Comparative Histology of Experimental Allergic Neuritis induced with Minimum Length Neuritogenic Peptides by Adoptive Transfer with Sensitized Cells or Direct Sensitization

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Abstract. Using synthetic peptides representing specific regions of the bovine myelin protein P2, the minimum peptide length requirement for the T-cell epitope necessary for successful production of experimental allergic neuritis (EAN) has been shown to involve residues 61-70 of the P2 protein. In this study, morphometric analysis was used to compare the histologic changes in sciatic nerves of Lewis rats after disease was induced by P2 specific neuritogenic T-cell lines (P3) or, alternatively, by direct inoculation of synthetic peptides representing residues 60-70 or 61-70 of the P2 protein sequence. Inoculation with cell line P3 stimulated with residue 61-70 failed to elicit demyelination in sciatic nerves. However, cells stimulated with residue 60-70 produced inflammation, endoneurial edema, mild demyelination and axonal degeneration within seven days. In contrast, disease induced with either peptide by direct sensitization was more severe. Morphometric analysis revealed that inflammation was most severe in animals sensitized to the decapeptide. In the sciatic nerve, axonal degeneration was proportional in frequency to the extent of inflammation. Inflammation was especially intense in spinal roots with extensive destruction of axons including unmethylated fibers. Spinal root changes were associated with Wallerian degeneration in the posterior white matter tracts of the spinal cord and were apparently secondary to endoneurial inflammation. Disruption of the blood-nerve-barrier (BNB), evident as physical separation of endothelial cells in association with severe perivascular inflammation, was observed.

Key Words: Adoptive transfer; Axonal degeneration; Direct sensitization; Edema; Experimental allergic neuritis; Inflammation; P3 cell lines.

INTRODUCTION

Recent developments in neurochemistry have provided more precise experimental models for inducing experimental allergic neuritis (EAN) than immunization with crude nerve homogenates. Direct sensitization with neuritogenic peptides or adoptive transfer with sensitized cells represent potent and reliable tools for producing EAN and assessing the impact of acute inflammation on the nerve microenvironment (1–6). The minimum peptide length for induction of EAN has been shown to be residue 61-70 of the P2 protein sequence (1). When this peptide was administered with Freund's complete adjuvant, physical signs of illness were detectable and histologic...
examination confirmed the presence of endoneurial inflammation and demyelination. By contrast, inoculation of rats with T-cell lines stimulated by the same peptide produced less morbidity than direct sensitization as determined by neurologic examination and qualitative assessment of light microscopic changes (1).

In this comparative study, adoptive transfer with lymphocytes stimulated either by P2.61-70 or P2.60-70 peptide fragments and direct sensitization with either of these fragments were used to induce EAN. Morphometric and ultrastructural studies were used to confirm differences in pathology induced by different length peptide fragments and methods of inoculation and, further, to explore the impact of inflammation and edema on the nerve microenvironment. Our findings demonstrate that with the more potent 60-70 peptide there is widespread axonal degeneration in nerves, roots and posterior tracts of the spinal cord suggesting that axonal injury is proportional to the severity of inflammation and secondary to it as lesions appear simultaneously in different parts of the nervous system during the peak of the inflammatory process. Finally, interendothelial cell separation in endoneurial vessels visualized by electron microscopy provides more evidence for the structural basis of altered vascular permeability in EAN.

MATERIALS AND METHODS

Peptides corresponding to sequences found within the bovine P2 protein (1) were synthesized by solid-phase stepwise elongation using an ABI 430 synthesizer (ABI, Foster City, CA, U.S.A.) as described elsewhere (7). The P2-specific cell line P3.3 was prepared as described by O'lee et al (2). Resting P2-specific cells (2 \times 10^5 cells/ml) from line P3.3 were stimulated with irradiated thymus cells (1 \times 10^7 cells/ml) and antigen (1 \mu M) for 72 hours in RPMI 1640 (GIBCO, Grand Island, NY, U.S.A.) enriched with L-glutamine (2 \mu M), 2-mercaptoethanol (5 \times 10^{-5} \text{ M}), penicillin (100 units/ml), streptomycin (100 \mu g/ml), fungizone (0.25 \mu g/ml) (GIBCO) and 1% normal rat serum.

