Serial Backcross Analysis of Genetic Resistance to Mousepox, Using Marker Loci for *Rmp-2* and *Rmp-3*

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At least three genes from C57BL/6 mice that mediate dominant resistance to lethal mousepox were isolated and transferred onto a susceptible DBA/2 background. Three [(C57BL/6 × DBA/2) F_1 × DBA/2] male mice that survived infection were selected as founders on the basis of different complements of marker loci for two resistance genes, *Rmp-2*^r (*Hc*⁺) and *Rmp-3*^r (*H-2D*^b). They were crossed with DBA/2 mice, male progeny were infected with ectromelia virus, and the cycle was repeated with surviving male progeny through seven backcross generations. Two founders carried a marker locus for *Rmp-2*^r or *Rmp-3*^r, and the third carried neither marker locus. Resistance pedigrees were analyzed for passage of marker loci. From the three founders, resistance was passaged through multiple generations, producing backcross lines with intermediate-male-resistance phenotypes (20% resistant). Females of backcross lines with intermediate male resistance had high resistance (>50%). High-resistance backcross lines (40% male resistance) also developed from the founders that carried marker loci for *Rmp-2*^r and *Rmp-3*^r, and marker loci were passaged through all generations of high resistance but not intermediate-resistance lines. About one-third of all resistant mice in high-resistance lines sired by mice that carried marker loci for *Rmp-2*^r and *Rmp-3*^r did not carry the respective marker locus. In lines that carried *Rmp-2*^r, this was apparently not the result of recombination between *Rmp-2*^r and *Hc*⁺, because *Rmp-2* was not in the predicted location on chromosome 2 and because mice that did not inherit *Hc*⁺ transmitted significantly less male resistance than *Hc*⁺-positive mice, although female resistance remained high. These results confirmed that C57BL/6 mice have redundant resistance mechanisms, two of which are controlled at least in part by *Rmp-2*^r and *Rmp-3*^r, and provided evidence for a fourth resistance gene, herein presumptively named *Rmp-4*, which protects females more than males and which may be epistatic to *Rmp-2*.

Poxviruses have become a focus of intense interest, in part because of the repertoire of proteins that they encode with the potential to interact with and modulate host antiviral responses (9, 39, 40). The spectrum of probable host-interactive proteins that poxviruses encode is without precedent, endowing them with a degree of self-determination not seen in other viruses. Attention has been directed to the role of poxvirus-encoded host-interactive proteins in promoting virulence and virus survival in the host. Little attention has been given to the possibility that these host-interactive proteins themselves are specific targets of host resistance mechanisms that diminish virulence and virus survival in the host.

Ectromelia virus, an orthopoxvirus, is a naturally occurring pathogen of mice and a paradigm for generalized poxvirus infections (14). Most inbred strains of mice are highly susceptible to the lethal effects of ectromelia virus infection, but C57BL and AKR strains are highly resistant (3, 5). Susceptible strains succumb to the acute necrotizing effects of ectromelia virus on liver, spleen, and bone marrow, whereas resistant strains develop mild, transient inflammatory changes in target organs (20). Genetic resistance to mousepox provides a means to study the relationship between resistance mechanisms and virus-encoded, host-interactive proteins at the genetic level.

Resistance to lethal mousepox is inherited as a dominant Mendelian trait (11, 34). Variable numbers of genes control resistance, depending on the genotypes of the crossed strains. Resistance in crosses between resistant C57BL/6

(B6) mice and susceptible A strain mice appears to be mediated by a single gene, presumptively named *Rmp-1* (41). Multiple genes determine resistance in crosses between B6 and other susceptible strains, such as DBA/2 (D2) and BALB/c (6, 41). Some of these genes are gonad dependent and protect female more than male mice (8). Two gonad-dependent genes, *Rmp-2* and *Rmp-3*, are linked, respectively, to the *Hc* locus on chromosome 2, the gene that encodes the fifth component of complement, and the major histocompatibility complex (*H-2*) on chromosome 17 (8). The mechanisms of action of *Rmp-1*, *Rmp-2*, and *Rmp-3* are unknown but are believed to function during the early stages of infection (21, 29). Studies of resistance in segregant crosses between resistant and susceptible strains suggest that the major resistance genes mediate resistance independently (6, 41).

