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Studies on the mechanism of protection from acute viral encephalomyelitis by delayed-type hypersensitivity inducer T cell clones

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SUMMARY

Previous studies have shown that mice can be protected from a lethal infection with the neurotropic JHM strain of mouse hepatitis virus (JHMV) by the adoptive transfer of delayed-type hypersensitivity (DTH)-inducer T cell clones specific for the virus. Protection does not involve the suppression of virus replication in the central nervous system (CNS) or via augmentation of the antiviral antibody response. In the present report we have compared the CNS lesions induced by JHMV in lethally infected and T cell clone protected mice. The presence of virus-specific T cell clones induced a transient increase in mononuclear cell infiltration into the parenchyma of the brains of protected mice, consistent with previous data suggesting that a DTH response was responsible for protection. Immunohistochemical studies suggested further that virus was not replicating in the ependyma or cellular infiltrate, but that the presence of the T cell clone prevented neuronal infection. While the mechanism of effectively altering the in vivo cellular tropism is unknown, survival is accompanied by increased specific destruction of target tissues with fulminant CNS demyelination and an increased incidence of persistent infection.

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INTRODUCTION

Primary demyelination, i.e., the loss of central nervous system (CNS) myelin with relative preservation of axons, is an important pathological process seen in several human diseases including multiple sclerosis. A number of viral infections induce primary demyelination in experimental animals (Martin and Nathanson 1979). One model system that has been studied extensively is the JHM strain of mouse hepatitis virus (JHMV) infection of mice. JHMV is a member of the coronaviridae family of enveloped RNA viruses. In general, these viruses produce acute disease via direct cellular cytolysis. These viruses infect a wide range of animal hosts, including man, and cause a variety of chronic diseases by virtue of their ability to persist in different tissues (Wege et al. 1984). In humans, this group of viruses represents one of the etiological agents causing the common cold; however, it also has been associated with acute gastroenteritis in infants and multiple sclerosis (Wege et al. 1984).

The pathogenesis of JHMV has been studied extensively in mice and to a lesser extent in rats and primates (Erlich and Fleming 1985). The pattern of disease induced by infection with JHMV depends upon a variety of parameters, including the haplotype of the animal used, the viral strain, amount of virus administered and the route of inoculation (Wege et al. 1984). Although the exact outcome of JHMV infection depends on a number of these variables, the general pathological picture of an acute encephalomyelitis with primary demyelination due to a cytolytic infection of oligo-dendroglia is preserved. The CNS pathology of the acute JHMV infection in mice was initially described by Bailey et al. (1949) and has subsequently been described by numerous investigators (Lampert et al. 1973; Nagashima et al. 1978; Erlich and Fleming 1985). The pathology of chronic infection with JHMV has been less well documented but in general consists of focal areas of primary demyelination in the CNS lasting more than one year after the acute infection (Erlich et al. 1987).

Immunosuppression of mice infected with JHMV does not prevent demyelination (Weiner 1973) indicating that the demyelination is not a direct result of the immune response, which is consistent with the finding that the virus is cytolytic for oligodendro-glial cells (Lampert 1973). The antiviral antibody response has been implicated as one protective mechanism because early passive immunization with both neutralizing and non-neutralizing monoclonal antibodies prevents death due to JHMV (Buchmeier et al. 1984; Wege et al. 1984; Nakanaga et al. 1986; Fleming et al. 1989). However, antibody-mediated protection from a lethal infection does not result from suppression of virus replication in the CNS, and the extent of demyelination is increased (Buchmeier et al. 1984). It was therefore suggested that antibody prevented death by altering the local environment within the CNS, preventing or minimizing the infection of neurons, which would be more likely to result in death than the infection of oligodendroglial cells (Buchmeier et al. 1984). This concept is consistent with recent data showing that variants of JHMV infect primarily oligodendroglial cells and cause severe acute demyeli-nation with little or no evidence of encephalomyelitis or neuronal infection (Fleming et al. 1986, 1987). These variants also replicate in the CNS to essentially the same extent as lethal strains of JHMV (Fleming et al. 1986, 1987).

Relatively little is known about the role of cell-mediated immunity to JHMV in preventing a lethal infection, suppressing virus replication in the CNS or contributing to chronic infection. Our laboratory has shown that natural killer (NK) cells are induced following JHMV infection, even in the absence of detectable interferon (Stohlman et al. 1983). However, NK cell induction could not be correlated with either the genetically natural resistance of SJL mice to JHMV or an altered pathogenesis in NK deficient beige mice (Stohlman et al. 1983; Fleming, personal communication), suggesting that NK cells probably do not play a critical role in protection. A macrophage has been implicated in the natural resistance of SJL mice to JHMV infection (Stohlman et al. 1980); however, in vitro analysis of macrophage-mediated antiviral activity was unable to substantiate either a direct or indirect role for macrophages in suppressing virus replication (Stohlman et al. 1982).

To better understand the role of cell-mediated immunity during JHMV infection, JHMV-specific T lymphocyte clones were isolated and characterized (Woodward et al. 1984). These clones proliferate in vitro in response to the E2 virion major envelope glycoprotein in an I-A restricted manner and have the cell surface phenotype Thy-1$^+$, Lyt-1$^+$, CD4$^+$. They induce an I-A restricted local delayed-type hypersensitivity (DTH) response in vivo, indicating they are of the DTH-inducer phenotype (Woodward et al. 1984). In addition all four clones tested secrete gamma-interferon and interleukin-2 suggesting they are of the Th1 subset (Mosman and Coffman 1987). Adoptive transfer of these T cell clones prevents an otherwise lethal JHMV infection (Stohlman et al. 1986). Preliminary experiments indicate that these cells can be transferred up to 4 days prior to infection and still provide protection to recipients (unpublished observation). This suggests that the clones may survive in vivo for 4 days. Similar to the results of antibody-mediated protection (Buchmeier et al. 1984), these DTH-inducer T cell clones did not suppress virus growth in the CNS of protected mice (Stohlman et al. 1986). This suggests that the antiviral immune response to JHMV may have at least 2 distinct components, one that prevents death during the acute phase of the infection and a separate component(s) that eliminates infectious virus from the CNS. In this paper, we have carefully compared the pathological changes in the CNS of mice succumbing to JHMV infection to those in mice protected from the lethal phase of the disease by the adoptive transfer of a DTH-inducer T cell clone to better understand the mechanism of protection afforded by the induction of a virus-specific DTH response in the CNS, especially as it relates to the establishment of a latent CNS infection with chronic demyelination.

