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Pathogenesis of Acute Murine Cytomegalovirus Infection in Resistant and Susceptible Strains of Mice

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We have characterized the progress of acute murine cytomegalovirus (MCMV) infection in the spleen, liver, and salivary gland of susceptible (BALB/c) and resistant (C3H) strains of mice after intraperitoneal inoculation. Viral replication was analyzed by virus titration, infectious-center assays, and in situ cytohybridization with cloned subgenomic fragments of the MCMV genome. The most striking differences between strains were observed in the spleen. At 24 h postinfection (p.i.), both strains had a similar number of infected spleen cells. At 48 h p.i., BALB/c mice showed marked dissemination of the splenic infection which continued until 96 h p.i. In contrast, the number of infected C3H spleen cells did not increase from the 24-h level but declined later on. This early block in dissemination of MCMV infection in C3H mouse spleens was not a result of the $H\text{-}2^k$ haplotype, as BALB.K ($H\text{-}2^k$) mice, which show an intermediate level of resistance to MCMV infection, exhibited dissemination of the infection between 24 and 48 h p.i., albeit at a reduced level. However, between 72 and 96 h p.i., we observed a decline in the number of infected spleen cells in BALB.K mice similar to that observed in C3H mice. We also demonstrated by Southern blot analysis of DNA from the infected spleen cells that the termini of the MCMV genome fuse after in vivo infection.

In the last 20 years, cytomegalovirus has been recognized as a serious pathogen in humans. The genetic background and immunological status of the host both appear to be critical factors in determining the outcome of the infection. Past studies have focused primarily on describing the pathogenesis and immunology of the infection. However, it is clear that an understanding of the multiple interactions of this virus with its host requires molecular studies. Although recent advances in molecular biology have made it possible to study human cytomegalovirus replication and gene expression in vitro, in vivo studies have been hindered by the species specificity of the virus.

The infection of mice with murine cytomegalovirus (MCMV) presents a useful model of human cytomegalovirus pathogenesis in acute infection, establishment of latency, and reactivation after immunosuppression (for reviews, see references 11, 12, and 20). In addition, the mouse model is especially attractive because inbred strains differ markedly in their resistance to the lethal effects of MCMV. Although the underlying mechanisms of resistance are not fully understood, they are probably manifested early in the infection, because susceptible mice usually die 3 to 8 days after inoculation with the virus. Studies on the genetics of resistance indicate an association with the *H-2* haplotype, the ability to induce high levels of interferon, and natural killer (NK) cell activity after infection (1, 4, 5, 7, 8).

In this paper, we examine the molecular events which occur after intraperitoneal (i.p.) inoculation of the following three strains of mice: the susceptible strain BALB/c ($H\text{-}2^d$), the resistant strain C3H ($H\text{-}2^k$), and the congenic strain BALB.K ($H\text{-}2^k$), which shows an intermediate level of resistance. We focused primarily on the spleen and liver, which are major sites of MCMV replication after i.p. inoculation, and on the salivary gland, a site of persistent MCMV infection.

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MATERIALS AND METHODS

Virus and mice. The Smith strain of MCMV was provided by Michael Oldstone. All virus used in these experiments was prepared as a 10% homogenate of BALB/c salivary glands in Dulbecco modified Eagle medium (Irvine Scientific, Santa Ana, Calif.) containing 10% calf serum and 10% dimethyl sulfoxide. Specific-pathogen-free BALB/c and C3H/HeN mice were obtained from Simonsen Laboratories. C3H/St mice were obtained from West Seneca Labs, West Seneca, N.Y. No differences were observed in the early kinetics or extent of infection between C3H/HeN and C3H/St mice. BALB.K mice were provided by Richard Dutton. All mice were infected i.p. at 37 to 38 days of age with 10^5 PFU of salivary gland-passaged MCMV.

Preparation of PEC. Unstimulated peritoneal exudate cells (PEC) were prepared by lavage of the peritoneal cavity with 5 ml of RPMI 1640 plus 15% fetal calf serum. The lavage fluid was stored at −70°C with 1% dimethyl sulfoxide for later plaque assays.

Plaque assays. Organ homogenates (10% wt/vol) were prepared in Dulbecco modified Eagle medium containing 10% calf serum and 10% dimethyl sulfoxide and stored at −70°C. Dilutions of the homogenates were adsorbed in 0.3 ml of Dulbecco modified Eagle medium plus 10% calf serum on mouse embryo cells (18) in 6-well dishes. After a 2-h adsorption period, the inoculum was removed and replaced with 2 ml of Dulbecco modified Eagle medium containing 10% calf serum and 0.2% agarose. Plaques were counted 4 days later.

Infectious-center assays. Cells were washed twice in RPMI 1640 plus 15% fetal calf serum, cocultivated with mouse embryo cells overnight, and overlaid as described above for plaque assays. Plaques were counted 6 to 7 days later.