Because of the genetic complexity of resistance to mousepox in B6 mice and the apparent redundancy of resistance mediated by the major genes, it appeared feasible and desirable to generate bilineal congenic resistant strains of mice each with a different resistance gene on a susceptible background. Such strains would greatly facilitate efforts to map resistance loci and to characterize their mechanisms of action.

In this study, we report on the independent and interdependent passage of resistance genes of B6 origin through multiple generations of backcrossing to D2 mice by using survival from infection as the selection method. Three [(B6 × D2) F_1 × D2] first-backcross mice that survived infection were selected as progenitors on the basis of their genotypes for *Hc* and *H-2D* and therefore presumably for *Rmp-2* and

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Rmp-3. *Hc* and *H-2D* were used as marker loci to monitor the passage of *Rmp-2* and *Rmp-3* in pedigree analyses.

MATERIALS AND METHODS

Mice. Male and female DBA/2NCr (D2) and female (C57BL/6NCr × D2) F_1 (F_1) mice were obtained from the Frederick Cancer Research and Development Center, Frederick, Md. The mice were specific pathogen free and were maintained under specific-pathogen-free conditions. First-backcross mice were produced from male D2 and female F_1 mice. Subsequent generations were produced by backcrossing surviving male backcross mice to female D2 mice. To avoid passive immunity of the progeny, only male backcross mice were used. Micro-isolator (Lab Products, Maywood, N.J.) cages, changed under a laminar flow hood, were used to house mice. Serological monitoring of dams and room sentinels confirmed that ectromelia virus was restricted to cages that contained inoculated mice.

Virus. Stocks of the Moscow strain of ectromelia virus were prepared, titers for them were determined, and they were stored as previously described (3). The titer of the stock virus was 2×10^9 PFU per ml as determined on BS-C-1 cell culture monolayers.

Mouse inoculations and clinical observations. The mice were lightly anesthetized with methoxyfluorane (Pitman-Moore, Washington Crossing, N.J.) and were inoculated subcutaneously between the shoulders (0.1 ml) with 10^3 PFU of ectromelia virus. This dose of virus consistently killed 100% of male D2 mice and less than 5% of male F_1 mice, as reported previously (6). The mice were examined daily for clinical signs, and when death occurred, gross examinations were made to confirm that mousepox lesions (hepatic and splenic necrosis) were present. Four weeks after inoculation, surviving mice were bled and their sera were tested for antibody to vaccinia virus by an immunofluorescence assay to confirm infection (22). All such mice seroconverted.

Assays for *Hc*¹, *H-2D*^b, and *B2m*^b. Blood samples were obtained from the periorbital venous plexus in heparinized capillary tubes. Hemolytic complement was assayed in plasma samples as previously described (8). Mononuclear blood cells were obtained by centrifugation of blood samples on Ficoll and sodium diatrizoate (density, 1.0875; Cedarlane Laboratories, Hornby, Ontario, Canada). Residual erythrocytes were lysed by hypotonic shock. Cells were assayed for the presence of *H-2D*^b by a microcytotoxicity assay in flat-bottom, 96-well plates, as described previously (8). Cells were assayed for the presence of *B2m*^b, the alloantigen for β_2 -microglobulin carried by B6 mice, under the same assay conditions, except that monoclonal anti-mouse *B2m*^b (New England Nuclear Research Products, Boston, Mass.) was used. Cells from alloantigen-negative (D2) and -positive (F_1) mice were included with each run.

