

HISTOCOMPATIBILITY-LINKED GENETIC CONTROL OF DISEASE SUSCEPTIBILITY

MURINE LYMPHOCYTIC CHORIOMENINGITIS VIRUS INFECTION*

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One of the most interesting observations in microbiology is natural host variation in the ability to resist infectious diseases. Although they have long been recognized in many species, the precise mechanisms have not been clearly defined. For example, in both the acute and chronic disorders associated with lymphocytic choriomeningitis (LCM)¹ virus infection, there is a wide variation in susceptibility among murine strains (1-5). In general, those strains producing apparently the greatest amount of virus and the highest immune response are the most susceptible, while strains producing less virus and a weaker immune response are more resistant (2).

Whether or not tissue injury accompanies LCM virus infection depends, in large part, on the host immune response. Intracerebral (i.c.) injection of a large dose of virus causes an acute, disseminated, necrotizing, inflammatory disease only in immunocompetent mice (6-14), whereas a similar inoculum in cultured cells causes little or no injury, although virus replication continues to occur and infectious virus is made.

Recently, there has been an increasing awareness that genetic control of several specific immune responses in mice is linked to the major histocompatibility (*H-2*) locus (15, 16). Thus, histocompatibility-linked genetic control of the immune response might play an important role in determining resistance

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¹ Abbreviations used in this paper: EBM, Eagle's basic medium; FCS, fetal calf serum; LCM, lymphocytic choriomeningitis; PBS, phosphate-buffered saline; SW, Swiss Webster.

or susceptibility in disease states associated with specific immune response (17). With this background, we examined: (a) whether susceptibility to acute LCM virus infection was genetically determined, and (b) whether the tendency for increased susceptibility was linked to *H-2*.

Materials and Methods

Mice.—SWR/J and C3H/HeJ mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. They were mated to produce (SWR/J × C3H/HeJ)F₁ and (C3H/HeJ × SWR/J)F₁, and the hybrids were backcrossed with their parents at Stanford University Medical School, Stanford, Calif. All backcross mice were *H-2* typed, ear coded, and shipped along with parental and F₁ strains to Scripps Clinic and Research Foundation, La Jolla, Calif., for experimentation. SWR/J mice are *H-2^a* while C3H/HeJ are *H-2^k*. Additionally, C3H.Q breeders were obtained from D. Shreffler, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Mich. Such mice are *H-2^a*, being congenic with C3H/HeJ mice, and differ only for the *H-2* complex. Breeding was performed both at Stanford and Scripps. Adult Swiss Webster (SW) mice, 10–14 g, were obtained from a local breeder. Random testing of all mice indicated that they were free of LCM virus infection.

Tissue Culture.—Cells from SWR/J, C3H/HeJ, and C3H.Q mouse embryos were cultured in our laboratory. All cultured cells were initially grown in Eagle's basic medium (EBM) supplemented with 10% fetal calf serum (FCS), glutamine, penicillin, and streptomycin. Maintenance media contained 5% FCS, glutamine, and antibiotics.

LCM Virus.—Original seed virus was mouse brain passage strain CA1371 provided by Dr. Wallace Rowe, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Various virus stocks have been maintained in our laboratory by either i.c. passage through SWR/J, C3H/HeJ, C3H.Q, or SW mice, or tissue culture passages through L929 cells. In addition, the E-350 strain of LCM virus was obtained through Dr. Neal Nathanson, Department of Epidemiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md. This virus pool was maintained by i.c. passage in BALB/c mice. Methods of handling and diluting virus for inoculation have been described previously (1, 2). Virus titrations were carried out by i.c. inoculation of serial 10-fold dilutions of virus into groups of 8–12 mice, and LD₅₀ titration end points were calculated by the method of Reed and Muench (18). In other experiments, one specific dose of LCM virus was given i.c.