In situ cytohybridization. Cell suspensions were deposited onto pretreated slides with a cytocentrifuge, or 10-μm frozen sections were cut with a cryostat and hybridized as described by Haase et al. (9). In some experiments, RNase

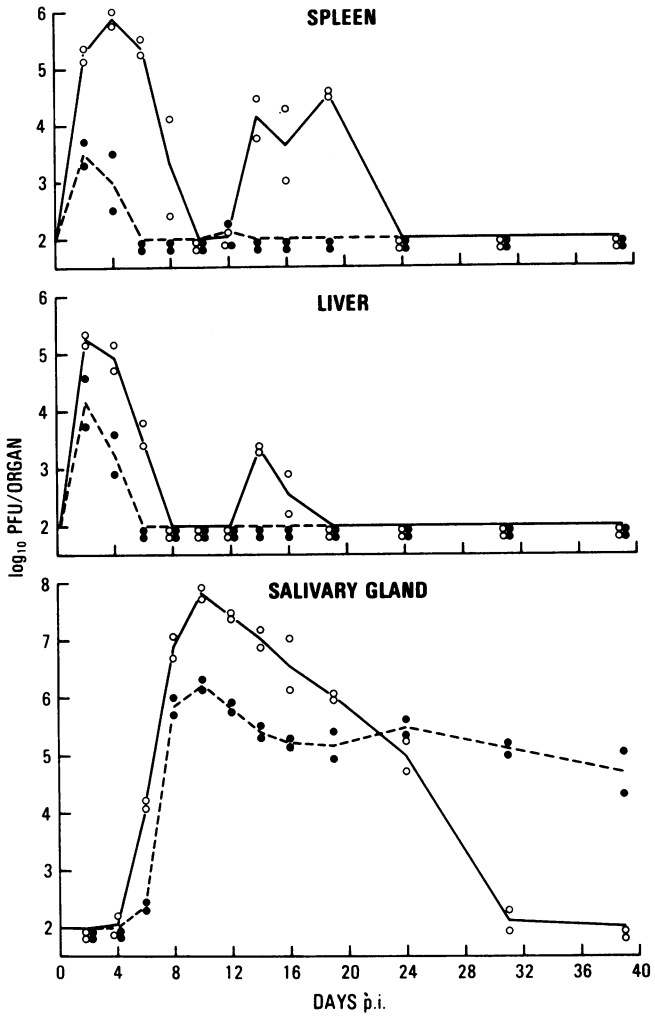


FIG. 1. Virus titers in spleen, liver, and salivary gland homogenates from BALB/c (○) and C3H (●) mice. Mice were infected at 37 to 38 days of age with 10^5 PFU of MCMV i.p. Each point represents an individual animal. The limit of sensitivity of the assay was 10^2 PFU per organ.

treatment was eliminated from the protocol for detecting DNA, allowing the detection of both RNA and DNA. Cloned MCMV *Eco*RI fragments E, P, and V (18), gel-purified from the cloning vehicle pACYC184 and labeled with [^{3}H]deoxynucleoside triphosphates to a specific activity of 1×10^8 to 2×10^8 dpm per μg by nick translation as previously described (25), were used as hybridization probes either individually or as an equimolar mixture. Negative controls were performed with pACYC184 as the probe.

DNA isolation and Southern blot analysis. To obtain spleen DNA, homogenates were incubated at 37°C for 1 h with 1% sodium dodecyl sulfate and 500 μg of pronase per ml in 0.1 M NaCl–10 mM Tris hydrochloride–10 mM EDTA (pH 8.0). The lysate was extracted with phenol-chloroform-isoamyl alcohol (50:48:2) and ethanol precipitated at −20°C. DNA was cleaved with the restriction endonuclease *Eco*RI in the buffer recommended by the supplier (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Agarose gel electrophoresis, transfer of DNA to nitrocellulose paper, preparation of ^{32}P-labeled DNA by nick translation, hybridization and washing, and autoradiography were performed as described by Tamashiro et al. (25).

RESULTS

Comparison of acute infection in susceptible and resistant mice. As described above, inbred strains of mice differ in their susceptibility to the lethal effects of MCMV. In the following series of experiments, we compared acute infection in a resistant strain, C3H, with that in a susceptible strain, BALB/c. Mice were infected as described in Materials and Methods. Two mice of each strain were sacrificed at 2, 4, 6, 8, 10, 12, 14, 16, 19, 24, 31, and 39 days postinfection (p.i.). Livers, spleens, and salivary glands were removed and processed for analysis. At the dose of virus used (10^5 PFU), none of the resistant or susceptible mice were killed by the infection. BALB/c mice exhibited ruffled fur, hunching, and weight loss at 4 to 9 days p.i. No symptoms were observed in the C3H mice.

A comparison of the virus titers for the various organs of the resistant and susceptible strains of mice is shown in Fig. 1. Although the kinetics of the infection in the resistant C3H mice were similar to those observed in the susceptible BALB/c mice, there were several notable differences. At all times in the early stages of the infection, the titer of the virus in the spleen, liver, and salivary gland was higher in the susceptible strain than in the resistant strain. In the spleens of the BALB/c mice, the virus titer reached values 100 to 1,000-fold higher than it did in the spleens of the C3H mice. In contrast, the titer of the virus in the liver and salivary gland during days 6 to 20 p.i. was only 10- to 50-fold higher in the BALB/c mice. We also observed a second peak of replication in the spleens and livers of the BALB/c mice, consistent with the results of Booss (2). One striking difference between the strains, although it occurred too late in the infection to account for differences in lethality, was the disappearance of detectable MCMV from the salivary glands of the BALB/c mice compared with the persistent salivary gland infection observed in the C3H mice.

