Acute Optic Neuritis Associated With Immunization With the CNS Myelin Proteolipid Protein

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Optic nerve tissue from SJL/J mice immunized with the central nervous system (CNS) myelin-specific proteolipid protein (PLP) was examined for histopathologic evidence of optic neuritis. Optic nerves isolated 17 d after immunization with PLP revealed an interstitial and submeningeal inflammatory infiltrate consisting of neutrophils and monocytes. In all cases, histologic evidence of optic nerve involvement correlated serologically with the presence of circulating anti-PLP antibodies. Control animals had no histopathologic evidence of disease or anti-PLP antibody. In many respects, the observed histopathologic profile of PLP-induced optic neuritis is similar to that associated with human inflammatory demyelinating diseases such as multiple sclerosis (MS). Because optic neuritis frequently is associated with some of the earliest clinical symptoms of MS, the acute nature of optic nerve involvement in this animal model suggests that immune recognition of the myelin PLP may play a significant role in the pathophysiology of optic nerve damage associated with sensitization to CNS-specific antigens. Invest Ophthalmol Vis Sci 33:1717-1722, 1992

The CNS-specific myelin proteolipid protein (PLP) is the major protein constituent of white matter. It makes up 50% of the total protein of myelin in the brain, spinal cord, and optic nerve.1 Its abundant distribution suggests that its primary function is to provide the structural integrity for the myelin lamellae.1 The protein is not expressed in the myelin sheath of the peripheral nervous system (PNS).2 Recent observations have demonstrated unequivocally a pathologic role of PLP immune recognition in several animal models of CNS inflammatory demyelination.3-6 Immunization of susceptible strains of animals with purified PLP6-8 or strain-specific PLP peptides9-11 results in the induction of experimental allergic encephalomyelitis (EAE), an autoimmune mediated inflammatory demyelinating disease of the central nervous system that, in many ways, shares a number of clinicopathologic features with multiple sclerosis (MS).

Although histopathologic studies of PLP-and PLP peptide-induced EAE have demonstrated a prominent inflammatory demyelination in brain and spinal cord,5,12 there have been no reported investigations of optic neuritis (ON) in PLP-induced disease. The optic nerves and chiasma are vulnerable to immunologic attack in MS, particularly in the early stages of the disease,13-15 so the development of an animal model that mimics the ocular changes seen in MS would be valuable. This is particularly true in light of the numerous prospective and retrospective studies that have noted an association between isolated ON and the eventual development of MS,13 suggesting that ON might represent one of the earliest clinical signs of the disease.

As described in this report, the ocular histopathologic findings observed in PLP-induced disease are particularly prominent in the acute or early forms of disease. Thus, it would appear that the histopathologic features of PLP-induced EAE make it an attractive model with which to study the ocular manifestations of sensitization to CNS-specific antigens.

Materials and Methods

Mice

Five-week-old male SJL/J mice were obtained from the Jackson Laboratories (Bar Harbor, ME). Animals were housed singly and were provided food and water ad libitum. All animals were immunized at 8 wk of age. The use of animals in these investigations was approved by the Animal Care Committee of the University of Connecticut Health Center, and their use adhered to the ARVO Resolution on the Use of Animals in Research.
Purification of the Myelin Proteolipid Protein

Myelin PLP was purified from bovine, rat, and human white matter following the modified procedure of Sakura and Lees.16 Briefly, PLP was prepared from chloroform:methanol extracts of CNS white matter followed by partial delipidation, emulsification, and centrifugation. Quantitative removal of all noncovalently bound lipid was achieved by two passages through a Sephadex LH-60 gel filtration column (Sigma Chemical Co., St. Louis MO.) eluted with chloroform:methanol:acetic acid (2:1:1%). Conversion of the protein to a water soluble form was carried out by the slow evaporation of the organic solvent with N₂ and its gradual replacement with water.16 The water soluble apoprotein was dialyzed extensively against several changes of distilled water and protein content determined spectrophotometrically. Purity of the PLP preparation was assessed by SDS-PAGE and Western blotting with CNS-specific antibody reagents17-18. The PLP preparation was determined to be free of contaminating myelin basic protein (MBP).

Immunizations

Five experimental and five control animals were used for these investigations. On day 0 each experimental animal received a single 400 μl subcutaneous injection into the abdominal flank consisting of an emulsion of 200 μg bovine PLP in Incomplete Freund’s Adjuvant (IFA) supplemented with 25μg M. tuberculosis H₃₇Ra (Difco, Detroit MI). Animals also received, on days 0 and 3, an intravenous injection of 0.75 X 10¹⁰ Bordetella pertussis bacilli (lot 94; Michigan Department of Public Health, Lansing MI); to increase blood-brain barrier permeability.10 Control animals followed an identical immunization protocol (adjuvant + pertussis) without added PLP.

