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Resistance to Fatal Central Nervous System Disease by Mouse Hepatitis Virus, Strain JHM

I. Genetic Analysis

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Introduction

Naturally occurring, genetically determined variation of resistance to infectious disease is one of the most interesting observations in immunobiology. For the past 40 years, virologists have been studying the genetic basis for resistance to viral infection (Webster 1937). For the majority of these studies, the mouse has been used as the model host because of the availability of inbred strains and the variety of viral agents capable of establishing lethal infections. Single-gene (Goodman and Koprowski 1966, Sabin 1952, Bang and Warwick 1960), including *H-2* (Lilly and Pincus 1973), and polygenic (Lopez 1975, Ross *et al.* 1976) inheritance of resistance or susceptibility to viruses have been described.

Mouse hepatitis virus (MHV), a member of the *Coronaviridae* (Tyrell *et al.* 1968), occurs naturally as a latent infection, generally causing hepatitis under laboratory conditions (Gledhill and Niven 1955). A previously described study of genetic susceptibility of mice to MHV-induced mortality resulting from hepatitis (Bang and Warwick 1960) showed a single recessive gene was responsible for susceptibility.

We have studied the neurotropic strain of MHV, JHM (Cheever *et al.* 1949, Weiner 1973). JHM causes a lethal central nervous system (CNS) disease, characterized by acute encephalomyelitis. The mice also exhibit myelin loss (Weiner 1973), and survivors have evidence of chronic demyelination (Herndon *et al.* 1975). JHM, in contrast to other MHV strains, causes minimal or no hepatitis. In this communication, we describe the genetic basis of resistance to CNS disease resulting from intracranial (i.c.) inoculation of JHM virus, which, in contrast to the results of Bang and Warwick (1960), requires two genes for resistance. One of these genes is dominant, tentatively designated *Rhv-1* (resistance to hepatitis virus), and one is recessive, designated *rhv-2*.

Materials and Methods

Mice. B10.S/Sf mice, as well as all F_1, backcross, and intercross mice, were bred in the immunogenetics mouse colony, University of Southern California School of Medicine. SJL-*Ig-1*^{a} were a gift of Dr. Roy Riblet, Institute for Cancer Research, Philadelphia. All other mouse strains (Table 1) were purchased from Jackson Laboratory, Bar Harbor, Maine, at 4–8 weeks of age and tested for susceptibility at 12

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Table 1. Mortality of Various Strains of Inbred Mice Following Intracerebral Inoculation with 1000 SMB LD_{50} of JHM Virus

Strain	*H-2* Haplotype	*Ig-1*	Survivors/Total	Percent Resistance
A	*a*	*e*	0/10	0
C57B1/6	*b*	*b*	0/25	0
C57B1/10	*b*	*b*	0/10	0
BALB/c	*d*	*a*	0/20	0
NZB	*d*	*e*	0/10	0
AKR	*k*	*d*	1/20[a]	5
CBA	*k*	*a*	0/10	0
C3H	*k*	*a*	0/10	0
DBA/1	*q*	*c*	0/10	0
RIII	*r*	*c*	0/10	0
B10.S	*s*	*b*	0/20	0
SJL	*s*	*b*	80/100[b]	80
SJL-*Ig-1*[a]	*s*	*a*	2/2	100

[a] One AKR survived challenge but showed no antibody response to JHM
[b] Total of six experiments. All survivors tested had antibody against JHM

weeks of age by intracranial inoculation with 1000 LD_{50} (determined in suckling Swiss-Webster mice) of the JHM strain of MHV in 0.032 ml. Following injection, mice were observed daily for disease and death. Mice surviving longer than 3 weeks following challenge were classified as resistant. No deaths occurred later than 14 days following CNS challenge. Adult mice from each group were bled and tested for anti-JHM neutralizing antibody by microneutralization before use in experiments. Only serum antibody-negative mice were used in this study. In addition, mice surviving challenge were tested for antibody to JHM virus to insure infection. All survivors tested produced antibody to JHM.

