plant pathogenic fungi, *M. grisea* produces a mucilage around the growing germ tube and appressorium (*20, 21*). Conidial attachment may thus represent the first step in a multicomponent attachment process.

REFERENCES AND NOTES

1. M. E. Barr, *Mycologia* **69**, 952 (1977).
2. B. Valent *et al.*, *Iowa State J. Res.* **60**, 569 (1986); M. S. Crawford *et al.*, *Genetics* **114**, 1111 (1986).
3. S. H. Ou, *Rice Diseases* (Commonwealth Agricultural Bureaux, Slough, U.K., 1985), p. 380.
4. H. D. Thurston, *Tropical Plant Diseases* (American Phytopathological Society, St. Paul, MN, 1984), p. 207.
5. R. L. Nicholson, in *Infection Processes in Fungi*, D. W. Roberts and J. R. Aist, Eds. (Rockefeller Foundation, New York, 1984), pp. 74–89.
6. R. W. Emmett and D. G. Parberry, *Annu. Rev. Phytopathol.* **13**, 147 (1975).
7. *Magnaporthe grisea*, strains 4091-5-8 and 0-42 (*2*), was grown and maintained as previously described (*2*). Conidia were harvested in 0.25% gelatin solution by gentle scraping of plate cultures, washed once by centrifugation at 2000*g*, suspended in 10 m*M* phosphate buffer, *p*H 6.5, and deposited in 100-μl portions containing 50,000 conidia on 2 by 2 cm squares of 10-mil Teflon-PFA film or on clean glass microscope cover slips and incubated at room temperature. The extent of conidial germination was determined by inverting spore suspensions onto a microscope slide and observing them at ×200 by phase-contrast light microscopy. The percentage of conidia with germ tubes was determined by counting the first 200 spores and recording the number of conidia with germ tubes. The average number of appressoria per field was determined for five microscopic fields observed at ×200. The extent of conidial attachment was determined by removing the 100-μl droplet of conidia from the Teflon-PFA surface at indicated times by pipetting. A further 100-μl droplet of sterile 0.25% gelatin solution was used to wash off any unattached conidia. The spores that had been removed by these two pipettings were combined, diluted, and plated in duplicate in complete medium. Colonies were counted after 1 week.
8. Teflon-PFA is tetrafluoroethylene copolymerized with perfluoroalkoxy pendant groups. Teflon polymers are chemically inert, have negligible moisture absorption, and are known for their nonstick characteristics. As thin films, these polymers have excellent optical clarity. Further details can be found in *Encyclopedia of Polymer Science and Technology* (Wiley, New York, 1976), vol. 1 (suppl.), pp. 260–267.
9. Rice leaf surfaces are covered by waxes and are resistant to wetting [S. Y. Zee, *Wheat and Rice Plants, a Scanning Electron Micrograph Survey* (Cosmos Books, Hong Kong, 1981), p. 58]. In this way they resemble the hydrophobic polymer surfaces that elicit appressorium formation.
10. W. J. Liang, *Natl. Sci. Counc. Monogr.* 7, 810 (1979).
11. Conidia, grown and harvested as described above (*7*), were resuspended at 5×10^5 conidia per milliliter in sterile distilled water and were allowed to settle over the surface of glass microscope slides or similar sized strips of 10-mil Teflon-PFA film. The surfaces with attached conidia were placed inside a flow chamber (Du Pont Engineering Technology Laboratory) equipped with a port for microscopy, and the chamber was attached to a centrifugal pump and flowmeter. Hydraulic shear was generated by increasing the flow of water through the opening in the chamber (cross-sectional area, 0.8 by 25 mm). Conidia in the chamber were viewed by light microscopy, and the response of conidia to increased flow rates was recorded on video tape. The percentage of conidia remaining attached after incremental increases in flow rate was determined from the tape recordings.
12. Dilute conidial suspensions were prepared by gently harvesting conidia from culture dishes in 10 m*M* phosphate buffer, *p*H 6.5, containing either sodium azide (2 m*M*) or cycloheximide (300 μg/ml). A control suspension was prepared with phosphate buffer alone. Conidial suspensions were deposited in 100-μl portions onto plastic cover slips and allowed to incubate at 23°C for 20 minutes, labeled with Con A, and examined microscopically. All three conidial suspensions contained a majority (≥86%) of conidia with STM (Fig. 2a). After 2.5 hours of incubation, microscopic examination of control conidial suspensions showed uniform germ tube emergence. Suspensions containing sodium azide or cycloheximide did not produce germ tubes after 2.5 hours.
13. Electron microscope specimens were processed on squares of cellulose membrane substratum. For dry conidia, squares of dry substratum were gently wiped across the sporulating mycelium. For wet conidia, 20-μl droplets of conidial suspension (*11*) were held aseptically on hydrated substratum for 10 minutes. Specimens were frozen in liquid propane, substituted in 2% osmium tetroxide and 0.1% uranyl acetate in acetone, embedded in Quetol epoxy resin, and thin-sectioned [R. J. Howard and K. L. O'Donnell, *Exp. Mycol.* **11**, 250 (1987)].
14. L. E. Hawker and R. J. Hendy, *J. Gen. Microbiol.* **33**, 43 (1964).
15. R. Campbell, *New Phytol.* **69**, 287 (1970).
16. G. M. Murray and D. P. Maxwell, *Can. J. Bot.* **53**, 2872 (1975).
17. R. C. Evans, H. Stempen, P. Frasca, *Phytopathology* **72**, 804 (1982).
18. D. H. Young and H. Kauss, *Appl. Environ. Microbiol.* **47**, 616 (1984).
19. G. Rees and E. B. G. Jones, *Bot. Mar.* **27**, 145 (1984).
20. T. Uchiyama, N. Ogasawara, Y. Nanba, H. Ito, *Agric. Biol. Chem.* **43**, 383 (1979).
21. R. J. Howard, unpublished results.