Statistics. The goodness-of-fit test was used to compare the numbers of resistant mice observed in six backcross generations that carried the marker locus with the numbers that would be expected if there was no linkage and the numbers that would be expected if there was linkage with selected recombination proportions (23). Expected numbers were determined by a modification of the formula (given by Flaherty [15]) $P_n = (1 - c)^n - 1$, where P is the probability of a passenger gene being retained, c is the recombination frequency, and n is the number of backcross generations as defined below. This was modified to the power of $n - 2$ to account for the selection of first-backcross mice that carried the marker locus.

Chi-square analysis with Yates' correction was used to compare numbers of resistant mice in selected groups.

Nomenclature and definitions. Standard nomenclature was used to designate backcross generations (1). The F_1 generation was designated N1, and the first backcross generation was designated N2. Individual mice were identified by the generation designation followed by a subscript number (e.g., N2₁). Resistant mice were defined as mice that survived infection with ectromelia virus for 3 or more weeks. Susceptible mice were defined as mice that succumbed to infection within 3 weeks.

Experimental design. The three N2 progenitors survived infection with ectromelia virus and had the following genotypes: N2₁, *Hc*¹ (*Rmp-2*^r) and *H-2D*^d (*Rmp-3*^s); N2₂, *Hc*^o (*Rmp-2*^s) and *H-2D*^b (*Rmp-3*^r); and N2₃, *Hc*^o (*Rmp-2*^s) and *H-2D*^d (*Rmp-3*^s). Serial backcross lines were developed by crossing the N2 progenitors with D2 females, infecting the male progeny with ectromelia virus, and again crossing the survivors with D2 females. Resistant descendants of mouse N2₁ were typed for *Hc*¹, which served as a marker locus for *Rmp-2*^r. Resistant descendants of mouse N2₂ were typed for *H-2D*^b, which served as a marker locus for *Rmp-3*^r. Mice were challenged with ectromelia virus at 8 weeks of age. Surviving mice were placed in breeding harems 4 weeks after infection. Serological monitoring showed that D2 dams did not become infected while housed with seropositive males.

At the end of the experiment, each mouse was assigned a resistance index, which was the fraction of total male descendants that were resistant. These numbers were cumulative from N8 to N3 through the pedigrees.

RESULTS

Passage of resistance and marker loci through serial backcross generations. Three resistant N2 mice (N2₁ to N2₃), which carried different combinations of marker loci for *Rmp-2*^r and *Rmp-3*^r, transmitted resistance through serial backcross generations (Fig. 1 and 2). Backcross lineages in which resistance passed uniformly between generations developed from each, as shown by uniform resistance indices.

Mouse N2₁, which carried *Hc*¹, the marker locus for *Rmp-2*^r, was the founder of multiple resistance lineages. These could be placed in two broad classes, based on levels of resistance and on passage of *Hc*¹ (Fig. 1). High-resistance lines originated from two of five resistant N3 mice (N3₁ and N3₅). These produced males that were approximately 40% resistant and passed *Hc*¹ into N8. Intermediate-resistance lines originated from the other three N3 mice (N3₂ to N3₄). These produced males that were about 20% resistant and did not pass *Hc*¹ beyond N5.

From these results, it appeared that mouse N2₁ carried at least two resistance genes, that one was *Rmp-2*^r, and that *Rmp-2*^r contributed to the high-resistance phenotype.

Mouse N2₂, which carried *H-2D*^b, the marker locus for *Rmp-3*^r, had a more attenuated pedigree than mouse N2₁ but also produced phenotypically distinct backcross lines. These were similar to the high- and intermediate-resistance lines developed from mouse N2₁, but with a slightly higher fraction of resistant mice in the high-resistance line derived from mouse N2₂ (Fig. 2). One high-resistance and two intermediate-resistance lines developed from three resistant N5 mice. *H-2D*^b was passed to N8 in the high-resistance line and was not carried by the two intermediate-resistance lines.

