IN VIVO AND IN VITRO MODELS OF DEMYELINATING DISEASES - VIII: GENETIC, IMMUNOLOGIC AND CELLULAR INFLUENCES ON JHM VIRUS INFECTION OF RATS

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Introduction

A number of mouse hepatitis viruses, including MHV-A59, MHV-3, and JHM, have been used as in vivo models of neurotropic viral infections (Hirano et al., 1980; Knobler et al., 1981; Lampert et al., 1973; Le Prevost et al., 1975). The majority of experiments with these agents have involved mice as the host, a system which may be complicated by the viscerotropism of some of these coronaviruses. Previous work in our laboratory (Sorensen et al., 1980, 1982) and by others (Nagashima et al., 1978, 1979; Hirano et al., 1980) demonstrated that rats injected intracerebrally (IC) with JHM virus (JHMV) offer a challenging model for studies of neurotropic agents interacting with the central nervous system (CNS). The presence of anti-coronavirus antibodies in a high proportion of the human population (MacNaughton, 1982) give these models added relevance in regard to enquiries into the viral etiology of human diseases of the central nervous system (CNS), among them multiple sclerosis.

Age-related Genetic Resistance of Rats to CNS Disease

Our studies revealed that rat strains can be classified as either "sensitive" or "resistant" to an IC inoculation of JHMV (Sorensen et al., 1980, 1982). In our experience, the inbred Wistar Furth (WF) strain (obtained from Microbiological Associates, Bethesda, Maryland and Harlan Sprague Dawley, Walkersville, Maryland) is more sensitive at 5 to 21 days of age than the Wistar Lewis (WL) rat (obtained from Charles River Canada Inc., St.-Constant, Quebec). The WL strain used in these experiments was highly susceptible to experimental allergic encephalomyetitis (unpublished data). In a comparable study Nagashima et

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al., (1979) were able to infect weanling Chbb.THOM rats with JHMV suggesting that these and WF rats may be equally sensitive. Eventually, at >21 days of age, the WF strain becomes completely resistant. With WF rats inoculated at 2 days of age the mortality rate is over 90% and remains quite high at 5 days of age and beyond (Table 1, Sorensen et al., 1980, 1982). In contrast, when WL rats are inoculated at 2 days of age their mortality rate is only about 50%. By 5 days only about 10% of WL rats are killed by the injection and by 10 days the mortality rate declines further (Table 1). The increased resistance is, however, accompanied by a shift in neurologic disease, from an acute encephalomyelitis, involving primarily the grey matter of the cerebrum, to a demyelinating disease characterized by lesions confined to the myelinated tracts of the rhombencephalon and spinal cord.

Table 1 - AGE AND STRAIN DEPENDENT SUSCEPTIBILITY OF INBRED RATS TO PARALYSIS AND DEATH FOLLOWING INTRACEREBRAL INOCULATION WITH JHMV

Strain	Age at Inoculation (days)	Number Paralyzed/no. dead at post-inoculation days[a] 1-7	8-14	15-21	>21	No. Inoculated
WL	10	0	0	1/1	0	12
	15	0	0	0	0	17
	21	0	0	0	0	14
	30	0	0	0	0	15
WF	10	0	6/8	9/9	5/5	33
	15	0	0	3/3	2/2	14
	21	0	0	1/1	0	8
	30	0	0	0	0	18
WF (immuno-suppressed)	5				6/6[b]	9

a - Rats which died or were killed in extremis.
b - 2 rats recovered from paralysis and are not included in this column

The heritable control of the development of resistance to the disease was analyzed in the F_1 and F_2 generations of (WF x WL) and among the (F_1 x WF) and F_1 x WL) backcrosses. In all cases the animals were challenged IC with JHMV at 10 days of age. The results of these breeding experiments are summerized in Figure 1. Along with the experimental data are the predicted (p) values calculated from a genetic model which assumes that (a) a single gene controls the resistance trait and (b) in the WF strain the

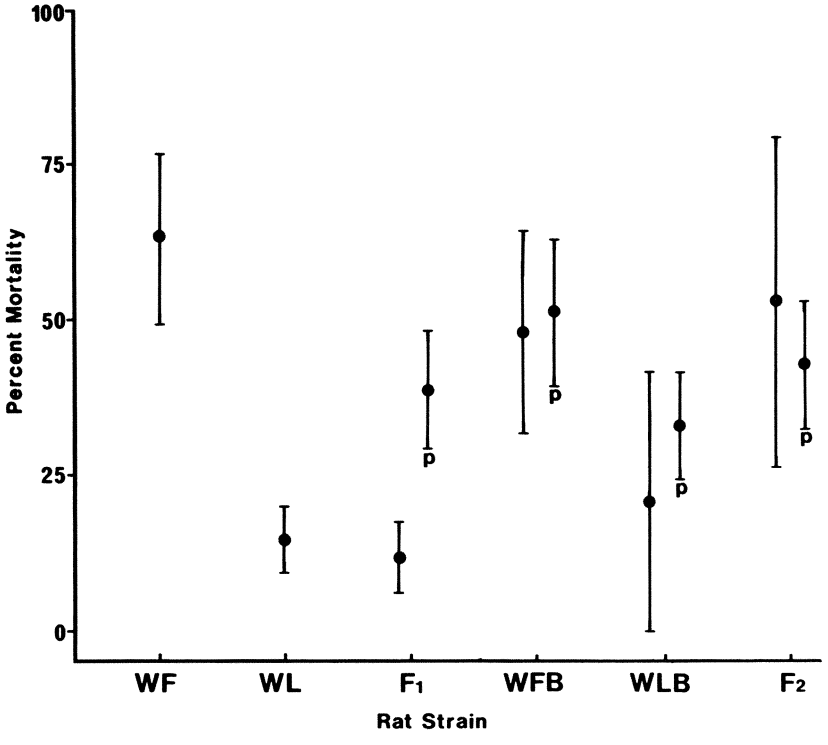

Fig. 1 - The average percent mortalities (with standard errors) of 10 day old WF or WL rats and various cross matings of these strains following intracerebral inoculation with JHMV. Bars desginated "p" indicate values predicted on the assumption that a single gene controls the acquisition of resistance and WF rats were homozygous recessive (rr) and the WL strain was heterozygous (RR,Rr,rr).