22. We gratefully acknowledge the technical assistance of T. Bourett and the assistance of H. Turchin and W. Jobst (Du Pont Engineering Technology Laboratory) with the flow chamber experiments. R. Uschold first suggested the use of Teflon films and R. B. Williams generously supplied us with Teflon films and information on their technical properties.

14 September 1987; accepted 18 December 1987

Perivascular Microglial Cells of the CNS Are Bone Marrow–Derived and Present Antigen in Vivo

WILLIAM F. HICKEY AND HIROMITSU KIMURA

A crucial question in the study of immunological reactions in the central nervous system (CNS) concerns the identity of the parenchymal cells that function as the antigen-presenting cells in that organ. Rat bone marrow chimeras and encephalitogenic, major histocompatability–restricted T-helper lymphocytes were used to show that a subset of endogenous CNS cells, commonly termed "perivascular microglial cells," is bone marrow–derived. In addition, these perivascular cells are fully competent to present antigen to lymphocytes in an appropriately restricted manner. These findings are important for bone marrow transplantation and for neuroimmunological diseases such as multiple sclerosis.

THE CENTRAL NERVOUS SYSTEM (CNS) of mammals has long been considered an immunologically privileged site (*1*). In healthy animals the CNS is virtually devoid of lymphocytes. The blood-brain barrier also excludes immunoglobulin, and molecules of the major histocompatibility complex (MHC) necessary for restricted antigen recognition are nearly undetectable in the CNS (*2*). However, during immunopathological processes that involve the CNS, this organ must allow the entry of immunospecific effector cells and permit antigen recognition by T cells in an MHC-restricted fashion. In multiple sclerosis, there is a malfunction of mechanisms that ordinarily protect the CNS from immune attack, and an autoimmune disease results. One of the persistent questions central to our understanding of this disease, and CNS immunology in general, concerns the identity of the cell or cells in the nervous system that can present antigen to T cells. Experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis, has been extensively studied in an attempt to answer this question, but to date no specific cell type has been functionally identified in vivo as an endogenous CNS antigen-presenting cell (APC).

Three primary candidates for the role of APC in the nervous system are endothelial, astrocytic, and microglial cells. In vitro studies have shown that both the astrocyte and the endothelial cell can express MHC-encoded molecules on their cell surfaces and present CNS-related antigens to T lymphocytes in an MHC-restricted manner (*3*). Nevertheless, the possibility remains that these cells in vitro may be exhibiting secondary functions not normally performed in vivo. In both multiple sclerosis and experimental allergic encephalomyelitis (EAE), immunohistochemical studies have shown that class I and class II MHC antigens are exhibited by both endothelial cells and microglial cells (*4, 5*). Astrocytes appear to

W. F. Hickey, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104-6079.
H. Kimura, Department of Surgery, University of Pennsylvania, Philadelphia, PA 19104-6079.