To investigate the pathogenesis of the infection at the molecular level, we prepared frozen sections of the spleens, livers, and salivary glands obtained from the mice described above for in situ cytohybridization. We used as the hybridization probe a ^{3}H-labeled equimolar mix of three MCMV *Eco*RI fragments, E, V, and P, which represent major regions of immediate early, early, and late transcription, respectively, during the permissive infection of mouse embryo cells (14, 16). Hybridizations were initially performed with methods which would allow us to detect MCMV RNA and DNA simultaneously (Fig. 2). Control hybridizations with labeled pACYC184 as the probe were all negative (data not shown).

In situ cytohybridization of BALB/c and C3H spleens confirmed the marked difference in splenic infection between strains (Fig. 2a to h). At 2 days p.i., the first time point examined, the entire red pulp in the BALB/c mouse spleens was positive; the perifollicular areas hybridized most strongly. Little hybridization to follicular areas was seen throughout the infection in either strain. At 2 days p.i., the virus titer in the C3H spleens was maximal, yet only 10 to 50 foci per section were observed. At 4 days p.i., the infection reached its peak in the BALB/c spleens and had begun to decline in the C3H spleens, which showed only 5 to 10 foci per section. At 6 to 19 days p.i., few foci of replication were observed in the BALB/c spleens, but the necrotic portions of the red pulp were still weakly positive. No hybridization to C3H spleen sections was observed at these later time points.