WF - Wistar Furth
WL - Wistar Lewis
F_1 - WF x WL
F_2 - F_1 x F_1
WFB - F_1 x WF (Wistar Furth Backcross)
WLB - F_1 x WL (Wistar Lewis Backcross)

gene behaves as a homozygous recessive (rr) while in WL rats it is present in the heterozygous form i.e. RR, Rr, or rr. Generally, the observed values fit reasonably well with those predicted by this model. Only the F_1 generation appeared to be more resistant than predicted (Fig. 1). Although the reason for departure from the predicted value is unknown it is conceivable that among the relatively few WL rats which were bred to produce the F_1 generation, those of the RR genotype predominated. The model, furthermore, presumes an equal representation of homozygous and heterozygous genotypes, an assumption which may not reflect accurately the genetic makeup of the general population or of those individual WL rats selected to produce the F_1 hybrids. These findings suggest that, in the rat, susceptibility to CNS disease provoked by JHMV is under genetic control.

Humoral and Intracerebral Antibody Responses.

A survey of serum JHMV-neutralizing antibody titers indicated that, upon a single IC or intraperitoneal (IP) injection of JHMV or MHV-3, the neutralizing antibody in the serum was not significantly different from that present in uninoculated controls (Sorensen et al., 1982). The low titers, usually <1:10, of endogenous antibodies were most probably the consequence of an endemic infection with SDA or Parker's rat coronavirus, agents possessing some serologic cross-reactivity with members of the mouse hepatitis group (Robb and Bond, 1979). A single injection might be expected to function as a booster to an endogenous coronavirus response. However, inoculation of small amounts of antigen, only 10^4-10^5 pfu/animal and absence of replication outside the CNS may explain the lack of a secondary-type increase in serum antibody titers. Furthermore, the injections were made at about the age when an initial exposure to the endemic agents would be expected to occur.

To determine whether virus introduced IC could elicit localized production of antibody, the cerebrospinal fluids (CSF) of control and infected rats were analyzed for the presence of anti-JHMV IgG. All CSF samples were checked for the presence of erythrocytes and those CSF's contaminated with blood were discarded. The data from radioimmune assays (RIA), summarized in Table 2, revealed that when symptoms of the acute or chronic virus-induced disease were evident, an accompanying 300-600 fold increase in anti-JHMV IgG CSF:serum ratio occurred, implying local production within the CNS (Schliep and Felgenhauer, 1978; Fleming et al., 1983). Among injected rats without clinical or histological indications of disease, including all animals examined after IC inoculation with MHV-3 and some inoculated IC with JHMV, increases in CSF anti-JHMV IgG concentrations were small (Table 2) when compared with control rats inoculated IP with JHMV. Thus, CSF antibody appeared to be formed only in those animals in which JHMV-induced disease occurs, presumably

contingentupon, and associated with, viral replication in the CNS. Nevertheless, localized production of I_gG in the CNS was unable to protect rats from either the acute or chronic neurologic disease

The RIA method employed here detected all subclasses of IgG reactive against all JHMV antigens. Thus, those antibodies relevant to neutralization probably comprised only a fraction of the total IgG detected. The levels reported here for CSF IgG are comparable to those reported for other viral infections of the CNS (Schliep and Felgenhauer, 1978; Martin et al., 1982). By

Table 2 - Anti-JHMV I_gG Concentrations in Cerbrospinal Fluid (CSF) and Serum of Rats after Virus Inoculation

Virus and Inoculation Route	Mean Concentration of Anti-JHMV IgG (ug/ml) Serum	CSF	Ratio $\frac{CSF}{Serum} \times 100$
Controls JHMV 1P No Disease[1] (21 dpi)	1370.0 ± 383.8[2]	0.019 ± 0.016	0.003 ± 0.002
MHV_3 IC No Disease (7-21 dpi)	2225.7 ± 940.6	0.30 ± 0.20	0.032 ± 0.028
JHMV IC No Disease (7-40 dpi)	2384.3 ± 766.0	1.60 ± 0.70	0.071 ± 0.017
JHMV IC Acute Disease (<15 dpi)	3018.0 ± 1235.1	50.5 ± 24.2	1.97 ± 0.89
JHMV IC Chronic Disease (>21 dpi)	1408.3 ± 588.7	6.1 ± 1.7	1.11 ± 0.35

1- No disease judged by lack of clinical and histological indications
2- Standard error of the mean.

contrast, serum antibody levels were much lower than those prevailing during the infection of sheep CNS by the neurotropic Visna virus (Martin et al., 1982).

The latency of JHMV in the CNS of some asymptomatic rats has been demonstrated following abrogation of the immune response by immunosuppression with cyclophosphamide (Sorensen et al., 1982). Thus, when 9 WF rats were immunosuppressed 28 days after IC inoculation with JHMV, 8/9 animals developed clinical signs of disease and infectious virus could be isolated (Sorensen et al., 1982). Immunosuppression, however, was unable to negate the genetically controlled age-related resistance of WL rats, when placed on the immunosuppressive regimen either 7 days prior to or at the time of virus injection at 17 days of age. These observations are consistent with those on mice, which reveal that cell-mediated immunity is of paramount importance in the development of resistance to infections by mouse hepatitis viruses (Sorensen et al., 1982).

Histopathology

Our previous work, based on electron microscopic examination, suggested that in the more prolonged demyelinating disease, viral infection was confined to the glia (Sorensen et al., 1980). However, analysis of viral antigen distribution by indirect immunofluorescent microscopy has demonstrated that cells with the morphology of neurons, contain viral antigen (Fig. 2b). Similarily, Knobler et al., (1980) showed that the neuron was an essential target cell for JHMV infection of SJL mice, a strain shown to have an age-dependent resistance to IC JHMV inoculation. The neuron may also be the initial target or a repository for JHMV during the CNS disease process in rats.