These results suggested that mouse N2₂ also carried at

FIG. 1. Passage of resistance from mouse N2₁, which carried *Hc*⁺, the marker locus for *Rmp-2*^r, through male serial-backcross mice. Five backcross lines composed of individuals with similar resistance indices are shown originating from individual resistant N3 mice. To the right of the arrows are the numbers of mice in each generation that contributed resistant progeny. The numerators of the fractions indicate the numbers of mice that also carried the marker locus. The cumulative resistance indices (in percentages) are between arrows. High (H) and intermediate (I) male and female resistance phenotypes for each backcross line are shown.

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Mouse N2₃, which did not carry marker loci for *Rmp-2*^r or *Rmp-3*^r, transmitted an intermediate level of resistance through N4. Backcrossing was discontinued at N4 because

TABLE 1. Observed and expected numbers of resistant backcross mice carrying *Hc*⁺ based on nonlinkage and linkage with selected recombination frequencies

Generation	No. of <i>Hc</i> ⁺ /total	Expected no. carrying <i>Hc</i> ⁺ based on the following recombination frequencies:				
		0.5	0.4	0.3	0.2	0.1
3	3/5	2.5	3.0	3.5	4.0	4.5
4	4/14	3.5	5.0	6.9	9.0	11.3
5	7/22	2.8	4.8	7.5	11.3	16.0
6	12/35	2.2	4.5	8.4	14.3	23.0
7	20/61	1.9	4.8	10.3	20.0	36.0
8	21/84	1.3	3.9	9.9	22.0	45.0
Goodness of fit χ^2 (5 df)		521	137	24.4	5.0	35.5
<i>P</i>		<0.001	<0.001	<0.001	>0.05	<0.001

of phenotypic similarities with intermediate-resistance backcross lines developed from the two other progenitors. In this case, the absence of marker loci for *Rmp-2*^r or *Rmp-3*^r was associated with the absence of a high-resistance phenotype.

Resistance in N8 female mice. Female N8 mice were challenged with ectromelia virus along with male mice to determine whether resistance in any of the backcross lines had a sex bias. Female N8 mice of intermediate-resistance lines developed from mice N2₁ and N2₂ were three- to sixfold more resistant than their male counterparts (Fig. 1 and 2). Females of high-resistance lines were slightly more resistant than their male counterparts.

Confirmation of linkage between the *Hc* locus and resistance. Genotypes for *Hc* in resistant descendants of mouse N2₁ (*Hc*⁺) were determined. The numbers of *Hc*⁺-positive mice in all generations were compared with the numbers that would be expected if there was no linkage and with the numbers that would be expected if there was linkage with selected recombination frequencies (Table 1). This comparison assumed that only one resistance gene was passed. The number of *Hc*⁺-positive resistant mice was not consistent with nonlinkage between resistance and the *Hc* locus ($P < 0.001$) and was most consistent with linkage at a recombination frequency of 0.2. This result confirmed that resistance was mediated at least in part by a gene linked to the *Hc* locus (*Rmp-2*), because the alternative assumption, that more than one resistance gene was being passed, would have only added to the pool of resistant mice that did not carry *Hc*⁺.

Evidence for selection of a second resistance locus in backcross lines carrying *Rmp-2*^r. Independent gene assortment in each backcross generation made it unlikely that two unlinked resistance genes would be copassed unless they were interactive (epistatic). Evidence that more than one resistance gene was being passed in high-resistance lines that carried *Rmp-2*^r was sought in two ways. First, if *Rmp-2*^r was the only resistance gene being passed in high-resistance lines, then resistant *Hc*⁺-negative mice sired by *Hc*⁺-positive mice were recombinants between *Rmp-2* and *Hc*. In this case, they would be expected to transmit the same level of resistance to their descendants as *Hc*⁺-positive mice, most of which presumably carried *Rmp-2*^r. This was not the case, however. The resistance indices for the high-resistance lines derived from mice N3₁ and N3₅ included the descendants of *Hc*⁺-negative mice. When these data were extracted from the cumulative data, they showed that 172 descendants of mouse N3₅ were sired by *Hc*⁺-negative mice, of which 51 were

FIG. 2. Passage of resistance from mouse N2₂, which carried *H-2D*^b, the marker locus for *Rmp-3*^r, and mouse N2₃, which carried no marker loci, through male serial-backcross mice. See the legend to Fig. 1 for further explanation.

