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Demyelination in chronic relapsing experimental allergic encephalomyelitis induced in the Lewis rats

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Chronic relapsing experimental allergic encephalomyelitis (CREAE) was induced in Lewis rats by inoculation with guinea-pig myelin and complete Freund's adjuvant followed by treatment with low dose cyclosporin A. Histological, histochemical and electronmicroscopic studies of the lesions during the first and the second clinical episodes, as well as during the first and the second remissions, revealed inflammation, prominent demyelination, remyelination and gliosis in the central nervous system (CNS). This model of CREAE in the Lewis rats may be a useful model of demyelinating diseases because of the marked demyelination in the CNS.

Key words: chronic relapsing experimental allergic encephalomyelitis, demyelination, Lewis rats.

Experimental allergic encephalomyelitis (EAE) is an autoimmune demyelinating disease of the central nervous system (CNS). It can be induced in genetically susceptible animals by immunization with nervous tissue homogenate or encephalitogenic compounds, together with a potent adjuvant. Clinical and pathological changes in EAE resembles those in the human demyelinating disease multiple sclerosis (MS), for which EAE is proposed to be a model [1, 6, 11]. EAE may have an acute or chronic relapsing form. The clinical form and amount of demyelination differ among species and antigen used. In particular the chronic relapsing form of EAE (CREAE), characterized clinically by periods of exacerbation and remission, more closely resembles MS. The main pathological features of EAE are inflammation and demyelination. In general, only minor loss of myelin sheaths is noted in the CNS of animals with acute EAE, whereas demyelination is much more pronounced in CREAE. It has been shown that in CREAE the conduction failure was mainly due to a demyelination-induced conduction block [15]. Since the extent of demyelination vary from model to model of CREAE, it is of great importance the destruction of the myelin sheaths to be analysed in every model.

The Lewis rat is highly susceptible to acute EAE [5, 7, 8], but it has been more difficult to induce CREAE in this animal. P o l m a n et al. [9] have succeeded to

induce CREAE in the Lewis rats with guinea-pig spinal cord and treatment with low-dose cyclosporin A (CsA).

In the present study CREAE was induced in the Lewis rats by inoculation with highly purified guinea-pig myelin, together with complete Freund's adjuvant and treatment with low-dose CsA. The neuropathology, and more specifically the demyelination, in this model of CREAE was investigated.

Material and Methods

Chronic relapsing experimental allergic encephalomyelitis was induced in Lewis rats (JC strains). Each batch of inoculum was prepared by homogenizing a mixture of 1 mg guinea-pig myelin, 0,75 ml complete Freund's adjuvant (Difco) and 100 mg Mycobacterium tuberculosis H37R (Difco). Rats, 7-12 - week-old, were injected intradermally with 0.1 ml inoculum into the two rear foot pads. Commencing on the day of inoculation the rats were given subcutaneous injection of CsA (Sandoz; 4mg/kg) on alternate days until 22 days post-inoculation (DPI) inclusive [9]. Control rats were inoculated and treated as above except that the inoculum did not contain guinea-pig myelin. The animals were weighed and examined daily from the seventh DPI for clinical symptoms of EAE which included evidence of weakness, loss of tail tonicity, hindlimb paraparesis and paralysis, incontinence, quadriparesis, quadriparalysis.

Light- and electronmicroscopic investigations were carried out on brains and spinal cords of the experimental animals during the first and the second clinical episodes (6 rats) and during the first and the second remissions (6 rats), as well as of control rats (3 animals). Routine histological and histochemical methods were applied: staining with hematoxyline and eosine and Baker's histochemical reaction for phospholipids. For electronmicroscopic studies the rats were perfused with 2.5% glutaraldehyde/ 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3 — 7.4). The brain, optic nerve and the spinal cord (cervical, thoracic and lumbar) were removed, immersed in fixative, postfixed with 2% osmium tetraoxide and embedded in Epon. Ultrathin sections were stained with uranylacetate and lead citrate and examined with an Opton 109 electron microscope.

Results

Clinical findings

The majority of rats inoculated with myelin and complete Freund's adjuvant and given CsA developed neurological signs commencing 11-16 DPI. Most of the affected animals recovered fully by 18-27 DPI. Of those that recovered 90% had a second episode by 25-30 DPI. The neurological signs in the second episode were similar to those in the first episode. Usually the clinical recovery from the second episode was complete by 29-36 DPI.