Histopathologic and Immunopathologic Techniques.—Central nervous system (CNS) tissue taken from mice inoculated with 100 LD₅₀ was harvested 6–7 days after i.c. injection. For histology, tissue was fixed in formalin and paraffin sections were stained with hematoxylin and eosin. Tissue taken for immunofluorescent evaluation was snap frozen in liquid nitrogen and handled as previously reported (2). Sections 4 μm thick were stained for the presence of viral antigen, host IgG, C3, and fibrinogen by a direct immunofluorescent technique that used fluorescein isothiocyanate conjugated to guinea pig antibody to LCM virus, rabbit antibody to mouse IgG, rabbit antibody to mouse C3, and rabbit antibody to rat fibrinogen, respectively. Production methods and specificity determinations of the reagents used have been published (2). The amount of virus carried by animals at the time of sacrifice was measured in 10% suspensions of brain tissue (wt/vol) from individual infected mice which were diluted serially 10-fold in EBM with 2.5% heat-inactivated FCS. Various dilutions were inoculated i.c. into SW mice.

Virus Adsorption.— 5×10^5 mouse embryo fibroblasts from primary cultures were plated in 60 × 15 mm Petri dishes. 48 h later, medium was removed and the cells were washed twice in phosphate-buffered saline (PBS). L929 cell-adapted LCM virus (LD₅₀ 10^{-5.5}/0.03 ml i.c. dose) diluted 1/50 in EBM containing glutamine, antibiotics, and 2.5% heat-inactivated FCS was added, and the resultant mixture was slowly rocked on a Bellco Glass rocker platform (Bellco Glass, Inc., Vineland, N. J.) at 37°C in a CO₂ incubator. After a 1 h incubation

the supernatant was collected; cells were washed three times in PBS, trypsinized, and counted. Serial 10-fold dilutions of supernatant fluid and cells were inoculated i.c. into SW mice. In other experiments, 10×10^6 cultured cells in suspension were incubated with LCM virus under the same conditions described above and then inoculated i.c. into SW mice. Quadruple samples were run during each experiment with one sample being utilized to determine cell protein concentration (19). Acute LCM disease is defined as death of animals from the disseminated, necrotizing, inflammatory effects accompanying LCM virus infection.

RESULTS

Susceptibility of SWR/J, C3H/HeJ, (SWR/J × C3H/HeJ)F₁, and C3H.Q Mice to Acute LCM Viral Disease.—When LCM virus that had been passed through SW brain was inoculated i.c., significantly less virus was needed to kill SWR/J than C3H/HeJ mice. Fig. 1 shows that while $10^{-5.4}$ and $10^{-6.6}$

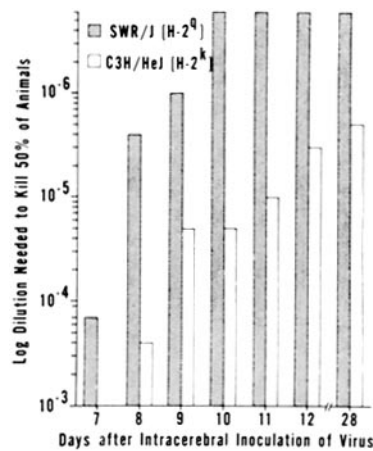


FIG. 1. Susceptibility of 6-wk-old SWR/J and C3H/HeJ mice to LCM virus. 10-fold dilutions of L929 cell-passed virus were inoculated i.c. and 10 mice received each dilution. The log dilution giving a 50% lethal end point per 0.03 ml i.c. injection was calculated by the method of Reed and Muench (18).

dilutions of virus killed 50% of SWR/J mice 8 and 28 days after viral challenge, only $10^{-3.4}$ and $10^{-5.5}$ dilutions of virus were needed to kill 50% of C3H/HeJ mice during the same time periods. Similar results occurred when either E-350 brain-passed or L929 cell-adapted LCM viruses were used. (SWR/J × C3H/HeJ)F₁ or (C3H/HeJ × SWR/J)F₁ hybrids were as susceptible to the lethal effects of acute LCM virus infection as their SWR/J parents. Table I shows that 1.6 logs less virus were required to kill 50% of (SWR/J × C3H/HeJ)F₁ combined male and female hybrids than the amount needed to kill 50% of C3H/HeJ mice. In contrast, similar amounts of virus killed (SWR/J × C3H/HeJ)F₁ hybrid and SWR/J mice. SWR/J and (SWR/J × C3H/HeJ)F₁ or (C3H/HeJ × SWR/J)F₁ hybrids are *H-2^{q/a}* and *H-2^{q/k}*, respectively, while

