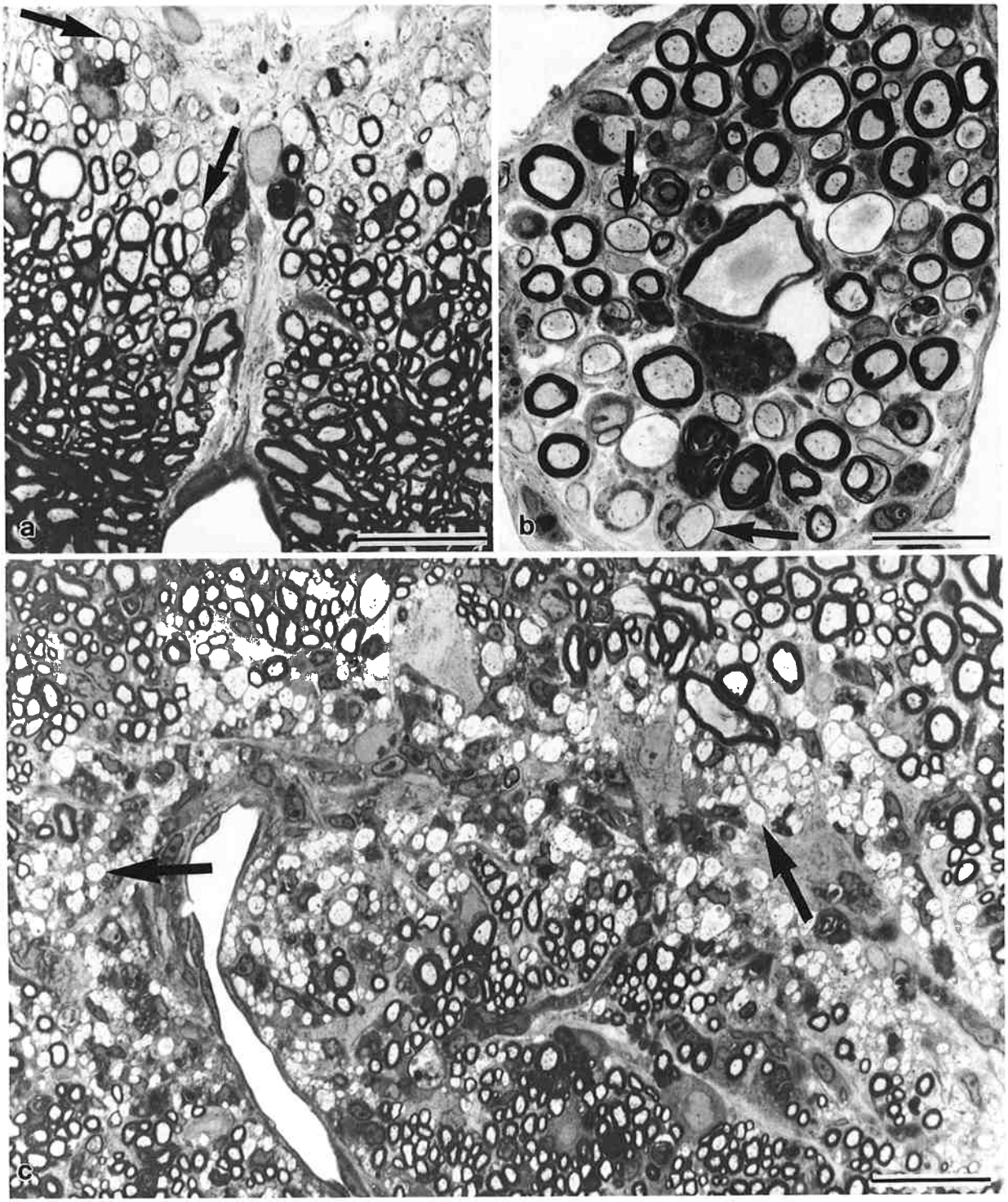

Fig. 3a–c. First remission of EAE (20 DPI). **a** L5 spinal cord showing remyelination (*arrows*); **b** cauda equina showing remyelination (*arrows*); **c** C5 spinal cord showing mononuclear infiltration and demyelination (*arrows*). HistoResin sections stained with cresyl fast violet. *Bars* = 25 µm