Antigen-containing cells were usually not present in areas with lesions, identified in hematoxylin and eosin (H & E) stained sections (Fig. 2a,3a). Immunofluorescent microscopy revealed that cells with viral antigen were distributed in tissue of relatively normal appearance (Fig. 2a) or adjacent to a lesion (Fig. 3b). Electron microscopic surveys, described previously (Sorensen et al., 1980), were likewise unsuccessful in demonstrating virus particles within lesion areas identified in 1 μm toluidine blue stained sections, but were successful in locating coronavirus particles in cells of adjacent apparently normal tissue.

The current observations imply that JHMV can persist and replicate within neurons and perhaps oligodendrocytes of the CNS without causing rapid death of the cell or eliciting an immune reaction leading to tissue necrosis and possible suppression of the infection. The absence of antigen-positive cells within areas of necrosis implies that virus infection may occur at a time preceeding the development of lesions. It is also conceivable that the demyelination evident in the chronic disease is the

consequence of oligodendrocyte killing by the host's immunologic response rather than cellular dysfunction and death provoked directly by the virus.

Detection of Virus-Related RNA

Detection of antigen-positive cells in the CNS enabled us to pinpoint cells expressing viral protein(s) but provided no data concerning cells which may be latently infected without antigen expression. To examine latency, CNS tissues were surveyed employing a cDNA probe against the murine coronaviruses. The probe was prepared against the smallest mRNA (RNA-A) of MHV-A59 because this molecular species possesses a high degree of homology with JHMV mRNA A. This mRNA species is homologous with the 3' end of all 7 mRNA species found in murine coronavirus infected cells (Cheley et al., 1981a). The isolated and purified mRNA was copied by reverse transcription into cDNA with ^{32}P-dCTP as described

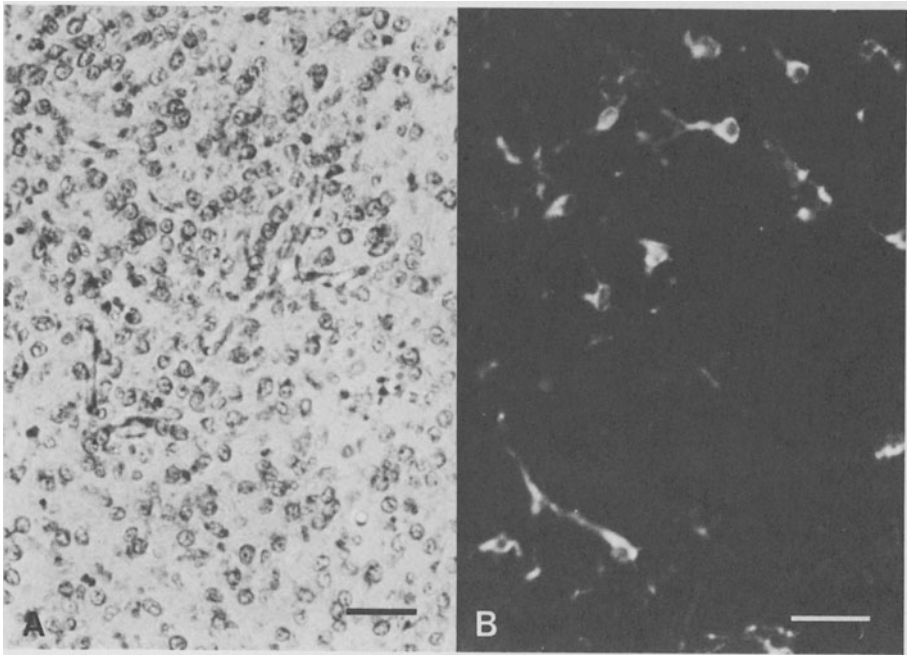

Fig. 2 - A sagittal section through the cerebrum of a 6 day old rat 4 days after IC inoculation with JHMV. H & E staining (a) reveals an apparently normal area of grey matter. However, indirect immunofluorescence staining for JHMV antigens (b) reveals a number of cells, probably neurons, containing viral antigens. Bar - 25 μm.

previously by Cheley et al., (1981b). The product had a specific activity of about 10^8 cpm/μg DNA and was deemed to be adequate for detecting subpicogram quantities of RNA by the dot-blotting procedure.

Animals killed for conducting dot-blot hybridizations on CNS tissues were anesthetized, then dissected to expose and remove the CNS. Selected regions of tissue from half the CNS were cut into samples and snapfrozen. Included were the telencephalon, diencephalon and mesencephalon, cerebellum, pons and myelencephalon, cervical spinal cord, lumbar spinal cord, and whenever possible, the optic nerves. The contralateral half of the CNS was fixed and prepared for histological and immunofluorescent microscopy. RNA extracts from the CNS were