C3H/HeJ mice are $H-2^{k/k}$. To evaluate the relationship of the $H-2^a$ and $H-2^k$ alleles to acute LCM virus disease, we compared the susceptibility of C3H.Q mice with that of C3H/HeJ mice. C3H.Q mice are congenic with C3H/HeJ mice, differing only at the $H-2$ complex, C3H.Q being $H-2^{a/a}$ whereas C3H/HeJ are $H-2^{k/k}$. Table I shows that C3H.Q mice were significantly more susceptible to the lethal effects of LCM virus than C3H/HeJ mice; the difference between $H-2^{a/a}$ or $H-2^{a/k}$ and $H-2^{k/k}$ mice was significant in the χ^2 test, with $\chi^2 = 15.5$ and 23.4 in two experiments, and $P < 0.005$.

Susceptibility of (SWR/J × C3H/HeJ)F₁ Hybrid × Parental Backcross Mice to Acute LCM Disease.—Various groups of mice derived from backcrossing (SWR/J × C3H/HeJ)F₁ to SWR/J or C3H/HeJ mice were tested for their susceptibilities to acute LCM viral disease. Table II shows that all mice con-

TABLE I
*Susceptibility of (SWR/J × C3H/HeJ)F₁ Hybrids and C3H.Q Mice to Acute LCM Disease**

Strain	$H-2$	Sex†	LD ₅₀ ‡
SWR/J	q/q	M	6.2
C3H/HeJ	k/k	M	4.3
(SWR/J × C3H/HeJ)F ₁	q/k	M	5.9
C3H.Q	q/q	M	5.8
SWR/J	q/q	F	6.0
C3H/HeJ	k/k	F	4.6
(SWR/J × C3H/HeJ)F ₁	q/k	F	6.2
C3H.Q	q/q	F	5.6

* 10-fold dilutions of SW brain-passed virus were inoculated i.c. Acute LCM disease is defined as specific deaths of animals from the disseminated, necrotizing, inflammatory effects of LCM virus infection.

† M, male; F, female.

‡ Reciprocal of log dilution giving a 50% lethal end point per 0.03 ml i.c. inoculation.

taining one or more $H-2^a$ alleles were more susceptible, whereas mice lacking the $H-2^a$ allele (homozygous $H-2^{k/k}$) were comparatively resistant. Whereas 73% of all mice having the $H-2^{a/a}$ or $H-2^{a/k}$ genotype died after one LD₇₅ limiting dose of virus, only 30% of $H-2^{k/k}$ mice succumbed. These differences were significant at a P level of <0.005 . No major differences between sexes in susceptibility to LCM disease were seen.

Histopathology and Immunopathology of Acute LCM Virus Infection in $H-2^a$ and $H-2^k$ Mice.—30 C3H.Q mice and 30 C3H/HeJ mice, 6–8-wk-old, were inoculated i.c. with a lethal dose of E-350 strain of LCM virus. On the 6th day after injection, half of the C3H.Q mice but none of the C3H/HeJ mice had died. 10 C3H.Q mice and 10 C3H/HeJ mice were sacrificed, and CNS tissue was taken for histopathological evaluation. The remaining C3H.Q mice died

on the 8th day, whereas half of the C3H/HeJ mice did not die until the 11th day. On the 11th day, six additional C3H/HeJ mice were sacrificed and tissues processed. By the 14th day after i.c. inoculation, the remaining C3H/HeJ mice died.

Histopathologically, brain tissue from all C3H.Q mice showed a heavy cellular infiltrate in the leptomeninges and choroid plexus (Fig. 2). An abundance of polymorphonuclear cells accompanying both round cells and macrophages comprised the inflammatory infiltrate. In contrast, brain tissues from C3H/HeJ mice sacrificed 6 or 11 days after i.c. injection of LCM virus showed

TABLE II
*Relationship of q and k H-2 Alleles in Susceptibility to Acute LCM Disease**

	<i>H-2</i>	Sex†	No. of mice		Mortality %
			Injected	LCM disease‡	
F ₁ × SWR/J	<i>q/k</i>	M	22	17	77
	<i>q/k</i>	F	18	15	83
	<i>q/q</i>	M	12	8	66
	<i>q/q</i>	F	20	12	60
F ₁ × C3H/HeJ	<i>q/k</i>	M	12	10	83
	<i>q/k</i>	F	15	10	66
	<i>k/k</i>	M	17	7	41
	<i>k/k</i>	F	20	4	20

* 1 × LD₇₅ dose (for SWR/J mice) of L929 cell-passed virus was inoculated i.c. into various groups of mice. All mice were ear coded, and *H-2* type was not known until the conclusion of the experiment.