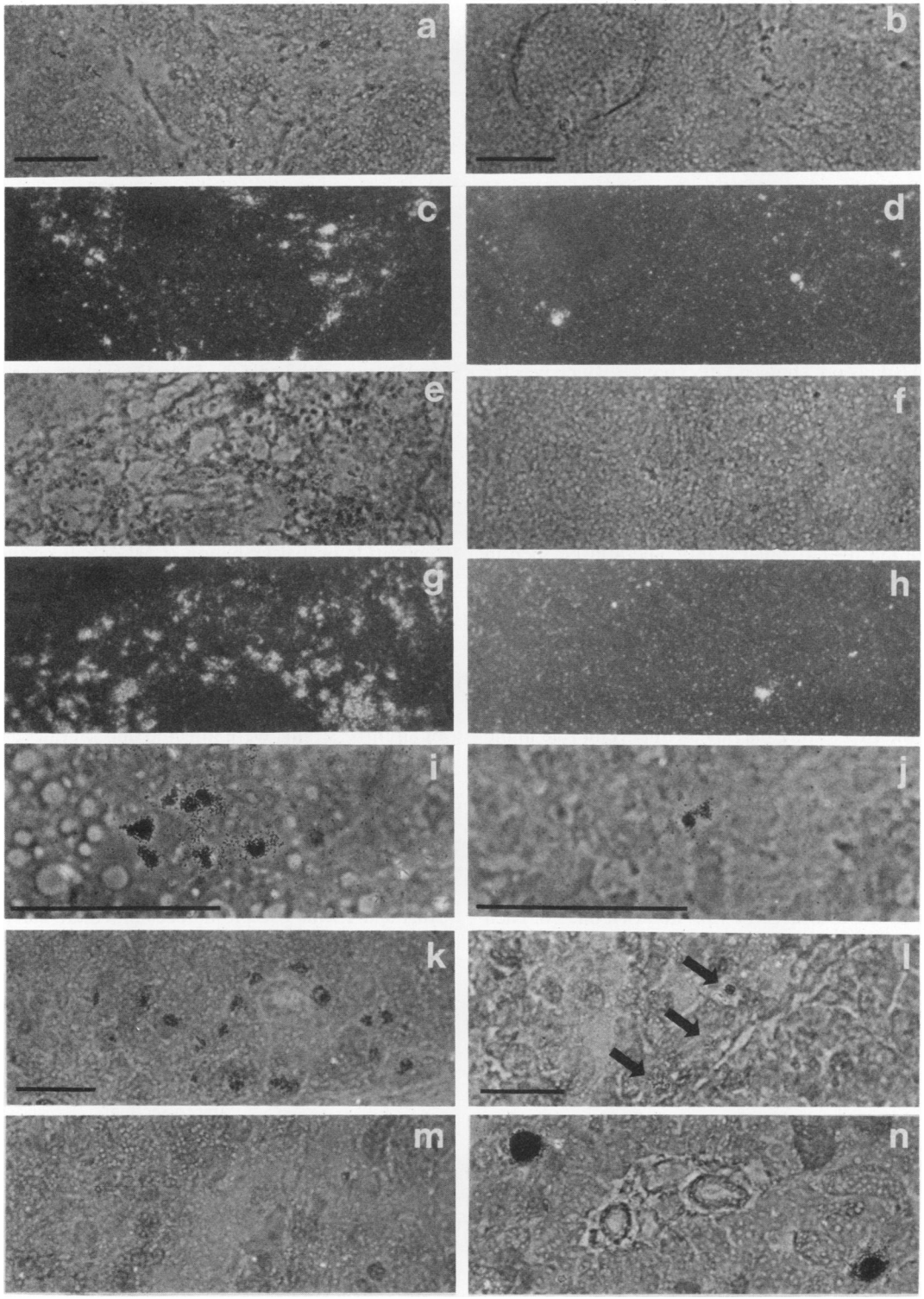


FIG. 2. Photomicrographs of organ sections from BALB/c (left side) and C3H (right side) mice hybridized for DNA plus RNA with *Eco*RI fragments E, P, and V as the probe. Panels: a to h, bright-field (a,b,e,f) and dark-field (c,d,g,h) photomicrographs of spleen sections at days 2 (a,b,c,d) and 4 (e,f,g,h) p.i.; i and j, bright-field photomicrographs of liver sections at day 4 p.i.; k to n, bright-field photomicrographs of salivary gland sections at days 10 (k,l) and 39 (m,n) p.i. Arrows indicate virus-positive cells. The autoradiographic exposure time was 9 (a to h) or 19 (i to n) days. Bars = 100 μm. Photomicrographs a to h and k to n were taken at the same magnification.

TABLE 1. MCMV replication in PECs of BALB/c and C3H mice

Time (h p.i.)	MCMV titer (PFU/ml) in peritoneal lavage fluid		No. of positive PECs/10^5 PECs[a]	
	BALB/c	C3H	BALB/c	C3H
12	<10	<10	<3	<3
24	<10	<10	5	8
48	<10	<10	50	7
72	100	<10	40	<3
96	200	<10	50	<3

[a] A cell was considered positive for MCMV nucleic acid if it contained >10 grains over background after in situ cytohybridization and autoradiography.

In situ cytohyridization of liver sections showed less dramatic differences between strains (Fig. 2i and j). Hybridization was observed in BALB/c liver sections at days 2 to 6 p.i. and in C3H liver sections at days 2 and 4 p.i. BALB/c livers at 4 and 6 days p.i. showed fatty change, which was far less severe at 8 days p.i.

The patterns of hybridization in salivary gland sections (Fig. 2k to n) were consistent with the results of the plaque assays. At 10 days p.i., the peak of salivary gland infection for both strains, replication was confined to individual acini, and the number of positive foci was significantly higher in the salivary glands of the BALB/c mice. However, at day 39 p.i., hybridization to individual acini could still be seen in C3H salivary glands, but not in BALB/c salivary glands.

Early block to spread of infection in C3H spleens. The difference in the MCMV infection for the two strains, particularly in the spleen, was evident by 2 days p.i. Among the various possibilities that could account for the low level of viral replication in the spleens of the resistant mice are the following. First, it was possible that since the mice were infected i.p., the PECs were playing an important role in the initial dissemination of the virus to the spleen and liver. Second, the spleen cells of the resistant mice could be refractory to the early stages of the infection. Finally, it was possible that in the spleens of the resistant mice the initial stages of the infection could proceed, but viral spread was inhibited. To address these possibilities, we looked at earlier time points in the infection. BALB/c and C3H mice were infected with 10^5 PFU of MCMV i.p., and two mice of each strain were sacrificed at 12, 24, 48, 72, and 96 h. PECs were isolated, washed, and cytocentrifuged onto pretreated slides for in situ cytohybridization. The titer of the peritoneal lavage fluid was determined, and spleens were removed and frozen for sectioning.

We measured the titer of MCMV in the peritoneal lavage fluid and determined by in situ cytohybridization the number of PECs that contained MCMV nucleic acid (Table 1). Free virus could not be detected in the peritoneal lavage fluid of the C3H mice at any of the time points examined. In the BALB/c mice, there was also no detectable MCMV at the early time points, but virus was detected at later times (72 and 96 h p.i.). As measured by in situ cytohybridization, the PECs of both strains were negative for MCMV RNA and DNA at 12 h p.i. but showed a low frequency of positive cells at 24 h p.i. At 48 h, the number of positive cells in the C3H mice did not change, although the number of positive cells in the BALB/c mice increased 10-fold. For the BALB/c mice, the number of positive cells remained at this increased level at the late time points, while the number of positive cells in the C3H mice declined to undetectable levels.

For in situ cytohybridization of the spleen sections, three different hybridization conditions were used. In the first two, sections were hybridized with an equimolar mixture of MCMV *Eco*RI fragments E, V, and P under conditions in which either DNA alone or RNA plus DNA could be detected. In the third, the sections were hybridized with MCMV *Eco*RI fragment E (the region of major immediate-early transcription) under conditions in which only RNA could be detected. For all three conditions, the results were similar (Table 2). No positive cells were detected at 12 h p.i. in either the C3H or BALB/c spleen sections. At 24 h p.i., an equivalent number of positive cells was seen for each strain. However, at 48 h p.i., a striking difference was observed between strains. The C3H spleens showed no spread of the infection, whereas extensive spread of the infection occurred in the BALB/c spleens. This difference was also evident at 72 and 96 h p.i.

The results of the experiments described above suggested that the block to MCMV infection in the resistant C3H spleens probably occurred after or during the first round of replication in the spleen. The observation that, at 48 h p.i., the number of positive cells in the C3H spleens was the same under all three hydridization conditions also indicated that after the initial round of replication during the first 24 h, the infection was subsequently blocked before significant viral transcription occurred. Furthermore, there was no evidence that the markedly higher MCMV titers seen in the susceptible BALB/c spleens at 48 h p.i. were caused by preferential replication or dissemination of MCMV from the PECs.

