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*

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Several human diseases whose etiologies and pathogeneses were formerly unknown have now been attributed to viruses alone or in concert with host immune responses (reviewed in 1, 2). For example, the cause of degenerative nervous system disorders like Jakob-Creutzfeldt disease and kuru is now known to be a transmissible "atypical slow virus" (3), and some instances of diabetes in man follow the direct attack of viruses on insulin-forming beta cells (4, 5). Further, antibody responses mounted against virus antigens and virions can result in the formation of virus-antibody immune complexes in the circulation. Subsequent trapping of these complexes in appropriate tissues lead to glomerulonephritis, arteritis, and disorders of the choroid plexus (6). Disorders of this kind are frequently identified in humans only after a similar, naturally occurring or experimentally induced disease has been studied in animal models. Thus, there is continuing interest in defining the parameters of virus-induced diseases in animals for application to related human disorders.

Mouse hepatitis virus (MHV),[1] a positive strand RNA virus of the coronavirus group, provides such a model. There are several stains of MHV, one of which, type 4 (MHV-4, strain JHM), causes primary demyelination as well as encephalomyelitis in its natural murine host (7-11). The recent ability to generate mutants of MHV-4 that cause either primary demyelination (D^+) or encephalitis (E^+) (11) and the technical advance in establishing homogeneous cultures of specialized cells from the central nervous system (CNS) (12-14) have spurred interest in the experimental usefulness of this agent. Wild-type MHV-4 is predominantly associated with an E^+ phenotype. We have used wild-type MHV-4 to infect a wide variety of inbred mouse strains or their primary neuronal cells and then have evaluated the incidence of disease as well as the mechanism of susceptibility or resistance. Our results indicate:

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[1] *Abbreviations used in this paper:* CNS, central nervous system; D^+, demyelinating phenotype; E^+, encephalomyelitis phenotype; LD_{50}, lethal dose of virus required to kill 50% of animals tested; MHV, mouse hepatitis virus.

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first, that the E^+ phenotype is controlled by a single gene; second, that this gene is dominant (for susceptibility) and is not linked to the major histocompatibility complex; and third, that susceptibility resides at the level of the neuronal cell.

Materials and Methods

Virus. Wild-type MHV-4 initially provided by Dr. L. Weiner (Department of Neurology, University of Southern California) was plaque purified three times in NCTC-1469 cells, grown as a stock, and used in subsequent studies. The infectious titer, expressed as plaque-forming units per gram of tissue or per milliliter of cultured cells, was determined by plaque assay on either NCTC-1469 or L-24-1 cells. The handling of virus, culture, and plaque assay conditions have been reported elsewhere (11, 15).

Mice. The inbred mouse strains listed in Table I were obtained from the Scripps Clinic and Research Foundation vivarium, La Jolla, Calif.; the L. C. Strong Research Foundation, La Jolla; or The Jackson Laboratory, Bar Harbor, Maine. 4-wk-old mice were inoculated intracerebrally with 0.05 ml of virus. To quantitate the amounts of infectious virus in various tissues, mice were exsanguinated and their tissues removed aseptically, then stored at −70°C until assayed. A 10% homogenate of various tissues, clarified by centrifugation at 700 *g*, was serially diluted 10-fold, and the amounts of infectious virus were quantitated by plaque assay on susceptible cells.

Cultured Cells. To obtain primary cultures of neurons, spinal cords were removed from 11–12-d-old embryos and dissected free of meningeal coverings, dissociated through steel or nylon mesh with 60–100 openings/in and plated on 0.01% poly(D-lysine)-coated 35-mm dishes at a concentration of 1×10^6 cells/dish. Media consisted of 10% fetal bovine serum in Eagle's minimal essential media that contained 1% glutamine and 0.5% additional glucose. The purity of various neuronal and glial cells in the cultures was assayed by detecting plasma membrane receptors for tetanus toxin, surface galactocerebroside, and cytoplasmic accumulation of glial fibrillary acid protein (12, 14, 16). Neuronal cells have tetanus-toxin receptors but lack the glial markers of galactocerebroside and glial fibrillary acid protein (12). Macrophages were obtained from peritoneal cavities of mice inoculated intraperitoneally with 2 ml of 3.8% thioglycollate broth. Peritoneal exudate cells harvested 5 d later were plated, and nonadherent cells removed 1–2 h later after vigorous shaking. Details concerning stimulating, obtaining, and culturing these cells are published elsewhere (17, 18). Adherent cells were macrophages, as judged by their ability to phagocytize zymosan particles, or sheep erythrocytes previously reacted with antibody and C5-deficient mouse sera, and by their morphology (19). Homogeneity was usually >95% with a range of 85–98%.

