Lymphocyte–myelin sheath interactions in acute experimental allergic encephalomyelitis

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Summary

Using a passively transferred acute model of experimental allergic encephalomyelitis (EAE) in the rat, inflammatory central nervous system (CNS) lesions were shown to develop rapidly, peak and then resolve. An unusual feature of the lesions in the CNS was the presence of pyknotic cells within myelin sheaths. A sequence of observations indicated that such cells were lymphocytes which had insinuated themselves into the myelin sheath by passage along the interperiod line. The presence of lymphocytes within myelin sheaths, a process which did not lead to demyelination, was considered to represent a change which reflects the specificity of the immune response in this disease. The detection of this change in other CNS autoimmune diseases, notably those associated with virus infections, may be important as an indicator of pathogenetically relevant lymphocyte–myelin interactions.

Introduction

Acute experimental allergic encephalomyelitis (EAE) in the rat, when induced by purified myelin basic protein (MBP) or MBP-specific CD4^{+} T cells, is a disease characterized by leukocyte in-

filtration and edema (Raine, 1984; Kerlero de Rosbo et al., 1985; Vandenbark et al., 1985; Sedgwick et al., 1986; Simmons et al., 1987). Although there may be demyelination in the spinal roots (Pender, 1987), there is little if any demyelination of central axons (Kerlero de Rosbo et al., 1985; Vandenbark et al., 1985; Pender, 1987). Clinical signs appear to correlate best with the extent of edematous changes in the spinal cord suggesting that effector T cell–central nervous system (CNS) vasculature interactions may be particularly important; however, in some species peripheral nervous system (PNS) demyelination may also contribute to the clinical disease (Pender, 1988). The number of ultrastructural studies carried out on this variant of EAE has been limited

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and we have been unable to locate documentation of specific interactions between lymphocytes and myelin sheaths, the latter being the site of the highest concentration of the antigen (MBP) for which some encephalitogenic effector T cells are specific in this disease model.

In this study we examined the lesions of passively transferred acute EAE in the Lewis rat for unusual lymphocyte–myelin sheath interactions which might reflect the specificity of the immune response of this disease. The possible relevance of our observations to the pathogenesis of other CNS autoimmune diseases, notably those associated with viral infections, is briefly discussed.

Materials and methods

Animals

Specific pathogen-free inbred male Lewis (RT1′) rats between 8 and 12 weeks of age were used in all experiments. Rats were bred at the MRC Cellular Immunology Unit, Oxford, U.K.

Passively transferred EAE

For all experiments, EAE was produced by the transfer, to either irradiated (see below) or non-irradiated recipient rats, of MBP-reactive splenocytes as described elsewhere (Richert et al., 1979; Sedgwick and Mason, 1986). Briefly, donor cells were obtained from the spleens of rats immunized subcutaneously 12 days previously with 50 μg MBP emulsified in Freund's complete adjuvent. Splenocytes were stimulated in vitro with 2–5 μg/ml MBP for 3 days before the intravenous injection of 4–6×10^7 viable leukocytes to each recipient animal.

Irradiation

Rats received 600 rad on two occasions, 3 days before cell transfer and again 4 h before receiving cultured cells (^{137}Cs, 88 rad/min; Gamma cell 40). This dose induced considerable leukopenia (peripheral blood leukocyte equal to 3–5% of untreated rats) when measured 3 days after treatment.

Histological examination of recipient rats

Animals were selected for histological examination from 3 to 10 days post-injection of effector cells which clinically represented early disease through to complete recovery. Animals were killed at 3 days (six rats), 4 days (three rats), 5 days (five rats), 7 days (two rats), and 10 days (three rats). Five irradiated recipient animals were killed on days 3 or 4 and two control animals (receiving no cells) were also killed for examination. Animals were anesthetized and perfused via the descending aorta with 4% phosphate-buffered glutaraldehyde. The spinal cord was removed and 1 mm coronal slices cut from the cervical, thoracic and lumbar spinal cord and spinal nerve roots from the lumbar region were post-fixed in osmium tetroxide, dehydrated with ethanol and embedded in resin. 1 μm sections were cut and stained with toluidine blue for light microscopy and selected areas prepared for electron microscopic examination.

Results

The clinical course of the disease followed the pattern typical for this model of EAE in the rat (Sedgwick et al., 1987), namely flaccid tail at 3–4 days, hind limb weakness at 4 days, hind limb paralysis by 5 days, restoration of hind limb function by 7 days, and full clinical recovery by 10 days post-injection of cells. The pathological changes which accompanied these signs were leukocyte infiltration and edema of the white and gray matter of the spinal cord, leptomeninges and the spinal roots. These changes reached their max-

Fig. 1. *a–c*: Examples of pyknotic lymphocytes adjacent to myelin sheaths in the white matter of the spinal cord in animals showing clinical signs of acute EAE. Plastic sections stained with toluidine blue. ×1500.

