INFECTION AND IMMUNITY, Apr. 1981, p. 277-286
0019-9567/81/040277-10$02.00/0
Vol. 32, No. 1

Influence of *H-2* and Non-*H-2* Genes on Resistance to Murine Cytomegalovirus Infection

JANE E. GRUNDY (CHALMER),†* J. S. MACKENZIE, AND N. F. STANLEY

Department of Microbiology, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, 6009, Western Australia

The resistance of adult mice to acute lethal infection with murine cytomegalovirus is controlled by genes linked to the *H-2* complex. The *k* haplotype is approximately 10 times more resistant than the *b* or *d* haplotypes. Susceptibility is inherited as a completely dominant trait. At least two genes within the *H-2* complex are involved, one mapping to the *K/IA* subregion and the other to the *D* subregion. The data suggest that interactions may occur between these *K*- and *D*-end genes which further affect resistance to the virus. The precise mechanism of *H-2* gene control of resistance to murine cytomegalovirus remains to be elucidated. Non-*H-2*-linked genes also affect resistance to the virus, particularly in the C57BL genetic background, which is associated with an increased resistance to murine cytomegalovirus. Newborn mice of all strains are equally susceptible: both the *H-2*- and the non-*H-2*-associated resistances develop in the first few weeks of life and are retained up to at least 18 months of age.

It has been recognized for many years that the genetic constitution of the host influences susceptibility to infection with various agents. The discovery of genes within the major histocompatibility complex of the mouse (*H-2* complex) which controlled the specific immune response to a variety of antigens posed the question as to whether such genes might control the host's response to infectious agents, including viruses. This stimulated a search for association between histocompatibility type and susceptibility to virus-induced disease. Such associations were indeed found between certain *H-2* haplotypes and susceptibility to viral leukomogenesis, involving a variety of leukemia viruses (reviewed in reference 5). Studies in this laboratory have previously demonstrated that an association exists between the susceptibility of adult mice to lethal infection with the herpesvirus murine cytomegalovirus (MCMV) and *H-2* haplotype (3). In congenic strains of BALB/c mice, possession of the $H\text{-}2^k$ haplotype was found to be associated with relative resistance, whereas the $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes conferred susceptibility. We believe this to be the only association at present known between *H-2* haplotypes and resistance to acute infection with a nonleukemic virus. The association originally reported by Oldstone and co-workers between resistance to lymphocytic choriomeningitis virus and particular *H-2* haplotypes (10) has not been confirmed by other workers (9).

† Present address: Immunology Branch, National Cancer Institute, Bethesda, MD 20205.

The studies presented in this report confirm the *H-2* association of resistance to MCMV in congenic strains of mice with the B10 genetic background. In addition, the pattern of resistance in recombinant strains of mice suggested that this was due to the existence of at least two loci within the *H-2* complex which controlled resistance to MCMV: one mapping to the *K* end and the other mapping to the *D* end. Non-*H-2*-linked genes were also found to influence resistance to the virus. The pattern of inheritance of these various genes was investigated, and preliminary studies were undertaken to determine their mechanism of action. The effect of *H-2*- and non-*H-2*-linked genes on the pathogenesis of MCMV infection is described in the following papers in this series.

MATERIALS AND METHODS

Mice. BALB/c, C57BL, and C3H/HeJ mice were provided by the Queen Elizabeth II Medical Centre Animal Breeding Unit, Perth, Western Australia. The congenic strains BALB/c ($H\text{-}2^d$), BALB.B ($H\text{-}2^b$), BALB.G ($H\text{-}2^g$), and BALB.K ($H\text{-}2^k$) were obtained from the Walter and Eliza Hall Institute, Melbourne, Australia, and were maintained in this department by brother-sister mating for several generations. Additional inbred strains of mice, including some recombinant strains, were kindly provided by the John Curtin School for Medical Research, Canberra, Australia. These were B10, B10.A, B10.A(2R), B10.A(4R), B10.A(5R), BALB/c, CBA, C3H.OH, C3H.OL, A/Tb, A.TL, and A.TH. The Royal Perth Hospital Animal Breeding Unit, Perth, Western Australia, supplied the congenic strains B10 and B10.BR.

Mice of the same strain obtained from different

breeding units showed identical resistance to MCMV infection, and distinction between them will not be made in this paper. However, the BALB/c strain obtained from the Walter and Eliza Hall Institute was always used in experiments when the other congenic strains on this background were used. Similarly, the B10 strain was consistently used in conjunction with the recombinant or congenic strains on this background, and not the C57BL strain obtained from the Queen Elizabeth II Medical Centre.

Virus. The Smith strain of MCMV was used in all experiments. The origin of this strain and the preparation of virus stocks have been described previously (3). Only virulent salivary gland virus was used in animal experiments.

MCMV infection of mice. Adult mice received 0.1 ml of MCMV in mouse osmolality-buffered saline (13) by the intraperitoneal (i.p.) route. Newborn mice received only 0.02 ml of virus intraperitoneally.

LD_{50} determination. The minimum dose which killed 50% of the animals inoculated (LD_{50}) was determined after inoculation of serial twofold dilutions of virus in adult mice or serial half-log dilutions of virus in newborn mice. The LD_{50} values were calculated by the Kaerber method: log $LD_{50} = 0.5/n +$ log of the highest concentration of virus used $- 1/n \times$ (sum of percent of dead animals)/100, where n = number of dilutions per log interval, i.e., $n = 1$ for log dilutions and $n = 2$ for half-log dilutions. The LD_{50} was calculated using the $\log_2$ if serial twofold dilutions of virus were used. The range of dilutions used covered the range from 0 to 100% mortality in most cases.

Cell culture. Mouse embryo cell cultures (MECC) were prepared as described previously (3). Cells were grown in medium 199 (GIBCO, Grand Island, N.Y.), adjusted to mouse osmolality (333 mosmol), and supplemented with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer and 10% fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Victoria). Maintenance medium contained only 2% fetal calf serum.