The following experiments were carried out in 24 Lewis rats aged 8-12 weeks at the time of inoculation. Experimental animals were divided into four groups, two that received autoreactive T-cells sensitized either to residues 61-70 (n = 6) or 60-70 (n = 6) of the P2 protein (AT P2.61-70 and AT P2.60-70, respectively) and two that were directly inoculated with either residues 61-70 (n = 6) or 60-70 (n = 6) (DS P2.61-70 and DS P2.60-70, respectively). The method of inducing EAN with sensitized T-cells has been documented in greater detail elsewhere (1). Animals receiving the P3.3 cell lines by adoptive transfer (AT) were given an intraperitoneal injection in which approximately 10^7 cells were present in 2.0-2.5 ml phosphate buffered saline. Animals in which EAN was induced by direct sensitization (DS) received intradermal injections of an emulsion containing either the peptide residue 60-70 or 61-70. The inocula were prepared by emulsifying equal volumes of the peptide (1 mg/1 ml) in saline and Freund's adjuvant containing Mycobacterium tuberculosis (Difco H37ra, 10 mg/ml). Each animal was given a total of 0.1 ml divided equally between each hind limb.

All rats were inspected daily for evidence of weight loss, weakness or other abnormalities. Tail tone was assessed by inverting the animal and studying tail movements as it attempted to right itself.

Tissue was harvested seven days after adoptive transfer or 14 days after direct sensitization because inflammation is most intense at these two time points with the respective methods of inoculation. Under anesthesia, the sciatic nerves were removed and fixed by immersion in 2.5% phosphate-buffered glutaraldehyde. The rats were then perfused by cardiac injection of glutaraldehyde at 37°C and the spine was removed and allowed to fix overnight before removing the spinal cord, nerve roots and plexi. The specimens were then rinsed in phosphate buffer, post-fixed in osmium tetroxide, dehydrated in serial alcohol solutions and propylene oxide prior to embedding in araldite resin. One-micron thick sections were cut and stained with p-phenylenediamine for light microscopy. Additional tissue was processed in paraffin for routine histologic examination and assessment of inflammation.
Fig. 1. Histologic appearance of rat sciatic nerves from each of the four groups studied. Plastic sections stained with p-phenylenediamine. a, b) Cell lines sensitized to minimum length neuritogenic peptides 61-70 and 60-70, c, d) direct sensitization to these P3 peptides. a) No demyelination or inflammation is present in rat sciatic nerve seven days after inoculation with T-cells sensitized to 61-70. ×140. b) Perivascular inflammatory infiltrates are present seven days after inoculation with T-cells sensitized to 60-70. ×270. c) Inflammation and
Morphometric analysis was performed on one-micron thick plastic sections taken from the sciatic nerve at the midpoint between the sciatic notch and the popliteal fossa. Only sections in which the full thickness of the tibial fascicle could be visualized were analyzed. Using a video morphometry system consisting of a high resolution Cohn video camera attached to an Olympus optical microscope with a ×40 Olympus plan achromat objective, adjacent non-overlapping fields along the radius of the longest diameter of the tibial fascicle and the radius at right angles to this axis were systematically sampled using a grid with a grid-bar spacing of 20 microns. With this sampling scheme approximately 23% of each tibial fascicle was sampled. Point-counting techniques were used to obtain estimates of the volume fraction of myelinated fibers, Remak fibers, vessels, extracellular space, inflammatory infiltrate, darkly staining axons, residual myelin sheaths, myelin ovoids and demyelinated fibers within the endoneurium by an investigator blinded as to the identity of the experimental group. Categories were defined as follows: myelinated fibers included normal myelinated fibers as well as those with "reactive" Schwann cell cytoplasm; Remak fibers included clusters of unmyelinated fibers; vessels included all layers of the vessel wall as well as the lumen; infiltrate included inflammatory cells, macrophages, mast cells and fibroblasts; darkly staining axons consisted of fibers with swollen, darkly staining axonplasm; residual myelin sheaths were devoid of axonplasm; myelin ovoids consisted of clumps of collapsed myelin; and demyelinated fibers included axons without any myelin sheath or those in which the myelin sheath was undergoing phagocytic digestion. The percentage volume density (100 × the ratio of the number of grid intersections of each category to the total number of intersections of all categories) was calculated for each category. Some categories were summed for data presentation and analysis (i.e. abnormal axons = darkly staining axons + residual myelin sheaths + myelin ovoids; edema and infiltrate = extracellular space + infiltrating cells). Only those categories representing nerve microenvironment pathology (i.e. abnormal axons, demyelination, edema and infiltrate) were analyzed with a two factor ANOVA to determine if differences in pathology induced by P2,61-70 and P2,60-70 and differences in pathology attributable to the method of disease induction (i.e. adoptive transfer and direct sensitization) were significant. Multiple comparisons were made with the Tukey test.