Table 1. Results of adoptive transfer of EAE. These results were obtained with encephalitogenic cells of a single GP-MBP- and EP-reactive line derived from a Lewis rat.

Recipient rat strain	Number with clinical EAE	Number with histological EAE
Lewis	3/3	3/3
BN*	0/1	0/1
DA*	0/2	0/2
(Lewis × BN)F_1 → BN	3/5	5/5
(Lewis × DA)F_1 → DA	5/6	6/6

*A number of other rats of these strains were tested for EAE development with similar encephalitogenic Lewis T lymphocyte cell lines with consistently negative results.

express these antigens only rarely in EAE, and to a greater extent in multiple sclerosis; yet in both situations the inflammatory reaction is already fully developed or resolving by the time these molecules are detected (*4, 5*). The identification of an endogenous APC prior to the development of inflammation in an organ that does not normally express MHC-encoded antigens presents a dilemma.

EAE in the rat is an acute, transient paralytic illness that can be induced by the injection of activated lymphocytes of the T-helper phenotype specific for myelin basic protein (*6, 7*). These effector cells require antigen recognition in association with MHC class II molecules (*8*). Singer *et al.* (*9*) demonstrated that EAE could be induced in rats made chimeric with bone marrow derived from an appropriate F_1 hybrid. Their work, however, was not directed at identifying this cell and did not permit a conclusion concerning the nature of this APC, since the encephalitogenic T lymphocytes they used could have been restricted by the MHC class II molecule of either parental rat strain (*8, 10*). In unrelated studies, Ting *et al.* (*11*) found that the detectable MHC molecules from the CNS of chimeric mice were bone marrow–derived; however, cells bearing these marrow-derived molecules could not be specified by their methods. We used the EAE model and graft-versus-host (GvH) disease in chimeric rats to identify an endogenous CNS cell derived from the bone marrow that can express MHC class I and II antigens and that functions in vivo as an APC.

Rats were made chimeric with bone marrow from F_1 hybrids as previously described (*9*). In brief, DA rats (MHC type $RT1^a$) and BN rats ($RT1^n$) were lethally irradiated (1000 rads) and given 10^8 bone marrow cells from (Lewis × DA)F_1 and (Lewis × BN)F_1 hybrid rats, respectively. After waiting 2 months for chimerism to become established, we determined the percentage of chimerism on dissociated lymph node cells by immunofluorescence with Il-69, a murine monoclonal antibody specific for Lewis class I molecules (*12*). In all chimeras, 80% to 90% of the lymph node cells expressed the Lewis class I molecule, an indication that a high percentage of the immune system was repopulated with donor F_1 hybrid cells. In these chimeras only bone marrow–derived cells of the F_1 hybrid donor could express molecules of the Lewis MHC ($RT1^l$); somatic tissues, including endothelial cells and astrocytes, could not elaborate the Lewis MHC molecules.

A long-term T lymphocyte cell line of the T-helper phenotype ($CD4^+CD8^-$) specific for guinea pig myelin basic protein (GP-MBP) was established. [^{3}H]Thymidine incorporation assays demonstrated a highly specific antigen reactivity to GP-MBP and to the encephalitogenic peptide (EP) (amino acid residues 68 to 88) derived from GP-MBP (*13*). Antigen presentation was restricted to the Lewis class II MHC molecules. No antigen reactivity or alloreactivity occurred with BN or DA accessory cells. Ten million of these cells produced typical symptoms of EAE in a Lewis rat.

Activated T lymphocytes (1.5×10^7) from a single line were injected intravenously into the bone marrow chimeras and control nonchimeric rats (Table 1). The recipients were observed daily for the appearance of clinical EAE which became manifest in 4 to 6 days. The severity of clinical EAE was scored as previously described (*14*). At the point of paralysis or on the 8th day after injection the rats were killed and their CNS was examined histologically to determine the presence of inflammation (Table 1). All Lewis controls and many of the chimeras exhibited clinical EAE. Histologically, all chimeras and Lewis rats had CNS inflammation indicative of EAE. The distribution of the EAE infiltrates was the same as in typical EAE, being more severe in the lower spinal cord and brainstem with few infiltrates in the high spinal cord and cerebrum. None of the nonchimeric DA or BN rats had either clinical or histological evidence of disease.