$TCID_{50}$ determination. MECC were seeded into 96-well Microtest II trays (Falcon Plastics, Oxnard, Calif.) and used within 24 h of reaching confluency. Growth medium was removed, and 0.025 ml of virus dilutions in maintenance medium was added to each well. Virus was allowed to adsorb at 37°C for 1 h. Maintenance medium was then added, and the trays were incubated for 3 weeks. Medium was changed every 7 days, and the trays were examined daily for cytopathic effect (CPE). The dose of virus which produced CPE in 50% of the inoculated cells ($TCID_{50}$) was calculated by the Kaerber method using the equation described for the LD_{50} above, except that percentage of cells showing CPE replaced percentage of dead animals.

Generally, one 96-well tray was infected with each half-log dilution of virus, and the range of dilutions used resulted in from 0% to 100% CPE. $TCID_{50}$ titers on MECC from different strains of mice were compared statistically using the χ^2 test. This was done by calculating the total number of wells showing CPE and the total number of wells infected over the whole range of virus dilutions used (this range was the same in the two groups being compared.)

Neutralization test. Neutralizing antibody was assayed by a complement-dependent neutralization test using guinea pig complement (C′). Secondary or tertiary cultures of cells from Prince Henry inbred embryos were seeded into 96-well Microtest II trays and used within 24 h of reaching confluency. Growth medium was removed, and 0.05 ml of maintenance medium was added to each well. Sera to be tested were diluted 1:5 with maintenance medium and heat inactivated at 56°C for 30 min. Serial twofold dilutions of sera were made in maintenance medium containing a 1:10 dilution of C′ (this represented a C′ excess). A 0.02-ml sample of the serum plus C′ mixture was incubated with an equal volume of virus (containing 100 $TCID_{50}$) for 1 h at 37°C. This was then added to the MECC in the Microtest trays. The trays were incubated at 37°C for 3 weeks. Medium was changed every 7 days, and the cells were inspected daily for CPE. The neutralizing antibody titer of sera was expressed as the reciprocal of the last dilution which inhibited the appearance of CPE.

Cortisone treatment. Hydrocortisone acetate (Hydrocortone) was obtained from Merck Sharp & Dohme (West Point, Pa.). A dose of 3.5 mg of cortisone per kg of body weight was given subcutaneously in 0.1 ml of mouse osmolality-buffered saline 2 h after i.p. inoculation with virus and thereafter by the i.p. route daily for 1 week or until the death of the animal.

RESULTS

Resistance of recombinant strains of mice to MCMV. The relative resistance of various recombinant and congenic strains of mice to lethal infection with MCMV is shown in Table 1. Differences were observed between the resistance of strains with the same *H-2* map but different genetic background, for example, B10.A and A/Tb, BALB.B and B10, BALB.K and C3H. Thus, direct comparisons should only be made when recombinants possess the same background genes. Accordingly, the data have been presented as groups of recombinants on a particular background.

Table 1 shows the relative resistance of strains with the BALB/c background. As we have previously reported (3), the *k* haplotype was approximately 10 times more resistant than the *b* and *d* haplotypes. It should be stressed that on this background, the *b* and *d* haplotypes were equally susceptible and that the BALB.G recombinant, in which *b* has been substituted for *d* at the *D* subregion, was unaltered in resistance as compared to BALB/c.

The relative resistance of recombinants with the B10 genetic background can also be seen in Table 1. The following points should be noted.

(i) A comparison of B10 and B10.BR shows that replacement of *b* alleles at all loci by *k* alleles significantly increased the resistance by 10-fold. Thus, as on the BALB/c background,

TABLE 1. *Relative resistance of various recombinant strains of mice to lethal infection with MCMV*[a]

Background	Strain	*H-2* map									$-\log_2 LD_{50}$	Relative LD_{50}[b]
		K	*IA*	*IB*	*IE*	*IJ*	*IC*	*S*	*D*	Tla		
BALB/c	BALB.K	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	—	3.4	10.2
	BALB.B	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	—	0.32	1.3
	BALB.G	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*b*	—	0.28	1.2
	BALB/c	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*c*	0	1.0
B10	B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*a*	−0.78	17.2
	B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	<0	<8.2
	B10.A(2R)	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*b*	*b*	<0	<8.2
	B10.A	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*a*	1.13	3.8
	B10.A(5R)	*b*	*b*	*b*	*k*	*k*	*d*	*d*	*d*	*a*	1.30	3.3
	B10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	2.23	1.8
C3H	C3H	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*b*	<0	<8.2
	C3H.OH	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*k*	*b*	0.7	5.1
	C3H.OL	*d*	*d*	*d*	*d*	*d*	*d*	*k*	*k*	*b*	1.3	3.3
A	A/Tb	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*a*	2.50	1.5
	A.TL	*s*	*k*	*k*	*k*	*k*	*k*	*k*	*d*	*c*	3.55	0.7
	A.TH	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*d*	*a*	>4.50	<0.4

[a] Mice received serial twofold dilutions of virus by the i.p. route. Five to 10 mice were used for each dilution. The differences between the LD_{50} titers of the various strains were analyzed statistically by the χ^2 test, and the following results were obtained. Significant at $P < 0.05$ level or below: BALB/c, BALB.B or BALB.G, and BALB.K; B10, B10.A, or B10.A(5R) and B10.BR, B10.A(2R), or B10.A(4R); C3H and C3H.OH or C3H.OL; A/Tb and A.TH. Not significant at $P < 0.05$ level; BALB/c and BALB.B or BALB.G; BALB.B and BALB.G; B10 and B10.A or B10.A(5R); B10.A and B10.A(5R); C3H.OH and C3H.OL; A.TL and A/Tb or A.TH.

[b] Relative LD_{50} refers to the LD_{50} of the particular strain compared with that of the BALB/c strain arbitrarily assigned the value 1.0.

relative resistance was associated with the *k* haplotype.