Immunochemical Labeling of MHV Antigens. Cultured cells were fixed with 4% paraformaldehyde and 0.5% glutaraldehyde in cacodylate buffer, pH 7.3, incubated with phosphate-buffered saline containing 1% bovine serum albumin and subjected to one cycle of freeze-thawing in a cryo-protective medium composed of 25% sucrose and 10% glycerol as described earlier (20),[2] or were used unfixed. For in vivo studies, tissues were obtained from mice perfused with 4% paraformaldehyde and 0.5% glutaraldehyde by the intracardiac route. Both uninfected and MHV-4 inoculated mice were analyzed. Perfused tissues were collected in petri dishes containing fixative (see above), and 8–14-μm sections were cut with an Oxford vibratome (Oxford Instruments Inc., Columbia, Md.). Thereafter, cells or tissue sections were incubated with monospecific antibodies to purified MHV virions or MHV antigens initially prepared in rabbits (kindly supplied by Dr. K. Holmes, Uniformed Services University of the Health Sciences, Bethesda, Md.), or in mice kindly provided by the National Cancer Institute, Bethesda, Md. resource program, or generated by immunization in this laboratory. All reagents gave equivalent results, and specificity was determined by appropriate absorption and blocking studies (21, 22). After 30 min, cells or tissues were washed gently in buffer and stained with protein A conjugated with peroxidase or with antibody to mouse or rabbit IgG conjugated with fluorescein-isothio-

[2] Knobler, R. L., M. Dubois-Dalcq, M. V. Haspel, A. Claysmith, P. W. Lampert, and M. B. A. Oldstone. Selective localization of wild type and mutant mouse hepatitis virus (JHM strain) antigens in CNS tissue by fluorescence, light and electron microscopy. *J. Neuroimmunol.* In press.

cyanate as reported elsewhere (21, 22). In some instances cultured macrophages or neurons were fixed with acid alcohol or with ether-alcohol as described (22).

Results

Genetic Susceptibility to MHV-4 Among Various Inbred Mouse Strains. 11 inbred strains of mice were tested for susceptibility to acute fatal encephalomyelitis after intracerebral inoculation of MHV, as shown in Table I. The mean lethal doses (LD_{50}) for A/J, A.SW, BALB/c, BALB/WEHI, B10.D2 new, B10.D2 old, C3H/HeJ, C3H/St, C57BL/6J, and SWR/J mice were ≤5 plaque-forming units needed to kill 50% of animals (LD_{50}) in each group. Death occurred as early as the second day after inoculation with the majority of animals dying by day 5. Male and female mice were equally susceptible (data not shown) to a MHV-4 infection. In contrast to these strains, SJL/J mice were resistant to the lethal effect of virus; 30- to 75-fold more virus was needed to reach the equivalent LD_{50} end point. Some SJL/J mice died at 5 d but most died 8–14 d after viral inoculation. Death from MHV-4 was not linked to the major histocompatibility locus inasmuch as A.SW mice, which are histocompatible with SJL/J mice (H-2SS), were susceptible.

The C3H/HeJ, C3H/St, and BALB/WEHI strains of mice, which are ordinarily susceptible to MHV-4 administered by the intracerebral route, were all relatively resistant to challenge by intraperitoneal route. Death occurred in some of these mice, especially BALB/WEHI, but only those receiving large doses of virus. After intraperitoneal inoculation, the LD_{50} for C3H/St and C3H/HeJ mice was reached with $>10^4$ PFU/ml and for BALB/WEHI mice with $10^{-2.2}$ dilution of stock virus titering 5×10^5 PFU/ml.