Effect of *H-2*^{k} haplotype on early block to MCMV replication in the spleen. To determine whether the lack of viral spread in the spleens of the C3H mice (*H-2*^{k}) compared with that in BALB/c mice (*H-2*^{d}) was due to differences in the *H-2* or background loci, we repeated the above experiment with BALB.K mice (*H-2*^{k}), which show an intermediate level of susceptibility to the lethal effects of MCMV infection. Mice of all three strains were infected and sacrificed as described above. Portions of each of the spleens were frozen for sectioning and processed for infectious-center assays.

Spleen sections from the three strains of mice were hybridized under the conditions described above to detect MCMV RNA plus DNA, MCMV DNA alone, or immediate-early RNA. The results were similar for the three hybridization conditions and are shown for the case in which MCMV fragment E was used to detect immediate-early RNA. Representative photomicrographs of the spleen sections are shown in Fig. 3, and the data are summarized in Fig. 4. At 12 and 24 h p.i., the number of positive cells was similar for all three strains of mice. As in the experiments described

TABLE 2. In situ cytohybridization to BALB/c and C3H mouse spleen sections

Time (h p.i.)	No. of positive cells/spleen section[a]					
	BALB/c			C3H		
	RNA + DNA	DNA	IE RNA[b]	RNA + DNA	DNA	IE RNA
12	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
24	14, 18	14, 35	13, 23	32, 9	41, 16	9, 17
48	133, 266	148, 279	261, 327	15, 7	22, 17	15, 16
72	>1,000	>1,000	>1,000	7, 22	8, 30	4, 27
96	>1,000	>1,000	>1,000	2, 0	2, 1	2, 2

[a] A cell was considered positive for MCMV nucleic acid if it contained >10 grains above background after in situ cytohybridization and autoradiography. Data are shown for sections from two mice of each strain for each time.

[b] Hybridization was performed for RNA only by using MCMV *Eco*RI fragment E (the region of major immediate-early [IE] transcription) as probe.