(ii) The greater than fivefold significant increase in resistance of B10.A(4R) mice over that of B10 was a result of the substitution of the *b* allele at the *K* and *IA* loci by the *k* allele. The highest dose of virus available in this experiment failed to kill any B10.A(4R) [or B10.A(2R)] mice, and so the extent of the resistance of this strain could not be assessed, nor could it be compared with that of B10.BR mice (which were examined in a separate experiment using a higher-titer virus stock). However, the data do indicate that at least one gene in the *K/IA* subregion of the *H-2* complex controls resistance to MCMV.

(iii) A comparison of the resistance of B10.A and B10.BR shows that the substitution of the *k* for the *d* allele at *IC, S,* and *D* loci significantly increased resistance by approximately fivefold. This observation suggests the existence of a second gene controlling resistance to MCMV at the *D* end of the *H-2* complex, somewhere to the right of *IC*.

(iv) The changing of the *d* allele at the *D* subregion of B10.A mice for the *b* allele as in B10.A(2R) resulted in a significant increase in resistance, in excess of 2.5 times. (Later experiments showed this difference to be 6.5 times.) Thus, although this result implies the involvement of the *D* locus (or a gene mapping in the *D* subregion) in controlling resistance to MCMV, the reason for the difference in the resistance of these two strains is unclear. On this B10 background, the presence of *b* alleles did not seem to confer susceptibility in a manner similar to the presence of *d* alleles, yet B10 mice with the *b* allele at all loci are susceptible. Furthermore, we observed that, on the BALB/c background, the *b* and *d* haplotypes were equally susceptible and that a similar substitution of *b* for *d* at the *D* locus in the BALB.G recombinant did not affect resistance.

(v) Replacement of the *b* allele by the *k* allele at *K, IA,* and *IB* loci failed to increase significantly the resistance of B10.A mice as compared with B10.A(5R). This is in contrast to the findings in point (ii), but it should be noted that the presence of *d* alleles at the *D* end in B10.A and B10.A(5R), as opposed to *b* alleles in B10.A(4R) and B10, may have influenced the resultant resistance.

(vi) The Tla region did not appear to be involved in controlling resistance to MCMV.

Table 1 shows the relative resistance of recombinant strains of mice with the C3H genetic

background. The results indicate that possession of the *k* allele at the *K* and *I* subregions of the *H-2* complex (in C3H mice) is associated with significantly greater resistance than the *d* allele (as in C3H.OH and C3H.OL mice). Thus, this supports the finding discussed above for mice of the B10 background, that a gene in the *K* end of *H-2* controlled resistance to MCMV.

The relative resistance to MCMV of recombinant strains of mice with the A genetic background is shown in Table 1. The lowest dose of virus given killed all A.TH mice, which have proven to be the most susceptible strain we have tested. Hence, the accurate LD_{50} for A.TH mice cannot be calculated, but it is less than 0.4 times that of BALB/c. Mice with the $H\text{-}2^s$ haplotype were not available for testing at this time, but later studies (with ASW mice) showed that *s* was a susceptible haplotype. Table 1 shows a small but not significant difference between the resistances of A/Tb and A.TL, which probably resulted from the loss of the resistant *k* allele at the *K* subregion (rather than the acquisition of *k* alleles at *IC* and *S*) in A.TL mice. There is a much more striking decrease in resistance in A.TH mice which have further loss of *k* alleles in the *I* and *S* subregions. The difference between A.TH and A/Tb is statistically significant. In view of the finding in the B10 recombinants that a gene in the *K/IA* subregion controls resistance to MCMV, it is tempting to speculate from the A-strain recombinants that this gene is located closer to the *IA* than to the *K* subregion. However, further studies with other recombinants are necessary to clarify this point.

Resistance of F_1 hybrids. We have previously examined the resistance of F_1 hybrids between resistant C3H ($H\text{-}2^k$) and susceptible BALB/c ($H\text{-}2^d$) parents (3), and found that the F_1 hybrid was relatively susceptible. This finding conflicts with the report of Selgrade and Osborn (12) that resistance was expressed dominantly in F_1 hybrids between resistant CBA ($H\text{-}2^k$) and susceptible C57BL ($H\text{-}2^b$) parents. In view of the differences observed in some of the B10 recombinants between the effects of *d* and *b* alleles, these apparently conflicting results in F_1 hybrid studies may be due to the use of different haplotypes (*d* or *b*) to represent a susceptible strain. We therefore extended our F_1 hybrid studies to include the *b* haplotype of C57BL mice. Accordingly, F_1 hybrids were obtained between C3H and C57BL, C57BL and BALB/c, and for comparison, as previously, C3H and BALB/c. The resistance of these various F_1 hybrids to MCMV was examined to determine the patterns of inheritance (Fig. 1). As previously reported (3), the *k/d* F_1 hybrid between C3H and BALB/c was more than twice as resistant as the BALB/c parent, but significantly more susceptible than the C3H parent. In contrast, however, the *b/k* F_1 hybrid between C57BL and C3H was almost as resistant as the C3H parent; indeed, the difference between them is not statistically significant. This F_1 hybrid was significantly more resistant than the C57BL parent. Of interest is the resistance of the *d/b* F_1 hybrid between the two susceptible strains, BALB/c and C57BL, which was greater than that of either parent. This increase in resistance over the BALB/c parent was statistically significant, whereas that over the C57BL parent was not. This finding suggested that the reasons for susceptibility in these two strains might be different and that some sort of complementation might have been occurring in vivo. However, because differences had been noted between the resistance of strains with the same *H-2* type and different background genes, the possibility existed that the genetic background might have been affecting the observed patterns of resistance in the various F_1 hybrids. For this reason, these experiments were repeated using F_1 hybrids between *d*, *b*, and *k* haplotypes, but all on the BALB/c background, and the results (Fig. 2) contrasted with those just described. The *d/b* F_1 hybrid (BALB/c × BALB.B) was more susceptible than either parent, although this difference was not statistically significant. The *k/d* F_1 hybrid between BALB/c and BALB.K was equally as susceptible as the $H\text{-}2^d$ (BALB/c) parent, but significantly more susceptible than the $H\text{-}2^k$ (BALB.K) parent. The *b/k* F_1 hybrid (BALB.B × BALB.K) on the BALB/c background was more susceptible than either parent. This difference was statistically significant with $P < 0.001$ ($\chi^2 = 2.95$) between the F_1 hybrid and the $H\text{-}2^k$) parent, but was not significant ($P < 0.1$; $\chi^2 = 2.95$) when the F_1 hybrid was compared with the $H\text{-}2^b$ parent.