Plasma cells were prominent (Fig. 6c, e). There were many ballooned myelin sheaths with massive dilatation of the periaxonal space (Fig. 5d). In some of these fibres the axons were intact; in others they were degenerating. Active demyelination was present within regions of remyelination but it was difficult to determine whether remyelinated fibres were being attacked. There was prominent gliosis and many astrocytes had irregular pleomorphic nuclei. Mitotic figures were present in some glial cells, but the exact nature of these cells was not determined. In some demyelinated gliotic regions there was a marked reduction in the number of axons and there was a paucity of oligodendrocytes. In some regions not affected by demyelination there were many collapsed

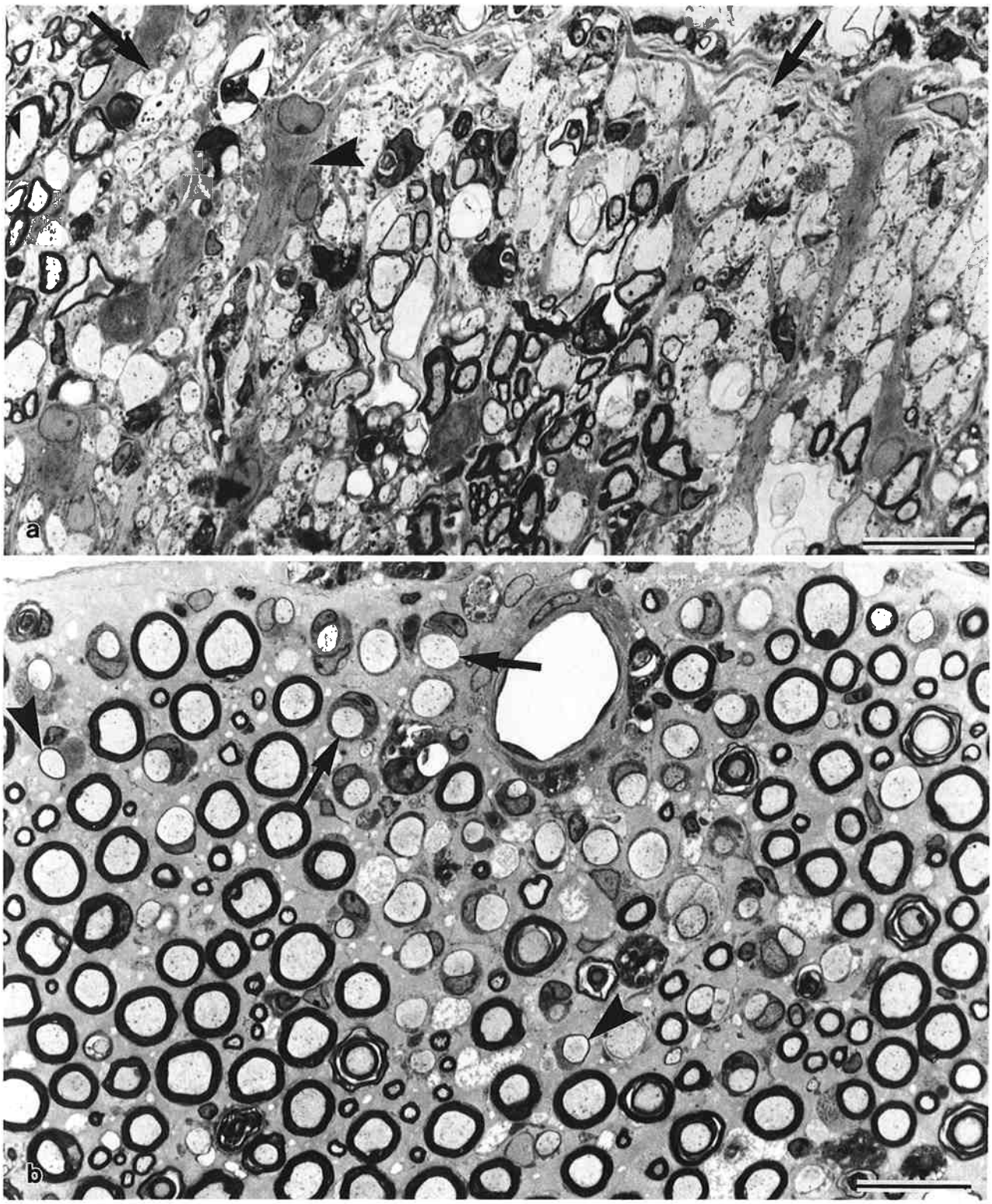

Fig. 4a, b. Second episode of EAE (23 and 19 DPI). **a** T6 spinal cord showing demyelination (*arrows*) and gliosis (*arrowhead*); **b** L5 ventral root showing oedema, demyelination (*arrows*) and remyelination (*arrowheads*). HistoResin sections stained with cresyl fast violet. *Bars* = 25 μm