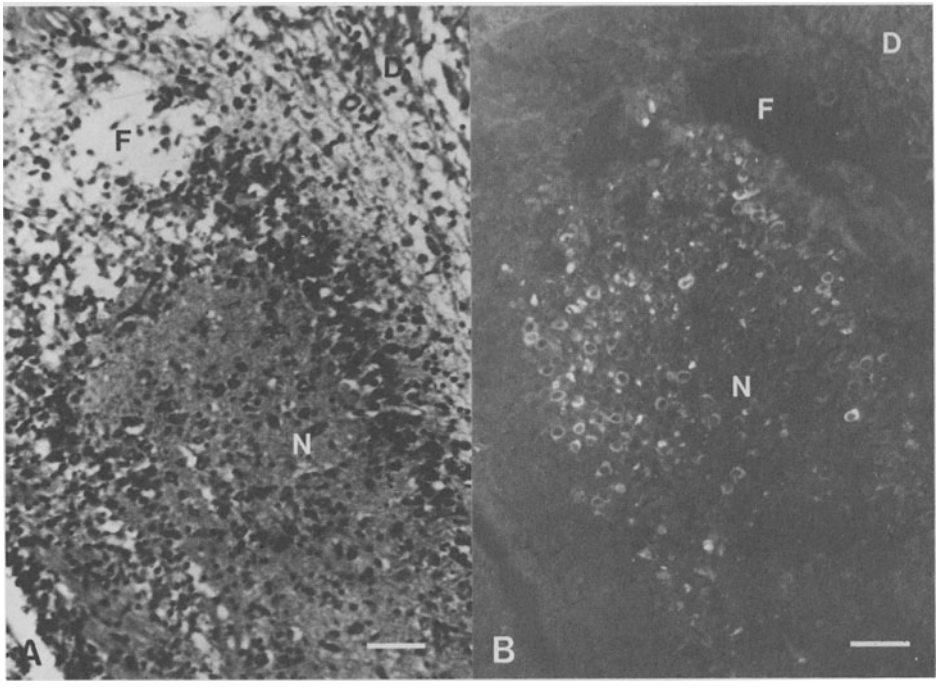

Figure 3

Fig. 3 - A sagittal section showing the hippocampus of a 6 day old rat 4 days after ic inoculation with JHMV. H & E staining (a) reveals a focus of necrosis (F) as well as considerable demyelination (D) while unaffected tissue is seen at N. Indirect immunofluorescent staining for JHMV antigens of an adjacent section (b) reveals presence of a large numer of infected cells in the apparently normal tissue. Note also that the necrotic (F) and demylinated (D) areas are devoid of cells containing JHMV antigens. Bar - 25 μm.

obtained by a modification of the method previously reported (Cheley et al., 1981a). Briefly, frozen tissues were defrosted in 7.6M guanidine-HCl, 0.1M potassium acetate, pH 7.5, to give a final concentration of 100 mg or less tissue/ml. The tissue was disrupted by forcing it several times through a 21g needle at -20°C. The homogenate was then mixed with 0.6 volumes of 95% ethanol, allowed to stand overnight at -20°C, then centrifuged at 5800xg for 20 min. The resultant pellet was dissolved in 1.5 ml 20 mM EDTA, pH 7.0, and extracted with 4.5 ml chloroform/butanol (4:1 v/v). The solvent phase was re-extracted twice more and the 3 aqueous extracts were pooled, made 3M with respect to sodium acetate, pH 6.0, and allowed to stand overnight at -20°C. The suspension was centrifuged at 1,800xg for 1 hour and the RNA pellet was washed with 95% ethanol and dried at -20°C. The dry RNA pellet was then dissolved in 150 µl water, 100 µl formalin and 250 µl of 20 x SSC (300 mM sodium chloride, 30 mM sodium acetate, pH 7.6), heated at 50°C for 15 min and stored at -70°C. For RNA-cDNA hybridization, samples were applied undiluted or at dilutions of 0.5, 0.25, or 0.1 to cellulose acetate then hybridized under stringent conditions.

In general, RNA samples from uninoculated control rats failed to specifically bind the ^{32}P-cDNA. Each extract applied at the cited dilutions was examined for the intensity and size of the dot produced by autoradiography. The data were deemed valid only when an appropriate dose response was obtained, judged by gradation in the size and intensity of dots. The wet weight of tissue in the original sample extracted for RNA in these analyses ranged from about 5 mgm of spinal cord or optic nerves to 50 mgm of telencephalon.

Previous examinations of CNS from diseased rats (Sorensen et al., 1980, 1982) revealed that histopathological lesions frequently had a bilateral distribution although usually not at identical positions in the CNS. Thus, we believe that the histopathology on one hemisphere of the CNS and the detection of viral RNA from the contralateral side provided a valid comparison of these two parameters of the infectious process. The selected examples of dot-blotting data, shown in Figure 4, illustrate the results achieved from uninoculated rats, animals with acute encephalomyelitis, or those in which a chronic, prolonged, demyelinating disease prevailed. Although the data are not shown in this figure, extracts of spinal cord from the animal with acute encephalomyelitis were also positive. It appears that during acute encephalomyelitis JHMV RNA can be detected throughout the CNS, perhaps with the exception of the cerebellum. By contrast, during chronic demyelinating disease viral RNA is present predominantly in the posterior portions of the brain and in the spinal cord (Fig. 4). With respect to the concordance between the presence of viral RNA (determined by dot-blots) and histopathology, ascertained on the opposite side of the brain,

Figure 5 illustrates that correlation could not always be demonstrated. In the selected example of an animal with acute encephalomyelitis there was no agreement between the presence of JHMV RNA and the histopathology observed in the telencephalon, cerebellum or spinal cord. In another rat, with chronic demyelinating disease, no correlation could be demonstrated in the diencephalon or mesencephalon. It should, however, be emphasized that the lack of concordance might be due to the limitation on the number of sections selected for histopathological evaluation.