Effect of genetic background on resistance. The marked differences between the results obtained with crosses among C3H, BALB/c, and C57BL (Fig. 2) compared with those among theBALB/c congenics (Fig. 3) emphasized the strong effect of background genes on the resistance determined by *H-2* type. To further clarify background effects, the relative resistance of *d*, *b*, and *k* haplotypes on various backgrounds was directly compared. The results are seen in Fig. 3, which shows the relative LD_{50} titers for the same virus stock tested at the same time in the various strains of mice. The relative LD_{50} titers of the three $H\text{-}2^k$ strains, BALB.K, C3H, and B10.BR, were 10, 26, and 34 times that of BALB/c, respectively. Thus, with the *k* hap-

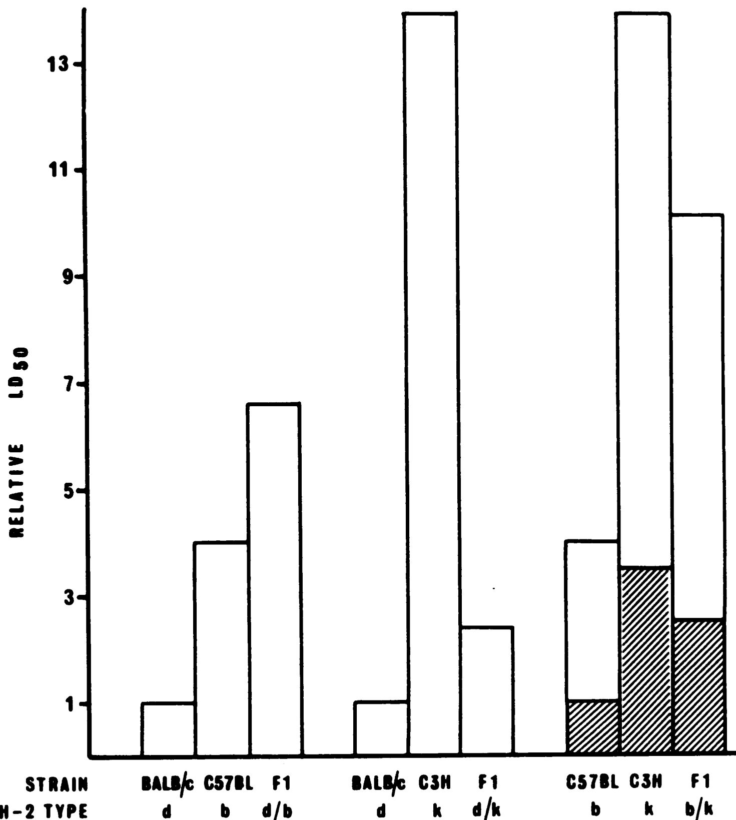

FIG. 1. *Relative resistance of adult BALB/c, C57BL, and C3H mice and their various F_1 hybrids to i.p. inoculation with MCMV. The figure shows the relative LD_{50} of each strain or F_1 hybrid compared with the LD_{50} of BALB/c mice (equal to 1.0). The shaded area shows the relative LD_{50} compared with the LD_{50} of C57BL mice (equal to 1.0). The LD_{50} values of the various groups were compared statistically by the χ^2 test, and the results obtained were as follows: (BALB/c × C57BL)F_1 and BALB/c: $P < 0.02$, $\chi^2 = 6.02$; and C57BL: P = NS, $\chi^2 < 1$. (BALB/c × C3H)F_1 and BALB/c: $P < 0.025$, $\chi^2 = 5.03$; and C3H: $P < 0.005$, $\chi^2 = 9.94$. (C57BL × C3H)F_1 and C57BL: $P < 0.02$, $\chi^2 = 5.54$; and C3H: P = NS, $\chi^2 < 1$.*

lotypes the C3H background increased resistance by a factor of 2.6 times and the C57BL/10 background increased resistance by 3.4 times as compared with the BALB/c background. Similarly with the two H-2^b strains, BALB.B and C57BL/10, the relative LD_{50} titers were 1.3 and 4.1 times that of BALB/c; hence the C57BL/10 background increased resistance of the *b* haplotype by a factor of 3.2 times over that of the BALB/c background.

Taken together, the results shown in Fig. 1, 2, and 3 indicate the following.

Effect of *H-2* linked genes. (i) On BALB/c background, *d* and *b* haplotypes are associated with equal susceptibility to MCMV. (ii) On BALB/c and B10 backgrounds, the *k* haplotype confers relative resistance (8- to 10-fold that of *d* and *b* haplotypes). (iii) On BALB/c background, susceptibility is a completely dominant trait.

Effect on genes not linked to *H-2*. (i) The genetic background of C3H mice contains a gene(s) which enhances the resistance of the *k* haplotype (compared to the BALB/c background) and modifies the dominant susceptibility associated with *b* and *d* haplotypes, so that susceptibility is only partially dominant in *k/b* and *k/d* heterozygotes. (ii) The C57BL genetic background appears to contain a gene(s) which complements *H-2* genes such that in F_1 hybrids resistance is markedly enhanced compared with that predicted according to their *H-2* type. This genetic background is also associated with a three- to fourfold enhancement of resistance of *b* and *k* haplotypes compared with the BALB/c background.