MATERIALS AND METHODS

Mice

C57BL/6J mice were purchased from The Jackson Laboratory, Bar Harbor, ME, at 6 weeks of age. Mice were used as recipients in the adoptive transfer experiments within 5 days of arrival. Pre-bleeds obtained from representative mice tested seronegative for MHV by ELISA (Fleming et al. 1986).

Virus

The DS small plaque variant of the neurotropic JHMV strain of mouse hepatitis virus was used throughout (Stohlman et al. 1982). DS produces a very high incidence of persistent CNS infection at sublethal doses (Stohlman et al. 1982). The virus was propagated on DBT cells, a continuous murine astrocytoma, as previously described (Stohlman et al. 1982). Mice were inoculated intracerebrally (i.c.) with 0.03 ml PBS containing approx. 5×10^3 plaque-forming units (PFU) of JHMV.

T cell clones

The isolation and characterization of the JHMV-specific T cell clones have been previously reported (Woodward et al. 1984). Briefly, the cells were cloned by limiting dilution from popliteal lymph node cells of JHMV immunized C57BL/6 mice. The T cell clones were maintained in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10 mM Hepes, 100 U/ml penicillin, 50 μg/ml streptomycin (Gibco Laboratories), 2 mM glutamine (Gibco Laboratories), 5×10^{-5} M 2-mercapto-ethanol, 10% prescreened fetal bovine serum (FBS; Gibco Laboratories), UV-inactivated JHMV antigen, irradiated (2000 rad) syngeneic spleen cells and partially purified interleukin-2 (IL-2) as described by Kimoto and Fathman (1980). The clone used in this report is designated 4B10 (Woodward et al. 1984). This clone was in continuous in vitro culture for approx. 7 months prior to use in these experiments. The cell surface phenotype of 4B10 is Thy-1.2$^+$, Lyt-1.1$^-$, Lyt-1.2$^+$, Lyt-2.1$^-$, Lyt-2.2$^-$ and CD4$^+$ (Woodward 1984; Stohlman et al. 1986).

Adoptive transfer procedure

4B10 cells were expanded into 75-cm^2 flasks (Falcon Plastics, Oxnard, CA). Viable cells were separated by centrifugation on lympholyte-M (Accurate Chemical and Scientific, Westbury, NY). The cells were washed twice with RPMI 1640 medium supplemented with 5% FBS and 10 mM Hepes, pH 7.4. They were suspended in RPMI to a concentration of 3×10^7 cells/ml. Recipients were injected i.c. with 0.03 ml complete medium containing 10^6 cells.

Serum antibody determinations

Virus-specific IgM and IgG were determined by enzyme-linked immunoadsorbent assay (ELISA) as previously described (Stohlman et al. 1986) using goat anti-mouse class specific antibodies (Cooper Biomedica, Cockrenville, PA). Reactions were read at 410 nm with an automated ELISA reader (Dynatech Minireader II). Concentrations were determined by probit analysis in comparison with class-specific monoclonal antibodies (Cooper Biomedical).

Histology

Mice which had received JHMV only were examined at days 4, 5, 7 and 10 post-infection (p.i.). Mice which had received JHMV and 4B10 cells were examined at days 4, 5, 7, 10, 12, 14, 18, 25, 32, 40, 50, 90 and 97 days p.i. All mice were killed by decapitation. Brains were removed and fixed in Clark's fixative (25% acetic acid, 75%

absolute ethanol) for 3 h. Coronal sections of cerebrum and brainstem were dehydrated and embedded in paraffin. All sections were cut at 7 μm and stained with hematoxylin and eosin (H&E).

Immunohistochemistry

Representative sections were stained with the peroxidase–antiperoxidase (PAP) technique to demonstrate JHMV antigen. Sections were deparaffinized with xylene, rehydrated through graded alcohols and pretreated with 0.3% H_2O_2 in methanol. Sections were reacted successively with normal horse serum, anti-JHMV nucleocapsid monoclonal antibody J.3.3. (Fleming et al. 1983), avidin biotinylated horseradish peroxidase complex and 0.01% H_2O_2/diaminobenzidine tetrachloride (DAB) (0.05 mg/ml) prepared in 0.05 M Tris-HCl (pH 7.2). Biotinylated anti-rabbit antiserum and avidin biotinylated horseradish peroxidase complex were purchased from Vector Laboratories (Burlingame, CA); conditions used were those recommended by the manufacturer. Slides were counterstained with Ehrlich's hematoxylin.

RESULTS

At days 4 through 10 p.i. mice infected with JHMV alone were compared to JHMV-infected mice which were protected via the adoptive transfer of 10^6 virus-specific DTH-inducer T cell clones (JHMVT) administered on the same day as the infection. All mice in the unprotected group succumbed to infection by 10–12 days p.i. (Stohlman et al. 1986). Therefore, control mice were examined only at the early time points in infection. At day 4 p.i. a very slight periventricular and perivascular infiltrate was seen in both the JHMV and JHMVT groups. The degree of inflammation was more pronounced at day 5, with mild perivascular cuffing present in both groups (Fig. 1A). The infiltrate, which consisted of both polymorphonuclear and mononuclear cells, penetrated into the parenchyma of both gray and white matter and also involved the leptomeninges. Surprisingly, no differences between the 2 groups were noted at day 4 or 5 p.i. even though maximum virus replication is found at day 5 (Stohlman et al. 1986), which should provide maximum antigenic stimulation to the JHMV-specific DTH-inducer T cell clones.

Meningoencephalitis reached its peak intensity at day 7 p.i. with the presence of thick perivascular cuffs (Fig. 1B). The meningoencephalitis was still prominent at day 10 (Fig. 1C), although less dramatic than at day 7. At both days 7 and 10, perivascular cuffing was more pronounced in the JHMVT group (Fig. 1D) than in the JHMV group, indicative of the induction of a DTH response. Besides showing a severe inflammatory infiltrate, the parenchyma also showed an increased number of microglia and foci of necrosis. No difference in the types of inflammatory cells was seen between the groups. These data suggest that the adoptively transferred T cell clone was able to induce the migration of additional cells into the parenchyma, consistent with the biological and cell surface phenotype of DTH inducer cells.