Electron microscopy was performed on blocks from severely inflamed tissue from sciatic nerve, spinal roots and adjacent regions of the spinal cord for qualitative observations assessing the impact of inflammation on axons and the blood-nerve-barrier (BNB).

RESULTS

In rats treated with the nonapeptide (AT P2,61-70), the only physical sign of illness appeared four days after intraperitoneal injection of P2,3 activated T-cells and was restricted to loss of tail tone. By contrast, rats receiving the decapetide (AT P2,60-70) showed signs of illness at four days that included limp tail in all rats and hind limb weakness in 5/6. In the AT P2,61-70 animals seven days postinoculation, slight to moderate inflammation was detected in paraffin-embedded sections of spinal ganglia and roots in 2/6 rats while no inflammatory changes were observed in sciatic nerves (Fig. 1a). Vessels were prominent in resin-embedded sections but other than mural thickening, no specific changes were seen. In the AT P2,60-70 animals, inflammatory changes were detected in 5/6 rats and were not limited to perivenular demyelination. One-micron thick sections stained with p-phenylenediamine showed evidence of endoneurial edema, perivenular and endoneurial inflammation and demyelination (Fig. 1b). Demyelinated fibers were usually perivenular and fibers un-

endoneurial edema in rat sciatic nerve 14 days after inoculation with 61-70 in Freund's complete adjuvant. ×140. d) Severe inflammation, edema and demyelination 14 days after inoculation with 60-70. ×270.
dergoing axonal degeneration were also noted. Axonal degeneration was also noted in the spinal cord in 2/6 rats from this group.

Physical signs and histologic changes were more severe in the experimental groups in which disease was induced by direct sensitization with either the nonapeptide (DS P261-70) or decapeptide (DS P260-70) and were detectable 12–14 days post-inoculation. Inflammatory changes were detected in sciatic nerves of 5/6 DS P261-70 rats and all the DS P260-70 animals (Fig. 1c and d, respectively). These changes were milder in rats sensitized with the 61-70 residue as perivascular inflammation characteristically involved fewer vessels. Sensitization with the 60-70 residue evoked severe endoneurial inflammation in association with edema, and, while demyelination was prominent, destruction of the entire nerve fiber was also noted.

In all groups, destruction of the endoneurial contents of spinal roots, both dorsal and ventral, was commonplace (Fig. 2a–d) with inflammatory changes usually more severe in the roots than nerves. In one-micron sections of the cauda equina, 63%–88% of roots were severely inflamed. In mildly affected spinal roots, perivascular demyelination was evident; however, when severe inflammation spanned the entire endoneurium, axons could no longer be detected. Axonal injury was widespread in the spinal cord, being most severe in rats in which EAN was induced by direct sensitization. Wallerian degeneration was detected in posterior spinal columns of 5/6 DS P261-70 rats where affected axons were vacuolated and myelin sheaths swollen or collapsed (Fig. 3).