Results

The strain distribution (Table 1) suggests no obvious association of resistance to JHM with either *H-2* haplotype or immunoglobulin allotype. Of the 12 strains tested, only SJL showed any appreciable resistance to CNS challenge by JHM.

Susceptible animals receiving this dose of JHM virus were found to die of acute encephalomyelitis with no evidence of hepatitis. At this dose, no virus replication has been detected in 12-week-old SJL; however, six-week-old animals have significant levels of virus in CNS tissues, and dead animals show histological evidence of acute encephalomyelitis. No virus replication has been detected in any SJL animal in extra-CNS tissues.

To test for *H-2*-linked resistance, we tested B10.S mice, which share the $H\text{-}2^s$ haplotype with the resistant strain SJL but derive the remainder of the genome from the susceptible strain B10. Strain B10.S is susceptible, suggesting that non-*H-2* genes are important in resistance. To determine the possible role of immunoglobulin-linked *Ir* genes, SJL-$Ig\text{-}1^a$ mice were tested. This strain derives its *Ig-1* genes from the susceptible strain BALB/c, and its remaining genome is derived from the resistant strain SJL. Although only two mice were tested, both SJL-$Ig\text{-}1^a$ mice were resistant, as is strain SJL (Table 1). Thus, neither *Ig-* nor *H-2*-linked genes appear to produce significant resistance alone.

SJL mice were crossed to mice of the susceptible strain B10.S. B10.S mice were chosen as the susceptible parents to eliminate segregation of *H-2*-linked *Ir* genes, since B10.S and SJL share the $H\text{-}2^s$ haplotype. When (B10.S × SJL)F_1 mice were

Table 2. Mortality of F_1, F_2, and Backcrosses Following Intercerebral Inoculation with 1000 SMB LD_{50} of JHM Virus

	Number of Survivors[a]/Total	Percent Resistance
(B10.S × SJL)F_1	0/20	0
(B10.S × SJL)F_1 × B10.S	0/34	0
(B10.S × SJL)F_1 × (B10.S × SJL)	9/95	9
(B10.S × SJL)F_1 × SJL	15/31	48

[a] All survivors had antibody to JHM virus following challenge

challenged with lethal doses of JHM virus, all were susceptible (Table 2). This result suggests that resistance to JHM challenge is inherited as a recessive trait.

In order to explore further the genetic control of resistance to JHM, F_1 animals were backcrossed to both parental strains and intercrossed (Table 2). All tested animals from the (SJL × B10.S)F_1 × B10.S backcross were susceptible to CNS challenge with JHM virus. This result is consistent with a single, recessive gene responsible for resistance. In contrast, among the F_2 mice, only nine of 95 survived challenge. This differs significantly from the expected 23.75 survivors for resistance mediated by a single recessive Mendelian gene ($\chi^2 = 11.69, P < .005$).

The survival of nine of 95 F_2 mice suggests two possibilities: Resistance is mediated by a single recessive gene with variable penetrance or two segregating genes are responsible for resistance. We believe that the two-gene model is correct, as will be discussed below. For two-gene models, we know that at least one is recessive, since F_1 mice are susceptible. We can therefore postulate that either two recessive genes or one recessive and one dominant gene are required for resistance. The action of one dominant and one recessive gene predicts that 17.81 mice of the 95 tested would have survived, and the existence of two recessive genes predicts six survivors. The data from the F_2 mice (9/95) do not allow rejection of either hypothesis (for two recessive genes $\chi^2 = 1.6, .3 > P > .2$; for one dominant and one recessive, $\chi^2 = 5.37, .05 > P > .02$.

When (SJL × B10.S)F_1 mice are crossed to the SJL parent, the pattern of resistance in these progeny allows us to state that one of the genes involved in resistance of SJL mice to JHM virus-induced CNS disease is inherited as a dominant trait and the other as a recessive. In these backcross progeny, one-half of the mice are resistant to CNS challenge with JHM (Table 2). We can reject the two-recessive gene model ($\chi^2 = 9.71, P < .005$) and the data are consistent with the prediction of a one-dominant/one-recessive model ($\chi^2 = .03, .9 > P > .7$). The simple one-to-one segregation in the backcross to SJL argues strongly against a model of a single gene with variable penetrance, and in favor of a two-gene model. Thus, for resistance to JHM, a mouse must be homozygous for the recessive resistance allele and possess at least one copy of the dominant resistance allele.

