

## *Cmv-1*, A GENETIC LOCUS THAT CONTROLS MURINE CYTOMEGALOVIRUS REPLICATION IN THE SPLEEN

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The genetically determined resistance of inbred mouse strains to lethal infection with murine cytomegalovirus (MCMV)<sup>1</sup> is under multigenic control and is determined by both H-2 and non-H-2 genes (1, 2). Mouse strains bearing the H-2<sup>k</sup> haplotype are 10 times more resistant to MCMV infection than are congenic mouse strains bearing the H-2<sup>b</sup> or H-2<sup>d</sup> haplotypes. In addition, increased resistance is associated with non-H-2 or background genes in the C57BL/6, C3H/HeJ, and CBA/CaH inbred mouse strains (2). Although the mechanisms of resistance to MCMV infection are not fully understood, the death of mice of susceptible strains by 3 d postinfection suggests that they must be effective early. In vitro infection studies have shown that the level of MCMV infection of isolated cells from a range of murine tissues reflects the patterns of H-2 determined resistance observed in vivo (3-5). These results suggest that the H-2-associated resistance observed during infection may operate at the level of individual target cells.

Although the number and chromosomal location of non-H-2 genes associated with resistance to MCMV is unknown, they have been shown to exert their influence early during MCMV infection by regulating the nonadaptive host responses mediated by interferon (6-9), NK cells (10-13), and inflammatory cells (14). Non-H-2 genes have also been shown to regulate the amount of MCMV replication in critical murine target organs after intraperitoneal infection (15). MCMV infection of strains with the C57BL background [C57BL/6(H-2<sup>b</sup>) and B10.BR(H-2<sup>k</sup>)] resulted in very low levels of splenic virus replication relative to H-2 identical strains with the BALB/c background [BALB.B(H-2<sup>b</sup>) and BALB.K(H-2<sup>k</sup>)]. However, H-2 genes can also regulate MCMV replication in the spleen, since resistant BALB.K(H-2<sup>k</sup>) mice exhibit lower levels of splenic virus replication and clear virus more rapidly than do the susceptible congenic BALB/c(H-2<sup>d</sup>) and BALB.B(H-2<sup>b</sup>) mouse strains (15, 16). The latter H-2<sup>d</sup> and H-2<sup>b</sup> strains both exhibit similar high levels of splenic virus replication.

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<sup>1</sup> Abbreviations used in this paper: MCMV, mouse cytomegalovirus; MEF, mouse embryo fibroblast; RI, recombinant inbred; SDP, strain distribution pattern.

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The primary aim of the present study was to determine the genetic basis of the non-H-2 component of control of splenic replication of MCMV that is associated with the C57BL background. Investigations using F<sub>1</sub> and F<sub>2</sub> hybrids, backcross mice, and the CXB set of recombinant inbred (RI) mouse strains indicate the operation of an autosomal dominant non-H-2 gene to which we assign the symbol *Cmv-1*.

Non-H-2 genes also influence host susceptibility to infection with HSV-1 and the C57BL background is associated with greatest resistance (17, 18). HSV-1 replicates initially in the skin from where it invades the sensory nervous system. Using a murine model of acute herpes simplex that closely mimics human disease (19), we recently showed that resistance to neural infection, after cutaneous inoculation with virus, is under complex host genetic control, involving up to four independently segregating non-H-2 genetic loci (20). Initial control of HSV-1 may be mediated in part by interferons (21, 22) and NK cells (23), both of which have been implicated in resistance to MCMV infection (6-13). It is therefore possible that common genes are involved in determining susceptibility to these herpesviruses, despite the dissimilarities in their organ tropism. Because the C57BL background conveys early acting resistance to the replication of both MCMV and HSV in their major target organs, we investigated the hypothesis that *Cmv-1* alleles are involved in resistance to herpes simplex virus. Using the CXB RI mouse strains we confirmed the multifactorial nature of host resistance to HSV-1, and showed that *Cmv-1* is unlikely to be involved.

### Materials and Methods

*Mice.* 8-wk-old female mice, bred under specific pathogen-free conditions, were obtained from the Animal Resources Centre (Murdoch, Western Australia) and housed under minimal disease conditions. Mice used in this study were the inbred strains A/J, BALB/cLac, BALB/cBy, BALB.B, BALB.K, C57BL/6J, C57BL/6By, B10.A, B10.BR, B10.D2, CBA/CaH, DBA/1J, DBA/2J, SJL/J, the F<sub>1</sub> generation (BALB/c × C57BL/6)F<sub>1</sub>, the backcross generations (BALB/c × C57BL/6)F<sub>1</sub> × BALB/c and (BALB/c × C57BL/6)F<sub>1</sub> × C57BL/6, the F<sub>2</sub> generation (BALB/c × C57BL/6)F<sub>1</sub> × (BALB/c × C57BL/6)F<sub>1</sub>, and the CXB/By recombinant inbred (RI) strains CXBD/By, CXBE/By, CXBG/By, CXBH/By, CXBI/By, CXBJ/By, and CXBK/By. The BALB/cBy and C57BL/6By strains were only used in experiments that involved the CXB/By RI strains. The CXB/By RI strains and the progenitor BALB/cBy and C57BL/6By were originally obtained from The Jackson Laboratory, Bar Harbor, ME.

*Virus.* The Smith strain of MCMV, originally obtained from Dr. D. Lang (Duke University, Durham, NC), was used in all experiments. The method of maintenance of this stock by salivary gland passage in weanling female BALB/c mice has been described elsewhere (15). Virus stocks were stored in the gas phase of liquid nitrogen and were regularly monitored to exclude contamination with other infectious agents.