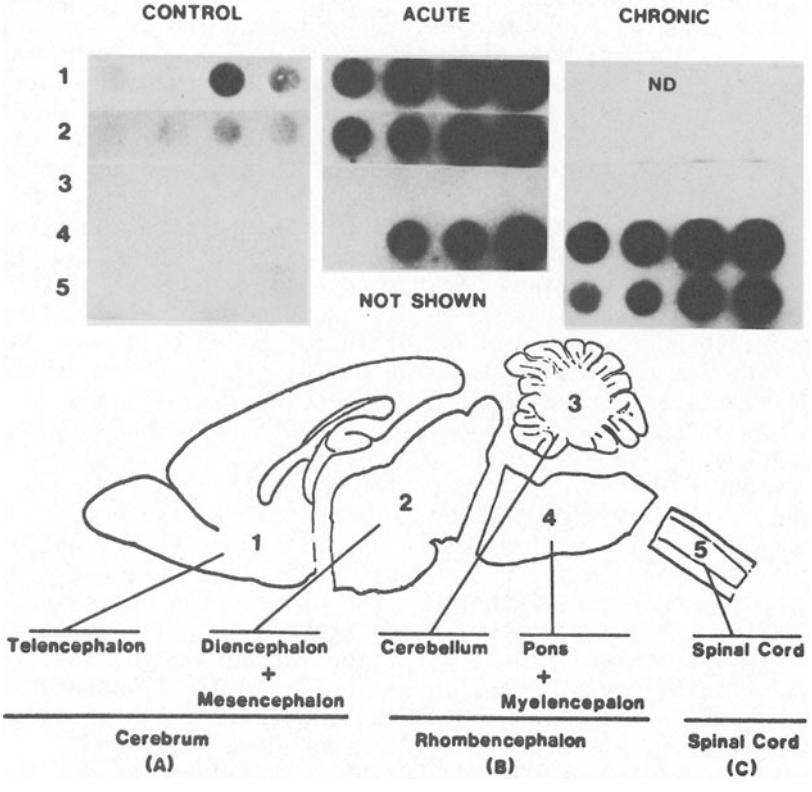

Fig. 4 - Autoradiograms of dot-blot cDNA hybridizations with extracts of the 5 tissues from rat brain identified in the diagram. Representative blots are presented from an uninoculated control animal, a rat suffering an acute encephalomyelitis and an animal with a chronic demyelinating disease. The 4 dots represent 1/10, 1/5, 1/2 and undiluted samples of each extract. The spinal cord of the animal with acute encephalomyelitis (not shown) was also JHMV RNA positive.
ND - not determined.

More likely, it is due to an absence of viral antigens in necrotic lesions and the presence of viral proteins in cells located within tissues with a histologically normal appearance, as documented by immunofluorescent microscopy. It was, therefore, not surprising to encounter acutely or chronically affected animals, like those exemplified in Figure 5, in which regions such as the diencephalon, mesencephalon, and the cerebella were negative for JHMV RNA but positive in histopathological analysis. Conversely, in areas with a histologically normal appearance, such as the telencephalon of the acutely affected rat, viral RNA was found. Similarily, the presence of JHMV RNA in the histologically normal cervical spinal cord of a rat with acute encephalomyelitis implies that virus infection may precede the development of lesions.

Our previous work revealed that there are profound time-related changes in the distribution of grey and white matter lesions in the CNS due to JHMV infection (Sorensen et al., 1980). The current survey, using JHMV specific cDNA in the dot-blotting

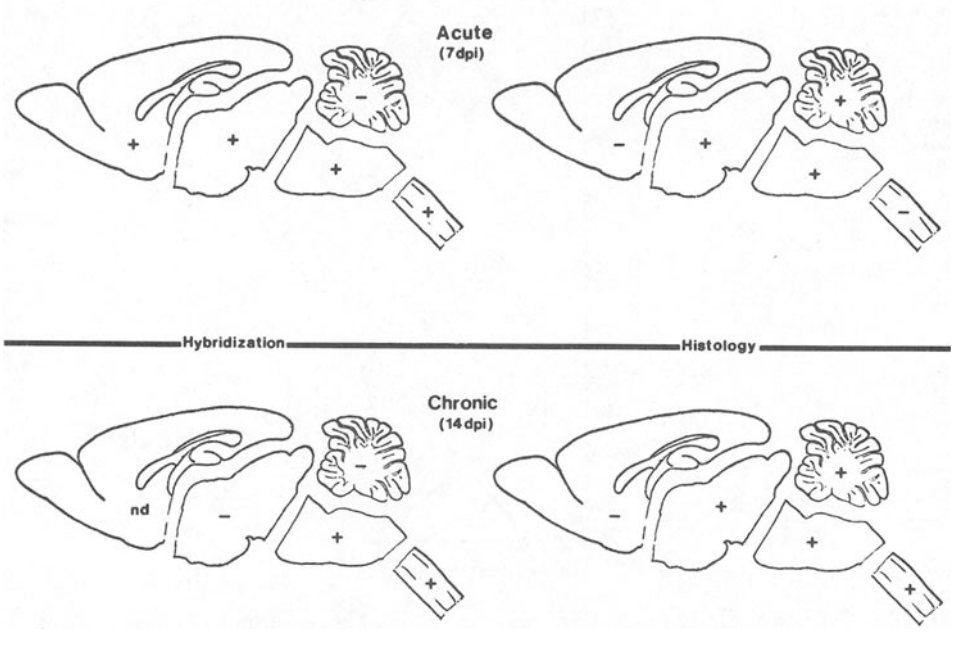

Fig. 5 - A comparison of results from cDNA hybridizations on tissue extracts prepared from half the rat brain with histological examination of the contralateral hemisphere. In hybridization "+" indicates a positive series of dot-blots. In the histology a "+" indicates the presence of lesion(s), identified in of H & E stained sections.
ND - not determined.

hybridization analysis revealed that 7 to 17 days post-inoculation the CNS of animals succumbing to JHMV infection contained virus specific RNA in regions where neuropathology was not evident (Fig. 6) also suggesting that the infection precedes lesion formation.

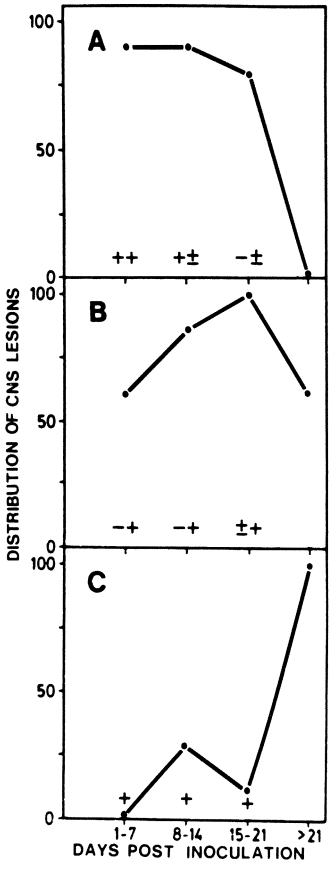

Fig. 6 - Concordance between presence (+) or absence (-) of JHMV RNA and the distribution of histological lesions (the percent of the animals examined with lesion(s) in a particular region of the CNS) plotted as a function of the number of days after IC inoculation.
Panel A - Cerebrum (telencephalon, diencephalon and mesencephalon)
Panel B - Rhombencephalon (Cerebellum, Pons and Myelencephalon)
Panel C - Spinal cord.