Lymphocytes of the T-helper phenotype ($CD4^+$) typically require antigen recognition in the context of the appropriate MHC class II molecule (*15*). Since the chimeric animals developed EAE, whereas the syngeneic controls did not, we concluded that a bone marrow–derived cell must have become established in the CNS. Furthermore, this cell must be functioning as an APC in that organ, if, indeed, MHC-restricted antigen presentation is required in the target organ. Nevertheless, identification of the specific APC by immunohistochemical examination of the CNS of EAE rats could not be performed because of the extensive nature of the inflammatory reaction. Most of the cells infiltrating the CNS of the chimeras bore Lewis MHC molecules because most of the bone marrow–derived elements of the immune system participating in the infiltrates were of F_1 hybrid origin.

Since it was impossible to identify this $RT1^l$-expressing donor-derived APC amid the extensive inflammatory infiltrates, a method of inducing MHC molecules throughout the CNS of the chimeras without producing inflammation was required. We recently showed that GvH disease elicits strong MHC molecule expression in the nervous system without any attendant inflammation (*16*). Chimeric rats in which EAE had not been induced were given 5×10^8 splenocytes and lymph node cells syngeneic to the host [(Lewis × DA)F_1 → DA chimeras were given cells from naïve DA rats]. This intravenous injection of a large number of syngeneic lymphocytes produced a systemic immune reaction similar to classical GvH disease because of the alloantigens recognized on the marrow-derived cells

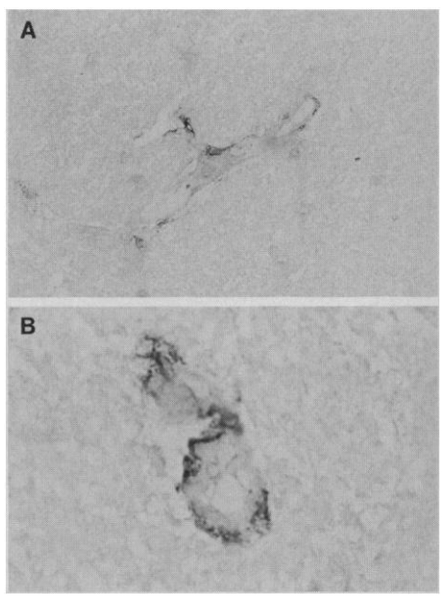

Fig. 1. Immunohistochemical staining of the spinal cord of DA rats given (Lewis × DA)F_1 bone marrow in which GvH disease had been induced. (**A**) Elongate cells external to a tangentially cut vessel; these cells are positive for Lewis class I MHC molecules (I1-69 antibody, ×200). (**B**) Cells around a vessel cut in cross section; these cells are positive for the Lewis Ia molecule (MRC OX-3 antibody, ×400).

of the immune system in the chimeras. Seven to 10 days after such injections the chimeras had clinical GvH disease. Immunohistochemical staining (*14, 16*) with murine monoclonal antibodies specific for the Lewis Ia molecule [RT1B^l, MRC was OX-3 (*17*)] and Lewis class I molecule [RT1A^l, I1-69 (*12*)] was used to demonstrate which cells in the CNS of these chimeras with GvH disease were of F_1 hybrid origin. Cells of the DA or BN hosts do not express the epitopes recognized by these antibodies. The only cells expressing these Lewis rat MHC molecules were elongated perivascular cells lying external to blood vessels of small diameter (Fig. 1). Perivascular cells with the Lewis-derived antigens were also found associated with the leptomeninges, and very rare arborizing parenchymal cells were observed. The perivascular cells corresponded in location to those termed "perivascular microglial cells" (*18*). They were found around blood vessels at all levels of the CNS.

These experiments were also performed with (Lewis × DA)F_1 → Lewis chimeras and an encelphalitogenic cell line derived from DA rats. The results of EAE susceptibility were similar. Again, in these chimeric Lewis rats given GvH disease, perivascular cells were the only cells in the CNS that expressed DA-derived MHC antigens.