For HSV-1 experiments, a well-characterized oral isolate, strain SC16, was used (24). The virus stock was grown and titrated in Vero cells and stored at -70°C until required.

*Cell Culture.* Primary mouse embryo fibroblast (MEF) cultures were prepared by trypsin dispersion of 14-15-d-old embryos from outbred Arc Swiss mice by the method described previously (1). MEFs and Vero cells were cultured in Eagle's MEM (Gibco Laboratories, Grand Island, NY) and 10% FCS. Maintenance medium contained 2% FCS.

*Infection of Mice.* Mice were inoculated with MCMV by the intraperitoneal route with 0.1 ml of salivary gland-derived virus diluted in PBS (333 mosM) and containing 0.5% FCS. The minimum intraperitoneal dose that killed 50% of animals inoculated (LD<sub>50</sub>) was calculated using the Kaerber equation as described elsewhere (2). Five mice per group were inoculated over a range of serial twofold dilutions of MCMV giving from 0 to 100% mortality.

A zosteriform model of HSV-1 infection was used in which mice were infected with  $5 \times 10^4$  PFU of virus by scarification of the left flank as described previously (19).

**Sample Collection.** Organs to be titrated for MCMV or HSV-1 by plaque assay were aseptically harvested, weighed, and then individually homogenized at 10% wt/vol in ice cold MEM containing 2% FCS, using chilled 2-ml Pyrex tissue grinders (Corning, NY). After clarification by centrifugation at 2,000 *g* for 20 min at 4°C, the supernatants were stored at -70°C until use.

**Virus Titrations: (i) MCMV Plaque Assay.** The method used was a modification of that described previously (1). Briefly, secondary MEF cultures were seeded into the wells of 24-well tissue culture trays (Costar, Cambridge, MA) at  $2 \times 10^5$  cells/well in MEM containing 10% FCS 48 h before virus assay. Confluent monolayers were washed with 1 ml of maintenance medium and 0.2 ml volumes of serial twofold dilutions of organ homogenates prepared in maintenance medium were added to the wells. After incubating for 1 h at 37°C in 10% CO<sub>2</sub> in air, virus inocula were removed, and replaced with 2 ml of 2% wt/vol methylcellulose (Fisher Scientific, Fairlawn, NJ) in maintenance medium. Infected cultures were incubated for 5 d at 37°C in 10% CO<sub>2</sub> in air and then stained with 1% methylene blue containing 10% formalin.

**(ii) HSV Plaque Assay.** Serial 10-fold dilutions of tissue homogenates were tested for infectivity in Vero cells using a standard plaque assay (25).

**(iii) Infectious Center Assay.** Productively infected cells present in the spleens of MCMV-infected mice were quantitated by an infectious center assay based on the method of Winkler and Booss (26). Briefly, single cell suspensions of spleens prepared as previously described (27) were adjusted to  $10^6$  cells/ml and serial twofold dilutions were prepared in 0.5 ml volumes of maintenance medium in 24-well tissue culture trays. The trays were incubated for 1 h at 37°C in 10% CO<sub>2</sub> in air, the supernatant was then aspirated and  $2 \times 10^5$  secondary MEFs in 1 ml maintenance medium were added to each well. After incubation for 1 h at 37°C in 10% CO<sub>2</sub> in air, the supernatant was aspirated and replaced with 2 ml of 2% methylcellulose in maintenance medium. Trays were then incubated for a further 5 d before staining with 1% methylene blue containing 10% formalin.

**Statistical Analysis.** Where appropriate, comparisons of virus titers or LD<sub>50</sub> titers between strains were performed using Student's *t*-test. The patterns of segregation of parental phenotypes in backcross and F<sub>2</sub> mice were analyzed using the  $\chi^2$  test and nonparametrically using the Kolmogoroff-Smirnoff test (28, 29). The relationship between splenic virus titers and spleen weight was analyzed nonparametrically using the Spearman rank correlation test (30).

## Results

**Restricted Replication of MCMV in the Spleens of (BALB/c × C57BL/6)F<sub>1</sub> Mice.** We have previously shown that inbred mouse strains with C57BL background genes (C57BL/6, H-2<sup>b</sup>; B10.BR, H-2<sup>k</sup>) support significantly lower levels of MCMV replication in their spleens than do H-2 syngeneic mouse strains with the BALB/c background (BALB.B, H-2<sup>b</sup>; BALB.K, H-2<sup>k</sup>) (15). To further assess the genetic basis for this non-H-2-determined control of splenic virus replication, the replication of MCMV in the spleens of (BALB/c × C57BL/6)F<sub>1</sub> hybrid, BALB/c, and C57BL/6 mice was compared. All mice were inoculated by the intraperitoneal route with a dose of virus that was sublethal for BALB/c mice ( $2.0 \times 10^4$  PFU, a 0.3 LD<sub>50</sub> dose for BALB/c). Relative to the parental BALB/c strain the level of MCMV replication in the spleens of (BALB/c × C57BL/6)F<sub>1</sub> hybrid mice was very low like that observed for the parental C57BL/6 strain (Fig. 1 A). In contrast to the spleen the levels of MCMV replication in the livers of the two parental strains and the F<sub>1</sub> hybrid mice were all similar (Fig. 1 B). In contrast to marked differences in the splenic MCMV titers between BALB/c and C57BL/6 mice, only very small differences in spleen titers were observed between male and female mice of each strain (data not