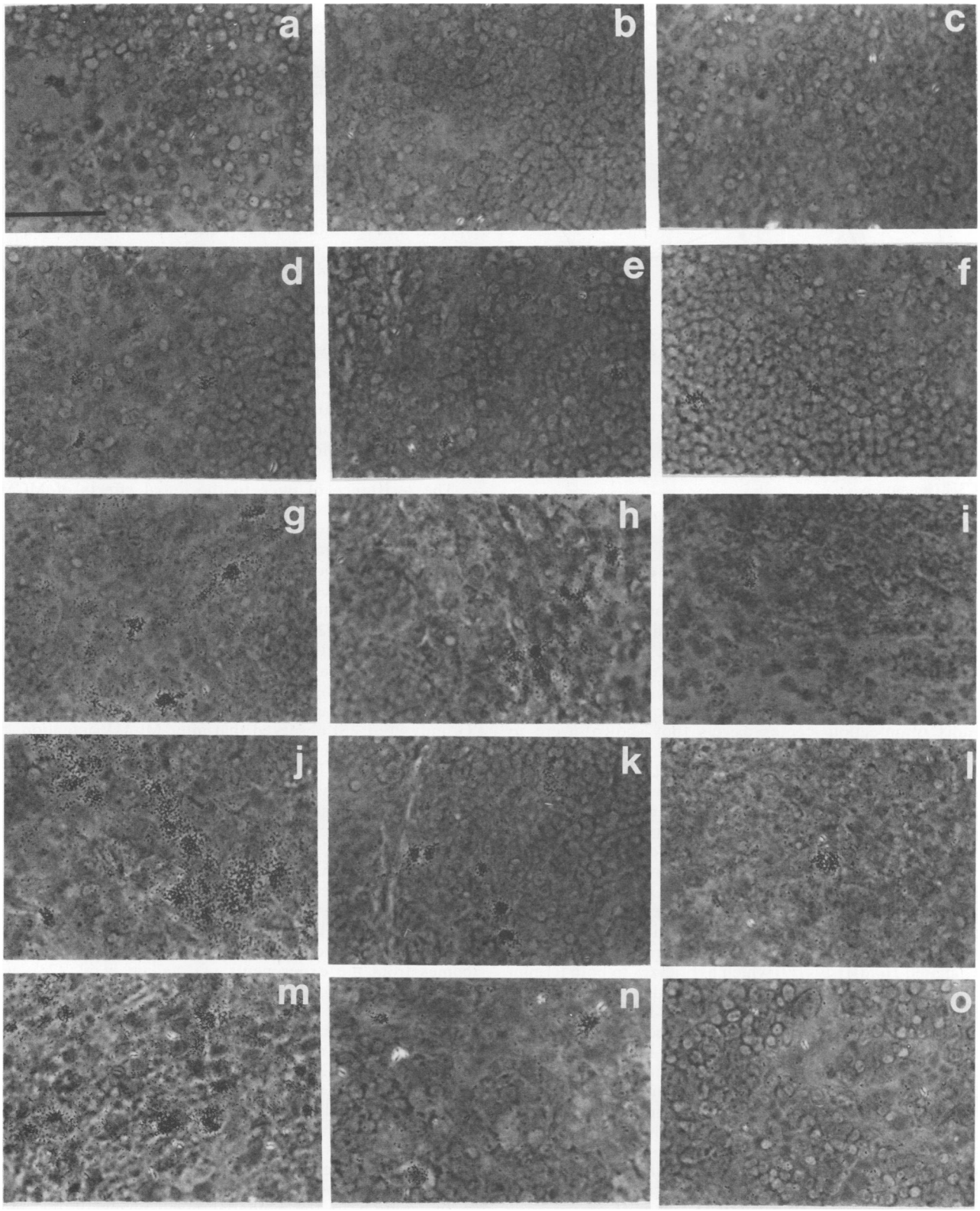


FIG. 3. Photomicrographs of spleen sections from BALB/c (left panels), BALB.K (center panels), and C3H (right panels) mice sacrificed at 12 (a,b,c), 24 (d,e,f), 48 (g,h,i), 72 (j,k,l), and 96 (m,n,o) h p.i., hybridized for RNA with *Eco*RI fragment E as the probe, with an exposure time of 8 days. Bar = 50 μm.

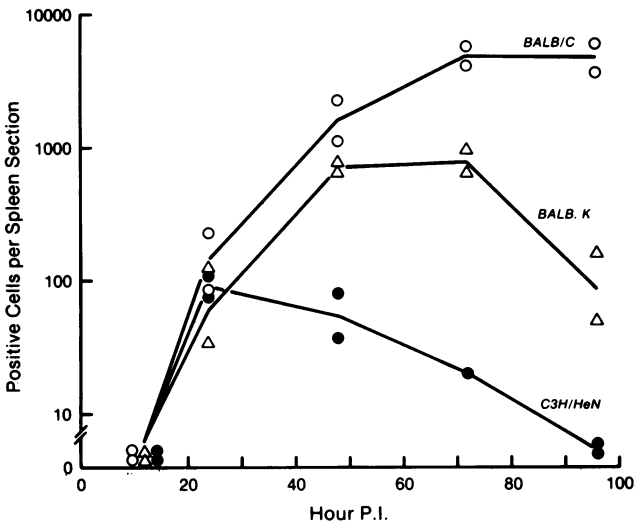


FIG. 4. Number of positive cells (>10 grains above background) per spleen section as determined by in situ cytohybridization for RNA with *Eco*RI fragment E as the probe, with an exposure time of 8 days. Each point represents the average number of positive cells in two spleen sections from an individual BALB/c (○), BALB.K (△), or C3H (●) mouse.

above, the number of positive cells in the C3H spleens decreased at later times, in contrast to the marked increase in the number of positive cells observed in the BALB/c spleens. Between 24 and 72 h p.i., the BALB.K mice showed an increase in the number of positive cells, but the increase was less than that for the BALB/c mice. Between 72 and 96 h p.i., however, there was a marked decrease in the number of infected cells in the BALB.K spleens, whereas there was no significant difference for the BALB/c spleens. Results of infectious-center assays (not shown) were in agreement with the results of the in situ cytohybridization.

Characterization of viral DNA in BALB/c spleens after acute infection. In a previous study, we presented evidence that MCMV DNA replication in mouse embryo cells proceeds via circular or concatenated intermediates or both (17). To determine whether the MCMV termini fuse during in vivo infection, DNA was extracted from infected BALB/c spleens at 24, 48, 72, and 96 h p.i. The DNA was digested with *Eco*RI and subjectd to agarose gel electrophoresis. The separated fragments were transferred to nitrocellulose filters and hybridized with a ^{32}P-labeled cloned fragment of MCMV (designated *Hin*dIII-E′-1.9) which hybridizes to both termini of the genome (18) (Fig. 5). At 24 h p.i., the amount of viral DNA present in the spleen was below the limits of detection by Southern blot analysis, probably because of the low percentage of cells infected. However, at 48 h p.i. and at the later time points, we detected bands which had the expected mobility of both the fused termini (5 kilobase pairs) and the free termini (3.6 kilobase pairs for *Eco*RI fragment X and 1.4 kilobase pairs for *Eco*RI fragment c). These data indicate that end-to-end linkage of the termini of the MCMV genome occurs during both in vitro and in vivo infection.