Moderate to mild encephalitis continued in the surviving JHMVT mice from days 12 to 18 p.i. Perivascular cuffing was generally less marked than at the earlier time
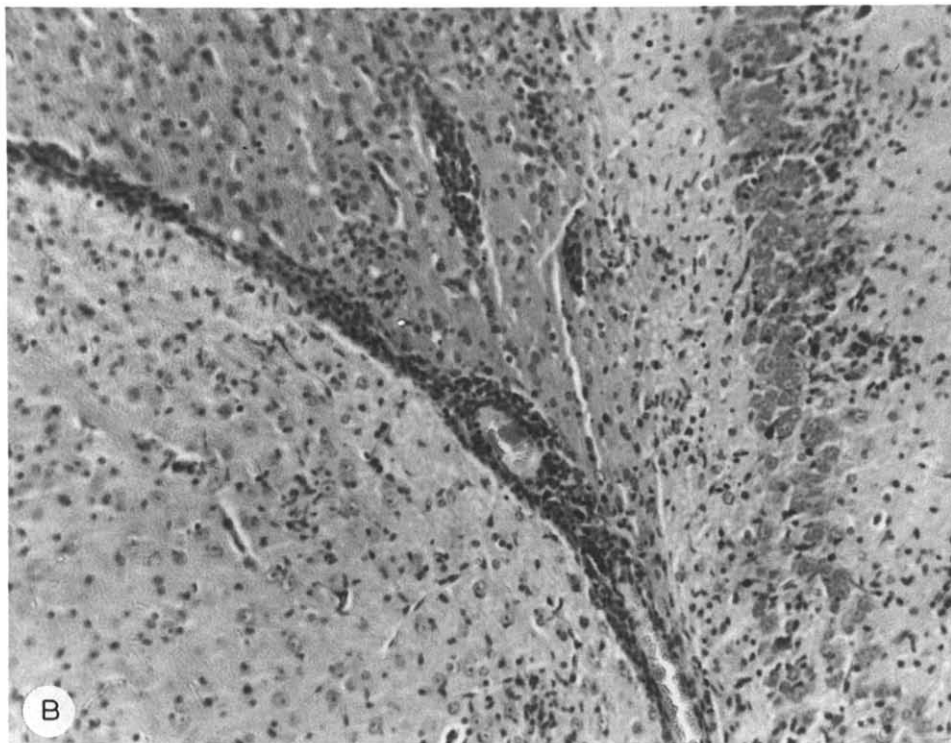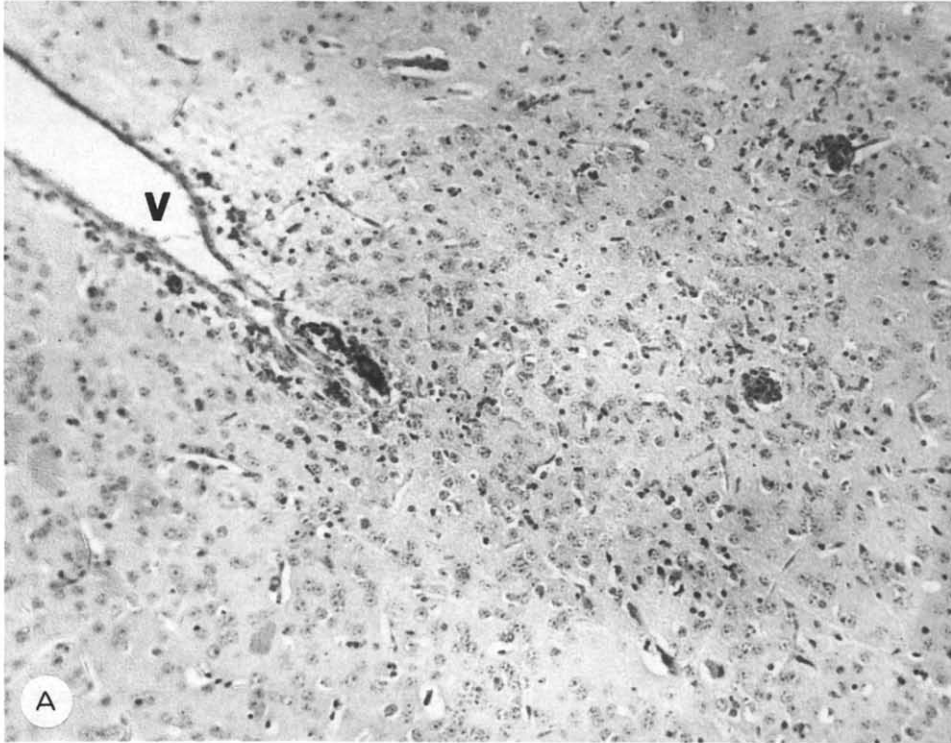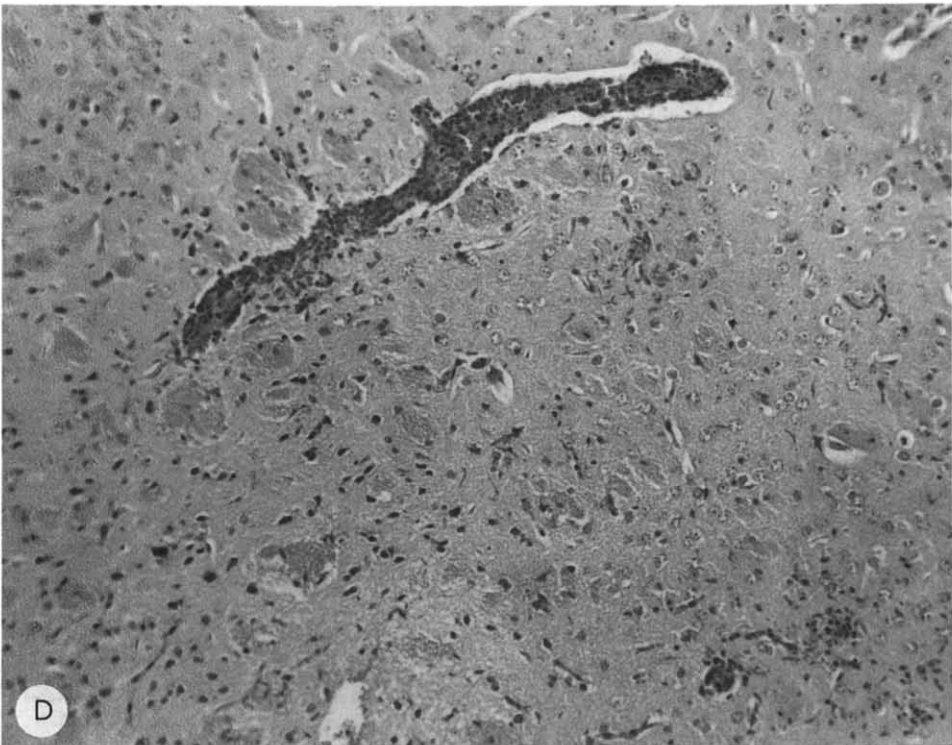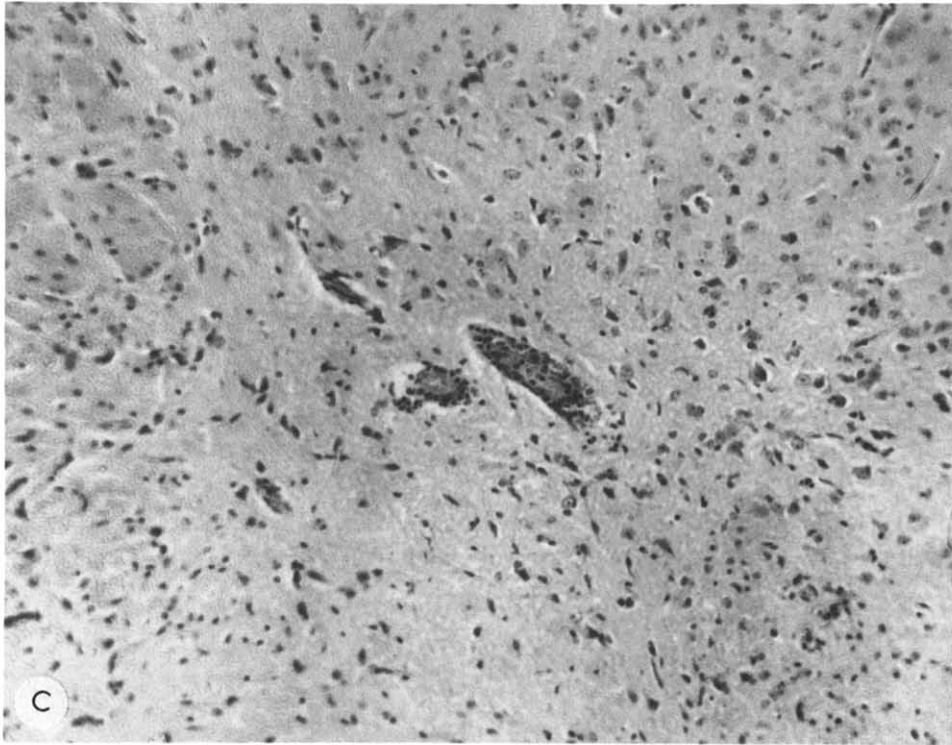
Fig. 1. Encephalitis during the acute phase of JHMV infection. (A) Day 5 p.i., JHMV group. A mild perivascular infiltrate and scattered periventricular inflammatory cells are present. V (ventricle). H&E × 200. (B) Day 7 p.i., JHMV group. A severe parenchymatous infiltrate with thick perivascular cuffs is seen.

