HOST AND VIRUS FACTORS ASSOCIATED WITH CNS CELLULAR TROPISM LEADING TO ENCEPHALOMYELITIS OR DEMYELINATION INDUCED BY THE JHM STRAIN OF MOUSE HEPATITIS VIRUS

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INTRODUCTION

Infection of mice with the neurotropic JHM strain (type 4) of mouse hepatitis virus (MHV), produces a spectrum of disease ranging from acute fatal encephalomyelitis (E+) to demyelination (D+) (Bailey et al., 1949; Haspel et al., 1978; Lampert et al., 1973; Weiner, 1973). The pattern of disease which prevails is affected by such factors as the age of the animal, the dose of virus administered, the route of infection (Weiner, 1973), the genetic strain of the mouse (Stohlman and Frelinger, 1978), and the nature of the virus (Haspel et al., 1978).

Intracerebral inoculation of two plaque forming units (PFU) of wild type (wt) MHV in susceptible four week old mice, produces a high frequency of fatal encephalomyelitis (E+), but infrequent demyelination (D-) due to virulence of the virus (Haspel et al., 1978). Intracerebral inoculation of 10^4 PFU of an attenuated mutant, designated ts8, rarely results in fatal encephalomyelitis (E-), but regularly produces demyelination (D+) (Haspel et al., 1978). We have developed a model system to dissect factors associated with the E+ and the D+ phenotypes.

GENETIC CONTROL OF SUSCEPTIBILITY TO MHV ENCEPHALOMYELITIS

To dissect factors associated with an E+ phenotype, we screened several inbred strains of mice for susceptibility to fatal disease:

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A/J($H\text{-}2^{a/k}$), A.SW($H\text{-}2^{ss}$), BALB/c($H\text{-}2^{dd}$), BALB/WEHI($H\text{-}2^{dd}$), B10.D2 OLD($H\text{-}2^{dd}$), B10.D2 NEW($H\text{-}2^{dd}$), C3H/ST($H\text{-}2^{kk}$), C57BL/6J($H\text{-}2^{bb}$), SJL/J($H\text{-}2^{ss}$) and SWR/J($H\text{-}2^{qq}$). The sex of the animal was found to have no influence on susceptibility to MHV (Haspel, unpublished data). The mice were obtained from Jackson Laboratories, Bar Harbor, ME, or from the breeding colony of the Research Institute of Scripps Clinic, and were screened for antibodies to MHV. The LD_{50} for each strain was calculated by the Reed-Muench method at the end of the 14 day assay period. Only SJL/J mice showed resistance to fatal encephalomyelitis.

To investigate the pattern of inheritance of susceptibility, BALB/c mice were selected as a susceptible strain and bred with resistant SJL/J mice to test the susceptibility of progeny to fatal encephalomyelitis in a 14 day assay. Hybrid F1 progeny of BALB/c x SJL/J, and SJL/J x BALB/c matings, F2 generation mice, and progeny of backcrosses of F1 mice to either BALB/c or SJL/J parental mice were studied at four weeks of age. In this experiment, the infecting dose of virus for intracerebral inoculation was 100 BALB/c LD_{50}. The results of these experiments support the conclusion that susceptibility is inherited as an autosomal dominant trait.

CELLULAR BASIS OF SUSCEPTIBILITY TO MHV REPLICATION

Although four week old SJL/J mice are resistant to fatal encephalitis compared to other strains, there is replication of MHV in brain tissue from SJL/J mice following intracerebral inoculation. Four week old SJL/J mice develop fatal encephalomyelitis only if very high infectious doses are used, but not under the conditions of these experiments. Table 1 contains virus titers expressed as PFU per gram of tissue from SJL/J and BALB/c mice at 1, 2 and 3 days after inoculation. Brain tissue from SJL/J mice, however, contains at least two logs less virus than brain tissue from similarly infected BALB/c mice. Deaths in BALB/c mice usually begin to occur on day 2 at this infectious dose. Since fatal encephalomyelitis is due primarily to neuronal involvement, it is important to determine whether the observed difference in mortalities and virus titers between SJL/J and the BALB/c mice reflects fewer infected neurons or less virus production per infected cell. In vitro systems were exploited to investigate this question.

Virus replication in thioglycollate stimulated macrophages or primary neuronal cultures from BALB/WEHI and SJL/J were compared, following infection with wt MHV at an MOI of 0.1. Table 2 contains the virus titers expressed as plaque forming units per ml from the supernatant culture fluids of the macrophages from each of the strains of mice. Macrophages derived from four week old BALB/WEHI mice produced 10^4 PFU/ml of MHV, while identical cell populations derived from four week old SJL/J mice had no detectable virus in the supernatant culture fluid. The phagocytic activity of macrophages

from both strains was assessed with antibody coated sheep erythrocytes or zymogen granules, and found to be equivalent. It was possible to demonstrate MHV antigens by immunofluorescence in both SJL/J and BALB/WEHI derived macrophages. However, the labeling pattern of the SJL/J macrophages consisted of intracellular globules, while the BALB/WEHI macrophages were labeled diffusely, but homogeneously. Virus release from infected macrophages was also assessed by overlaying the SJL/J or BALB/WEHI macrophages with L-241 cells, which form multi-nucleated giant cells when infected with MHV. SJL/J macrophage cultures, so treated, had a contiguous sheet of L-241 cells (Figure 1), with only one giant cell per 10^6 plated L-241 cells demonstrated. In contrast, BALB/WEHI macrophage cultures contained numerous giant cells, and very few L-241 cells remaining intact. These findings indicate that macrophages from MHV resistant SJL/J mice, although infected, as indicated by positive immunofluorescence, do not readily release virus, and thus, may play a role in limiting the spread of MHV infection.

