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14. We thank S. L. Patt, Varian Instrument Division, Florham, N.J., for carbon magnetic resonance data, J. L. Occolowitz, Lilly Research Laboratories, Indianapolis, Ind., for electron impaction mass spectrometry, I. Miura, Columbia University, for FT-PMR measurements, and G. Jordan, Lederle Laboratories, for the FT-IR spectrum. The studies were supported by NIH grants R01-GM23232 (to L.S.E.) and CA 11572 (to K.N.) and NIH fellowship F32 GM06464 (to D.L.). L.S.E. thanks J. Van't Hof for support during the early investigations of the G_2 factor.

7 July 1978; revised 6 October 1978

Multiple Sclerosis: Presence of Lymphatic Capillaries and Lymphoid Tissue in the Brain and Spinal Cord

Abstract. *Thin-walled channels resembling lymphatic capillaries and containing lymphocytes were observed in perivascular spaces throughout the central nervous system of patients with various neurological disorders. This suggests that immunological surveillance in the central nervous system may normally involve lymphocyte circulation through the perivascular compartment. In some old multiple sclerosis plaques, perivascular lymphoid tissue was found which was organized in a manner similar to the antibody-producing medullary region of lymph nodes. This may indicate continuous processing of the putative multiple sclerosis antigen in such lesions.*

Perivascular infiltrates of lymphocytes and plasma cells are a prominent histological feature of the discrete lesions (plaques) that develop periodically in different parts of the brain and spinal cord in typical cases of multiple sclerosis (MS) (*1*, *2*). This, together with the demonstration that some of the immunoglobulin G in the spinal fluid in this disease is synthesized within the central nervous system (CNS) (*3*, *4*), suggests that a local immune response directed against an (unidentified) antigen is involved in its pathogenesis. In the absence of definite evidence of a systemic immune response, the reasons just given for supposing that such an antigen exists further suggest that the highest concentration of expressed antigen visible to the immune system occurs in plaques. Because plasma cells may be present in large numbers in perivascular spaces within plaques (*2*, *5*), it has been suggested that these spaces may represent the major site for antigen processing and antibody production within the CNS of patients with MS (*4*–*7*). In the present study, perivascular spaces in old plaques were found to contain organized lymphoid tissue, suggesting the occurrence of persistent antigen expression in such lesions.

Epoxy-embedded CNS tissue, suitably fixed for electron microscopy, was available for study from three patients with MS, a patient with motor neuron disease, and a patient with adrenoleukodystrophy (*8*). In each of these cases, light and electron microscopy revealed that the perivascular spaces in unaffected CNS tissue commonly contained lymphocytes and macrophages. Plasma cells were also present in two MS cases. These three cell types were not randomly distributed within the perivascular spaces; the lymphocytes and macrophages tended to be confined within thin-walled channels (Fig. 1, A and B), whereas any plasma cells that were present were invariably located outside these channels (Fig. 2). The walls of the channels consisted of a single layer of flattened cells that were joined at their edges by desmosomes (Fig. 1C) or separated by gaps of varying width. Each cell had an oval nucleus that was surrounded by scanty cytoplasm containing a few profiles of granular endoplasmic reticulum, several small dense bodies, and modest numbers of microtubules and microfilaments. The thin cytoplasmic processes that formed the channel walls were often less than 0.1 μm thick; they contained numerous smooth and coated pinocytic vesicles; and on their abluminal surface there were numerous hemidesmosomes associated with bundles of fine collagenous fibrils and microfibrils (Figs. 1C and 2B). Cells of the same type were also observed lining the glial and vascular walls of some perivascular spaces, or occurring as isolated cells in the absence of distinct channels.

All of the MS plaques studied were typical old plaques with few fat granule cells present in the demyelinated zone and with only minor perivascular inflammatory cell cuffing apparent in routine histological sections. In these plaques the perivascular spaces surrounding the larger blood vessels in demyelinated tissue revealed similar thin-walled channels containing lymphocytes and macrophages. However, compared to the channels in normal white matter, the channels in this location were more numerous and more irregular in shape, and they were separated by collagenous trabeculae which contained isolated plasma cells or groups of plasma cells clustered around cells of the same type as those that formed the walls of the channels (Fig. 2). Where these two cell types touched, the cell membrane of the plasma cell exhibited a lentiform electron-dense undercoat (Fig. 2B). Intimate contact was also observed between lymphocytes and macrophages inside the thin-walled channels; this specialized contact involved the formation of a number of deep, cylindrical indentations in the macrophage plasma membrane, each indentation enclosing a slender cytoplasmic process extending from the body of the lymphocyte, as reported previously in lymph node sinuses (*9*). Active phagocytosis was also observed occasionally inside the channels (Fig. 2A).