† M, male; F, female.

‡ Deaths due to the disseminated, necrotizing, inflammatory effects of LCM virus infection. Mice observed for 28 days after viral inoculation.

lesions consisting only of minimal leptomeningeal and choroid plexus infiltrates. Such infiltrates contained scant polymorphonuclear cells (Fig. 3).

10 6-8-wk-old C3H.Q and C3H/HeJ mice were inoculated i.c. with a lethal dose of E-350 strain of LCM virus. On the 6th day after injection the surviving C3H.Q and all the C3H/HeJ mice were sacrificed. Brain tissue was removed, cut in half with one part made into a 10% suspension (wt/vol), diluted 10-fold serially, and then injected into 4-6-wk-old SW mice, while the other part of the brain was snap frozen in liquid nitrogen for immunofluorescent study. Table III shows that the amounts of infectious virus carried in the brains of C3H.Q and C3H/HeJ mice were similar. Brains from the four C3H.Q mice studied had an average LD₅₀ titer of 10^{-5.5} as compared with 10^{-5.7} for C3H/HeJ mice. Immunofluorescence of brain tissue from C3H.Q and C3H/HeJ mice revealed approximately the same amount of intracerebral and intracerebel-

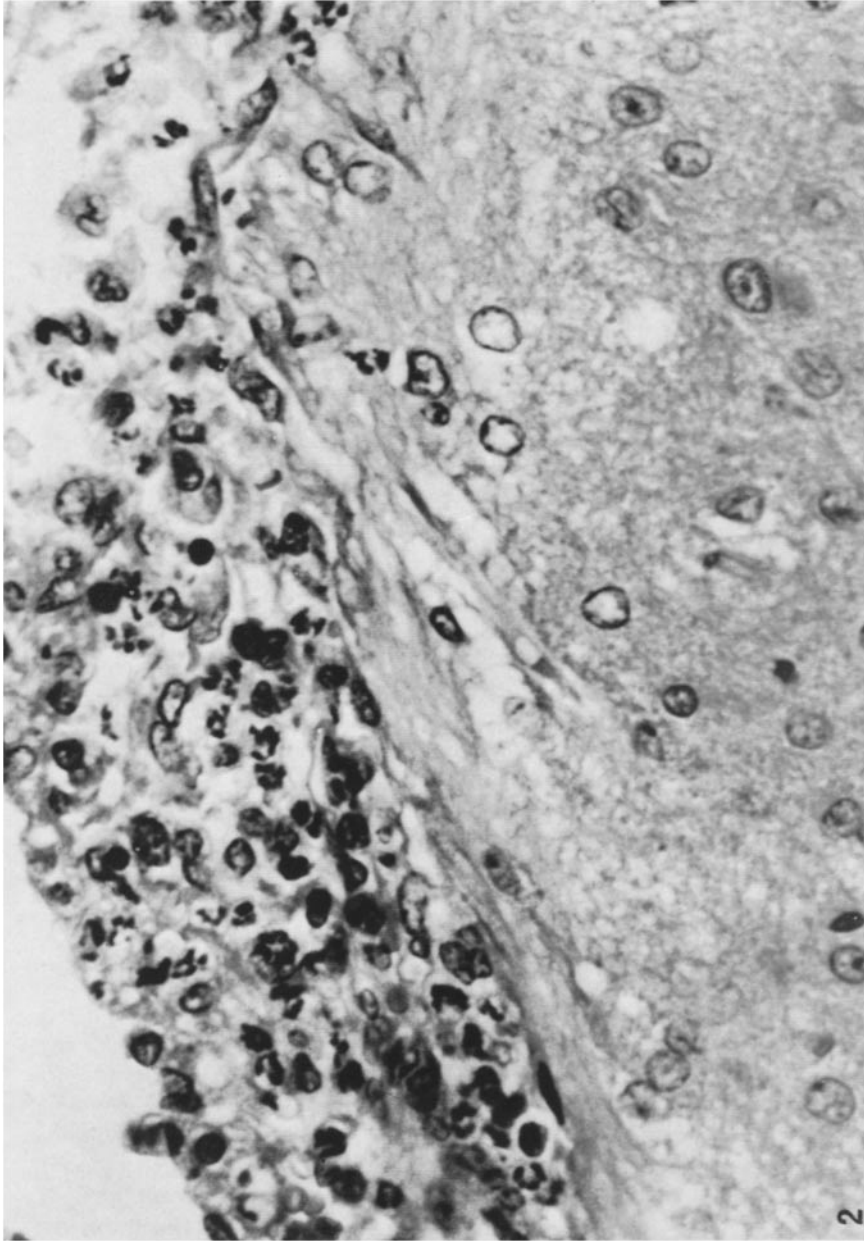


FIG. 2. Light photomicrograph of hematoxylin- and eosin-stained cerebral tissue from an 8-wk-old C3H.Q (H-2^q) mouse taken 6 days after i.c. injection of E-350 strain LCM virus. Heavy mixed cell infiltrates with a large number of polymorphonuclear cells are seen in the leptomeninges.

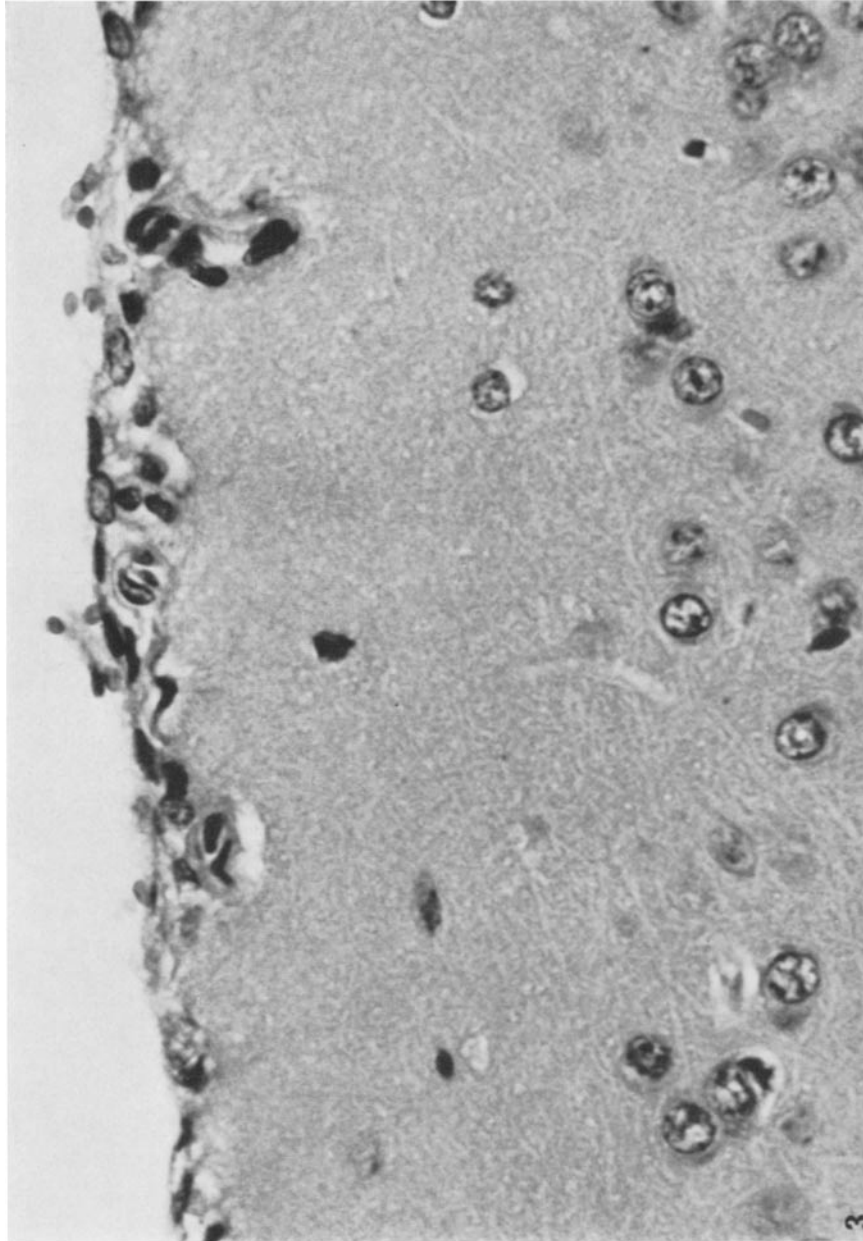


FIG. 3. Light photomicrograph of hematoxylin- and eosin-stained cerebral tissue from an 8-wk-old C3H/HeJ ($H-2^k$) mouse taken 6 days after i.c. injection of E-350 strain LCM virus. Few infiltrating cells are seen in the leptomeninges.