myelin figures indicating axonal degeneration, particularly in the midline dorsal column region (Fig. 5c). Longitudinal sections confirmed the presence of primary demyelination and also revealed markedly expanded axons laden with organelles and terminating in stumps bounded by astrocytic processes (Fig. 5f). Electron microscopy revealed macrophages invading and destroying myelin sheaths (Fig. 6a) and confirmed the presence of

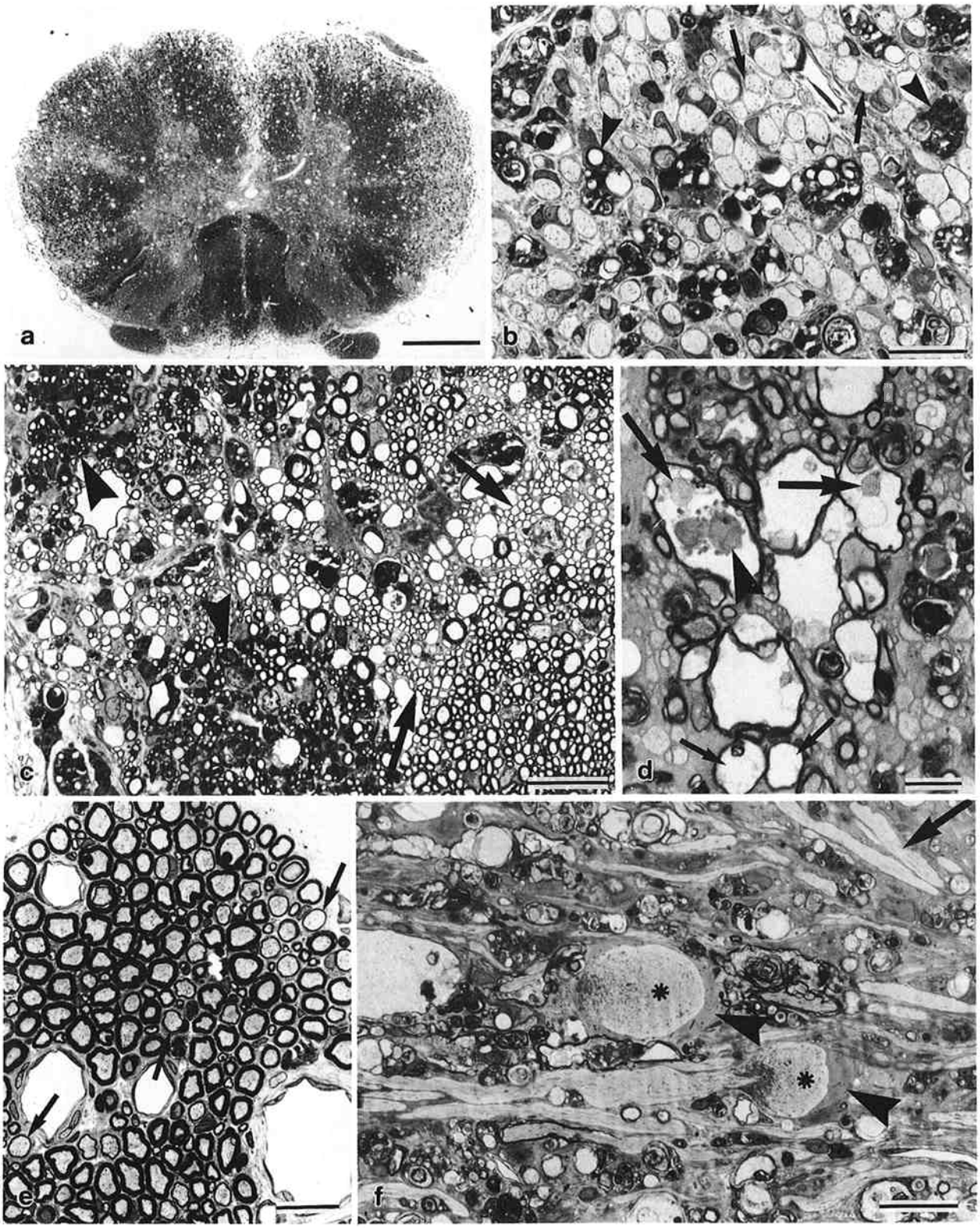

Fig. 5 a – f. Chronic persistent EAE (41 DPI). **a – d** Sections through T6 spinal cord. **a** Extensive myelin loss; **b** higher magnification of **a** showing demyelinated axons (*arrows*) and myelin debris (*arrowheads*); **c** higher magnification of **a** showing remyelination (*arrows*) and axonal degeneration (*arrowheads*); **d** dilatation of myelin sheaths. Axons (*large arrows*) appear preserved in some of these sheaths but absent in others (*small arrows*). Two macrophages can be seen within one sheath (*arrowhead*). **e** Cauda equina showing remyelination (*arrows*); **f** longitudinal section of T7 spinal cord showing demyelinated axons (*arrow*) and expanded axons (*asterisks*) bounded by astrocytic processes (*arrowheads*). HistoResin sections stained with cresyl fast violet (**a – c, e, f**) and epoxy section stained with toluidine blue (**d**). *Bars* **a** = 500 μm; **b, c** = 25 μm; **d** = 10 μm; **e, f** = 25 μm