Table 1. The Replication of Wild Type MHV (JHM Strain) in Brain Tissue from BALB/c and SJL/J Mice Following Intracerebral Inoculation of 100 BALB/c LD_{50}*

Days Post-Infection	BALB/c	SJL/J
1	++++	++
2	+++++	+++
3	+++++	++++

*(+) signifies logs of PFU/gm

Primary neuronal cultures were established by the method of Peacock et al. (1973), from 11-12 day old embryonic spinal cords taken from A.SW($H\text{-}2^{ss}$), BALB/WEHI($H\text{-}2^{dd}$) and SJL/J($H\text{-}2^{ss}$) mice. Neuronal cells were identified by the binding of tetanus toxin (Mirsky et al., 1978), to their surfaces and subsequent demonstration by indirect immunofluorescence. Primary neuronal cultures derived from SJL/J mice had no detectable virus in the supernatant culture fluid (Table 3), although they did contain MHV antigens by immunofluorescence. In contrast, primary neuronal cultures derived from BALB/WEHI mice had 10^5 PFU/ml of MHV in supernatant culture fluid. The restriction of MHV replication in SJL/J derived neurons is not related to the $H\text{-}2^{ss}$ haplotype; primary neuronal cultures derived from A.SW mice, which are also of the $H\text{-}2^{ss}$ haplotype produced just under 10^5 PFU/ml of MHV in supernatant culture fluid. Restricted replication in neurons is the most probable cause for

the resistance of SJL/J mice to fatal encephalomyelitis. Our data indicate that SJL/J neurons are capable of being infected with MHV, but that it is not a productive infection. Thus, the limited replication of MHV in the brains of SJL/J mice under the conditions of our experiments probably represents replication in non-neuronal cells.

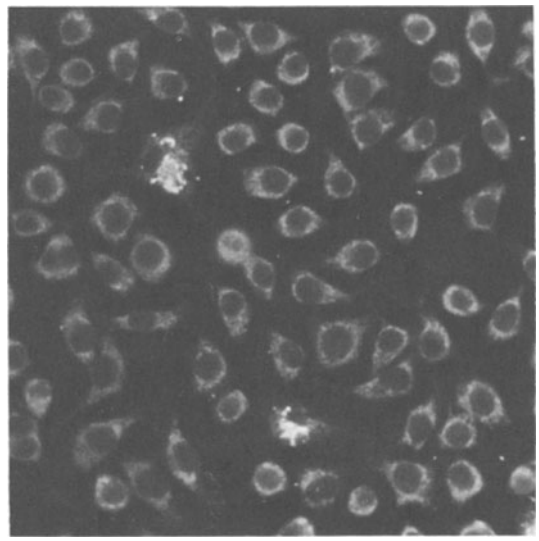

Fig. 1. There are two SJL/J macrophages with a globular pattern of MHV antigens demonstrated by immunofluorescence, surrounded by contiguous L-241 cells, which demonstrate autofluorescent granules. Light micrograph, 250x.

Table 2. The Replication of Wild Type MHV (JHM Strain) by Thioglycollate Stimulated Macrophages Derived from BALB/WEHI and SJL/J Mice, MOI = 0.1*

Days Post-Infection	BALB/WEHI	SJL/J
0	NIL	NIL
1	++++	NIL
2	++++	NIL
3	+++	NIL
4	+++	NIL

*(+) signifies logs of PFU/ml

Table 3. The Replication of Wild Type MHV (JHM Strain) by Primary Neuronal Cultures Derived from A.SW, BALB/WEHI and SJL/J Mice, Expressed as PFU/Ml of Supernatant Culture Fluid, MOI = 0.1*

Days Post-Infection	A.SW(H-2^{ss})	BALB/WEHI(H-2^{dd})	SJL/J(H-2^{ss})
1	++++	++++	NIL
2	+++++	+++++	NIL
3	+++++	+++++	NIL

*(+) signifies logs of PFU/ml

VIRUS TROPISM FOR DIFFERENT CNS CELLS CORRELATES WITH DISEASE PHENOTYPE

To dissect factors associated with a D+ (demyelinating) phenotype, we compared virus production and antigen localization following intracerebral inoculation of susceptible BALB/c mice with either wild type virus, which frequently produces fatal encephalomyelitis, or mutant ts8, which frequently produces demyelination. For this experiment, less than one PFU of wt virus (one half LD_{50}) was used, compared to 10^4 PFU of ts8. Animals were sacrificed at 2, 4, 7, 10 and 14 days after inoculation and tissues were taken for determination of virus titer or the animals were perfused for antigen localization studies. It should be noted that animals receiving wt virus did not survive past seven days after inoculation.