lar LCM antigen deposited predominately in choroidal and occassionally in neuronal cells. The amount of LCM viral antigen present in or on meningeal cells was difficult to assess because of the heavy infiltrative lesions in C3H.Q brains. In such lesions, abundant host Ig, C3, and fibrinogen were deposited. Of interest were the large number of infiltrating round cells that had Ig on their surfaces.

Amount of LCM Virus Adsorbed onto H-2^{q/a} and H-2^{k/k} Mouse Cells.—When added to cultures, approximately 1% of the total infectious virus adsorbed to either C3H.Q or C3H/HeJ mouse embryo cells. After a 1 h incubation of 10×10^6 C3H.Q or 10×10^6 C3H/HeJ embryo cells with LCM virus ($10^{-5.5}$ LD₅₀/0.03 ml i.c. inoculation) C3H.Q cells had an infectious LD₅₀ titer of $10^{-3.5}$ as compared with an LD₅₀ titer of $10^{-3.3}$ for C3H/HeJ cells.

TABLE III
*Amount of LCM Infectious Virus Carried in the Brains of 6-8-Wk-Old C3H.Q and C3H/HeJ Mice**

Strain	<i>H-2</i>	LCM virus titer†				Av
		No. 1	No. 2	No. 3	No. 4	
C3H.Q	<i>q/q</i>	5.4	5.7	5.7	5.2	5.5
C3H/HeJ	<i>k/k</i>	5.5	6.0	5.3	5.8	5.7

* Mice were sacrificed 6 days after receiving a lethal dose of E-350 strain of LCM virus i.c. An initial 10% suspension (wt/vol) and then 10-fold serial dilutions were made in 2.5% heat-inactivated FCS. Various dilutions were inoculated i.c. into 4-6-wk-old SW mice. Animals were observed for 28 days for deaths specifically due to LCM virus infection.

† Number represents the reciprocal of the log dilution needed to kill 50% of the animals.

DISCUSSION

These experiments clearly show that susceptibility to acute LCM virus disease is controlled in part by a dominant gene which is closely linked to the *H-2* locus. First, the F₁ hybrid (*H-2^{q/k}*) from mating a susceptible mouse (*H-2^{q/a}*) to a resistant mouse (*H-2^{k/k}*) is susceptible to LCM virus disease. Secondly, when such hybrids are backcrossed with susceptible parents, all backcross offspring (*H-2^{q/a}* and *H-2^{q/k}*) are highly susceptible, whereas F₁ backcrosses to resistant parents result in half of the F₂ offspring being susceptible (*H-2^{q/k}*) while the other half are relatively resistant (*H-2^{k/k}*). Thirdly, study of a congenic strain pair shows that *H-2^q* mice are relatively susceptible to acute LCM virus disease, whereas *H-2^k* mice are resistant.

Since segregation in the F₁ × C3H population does not show a strict 1:1 ratio, it is likely that susceptibility to LCM virus is a multigenic trait. While such a hypothesis is difficult to test because of the limitations of the assay (mortality), it is borne out by the observation that *H-2^{k/k}* mice from the

$F_1 \times C3H$ population are less susceptible to LCM than C3H/HeJ mice ($\chi^2 = 13.5$, $P < 0.005$). This result could be due to hybrid vigor, but is compatible with the existence of other genes controlling LCM susceptibility and segregating in the $F_1 \times C3H$ population.

Correlation between $H-2$ type and susceptibility to acute LCM disease was best seen among $H-2^{k/k}$, $H-2^{a/k}$, and $H-2^{a/a}$ backcross mice given a limited dose of virus. Vaz, Vaz, and Levine (20, 21) have reported a similar linkage between immune response to complex antigens given in limiting doses and the $H-2$ complex. When the dose of antigen was low, the correlation between $H-2$ type and immune responsiveness was clear, whereas with a high antigen dose this distinction became obliterated and all strains responded equally.