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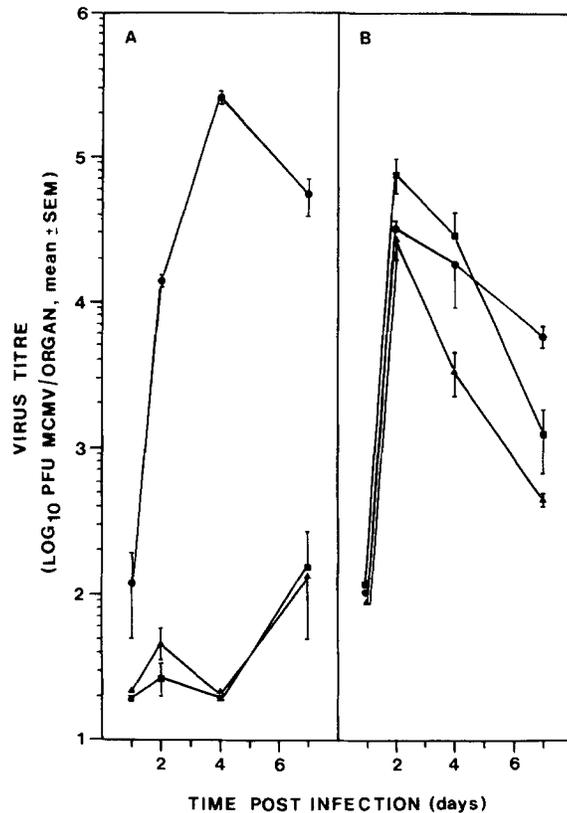


FIGURE 1. Replication of MCMV in the spleens (A) and livers (B) of BALB/c (●), C57BL/6 (■), and (BALB/c × C57BL/6)F<sub>1</sub> hybrid (▲) mice. Mice were inoculated by the intraperitoneal route with  $2.0 \times 10^4$  PFU (0.3 LD<sub>50</sub> dose for BALB/c) of MCMV. Each point represents the mean titer  $\pm$  SEM of virus present in organs from three individual mice. The limit of sensitivity of the plaque assay was 20 PFU/organ.

shown). Taken together, these results indicate that the restriction in splenic replication associated with the C57BL background is an autosomal dominant trait and does not extend to the liver, which is the other major target organ during acute MCMV infection.

*Enumeration of the Genes Responsible for Restricted Splenic Replication of MCMV in C57BL/6 Mice: (i) Backcross Study.* The number of genes involved in mediating the restriction of MCMV replication in the spleens of C57BL/6 mice was investigated by determining the pattern of segregation of high and low levels of MCMV replication in the spleens of (BALB/c × C57BL/6)F<sub>1</sub> × BALB/c backcross mice. Splenic titers in the backcross progeny mice at day 4 postinfection, the day of peak splenic replication, segregated into distinct groups (Fig. 2) and were either BALB/c-like (24 of 53 with titers  $>10^4$  PFU/spleen, 45.3%), or C57BL/6-like (29 of 53 with titers  $<10^4$  PFU/spleen, 54.7%). This distribution, which is not significantly different from a 1:1 ratio ( $\chi^2 = 0.472$ ,  $p < 0.5$ ), suggests the operation of a single dominant gene mediating the restriction in splenic MCMV replication during acute infection of C57BL/6 mice (legend, Fig. 2).

A comparison of the distribution of splenic virus titers and spleen weights in individual mice within the (BALB/c × C57BL/6)F<sub>1</sub> × BALB/c backcross population reveals that an inverse correlation exists between these two variables (Spearman rank

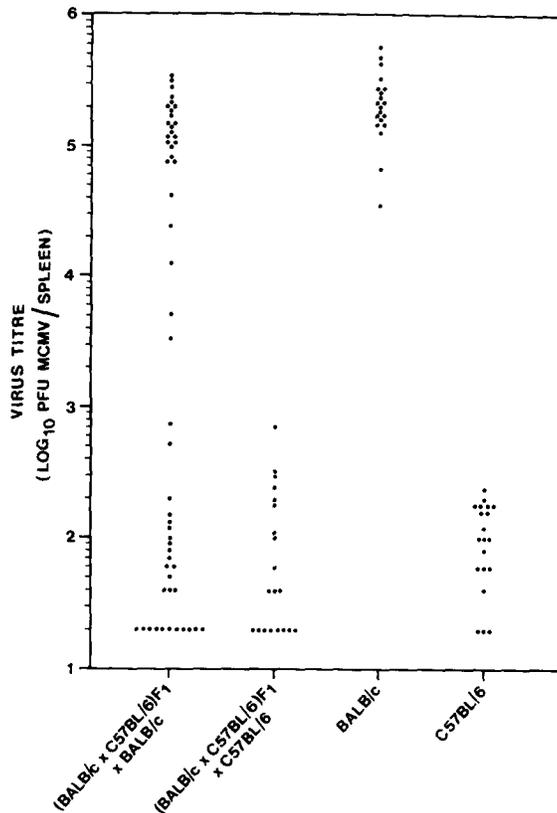


FIGURE 2. Genetic analysis of MCMV replication in the spleens of backcross generation mice. Mice were inoculated by the intraperitoneal route with  $3.2 \times 10^4$  PFU (0.5 LD<sub>50</sub> dose for BALB/c) of MCMV and spleens removed at day 4 after infection. Essentially identical results were obtained in a separate repeat experiment. Analysis of the cumulative frequency distribution of spleen titers in the (BALB/c  $\times$  C57BL/6)F<sub>1</sub>  $\times$  BALB/c backcross mice relative to the combined cumulative frequency of spleen titers in the parental BALB/c and C57BL/6 mice using the nonparametric Kolmogoroff-Smirnoff statistic as previously described (28, 29), revealed that  $D_{\max}$  (0.1641) was less than the critical tabulated value at  $\alpha = 0.05$  (0.1831 for  $n = 53$ ). On this basis the hypothesis of control by a single gene would not be rejected.

correlation coefficient,  $r_s = -0.855$ , corrected for tied data) (Fig. 3). Backcross mice with low levels of virus replication ( $<10^4$  PFU/spleen) had higher spleen weights (mean  $\pm$  SEM =  $0.124 \pm 0.004$  g), which were comparable to those seen in infected C57BL/6 parental mice ( $0.149 \pm 0.003$  g). Conversely, mice with high levels of virus replication ( $>10^4$  PFU/spleen) had low spleen weights ( $0.072 \pm 0.002$  g), which were comparable with those of infected BALB/c parental mice ( $0.086 \pm 0.003$  g).