The results of a comparative, morphometric analysis of nerve microenvironment pathology are shown in Table 1 and confirm that more severe disease was detectable in directly sensitized rats versus animals receiving cell lines. Furthermore, with each method of inoculation, rats exposed to the longer peptides (AT P260-70 and DS P260-70) were more severely affected (Table 1, Fig. 4). Specifically, in rats exposed to P23 cell lines activated by residues 60-70, the volume fraction representing edema and inflammatory cells was significantly greater than the response elicited by cells stimulated by the shorter, 61-70 residue (p < 0.05). Similarly, the edema and infiltrate volume fraction was significantly greater in the DS P260-70 group than the DS P261-70 group (p < 0.05). Low levels of demyelination were detected in all groups except AT P261-70 animals. Axonal injury was detected in 3/6 AT P261-70, 4/4 AT P260-70, 3/5 DS P261-70 and 4/4 DS P260-70 rats. The severity of endoneurial edema and inflammation elicited by neuritogenic peptides was proportional to the cell proliferation assay response (Table 2) and, in most instances, appeared to correlate with the extent of axonal injury (Fig. 5).

Electron microscopy of sciatic nerves from rats sacrificed after being directly sensitized to either residues 61-70 or 60-70 showed inflammatory infiltrates composed of lymphoid, mononuclear cells and polymorphonuclear cells (Figs. 6, 7).

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**Fig. 2.** Histologic appearance of the spinal roots from rats directly sensitized to neuritogenic peptides. Plastic sections stained with p-phenylenediamine. a) Perivascular inflammatory cells forming a dense infiltrate in which some disintegrating myelin sheaths can be observed. Demyelinated axons are not seen. ×270. b) Only a few viable axons remain in this severely inflamed spinal root in which laminated, spherical bodies of degenerating myelinated fibers are present. ×140. c) In a less severely inflamed root, perivascular demyelination can be visualized and is characterized by groups of naked axons (surrounding asterisks) as well as fibers in various stages of myelinolysis. ×270. d) In this severely affected nerve root, Bungner's bands are visible (arrow), but few viable axons remain. ×140.

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Fig. 3. Wallerian degeneration in the posterior white matter tracts of the spinal cord in a Lewis rat directly sensitized to neurogenic peptide 61-70. Plastic sections stained with p-phenylene diamine. Swollen, vacuolated axons and collapsing myelin sheaths reveal extensive damage in the posterior columns. $\times 420$. Low power insert from the same field shows involvement of the posterolateral tracts on both sides. $\times 140$. 

TABLE 1
Comparative Pathology of the Nerve Microenvironment in EAN Induced with Minimum Length Neuritogenic Peptides

<table>
<thead>
<tr>
<th>Inoculation method (n)</th>
<th>P2 peptide fragment</th>
<th>Myelinated fibers</th>
<th>Remak fibers</th>
<th>Vessels</th>
<th>Edema + infiltrate</th>
<th>Abnormal axons</th>
<th>Demyelinated fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adoptive transfer (6)</td>
<td>61-70</td>
<td>74.7 ± 3.3</td>
<td>6.0 ± 2.5</td>
<td>1.5 ± 0.7</td>
<td>18.4 ± 7.2</td>
<td>0.9 ± 1.2</td>
<td>0</td>
</tr>
<tr>
<td>60-70</td>
<td>(1.4)</td>
<td>(1.0)</td>
<td>(0.3)</td>
<td>(2.9)</td>
<td>(0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct sensitization (5)</td>
<td>61-70</td>
<td>54.8 ± 19.5</td>
<td>2.7 ± 1.9</td>
<td>2.7 ± 1.0</td>
<td>30.5 ± 10.1</td>
<td>7.6 ± 8.7</td>
<td>1.8 ± 2.4</td>
</tr>
<tr>
<td>60-70</td>
<td>(8.7)</td>
<td>(0.9)</td>
<td>(0.4)</td>
<td>(4.5)</td>
<td>(3.9)</td>
<td>(1.1)</td>
<td></td>
</tr>
<tr>
<td>Direct sensitization (4)</td>
<td>60-70</td>
<td>42.5 ± 10.4</td>
<td>2.1 ± 0.5</td>
<td>42.6 ± 4.8</td>
<td>11.2 ± 5.1</td>
<td>1.1 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5.2)</td>
<td>(0.5)</td>
<td>(0.2)</td>
<td>(2.4)</td>
<td>(2.6)</td>
<td>(1.0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD (SEM) of the % volume density determined by point counting. Statistical analysis was restricted to edema plus infiltrate and nerve fiber abnormalities. Data were analyzed with a two factor ANOVA after which multiple comparisons were made with the Tukey test: *p < 0.05 vs adoptive transfer P261-70, adoptive transfer P260-70 and direct sensitization P261-70; and *p < 0.05 vs adoptive transfer P261-70.
Occasional erythrocytes were also noted (Fig. 7a). Increased amounts of structureless space were noted between bundles of collagen (Fig. 6b, c). Edema appeared more severe in the subperineurial zone where there is less collagen than in the endoneurium. Granular, electron dense "proteinaceous" material accumulated in the interstitium (Fig. 6b). Mast cell degranulation was also noted. Damage to myelinated axons and proliferation of Schwann cells was observed (Fig. 6a) and loss of axons was also apparent in Remak fibers (Fig. 6b, c). Loss of tight junctions and separation

