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Genetics of Natural Resistance to Sendai Virus Infection in Mice

DAVID G. BROWNSTEIN

Section of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

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The genetics of resistance to a naturally occurring respiratory infection caused by Sendai virus was examined in F_1, F_2, and backcross progeny of resistant C57BL/6J and susceptible DBA/2J mice and in 25 recombinant inbred strains. An intranasal inoculum of 0.1 50% tissue culture infective dose (low dose) of Sendai virus caused 0% mortality in C57BL/6J and F_1 mice and 73% mortality in DBA/2J mice. An inoculum of 1.0 50% tissue culture infective dose (high dose) caused 3, 0, and 89% mortality in C57BL/6J, F_1, and DBA/2J mice, respectively. Low-dose infection caused 36% mortality in F_1 × DBA/2J hybrids and 0% mortality in F_2 hybrids. High-dose infection caused 29 and 32% mortality in F_1 × DBA/2J and F_2 hybrids, respectively. Resistance was not linked to H-2 haplotype, coat color, or sex. High-dose infection caused deaths in 12 recombinant inbred strains, and the strain distribution pattern was concordant with that of a chromosome 1 marker, Sas-1, in 20 of 25 strains ($P < 0.01$). Resistance therefore behaved as a simple Mendelian dominant trait which presumptively mapped to chromosome 1.

Genetic factors play a decisive role in resistance to many viral infections. These factors may be mono- or polygenic and may be expressed through a variety of mechanisms. The availability of inbred strains of mice, including mutant, congenic, and recombinant strains, has provided an important tool with which to evaluate the role and expression of genetic factors in natural resistance to viral infections.

There have been few studies concerning the role of genetic factors in resistance to acute viral respiratory infections, and these have been limited to influenza A virus infection. An autosomal dominant allele, Mx, confers resistance to the lethal effects of pneumotropic influenza A virus in mice (13–15). This allele has only been found in A2G mice (14, 15). Because mice are not natural hosts of influenza A virus, the significance of Mx-conferred resistance to this virus is unknown (3).

Sendai virus, a parainfluenza 1 virus, is a natural respiratory pathogen of laboratory mice. Resistance to lethal Sendai virus infection varies among inbred mouse strains, suggesting that genetic factors are important in natural resistance (19). In this study, the genetic basis of resistance to Sendai virus infection is confirmed, and the mode of inheritance is examined in F_1, F_2, and backcross progeny of resistant C57BL/6J and susceptible DBA/2J mice. In addition, possible linkage of resistance to typed genetic loci is examined in F_2 hybrids and in recombinant inbred (RI) strains derived from the parent strains.

MATERIALS AND METHODS

Mice. C57BL/6J and DBA/2J mice were obtained from Jackson Laboratory, Bar Harbor, Maine. These strains were selected because the former is resistant to and the latter is susceptible to lethal Sendai virus infection (19) and because they are the progenitors of a large set of RI strains (27).

Several hybrid crosses were studied. F_1 mice derived from C57BL/6J females and DBA/2J males ($B6D2F_1/J$) were obtained from Jackson Laboratory. F_2 mice and backcross progeny were produced at the Yale University School of Medicine in specific-pathogen-free isolators. F_2 mice were produced from breeding harems of $B6D2F_1/J$ mice. Backcross mice were produced from breeding harems of $B6D2F_1/J$ females and DBA/2J males.

RI strains are derived by inbreeding randomly selected pairs of mice from the F_2 generation of the cross between two disimilar progenitor strains (2). The BXD RI strains were obtained from B. A. Taylor, Jackson Laboratory, and were derived from the initial cross of C57BL/6J and DBA/2J mice (25).

Mice were used in experiments at 6 to 8 weeks of age. They were maintained in a biohazard containment facility and housed in polycarbonate or stainless steel cages with corn-cob bedding in a positive-pressure laminar flow cabinet. They were fed Purina Rodent Laboratory Chow 5001 and hyperchlorinated water (9 mg/liter) ad libitum. Sentinel mice from Jackson Laboratory and from breeding isolators were killed, bled,

and determined to be free of serum antibodies to Sendai virus by a complement fixation test (9).