FIG. 3. Resistance pedigree of mouse N2₁ showing distribution of *Hc*¹ carriers. Squares and circles symbolize resistant male and female backcross mice, respectively. Shaded symbols indicate mice that carried the marker locus. Female D2 mice to which resistant backcross mice were bred are not shown.

resistant (30%). This was significantly ($P < 0.001$) less than the 84 resistant mice among 165 (51%) descendants of *Hc*¹-positive mice. The results were similar for descendants of mouse N3₁. This result suggested that most resistant *Hc*¹-negative mice sired by *Hc*¹-positive mice in the high-resistance lines carried a different or diminished complement of resistance genes relative to *Hc*¹-positive mice; since they did not carry *Hc*¹, it was most likely that they did not carry *Rmp-2*⁺.

Next, we sought evidence through linkage analysis that *Rmp-2*⁺ was in the chromosome 2 location that would be predicted if *Rmp-2* was the only resistance gene being passed. Most resistant *Hc*¹-positive descendants of mice N3₁ and N3₅ that sired resistant mice had resistant *Hc*¹-positive and *Hc*¹-negative progeny (Fig. 3). In generations N5 to N8, a total of 95 resistant mice were sired by *Hc*¹-positive mice, of which 35 were *Hc*¹ negative. If *Rmp-2*⁺ was the only resistance gene carried by these high-resistance lines and resistant *Hc*¹-negative progeny of *Hc*¹-positive mice were recombinants, then the calculated map distance between *Rmp-2* and *Hc* was 36.8 ± 5.0 centimorgans (cM). The *Hc* locus is 26 cM from the terminal centromere on chromosome 2 (18). Therefore, the predicted map location of *Rmp-2*⁺ was probably distal to *Hc*. Linkage was tested between *Rmp-2* and *B2m*, a locus approximately 26 cM distal to *Hc* (18), in N5 to N7 mice of the high-resistance lines. Only 8 of the 39 *Hc*¹-positive mice carried *B2m*^b (B6), and 1 of the 21 resistant *Hc*¹-negative mice sired by *Hc*¹-positive mice carried *B2m*^b.

These results suggested that *Rmp-2* was nearer to *Hc* than to *B2m* and that virtually all *Hc*¹-negative mice sired by *Hc*¹-positive mice did not carry the 26-cM segment of B6 chromosome 2 between *Hc* and *B2m* or portions thereof that included *Hc* or *B2m*. Most resistant *Hc*¹-negative mice could not have carried *Rmp-2*⁺ if it was distal to *Hc* unless they had double crossovers which excluded *Hc*¹ and *B2m*^b. We concluded that most resistant *Hc*¹-negative mice probably carried a resistance gene that was not *Rmp-2*.

The uniform distribution of resistant *Hc*¹-negative mice sired by *Hc*¹-positive mice in all generations of the high-resistance lines suggested that the second resistance gene was coselected with *Rmp-2*⁺ because it was epistatic. We

considered the possibility that the second resistance gene carried in high-resistance lines was the same gene that was carried by intermediate-resistance lines derived from mouse N2₁. In the intermediate-resistance lines, N8 female mice had more than threefold greater resistance than male mice. In the high-resistance lines, 55 female N8 descendants of mouse N3₅ were sired by *Hc*¹-negative mice, of which 46 (84%) were resistant. Phenotypically, the second resistance gene in the high-resistance lines behaved like the gene carried by the intermediate-resistance lines.

***Rmp-2*⁺ augments resistance in male mice but is apparently not needed by female mice.** The preceding results suggested that intermediate-resistance lines and most resistant *Hc*¹-negative mice of high-resistance lines derived from mouse N2₁ carried a resistance gene that was highly protective of female mice while conferring a lower level of protection on male mice. Most of the male resistance in high-resistance lines therefore derived from *Rmp-2*⁺. That 63% (60 of 95; $P < 0.025$) of resistant N5-to-N8 male progeny of *Hc*¹-positive mice carried *Hc*¹ attested to that fact.