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of endothelial cells was evident in some vessels and provided evidence of disruption of the blood-nerve-barrier (Figs. 7b, 8). When separation of endothelial cells occurred, proteinaceous material accumulated on the subendothelial basal laminar surface (Figs. 7b, 8). Demyelination was observed with stripping of myelin lamellae from the surfaces of large axons. Remyelination was also prominent with abnormally thin myelin sheaths around axons. Qualitatively similar changes were observed in both groups of rats directly sensitized to neuromyogenic peptides.

Electron microscopy performed on spinal nerve roots confirmed the presence of demyelination and revealed that axonal damage was particularly severe in fascicles in which inflammation was extensive. Residual basal laminae from preexisting fibers were commonplace. As noted in light micrographs (Fig. 2c, d), demyelination was more readily appreciated in less inflamed fascicles while fascicles in which inflammation was severe showed extensive loss of axons. Ultrathin sections of white matter tracts in the spinal cord also showed axonal degeneration. These changes were extensive and were noted in areas of the spinal cord contiguous to inflamed roots in which axonal degeneration was severe.

**DISCUSSION**

These morphologic studies confirm that the minimum peptide length requirement for the T-cell epitope for EAN comprises residues 61-70 of the P2 protein with the sequence EISFKLQQEF. As noted by Olee et al (1), addition of a threonine at the NH2 terminus of this peptide, reproducing residues 60-70 of the P2 protein sequence, considerably enhances the neuromyogenic potential of this peptide. Indeed with the more potent peptide fragment, the onset of disease was earlier, the duration longer and histology revealed that inflammation was more intense and nerve fiber damage more severe (Figs. 1, 2, 4; Table 1). The contrast in severity of disease was most pronounced when EAN was induced by adoptive transfer of lymphocytes and it is interesting to note that the proliferative response of the P2 specific cell lines to the longer peptide was more intense predicting the behavior of these cells in vivo (1; Table 2). These experiments also suggest that direct sensitization provides a more effective means of inducing disease, although the onset in rats immunized by this method is later (12-14 days) than the onset in adoptive transfer (4-5 days). Although it should be noted that in an earlier study of P2 specific T-cell lines, doubling the dose of lymphocytes in the inoculum augmented the disease (4). In rats directly sensitized to whole myelin, Hahn and associates (8) showed that the extent of nerve fiber injury is dose-dependent. They also suggested that if EAN were induced by more precise peripheral nerve antigens a more severe disease could be anticipated.

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**TABLE 2**

<table>
<thead>
<tr>
<th>P2 peptide fragment</th>
<th>61-70</th>
<th>60-70</th>
<th>57-81</th>
</tr>
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<tbody>
<tr>
<td>Indexa of P3 cell line proliferative response</td>
<td>157</td>
<td>272</td>
<td>565</td>
</tr>
<tr>
<td>Volume density of edema + infiltrate (%)</td>
<td>18.4</td>
<td>28.4</td>
<td>41.6</td>
</tr>
</tbody>
</table>

a Stimulation assay data were taken from Olee et al (2) and calculated as the ratio of the mean cpm incorporated into triplicate stimulated culture to the mean cpm incorporated into triplicate unstimulated culture (no antigen present). Volume densities are taken from Table 1 for P2 peptide fragments 61-70 and 60-70 and from Powell et al (16) for fragment 57-81.
Indeed this appears to be the case as 50 μg/rat of either minimum length peptide appears to cause comparable pathologic change (Table 1) to that reported with 1.5 mg/rat of purified whole myelin (8). The newer methods of disease induction are not only more potent, but when the severity of inflammation is more predictable, the impact on the nerve microenvironment is more easily studied.