(ii) *F<sub>2</sub> Study.* To corroborate the conclusion that the restricted splenic MCMV replication associated with the C57BL/6 background is under unigenic control, the pattern of splenic virus replication in the F<sub>2</sub> progeny of a (BALB/c  $\times$  C57BL/6)F<sub>1</sub>  $\times$  (BALB/c  $\times$  C57BL/6)F<sub>1</sub> mating was assessed. Fig. 4 shows that 25 of 101 (24.8%) F<sub>2</sub> progeny had high splenic virus titers ( $>10^4$  PFU/spleen), while 76 of 101 (75.2%) had low titers ( $10^4$  PFU/spleen). This distribution, which is not significantly different from a 1:3 ratio ( $\chi^2 = 0.0033$ ,  $p < 0.9$ ), is consistent with the Mendelian segregation of a single dominant gene (legend, Fig. 4). Based on the rules and guidelines for standardized gene nomenclature (31), we propose to assign the symbol *Cmv-1*, to the non-H-2 gene which restricts splenic replication of MCMV. Accordingly, BALB/c mice would be designated *Cmv-1<sup>h</sup>* and C57BL/6 mice would be designated *Cmv-1<sup>l</sup>*.

As observed above for the (BALB/c  $\times$  C57BL/6)F<sub>1</sub>  $\times$  BALB/c backcross mice,

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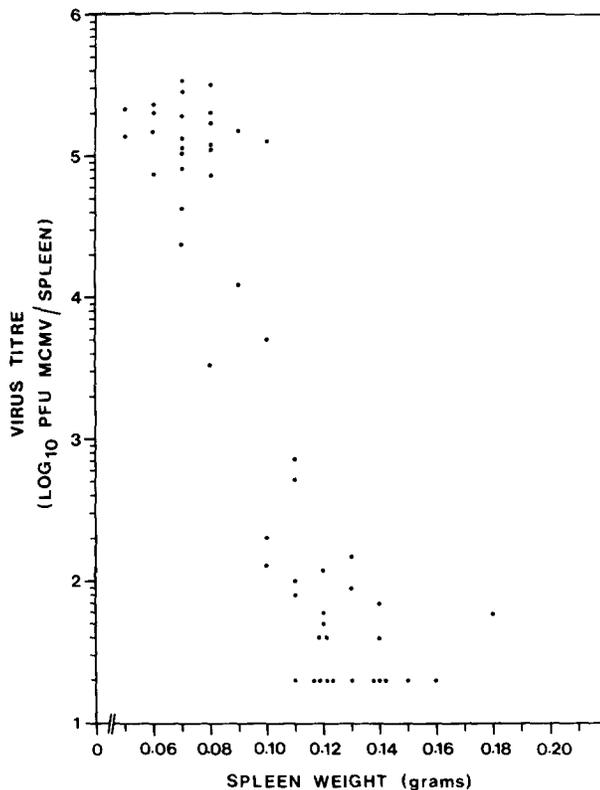


FIGURE 3. Splenic virus titers and spleen weights in MCMV infected (BALB/c  $\times$  C57BL/6) $F_1$   $\times$  BALB/c backcross mice. This figure shows a scatter plot of splenic virus titers versus spleen weights for the individual (BALB/c  $\times$  C57BL/6) $F_1$   $\times$  BALB/c backcross mice represented in Fig. 2. The mean  $\pm$  SEM of spleen weights of backcross mice with splenic virus titers  $>10^4$  PFU/spleen was  $0.072 \pm 0.002$  g and for mice with titers  $<10^4$  PFU/spleen was  $0.124 \pm 0.004$  g. In the parental strains the mean  $\pm$  SEM of spleen weights were for BALB/c (uninfected,  $0.091 \pm 0.003$  g; infected,  $0.086 \pm 0.003$  g) and for C57BL/6 (uninfected,  $0.084 \pm 0.007$  g; infected  $0.149 \pm 0.003$  g).

spleen weights in individual MCMV-infected  $F_2$  mice also reflected splenic virus titers. Mice with low splenic virus titers ( $<10^4$  PFU/spleen) had higher spleen weights ( $0.141 \pm 0.003$  g,  $n = 76$ ), compared with a mean weight of  $0.074 \pm 0.004$  g ( $n = 25$ ) in mice that had high spleen titers ( $>10^4$  PFU/spleen).

