Demyelination and Nerve Conduction Abnormalities in Acute and Chronic Experimental Allergic Encephalomyelitis in the Lewis Rat

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Abstract
We have been using histological and electrophysiological techniques to compare the pathology and pathophysiology in different forms of experimental allergic encephalomyelitis (EAE) in the Lewis rat. In acute EAE induced by sensitization to myelin basic protein (MBP) or by the passive transfer of MBP-specific lymphocytes, there is inflammation and demyelination in the ventral and dorsal spinal roots and inflammation and limited demyelination in the spinal cord. More extensive spinal cord demyelination as well as demyelination in the spinal roots and ganglia is observed in acute EAE induced by inoculation with whole central nervous system (CNS) tissue. In chronic relapsing EAE induced by inoculation with whole CNS tissue and treatment with low dose cyclosporin A, large plaques of spinal cord demyelination occur in rats with clinical episodes >28 days after inoculation. In rats with clinical disease <25 days after inoculation there is prominent demyelination in the spinal roots and ganglia as well as in the spinal cord. In all 4 forms of EAE, peripheral nervous system (PNS) remyelination by Schwann cells and CNS remyelination by oligodendrocytes occurs during clinical recovery. Electrophysiological studies revealed PNS and CNS nerve conduction abnormalities during clinical episodes, with improvement during remissions.

Keywords
demyelination; experimental allergic encephalomyelitis; nerve conduction; remyelination

Introduction
Experimental allergic encephalomyelitis (EAE) is an autoimmune T-cell-mediated demyelinating disease. It can be induced by active sensitization to whole central nervous system (CNS) tissue, purified myelin basic protein (MBP) or myelin proteolipid protein (PLP), or by the passive transfer of MBP-specific or PLP-specific lymphocytes. It may have either an acute or a chronic relapsing course. Acute EAE is a mono phasic disease and closely resembles the human demyelinating disease, acute disseminated encephalomyelitis; chronic relapsing EAE has many similarities with the human disease, multiple sclerosis.

In this study we have used histological and electrophysiological techniques to compare the pathology and pathophysiology of different forms of EAE in the Lewis rat.

Materials and Methods

Animals
Lewis rats (JC strain) were kept in cages of five and were fed rat and mouse cubes and water ad libitum.

Induction of EAE
Four different forms of EAE were induced.

Myelin basic protein (MBP)-induced acute EAE (actively induced acute MBP-EAE)
MBP was prepared from guinea pig spinal cord (after removal of the spinal roots) by the method of Deibler et al [1]. MBP in 0.9% saline was emulsified in an equal volume of incomplete Freund's adjuvant containing 4mg/ml of added Mycobacterium butyricum. Male rats, 8-10 weeks old, were inoculated with 0.1 ml of emulsion in one footpad of each hindfoot. The total dose of MBP was 50 µg/rat.
Passively transferred acute MBP-EAE
Passive EAE was induced as previously described [14]. 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 x 10^6 cells/ml in RPMI 1640 with added 5% fetal calf serum, 5 x 10^-3 M 2-mercapto-ethanol, 200mM L-glutamine, penicillin and streptomycin. Concanavalin A was added at 2µg/ml, and 50ml cultures were incubated at 37°C in an atmosphere of CO_2 (10%), O_2 (7%) and N_2. Cells were harvested after 72h and washed with Hanks' balanced salt solution. 5 x 10^7 viable cells were injected into a lateral tail vein of each recipient male rat.

Whole-spinal-cord-induced acute EAE
The inoculum was an homogenate of equal volumes of a 30% suspension of guinea pig spinal cord (the spinal roots having been removed) in 0.9% saline and a suspension of 4mg of killed and dried Mycobacterium butyricum (Difco) per ml of incomplete Freund's adjuvant (Commonwealth Serum Laboratories, Melbourne, Australia). Under anaesthesia, male rats 8-10 weeks old were given 0.05ml of inoculum in the footpad of each of the four feet or 0.1 ml in one footpad of each hindfoot.