Because microglial cells are the only native CNS cells with the membrane antigen recognized by the monoclonal antibody MRC OX-42, which identifies monocytes, macrophages, dendritic cells, granulocytes, and microglia (*19*), immunofluorescent double-labeling was performed with MRC OX-3 and MRC OX-42, and with I1-69 and MRC OX-42, as previously described (*5*). The perivascular cells positive for the Lewis rat MHC molecules were also positive for MRC OX-42 (Fig. 2). The close relation of these perivascular cells to other known types of APCs is further supported by their selective positivity with the murine monoclonal antibodies ED-1 and ED-2 that identify cells of this type in tissues of the immune system (*20*). Since OX-42$^+$, ED-1$^+$, and ED-2$^+$ cells are present in similar numbers and location in the CNS of healthy naïve rats, it seems certain that they are a normal CNS constituent and not merely macrophages entering the CNS in response to the chimeric state or GvH disease. Endothelial cells that expressed Lewis rat MHC molecules were not detected in DA or BN chimeras, and no cells doubly positive for the astrocyte marker protein glial fibrillary acidic protein and Lewis MHC molecules were found. No inflammation or structural alteration was noted in the nervous system of these chimeras with GvH disease. Thus, the perivascular microglia resident in the CNS of these chimeric rats were bone marrow–derived and were able to express the appropriate MHC restriction molecules required to present antigen to the encephalitogenic Lewis T-helper cells used to induce EAE.

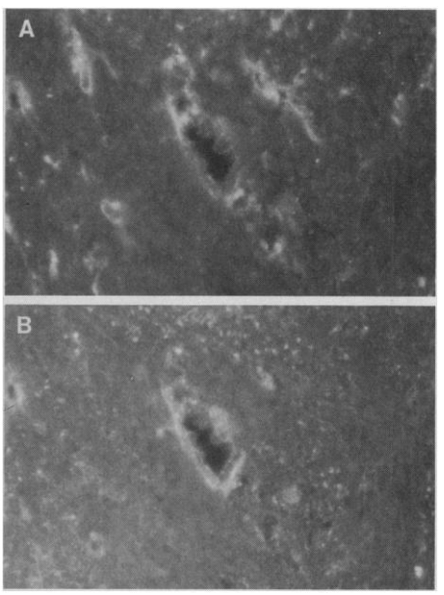

Fig. 2. Immunofluorescent double-labeling of the neural parenchyma in a chimeric DA rat with GvH disease demonstrating (**A**) microglial cells, some in a perivascular location, that are positive (MRC OX-42 antibody with rhodamine); (**B**) some of the same perivascular cells express Lewis Ia molecules [MRC OX-3 antibody with fluorescein isothiocyanate (FITC), ×300].

The terminology, origin, physiology, and role of these perivascular cells has long been the subject of debate that was based on data obtained from various experimental systems (*18*). Our experiments provide strong evidence that in the rat the perivascular microglia derive from a bone marrow precursor and enter the CNS to assume their positions within 2 months. In addition, it appears that they can function as APCs. It is probable that the rare arborizing parenchymal cells expressing Lewis MHC molecules are also microglial cells; however, we were unable to confirm this by double-labeling because of their rarity.

These studies do not necessarily exclude the possibility that astrocytes or endothelial cells or both may function as APCs in the CNS. Because of the way these chimeras were constructed, this possibility could not be explored. Nevertheless, our results do show that the perivascular microglia are sufficient for antigen presentation in the induction of EAE in vivo. In their anatomic arrangement, wrapping about blood vessels with diameters corresponding to those of venules or arterioles, the perivascular microglial cells are ideally situated to contact cells entering the CNS from the blood. Their relation to cells of the monocyte-macrophage-dendritic cell family, as demonstrated by their MRC OX-42, ED-1, and ED-2 positivity, is another feature in favor of their potential APC role.

On the basis of our findings, we propose that the perivascular microglia, and possibly their parenchymal counterpart, can function as the accessory APCs in the CNS. As such, they are appropriate subjects for in vitro investigation of their roles in antigen processing and interactions with T lymphocytes.