Fig. 2. A pyknotic cell surrounded by a small number of myelin lamellae from the myelin sheath which surrounds axon a. Oligodendrocyte loops can be seen at the point indicated by the arrow. ×12000.

Fig. 3. A lymphocyte in a position similar to the pyknotic cell illustrated in Fig. 2. Arrows indicate the myelin lamellae which surround the cell and are derived from the sheath which surrounds axon a. ×14000.
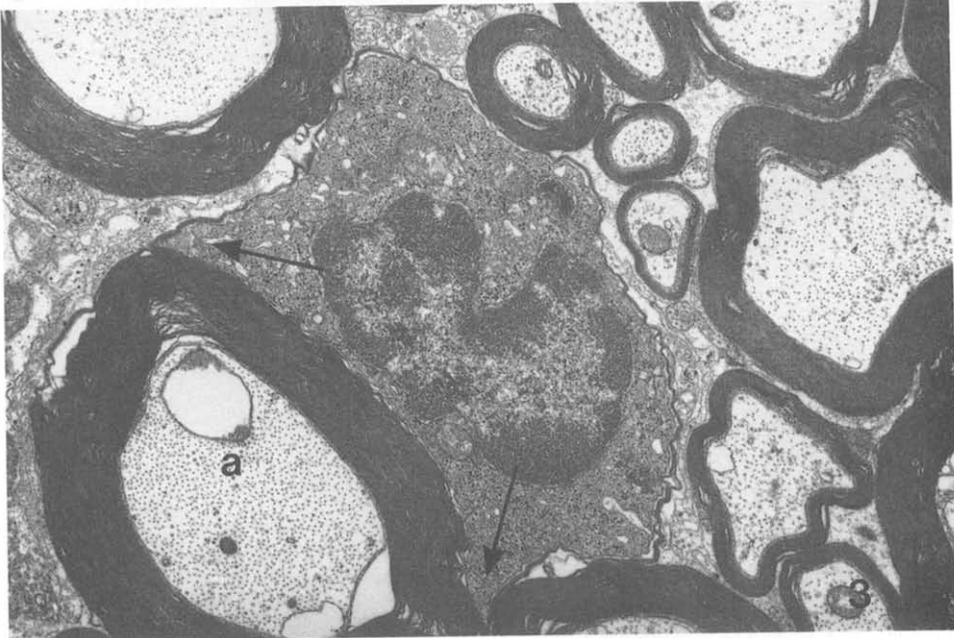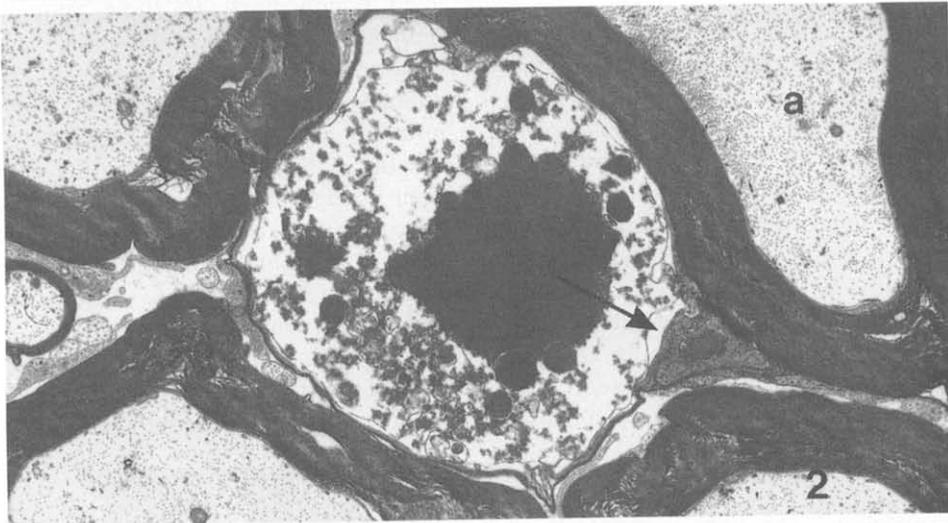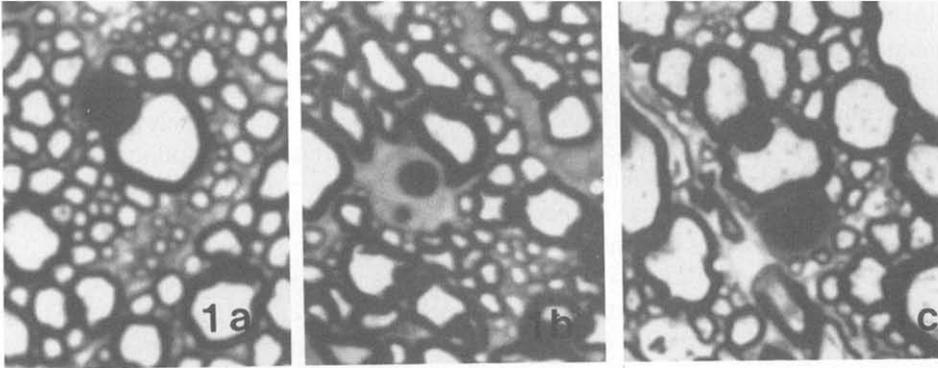
imal severity at 5 days post-injection. In clinically recovered animals there was little cellular infiltration of the spinal cord or meninges to be found by 7 days post-injection and by 10 days the spinal cord appeared normal except for sporadic lymphocytes and a generalized hypertrophy of astrocytes. The only evidence of central demyelination found in these animals was an occasional demyelinated axon in the white matter beneath the pial surface. In addition to cellular infiltration and edema of the spinal nerve roots there was also some demyelination of axons in these regions.