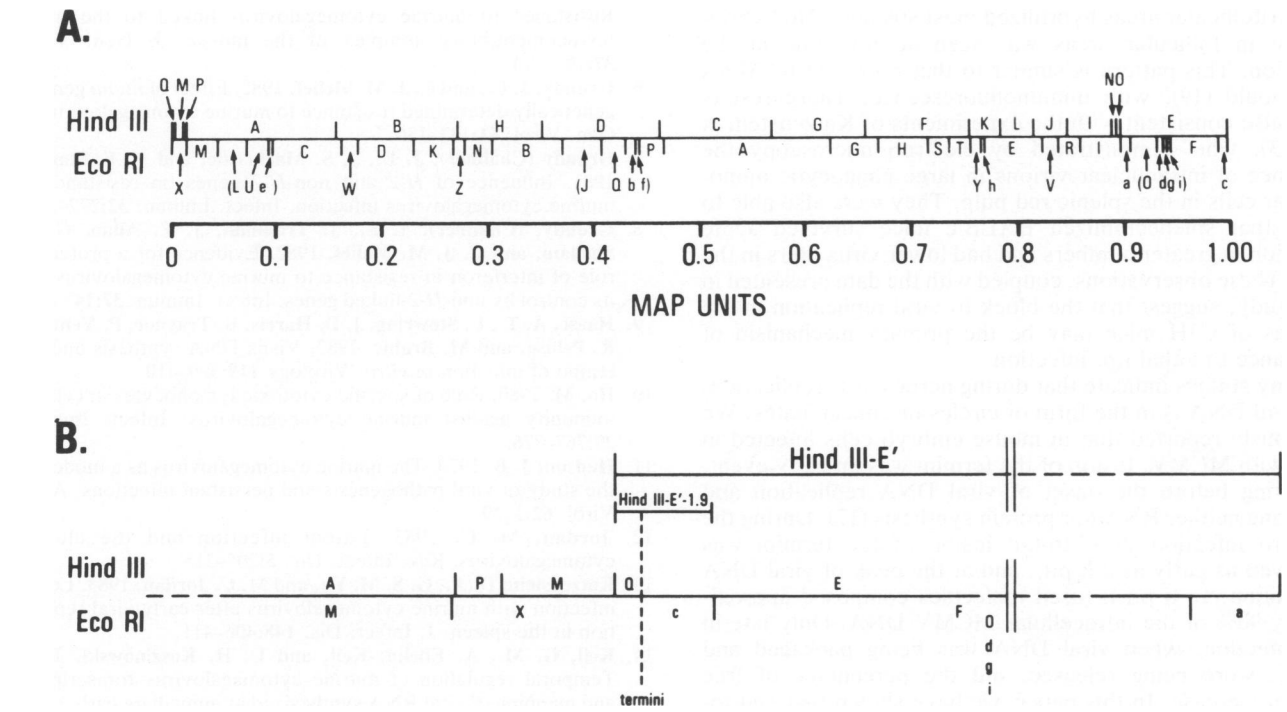
DISCUSSION

We followed the progress of the acute phase of MCMV infection in BALB/c, BALB.K, and C3H mice after i.p. inoculation at 37 to 38 days of age. The major difference observed between strains in the acute infection was the 1,000-fold-higher virus titer in the spleens of the BALB/c mice than in those of the C3H mice. One possible explanation for this difference in titers is that the virus first replicated in PECs and that this early differential replication in the PECs was responsible for the differences seen in the spleen. Two observations argued against this hypothesis. First, both in situ cytohybridization and infectious-center assays showed similar numbers of infected cells in the C3H and BALB/c spleens at 24 h p.i., suggesting that there was little difference between strains in the primary splenic infection. Second, we found that the kinetics of viral replication in the PECs of the BALB/c mice paralleled that in the spleen. These results suggest that similar mechanisms of resistance may be operating at both sites.

Dissemination of the infection between 24 and 48 h p.i. was not observed in the spleens of the C3H mice. This block in dissemination was not a result of the *H-2*^{k} haplotype, since viral spread in the spleens of the BALB.K mice was observed but was less than that observed in the BALB/c mouse spleens. At 48 h p.i., we observed the same number of positive cells in the C3H spleens whether in situ cytohybridization was done to detect MCMV DNA alone, DNA plus RNA, or immediate-early RNA, indicating that the block occurred before the onset of significant viral transcription.

The resistance of mice to the lethal effects of MCMV infection is probably the result of a complex interaction of multiple factors involving both *H-2*- and non-*H-2*-associated defense mechanisms (1, 4, 5, 7, 8). It has been observed that after infection with MCMV, there is a stimulation of NK cell activity at 3 to 5 days p.i. (1, 22), whereas the virus-specific *H-2* restricted cytotoxic T lymphocyte response does not peak until 6 to 20 days p.i. (10, 21). The finding that resistance to infection in the C3H spleens was manifested so early suggests that it is probably mediated by a defense mechanism such as interferon production or response, NK cell activity, or macrophage antiviral activity. In this regard, Grundy et al. (8) observed that after MCMV infection, the serum interferon levels in C3H mice were 5- to 10-fold higher than those in the BALB/c and BALB.K mice. In addition, the resistance of various strains of mice to the early lethal effect of MCMV infection correlates well with the magnitude of the NK cell response (1, 5). The observation that the stimulation of NK cell activity in BALB.K mice is intermediate to that in BALB/c and C3H mice (1) is consistent with the results of our experiments, in which early viral spread in the spleens of the BALB.K mice was less than that in the spleens of the BALB/c mice. Other support for the involvement of NK cell activity in this early resistance to infection comes from the experiments of Shellam et al. (23), who reported that NK cell-deficient homozygous beige mice were more susceptible to MCMV infection than were their heterozygous littermates. Bukowski et al. (4) also showed that treatment of mice with antibody to asialo GM1, which selectively depletes NK cell activity in vivo, results in an MCMV infection which is more severe and of longer duration. The NK cell depletion was most effective early in the infection and resulted in a 500- to 1,000-fold increase in spleen virus titers by day 3 p.i. In another study, Bukowski et al. (3) protected suckling mice from lethal infection by adoptive transfer of NK cells from adult mice.