**Chromosomal Location of the *Cmv-1* Gene.** To determine the genetic linkage and chromosomal location of *Cmv-1*, the level of splenic MCMV replication in the CXB/By set of recombinant inbred mice was assessed. Fig. 5 A shows that all seven CXB/By RI mouse strains clearly segregate into one or other of the progenitor strain patterns. The CXBD/By, CXBE/By, and CXBJ/By RI strains exhibited very low spleen titers, like the C57BL/6By progenitor strain, whereas the CXBG/By, CXBH/By, CXBI/By, and CXBK/By RI strains were all BALB/cBy-like, exhibiting high spleen titers that peaked at  $>10^5$  PFU/spleen at day 4 postinfection. This strain distribution pattern (SDP) was reflected in an identical SDP for the numbers of infected splenic cells at day 2 after infection, as determined by infectious center plaque assay (Table I). In contrast to the spleen, the virus titers present in the livers of the CXB RI strains showed less variation, and did not clearly segregate into progenitor strain patterns (Fig. 5 B). The CXBK/By strain differed from all the other strains by failing to show clearance of virus in the liver at day 6 after infection.

The clear-cut SDP of the *Cmv-1<sup>l</sup>* (C57BL/6-like) and *Cmv-1<sup>h</sup>* (BALB/c-like) alleles

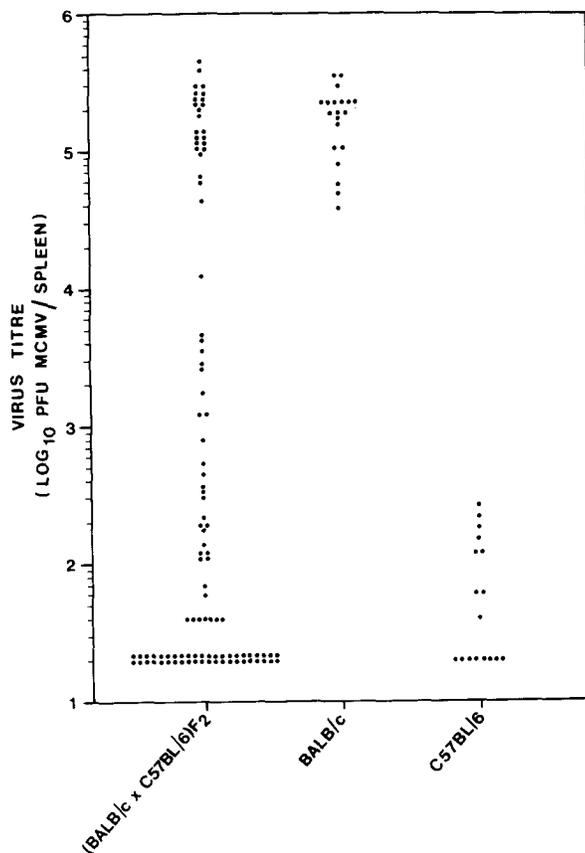


FIGURE 4. Genetic analysis of MCMV replication in the spleens of  $F_2$  generation mice. Mice were inoculated by the intraperitoneal route with  $3.2 \times 10^4$  PFU (0.5  $LD_{50}$  dose for BALB/c) of MCMV and spleens were removed at day 4 after infection. Comparison of the cumulative frequency distribution of the  $F_2$  spleen titers with the combined frequency distribution of the parental strains (3/4 of cumulative frequency of C57BL/6 + 1/4 of cumulative frequency of BALB/c) using the Kolmogoroff-Smirnoff statistic (28, 29), reveals that the value of  $D_{max}$  (0.1080) was less than the critical value at  $\alpha = 0.05$  (0.1334, for  $n = 101$ ).

among the seven CXB RI strains permitted comparison with the SDPs of other genes in these mice. The pattern was identical only to that of the proline-rich protein (*Prp*) and erythrocyte alloantigen-10 (*Ea-10*) genes (Table II), which are linked on chromosome 6 (32). The pattern showed that the *Cmv-1* gene was not located within the H-2 complex. Although the available evidence suggests that *Cmv-1* is linked to *Prp* and *Ea-10* on chromosome 6, a more detailed analysis in a larger set of RI strains is required, and this is in progress.

**HSV-1 Replication in the Ganglia of the CXB Recombinant Inbred Mouse Strains.** Replication of HSV-1 in the sensory ganglia of CXB mice was examined 2 and 3 d after cutaneous inoculation with virus (Fig. 6). The progenitor strains were clearly distinguishable and most recombinant inbred strains were of an intermediate phenotype. However, CXBD/By and CXBG/By mice segregated with the C57BL/6By progenitor.

**Resistance of CXB Recombinant Inbred Mouse Strains to Lethal MCMV Infection.** Previous studies have shown that mouse strains possessing the C57BL background are reproducibly about four times more resistant to lethal infection with MCMV than are mice with the BALB background (1, 2). To investigate if the low levels of splenic virus replication seen in the CXBD/By, CXBE/By, and CXBJ/By RI strains are associated

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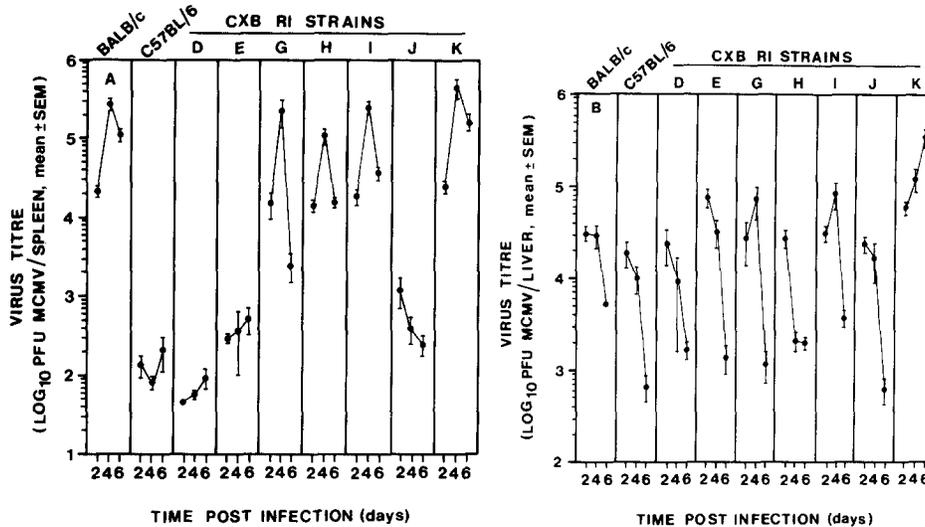