Clinical and Histologic Evaluation

All animals were inspected daily for signs of disease and were clinically assessed for EAE according to the following criteria: 0, no disease; 1, decreased tail tone or slightly clumsy gait; 2, tail atony, moderately clumsy gait, or poor righting ability; 3, limb weakness; 4, limb paralysis; 5, moribund state. On day 17, under ketamine-xylazine anesthesia, all animals were exsanguinated by cardiac puncture. Brain, spinal cord, and optic nerves were removed, fixed in 10% phosphate-buffered formalin, and embedded in paraffin for microscopic evaluation. Optic nerves were severed close to the optic chiasma as the brain was removed, and the entire eye with attached nerve was fixed as described above. For these investigations, proximal segments of optic nerve were used for histologic review. For paraffin-embedded tissue, 5 μm sections were cut and stained with hematoxylin and eosin or toluidine blue, and degree of inflammation was noted. The lesions were reported on a scale of 0 to 4+ and were based on the number of lesions found in the cross-section of optic nerve. The absence of any lesion was designated as 0.19

ELISA

A direct binding ELISA format was employed to measure circulating anti-PLP antibody levels in experimental and control animals following the procedure of Potter and Lees.18 Briefly, purified bovine, rat, and human PLP apoprotein (100 ng diluted in 0.1 M carbonate buffer pH 9.0) was coated in a volume of 50 μl to the wells of a microtiter plate (Corning Glass Works, Corning NY). After an overnight incubation at 4°C, unbound material was aspirated off and the plates were washed three times with phosphate buffered saline (PBS) containing 0.05% Tween-20. Plates then were blocked with a solution of 1% bovine serum albumin (BSA) in PBS-Tween for 1 hr at 37°C. Plates were washed and individual wells were probed with a 1/200 dilution of serum ± SD (N = 5).

Table 1. Development of EAE in mice immunized with bovine CNS myelin PLP (bPLP)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. animals with EAE (day 17)</th>
<th>Clinical signs*</th>
<th>Histopathology†</th>
<th>Anti-PLP antibodies‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>bPLP + <em>M. tuberculosis + B. pertussis</em></td>
<td>4/5</td>
<td>3</td>
<td>2+</td>
<td>0.484 ± 0.106 0.422 ± 0.065 0.397 ± 0.024</td>
</tr>
<tr>
<td>bPLP + M. tuberculosis + B. pertussis</td>
<td>0/5</td>
<td>0</td>
<td>0</td>
<td>0.111 ± 0.056 0.159 ± 0.045 0.130 ± 0.064</td>
</tr>
</tbody>
</table>

* Clinical grading as described in Materials and Methods.
† Grading of optic nerve sections as described in Materials and Methods.
‡ OD 410 nm determined at 1/200 dilution of serum ± SD (N = 5).
Results

A summary of all experimental data is given in Table 1. On day 17, four of five experimental animals had definite clinical signs of EAE. These included weight loss, lack of tail tonicity (tail atony), hind limb weakness, and poor righting ability. Control animals remained clinically asymptomatic. Demonstrable titers of circulating PLP antibody to the immunizing antigen bovine PLP, as well as to PLP from rat and human CNS white matter, were detected in the sera of all animals that had developed EAE. Sera from adjuvant-treated controls showed minimal binding to PLP. The results of histologic examination of optic nerve sections are presented in Figures 1–3. In contrast to the normal optic nerve histology in adjuvant-immunized animals killed on day 17 (Figs. 1A and 1B), mice that received one injection of bovine PLP had prominent inflammation of the optic nerve. Inflammation was characterized as interstitial—appear-

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Fig. 1. Paraffin-embedded sections of optic nerve from SJL/J mouse immunized with adjuvant alone and pertussis and killed on day 17 as described in Materials and Methods. Note normal optic nerve histology, with no increased cellularity or signs of inflammatory infiltrates in any region. A and B, toluidine blue, original magnification ×25.)
ing as a circular “whirl” of cells in areas devoid of blood vessels—and submeningeal (Fig. 2). In general, light microscopic examination of optic nerves in all experimental animals demonstrated a definite increase in cellularity accompanied by discrete focal areas of inflammation. Higher magnification of regions of submeningeal inflammation (Fig. 3) revealed an inflammatory infiltrate consisting of polymorphonuclear leukocytes, monocytes, and small lymphocytes.