The type of tissue organization just described—with clusters of plasma cells together with free-lying collagen and reticular cells surrounding collagen-free channels containing lymphocytes and macrophages—is similar to the immunoglobulin-secreting medullary region of a lymph node (*10*–*12*). This was confirmed in one MS patient in whom lymph node tissue was available for study. In this patient, a comparison of the fine structure of plaque perivascular spaces and lymph node medulla revealed the same general arrangement of various cell types; the chief differences in the two tissues were the larger number of cells present in the medullary cords, which also contained a specialized vascular endothelium, and the fact that the reticular cells in the lymph node medulla exhibited certain structural differences depending on whether they protruded into the medullary sinuses, formed the walls of the sinuses, or were located among plasma cells outside the walls of the sinuses (*10*, *11*).

The presence of lymphoid tissue of

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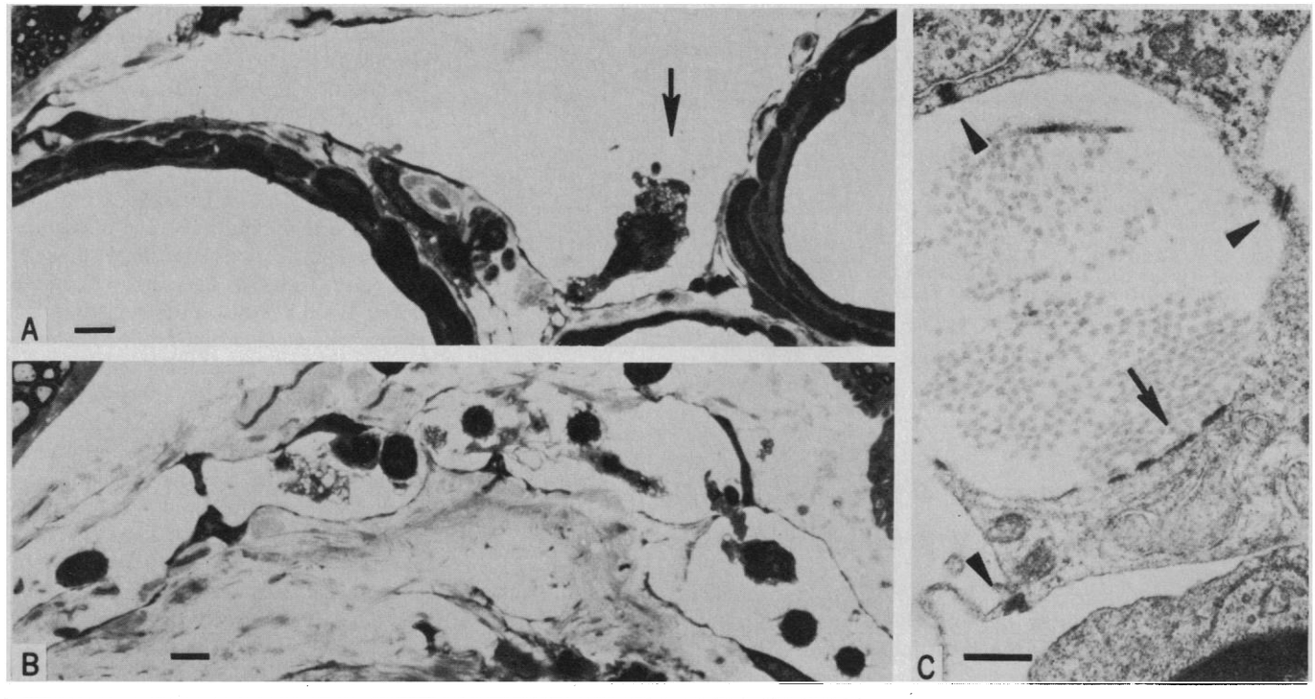