FIGURE 5. Genetic analysis of MCMV replication in the spleens (A) and livers (B) of the CXB recombinant inbred mouse strains. Mice were inoculated by the intraperitoneal route with  $2.0 \times 10^4$  PFU (0.3 LD<sub>50</sub> dose for BALB/c) of MCMV and organs were removed at the times indicated. Each point represents the mean  $\pm$  SEM of virus titers from eight individual animals.

with enhanced resistance, the relative resistance status of each of the CXB RI mouse strains was determined. Table III shows the mean LD<sub>50</sub> values from three separate experiments. The CXBG/By, CXBH/By, CXBI/By, and CXBK/By RI strains were at least as susceptible to lethal MCMV infection as the progenitor BALB/cBy strain. In contrast, the CXBD/By, CXBE/By, and CXBJ/By RI strains were all significantly more resistant than the BALB/cBy mouse strain ( $p < 0.025$ ,  $p < 0.025$ ,  $p < 0.01$ , respectively), but not as resistant as the progenitor C57BL/6By strain. This pattern resembles

TABLE I  
Numbers of MCMV-infected Spleen Cells after Acute Sublethal  
Infection of CXB RI Strains

Mouse strain	Numbers of infected cells/spleen*
BALB/cBy	20,830
C57BL/6By	<110
CXBD/By	<70
CXBE/By	210
CXBG/By	36,290
CXBH/By	8,230
CXBI/By	7,070
CXBJ/By	420
CXBK/By	34,960

\* Pooled spleen cell suspensions were prepared from groups of three mice per strain infected 2 d previously by the intraperitoneal route with  $2.5 \times 10^4$  PFU of MCMV. The numbers of infected splenocytes were quantitated by infectious center plaque assay.

TABLE II  
Concordance of the SDPs of *Cmv-1* and Chromosome 6 Markers  
in the CXB/By Recombinant Inbred Strains

Locus	Chromosome	CXB RI strains						
		D	E	G	H	I	J	K
<i>Cmv-1</i>		B*	B	C*	C	C	B	C
<i>Prp</i> <sup>‡</sup>	6	B	B	C	C	C	B	C
<i>Ea-10</i> <sup>‡</sup>	6	B	B	C	C	C	B	C
<i>H-2</i> <sup>§</sup>	17	C	B	B	C	B	B	B

\* The generic symbols B and C are used here to indicate alleles inherited from the progenitor strains C57BL/6By and BALB/cBy, respectively (33).

<sup>‡</sup> Reference 32.

<sup>§</sup> Reference 33.

the strain distribution pattern observed for regulation of splenic MCMV replication by the *Cmv-1* gene in the CXB RI strains. These results suggest that the low levels of splenic MCMV replication associated with the *Cmv-1*<sup>l</sup> allele may in part be responsible for the enhanced resistance to lethal MCMV infection seen in mouse strains with the C57BL genetic background.

*Comparison of Splenic MCMV Replication in a Range of Inbred Mouse Strains.* To determine if the restriction in splenic MCMV replication observed in mouse strains with the C57BL background was a characteristic of other genetic backgrounds, the ability of MCMV to replicate in the spleens of a range of mouse strains was investigated. Mice were infected with a low dose ( $4 \times 10^4$  PFU; sublethal for BALB/c) or a high dose ( $4 \times 10^5$  PFU; lethal for BALB/c and C57BL/6) and spleens were harvested at early time points (day 2 after infection, Exp. 1; day 3 after infection, Exp. 2).

TABLE III  
Relative Susceptibility of CXB Recombinant Inbred Mouse Strains  
to Lethal Infection with MCMV

Mouse strain	Mean LD <sub>50</sub> dose $\pm$ SEM (PFU $\times 10^{-4}$ ) <sup>*</sup>	<i>p</i> Value <sup>‡</sup>	Relative LD <sub>50</sub> <sup>§</sup>
BALB/cBy	6.4 $\pm$ 0.1		1.0
C57BL/6By	31.7 $\pm$ 5.0	0.0005 < <i>p</i> < 0.005	5.0
CXBD/By	21.3 $\pm$ 4.6	0.01 < <i>p</i> < 0.025	3.3
CXBE/By	16.0 $\pm$ 3.2	0.01 < <i>p</i> < 0.025	2.5
CXBG/By	3.3 $\pm$ 0.8	0.005 < <i>p</i> < 0.01	0.5
CXBH/By	7.4 $\pm$ 1.4	0.1 < <i>p</i> < 0.375	1.2
CXBI/By	6.0 $\pm$ 0.7	0.1 < <i>p</i> < 0.375	0.9
CXBJ/By	18.0 $\pm$ 2.8	0.005 < <i>p</i> < 0.01	2.8
CXBK/By	2.7 $\pm$ 0.2	<i>p</i> $\leq$ 0.0005	0.4

\* Groups of five mice per dilution received serial twofold dilutions of MCMV by the intraperitoneal route. The results represent the means  $\pm$  SEM of three separate determinations.