There are three principal mechanisms by which histocompatibility antigens might alter susceptibility to a viral infection. First, histocompatibility antigens may represent specific receptor sites for attachment of LCM virus. This possibility can be ruled out since cells from $H-2^a$ mice (which are more susceptible to LCM virus disease than $H-2^k$ mice) adsorb the same amount of LCM virus as $H-2^k$ cells. Secondly, histocompatibility antigens and LCM virus may share antigenic determinants. The host would then fail to respond immunologically to the virus and would exhibit resistance to LCM virus (since death requires an effective immune response in this infection). Again this appears not to be the mechanism since infected C3H/HeJ mice ($H-2^k$, resistant) are capable of mounting both humoral and cellular responses against LCM virus (2, 22–24); susceptibility (responsiveness) is dominant in the F_1 when this mechanism would require it to be recessive, and antibody against $H-2^k$ determinants does not stain other cells infected with LCM virus.²

The third possibility is that an immune response gene analogous to the Ir genes described by McDevitt and Benacerraf (15, 16) controls the ability of the host to respond to LCM virus antigens. There are a number of reasons for believing that the $H-2$ -linked control of susceptibility to LCM virus is an example of such a mechanism. Since the $H-2$ -linked immune response genes are dominant, susceptibility would be expected to be, and is, dominant in the heterozygote. Mice that are homozygous or heterozygous for $H-2^a$ are susceptible, whereas those homozygous for $H-2^k$ are resistant. In addition, inflammatory responses against the virus mounted by $H-2^a$ (susceptible) mice were greater than those of $H-2^k$ (resistant) mice, yet the amounts of virus carried in the brain were similar in mice of both $H-2$ types. It is not yet known whether the distribution of virus antigens on cell surfaces is different in $H-2^a$ than in $H-2^k$ cells, or whether activation of pharmacologic mediators of tissue injury is greater in $H-2^a$ than in $H-2^k$ cells.

Resistance or susceptibility to infection involves many complex processes

² Oldstone, M. B. A. Unpublished data.

affected by immune and nonimmune mechanisms. The available evidence suggests that LCM virus infection represents an example of histocompatibility-linked genetic control of specific disease susceptibility. Earlier reported examples of this phenomenon include *H-2*-linked control of susceptibility to Gross virus leukemogenesis in the mouse (25, 26) and *H-2*-linked control of induction of autoimmune thyroiditis in the mouse (27). In all of these instances, immune responsiveness plays a vital role in pathogenesis, and the pattern of inheritance of specific disease susceptibility with particular *H-2* alleles is compatible with the expected action of an *H-2*-linked specific immune response gene. Unfortunately, there is as yet no direct evidence showing that immune response to the Gross virus, LCM virus, or thyroid antigens separate with *H-2* type and with disease incidence in individual members of a segregating population. This evidence is crucial to the hypothesis that *H-2*-linked genetic control of specific disease susceptibility operates via the mechanism of specific immune response genes, and is of further importance because there is as yet no estimate of how widespread in nature such a mechanism may be.

SUMMARY

Acute necrotizing inflammatory disease after intracerebral injection of LCM virus is largely dependent on the host immune response to the virus and is controlled, in part, by a dominant gene which is closely linked to the *H-2* locus. The F_1 hybrid ($H-2^{a/k}$) from mating a susceptible SWR/J mouse ($H-2^{a/a}$) to a resistant C3H/HeJ mouse ($H-2^{k/k}$) is susceptible to LCM virus disease. When such hybrids ($H-2^{a/k}$) are backcrossed to susceptible parents ($H-2^{a/a}$), all F_2 offspring ($H-2^{a/a}$, $H-2^{a/k}$) are highly susceptible. In contrast, hybrid ($H-2^{a/k}$) backcross to resistant parents ($H-2^{k/k}$) results in half of the F_2 offspring being susceptible ($H-2^{a/k}$) while the other half are resistant ($H-2^{k/k}$). Similarly, in congenic $H-2^{a/a}$ and $H-2^{k/k}$ mice, $H-2^{a/a}$ mice are relatively susceptible to acute LCM disease, whereas $H-2^{k/k}$ are resistant.

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