Effect of age on resistance. The studies presented so far were all conducted in adult mice, usually 10 to 20 weeks old. Resistance to MCMV is known to increase with age, presum-

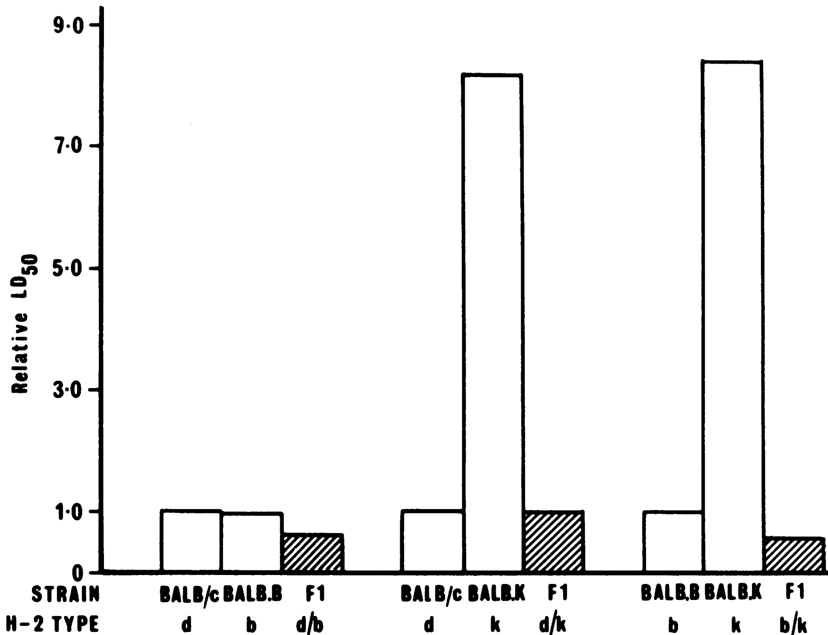

FIG. 2. *Relative resistance of adult BALB/c, BALB.B, and BALB.K mice and their various F_1 hybrids (shaded areas) to i.p. inoculation with MCMV. The figure shows the relative LD_{50} of each strain of F_1 hybrid compared with the LD_{50} of BALB/c mice (equal to 1.0). The LD_{50} values of the various groups were compared statistically by the χ^2 test, and the results obtained were as follows. (BALB/c × BALB.B)F_1 and BALB/c: P = NS, $\chi^2 = 1.41$; and BALB.B: P = NS, $\times^2 = 1.63$. (BALB.B × BALB.K)F_1 and BALB/c: P = NS, $\chi^2 < 1$; and BALB.K: $P < 0.02$, $\chi^2 = 6.22$. (BALB.B × BALB.K)F_1 and BALB.B: P = NS, $\chi^2 = 2.95$; and BALB.K: $P < 0.001$, $\chi^2 = 12.95$.*

ably due to maturation of the immune system (2, 7). To determine whether the relative resistance seen in some strains was a property of those strains throughout their life-span or whether it developed with age, the resistance to lethal infection with MCMV was determined in BALB/c, C3H, and C57BL mice from 3 days to 18 months old. Table 2 shows that at 3 to 4 days of age there were no significant differences in resistance between the three strains. Note also that the susceptibility of the 3- to 4-day-old mice compared with adult BALB/c mice was about 1:560 to 1:180 (relative LD_{50} of $10^{-2.75}$ to $10^{-2.25}$). We have previously shown (3) that by 21 days of age, C3H mice are significantly more resistant than BALB/c mice of equal age or adult BALB/c mice. Table 3 shows that the differences in resistance between BALB/c, C57BL, and C3H mice, seen at 10 to 20 weeks of age, were still apparent at 18 months of age. Within each strain, there was no significant difference in resistance between the two age groups.

Cortisone treatment. To evaluate the role of the inflammatory response in the resistance of C3H mice, the effect of cortisone treatment on the outcome of MCMV infection was examined. In addition, BALB/c and C57BL mice were also treated with cortisone to see whether the inflammatory response during MCMV infection was beneficial or contributed to illness and death in these more susceptible strains. The regime used had been shown to make ICR/HA mice more susceptible to MCMV (6). The results (Table 4) show that cortisone treatment significantly increased the susceptibility of BALB/c, C57BL, and C3H mice to lethal infection with MCMV.

Virus replication in vitro. We have previously reported that MCMV replicates equally efficiently in vitro in MECC derived from strains of susceptible (*d* and *b*) or resistant (*k*) *H-2* haplotypes (3). To determine whether differences in efficiency of viral replication might explain the effect of non-*H-2*-linked genes on resistance to MCMV, we have examined the in vitro replication of the virus in MECC derived from BALB/c, C57BL, and C3H mice. Table 5 shows that, in a highly sensitive assay, there was no significant difference in the ability of the virus to replicate in MECC from the susceptible or resistant strains.

Neutralizing antibody. The appearance of neutralizing antibody was examined in an infection which was lethal in BALB/c mice but sub-