H&E × 200. (C) Day 10 p.i., JHMV group. Perivascular cuffing is still prominent, though less striking than on day 7 p.i. H&E × 200. (D) Day 10 p.i., JHMVT group. Perivascular inflammation is more pronounced than in the JHMV group. H&E × 200.

points, consistent with the transient nature of a DTH response (Liew 1982). Inflammatory cells were still present in the parenchyma at days 25, 32, 40 and 50 p.i.; however, perivascular cuffing was minimal. Ongoing periventricular white matter demyelination was seen at days 25, 32 and 50 p.i. (Fig. 2A). At these later time points many subarachnoid space (SAS) plasma cells were found (Fig. 2B), possibly indicating a continuing immunologic response to viral antigens. At day 97 p.i., mild perivascular cuffing by mononuclear cells was still found, though very rarely.

Immunohistochemical analysis

The histological evidence described above suggests that the presence of mononuclear infiltrates in the CNS are a consequence of the adoptive transfer of DTH inducer T cell clones and that during the initial phase of infection (prior to day 7) there is little difference, in the findings between the 2 groups. We have previously shown that the replication of JHMV in the CNS is not decreased in the protected mice (Stohlman et al. 1986). Sections were examined for the distribution of JHMV antigen to determine
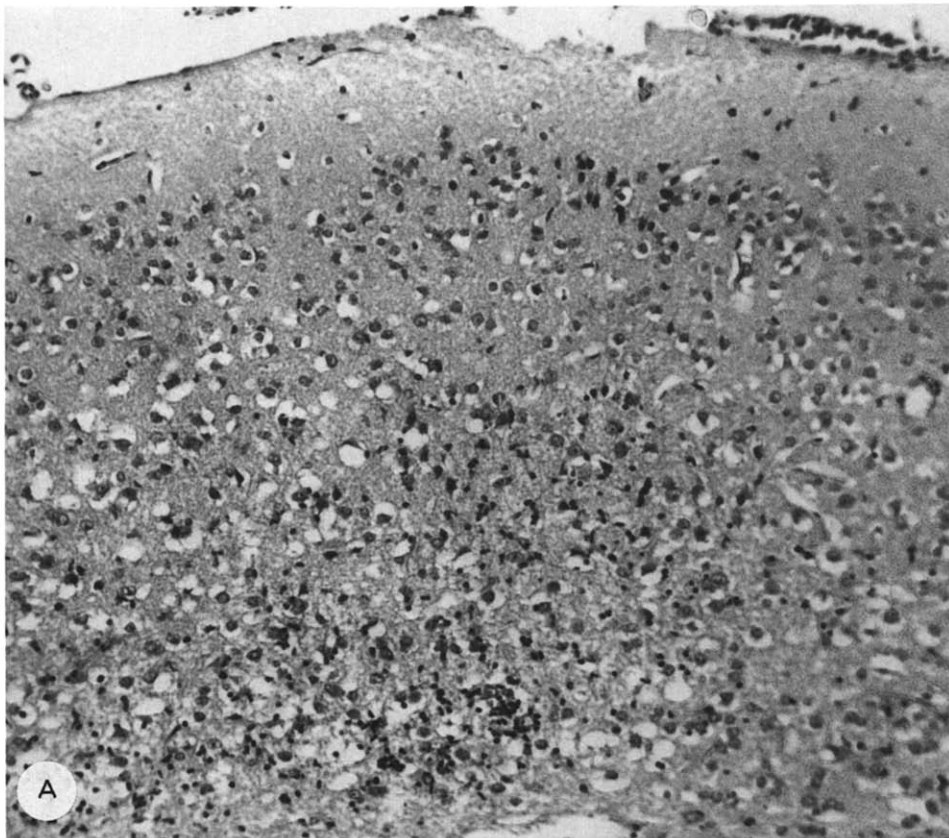

