Genetic Determinants of Resistance to Ectromelia (Mousepox) Virus-Induced Mortality

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Resistance to ectromelia (mousepox) virus-induced mortality was examined in crosses between susceptible DBA/2J, A/J, and BALB/cByJ mice and resistant C57BL/6J and AKR/J mice. Depending on the cross, resistance to mousepox virus was shown to be determined by one or more independently assorting autosomal loci with dominant alleles for resistance in AKR/J and C57BL/6J mice and recessive alleles in A/J, BALB/cByJ, and DBA/2J mice. A sexual dimorphism in resistance to disease was also observed.

Ectromelia virus, a member of the orthopoxvirus family, is a natural pathogen of mice that causes lethal disease in some strains of inbred mice but not in others (10, 13). The mechanisms responsible for differences in susceptibility to the lethal effects of this agent are poorly understood. However, a clear genetic contribution to these strain differences was established in an earlier study of a cross between resistant C57BL mice and susceptible outbred mice (10, 11) as well as in evaluations of C57BL/10 mice congenic for different *H-2* haplotypes (8). The results of these studies demonstrated that, in the cross examined, a single autosomal dominant gene determined the resistant phenotype of C57BL mice (11) and that different *H-2* haplotypes had a comparatively minor but still detectable influence on the development of disease in C57BL/10 mice (8). The latter effect was attributed to the influence of *H-2* genes on the development of an effective cell-mediated immune response (1-3).

In more recent studies, it was shown that AKR/J(AK) mice (like C57BL/6J[B6]mice) were resistant to the lethal effects of ectromelia virus, whereas BALB/cByJ(C), DBA/2J(D2), A.By/SnJ, and C3H/HeJ mice were all highly susceptible, although to various degrees (13). To determine if host genetic influences were responsible for the strain differences in susceptibility to ectromelia virus, we crossed resistant and susceptible strains and backcrossed the F_1 s to the susceptible parent (Table 1).

Mice 6 to 8 weeks of age were inoculated in the footpads with 5×10^3 to 1×10^4 PFU of the NIH 79 strain of ectromelia virus in 0.05 ml of phosphate-buffered saline (without Ca^{2+} and Mg^{2+} [13]) and observed for 21 days. This dose of virus was chosen because preliminary studies indicated that it killed 100% of male A/J (A) mice tested. In agreement with a previous study, no mortality was observed in B6 or AK mice (13). For each of the susceptible strains, mortality was higher in males than in females, although these differences were not statistically significant. However, when the deaths of 22 male and female A mice were analyzed by the Wilcoxon two-sample test (12), the male mice were shown to have a significantly shorter median survival time ($P < 0.001$; R. M. L. Buller, unpublished data). No mortality was observed among any of the F_1 female mice; among the males, mortality was observed only in (B6 $\times$ A) F_1 mice (7%).

These results demonstrated that resistance to ectromelia virus-induced mortality was a dominant characteristic and that susceptibility was recessive in crosses between B6 and A mice.

The genetic contribution to resistance to ectromelia virus-induced mortality was evaluated in studies of infected mice from crosses between resistant F_1 female mice and susceptible A, C, and D2 male mice. The results of these tests (Table 1) demonstrated the following points. First, ca. 50% of all (B6 $\times$ A) F_1 $\times$ A backcrossed mice died, indicating that in this backcross resistance to ectromelia virus-induced mortality was controlled by a single autosomal dominant gene. By chi-square analysis, the differences in mortality between male (51%) and female (40%) mice in this backcross were not significant ($\chi^2 = 1.91$; $P = 0.17$). This gene was provisionally termed *Rmp-1* (resistance to mousepox), with an *r* (resistance) allele carried by B6 mice and an *s* (susceptibility) allele carried by A mice. A strain of A mice (A.B6-*Rmp-1'*) congenic for the resistance locus is being developed and is now at the fifth backcross generation.

By comparison, overall mortality in the three other sets of backcrossed mice tested ranged from 0 to 21%, and there were sex-related differences in the frequencies of deaths in each backcross population (Table 1). Combined male and female mortality rates of (B6 $\times$ C) F_1 $\times$ C, (B6 $\times$ D2) F_1 $\times$ D2, and (AK $\times$ D2) F_1 $\times$ D2 mice were significantly different from those of (B6 $\times$ A) F_1 $\times$ A mice ($\chi^2 = 68$; 3 df; $P < 0.001$), and the male-female differences in mortality were all significant at the $P < 0.05$ level. The discrepancies in mortality rates of male and female mice made it difficult to determine accurately the number of B6 or AK genes contributing to ectromelia virus resistance in the crosses with C or D2 mice, but in each population at least two and probably more independently assorting loci were involved.

Taken together, these data demonstrate that mortality in susceptible mice induced by ectromelia virus is determined by one or more independently assorting autosomal loci with dominant alleles for resistance in AK and B6 mice and recessive alleles in A, C, and D2 mice. Sexual dimorphism in resistance, although evident in some of the parental strains, was much more apparent in the backcrossed populations. At present, it has not been established if these sex-related differences are determined by a gene(s) mapping to the X or Y chromosome or if they are hormonally determined. Studies designed to answer this question are planned. An earlier study demonstrated that the 50% infectious doses of virus

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TABLE 1. Ectromelia virus-induced mortality in inbred, selected F₁, and backcrossed mice

Mice	Female			Male			Total % mortality
	No. inoculated	No. dead	% Mortality	No. inoculated	No. dead	% Mortality	
B6	21	0	0	22	0	0	0
AK	10	0	0	26	0	0	0
A	20	18	90	43	43	100	97
C	10	5	50	6	6	100	69
D2	10	8	80	17	14	82	81
(B6×A)F ₁	60	0	0	30	2	7	2
(B6×C)F ₁	10	0	0	10	0	0	0
(B6×D2)F ₁	10	0	0	10	0	0	0
(AK×D2)F ₁	30	0	0	30	0	0	0
(B6×A)F ₁ ×A	68	27	40	70	36	51	46
(B6×C)F ₁ ×C	26	0	0	26	5	19	10
(B6×D2)F ₁ ×D2	91	6	7	81	14	17	12
(AK×D2)F ₁ ×D2	98	9	9	70	15	21	14

for all the parental strains used in this study were quite similar (13). Thus, the effects of *Rmp-1* alleles do not involve resistance to infection, and the exact mechanism remains to be elucidated.

The detection of innate resistance determinants in inbred mice raises the possibility that, by analogy, similar genetic factors may be present in the human population. Genes such as these could have influenced the susceptibility of individuals to the now eradicated disease smallpox, which was caused by a related orthopoxvirus. There is some indirect epidemiological evidence to support this contention (4). With the possible reintroduction of vaccinia virus (an orthopoxvirus of unknown origin) as a vehicle for immunizing humans with heterologous antigens (6, 7, 9), understanding the genetics of resistance to diseases caused by orthopoxviruses takes on renewed importance. The significance of this subject is underscored by the occurrence of 2.9 cases of postvaccine encephalitis per million primary vaccinations administered during the smallpox eradication program in the United States (5).

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