Virus. Sendai virus (isolate 771076) was isolated in our laboratory from a 1-year-old C3H mouse with spontaneous Sendai virus pneumonia. Inoculated BHK-21 cells adsorbed guinea pig erythrocytes, and specific anti-Sendai serum inhibited this adsorption. A 10% (wt/vol) lung suspension containing 10^7 50% tissue culture infective doses ($TCID_{50}$) per 0.1 g of tissue was stored at −70°C. Dilutions were made in balanced salt solution containing 5% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Six-week-old CD_1 mice, previously determined to be free of serological evidence of infection with Sendai virus, pneumonia virus, mouse hepatitis virus, mouse encephalitis virus, ectromelia virus, reovirus 3, mouse rotavirus, lymphocytic choriomeningitis virus, minute virus, and *Mycoplasma pulmonis*, only seroconverted to Sendai virus 14 days after intranasal inoculation of diluted stock lung suspension.

H-2 typing. Mice for typing were individually identified with toe clips and bled from the periorbital venous plexus before infection. Blood samples were placed in 3.8% sodium citrate and centrifuged at 4,500 × *g* for 2 min. The buffy coat was transferred to a tube (12 by 75 mm) and centrifuged at 1,500 × *g*, and the erythrocytes were lysed by hypotonic shock. Leukocytes were suspended in medium 199 with 5% fetal bovine serum. The H-2 haplotype (b/b, b/d, d/d) of peripheral blood lymphocytes was determined by Donal Murphy, Yale University School of Medicine, with a dye exlusion assay described elsewhere (17).

Mode of infection. Mice were infected under light methoxyflurane (Metophane; Pitman-Moore, Washington Crossing, N.J.) anesthesia by placing 10 μl of diluted infected mouse lung suspension on the external nares.

Evaluation of resistance. F_2 and backcross hybrids were segregated by sex but randomly grouped by coat color at five mice per cage. Only male RI mice were used, and these were housed at three mice per cage by strain. Mice were infected with either of two virus doses (1.0 and 0.1 $TCID_{50}$ per 0.01 ml) which were previously determined to elicit maximal phenotypic differences between the parent strains. For most of the studies of F_1, F_2, and backcross hybrids 10 to 20 mice of the parent strains were included. Because of the consistency of the parental responses to these challenge doses, the parent strains were not included in some of the later studies. Mortality was scored daily for 21 days after infection. Preliminary studies indicated that only sporadic deaths occurred after this interval. Dead mice were necropsied and inspected for gross evidence of pneumonia. Lungs from dead animals with equivocal gross findings and 10% of randomly selected dead mice with unequivocal gross findings were perfused with 10% buffered Formalin, routinely processed for histopathology, and examined for the presence of the characteristic histopathological changes of Sendai virus pneumonia. These included necrotizing or hyperplastic endobronchiolitis and bronchogenic alveolitis (8). Sendai virus antigens were demonstrated in the lungs of selected mice by indirect immunofluorescence of Formalin-fixed tissues by trypsin digestion as described elsewhere (24). Goat antiserum to Sendai virus (M. A. Bioproducts, Walkersville, Md.) and fluorescein-labeled rabbit antiserum to goat immunoglobulin G (Miles Laboratories, Elkhart, Ind.) were used.

Surviving mice were killed with CO_2 gas at 21 days, bled by cardiac puncture, and examined for gross or microscopic evidence of pneumonia. Selected mice were also examined for the presence of Sendai virus antigens in pneumonic foci as described above. Any mice without pathological evidence of pneumonia were assessed for the presence of serum complement fixing antibodies to Sendai virus. All mice inoculated intranasally developed pathological or serological evidence of infection.

Datum analysis. The normal difference (z) test was used to compare mortality proportions in the parent strains. The chi-square test was used to compare observed deaths with expected deaths in conventional genetic analyses of groups of over 100 animals, to assess linkage to H-2 haplotype, sex, and coat color in hybrid mice, and to assess linkage to typed genetic loci in RI strains. Yates' correction was applied to comparisons with one degree of freedom. Binomial distributions were used to compare observed deaths with expected deaths in conventional genetic analyses of groups of under 100 animals. Differences that yielded probability values of <0.01 were considered statistically significant.