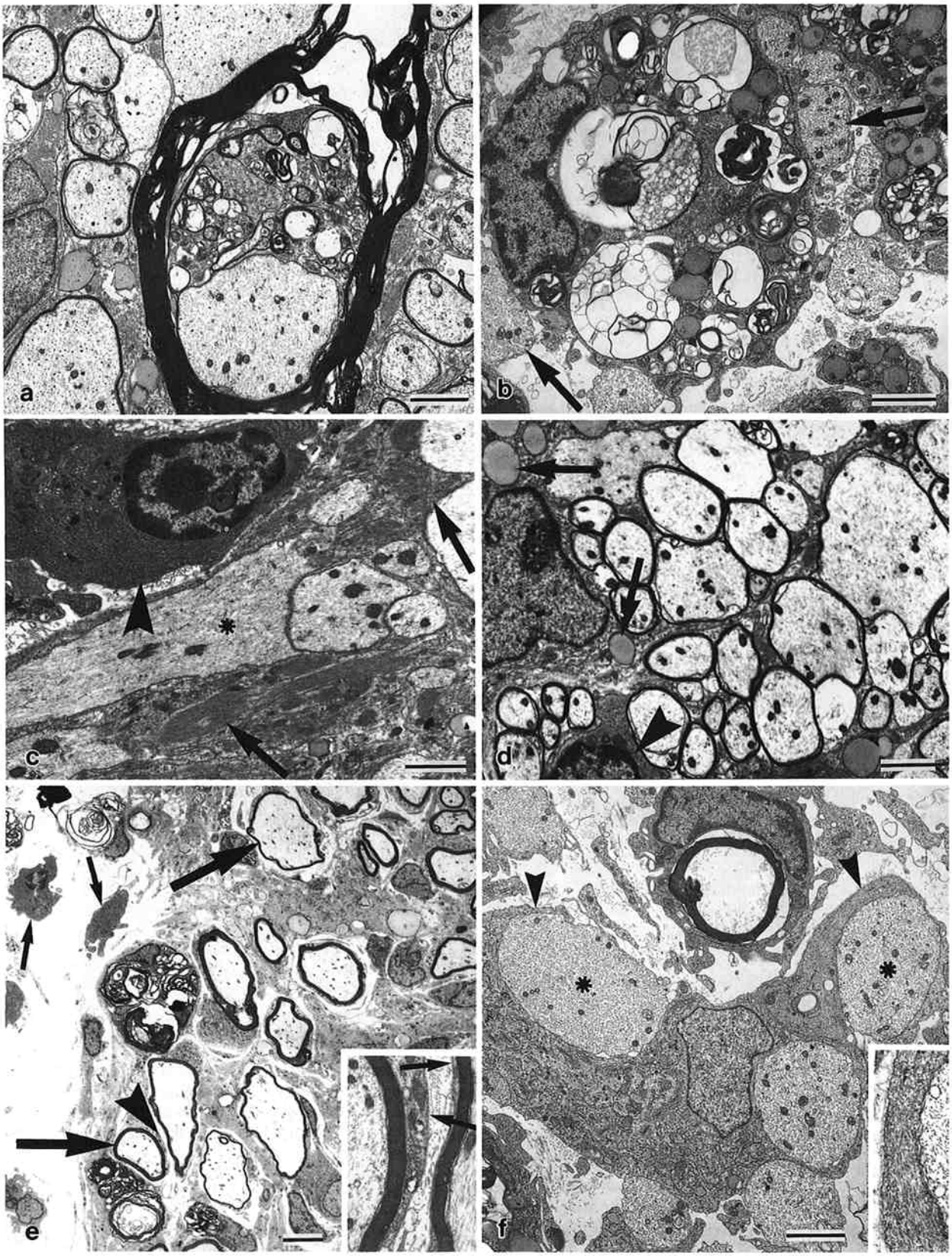

Fig. 6a–f. Chronic persistent EAE (33 and 41 DPI). **a–f** Electron micrographs of T6 spinal cord. **a** Macrophage invading a myelin sheath; **b** primary demyelination (*arrows*) and a macrophage containing myelin debris; **c** primary demyelination (*asterisk*), gliosis (*arrows*) and a plasma cell (*arrowhead*); **d** remyelination by oligodendrocytes. An oligodendrocyte is indicated by the *arrowhead*. Lipid droplets (*arrows*) are present within astrocytes. **e** Schwann cell remyelination (*large arrows*). Plasma cells (*small arrows*) can also be seen. *Inset* shows higher magnification of region indicated by *arrowhead* and reveals Schwann cell basement membranes (*arrows*) and collagen. **f** Demyelinated fibres (*asterisks*) invested by astrocytic processes (*arrowheads*). *Inset* shows higher magnification of region indicated by *right arrowhead* and reveals intermediate filaments. *Bars* **a–d** = 2 μm; **e** = 5 μm; **f** = 2 μm