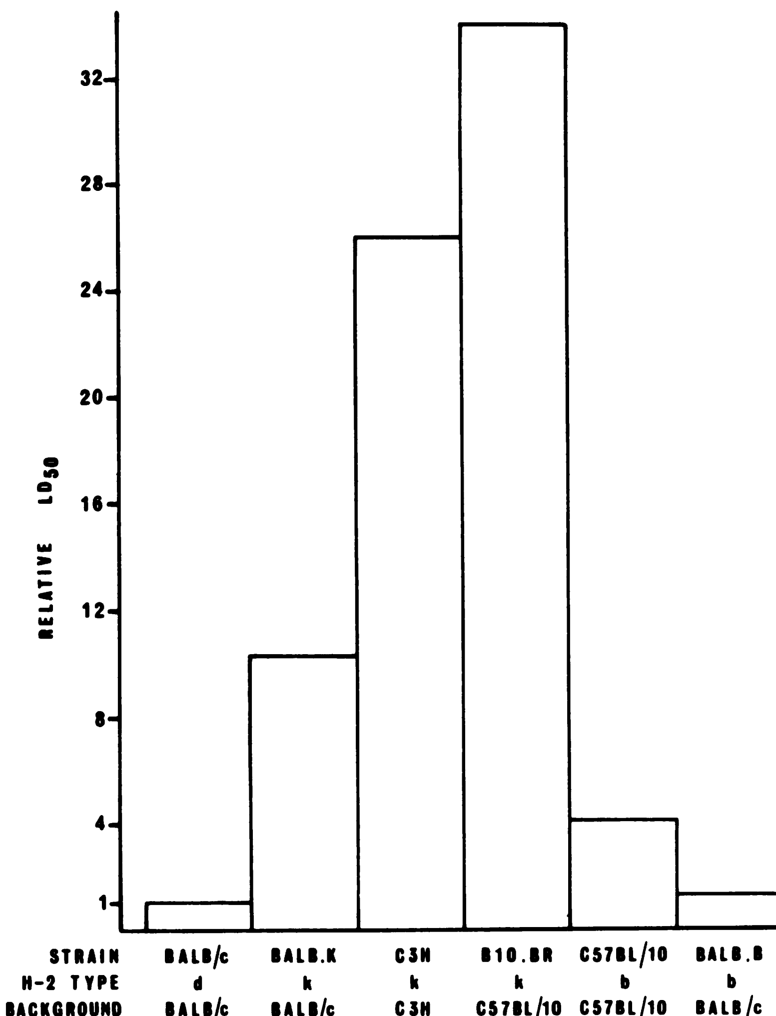

FIG. 3. *Effect of H-2 haplotype and genetic background on resistance of adult mice to i.p. inoculation with MCMV. The figure shows the relative LD_{50} values for BALB.K, C3H, B10.BR, C57BL/10, and BALB.B mice compared with the LD_{50} of BALB/c mice (equal to 1.0). The LD_{50} values of the various groups were compared statistically by the χ^2 test, and the results obtained were as follows. BALB/c and BALB.K: $P < 0.05$, $\chi^2 = 4.5$; and BALB.B: P = NS, $\chi^2 < 1$. BALB.K and C3H: $P < 0.05$, $\chi^2 = 3.86$; and B10.BR: $P < 0.05$, $\chi^2 = 4.32$. C57BL/10 and B10.BR: $P < 0.025$, $\chi^2 = 5.79$; and BALB.B: P = NS, $\chi^2 = 2.62$. C3H and B10.BR: P = NS, $\chi^2 = 2.67$.*

lethal in the more resistant BALB.K and C3H mice, in an attempt to correlate the appearance, or lack of, neutralizing antibody with the time of death in the more susceptible mice. Groups of 6 to 9 mice from each strain were bled at 24 h and also at 58 h postinfection, at which time deaths had begun to occur in the BALB/c group. Samples from each mouse were assayed individually for neutralizing antibody in a complement-dependent neutralization test. All samples were assayed in parallel. Of 22 samples taken at 24 h postinfection, only one, from a BALB.K mouse, showed any evidence of neutralizing antibody. Similarly, of 23 serum samples from the 58-h bleed, only two had any activity: one was from a C3H mouse, and the other was from a BALB/c mouse. The three positive samples gave only a weak reaction at 1:10 dilution of serum. Thus, by the time BALB/c mice began to die, neutralizing antibody was not generally demonstrable in their serum by the assay used, nor was it present in the sera of C3H or BALB.K mice. Hence, it is unlikely that the early appearance of neutralizing antibody is involved in the increased resistance of C3H and BALB.K mice to lethal infection with MCMV.

DISCUSSION

The resistance of adult mice to lethal infection with MCMV is under genetic control. Part of this control is mediated by genes linked to the major histocompatibility complex of the mouse. Of the three *H-2* haplotypes studied, susceptibility was associated with two, *d* and *b*, whereas the third haplotype, *k*, was relatively resistant. In mice of the BALB/c background the increase in resistance conferred by the *k* haplotype was about 8- to 10-fold that of the *d* and *b* haplotypes, which were equally susceptible. Susceptibility was expressed as a completely dominant trait. The data indicate that at least two loci within the *H-2* complex are involved in determining resistance to MCMV; one (in mice of B10 background) maps to the *D* end, close to the *D* locus, and the other (in mice of B10 or C3H background) maps to the *K* end, close to the *K* and *IA* loci. Generally, the presence of *d* and *b* alleles at these loci was associated with susceptibility, whereas that of *k* alleles conferred increased resistance; there were, however, two exceptions to this general trend. First, we observed unexpected resistance of B10.A(2R) mice compared with B10.A (see above), resulting only from a replacement of the *d* allele (in B10.A) for the *b* allele [in B10.A(2R)] at the *D* locus. The second exception was the failure of the replacement of *b* alleles at *K, IA,* and *IB* loci in B10.A(5R) by *k* alleles in B10.A to increase resistance (see above). It is possible that the D^d allele codes very strongly for susceptibility, overriding any protective effect afforded by *k* alleles at *K* and *IA,* whereas in the presence of the D^b allele such a protective effect of *k* alleles at the *K* end is apparent. These observations, however, could also be explained by the existence of an interaction between K^k or IA^k and D^b gene products which increased resistance, such

TABLE 2. *Comparison of the relative resistance of 3- to 4-day-old BALB/c, C57BL, and C3H mice to i.p. infection with MCMV*[a]

Strain	No. of mice inoculated	No. of deaths	$-\log_{10}$ LD$_{50}$	$-\log_{10}$ relative LD$_{50}$[b]
BALB/c	89	69	2.6	2.25
C57BL	38	31	2.6	2.25
C3H	71	68	3.1	2.75

[a] Virus was given in 0.02 ml in half-log dilutions. There was no sigificant difference by the χ^2 test between the number of deaths in any strain pair combination ($\chi^2 < 1$).