Discussion

The results of these investigations have demonstrated definite optic nerve pathology (inflammation) in animals actively immunized with the CNS-specific antigens.
PLP. Furthermore, it has been noted that these pathologic changes can occur in the acute or early stages of PLP-induced EAE. Collectively, these observations suggest that the PLP model of neuroantigen-induced inflammatory demyelinating disease may mimic some of the early clinicopathologic sequela observed in the human inflammatory demyelinating disease, MS, making this animal model an attractive system in which to study early histopathologic changes associated with immune-mediated CNS damage.

Although an association between ON and MS have been well established for almost 100 yr,\(^{20-22}\) the fact that MS is rarely complicated by death has limited the number of histopathologic reports of isolated ON. However, in the few published studies,\(^ {23-24}\) the microscopic and ultrastructural changes found in optic nerve and brain white matter appear to be identical, suggesting that ON might be a restricted form of the disease. This latter association has some support from the numerous retrospective and prospective studies that have suggested a predilection for the eventual development of MS in those patients that have presented earlier with clinically isolated ON.\(^ {25}\) Whether ON is a harbinger of MS still remains enigmatic. How-ever, because recent magnetic resonance imaging studies have identified neurologically silent lesions in the white matter of patients with acute ON,\(^ {26-27}\) the strong association between the two should not be overlooked. Some support of this association has been provided by studies of T lymphocyte subpopulations in ON. In a prospective examination of nine cases of isolated acute unilateral ON, Guy et al\(^ {28}\) found that one out of nine patients had a concomitant increase in the CD4/CD8 ratio of peripheral blood T cells. Interestingly, this patient also developed clinical MS.

These data suggest that further investigations of immunoregulatory T cells may yield information regarding the relationship between ON and MS.

Despite numerous histopathologic studies of the classic cerebro-spinal lesions associated with neuroantigen-induced EAE,\(^ {29}\) few reports have specifically examined optic nerve pathology in experimental animals. In one early study published on the subject, Raine et al\(^ {30}\) examined the relationship between ON and chronic relapsing EAE in strain 13 guinea pigs actively immunized with isogenic spinal cord homogenates. In those studies, animals were sampled between 2.5 and 35 mo after immunization at points that generally correlated with a relapse of clinical disease. ON was found to be a consistent finding in this model of EAE. However, pathologic changes (new lesion formation) did not appear to correlate with the clinical course of disease. Early pathologic changes not associated with clinical symptomatology may have been overlooked in that study because there were no histologic examinations of optic nerve tissue before the first relapse. Evidence for optic nerve involvement early in the pathogenesis of neuroantigen-induced EAE has been reported in two recent studies. Raine et al,\(^ {31}\) studying chronic relapsing EAE in the SJL/J mouse, reported that adoptive transfer of MBP-specific lymph node cells into naive syngeneic recipients could induce extensive mononuclear cell infiltration and optic nerve fiber damage as early as 14 d after transfer. More recently, Jones et al\(^ {32}\) demonstrated in the Buffalo rat model of MBP-induced EAE that the transfer of MBP-specific T cell lines can result in an inflammatory demyelination of the optic nerve as early as 11 d after transfer. Collectively, these studies underscore the significance of ON and demyelination in EAE and demonstrate the susceptibility of the optic tracts in the early stages of disease.

Unlike the more traditional and well characterized encephalitogenic antigen MBP, PLP fulfills several important and unique criteria that establish it as a potential target for direct involvement in immune-mediated inflammatory demyelination. First, unlike the cytoplasmically located MBP, PLP is an integral transmembrane protein with exposed extracellular domains that are theoretically accessible to direct interaction with the products of an immune response.\(^ {33}\) Second, expression of PLP is restricted to CNS myelin,\(^ {2}\) in contrast to MBP, which is present in both CNS and PNS.\(^ {1}\) This is important because PNS involvement is known to be minimal in MS.\(^ {13}\) These characteristics underline the suitability of examining the role of PLP in CNS inflammatory demyelination.

The immunologic and pathologic characterization of the PLP model of neuroantigen-induced EAE is currently the subject of considerable investigation. However, the contribution of optic nerve involvement in PLP-induced EAE has not been studied nor is the role of immune recognition of PLP in these processes known. Our data suggest that in the early stages of disease, optic neuritis is a prominent feature. However, in our hands the degree of demyelination varied. Whether demyelination in this model plays an important immunopathologic role has yet to be adequately determined. However, because acute and chronic relapsing PLP-induced EAE can be produced in the SJL/J mouse,\(^ {6-8,34}\) we believe this model system will lend itself to long-term longitudinal studies of the association between ON and CNS inflammation and demyelination across the entire clinical spectrum of disease.

Key words: central nervous system, myelin, proteolipid protein, optic nerve, inflammation
References