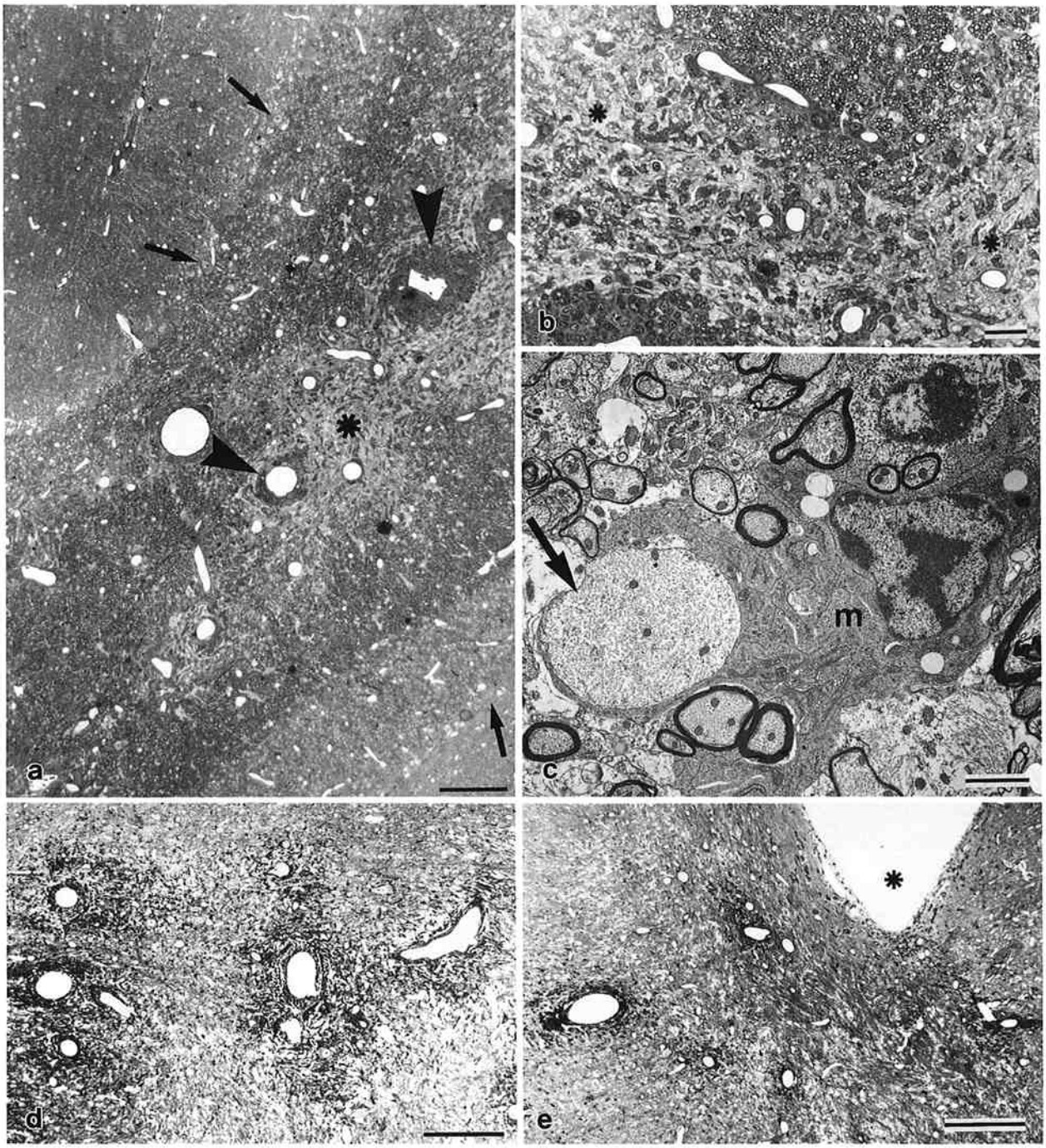

Fig. 7a–e. Chronic persistent EAE (33 and 41 DPI). **a** Epoxy section of cerebellar white matter showing inflammation (*arrowheads*) and demyelination (*asterisk*). The Purkinje cell layers are indicated by *arrows*. **b** Higher magnification of **a** showing area of demyelination (*asterisks*). Toluidine blue. **c** Electron micrograph of same region showing a demyelinated axon (*arrow*) adjacent to a macrophage. (*m*). **d** HistoResin section of pons showing inflammation. Cresyl fast violet. **e** HistoResin section of cerebrum showing inflammation in periventricular region. The lateral ventricle is indicated by an *asterisk*. Cresyl fast violet. *Bars* **a** = 100 μm; **b** = 25 μm; **c** = 2 μm; **d, e** = 200 μm

primary demyelination, remyelination by oligodendrocytes and gliosis (Fig. 6b–d, f). Groups of CNS fibres remyelinated by Schwann cells were occasionally seen near the ventral root exit zones (Fig. 6e). The cerebrum, brainstem and cerebellum were also involved, particularly the cerebellar white matter (Fig. 7a–e). Within the cerebrum the periventricular regions were a site of predilection (Fig. 7e). There was minimal involvement of the optic nerves.

PNS. In the spinal roots and dorsal root ganglia there was prominent remyelination by Schwann cells, slight demyelination and minimal inflammation (Fig. 5e). Occasional degenerating axons were present. There was