If female mice in high-resistance lines did not require *Rmp-2*⁺ to express high resistance, then there should have been no survival advantage for female mice that inherited *Hc*¹ over females that did not inherit *Hc*¹. Unlike male mice, only 13 of 38 (34%) resistant N8 female mice sired by *Hc*¹-positive mice in high-resistance lines carried *Hc*¹ (Fig. 3). The resistant female carrier rate for *Hc*¹ was significantly lower than the male carrier rate ($P < 0.005$).

Confirmation of linkage between the *H-2D* locus and resistance. Mice in the resistance pedigree of mouse N2₂ (*H-2D*^b) were typed for *H-2D* haplotypes. The numbers of *H-2D*^b-positive mice in all generations were compared with the number that would be expected if there was linkage with selected recombination frequencies or nonlinkage and on the assumption that one resistance gene was passed (Table 2). The number of *H-2D*^b-positive mice was not consistent with nonlinkage between resistance and the *H-2D* locus ($P < 0.001$) and was most consistent with linkage at recombination frequencies of 0.2 to 0.3. This result confirmed that resistance was mediated at least in part by a gene linked to the *H-2D* locus (*Rmp-3*).

Pedigree analysis of backcross lines founded by mouse N2₂.

TABLE 2. Observed and expected numbers of resistant backcross mice carrying *H-2D^b* based on nonlinkage and linkage with selected recombination frequencies

Generation	No. of <i>H-2D^b</i> /total	Expected no. carrying <i>H-2D^b</i> based on the following recombination frequencies:				
		0.5	0.4	0.3	0.2	0.1
3	1/1	0.5	0.6	0.7	0.8	0.9
4	1/1	0.3	0.4	0.5	0.6	0.8
5	2/5	0.6	1.1	1.7	2.6	3.6
6	2/9	0.6	1.2	2.2	3.7	5.9
7	5/20	0.6	1.6	3.4	6.6	11.8
8	7/29	0.5	1.4	3.4	7.6	15.4
Goodness of fit χ^2 (5 df)		135	34.0	5.3	1.6	11.9
P		<0.001	<0.001	>0.1	>0.1	<0.05

The high-resistance line that developed from mouse N2₂ was descended from an N5 mouse (N5₁) that carried *H-2D^b*. A total of 5 of 20 resistant male mice in the high-resistance line sired by *H-2D^b*-positive mice did not carry *H-2D^b* (Fig. 4). A total of 4 of 10 resistant N8 female mice sired by *H-2D^b*-positive mice in the high-resistance line also did not carry *H-2D^b*. Because of the limited number of mice in this pedigree, a statistical comparison of the passage of resistance from *H-2D^b*-negative versus *H-2D^b*-positive mice in the high-resistance line was not possible. Such a comparison and a search for other chromosome 17 markers of B6 origin in the *H-2D^b*-negative mice could distinguish recombination between *Rmp-3* and *H-2D* from the passage of a second resistance gene. Nevertheless, 17 male mice were sired by *H-2D^b*-negative mice in the high-resistance line, and 16 were resistant. This suggested that, unlike the *Hc¹*-negative mice in backcross lines that carried *Rmp-2^r* which passaged intermediate resistance, the resistance component of most *H-2D^b*-negative mice was the same as that of *H-2D^b*-positive mice. This supported the interpretation of recombination as the principal cause of resistant *H-2D^b*-negative mice in the high-resistance line.

FIG. 4. Resistance pedigree of mouse N2₂ showing distribution of *H-2D^b* carriers. See the legend to Fig. 3 for an explanation of symbols.