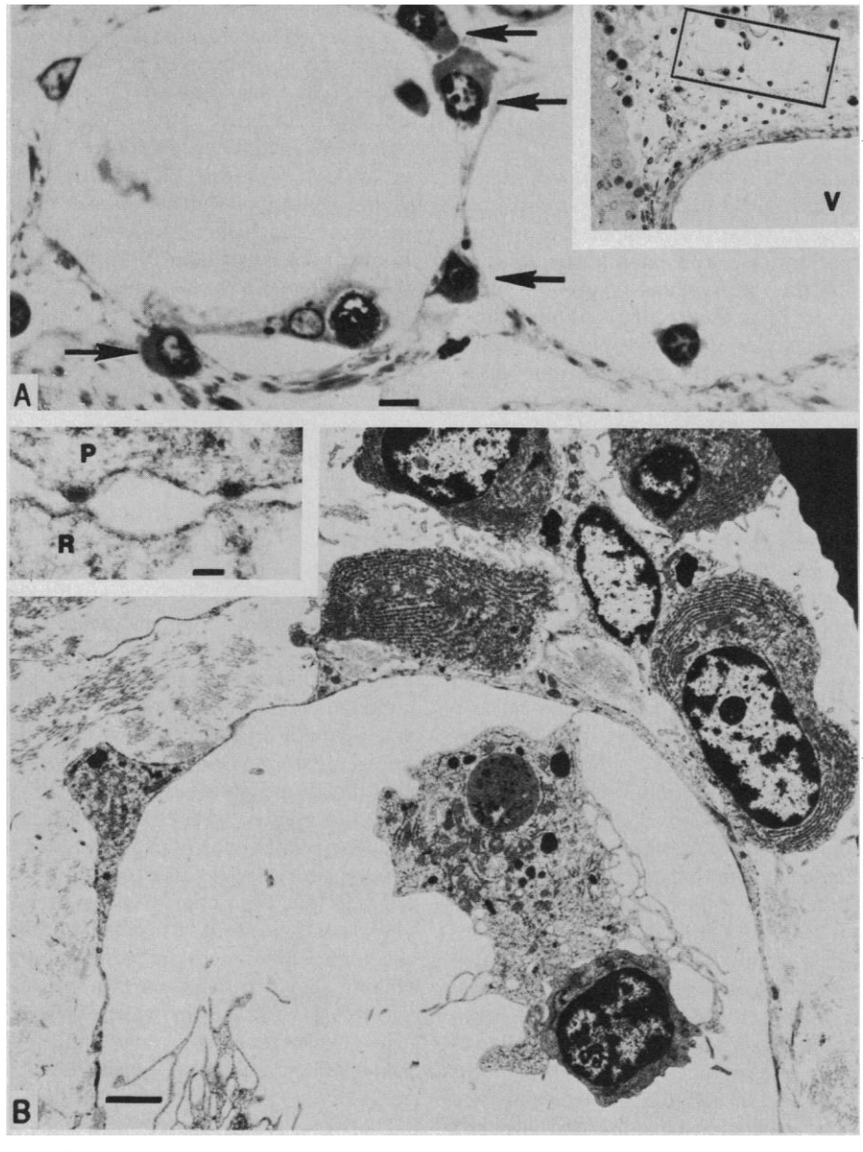

Fig. 1 (top). Perivascular spaces in unaffected white matter from patients with motor neuron disease and MS. (A) Macrophage (arrow) can be seen inside a thin-walled channel in the perivascular space related to an artery in the spinal cord of a patient with motor neuron disease. The gray material on the abluminal surface of the channel is composed of collagen and reticular fibers (toluidine blue; scale bar, 5 μm). (B) A channel similar to that shown in (A) contains a number of lymphocytes and macrophages (from patient with motor neuron disease; toluidine blue; scale bar, 5 μm). (C) Electron micrograph of the wall of a perivascular channel (lumen of the channel located on the right and also at upper left) illustrating desmosomes between adjacent cells (arrow points), and hemidesmosomes (arrow) on the abluminal surface (from patient with MS; scale bar, 0.5 μm). Fig. 2 (left). Perivascular spaces in MS plaques, illustrating the structure of the microenvironment likely to be responsible for immunoglobulin production and possibly antigen processing in this disease. (A) The perivascular space around a large vein (v) in an old MS plaque (see inset) contains a number of thin-walled channels. A lymphocyte can be seen inside the channel on the right, and a macrophage phagocytosing a degenerate cell is present inside the channel on the left. Four plasma cells (arrows) can be seen outside the channels (toluidine blue; scale bar, 5 μm. (B) The lumen of a perivascular channel in an MS plaque contains a macrophage in contact with a lymphocyte. Plasma cells can be seen clustered around a reticular cell outside the channel. The inset illustrates junctions of the type commonly observed between plasma cells (P) and reticular cells (R) (scale bar, 1 μm; inset scale bar, 0.1 μm).

this type in some old MS plaques is further evidence that the putative antigen may be continuously expressed in such lesions (*5*). Also, depending on the efficiency of antigen trapping by this tissue, it is possible that little or no antigen escapes from this region to induce systemic immunity. The present findings further suggest that the antigen may be processed and exhibited to passing lymphocytes by perivascular macrophages in a manner similar to that proposed for lymph nodes where B lymphocytes are thought to be arrested at this point in their circulation by contact with macrophage-processed, membrane-associated antigen to develop locally into antibody-forming plasma cells (*13*).