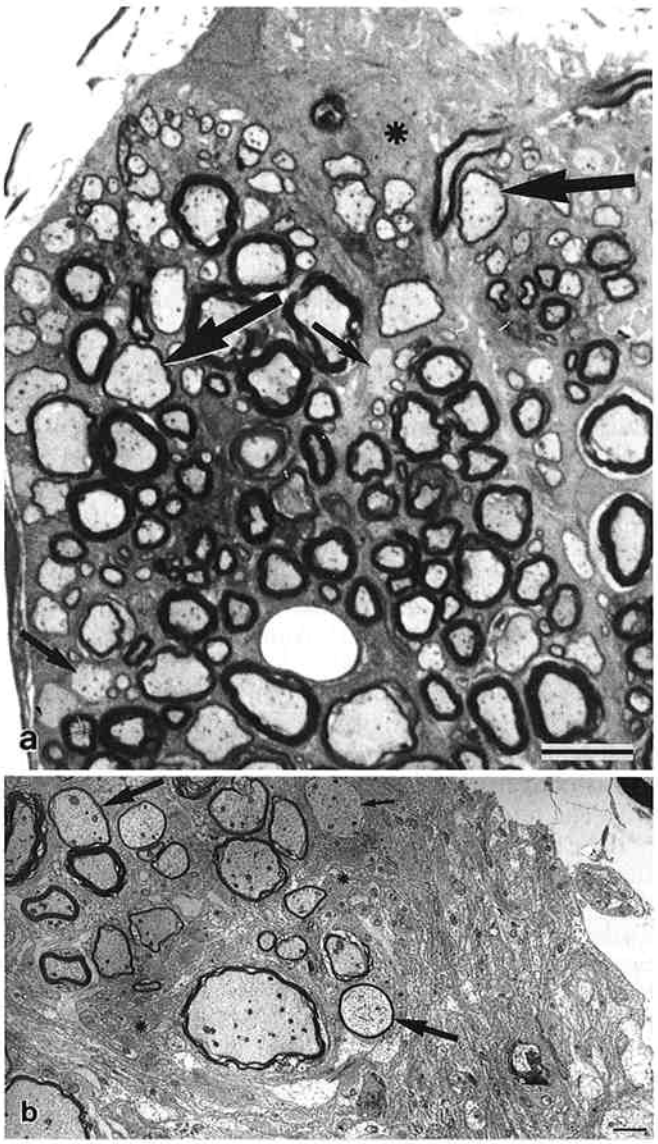

Fig. 8a, b. Late remission (70 DPI). **a** Epoxy section of T6 spinal cord showing remyelination (*large arrows*), persistently demyelinated fibres (*small arrows*) and gliosis (*asterisk*). Toluidine blue. **b** Electron micrograph of same region showing remyelination (*large arrows*), a persistently demyelinated fibre (*small arrow*) and gliosis (*asterisks*). *Bars* **a** = 10 μm; **b** = 2 μm

minimal involvement of the spinal and sciatic nerves except in one animal in which one sciatic nerve fascicle contained many collapsed myelin figures and swollen dark axons associated with apparently occluded blood vessels.

Third and late first and second remissions (40, 52, 61 and 70 DPI). There was remyelination, slight demyelination and inflammation and some gliosis in the CNS and remyelination and minimal demyelination and inflammation in the PNS.

CNS. In the spinal cord the predominant feature of all animals in late remission (> 40 DPI) was remyelination by oligodendrocytes (Fig. 8a, b). The extent of demyelination and degree of inflammatory cell infiltration decreased with time post-inoculation. At 40 DPI there was some demyelination and overall only slight inflammatory cell infiltration, although there were regions of prominent inflammatory cell infiltration associated with demyelination. Some of these regions were adjacent to remyelinated areas. Some gliosis and axonal degeneration were also present. At 70 DPI there was prominent remyelination of the spinal cord by oligodendrocytes. Gliosis was often present in the regions of remyelination (Fig. 8a, b). In the regions of remyelination there were also some demyelinated fibres embedded in an astrocytic matrix (Fig. 8a, b). Collapsed myelin figures indicating axonal degeneration were present. Less frequently there were dilated myelin sheaths containing necrotic material, also indicating axonal degeneration. There were occasional foci of inflammatory cell infiltration but overall there was minimal inflammation. There was minimal inflammation of the cerebrum, brainstem and cerebellum.