RESULTS

Death rates were higher in DBA/2J mice that were challenged with 0.1 $TCID_{50}$ ($P < 0.0001$) and 1.0 $TCID_{50}$ ($P < 0.0001$) of Sendai virus than they were in C57BL/6J mice similarly challenged (Table 1). F_1 hybrids exhibited the same phenotype as the resistant parent, suggesting that resistance was inherited as a dominant trait. Hybrids produced from F_1 mice backcrossed to the susceptible parent strain showed 36 and 29% mortality after low- and high-dose challenges, respectively (Table 2). F_2 hybrids showed 0 and 32% mortality after low- and high-dose challenges, respectively. These values were in agreement with values predicted by a one-gene hypothesis of resistance but not with values predicted for two or more genes. Coat color differences among the hybrid mice were determined by the brown (b) locus on chromsome 4 and the dilution (d) locus on chromosome 9. The influence of these two loci, as well as H-2

TABLE 1. Occurrence of mortality in C57BL/6J, DBA/2J, and F_1 mice infected with Sendai virus

Strain	Dose ($TCID_{50}$)	No. (%) dead/ no. tested
C57BL/6J	0.1	0/30 (0)
	1.0	1/29 (3)
DBA/2J	0.1	22/30 (73)
	1.0	24/27 (89)
F_1	0.1	0/30 (0)
	1.0	0/28 (0)

TABLE 2. Observed and expected mortality in C57BL/6J and DBA/2J hybrid mice infected with Sendai virus

Cross	Dose ($TCID_{50}$)	Observed no. dead (no. tested)	Expected no. dead[a] (*P* value)	
			1 Gene	2 Genes
F_1 × DBA/2J	0.1	54 (150)	55 (>0.10)[b]	83 (<0.01)[b]
	1.0	9 (31)	14 (>0.05)[c]	21 (<0.01)[c]
F_1 × F_1	0.1	0 (14)	3 (>0.10)[c]	5 (<0.01)[c]
	1.0	63 (200)	50 (>0.025)[b]	82 (<0.01)[b]

[a] Based on the genetic hypothesis of resistance where $(1/2)^n$ backcross mice exhibit the resistant phenotype $(3/4)^n$ F_2 mice exhibit the resistant phenotype, and *n* equals the number of genes (11).
[b] Calculated by the chi-square test.
[c] Calculated by binomial distribution.

haplotype and sex, on resistance is shown in Table 3. There was no evidence that resistance was linked to any of these loci.

Resistance was examined in 25 RI strains (three animals per strain) derived from C57BL/6J and DBA/2J progenitors. After infection with 1.0 $TCID_{50}$ of virus, one or more animals of 12 strains died (Table 4). This was in agreement with the number of strains expected to be susceptible based on single-gene inheritance ($\chi^2 = 0.0$). There was, however, equivocal mortality for 5 of the 12 strains in which only a single animal died. The strain distribution pattern for resistance was compared with those for typed genetic loci in the BXD strains. If all strains with one or more deaths were assigned the genotype of the susceptible parent (D) and all strains without mortality were assigned the genotype of the resistant parent (B), there was significant concordance (20 of 25) with the serum antigenic substance (Sas-1) on chromosome 1 ($\chi^2 = 7.83$, $P < 0.01$) (Table 5) (23). If the five strains with equivocal mortality were eliminated from the data, concordance with Sas-1 approached significance (16 of 20, $P < 0.025$).

TABLE 3. Linkage assessment between resistance to Sendai virus infection and H-2 haplotype, coat color, and sex in F_2 mice

Locus	Genotype	No. (%) dead/no. tested	Chi-square value	*P* value
H-2[a]	d/d	13/51 (25)	0.87[b]	>0.10
	d/b	30/94 (32)		
	b/b	17/50 (34)		
b (Brown)	b/b	22/50 (44)	2.73[c]	>0.05
	b/+, +/+	41/150 (27)		
d (Dilution)	d/d	18/47 (38)	0.64[c]	>0.10
	d/+, +/+	45/153 (29)		
Sex	xx	35/107 (22)	0.57[c]	>0.10
	xy	28/93 (30)		

[a] Determined by cytotoxicity testing of peripheral blood lymphocytes.
[b] Calculated by the chi-square test with two degrees of freedom.
[c] Calculated by the chi-square test with Yates' correction.

DISCUSSION

This study confirms that genetic factors are responsible for the difference in resistance to lethal Sendai virus infection exhibited by C57BL/6J and DBA/2J mice, since resistance followed the predictions of Mendelian genetics and acted as a single autosomal dominant trait. This result is surprising because of the reported response patterns of inbred mouse strains to lethal Sendai virus infection (19). Inbred strains do not fall clearly into resistant and susceptible groups as predicted by a single autosomal trait but exhibit a continuum of resistance and susceptibility, suggesting multiple determinants. This apparent disparity may be explained in several ways. The previously reported response pattern was for inbred mice 4 to 6 weeks old,

TABLE 4. Occurrence of mortality in BXD RI strains infected with Sendai virus

RI strain	No. dead[a]	RI strain	No. dead[a]
1		18	3
2		19	
5	3	21	2
6	1	22	
8		23	3
9		24	
11		25	
12		27	3
13	1	28	2
14	1	29	3
15		30	1
16		31	
		32	1

[a] Number dead out of three mice per strain infected with 1.0 $TCID_{50}$ of Sendai virus.