[b] Relative LD$_{50}$ compared with that of adult BALB/c mice = 1.0 (i.e., $-\log_{10}$ LD$_{50}$ = 0).

TABLE 3. *Comparison of the relative resistance of 19- to 24-week-old and 18-month-old BALB/c, C57BL, and C3H mice to lethal infection with MCMV*[a]

Strain	Age	Mean wt (g)	$-\log_2$ LD$_{50}$	Mean time of death (days)
BALB/c	24 wk	24.4	>5.50	5.28
	18 mo	25.7	4.83	5.17
C57BL	25 wk	20.6	4.10	5.01
	18 mo	22.6	3.10	5.33
C3H	19 wk	22.2	0.83	6.92
	18 mo	29.0	1.13	3.50

[a] The resistance of 18-month-old mice was not significantly different by the χ^2 test from that of the 19- to 25-week-old mice in any strain ($\chi^2 < 1$ in each case).

TABLE 4. *Effect of cortisone treatment on the resistance of adult BALB/c, C57BL, and C3H mice to lethal infection with MCMV*[a]

Strain	Group	Cortisone	Virus	No. of mice inoculated	No. of deaths	Mean time of death (days)
BALB/c	1	+	+	10	8	5.75
	2	+	−	5	0	—
	3	−	+	8	0	—
C57BL	4	+	+	10	9	5.67
	5	+	−	5	0	—
	6	−	+	10	2	6.00
C3H	7	+	+	10	9	5.44
	8	+	−	5	0	—
	9	−	+	10	0	—

[a] Cortisone was given subcutaneously at 3.5 mg/kg in 0.1 ml of mouse osmolality-buffered saline 2 h after i.p. inoculation with virus and thereafter i.p. daily for 1 week or until the death of the animal. Sham control mice received 0.1 ml of mouse osmolality-buffered saline. The differences between the numbers of deaths in various groups were analyzed statistically by the χ^2 test and were significant with $P < 0.02$ in the following pair combinations: 1 and 2, 1 and 3, 4 and 5, 4 and 6, 7 and 8, 7 and 9, 1 and 2 (or 3), 4 and 5 (or 6), 7 and 8 (or 9). —, Not applicable.

TABLE 5. *Comparison of the ability of MCMV to replicate in MECC derived from BALB/c, C57BL, and C3H embryos*[a]

−log virus dilution	Wells with CPE/total wells of MECC from:		
	BALB/c	C57BL	C3H
3.5	96/96	96/96	96/96
4.0	89/96	92/96	79/83
4.5	59/96	67/95	52/85
5.0	27/96	28/96	23/88
5.5	9/96	11/94	9/95
6.0	5/96	8/96	4/95
6.5	2/96	1/96	0/96
7.0	1/96	0/96	0/96
7.5	0/96	0/96	0/96
−log $TCID_{50}$	4,500	4,579	4,481

[a] The virus used was a salivary gland stock which had been passaged once in MECC derived from C3H mice. The $TCID_{50}$ titers of the various strains were not significantly different from each other by the χ^2 test ($\chi^2 < 1$ in all cases).

an interaction being lacking with the D^d gene product.

The effect of background genes makes the interpretation of *H-2* effects more difficult. Of the three backgrounds studied in detail, BALB/c was the most susceptible and C57BL was the least susceptible. The existence of a recessive gene(s) in the background of BALB/c mice which increased susceptibility would explain some of the background effects seen in the F_1 hybrid studies. Thus, the C3H and BALB/c (H-$2^{k/d}$) heterozygote would not express this recessive trait and would be expected to be (and indeed was) more resistant than the BALB.K and BALB/c (H-$2^{k/d}$) F_1 hybrid which was homozygous for the proposed BALB/c background genes. Similarly, the C57BL and BALB/c (H-$2^{b/d}$) heterozygote would be predicted to be more resistant than the BALB.B and BALB/c (H-$2^{b/d}$) F_1 hybrid which, although having the same *H-2* type, was homozygous for the BALB/c background genes; the results were in agreement with this prediction. This hypothetical gene, however, would not explain why the C57BL × BALB/c F_1 hybrid was more resistant than the C57BL parent, and the existence of another gene(s) in the C57BL background, which acted in a complementary manner, is suggested. The effects of a C57BL background gene were also seen in the C3H × C57BL (H-$2^{k/b}$) F_1 hybrid, which was markedly more resistant than the C3H × BALB/c (H-$2^{k/d}$) F_1 hybrid or the BALB.K × BALB.B (H-$2^{k/d}$) F_1 hybrid. Although the susceptibility of the latter may be due to the homozygous BALB/c background genes, that of the C3H × BALB/c heterozygote would not be, and because *b* and *d* haplotypes are associated with equal susceptibility, the increased resistance of the C3H × C57BL F_1 hybrid must be attributed to C57BL background genes. These results also suggest that such genes must be expressed in a dominant fashion. The increased resistance of B10.BR compared with other H-2^k strains, such as C3H or BALB.K, may also be due to such genes. The existence of autosomal dominant genes in the background of C57BL mice which increase resistance to other viruses has been described (6, 11). It cannot be determined from these experiments whether these latter genes are identical to those postulated here to be involved in resistance to MCMV. Blanden found (R. V. Blanden, personal communication) that these C57BL background genes interacted with particular *H-2* genes and affected the resultant resistance to ectromelia virus infection. This raises the possibility that such an interaction might be occurring in the studies presented here. Thus, the observation noted above, that on the C57BL background the substitution of the D^d allele with D^b allele increased resistance, might reflect an interaction between the D^b gene product and a C57BL background gene in a manner similar to that seen with ectromelia virus infection. Recombinants with the same *H-2* map on different genetic backgrounds would be needed to test the possibility of such interactions affecting the resistance to MCMV.