Chronic relapsing EAE
Chronic relapsing EAE was induced as previously described [15]. Each batch of inoculum was prepared by homogenizing a mixture of 1g guinea pig spinal cord, 1 ml 0.9% saline, 1 ml complete Freund's adjuvant (Difco) and 10mg Mycobacterium tuberculosis H37RA (Difco). Female rats, 7-10 weeks old, were inoculated by the intradermal injection of 0.05ml inoculum into the medial footpad of the right hindfoot. Commencing on the day of inoculation, the rats were given subcutaneous injections of cyclosporin A (Sandoz; 4mg/kg) on alternate days until 22 days post-inoculation inclusive.

Histological studies
Under anaesthesia rats were perfused through the left ventricle with 0.9% saline followed by 2.5% glutaraldehyde/2% formaldehyde in 0.1M sodium cacodylate buffer (pH 7.3-7.4). After removal, the spinal cord was immersed in fixative and postfixed with 1% osmium tetroxide (Dalton's solution), embedded in HistoResin (LKB, Bromma, Sweden) or Epox 812 (Ernest F Fullam, Schenectady, NY), sectioned (1µm) and stained with cresyl fast violet or toluidine blue respectively. Ultrathin Epox 812 sections were double-stained with uranyl acetate and lead citrate and examined with an Hitachi H-300 electron microscope.

Electrophysiological studies
These methods have been described in detail previously [7,9,13,17]. Under anaesthesia, the left sciatic nerve was exposed in the posterior thigh and a lumbosacral laminectomy was performed. In some animals a right craniectomy was also performed. Nerve conduction in the peripheral nervous system (PNS) and in the central nervous system (CNS) was studied by direct stimulation of, and recording from, the exposed nervous tissues.

Results
Actively induced acute MBP-EAE
Distal tail weakness commenced 8-13 days after inoculation and was followed by flaccid tail paralysis, hindlimb weakness and sometimes hindlimb paralysis. Clinical recovery occurred rapidly and by 18 days after inoculation there was only mild tail weakness. Histological studies revealed inflammation and limited demyelination in the spinal cord [10]. The CNS parts of the dorsal root entry and ventral root exit zones were sites of predilection for demyelination. In the PNS there was perivascular mononuclear infiltration and demyelination in the lumbar, sacral and coccygeal ventral and dorsal roots [10,11]. The dorsal root ganglia were mildly affected and the spinal and peripheral nerves showed no or minimal involvement. Electrophysiological studies revealed conduction block in the dorsal roots [7], reduced conduction velocities between the lumbar ventral roots and sciatic nerve, conduction block in a small proportion of fibres at the lumbar ventral root exit zones of the spinal cord and a
markedly reduced H reflex [10]. These nerve conduction abnormalities could be explained by
demyelination-induced conduction block in the PNS and CNS. During clinical recovery there
was restoration of the H reflex and ensheathment and remyelination of the PNS by Schwann
cells and of the CNS by oligodendrocytes[12].

**Passively transferred acute MBP-EAE**
The clinical course was similar to that in rats with actively induced acute MBP-EAE except
that the neurological signs commenced and resolved 6 days earlier. The neuropathological
findings were similar to those in rats with actively induced acute MBP-EAE. There was
inflammation and demyelination in the dorsal and ventral spinal roots and inflammation and
limited demyelination in the spinal cord [14]. Demyelination in the spinal cord was
concentrated in the dorsal root entry and ventral root exit zones. During clinical recovery
there was ensheathment and remyelination of the PNS by Schwann cells and of the CNS by
oligodendrocytes [14]. Formation of new compact myelin lamellae by Schwann cells was first
observed 9 days after passive transfer (5 days after the onset of tail weakness) and by
oligodendrocytes 10 days after passive transfer.