TABLE 5. Segregation of resistance to Sendai virus and chromosome 1 marker Sas-1 in BXD RI strains

RI strain	Sendai virus[a]	Sas-1[b]	RI strain	Sendai virus[a]	Sas-1[b]
1	B	B	18	D	B
2	B	B	19	B	B
5	D	D	21	D	B
6	(D)[c]	D	22	B	B
8	B	B	23	D	D
9	B	B	24	B	D
11	B	D	25	B	B
12	B	B	27	D	D
13	(D)	D	28	D	D
14	(D)	B	29	D	D
15	B	B	30	(D)	D
16	B	B	31	B	B
			32	(D)	D

[a] Strains with one or more deaths were assigned the genotype of the susceptible parent (D). Strains with no deaths were assigned the genotype of the resistant parent (B).

[b] From Rosenstreich et al. (23).

[c] Letters in parentheses are for strains in which only one of three mice died.

whereas 6- to 8-week-old mice were used in the present study. A genetic analysis of the differential resistance of C57BL/6J and DBA/2J mice to Sendai virus infection performed at this laboratory with 4- to 6-week-old mice was in agreement with polygenic rather than monogenic inheritance (D. G. Brownstein, Lab. Anim. Sci. 32:419–420, 1982). Certain genetically determined resistance factors are apparently only expressed in animals under 6 weeks of age and are therefore involved in the ontogeny of natural resistance. Alternatively, multiple genetically determined resistance factors may be distributed among inbred mouse strains, but only one of these factors determines the difference in susceptibility between mature C57BL/6J and DBA/2J mice.

Resistance of mice to several conventional virus infections has been linked to the major histocompatibility complex, H-2 (10, 12, 16). The mechanism of the H-2 effect on host responses to these viruses is unknown but may involve immune responsiveness. There is evidence that genetic resistance to Sendai virus infection is expressed through the immune system: (i) the immune response is of prime importance in terminating a primary Sendai virus infection (1, 4–6, 21, 22); (ii) immunosuppression abrogates genetic resistance to Sendai virus infection (1, 4, 22); and (iii) genetically resistant (C57BL/6J) mice recruit large numbers of lymphoid cells to infected airways where virus replication is terminated, whereas susceptible (DBA/2J) mice recruit relatively few lymphoid cells to infected airways and sustain severe parenchymal injury resulting from the extension of virus replication to alveolar lining cells (8). Resistance to Sendai virus was not linked to the major histocompatibility complex. The H-2^d haplotype of the susceptible strain was not disproportionately represented among F_2 mice that died or among susceptible RI mice. If the gene controlling resistance to Sendai virus infection controls immunological events, this is another example of non-H-2-linked immune responsiveness.

Presumptive evidence was provided that resistance to Sendai virus infection was linked to the chromosome 1 marker Sas-1 with BXD RI strains. Resistance segregated concordantly with this marker in 20 of the 25 strains tested. Since only three mice per strain were used, there was some ambiguity concerning the susceptibility of 5 of the 25 strains. This linkage approached significance ($P < 0.025$), however, after ambiguous strains were eliminated from the calculations. Further studies to confirm this linkage should include larger RI cohorts and linkage analysis among backcross populations.

The presumptive linkage of resistance with a chromosome 1 marker is of interest because resistance to two other intracellular pathogens, *Salmonella typhimurium* (20) and *Leishmania donovani* (7), is also linked to chromosome 1 markers. The locus (*Lsh*) controlling susceptibility to visceral leishmaniasis has been mapped between the centromere and the marker isocitrate dehydrogenase-1, which is proximal to Sas-1 (7, 23). The locus controlling resistance to *S. typhimurium* is closely linked to but distinct from the *Lsh* locus (18, 26).

Recent advances in genetics, immunology, and molecular biology make the analysis of genetic factors that control resistance to viral infections tractable. The non-H-2-linked, unifactorial nature of genetic resistance of mice to Sendai virus infection makes this a relatively simple and therefore attractive system for the study of genetic factors that control natural resistance to acute viral respiratory infections.

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