Resistance to MHV-4 Is Controlled by a Single Recessive Gene. To study the dominance and number of genes controlling resistance to MHV-4, we inoculated MHV-4 intracerebrally into BALB/c (susceptible) mice, SJL/J (resistant) mice, their F_1 and

TABLE I
*Susceptibility of Inbred Mouse Strains to Acute Fatal Encephalomyelitis Caused by MHV-4**

Strain	H-2	LD_{50}‡
		PFU
A/J	aa	<5
A.SW	ss	<5
BALB/c	dd	<2
BALB/WEHI	dd	<3
B10.D2 new	dd	<3
B10.D2 old	dd	<2
C3H/HeJ	kk	<2
C3H/St	kk	<5
C57BL/6J	bb	<5
SJL/J	ss	149
SWR/J	qq	<5

* 4-wk-old mice were inoculated intracerebrally with varying log dilutions of MHV-4 virus. Each group contained a minimum of 10 mice per inoculation dose.

‡ LD_{50} in plaque-forming units (PFU) calculated by Reed-Muench method (35).

F_2 hybrids, and the F_1 backcross to resistant and susceptible parents. As seen in Table II, the F_1 hybrids were as susceptible to acute encephalitis as the BALB/c parents. All F_1 hybrids inoculated with 100 BALB/c LD_{50} died, thus indicating that resistance is recessive. Among mice bred by mating F_1 hybrids to each parental stock, offspring of F_1 mice crossed with susceptible BALB/c partners had a mortality rate of 96% as compared with a 45% mortality rate in mice parented by F_1 mice crossed with SJL/J mice. These values obtained experimentally are close to the theoretical values of 100% and 50%, respectively, that should hold true if resistance is inherited as a single recessive gene. Similarly, the mortality of F_2 mice was 65%, close to the theoretical value of 75% for a one-recessive-gene model.

Replication of MHV In Vivo Is Less Efficient in SJL/J Mice Than In Susceptible Strains and Occurs Infrequently in Neuronal Cells. To evaluate replication of MHV in vivo SJL/J and BALB/c mice were inoculated intracerebrally with 100 BALB/c LD_{50} of virus, and after killing at various intervals, their tissues were assayed for amounts of infectious virus. Virus replicated to some degree in SJL/J brains, but the titers were 100- to 1,000-fold lower than in BALB/c mice tested within the first 2 d after inoculation (Table III). Thereafter, the majority of BALB/c mice died, but surviving

TABLE II

Genetic Basis of Susceptibility to Acute Fatal Encephalomyelitis Caused by MHV-4

Strain	Mortality	
	Experimental*	Theoretical‡
BALB/c	48/49	49/49
SJL/J	2/50	0/50
(BALB/c × SJL/J)F_1	12/12	12/12
(SJL/J × BALB/c)F_1	12/12	12/12
F_1 × BALB/c	48/50	50/50
F_1 × SJL/J	25/55	27/55
F_2	36/55	41/55

* Deaths recorded after intracerebral inoculation of 100 BALB/c LD_{50} of MHV-4.

‡ Expected mortality if resistance were controlled by single recessive gene.

TABLE III

*Replication of MHV-4 in BALB/c and SJL/J Mice after Intracerebral Inoculation of 100 BALB/c LD_{50}**

Days post-infection	PFU/gram of brain		PFU/gram of liver	
	BALB/c	SJL/J	BALB/c	SJL/J
1	4.2×10^4	3.1×10^2	6.4×10^2	1.0×10^2
2	1.7×10^6	1.0×10^3	1.0×10^3	2.9×10^1
3	3.5×10^5	6.3×10^4	6.2×10^3	4.9×10^1
4		5.3×10^4		2.1×10^1

* By the 3rd d after inoculation, the majority of BALB/c mice were dead (>95%). The majority of SJL/J mice were alive 7 d after inoculation (>90%). Tissues from three mice were individually assayed at each day. Number reflects the mean value. Each assay plate was run in duplicate. Similar results were obtained in a repeat experiment. PFU, plaque-forming units.

SJL/J mice consistently had lower titers of virus than BALB/c mice killed during the peak of infection. Virus replicated to higher titers, 1 to 3 logs more, in CNS tissues (brain and spinal cord) than in livers, spleens, or thymuses. Again, virus titers were significantly higher in all tissues from BALB/c mice when compared with those from SJL/J mice. Virus replication in inoculated BALB/WEHI mice were equivalent to that seen in BALB/c mice.

The use of a specific fixative and unembedded sections thinly sliced with a vibratome enabled us to preserve sufficient viral antigen and morphologic structure to assess the CNS cell type involved and the sites of antigen localization (Fig. 1). The study of spinal cord and brain tissue from BALB/c mice showed evidence of MHV-4 antigen expression both in neurons (Fig. 1) and in glial cells. To the contrary, it was uncommon to find MHV-4 antigens in neuronal cells of SJL/J mice. However, when the viral dose was increased 100-fold, occasional neuronal cells from SJL/J mice contained MHV antigens.