Although the pathognomonic lesion of EAN is segmental demyelination (9), axonal injury represents an important component of this inflammatory disorder (10, 11). Demyelination, while prominent in nerve roots (Fig. 2c), is less frequent in the sciatic nerves (Fig. 1c) where axonal injury is common (Figs. 1d, 6a). Increasing the dose of antigen augmented the inflammatory process and higher doses were associated with increased axonal injury in lumbar roots and sciatic nerves (8). Morphometric analysis of rat sciatic nerves allows one to discriminate between pathologic abnormalities and provides further support for the suspected relationship between inflammation and axonal injury (Fig. 5). One of the more striking morphologic findings was the destruction of the entire contents of nerve root fascicles in which inflammation and endoneurial edema were especially severe (Fig. 2d). In these areas, axonal injury over-shadowed primary demyelination and its significance was underlined by associated destruction of nerve fibers in the posterior horns of the spinal cord. Damage to axons in the posterior funiculi of the spinal cord has been noted previously (4) in rats inoculated with autoreactive T-cells where the intensity of changes ap-

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**Fig. 6.** Axonal injury in an inflamed sciatic nerve induced by direct sensitization to P1 (60-70). Uranyl acetate and lead citrate. a) Mitosis occurring in a Schwann cell in the rat sciatic nerve after axonal degeneration. Note the absence of normal axoplasm and layers of ruffled
basal lamina (arrowheads), ×12,000. b) A polymorphonuclear leukocyte, proliferating fibroblasts and electron-dense "proteinaceous material" are present in the endoneurium. Axonal degeneration has occurred in the unmyelinated fiber at the bottom of the picture (UF). ×7,250.

c) No axons are found in the Remak fiber (UF) in this electron micrograph from the same nerve illustrated in (b). ×8,900.
peared dose-dependent. These changes, occurring in 10–12 week old Lewis rats, may be distinguished from dystrophic axonopathies in aging Vitamin E deficient and control Fischer rats (12, 13). The axonal changes reported in control Fischer rats involved scattered dystrophic fibers, in contrast to the rapidly evolving Wallerian degeneration noted in animals suffering from EAN in this study (Fig. 3). Additionally, severe endoneurial edema was present in these animals and spinal cord injury may be related to severe inflammation in the dorsal root. While primary demyelination is the pathognomonic abnormality in the pathology of EAN, axonal degeneration is of equal importance (10).

The pathogenesis of axonal injury in EAN is not completely understood and, given the complex nature of this model, may result from several factors. Madrid and Wisniewski showed that the axonal lesions correlated with the distribution of inflammatory lesions (14). Based on this correlation, they suggested axonal degeneration could be due to toxic factors released by activated lymphocytes in the presence of target antigen (“bystander damage”) or, alternatively, that inflammatory edema might result in compressive damage to axons. Hahn and others noted a correlation between axonal injury and the severity of inflammation (8) noting centrifascicular nerve fiber damage reminiscent of ischemic nerve injury (15). Other EAN studies correlating endoneurial fluid pressure (EFP) measurements with histologic and morphologic changes detected axonal degeneration when edema and EFP were greatest (16, 17). It is worth noting that in hexachlorophene neuropathy, another demyelinating neuropathy in which EFP is increased, axonal injury appears only after myelin swelling, endoneurial edema, increased EFP and reduced nerve blood flow (NBF) are well established (18). Any ischemic changes occurring in EAN would probably be exacerbated by increased demand for oxygen associated with the respiratory burst of infiltrating macrophages (19). However, assessment of the relative contribution to axonal injury in EAN of “bystander damage”, the direct compressive effect of inflammatory edema or ischemia, awaits further studies.