DISCUSSION

This investigation provided several new insights into the genetic control of resistance to mousepox. First, the passage of incremental resistance and different marker loci through multiple generations of backcrossing proved that resistance in B6 mice is redundant. Second, the passage of the two marker loci through seven generations of backcrossing confirmed that two resistance genes are linked to the *Hc* locus on chromosome 2 and the *H-2D* locus on chromosome 17. Third, evidence for the passage of a previously undescribed resistance gene that mediated greater resistance in female than in male mice indicated that sex differences in resistance are mediated by differential expression of the same gene. Finally, evidence for the simultaneous passage of a second resistance gene with *Rmp-2^r* that expressed greater resistance in female than in male mice suggested that the gonad dependence of *Rmp-2^r* is due to the gonad dependence of a female-bias epistatic gene and that the female bias of this epistatic gene is offset by the male bias of *Rmp-2^r*.

Previous studies of the genetics of resistance to mousepox by the present authors (6) and by others (34, 41) showed that more than 50% of first backcrosses to most susceptible strains of mice are resistant. The most likely explanation for this level of resistance is that there are multiple, independently acting resistance genes. Similar evidence came from a study of the resistance to mousepox of recombinant inbred strains of mice derived from B6 and D2 mice in which 21 of 25 strains were variably resistant, again suggesting that there are multiple, independently acting resistance genes (6). The passage of resistance and two marker loci through serial backcross generations in the present study during which unlinked resistance genes segregated randomly in each generation provided additional evidence for the independence of these genes.

The question that arises from these results is whether these redundant, unlinked resistance genes control the same or different mechanisms. Resistance mechanisms differ in at least one fundamental aspect, in that some are gonad dependent and others are gonad independent (8). Female mice express greater gonad-dependent resistance than male mice (8). *Rmp-3* is a gonad-dependent resistance gene that is linked to the major histocompatibility complex (8). Because sex steroids have quantitative effects on immune responsiveness (10, 38) and because specific cytotoxic T-cell precursors have been shown to appear earlier in regional lymph nodes of resistant strains of mice infected with ectromelia virus than in susceptible strains (28, 30), it is tempting to speculate that some of these mechanisms are immune mediated, and this has been proposed elsewhere (30, 33). There is substantial evidence, however, that some or all of the major resistance genes are expressed during the earliest stages of infection. For example, we recently showed that ectromelia virus replication in the spleens of B6 mice after intravenous inoculation was clearly suppressed relative to that in D2 mice by 48 h, with a similar trend present at 24 h (7). O'Neill and Blanden reported similar temporal differences in ectromelia virus replication kinetics in regional lymph nodes of resistant and susceptible strains after peripheral inoculation (29). Such early intervals preclude specific immune-mediated resistance mechanisms. However, because these studies have been conducted on B6 mice which carry multiple resistance genes, it is possible that the onset of expression of these genes is temporally staggered and that early-acting genes mask the later onset of other resistance genes. Studies of congenic resistant mice can address this possibility.

In a previous study, we used recombinant inbred strains of mice derived from B6 and D2 mice and first-backcross mice to show that there was significant linkage between resistance to mousepox and the *Hc* and *H-2D* loci (8). These associations were confirmed in the present study by the passage of B6 alleles for these marker loci through seven generations of backcrossing, during which B6 genes that were not linked to resistance were reduced to less than 1%. The number of resistant mice in each generation that carried the marker loci significantly exceeded expectations if the markers were contaminant passenger genes. In only three other cases of which we are aware have resistance genes to acute viral diseases been successfully passed through serial backcrosses. One of these is *Mx-1* on chromosome 16, which protects against acute mortality caused by influenza A virus through an interferon-dependent pathway (19). Another is a gene that controls resistance to yellow fever virus, which Groschel and Koprowski passed from resistant PRI mice to susceptible C3H mice through eight generations of backcrossing (17). This gene has not been mapped, and its function is not known. The third of these cases is a dominant gene that controls susceptibility to mouse hepatitis virus strain 2, which Weiser and coworkers successfully transferred from susceptible PRI mice to resistant C3H mice through 20 generations of backcrossing (42). Although this gene has not been mapped and its function is not known, subsequent studies with other crosses suggest that this was the receptor for mouse hepatitis virus located on chromosome 7 (4, 37). In these cases, resistance is unifactorial. The present study is the first report of successful passage of increments of a genetically complex resistance phenotype through serial backcrosses.