<sup>‡</sup> The differences in the LD<sub>50</sub> titers of the CXB/By RI strains and the C57BL/6By strain from the BALB/cBy strain were statistically analyzed by Student's *t*-test.

<sup>§</sup> Relative LD<sub>50</sub> refers to the ratio of the LD<sub>50</sub> dose for a particular strain relative to that of BALB/cBy.

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Table IV shows that all mouse strains, except those with the C57BL background, gave elevated levels of splenic virus replication when inoculated with  $4 \times 10^4$  PFU of MCMV. When the virus dose was increased to  $4 \times 10^5$  PFU even mice of the C57BL background exhibited moderately elevated titers, although these were still significantly less ( $p < 0.005$ ) than those obtained from the H-2 syngeneic BALB.K (H-2<sup>k</sup>) and BALB.B (H-2<sup>b</sup>) strains (Table IV, Exp. 1).

## Discussion

Classical Mendelian analyses with backcross and F<sub>2</sub> progeny derived from BALB/c (susceptible) and C57BL/6 (resistant) progenitor strains disclosed an autosomal genetic locus that controls splenic replication of MCMV. At this locus, to which we have assigned the symbol *Cmv-1*, alleles conveying resistance are dominant. The concordance, in CXB RI mice, between the SDP's of *Cmv-1*, *Prp*, and *Ea-10* suggests that *Cmv-1* may be located on mouse chromosome 6.

The *Cmv-1*<sup>l</sup> allele associated with C57BL mice is not present in the A, BALB, CBA, DBA/1, DBA/2, or SJL genetic backgrounds (Table IV). However, the intermediate titers obtained for the CBA/CaH, DBA/2J and SJL/J strains do not permit a *Cmv-1*<sup>h</sup> genotype to be ascribed to these strains and a more extensive kinetic analysis is required to determine if these strains possess *Cmv-1* alleles that exert intermediate control of virus replication, or whether other non-H-2 genes also modulate the level of splenic MCMV replication. At the higher dose of  $4 \times 10^5$  PFU the B10.BR (H-2<sup>k</sup>) and C57BL/6J (H-2<sup>b</sup>) strains also showed elevated MCMV titers

TABLE IV  
*Splenic MCMV Replication in a Range of Inbred Mouse Strains*

Exp.	Mouse strain	H-2 haplotype	Splenic MCMV titer (mean PFU/spleen $\pm$ SEM) in mice inoculated with:	
			Low dose ( $4 \times 10^4$ PFU)	High dose ( $4 \times 10^5$ PFU)
1*	BALB.B	H-2 <sup>b</sup>	14,170 $\pm$ 830	186,880 $\pm$ 30,520
	C57BL/6J	H-2 <sup>b</sup>	60 $\pm$ 25	3,310 $\pm$ 1,245
	BALB.K	H-2 <sup>k</sup>	8,950 $\pm$ 1,250	133,760 $\pm$ 33,820
	B10.BR	H-2 <sup>k</sup>	<20	11,980 $\pm$ 745
	CBA/CaH	H-2 <sup>k</sup>	2,320 $\pm$ 390	51,305 $\pm$ 10,835
2*	A/J	H-2 <sup>a</sup>	27,510 $\pm$ 3,750	ND†
	B10.A	H-2 <sup>a</sup>	55 $\pm$ 30	ND
	BALB/c	H-2 <sup>d</sup>	24,890 $\pm$ 3,930	ND
	DBA/2J	H-2 <sup>d</sup>	10,090 $\pm$ 1,730	ND
	B10.D2	H-2 <sup>d</sup>	<20	ND
	BALB.B	H-2 <sup>b</sup>	39,105 $\pm$ 5,010	ND
	C57BL/6J	H-2 <sup>b</sup>	90 $\pm$ 35	ND
	BALB.K	H-2 <sup>k</sup>	7,180 $\pm$ 2,250	ND
	DBA/1J	H-2 <sup>a</sup>	20,130 $\pm$ 2,990	ND
	SJL/J	H-2 <sup>s</sup>	6,700 $\pm$ 1,730	ND

\* In both experiments groups of four mice per strain received intraperitoneal inoculations of the appropriate dose of MCMV. Spleens were taken at day 2 (Exp. 1) or day 3 after infection (Exp. 2) for assay of virus titers.

† ND, not done.

(Table IV, Exp. 1). This confirms previous results of Allan and Shellam (15) and indicates that the operation of *Cmv-1* can be overridden by increasing the virus inoculum.

The *Cmv-1* locus may substantially influence overall survival of the animal because mouse strains that exhibited low levels of splenic MCMV replication were significantly more resistant to lethal infection (Table III). Two additional observations support this hypothesis. Firstly, Katzenstein et al., (34) showed that splenectomy conferred resistance to BALB/c mice implying that replication of virus in this organ contributed significantly to death. Second, preliminary studies have shown that the *Cmv-1* locus may also regulate the level of in vivo infection in the bone marrow and thereby influence the degree of bone marrow suppression associated with MCMV infection (Gibbons, A. E., A. A. Scalzo, P. Price, and G. R. Shellam; unpublished observations). Indeed Bale et al. (35), showed that peak replication of MCMV in bone marrow and spleens of outbred Swiss mice was concurrent and coincided with leukopenia.

Nevertheless resistance to lethal infection cannot be influenced exclusively by *Cmv-1* because some RI strains (CXBG/By and CXBK/By) showed enhanced susceptibility compared with BALB/cBy, while others (CXBD/By, CXBE/By, and CXBJ/By) were intermediate in relation to the progenitor strains. In this context it is interesting to note that the effect of the *Cmv-1* locus is not manifested in the liver.