A feature of all animals killed while they were showing clinical signs was the presence of pyknotic cells within the spinal cord. Many of these cells seen in plastic sections to be next to myelin sheaths (Fig. 1) were found by electron microscopy to be surrounded by a small number of myelin lamellae (Fig. 2). In the animals killed between 3 and 5 days post-injection, lymphocytes were seen in similar locations to the pyknotic cells (Fig. 3). The cytoplasm of many of these lymphocytes contained electron-dense granular bodies. In animals killed at the time of maximal cell infiltration and edema (4–5 days) the following changes were also seen: lymphocytes processes directed towards single or multiple myelin sheaths, outer oligodendrocyte tongues separated from their myelin sheath by lymphocyte cytoplasm (Fig. 4), lymphocytes incompletely surrounded by a single myelin lamella, lymphocytes completely surrounded by a single myelin lamella and lymphocytes surrounded by several myelin lamellae (Fig. 3).

The lymphocyte–myelin sheath interactions were also seen in the spinal cords of irradiated animals which received injections of MBP-stimulated lymphocytes. In these animals, the total number of leukocytes in the spinal cord was greatly reduced compared to non-irradiated recipient animals although there appeared to be comparable numbers of lymphocytes associated with myelin sheaths. This is consistent with earlier observations (Sedgwick et al., 1987) that provided evidence that the relatively few cells present in the CNS of irradiated recipient animals were MBP-specific effector cells and suggests, furthermore, that in non-irradiated animals also, it is the MBP-specific effector cells, rather than non-specifically recruited cells, that are in association with myelin sheaths. It is noteworthy that the intramyelinic
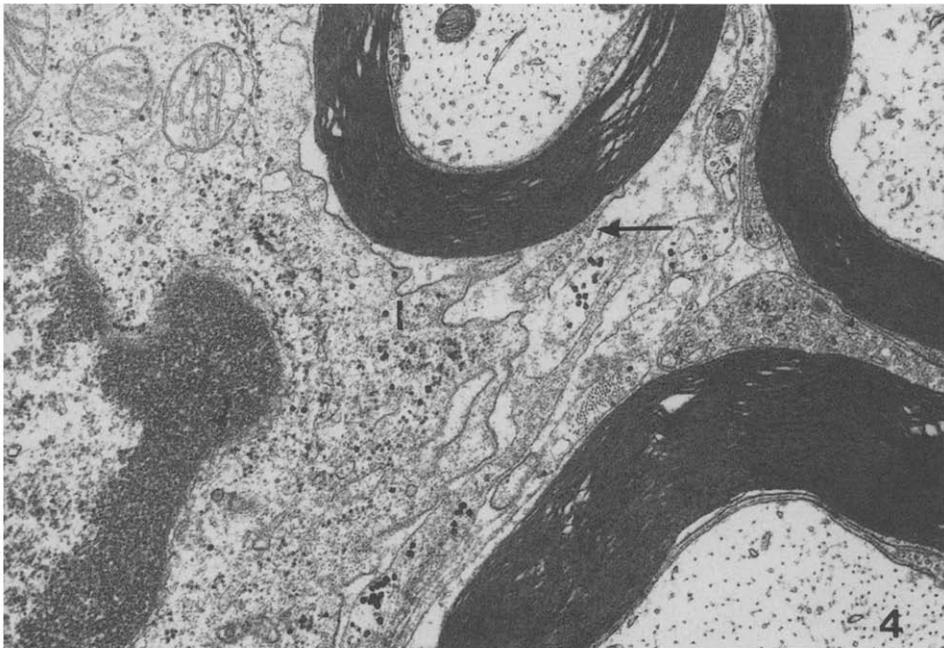

Fig. 4. A process from a lymphocyte (l) is present beneath an oligodendrocyte outer tongue (arrow) which is separated from the underlying myelin sheath. ×24 000.

pyknotic cells were not seen in the spinal roots and no changes of any sort were seen in the control animals.