An unexpected finding of this study is evidence for opening of the BNB due to retraction of adjacent endothelial cells leaving a space between them (Figs. 7b, 8). The issue of altered vascular permeability in EAN has been discussed for many years during which passage of hematogenous cells into the endoneurial interstitium has been documented. Migration of activated circulating lymphocytes was first documented by Astrom (20) who suggested that they pass through venular endothelia. Subsequently, Lampert demonstrated movement of mononuclear cells between endothelial cells as they immigrated into the endoneurium prior to destruction of

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**Fig. 7.** Electron micrographs illustrating perivascular inflammation and endothelial morphology in rat sciatic nerve after direct sensitization to the P₄,60-70 peptide residue. Uranyl acetate and lead citrate. a) The perivascular interstitium contains many mononuclear cells and a single erythrocyte (RBC) while an intravascular lymphoid cell is adherent to the abluminal endothelial surface. Intercellular tight junctions appear undisturbed. ×7,000. b) Endothelial abnormalities in a vessel surrounded by inflammatory cells. Three endothelial cell processes labeled 1, 2 and 3 exhibit intercellular dissociation with loss of tight junctions. A wide intercellular space has opened up between endothelial cells 1 and 2 and a platelet overlies but does not block the space. In the inset at lower left, electron-dense material appears to accumulate next to the basal lamina in the subendothelial space (arrowhead). ×14,600. Loss of tight junctions and endothelial separation appears between endothelial cells 2 and 3 (arrowhead). Inset on the lower right shows separation between endothelial cells 2 and 3 at higher magnification. ×14,600.

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Fig. 8. Separation of endothelial cells (1 and 2) of an endoacinar venule associated with accumulation of electron-dense material (asterisk) on the subendothelial basal lamina after direct sensitization to the P960-70 peptide residue. Uranyl acetate and lead citrate. × 10,000.
myelin (9). Proteinaceous fluid was thought to extravasate with the lymphoid cells or to be produced by other mechanisms (21). Intravenously injected tracer proteins have been shown to enter the endoneurial interstitium 10–12 days after direct sensitization (17, 21) and immunoglobulin deposits have been detected in the edematous superneurial spaces by immunocytochemistry (17). Mast cell degranulation induces edema and is an early event in EAN (17, 21, 22). Release of histamine by degranulating mast cells may contribute to altered vascular permeability. Hahn and associates suggested that transient opening of interendothelial junctions might occur but they did not feel that it was clearly demonstrated either in their material or prior published work (21). However, Claudio and her colleagues (23) describe disruption of the blood-brain-barrier in experimental allergic encephalomyelitis induced by autoreactive T-cell lines. In this study, disruption of the BNB was found only after direct sensitization to P360–70; however, we have also observed interendothelial separation in Lewis rat sciatic nerve venules in EAN induced by T-cells sensitized to P357–81 (16). Since the P357–81-stimulated cell line is a more potent inducer of inflammation (Table 2), it is possible that barrier disruption is limited to only the most severely affected animals. The wide interendothelial cell separation noted in these experimental models indicates that physical opening of the BNB is a possible mechanism for escape of macromolecules independent of cellular immigration and is of some interest because humoral immunity has long been suggested to play a role in the pathogenesis of EAN (24, 25).

A relationship between the inflammatory process and axonal injury has been noted in both clinical and experimental demyelinating neuropathies. As discussed by Hartung and associates (26) axonal damage can be attributed to intense inflammatory response associated with prominent edema formation. In their view, a similar mechanism may explain axonal injury in Guillain-Barré Syndrome (GBS) and is responsible for conduction failure and poor recovery in the subgroup of GBS patients in whom inflammation is especially severe. Further support for a role for axonal injury is chronic morbidity associated with GBS from the neuropathologic studies of Feasby and associates (27–29), from animal experiments (11, 14, 30) and from electrophysiologic documentation in patients with GBS (29, 31, 32). An improved understanding of the pathogenesis of axonal injury in GBS may help in devising therapeutic strategies for controlling inflammation and edema. Early diagnosis and timely intervention might forestall these events which contribute to many long-term complications of what is otherwise a self-limited, subacute disease (32, 33).

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