The mechanism by which the C57BL/6 *Cmv-1* allele conveys resistance is unknown, but several points are worthy of discussion. In (BALB/c × C57BL/6)F<sub>1</sub> × BALB/c backcross progeny, high splenic virus titers were associated with low spleen weight. This observation extends the findings of Mims and Gould (36) who showed that MCMV-infected BALB/c and A strain mice developed splenic atrophy and necrosis in association with a high level of virus replication. In contrast, low splenic virus titers were accompanied by splenomegaly that could be the result either of an influx of leukocytes from other lymphoid organs or the proliferation of cells already present within the spleen (Fig. 3). It is tempting to speculate that the as yet unidentified product of *Cmv-1* determines the efficacy of this early inflammatory response. Certainly the resistance conferred by the C57BL/6 allele is manifest early in the course of the disease, because restriction of splenic replication is evident 1-2 d after infection (Figs. 1 A and 5 A). This aspect, including the potential involvement of interferon and NK cells, is a focus of continuing work.

For both MCMV and HSV-1 similarities in the hierarchy of resistance exist, such that mice with the C57BL background show greater resistance relative to mice with the BALB background, although this effect is much more pronounced for HSV-1 (2, 17, 37). Indeed, C57BL/6 mice survive an intraperitoneal challenge with >1,000 times the amount of HSV-1 tolerated by a variety of other inbred strains (17). After cutaneous inoculation, restriction of the infection by resistant mice occurs primarily in the sensory nervous system (38). In the present study, at 2 and 3 d after infection of the flank skin, C57BL/6 and BALB/c progenitor mice could be clearly distinguished in terms of virus recovery from spinal ganglia (Fig. 6). Most CXB RI strains were intermediate in this respect suggesting that neural infection is under complex genetic control, which is in concordance with previously reported F<sub>2</sub> and backcross studies (20). However, the behavior of CXBG/By mice requires further comment. This strain has the BALB/c allele at the *Cmv-1* locus yet is completely resistant to

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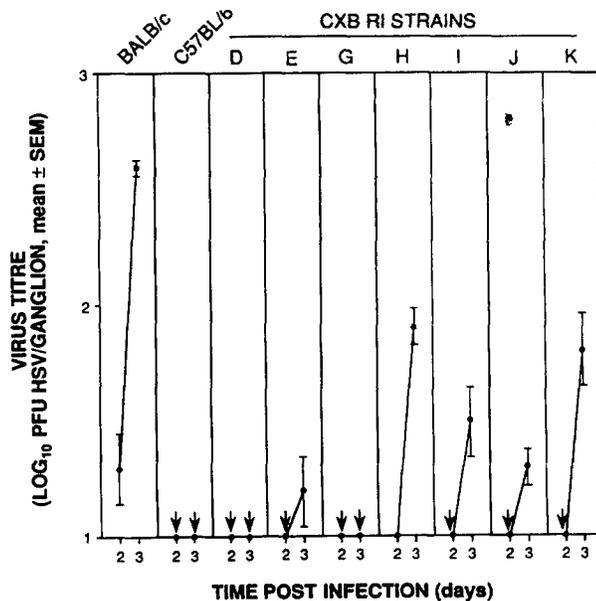


FIGURE 6. Genetic analysis of HSV replication in the peripheral nervous system of CXB recombinant inbred mouse strains. Mice were inoculated cutaneously with  $5 \times 10^4$  PFU of HSV-1 and the spinal ganglia were removed at the times indicated. Each point represents the mean  $\pm$  SEM of virus titers from 5 to 10 individual animals.

neural infection with HSV-1. This strongly suggests that *Cmv-1* is not involved in the restriction of HSV-1 infection, with the caveat that complementation between unrecognized genes cannot be excluded as the cause of CXBG/By resistance.

Two strategies will be valuable for the functional analysis of the *Cmv-1* locus. First, and most important, is the construction of a bilineal congenic strain in which the C57BL/6 allele *Cmv-1<sup>l</sup>* has been transferred to the BALB/c background. Second, localization of the gene may be of value. Its apparent association with chromosome 6 will be strengthened by determining the SDPs of splenic MCMV replication in the AXB and BXD RI sets, which are derived from A/J and C57BL/6J progenitors, and C57BL/6J and DBA/2J progenitors, respectively. Both of these approaches are underway in our laboratory. In addition, determination of recombination frequencies in hybrid mice may be used to estimate the distance between *Cmv-1* and genes of known location such as *Prp* and *Ly-2* (32, 39).

In conclusion, this study has identified a novel non-H-2 gene, designated *Cmv-1*, which regulates MCMV replication in an organ-restricted manner and contributes to host resistance to lethal MCMV infection.

### Summary

The genetic basis of the control of acute splenic MCMV infection was studied after intraperitoneal inoculation of the virus. Classical Mendelian analyses using C57BL/6 (resistant) and BALB/c (susceptible) parental strains disclosed an autosomal dominant non-H-2 gene that regulates splenic virus replication. The probable location of this gene, to which we have assigned the symbol *Cmv-1*, is on chromosome 6 as defined by the strain distribution pattern of splenic MCMV replication in CXB recombinant inbred mice.

Although there is a similar hierarchy of resistance to MCMV and HSV-1 with respect to the C57BL and BALB genetic backgrounds, the strain distribution pattern of HSV-1 replication in recombinant inbred mice suggests that *Cmv-1* is not involved in restricting the spread of this virus.

This is the first clear identification of a non-H-2 gene regulating the magnitude of MCMV infection. Elucidation of the function of this gene may be a fundamental step towards understanding the control of systemic CMV infection.

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