Discussion

The images we have observed in this study illustrate a lymphocyte–myelin sheath interaction which may be of importance in the pathogenesis of autoimmune disease in the CNS. In this passively induced model of EAE in the rat, inflammation of both the CNS and PNS was observed. Morphological evidence of this process was leukocyte influx, edema and astrocyte hypertrophy which of course have been reported by others (Bubis and Luse, 1964; Lampert, 1965; Hirano et al., 1970; Raine et al., 1980). However, within the spinal cord a novel feature of the inflammatory reaction was the passage and sequestration of lymphocytes into myelin sheaths. This process appeared to begin with the insertion of a lymphocyte process beneath the outer oligodendrocyte tongue which caused it to separate from the myelin sheath. The lymphocytes proceeded further along the interperiod line into the myelin sheath until they became surrounded by a single myelin lamella. With time the number of lamellae which surrounded the lymphocytes increased and the cells died.

It appears that this process per se does not lead to demyelination as evidence of primary myelin loss in the spinal cord was minimal. Perhaps paradoxically, these interactions were not observed in the PNS and yet mild segmental demyelination was clearly evident there. Thus, at least in the PNS this process is not obligatory for demyelination to occur and as demonstrated by others, central demyelination may be dependent on the presence of specific humoral factors (Schluesner et al., 1987; Lassmann et al., 1988). Although intramyelinic pyknotic cells have been observed in EAE (Bubis and Luse, 1964; Lassmann et al., 1988), their genesis has not been examined in detail. We have recently observed this change in association with the delayed demyelinating lesions of Theiler's virus infection in mice (Blakemore et al., 1988) and it is illustrated in a paper on Semliki Forest virus infection in mice by Gates and coworkers (1984) and in a study on Venezuelan equine encephalomyelitis in mice (Dal Canto and Rabinowitz, 1981). As insinuation of lymphocytes into myelin sheaths is a process which is unlikely to occur by chance it must represent a specific cell–cell interaction which can be related to the sensitizing antigen (MBP) used in the present study. In neurotropic virus infections this interaction may occur as a response to viral antigen expressed in the myelin sheath, or may indicate the presence of autoreactive lymphocytes which have been, in some way, amplified by the viral infection. It is therefore pertinent to examine all virally induced demyelinating diseases in which autoimmunity has been implicated (e.g. Watanabe et al., 1983) to see if these intramyelinic pyknotic cells are present.

It is known that activated T cells with specificity for antigens, such as ovalbumin, that are not normally found in the CNS may traverse the blood–brain barrier (Wekerle et al., 1986). However, previous studies at the light microscope level by one of us (Sedgwick et al., 1987) found no evidence of infiltrating lymphocytes in the CNS of animals injected with activated ovalbumin-specific splenocytes. We would conclude from this that the observations made here reflect a specific cell–cell interaction related to MBP. What is not clear is how MBP and major histocompatibility (MHC)-restricted T cells may be involved in such an interaction given the reported absence of MHC antigens on the bulk of cells in the CNS and the cytoplasmic location of MBP. With regard to the latter, it is now clear that T cells recognize peptide fragments of antigen and that these may be derived from cytoplasmic proteins (Townsend et al., 1986). The sequestered location of intact MBP is therefore not in itself a problem with regard to T cell recognition.

Although it is reasonable to suggest that the myelin sheath–lymphocyte interactions described in this paper represent a specific interaction one can only speculate why an intramyelinic location should result in the death of some of the lymphocytes. It may be that, once covered by several lamellae of myelin, the lymphocyte would be isolated from metabolites and trophic factors (e.g. interleukin 2 if the cells do not make it themselves) which are necessary for survival. Alterna-

tively, recent work has shown that a number of factors are produced by glial cells some of which have potent immunosuppressive properties (Fontana et al., 1982; De Martin et al., 1987; Oropeza et al., 1987).

The precise role of the lymphocyte–myelin sheath interaction described in this study in the pathogenesis of CNS viral infections and experimental allergic encephalomyelitis awaits further study.

Acknowledgement

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