REFERENCES AND NOTES

1. C. F. Barker and R. E. Billingham, *Adv. Immunol.* **25**, 1 (1971); D. E. Griffin, J. L. Hess, F. Moktarian, in *Neuroimmunology*, P. Behan and F. Spreafico, Eds. (Raven Press, New York, 1984), pp. 193–206.
2. L. A. Lampson and C. A. Fisher, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6476 (1984); R. I. Craggs and H. DeF. Webster, *Acta Neuropathol.* **68**, 263 (1985); L. A. Lampson and W. F. Hickey, *J. Immunol.* **136**, 4054 (1986).
3. A. Fontana, W. Fierz, H. Wekerle, *Nature (London)* **307**, 273 (1984); *Transplantation* **38**, 293 (1984); D. K. Male, G. Pryce, C. C. W. Hughs, *Immunology* **60**, 453 (1987); R. M. McCarron, O. Kempski, M. Spatz, D. E. McFarlin, *J. Immunol.* **134**, 3100 (1985); P. T. Massa, V. ter Meulen, A. Fontana, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4219 (1987).
4. U. Traugott, *Springer Seminars Immunopathol.* **8**, 71 (1985); ———, L. C. Sheinberg, C. S. Raine, *J. Neuroimmunol.* **8**, 1 (1985); R. A. Sobel, B. W. Blanchette, A. K. Bahn, R. B. Colvin, *J. Immunol.* **132**, 2393 (1984); Y. Matsumoto, N. Hara, R. Tanaka, M. Fujiwara, *ibid.* **136**, 3668 (1986).
5. W. F. Hickey, J. P. Osborn, W. M. Kirby, *Cell. Immunol.* **91**, 528 (1985).
6. A. Ben-Nun, H. Wekerle, I. R. Cohen, *Eur. J. Immunol.* **11**, 195 (1981); J. H. Holda and R. H. Swanborg, *ibid.* **12**, 425 (1982).
7. A. A. Vandenbark, T. Gill, H. Offner, *J. Immunol.* **135**, 223 (1985).
8. A. A. Vandenbark, G. Nilaver, G. Konat, P. Teal, H. Offner, *J. Neurosci. Res.* **16**, 643 (1986); E. Beraud, T. Reshef, A. A. Vandenbark, H. Offner, R. Fritz, C-H. J. Chou, I. R. Cohen, *J. Immunol.* **136**, 511 (1986).
9. D. E. Singer, M. J. Moore, R. M. Williams, *J. Immunol.* **126**, 1553 (1981).
10. I. R. Cohen, in *Progress in Immunology V*, Y. Yamamura and T. Tada, Eds. (Academic Press, New York, 1984), pp. 1129–1137.
11. J. P-Y. Ting, D. F. Nixon, L. P. Weiner, J. A. Frelinger, *Immunogenetics* **17**, 295 (1983).
12. H. Kimura and D. B. Wilson, *Nature (London)* **308**, 463 (1984).
13. F. G. H. Chou *et al.*, *Neurochemistry* **28**, 1051 (1977); M. D. Mannie, P. Y. Paterson, D. C. U'Pritchard, G. Flouret, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5515 (1985).
14. W. F. Hickey, N. K. Gonatas, H. Kimura, D. B. Wilson, *J. Immunol.* **131**, 2805 (1983).
15. E. R. Unanue, *Annu. Rev. Immunol.* **2**, 395 (1984).
16. W. F. Hickey and H. Kimura, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2082 (1987).
17. W. R. McMaster and A. F. Williams, *Eur. J. Immunol.* **9**, 426 (1979).
18. C. L. Dolman, in *Textbook of Neuropathology*, R. L. Davis and D. M. Robertson, Eds. (Williams and Wilkins, Baltimore, 1985), pp. 117–137; H. Hager, *Acta Neuropathol. Suppl.* **6**, 279 (1975); R. L. Schelper, E. Whitter, M. N. Hart, *J. Neuropathol. Exp. Neurol.* **44**, 332 (1985).
19. A. P. Robinson, T. M. White, D. W. Mason, *Immunology* **57**, 239 (1986).
20. C. D. Dijkstra *et al.*, *ibid.* **54**, 589 (1985).

21. Supported by grants from the National Multiple Sclerosis Society (RG-1685) and NIH grant KO7-NS0087.

17 July 1987; accepted 24 November 1987