Fig. 2. The chronic phase of JHMV infection. (A) Day 50 p.i., JHMVT group. The white matter infiltrate and vacuolation indicate ongoing demyelination. H&E × 200. (B) Day 50 p.i., JHMVT group. Numerous subarachnoid space plasma cells. H&E × 500.

if the adoptive transfer of the DTH inducer T cells had altered the distribution of infected cells or if the virus present in the CNS of protected mice was due to replication in the infiltrating monocytes. In both untreated and protected mice at day 7, viral antigen was present in neurons (Fig. 3A) and glia (Fig. 3B) as previously described (Fleming et al. 1986). At day 10, viral antigen was also found in glia in both groups and in neurons of the untreated mice (Fig. 3C); however, no viral antigen was found in neurons of the JHMVT group. Viral antigen was not detected in either the ependyma or in the cellular infiltrates in either group of mice. In addition, cells in the periventricular white matter having small round nuclei characteristic of oligodendrocytes were found to be positive for JHMV antigen at day 50 in the JHMVT group. This is consistent with previous reports of persistent JHMV in the CNS of mice surviving the acute disease (Erlich and Fleming 1986). Large PAP positive amorphous clumps were also found in the corpus callosum at this time point (Fig. 3D). Otherwise, no antigen was identified in either neurons or glia during the chronic phase of the infection, i.e., after day 50.
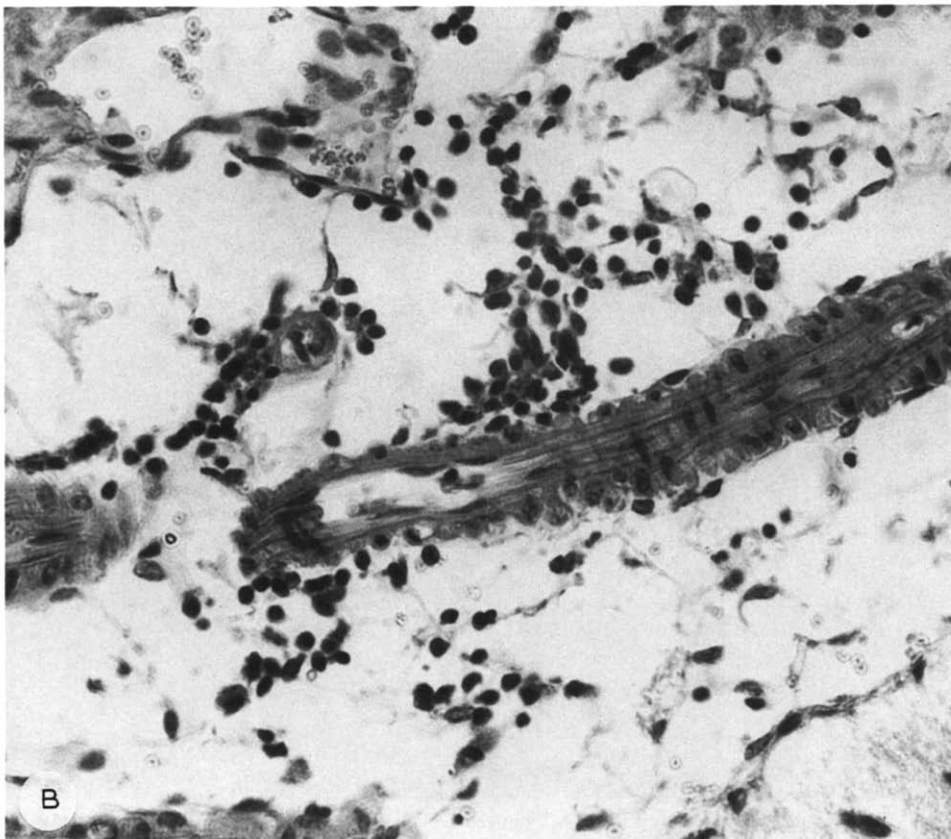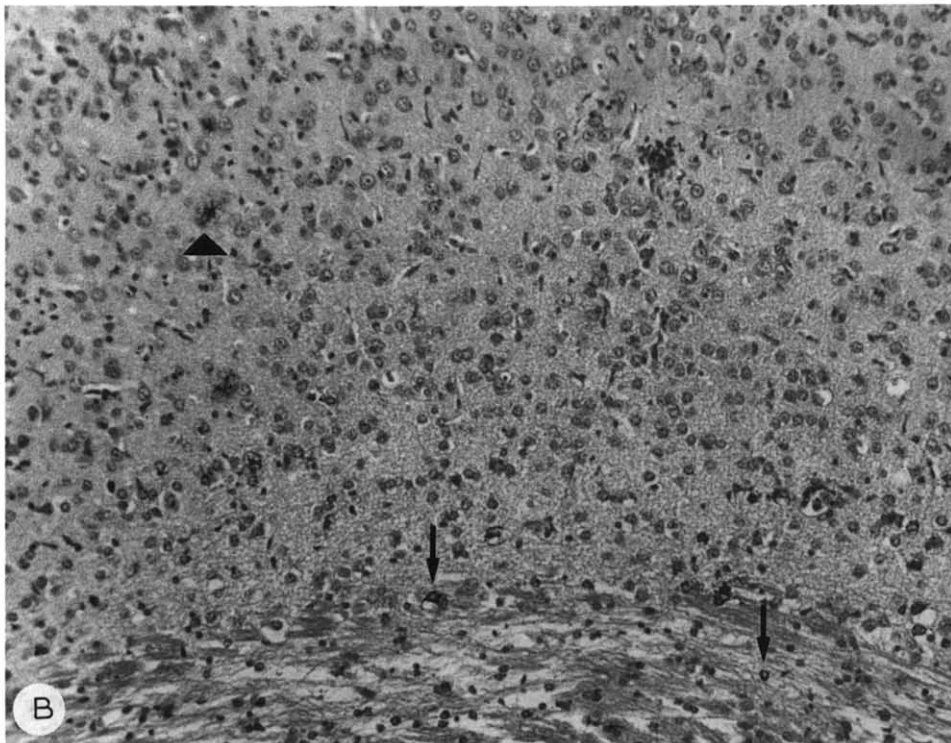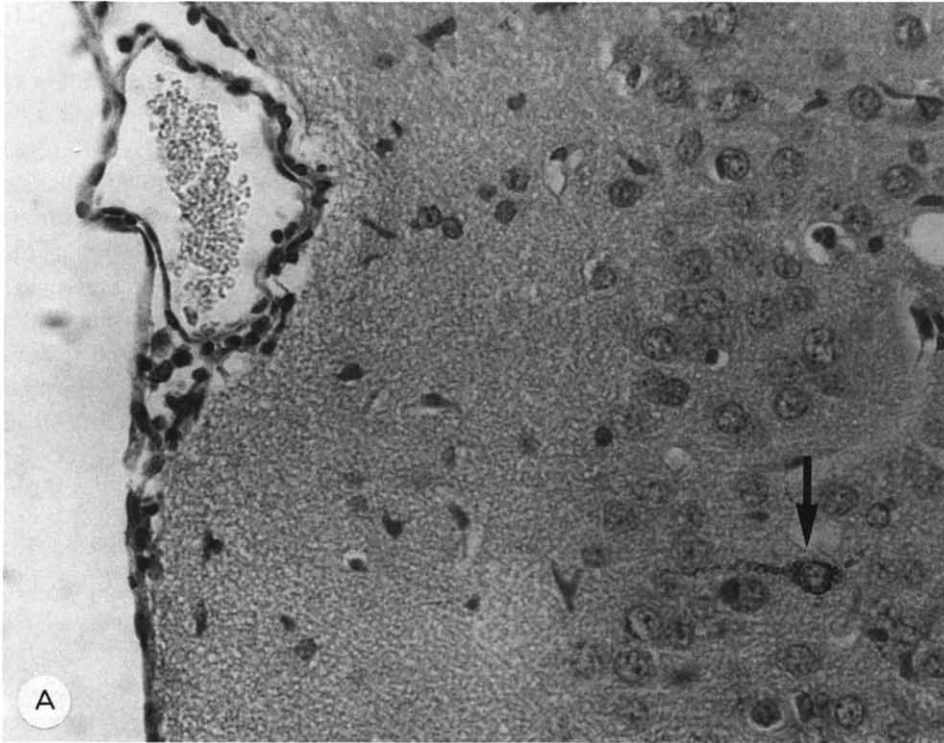
Fig. 3. Immunoperoxidase staining for JHMV antigen. (A) Day 7 p.i., JHMV group. The dark cytoplasmic staining of this cortical neuron (arrow) appears brown by light microscopy and indicates the presence of antigen. PAP × 200. (B) Day 7 p.i., JHMV group. Several small round cells, probably oligodendrocytes, show positive staining (arrows), as well as an occasional astrocyte (arrowhead). PAP × 200. (C) Day 10
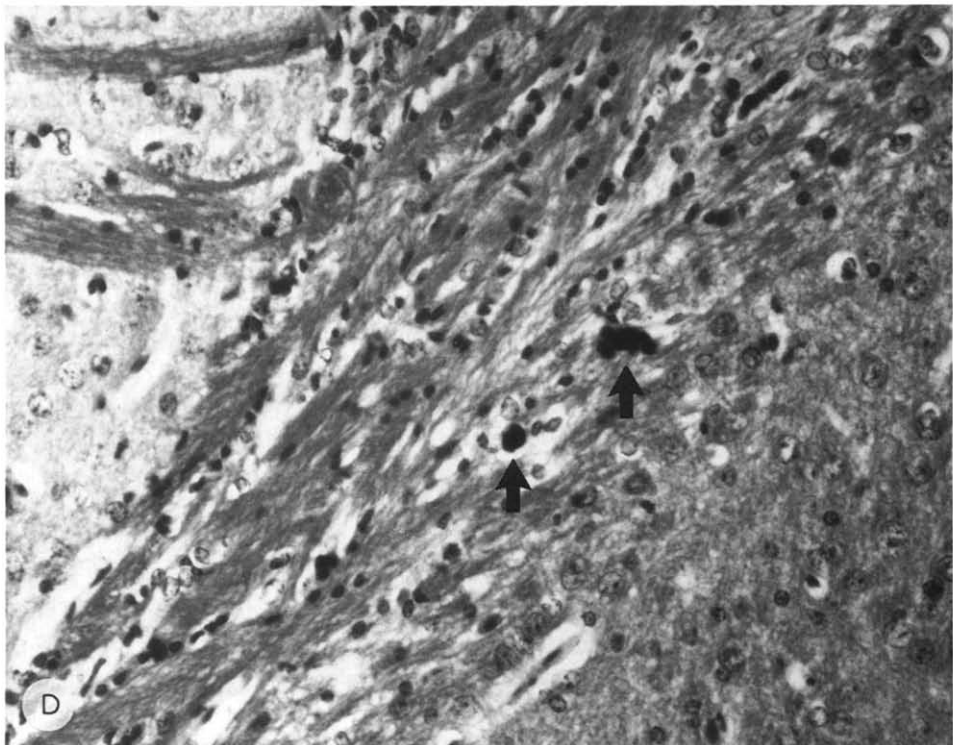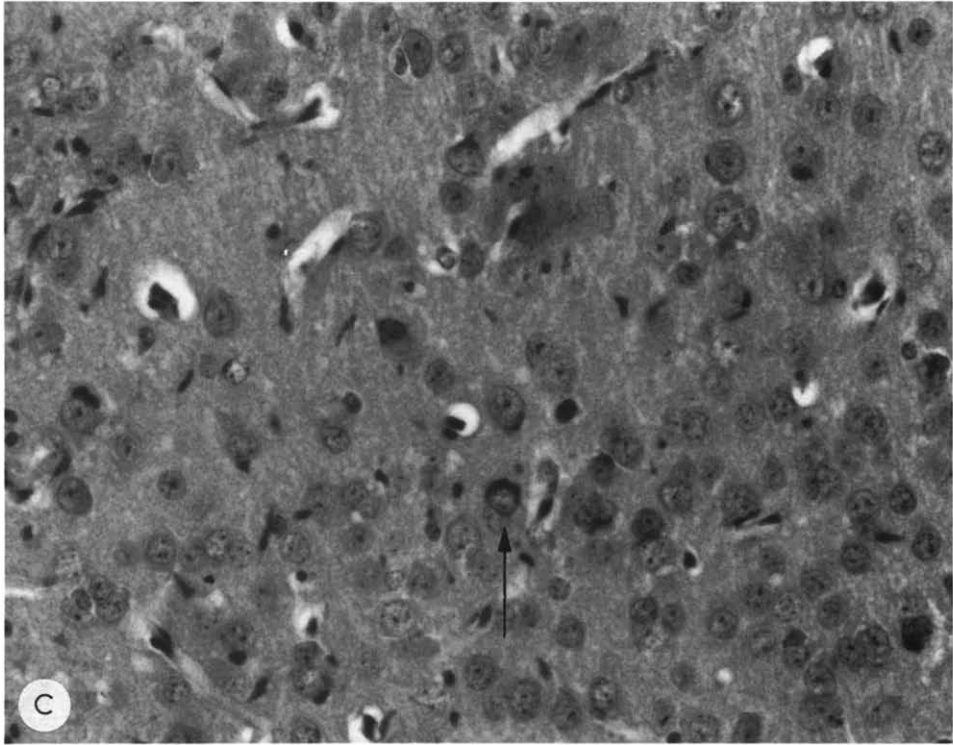
p.i., JHMV group. A small neuron containing antigen (arrow). PAP × 400. (D) Day 50 p.i., JHMVT group. Positive material is present (arrows) in the corpus callosum. The nuclei associated with these clumps are poorly discernible but probably are those of oligodendrocytes. PAP × 400.