The thin-walled channels observed in perivascular spaces in unaffected CNS tissue in each of the five patients studied were indistinguishable from lymphatic capillaries in other tissues in terms of both their structure and contents (*10, 14, 15*). The presence of such channels is not easily reconciled with the traditional view that the CNS lacks lymphatic vessels (*10, 16–19*) and that the perivascular spaces represent cul de sacs or backwaters of the subarachnoid space whose chief function is to act as a protective cushion between the expansile blood vessels and the parenchyma (*20–22*). The present findings are more in keeping with the view of Harriman and other neuropathologists that these spaces serve the same function in the CNS as lymphatic vessels serve in other tissues (*23, 24*). While this is not to say that the CNS has a lymphatic drainage which is equivalent to that in other tissues (*25*), it is not unreasonable to view the presence of lymphocyte-containing channels in the perivascular spaces in the CNS as evidence that lymphocytes normally circulate through these channels, possibly in the same manner and in the same numbers as lymphocytes circulate in other tissues, and that this may constitute the basis of immunological surveillance in the CNS.

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8. The CNS tissue in four of the five patients was fixed within 20 minutes of death by perfusion in situ with 3 percent glutaraldehyde (*7*). In the fifth case (MS), glutaraldehyde-fixed subcortical white matter obtained at biopsy was studied. The tissue was postfixed in Dalton's solution, embedded in Spurr's medium, and prepared for light and electron microscopy. Epoxy-embedded biopsy tissue and immersion-fixed CNS tissue obtained at routine autopsy were examined in six further MS cases but in none were perivascular structures sufficiently well-fixed for the purposes of the study.
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18. Previous descriptions of the normal histological appearance of the perivascular spaces in the CNS (*19, 21, 24*) refer chiefly to the presence of flattened mesenchymal or reticuloendothelial cells. Some authors also describe occasional phagocytic cells, but lymphocytes are rarely mentioned (*24*). With reticular stains, and in studies in which the perivascular spaces have been filled with India ink, it has been reported that the flattened cells line both sides of the perivascular space, and that this lining endothelium is continuous with the arachnoid and pia where the perivascular space opens into the subarachnoid space. Electron microscopical studies (mostly dealing with the cerebral cortex in rodents) also note that the perivascular spaces normally contain flattened cells—described as flat connective tissue cells or fibroblast-like cells—which ultrastructurally resemble piaarachnoid cells, some collagen, and occasional phagocytic cells "of pial origin." Lymphocytes seem not to have been described, and several of these studies report that no continuous endothelial lining could be demonstrated [see (*17, 22*); E. Nelson, K. Blinzinger, H. Hager, *Neurology* **11**, 285 (1961); E. G. Jones, *J. Anat.* **106**, 507 (1970); B. van Deurs, *J. Ultrastruct. Res.* **56**, 65 (1976)].
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25. The view that the CNS lacks lymphatic vessels (vessels through which tissue fluid and cells return toward the blood stream) is based not only on the fact that such vessels have not been seen in the brain by routine histological methods, but also because multiple injections into the brain substance of India ink or other suspensions of particulate material fail to show such vessels (*15*, p. 15). When particulate material is introduced in this way into the brain parenchyma, it does not pass freely into the perivascular spaces but accumulates outside the glial-limiting membrane in the artifact space of His. When the injections are made into the subarachnoid space, however, the particulate material can be induced to enter the perivascular spaces, and so pass deeply into the brain, if the brain is simultaneously dehydrated (by hypertonic intravenous infusions) (*19*). Regarding draining to regional lymph nodes, while most of the draining tissue (spinal) fluid enters the blood stream without passing first through a lymph node, as occurs in other tissues (*15*, p. 518), some passes to cervical, paravertebral, and mesenteric lymph nodes via lymphatics in the olfactory mucosa and along vessels and nerves entering and leaving the cranial and spinal cavities [see (*16, 20*); J. B. Brierley and E. J. Field, *J. Anat.* **82**, 153 (1948); D. H. M. Woollam and J. W. Millen, *Lancet* **1953-I**, 364 (1953); Yoffey and Courtice (*15*, p. 309)].
26. Supported by the Medical Research Service of the Veterans Administration and the Kroc Foundation. I thank C. S. Raine and D. M. Boehme for the use of their laboratory facilities, and F. Connell and E. Swanson for technical assistance.

29 August 1978; revised 24 October 1978

Red-Absorbing Visual Pigment of Butterflies

Abstract. *Noninvasive photochemical and physiological experiments with intact butterflies of 17 species showed that nine species have a rhodopsin absorbing maximally at 610 nanometers, contained in retinular cells that are maximally sensitive at 610 nanometers. This is the longest-wavelength visual pigment known for an invertebrate. Eight species of butterflies lack the 610-nanometers rhodopsin. All species possess a rhodopsin absorbing maximally in the green region of the spectrum.*

Compared with humans, most invertebrates have very low sensitivity and poor color discrimination in the red and orange regions of the spectrum. However, judging from behavioral studies (*1*) some butterfly species are an exception. The physiological basis for this sensitivity to long wavelengths is still unknown because the methods (*2–4*) that have been applied to the problem have serious weaknesses. For example, the electrical mass response of an eye, the electroretinogram (ERG), can not in general be used to infer the spectral sensitivity of

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