Replication of MHV-4 In Vitro

Replication in neurons. Because the death of mice inoculated with MHV-4 is associated with neuron destruction, and viruses replicate best in the CNS, we compared MHV-4 replication in primary neuronal cultured cells from resistant SJL/J and from susceptible C3H/St, BALB/WEHI, and A.SW mice. In these experiments, >90% of the cells cultured on poly(D-lysine)-coated surfaces were neurons as judged by the presence of binding sites for tetanus toxin on the cells' surfaces (Fig. 2), and the absence of surface galactocerebroside or cytoplasmic glial fibrillary acid protein.

The results indicated that SJL/J neurons failed to replicate infectious MHV-4 effectively. As Table IV shows, neurons from SJL/J mice formed <10 PFU of MHV, whereas primary neurons obtained from A.SW, BALB/WEHI, and C3H/St mice in contrast formed 10^4–10^7 PFU of MHV. Preliminary evidence indicated that some MHV antigens were made in SJL/J neurons using immunofluorescence assay.

We tested whether primary neurons obtained from F_1 mice made by breeding resistant (SJL/J) and susceptible (BALB/WEHI) mice were permissive to and replicated infectious MHV-4. Table V shows that neurons from (SJL/J × BALB/WEHI)F_1 or (BALB/WEHI × SJL/J)F_1 replicated as much or more virus than neurons from susceptible BALB/WEHI parents. These results obtained in vitro complemented those obtained in vivo (Table II) and again indicated that susceptibility to MHV-4 was dominant.

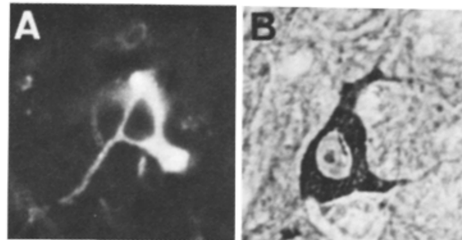

Fig. 1. Demonstration of MHV-4 antigens in spinal cord neurons of BALB/c mice. 2 d after intracerebral infection with MHV-4, the mouse was perfused and the spinal cord removed and sectioned on a vibratome (Materials and Methods). (A), MHV antigens by immunofluorescence (B) MHV antigens by immunoperoxidase. × 700.

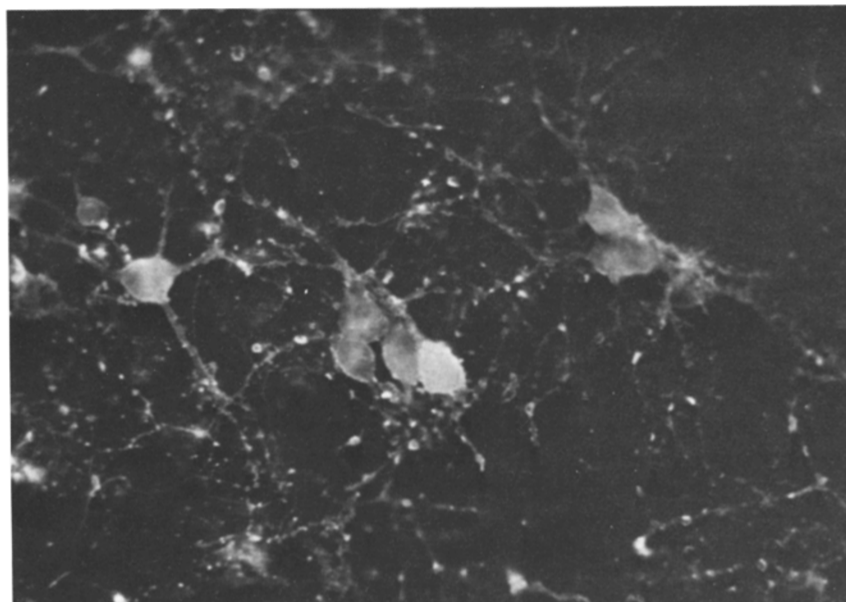

FIG. 2. Primary neuron cells in culture incubated first with tetanus toxin and then fluoresceinated monospecific antibody to tetanus toxoid (Materials and Methods). 11–12-d-old BALB/WEHI embryonic spinal cords were processed and resultant cultured cells analyzed 7–21 d later for a variety of neuron and glial cell markers. × 400.