TABLE 1

COMPARATIVE SUMMARY OF T CELL-MEDIATED PROTECTION

Clinical, virological and pathological findings	JHMV only	JHMV plus clone 4B10
Virus replication at day 5	No difference 10^5 PFU/g brain	No difference 10^5 PFU/g brain
Antibody response at day 10 IgM	No difference 260 μg/ml	No difference 260 μg/ml
IgG	100 μg/ml	100 μg/ml
JHMV antigen		
Day 7	No difference	No difference
Day 10	oligodendrocytes astrocytes neurons	oligodendrocytes astrocytes
Encephalitis	peaks 5–10 days	peaks 5–10 days
Perivascular cuffing	+ +	+ + + +
Death	10–12 days post-infection	Prevented

DISCUSSION

The protection of mice from an otherwise lethal infection with JHMV by the adoptive transfer of DTH inducer T cell clones occurs without alteration in either the antiviral antibody response or by suppressing virus replication in the CNS (Stohlman et al. 1986) (Table 1). The data in this paper show that the adoptive transfer of DTH inducer T cell clones results in an acute DTH response in the CNS of infected mice coincident with the appearance of viral antigen. However, the major components of the DTH response, i.e., the mononuclear cell infiltration, cytokines, lymphokines and breakdown of vascular permeability are antigen-nonspecific effector mechanisms (Liew 1982). We have recently addressed the role of these nonspecific effector mechanisms by inducing DTH responses to antigens other than JHMV in the CNS of infected mice (Stohlman et al. 1988). We demonstrated that the presence of a vigorous DTH response in the CNS was not able to alter the pathogenesis of a lethal JHMV infection, suggesting that these antigen-nonspecific cells and mediators are not sufficient for protection.

In addition to documenting the histological changes that occur in mice protected from death via the adoptive transfer of the virus-specific DTH inducer T cell clones, we used immunohistochemistry to determine the sites of JHMV replication. We have previously shown that JHMV will replicate in macrophages, including activated macrophages such as those elicited during the DTH response (Stohlman et al. 1982). Therefore, it was important to determine if the infectious virus recovered from the CNS of protected mice was derived from CNS cellular components or from the infiltrating mononuclear cells. The data clearly show no evidence of virus replication in the perivascular mononuclear cells, which comprise the majority of the DTH responding cells.

Protection from a lethal JHMV infection can also be mediated by the passive transfer of monoclonal antibodies specific for all 3 virus structural proteins (Buchmeier et al. 1984; Wege et al. 1984; Nakanaga et al. 1986; Fleming et al. 1989). Virus non-neutralizing antibodies protect just as well as antibodies that can neutralize infectivity in vitro (Wege et al. 1986). Although the mechanism of protection is not clear, even in those instances where neutralizing antibodies have been examined they do not exert their protective effect by suppressing the quantity of infectious JHMV present in the CNS of infected mice (Buchmeier et al. 1984). It has been suggested that protection is due to an alteration in target cell populations infected by the virus. Neurons are clearly infected in unprotected mice, while oligodendroglia are the cells infected predominant in mice receiving passively transferred anti-JHMV antibody (Buchmeier et al. 1984). Although we have previously shown that there is no difference in either the antiviral IgM and IgG responses in mice protected via the adoptive transfer of the virus-specific T cell clones (Stohlman et al. 1986), the data presented here show that the number of neurons infected at day 10 in the JHMVT mice was markedly reduced, if not altogether absent, compared to the control mice undergoing a lethal infection. Although the mechanism that alters the infection of neurons is unclear, in both the case of antibody and DTH inducer T cell mediated protection, immunomodulation clearly alters the effective tropism of JHMV for specific cells in the CNS. In both instances this results in a florid demyelination and paralysis without death of the mice. In addition, there was little or no change in the amount of infectious virus in the CNS compared to mice undergoing a lethal infection involving neurons. Taken together these data suggest that alteration of the specific virus–cell interaction, either by modulating the expression of the virus receptor or via immune therapy, leads to a circumstance in which the animals survive the lethal effects of the virus. However, protected mice exhibit increased evidence of demyelination and a propensity for chronic CNS viral infection.