JHMV and MHV-3 Infection of Dissociated CNS Cultures

Despite considerable progress with regard to the factors important to coronavirus-induced disease in rats (Bailey et al., 1949; Nagashima et al., 1978, 1979; Hirano et al., 1980; Sorensen et al., 1980, 1982), little is known about the initial cell-virus interactions that occur following IC inoculation, nor how these events might regulate the resulting neurologic disease. Electron microscopic studies of JHMV infected CNS tissues have demonstrated the presence of virus particles in oligodendrocytes and neurons during chronic demyelinating disease (Nagashima et al., 1978; Sorensen et al., 1980) and in astrocytes during acute encephalomyelitis in rats (Nagashima et al., 1978). The nature of the initial tropism of JHMV for particular cells in the CNS, however, remains to be elucidated. To examine these early cell-

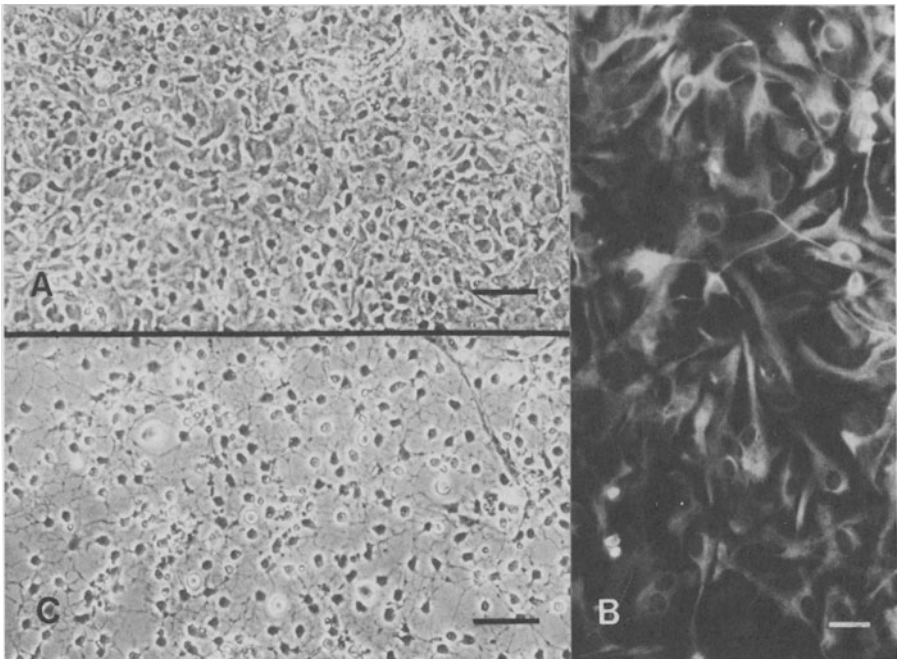

Figure 7

Fig. 7 - The appearance of a 10 day old primary dissociated CNS culture prepared from neomatal rats. A stratified mixed culture (a) consisting of a lower layer of adherent astrocytes and less adherent cells forming a superficial layer. Adherent astrocyte layer (b) stained, by the indirect method, with anti-GFAP antibodies. A shaken culture (c) established by plating less asherent cells removed from the mixed culture. Note the presence of fluorescing intracellular intermediate filamentous structures. Bar - 25 μm

virus interactions under controlled and defined conditions, dissociated primary CNS cultures were prepared from the cerebral corticies of neonatal rats by a modified procedure of McCarthy and de Vellis (1978), using a mechanical dissociation of tissue by serial passages through pipettes of decreasing bore diameter. The cell suspensions, containing astrocytes, oligodendrocytes and neurons, were seeded onto plastic tissue culture dishes and incubated at 37°C.

Such mixed cultures (Fig. 7a) when examined microscopically were observed to contain a layer of adherent cells identified as astrocytes, by presence of glial fibrillary acidic protein (GFAP)in them as revealed by immunofluorescent microscopy (Fig. 7b), and a superficial layer of less adherent cells presumed, on morphological criteria, to be neurons and oligodendrocytes (Bhatt et al., 1980). The less adherent superficial cells could be removed by shaking (McCarthy and de Vellis 1981) so as to produce two types of cultures, one of the adherent cells, enriched in astrocytes (Fig. 7b) and the other consisting of cells from the superficial layer, enriched in oligodendrocytes and neurons but devoid of astrocytes (Fig. 7c).

Since IC inoculation of very young rats with JHMV elicits neurological disease but a similar inoculation with MHV-3, even into the newborns, fails to do so (Hirano et al., 1980; Sorenson et al., 1981, 1982), it was surprizing to discover that in the mixed cultures MHV-3 could replicate at 32.5°C to high titers (Table 3, Fig. 8) and cause cytopathic effects (CPE) among the adherent astrocytic layer. This CPE was characterized by the formation of syncytia similar to those described for L cells and cells of neural origin (Lucas et al., 1977). MHV-3 replication was arrested when infected cultures were shifted to 39.5°C but resumed upon shift-down to 32.5°C (Fig. 9). A similar temperature restriction of coronavirus infection has been reported for cells of neural and other origin (Lucas et al., 1978). In contrast to MHV_3, JHMV replicated only briefly in mixed cultures yielding low titers and failing to cause any apparent CPE (Table 3, Fig. 9). JHMV replicated for more extended periods in only 2/12 cultures still to low titers and without apparent CPE. Variability in the duration from the time of birth, when the cultures were established, until infection was initiated did not influence the infectious process of mixed cultures, despite the continued in vitro maturation processes shown to occur among astrocytes, oligodendroyctes and neurons (Abney et al., 1981).