TABLE IV

*Replication of MHV-4 in Cultured Murine Neuron Cells**

Exp.	Mouse strain	H-2	PFU/1 × 10^6 neuron cells at h postinfection:					
			2	6	12	24	48	72
1	C3H/St	kk	<10	<10	3 × 10^4	3 × 10^5	6 × 10^6	9 × 10^6
	BALB/WEHI	dd	<10	<10	2 × 10^5	1.8 × 10^6	3 × 10^6	9 × 10^6
	A.SW	ss	<10	<10	ND	3 × 10^5	6 × 10^6	3 × 10^6
2	BALB/WEHI	dd	<10	<10	1 × 10^3	3 × 10^6	2 × 10^6	2 × 10^4
	SJL/J	ss	<10	<10	<10	<10	<10	<10
3	A.SW	ss	<10	<10	1 × 10^5	2 × 10^7	2 × 10^7	9 × 10^5
	SJL/J	ss	<10	<10	<10	<10	<10	<10

* Primary neuron cultures were inoculated with virus at a multiplicity of infection of 0.1. Neuronal cultures were >90% pure as judged by cells having surface receptors for tetanus toxin but lacking markers of glial cells (galactocerebroside and glial fibrillary acid protein). ND, not determined; PFU, plaque-forming units.

REPLICATION IN MACROPHAGES. Earlier studies by Bang et al. (23, 24) placed the host's genetic restriction over MHV-2 replication at the level of the macrophage. In contrast to MHV-4, MHV-2 caused a severe hepatitis after intraperitoneal inoculation into susceptible mice. We studied the replication of MHV-4 in thioglycollate-induced peritoneal macrophages harvested from resistant SJL/J and susceptible BALB/WEHI mice. MHV-4 replicated in >96% of BALB/WEHI macrophages inoculated with virus, and as seen in Table VI, infectious virus was produced. In contrast, macrophages from SJL/J mice failed to release detectable amounts of infectious virus into the

TABLE V

*Replication of MHV-4 in Cultured Neuron Cells from (SJL/J × BALB/WEHI)F_1 Hybrid Mice**

Source of neurons	PFU/1 × 10^6 neuron cell at h postinfection:				
	4	24	48	72	96
BALB/WEHI	<10	2 × 10^5	2 × 10^5	5 × 10^4	6 × 10^4
SJL/J	<10	<10	<10	<10	<10
(BALB/W × SJL/J)F_1	<10	1 × 10^7	1 × 10^5	6 × 10^4	1 × 10^5
(SJL/J × BALB/W)F_1	<10	2 × 10^6	3 × 10^5	7 × 10^4	6 × 10^4

* Primary neuron cultures were inoculated with virus at a multiplicity of infection of 0.1. See Materials and Methods and Table IV for characterization of neuron cells. PFU, plaque-forming units.

TABLE VI

*Replication of MHV-4 in Cultured Macrophages**

Exp.	Mouse strain	MOI	PFU/5 × 10^5 macrophages at h postinfection:				
			4	24	48	72	96
1	BALB/W	0.1	<10	1.9 × 10^4	3.6 × 10^4	—	—
	SJL/J	0.1	<10	<10	<10	<10	<10
	BALB/W	0.01	<10	<10	1.2 × 10^5	6.6 × 10^4	1.5 × 10^3
	SJL/J	0.01	<10	<10	<10	<10	<10
2	BALB/W	0.1	<10	3 × 10^4	3 × 10^4	<10	<3 × 10^1
	SJL/J	0.1	<10	<10	<10	<10	<10
	BALB/W	0.01	<10	2.2 × 10^3	1.1 × 10^5	1.2 × 10^4	<3 × 10^1
	SJL/J	0.01	<10	<10	<10	<10	<10

* Macrophages were obtained from peritoneal cavities of mice 4 d after inoculation with 3.8% thioglycollate. Cells were characterized on the basis of adherence and ability to phagocytize particles (Materials and Methods). MOI, multiplicity of infection; PFU, plaque-forming units.

culture fluids (Table VI), although the majority of cells expressed viral antigens. However, infectious virus was detected in a subsequent amplification assay when susceptible L cells were layered over SJL/J macrophages (Fig. 3). The occurrence of infected foci in the L cells layered over infected SJL/J macrophages was uncommon. 1–3 giant cell foci occurred per 35-mm dish as compared to >100 foci when infected BALB/WEHI macrophages were similarly assayed. Viral antigens detected in SJL/J macrophages were grouped in clusters in the cytoplasm as compared with a more diffuse and granular MHV antigen presentation in macrophages from BALB/WEHI mice. The presence of viral antigens in macrophages harvested from SJL mice again suggested that the block in replicating virus was likely not at the level of the receptor for the virus.