The infectious titers of virus produced in the brain or spinal cord following infection with either wt or mutant ts8 are equivalent (Table 4). Thus, the different pattern of disease with mutant ts8 is not due to a difference in the infectious titer produced in the tissues following infection. However, the localization of virus antigen by immunofluorescence in perfused (4% paraformaldehyde and 0.5% glutaraldehyde), vibratome sectioned spinal cords demonstrated an altered tropism for CNS cells of mutant ts8 compared to wt MHV, that closely correlated with the pattern of disease each produces. Animals inoculated with wild type MHV had MHV antigens localized principally in cells of the substantia gelatinosa and ventral horn neurons (Figure 2), as well as oligodendrocytes in the spinal cord. In marked contrast, animals inoculated with mutant ts8 had MHV antigens localized primarily in oligodendrocytes of the ventral median white matter of the spinal cord (Figure 3), while only rarely in neuronal cells. Thus, the high frequency of demyelination and infrequency of fatal encephalomyelitis observed with infection by mutant ts8 correlates with an altered cellular tropism within the CNS by ts8.

The demonstration of MHV antigens in oligodendrocytes processes extending to and surrounding intact myelin sheaths (Figure 3) is of further interest. The oligodendrocytes in MHV infection are hypertropic and have more notable connections to myelin sheaths than is usually observed (Powell and Lampert, 1975). Whether the MHV antigens are actually being incorporated into these myelin sheaths and the significance of this process for virus-induced demyelinating or immune-mediated demyelinating disease is under study.

SUMMARY AND CONCLUSIONS

1. Ten inbred strains of mice at four weeks of age were tested for susceptibility to fatal encephalomyelitis (E+) by intracerebral inoculation of ten-fold dilutions of wild type (JHM Strain) mouse hepatitis virus. Only SJL/J mice were resistant (E-) to fatal encephalomyelitis. This resistance is independent of the $H\text{-}2^{ss}$ haplotype.

2. By testing appropriate crosses between BALB/c and SJL/J mice, susceptibility was determined to be an autosomal dominant trait.

3. Thioglycollate stimulated macrophages, and primary neuronal cultures from SJL/J mice did not produce infectious virus. In contrast, these cell populations derived from BALB/WEHI mice released 10^4-10^5 PFU of infectious virus/ml of supernatant culture fluid.

4. The labeling pattern of virus antigens by immunofluorescence was globular and moderately positive in SJL/J cells. The pattern in BALB/WEHI cells was diffuse and markedly positive.

Table 4. The Replication of Wild Type and Mutant ts8 MHV (JHM Strain) in Brain Tissue from BALB/ST Mice Following Intracerebral Inoculation*

Days Post-Infection	Wild Type	ts8
2	++++++	++++++
4	++++++	++++++
7	+++++	+++++
10		++++
14		+++

*(+) signifies logs of PFU/gm

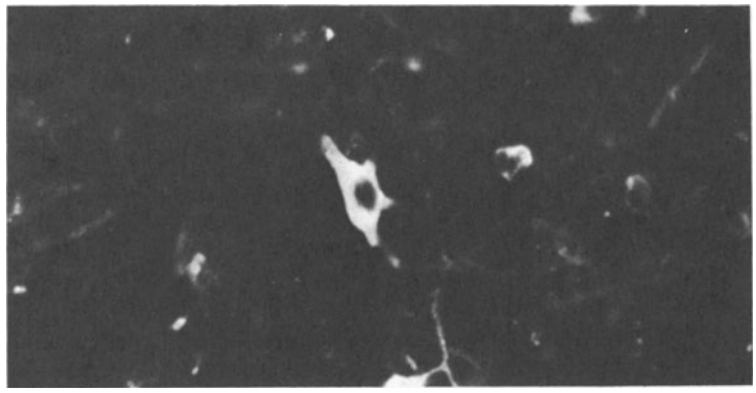

Fig. 2. Wild type mouse hepatitis virus antigens demonstrated in cervical spinal cord ventral horn neurons, two days after intracerebral inoculation by immunofluorescent staining of a vibratome section. Light micrograph, 400x.

Fig 3. Mutant ts8 mouse hepatitis virus antigens demonstrated in cervical spinal cord ventral median white matter oligodendrocyte two days after intracerebral inoculation by immunofluorescent staining of a vibratome section. Two planes of focus on the same section are shown. Light micrograph, 400x.

5. Susceptibility to fatal disease from infection with wild type MHV (JHM Strain) correlates best with the ability of the virus to replicate in neuronal cells. Wild type viral antigens are localized in neuronal cells, as well as oligodendrocytes.

6. Mutant ts8 in contrast to wild type, produces a high frequency of demyelination without fatal encephalomyelitis. The viral antigens of ts8 are localized primarily in oligodendrocytes, and rarely in neuronal cells. This difference in CNS cell tropism correlates well with the different phenotype (D+,E-) produced by mutant ts8.

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