The results reported here do not prove conclusively the two-gene hypothesis which we have suggested. Such a proof would entail a much more extensive genetic analysis of resistance. However, the data which we do present effectively rule out any simple single-gene models or the simplest two-gene models in which both resistant genes are either dominant or recessive. The data do not rule out any more complicated multigene models or models invoking variable penetrance of single

genes. These models, while not as simple as the hypothesis proposed here, cannot yet be excluded on any concrete experimental grounds.

Our results are in contrast to the observations of Bang and Warwick (1960), who have noted C3H were resistant to MHV and that there was a single recessive inheritance of resistance (Bang and Warwick 1960). We noted that C3H are susceptible to JHM. The reason for this difference may be the result of different virus, different routes of administration, or difference in the mechanisms of resistance to CNS versus liver disease. Experiments to examine the possibility that C3H and SJL differ only in the dominant resistance gene are in progress.

It is particularly important to state that our results do not preclude the existence of important *H-2*-linked *Ir* genes responsible for JHM resistance. Rather, the crosses performed were designed to preclude the observation of such *H-2*-linked effects. The components of the combination chosen were identical in the *H-2* gene complex to simplify these studies.

Although the segregation studies were conducted to minimize *H-2*-linked effects, the lack of obvious *H-2* control of disease resistance in the strain distribution (Table 1) is interesting, since SJL mice are resistant, and B10.S mice, which share the same $H\text{-}2^s$ haplotype, are susceptible. This finding argues against predominant effects of *H-2* on resistance to fatal disease. The lack of a strong *H-2* effect is consistent with our inability to transfer protection from old to young SJL mice by T cells (Stohlman, unpublished observations), as would be expected if a classical *Ir* gene were operating in a conventional manner. In addition, no obvious linkage with the coat color loci, *a* (agouti), *c* (albino), or *p* (pink-eyed, dilute), were seen.

Classically, genetic resistance to virus disease has been interpreted in two ways. Most dominant genes are assumed to function as do *H-2*-linked immune response genes. For example, resistance to Cross-virus leukemia leukemogenesis is apparently identical to an *H-2*-linked *Ir* gene (Lilly and Pincus 1973). Recessively inherited resistance genes have been interpreted as nonpermissiveness for viral replication. Bang and his coworkers showed that MHV could replicate in macrophages derived from susceptible, but not resistant, mouse strains (Bang and Warwick 1960). Another example of recessively inherited resistance would be host-range mutants of Rous sarcoma virus, which, because of altered virus glycoprotein, cannot adsorb to the host cell and so do not replicate in cells which lack the appropriate virus receptors on their surfaces (Mason and Yeater 1977). Recessive inheritance, however, does not rule out lack of immune etiology. The work of Oldstone and his colleagues (1973) in the LCM system has shown an unusual twist. In this case, they proposed that mice which are resistant to fatal disease are resistant because they failed to produce an appropriate immune response, and so do not suffer the consequences of the immune-mediated process. Genetic results themselves can never completely unravel the mechanism of resistance. They can only provide limitations on the possible mechanisms. Our own preliminary investigations into the immunobiology of SJL resistance to JHM have suggested that neither T nor B lymphocytes can mediate this protection. Rather, it is mediated by cells which have neither T nor B cell surface markers, but are adherent to Sephadex G-10, and are therefore probably macrophage-like cells.

Genetic analysis of the resistance of SJL mice to CNS challenge by JHM suggests that resistance is mediated by at least two genes—one dominant (*Rhv-1*),

and one recessive (*rhv-2*). Both genes are required for efficient resistance, and both genes function with nearly complete penetrance[1].

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[1] We have recently tested B10.M (*H-2*^{s}) mice for resistance to i.c. challenge, with JHM. In contrast to the results of Oth and coworkers (1976), we found that *H-2*^{s} mice are sensitive to JHM