In addition to the potential mechanisms of resistance described above, there are also *H-2*-associated differences, and at least some of these are probably manifested by a T lymphocyte-mediated response. The rapid decline in the number of infected cells in BALB.K spleens between 72 and



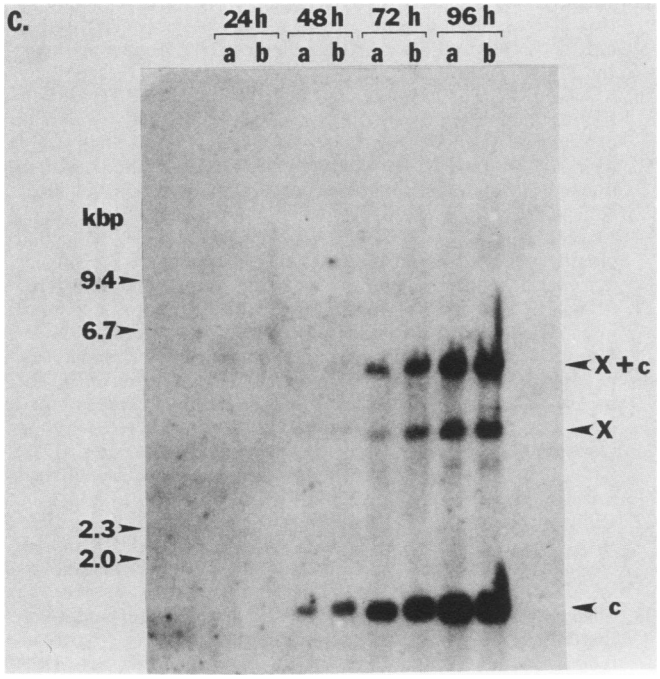


FIG. 5. Southern blot analysis of *Eco*RI-digested BALB/c spleen cell DNA hybridized with ^{32}P-labeled MCMV fragment *Hin*dIII-E'-1.9. (A) Restriction endonuclease map of the MCMV genome (18). (B) Expanded representation of the fusion fragment *Hin*dIII-E' and the *Eco*RI subfragment *Hin*dIII-E'-1.9. (C) DNA (5 μg) from each of two mice (lanes a and b, respectively) sacrificed at the given times p.i. was cleaved with *Eco*RI, electrophoresed through 0.8% agarose, blotted onto nitrocellulose, and hybridized with ^{32}P-labeled *Hin*dIII-E'-1.9. The fusion fragment X + c, and the authentic termini, X and c, are indicated. kbp, Kilobase pairs.

96 h p.i. may have been due to the cytotoxic T cell response. In support of this hypothesis, Grundy and Melief (6) found that the *nu/nu* genotype was more effective in increasing susceptibility in the BALB/c (*H-2*^{d}) background than in the resistant CBA (*H-2*^{k}) background. Of interest was their observation that the homozygous *nu/nu* mice died later than their *nu/+* littermates. This may be because the augmentation of NK cell activity induced by MCMV is greater in athymic *nu/nu* mice than in their *nu/+* littermates (1). NK or natural cytotoxic cell activity may also play a role in *H-2* associated resistance. Loci affecting NK and natural cytotoxic cell activity have been mapped to or near the *D* end of the *H-2* complex (15, 24). However, it has not yet been shown whether these loci are involved in the lysis of virus-infected targets. In summary, these results suggest an important role for NK cells, and possibly interferon, in early resistance to MCMV infection, with the *H-2*-associated T cell response playing a role at later times.

With in situ cytohybridization, we found that the primary site of viral replication in the spleen involved the red pulp;

the perifollicular areas hybridized most strongly. No hybridization in follicular areas was seen at any time in the infection. This pattern is similar to that observed by Mims and Gould (19), with immunofluorescence. These results were also consistent with the experiments of Katzenstein et al. (13), who demonstrated by electron microscopy the presence of intranuclear virions in large phagocytic mononuclear cells in the splenic red pulp. They were also able to show that splenectomized BALB/c mice survived acute infection in greater numbers and had lower virus titers in the liver. These observations, coupled with the data presented in this study, suggest that the block to viral replication in the spleens of C3H mice may be the primary mechanism of resistance to lethal i.p. infection.

Many studies indicate that during herpesvirus replication, the viral DNA is in the form of circles or concatenates. We previously reported that in mouse embryo cells infected in vitro with MCMV, fusion of the termini was an early event, occurring before the onset of viral DNA replication and requiring neither RNA nor protein synthesis (17). During the in vitro infection, head-to-tail fusion of the termini was observed as early as 2 h p.i., and at the peak of viral DNA replication (16 h p.i.), fused molecules composed approximately 90% of the intracellular MCMV DNA. Only late in the infection, when viral DNA was being packaged and virions were being released, did the percentage of free termini increase. In this paper, we have shown that end-to-end linkage of the termini can also be detected in the infected spleen cells of mice inoculated i.p. with MCMV. However, because of the low percentage of infected cells, we could not detect the presence of the fused termini until 48 h p.i., after the first round of replication. Since at this time both fused and free termini were present, we could not determine whether the fused molecules represented true replicative intermediates.

The molecular studies presented in this paper provide a basis for studying the underlying mechanisms of viral replication during the acute infection of resistant and susceptible strains of mice. In addition, they provide a framework for analyzing the subsequent molecular events associated with persistent and latent MCMV infections.

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LITERATURE CITED

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