The dominance of the *H-2*-associated susceptibility in F_1 hybrids is not consistent with an immune response (Ir) gene basis of resistance. The immune response to MCMV is generally believed to be beneficial, and this conclusion is supported by our finding here that cortisone treatment rendered all strains of mice more susceptible. Classically Ir genes are dominant for high response; thus, if resistance to MCMV were due to Ir genes, the F_1 hybrid between susceptible and resistant haplotypes should be resistant. Furthermore, the presumptive mapping of genes controlling resistance to MCMV to the *K* and *D* subregions does not support the involvement of Ir genes, although the possible involvement of the *IA* locus in determining resistance to MCMV leaves this question open.

The data presented here do indicate that neither *H-2-* nor non-*H-2*-linked genes exert control on resistance to lethal infection with MCMV by virtue of the early appearance of neutralizing antibody. Similarly, neither *H-2-* nor non-*H-2*-linked genes appear to control viral replication at the cellular level, at least as judged by the ability to replicate in mouse embryo fibroblasts.

We have previously suggested (3) that subtle differences between strains in the ability of the host to prevent viral spread may account for their differing susceptibilities to lethal infection, rather than inherent differences in viral replication. This conclusion is supported by the finding here that newborn mice of resistant and susceptible strains are equally susceptible to the virus. All strains become more resistant during the first few weeks of life and it is during this period that the relative differences in resistance between strains emerge, with the degree of resistance becoming a characteristic of each strain throughout its lifespan. Thus, some host function, possibly immunological (either specific or nonspecific) in nature, which matures in this period, is necessary for the expression of genetically determined resistance.

It has recently been reported (8) that tracheal ring organ cultures from susceptible strains produce more virus after 2 to 3 weeks of infection than do similar cultures from resistant strains. The authors concluded that mouse epithelial cells (rather than fibroblasts) are infected with MCMV in vivo and that in the tracheal ring organ cultures resistance may be based partly on the innate genetic susceptibility of epithelial cells to MCMV infection. However, in the acute, rapidly lethal infection examined in this study, epithelial cells are not a prime cell target, and such a basis of resistance is unlikely. Also, if such an innate form of resistance were operating, this should result in differences in susceptibility among newborn as well as adult mice. This was not the case. Furthermore, the observations of Nedrud et al. (8) on viral replication in the tracheal ring organ cultures may merely reflect differences in interferon production in organ cultures from the different strains, because those authors did not measure interferon production in their organ cultures. We have observed strong differences in interferon production in vivo during the first 24 h of MCMV infection in resistant and susceptible strains (J. E. Chalmer and J. Trapman, unpublished data). Viral replication in vivo and the pathogenesis of MCMV infection in resistant and susceptible strains is the subject of a second paper in this series. Other studies in this laboratory (1) have shown that augmentation of natural killer cell activity early in MCMV infection may play an important role in resistance to MCMV. However, genetic analyses of such responses to MCMV infection indicated that natural killer cell augmentation was primarily controlled by non-*H-2*-linked genes, and our preliminary studies indicate that this is also true for interferon production. The mechanism of the *H-2* gene control of resistance to MCMV thus remains to be elucidated.

ACKNOWLEDGMENTS

The help of G. Harnett in the $TCID_{50}$ and antibody determinations is gratefully acknowledged.

LITERATURE CITED

1. **Bancroft, G. J., G. R. Shellam, and J. E. Chalmer.** 1981. Genetic influences on the augmentation of natural killer (NK) cells during murine cytomegalovirus infection: correlation with patterns of resistance. J. Immunol. **126**:988.
2. **Booss, J., and F. Wheelock.** 1975. Correlation of survival from murine cytomegalovirus infection with spleen-cell responsiveness to concanavalin A. Proc. Soc. Exp. Biol. Med. **149**:433–446.
3. **Chalmer, J. E., J. S. Mackenzie, and N. F. Stanley.** 1977. Resistance to murine cytomegalovirus linked to the major histocompatibility complex of the mouse. J. Gen. Virol. **37**:107–114.
4. **Henson, D., and C. Neopolitan.** 1970. Pathogenesis of chronic cytomegalovirus infection in submaxillary glands of C3H mice. Am. J. Pathol. **58**:255–267.
5. **Klein, J.** 1975. Biology of the mouse histocompatibility-2 complex: principles of immunogenetics applied to a single system, Springer-Verlag, Berlin. p. 389–410.
6. **Lopez, C.** 1975. Genetics of natural resistance to herpes virus infection in mice. Nature (London) **258**:152–153.
7. **Mannini, A., and D. N. Medearis.** 1961. Mouse salivary gland virus infection. Am. J. Hyg. **73**:329–343.
8. **Nedrud, J. G., A. M. Collier, and J. S. Pagano.** 1979. Cellular basis for susceptibility to mouse cytomegalovirus: evidence from tracheal organ culture. J. Gen. Virol. **45**:737–744.
9. **Neustadt, P. M., T. A. Cody, and A. A. Monjan.** 1978. Failure to find H-2-associated susceptibility to LCM disease. J. Immunogenet. **5**:397–400.
10. **Oldstone, M. B. A., F. J. Dixon, G. F. Mitchell, and H. O. McDevitt.** 1973. Histocompatibility-linked genetic control of disease susceptibility to murine lymphocytic choriomeningitis virus infection. J. Exp. Med. **37**:1201–1212.
11. **Schell, K.** 1960. Studies on the innate resistance of mice to infection with mousepox. II. Route of inoculation and resistance and some observations on the inheritance of resistance. Austr. J. Exp. Biol. Med. Sci. **38**:289–300.
12. **Selgrade, M. K., and J. E. Osborn.** 1974. Role of macrophages in resistance to murine cytomegalovirus. Infect. Immun. **10**:1383–1390.
13. **Sheridan, J. W., and J. J. Finlay-Jones.** 1977. Studies on a fractionated murine fibrosarcoma: a reproducible method for the cautious and a caution for the unwary. J. Cell Physiol. **90**:535–552.