Female first-backcross mice of the cross that was used in the present study are about fivefold more resistant than their male siblings to lethal mousepox (6, 8, 41). This sex difference is mediated through autosomes and could arise by differential expression of the same genes or by sex-limited expression of additional genes by female mice. Backcross lines derived from mice N₂₁ and N₂₂ that expressed an intermediate-resistance male phenotype expressed a high-resistance female phenotype. It is likely, on the basis of this result, that sex differences in resistance to mousepox are at least partly due to differential expression of the same genes by males and females. Because neonatal gonadectomy eliminates sex differences in first-backcross mice (8), this differential expression may reflect differential effects of gonadal hormones.

The coselection of a second resistance gene with *Rmp-2'* that expressed a sex bias in the apparent absence of *Rmp-2'* is of interest because *Rmp-2'* is a gonad-dependent resistance gene (8). Neonatally gonadectomized first-backcross mice, unlike intact mice, have no evidence of linkage between the *Hc* locus and resistance to mousepox (8). The presence of resistant mice that probably did not carry *Rmp-2'* among the pedigree of mice that probably carried *Rmp-2'* suggests that this sex-biased second resistance gene was epistatic to *Rmp-2'*; otherwise, we would have expected to see evidence of the passage of *Rmp-2'* without the second gene. By contrast, there was ample evidence for the passage of the second gene without *Rmp-2'*, a condition that resulted in intermediate resistance in male mice and high resistance in female mice. Epistasis of the sex-biased gene to *Rmp-2'* could account for the gonadal dependence of *Rmp-2'* if the sex bias is indicative of gonadal dependence. We propose that this sex-biased gene that was coselected with *Rmp-2'* be provisionally named *Rmp-4*. The resistance allele, *Rmp-4'*,

mediated high resistance in female mice and intermediate resistance in male mice in the absence of *Rmp-2'* and with *Rmp-2'* mediated high resistance in both sexes. This relegated *Rmp-2'* to a protective role for male mice that had only a slight incremental effect or no effect on female mice.

Linkage between resistance to mousepox and *Rmp-3* is, like *Rmp-2*, gonad dependent (8). Other similarities between these two genes in the present study were that both were associated with male and female high-resistance phenotypes and that both segregated from other resistance genes carried by the progenitors that mediated intermediate resistance in male mice and high resistance in female mice. No evidence was found, however, for the copassage of an epistatic resistance gene with *Rmp-3'*. The passage of high male resistance by *H-2D^b*-negative mice in the high-resistance line suggested that these mice were *Rmp-3-H-2D* recombinants rather than carriers of a non-*Rmp-3* resistance gene.

The resistance mechanisms that are controlled by the genes isolated in the current study represent effective host responses to a virus that has evolved a sophisticated strategy for survival in the host. That strategy relies heavily upon virus-encoded host-interactive proteins to disrupt normal host antiviral responses. Ectromelia virus is known to encode a major secretory protein that inhibits the complement cascade in a manner similar to that of the 35-kDa major secretory protein of vaccinia virus, which has significant homology to a human protein that binds the C4b fragment of the fourth component of complement (24). Ectromelia virus doubtlessly encodes other host-interactive proteins because of its genomic sequence homology with vaccinia virus and other orthopoxviruses (12, 13, 26, 27), which encode many such proteins (2, 16, 24, 25, 31, 32, 35, 36, 43). Elucidation of the mechanisms of genetic resistance to mousepox will provide insights into the evolution of host strategies for minimizing the severity of diseases caused by viruses that elaborate host-interactive proteins. Bilineal congenic resistant strains derived from the mice in this study should facilitate efforts to map resistance genes and determine their mechanisms of action by providing a low background of unrelated variables.

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