Discussion

In this study of MHV-4 induced fatal central nervous system disease, we made two primary observations. First, resistance to MHV-4 is apparently controlled by a single recessive gene and, second, this gene probably acts at the level of neuronal cells.

The evidence for genetic control was obtained using inbred mouse strains, F_1 and

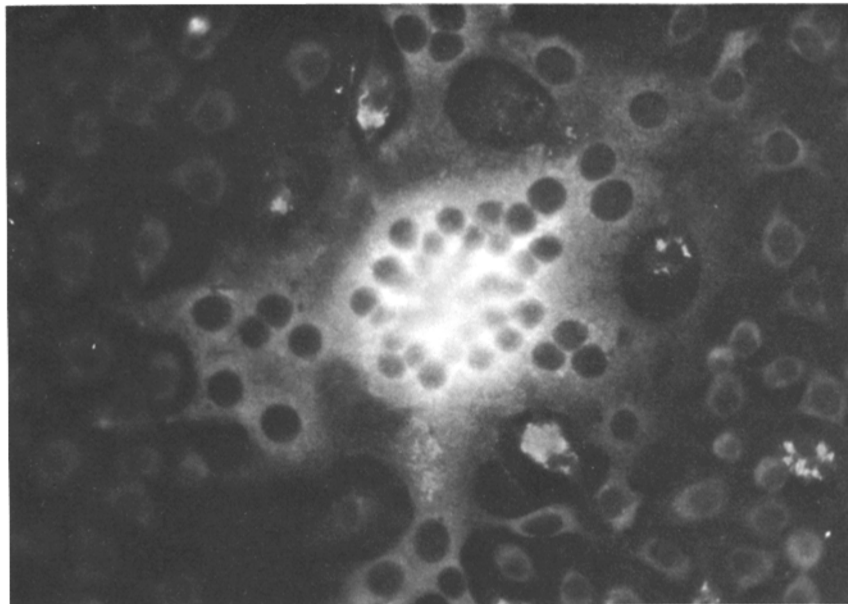

FIG. 3. Multi-nucleated giant cell produced after overlaying L-24-1 cells on top of SJL/J macrophages previously infected with MHV-4 (Materials and Methods). SJL/J macrophages, alone, failed to release infectious virus. Cells incubated first with mouse antibody to MHV-4 and second with fluorescein-conjugated rabbit antibody to mouse IgG to detect MHV-4 antigens. × 400.

F_2 mice generated from crossing susceptible and resistant mice, and mice obtained by backcrossing the F_1 mice to both susceptible and resistant parental strains. Of the eleven inbred strains studied, only one, SJL/J, was resistant to the effects of MHV-4 after intracerebral inoculation. The complete susceptibility of BALB/c mice and of the F_1 hybrids obtained from mating BALB/c × SJL/J indicates that resistance of MHV-4 is recessive. Mortality data obtained both from F_2 intercrosses and from F_1 backcrosses to each parent best fit a single recessive gene model (Table II). It should be emphasized that these results were obtained with 4-wk-old mice, a time when neurons are mature. Complementary data was obtained from in vitro studies when neurons from BALB/WEHI and the F_1 hybrids obtained from mating BALB/WEHI × SJL/J mice produced 4 logs more infectious virus than similarly prepared neurons from SJL/J mice (Table V).

Several investigators have studied host factors affecting resistance to other strains of MHV. The pioneering work of Bang et al. (23–28) focused on the nature of susceptibility to MHV-2 in causing fatal hepatitis in PRI mice but not in C_3H mice. These investigators showed that the ability of virus to replicate in vitro in macrophages correlated with production of fatal hepatitis and that resistance was inherited as a single autosomal recessive gene. Our studies complement and extend those of Bang by showing that MHV-4 resistance at the host level, like MHV-2, seem to be inherited as a single autosomal recessive gene. The apparent difference between MHV-2 and MHV-4 lies in the primary target tissue injured by viral replication—the former is liver cells and the latter, neurons. Interestingly, although replication of MHV-2 is restricted in macrophages from C_3H mice (23,26), macrophages from C3H mice are susceptible to replication of MHV-3 (29) and MHV-4 in vitro (R. Knobler and M. B.