PNS. In the spinal roots and dorsal root ganglia, remyelination by Schwann cells was the predominant feature in all animals in late remission. There was minimal inflammation and demyelination. In the spinal and peripheral nerves there were occasional remyelinated fibres and minimal or no inflammation and demyelination.

Discussion

The present study has revealed inflammation, primary demyelination, remyelination, gliosis and axonal degeneration in the CNS, and inflammation, primary demyelination and remyelination in the PNS of Lewis rats with chronic relapsing EAE induced by inoculation with whole spinal cord and treatment with low-dose CsA. In some animals, particularly those with a chronic persistent or chronic progressive clinical course, there were large spinal cord plaques of primary demyelination and gliosis with many of the features of the typical MS demyelinated plaque. In animals with this clinical course large plaques of demyelination and gliosis occurred early (29 DPI) compared to guinea-pigs with chronic relapsing EAE [7, 10]. However, cerebral and optic nerve involvement were mild, in contrast to guinea-pigs with chronic relapsing EAE [9] but similar to the Sprague Dawley rat with chronic EAE [10]. The limited extent of cerebral and optic nerve demyelination may have been due to the relatively short duration of disease activity, as it has been noted that, in guinea-pigs, higher regions of the neuraxis were affected with increasing duration of disease [8]. As in guinea-pigs and Sprague Dawley rats with chronic EAE [9, 10] the thoracic spinal cord was a site of predilection. The mechanism by which low-dose CsA facilitates the development of chronic (relapsing) EAE is unclear, but it is likely that it interferes with immunoregulation as demonstrated in other situations [5, 31]. One possible explanation is that low-dose CsA interferes with the activity of regulatory T cells such as anti-idiotypic T cells,

suppressor T cells or anti-ergotypic T cells which have been reported to have immunoregulatory effects on EAE [2, 13, 16, 34, 36]. We have recently shown that low-dose CsA also leads to the development of chronic relapsing experimental allergic neuritis when given to Lewis rats inoculated with bovine intradural root myelin and adjuvants [17].