REFERENCES

Bailey, O.T., A.M. Pappenheimer, F.S. Cheever and J.B. Daniels (1949) A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. II. Pathology. *J. Exp. Med.*, 90: 195–212.
Buchmeier, M.J., H.A. Lewicki, P.J. Talbot and R.L. Knobler (1984) Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated in vivo by monoclonal antibody. *Virology*, 132: 261–270.

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Erlich, S. S. and J. O. Fleming (1985) JHM virus infection: a model of viral-induced demyelinating disease. In: Adachi, M., A. Hirano and S. M. Aronson (Eds.), *The Pathology of the Myelinated Axon*, Igaku-Shoin, Tokyo, Chap. 11, pp. 276–307.

Erlich, S. S., J. O. Fleming, S. A. Stohlman and L. P. Weiner (1987) Experimental neuropathology of chronic demyelination induced by a JHM virus variant (DS). *Arch. Neurol.*, 44: 839–842.

Fleming, J. O., S. A. Stohlman, R. C. Harmon, M. M. C. Lai, J. A. Frelinger and L. P. Weiner (1983) Antigenic relationships of murine coronavirus: analysis using monoclonal antibodies to JHM (MHV-4) virus. *Virology*, 131: 296–307.

Fleming, J. O., M. D. Trousdale, F. A. K. El-Zaatari, S. A. Stohlman and L. P. Weiner (1986) Pathogenicity of antigenic variants of murine coronavirus JHM selected with monoclonal antibodies. *J. Virol.*, 58: 869–875.

Fleming, J. O., M. D. Trousdale, J. Bradburry, S. A. Stohlman and L. P. Weiner (1987) Experimental demyelination induced by coronavirus JHM (MHV-4): molecular identification of a viral determinant of paralytic disease. *Microbiol. Pathogen.*, 3: 9–20.

Fleming, J. O., R. Shubin, M. Sussman, N. Casteel and S. A. Stohlman (1989) Monoclonal antibodies to the matrix (E1) glycoprotein of mouse hepatitis virus protect mice from encephalitis. *Virology*, 168: 162–167.

Kimoto, M. and C. G. Fathman (1980) Antigen reactive T cell clones I. Transcomplementing hybrid I-A region gene products function effectively in antigen presentation. *J. Exp. Med.*, 152: 759.

Lampert, P. W., J. K. Sims and A. J. Kniaze (1973) Mechanism of demyelination in JHM virus encephalomyelitis. *Acta Neuropathol. (Berl.)*, 24: 76–85.

Liew, F. Y. (1982) Regulation of delayed-type hypersensitivity to pathogens and alloantigens. *Immunol. Today*, 3: 18–23.

Martin, J. R. and N. Nathanson (1979) Animal models of virus-induced demyelination. *Prog. Neuropathol.*, 4: 27–50.

Mosman, T. R. and R. L. Coffman (1987) Two types of helper T cell clones. *Immunol. Today*, 8: 223–228.

Nagashima, K., H. Wege, R. Meyermann and V. ter Meulen (1978) Coronavirus induced subacute demyelinating encephalitis in rats: a morphological analysis. *Acta Neuropathol.*, 44: 63–70.

Nakanaga, K., K. Yamanouchi and K. Fujiwara (1986) Protective effect of monoclonal antibodies on lethal mouse hepatitis virus infection in mice. *J. Virol.*, 59: 168–171.

Stohlman, S. A., J. A. Frelinger and L. P. Weiner (1980) Resistance to fatal central nervous system disease by mouse hepatitis virus, strain JHM II. Adherent cell-mediated protection. *J. Immunol.*, 124: 1733–1739.

Stohlman, S. A., P. R. Brayton, L. P. Weiner and M. M. C. Lai (1982a) Murine coronaviruses: Isolation and characterization of two plaque morphology variants of the JHM neurotropic strain. *J. Gen. Virol.*, 63: 265–275.

Stohlman, S. A., J. G. Woodward and J. A. Frelinger (1982b) Macrophage antiviral activity: Extrinsic or intrinsic activity. *Infect. Immun.*, 36: 672–677.

Stohlman, S. A., P. R. Brayton, R. C. Harmon, D. Stevenson, R. G. Ganges and G. K. Matsushima (1983) Natural killer cell activity during mouse hepatitis virus infection: Response in the absence of interferon. *Int. J. Cancer*, 31: 309–314.

Stohlman, S. A., G. K. Matsushima, N. Casteel and L. P. Weiner (1986) In vivo effects of coronavirus-specific T cell clones: DTH inducer cells prevent a lethal infection but do not inhibit virus replication. *J. Immunol.*, 136: 3052–3056.

Stohlman, S. A., M. A. Sussman, G. K. Matsushima, R. A. Shubin and S. S. Erlich (1988) Delayed-type hypersensitivity response in the central nervous system requires viral specificity for protection. *J. Neuroimmunol.*, In press.

Wege, H., S. Siddell and V. ter Meulen (1982) The biology and pathogenesis of coronaviruses. *Curr. Topics Microbiol. Immunol.*, 99: 165–200.

Wege, H., R. Dories and H. Wege (1984) Hybridoma antibodies to the murine coronavirus JHM: characterization of epitopes on the peplomer protein (E2). *J. Gen. Virol.*, 65: 1931–1942.

Weiner, L. P. (1973) Pathogenesis of demyelination induced by a mouse hepatitis virus (JHM virus). *Arch. Neurol.*, 28: 298–303.

Woodward, J. G., G. K. Matsushima, J. A. Frelinger and S. A. Stohlman (1984) Production and characterization of T cell clones specific for mouse hepatitis, strain JHM: in vivo and in vitro analysis. *J. Immunol.*, 133: 1016–1021.