Neuropathological findings

During the first clinical episode of CREAE inflammation and demyelination were present in the spinal cord, cerebellar white matter and brain stem, particularly in the medulla. Electronmicroscopic examination showed primary demyelination and invasion of myelin sheaths by macrophages (Fig. 1). Most frequently their processes

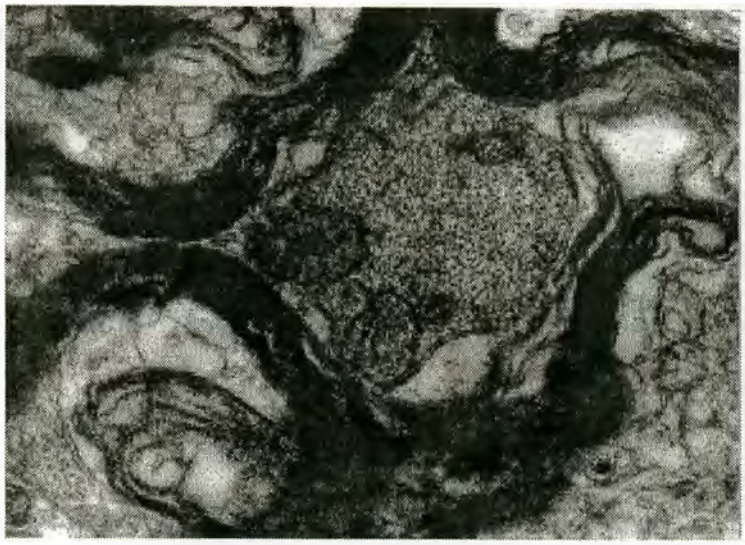

Fig. 1. First episode of CREAE. Lumbar spinal cord. Primary demyelination and invasion of myelin sheaths by a macrophage process (× 27 000)

invade between the myelin lamellae (myelin stripping). Finally the axon is left completely demyelinated.

During the first remission remyelination and demyelination are clearly present in the CNS (Fig. 2). During the second clinical episode there was prominent demyelination, remyelination, inflammation and gliosis. Massive dilatation of the myelin sheath and axonal degeneration were present in the spinal cord lesions (Fig. 3). Electron microscopy showed during the second recovery marked demyelination and many fibres in the stage of remyelination.

Discussion

The present study has revealed a marked demyelination in the CNS of Lewis rats with chronic relapsing EAE induced by inoculation with purified guinea-pig myelin and treatment with low-dose CsA. Furthermore, active demyelination could still be found in animals during the first and second remission, indicating ongoing disease activity. In our model of CREAE the Lewis rats were inoculated with highly purified guinea-pig myelin instead of guinea-pig spinal cord, used by P o l m a n et al. [9]. Lewis rats with acute EAE do not usually show as large demyelinative lesions as are seen in other species. Nevertheless demyelinated areas were observed frequently in myelin-immunized animals [13]. The mechanism by which low-dose CsA facilitates the development of CREAE is unclear, but it is likely that it interferes with immunoregulation as demonstrated in ohter cases [2, 14]. In the present study macrophages have been observed to invade and destroy myelin sheaths, as it has been previously described in EAE and MS [3, 10, 11]. Nevertheless whether macrophages directly causes demyelination by attacking myelin by instruction from T helper cells or they are merely subserving their general function as scavenger cells remains to be established. In our

Fig. 2. First remission of CREAE (43 DPI). Lumbar spinal cord. Note completely demyelinated axons (small arrows) and ongoing remyelination of an axon (large arrow) (× 33 000)

Fig. 3. First remission of CREAE (43 DPI). Lumbar spinal cord. Massive dilatation of the myelin sheath and axonal degeneration (× 70 000)

model of CREAE massive dilatation of the myelin sheath with axonal degeneration was present in the spinal cord. Such dilatation has been described in the spinal cord of Sprague Dawly rats with chronic EAE [4].

In conclusion, our study demonstrates that Lewis rats inoculated with myelin and treated with low-dose CsA develop CREAE with prominent demyelination. This model of CREAE may be a valuable animal model for studying the pathogenesis of demyelinating disorders.

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