Overall the degree of CNS inflammatory cell infiltration and demyelination correlated well with the clinical status of the animal. CNS inflammatory cell infiltration and demyelination were more marked during episodes of clinical signs than during clinical remission. Nevertheless regions of prominent inflammatory cell infiltration and demyelination could still be found in animals during clinical remission up to 40 DPI, indicating ongoing disease activity as previously reported in the first remission of recurrent EAE in the Lewis rat [18]. In contrast to the CNS involvement, PNS inflammation and demyelination were prominent in animals with clinical signs before 25 DPI but were minimal in animals with severe clinical signs at later times. Thus, in the early stages of this disease active autoimmune demyelination occurs in the CNS and PNS but later it is essentially restricted to the CNS. This suggests that, in the later stages of active disease, the targeted myelin antigen is restricted to the CNS and, therefore, not myelin basic protein or galactocerebroside which occur in both the CNS and PNS. Possible candidates for such a myelin antigen restricted to the CNS include myelin proteolipid protein [35] and M2/myelin oligodendrocyte glycoprotein [12, 15]. M2/myelin oligodendrocyte glycoprotein is a necessary antigen for the induction of chronic EAE in the guinea-pig [11] and is a target for antibody-mediated demyelination in vivo [32]. Furthermore, in sera from guinea-pigs with chronic relapsing EAE the anti-myelin oligodendrocyte glycoprotein antibody titre correlates with the in vivo demyelinating activity of the sera, indicating that these antibodies may be involved in the pathogenesis of demyelination in this model [14]. The prominence of plasma cells in the vicinity of large demyelinated plaques in rats with chronic EAE in the present study also suggests that antibodies are contributing to the genesis of large demyelinated lesions. The involvement of the PNS and CNS in the early stages and the restriction to the CNS in the later stages is similar to the situation in MS where PNS involvement is common in acute MS but rare in chronic MS [7].

CNS remyelination by oligodendrocytes and PNS remyelination by Schwann cells were prominent in all animals from the time of the first clinical remission onwards, as recently described during recovery from acute EAE [25, 27]. The findings in the present study are consistent with the hypothesis that the neurological signs in the early stages are due to CNS and PNS demyelination and that clinical recovery is due to repair by oligodendrocytes and Schwann cells, respectively [24, 25]. In the later stages the neurological signs and recovery therefrom are explained by CNS demyelination and repair by oligodendrocytes. Axonal degeneration in the spinal cord is likely to contribute to persisting neurological signs.

Axonal degeneration is a well-known feature of chronic relapsing EAE and MS [7] but the cause is unknown. Axonal degeneration was prominent in the present study. Longitudinal sections revealed expanded demyelinated axons laden with organelles and terminating in stumps bounded by astrocytic processes. Ischaemia (secondary to thrombosis or compression by oedema within a confined space) may have produced some of the axonal degeneration in the present study, for example the groups of degenerated fibres in the midline dorsal column region. However, ischaemia would not account for the many isolated degenerating fibres in other regions of the spinal cord white matter. The latter may have been due to immune attack (specific or non-specific) on the axonal membrane itself. The markedly expanded demyelinated axons laden with organelles resembled those described in the late stages of chronic relapsing EAE in the guinea-pig, where they were attributed to a longstanding disruption of normal axon-glial relationships [29]. However, in the present study these structures occurred too early for such a mechanism to be operative and we suggest that the expanded axons were due to stasis of axoplasmic flow due to loss of axonal continuity, as previously described after axonal transection [6] or ischaemia [20].

Massive dilatation of the myelin sheath was prominent in the spinal cord lesions of rats with a chronic persistent course, as previously described in the thoracic spinal cord of Sprague Dawley rats with chronic EAE [10]. The fate of these fibres is unclear. In at least some of these fibres the axons degenerate, leaving dilated empty sheaths. It remains unclear whether in some fibres the dilated myelin sheath is removed leaving an intact primarily demyelinated axon. Similar massive dilatation of the myelin sheath as well as oligodendrocyte destruction have been produced in vitro by the addition of tumor necrosis factor [33].

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