A. Oldstone, unpublished data). Although C3H mice were permissive to MHV-3 infection (29, 30), A/J mice were resistant. Genetic studies also mapped MHV3 resistance to a single recessive gene (29–31). Thus, our findings with MHV-4 resemble those with MHV-2 and MHV-3 in that resistance is controlled by a single recessive gene. Yet, in contrast with the resistance of A/J mice to MHV-3 (31), we noted that similarly aged A/J mice are susceptible to MHV-4 infection (Table I). Our results with MHV-4 differ from a recent report (32) suggesting that acute encephalitis caused by this virus is controlled by two genes, one recessive and one dominant. The cause of these differences is not yet apparent.

MHV-4 replicates in the neurons of susceptible mice (Fig. 1; and Tables IV and V). To do these studies, we utilized methods permitting critical assessment of various CNS cells in vivo by preserving their morphology and MHV-4 antigenicity (20)[2] and employed primary cultures of neurons. Although neuronal cells obtained from several strains of susceptible mice infected with MHV-4 expressed MHV-4 antigens, neurons from SJL/J mice given equivalent doses of virus only infrequently expressed viral antigen in vivo. Despite the relative inability of the neurons to express viral antigens, other cells in the CNS of SJL/J mice produced infectious virus (Table III), showing that virus could replicate there. When MHV-4 was used to infect cultured primary neuronal cells harvested from SJL/J mice, no infectious virus was detected in supernatant fluids. In contrast, cultured neuronal cells from several strains of susceptible mice made 4 to 7 logs of infectious virus. Hence, both in vivo and in vitro studies suggest that the majority of infectious virus found in brains of SJL/J mice after intracerebral inoculation of MHV-4 may reflect replication of virus in nonneuronal cells. This failure to replicate MHV in neurons is manifested as a low incidence of death after one exposes SJL/J mice to MHV-4. However, this restriction is not absolute, because larger doses of virus (an additional 2–3 logs) result in viral replication in their neurons in vivo and death. The stage of replication at which MHV is restricted in neurons is not clear. Studies with neurons and macrophages suggest that the block may take place in some cells after virus-receptor interaction and after some viral antigens are produced. Whether all the viral antigens are made satisfactorily in such cells, or the defect is in packaging and/or release is under investigation.

The tropism of neuronal cells explains the E^+ phenotype in susceptible MHV-4 infected mouse strains. This indicates the need for studying the specialized cells(s) involved in any disease process to understand the basic host restriction in susceptibility to disease. That principle is supported by our observations here and in studies with two other viruses. In an encephalomyocarditis virus model of diabetes, virus replication differed notably only in cultures of pancreatic beta cells from resistant and susceptible strains (33). Using cytomegalovirus, Nedrud et al. (34) found significant differences in the amount of virus replicated by resistant and susceptible mice, but only in tracheal ring cultures, not in fibroblast cultures. The ability to generate homogeneous cultures of primary neuronal cells and the expectation of obtaining similar cultures for oligodendrocytes, now offers the opportunity to dissect the molecular basis of host restriction for virus induced demyelinating and degenerative diseases of the CNS. Further, definition of the genetic restriction in vitro using cells from recombinant inbred strains of mice should allow for allelic localization of the gene(s) involved in susceptibility.

Summary

Mouse hepatitis virus (JHM strain) type 4 induces acute encephalitis followed by death in many strains of laboratory mice. Immunohistochemical study in vivo and analysis of mouse neuronal cells in vitro both indicate that the target cell in this infection is the neuron. Further, examination of several inbred mouse strains and neuronal cells from them shows that disease expression is controlled by a single autosomal gene acting at the level of the neuronal cell. Susceptibility is dominant but not H-2 linked. However, cultured neuronal cells and macrophages from SJL/J mice, which are resistant to this infection, fail to make significant amounts of infectious virus after an appropriate viral inoculation. Apparently the defect is not at the level of the virus-cell receptor, because these cells, in part, express viral antigens.

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