To determine which cell layer was associated with the replication of which agent the shaken and mixed cultures were inoculated separately with MHV-3 or JHMV. The data, summarized in Table 3, revealed that MHV-3 was unable to replicate efficiently in the shaken cultures consisting primarily of neurons and oligodendrocytes, whereas JHMV replicated equally well in the shaken and mixed cultures. By comparison MHV-3 was replicated for

Table 3 - IN-VITRO REPLICATION OF CORONAVIRUSES IN TOTAL OR SELECTED RAT CORTICAL CELL CULTURES

Days after inoculation	Mixed Cultures		Shaken Cultures	
	MHV_3 *	JHMV *	MHV_3 *	JHMV *
1	1,000-10,000	100-1,000	10-100	100-1,000
2	10,000	100	0-10	100
4	10,000-100,000	0-100	0	0-10
8	10,000-1,000,000	0	0	0

M.O.I.-0.1 pfu/cell
* pfu/ml of supernatant

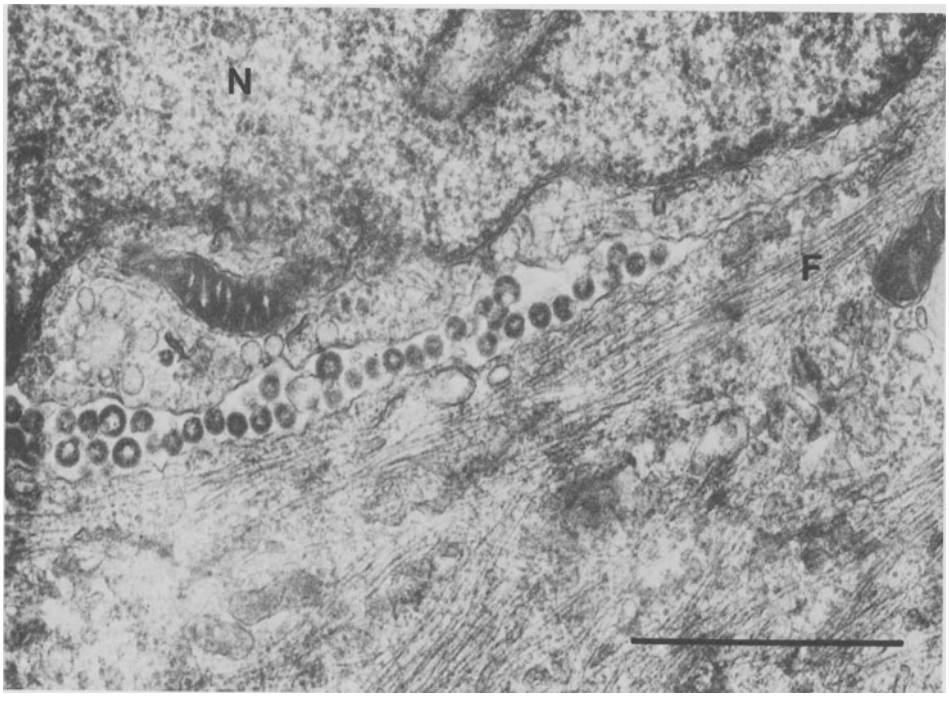

Fig. 8 - A representative electron micrograph of an adherent astrocyte from the lower layer of a mixed culture sampled 28 days after infection with MHV-3. Note numerous coronavirus particles within a cytoplasmic vesicle in the vicinity of a nucleus (N) and organized bundles of cytoplasmic intermediate filaments (F). Bar - 1.0 μm.

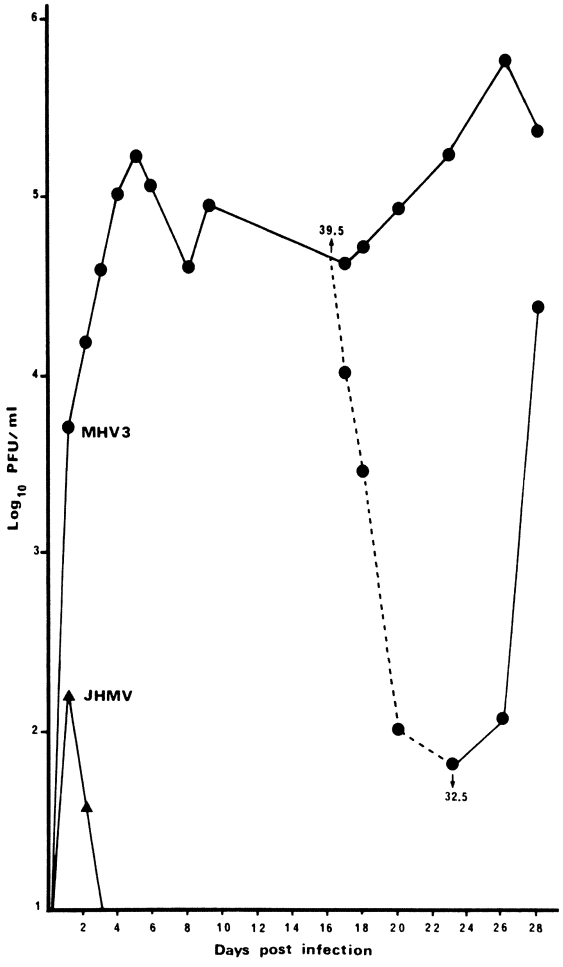

Fig. 9 - Replication of JHMV (▲) and MHV-3 (●) in mixed cultures at the permissive (32.5°C) and restrictive (39.5°C) temperatures. Arrows indicate temperature shifts (↑ up, ↓ down). The M.O.I. in each experiment was about 0.1 pfu/cell.

prolonged periods, >21 days, and to high titers ($\sim 10^5$ pfu/ml) in the adherent astrocytic cultures. Thus, an efficient and persistent MHV-3 replication appears to be confined to the primarily astrocytes while JHMV replication, limited in quantity and duration, appears to be associated with cells transferrable to the shaken cultures, presumed to be enriched in neurons and oligodendrocytes.