**Whole-spinal-cord-induced acute EAE**
The clinical course was similar to that in rats with actively induced acute MBP-EAE except
that the neurological signs lasted longer. Histological studies showed more demyelination in
the spinal cord than in rats with acute MBP-EAE [13]. The spinal cord demyelination was
more extensive caudally than rostrally, and the CNS parts of the dorsal root entry and ventral
root exit zones were sites of predilection. In the PNS there was inflammation and
demyelination in the dorsal and ventral roots and particularly in the sacro-coccygeal dorsal
root ganglia. There was minimal if any involvement of the spinal nerves and peripheral
nerves. Electro-physiological studies revealed nerve conduction abnormalities in the regions
of the dorsal root ganglia [13], conduction block at the lumbar ventral root exit zones of the
spinal cord and reduction of the H reflex [6,9] During clinical recovery there was evidence of
restoration of nerve conduction and remyelination of the PNS by Schwann cells and of the
CNS by oligodendrocytes [12].

**Chronic relapsing EAE**
Rats inoculated with whole spinal cord tissue and adjuvants and treated with low dose
cyclosporin A had a chronic relapsing or chronic progressive clinical course. Histological
studies during the early stages of clinically active disease (<25 days after inoculation)
revealed inflammation and primary demyelination in the CNS, particularly the spinal cord,
and in the PNS, specifically the dorsal and ventral roots and dorsal root ganglia [15]. 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 studies later than 18 days after inoculation (the time of the first remission, if it
occurred). Axonal degeneration was observed in the spinal cord and in the PNS. During
clinical remission there was CNS and PNS remyelination and much less inflammation.
Electrophysiological studies revealed conduction failure in both the CNS and PNS during the
early and later stages of chronic relapsing EAE [17] Conduction was restored in some CNS
and PNS fibres during remission but conduction abnormalities persisted in animals that were
in late remission and that had no neurological signs. The reversible conduction abnormalities
were explained by demyelination followed by remyelination in the CNS and PNS. The
persistent conduction failure in late remission was mainly due to axonal degeneration.
Table
Extent of Demyelination in Different Forms of EAE

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<thead>
<tr>
<th>Demyelination</th>
<th>PNS</th>
<th>CNS</th>
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<tr>
<td>Active MBP-induced acute EAE</td>
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<td>Passive MBP-induced acute EAE</td>
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<td>Whole-spinal-cord-induced acute EAE</td>
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<td>Chronic relapsing EAE – early</td>
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<td>Chronic relapsing EAE – late</td>
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Discussion

The present studies demonstrate that demyelination is present in the CNS and proximal PNS (spinal roots and ganglia) in 4 different forms of EAE in the Lewis rat. The extent of demyelination varies with the form of EAE and the stage of the disease process (Table). In acute MBP-EAE, either actively induced or passively transferred, there is limited CNS demyelination. However, this CNS demyelination together with the PNS demyelination in the ventral and dorsal roots is likely to be an important cause of neurological signs in acute MBP-EAE, as in the other types of EAE studied [8]. More extensive CNS demyelination occurs in [2] acute EAE induced by inoculation with whole CNS tissue than in acute MBP-EAE indicating that sensitization to additional myelin antigens is necessary to produce extensive CNS demyelination in Lewis rats. In the guinea pig the addition of galactocerebroside or total myelin lipids to MBP in the inoculum augments CNS demyelination [16] Furthermore, the administration of an antibody against myelin oligodendrocyte glycoprotein augments CNS demyelination in Lewis rats with passively transferred acute MBP-EAE [5]. In the early stages of chronic relapsing EAE active demyelination occurs in the CNS and PNS but later it is essentially restricted to the CNS and occurs in large plaques. This suggests that, in the later stages of active disease, the targeted myelin antigen is restricted to the CNS and therefore not MBP. Possible candidates for such a myelin antigen restricted to the CNS include PLP [18] and M2/myelin oligodendrocyte glycoprotein [3,4] M2 is a necessary antigen for the induction of chronic EAE in the guinea pig [2]. In all 4 forms of EAE in the Lewis rat, remyelination of the CNS by oligodendrocytes and of the PNS by Schwann cells occurs at the time of clinical recovery and is likely to be a major mechanism of this recovery. However, axonal degeneration occurs in EAE and is an important cause of residual neurological deficits.

References


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