Conclusions and Prospective

Data obtained to date show that JHMV-induced neurologic disease in rats represents an interesting model for CNS diseases and might have human relevance. In future analysis of this model it will be essential to (a) obtain explanations for the ability of JHMV but not MHV-3 to cause disease in rats despite the ability of both viruses to replicate in rat cells of neural origin; (b) establish the identity of the primary target cell(s) in the CNS for JHMV; and (c) elucidate the mechanisms for viral persistence and the maintenance of prolonged latency. Host factors are also important and must be investigated further, including the genetically controlled influence on age-related acquisition of resistance to this disease and the manner in which humoral and/or cell-mediated immunity affects the disease process, particularly that connected with the formation of demyelinating lesions.

REFERENCES

Abney, E.R., Bartlett, P.P., and Raff, M.C. 1981. Astrocytes, ependymal cells, and oligodendrocytes develop on schedule in dissociated cell cultures of embryonic rat brain Developmental Biol. 83:301-310.

Bailey, T.O., Pappenheimer, A.M., Cheever, F.S., and Daniels, J.B. 1949. A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. II. Pathology. J. Exp. Med. 90:195-231.

Brahic, M., and Haase, A.T. 1978. Detection of viral sequences of low reiteration frequency by in situ hybridization Proc. Natl. Acad. Sci. U.S.A. 55:6125-6129.

Cheley, S., Anderson, R., Cupples, M.J., Chan, E.C.M.L., and Morris, V.L. 1981a. Intracellular murine hepatitis virus-specific RNA's contain common sequences. Virology 112:596-604.

Cheley, S., Morris, V.L., Cupples, M.J., and Anderson, R. 1981b. RNA and polypeptides homology among murine coronaviruses. Virology 115:310-321.

Fleming, J.O., Ting, J.Y.P., Stohlman, S.A., and Weiner, L.P. 1983. Improvements in obtaining and characterizing mouse cerebrospinal fluid. Application to mouse hepatitis virus-induced encephalomyelitis. J. Neuroimmunol. 4:129-140.

Hirano, N., Goto, N., Ogawa, T., Ono, K., Murakawi, T., and Fujiwara, K. 1980. Hydrocephalus in suckling rats infected intracerebrally with mouse hepatitis virus, MHV-A59. Microbiol. Immunol. 24:825-834.

Knobler, R.L., Haspel, M.V., and Oldstone, M.B.A. 1981. Mouse hepatitis virus type 4 (JHM strain)-induced fatal central nervous system disease. I. Genetic control and the murine neuron as the susceptible site of disease. J. Exp. Med. 153:832-843.

Lampert, P.W., Sims, J.K., and Kiazeff, A.J., 1973. Mechanism of demyelination of JHM virus encephalomyelitis, Electron microscopic studies. Acta Neuropathol. 24:76-85.

Le Prevost, C., Virelizier, J.L., and Dupuy, J.M. 1975. Immunopathology of mouse hepatitis virus type 3. III. Clinical and virologic observations of a persistent viral infection. J. Immunol. 115:640-643.

Lucas, A., Coulter, M., Anderson, R., Dales, S., and Flintoff, W. 1978. In vivo and in vitro models of demyelinating diseases. II. Persistence and host-regulated themosensitivity in cells of neural derivation infected with mouse hepatitis and measles viruses. Virology 88:325-337.

Lucas, A., Flintoff, W., Anderson, R., Percy, D., Coulter, M., and Dales, S. 1977. In vivo and in vitro models of demyelinating diseases: Tropism of the JHM strain of murine hepatitis virus for cells of glial origin. Cell 12:553-560.

MacNaughton, M.R. 1982. Occurrence and frequency of coronavirus infections in humans as determined by enzyme-linked immunosorbent assay. Infect. Immun 38:419-423.

Martin, J.R., Goudswaard, J., Palsson, P.A., Georgsson, G., Petursson, G., Klein, J., and Nathanson, N. 1982. Cerebrospinal fluid immunoglobulins in sheep with Visna, a slow virus infection of the central nervous system. J. Neuroimmunol. 3:139-148.

McCarthy, K.D., and de Vellis, J. 1978. Alpha-adrenergic receptor modulation of beta-adrenergic, adenosine and protaglandin E_1 increased adenosine 3':5'-cyclic monophosphate levels in primary cultures of glia. J. Cyclic Nuc. Res. 4:15-26.

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

Nagashima, K., Wege, H., Meyermann, R., and ter Meulen, V. 1979. Demyelinating encephalomyelitis induced by long-term coronavirus infection in rats, A preliminary report. Acta Neuropathol. 45:205-213.

Robb, J.A., and Bond, C.W. 1979. Coronaviridae. Comp. Virol. 14:193-247.

Schliep, G., and Felgenhauer, K. 1978. Serum-CSF protein gradients, the blood-CSF barrier and the local immune response. J. Neurol. 218:77-96.

Sorensen, O., Dugre, R., Percy, D., and Dales, S. 1982. In vivo and in vitro models of demyelinating disease: Endogenous factors influencing demyelinating disease caused by mouse hepatitis virus in rats and mice. Infect. Immun. 37:1248-1260.

Sorensen, O., Percy, D., and Dales, S. 1980. In vivo and in vitro models of demyelinating disease. III. JHM virus infection of rats. Arch. Neurol. 37:478-484.

Stroop, W.G., Baringer, J.R., and Brahic, M. 1981. Detection of Theiler's virus RNA in mouse central nervous system by in situ hybridization. Lab. Invest. 45:504-509.