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Detection of Murine Cytomegalovirus DNA in Circulating Leukocytes Harvested during Acute Infection of Mice

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We used virus assay and in situ hybridization with a cloned fragment of the murine cytomegalovirus (MCMV) genome to study MCMV infection of circulating leukocytes harvested from 3-week-old BALB/c, C57BL/6, and C3H mice infected with MCMV intraperitoneally. Infectious virus or MCMV DNA was detected in leukocytes on days 1 through 21 of infection in BALB/c mice and on days 3 through 7 in C57BL/6 mice. On days 5 and 7, MCMV DNA or infectious virus was detected in the leukocytes of 17 (94%) of 18 BALB/c mice and 10 (59%) of 17 C57BL/6 mice. In both strains infection peaked on days 5 and 7, when as many as 0.01 to 0.1% of the circulating leukocytes contained MCMV DNA. In C3H mice, however, infectious virus was rarely recovered from leukocyte fractions and MCMV DNA was detected in the circulating leukocytes of only one animal. Circulating leukocytes may have an important role in the dissemination of CMV infections in susceptible hosts.

Human cytomegalovirus (HCMV) can frequently be recovered from the peripheral blood of patients with acute HCMV infections (12). In studies of patients with the HCMV mononucleosis syndrome, Rinaldo et al. isolated HCMV from the neutrophil and mononuclear cell-enriched fractions as late as 93 days after the onset of clinical symptoms (20). In immunosuppressed patients with acute HCMV infections such as those that occur after renal or marrow transplantation, HCMV can be isolated from the neutrophil-enriched fraction (8, 9, 23).

Subsequent molecular studies have confirmed that HCMV infects the circulating leukocytes of humans. Martin et al. detected HCMV DNA in blood leukocytes during fatal disseminated HCMV infection and calculated that these cells contained approximately 0.5 to 1.0 genome equivalent per leukocyte (15). More recently, Schrier et al. used in situ hybridization techniques to identify HCMV RNA in the lymphocytes of subjects who were seropositive for HCMV (21). Such observations suggest that circulating leukocytes infected with HCMV may play an important role in the pathogenesis of HCMV infections.

Murine cytomegalovirus (MCMV) infection of mice has been an excellent experimental model with which to study the interaction of CMVs and various tissues or cells, including leukocytes (6, 13, 17). Mice infected with MCMV develop a mononucleosislike syndrome with hematologic manifestations similar to those of HCMV disease (6). In previous experiments from this laboratory, we used in situ hybridization with a subgenomic fragment of the MCMV genome to identify MCMV DNA in bone marrow cells of immunocompetent mice undergoing sublethal infection (3). In the current experiments, we have used similar techniques to study MCMV infection of circulating leukocytes in susceptible (BALB/c and C57BL/6) and resistant (C3H) strains of mice. Our data suggest that MCMV-infected leukocytes serve to disseminate infectious virus in susceptible hosts.

MATERIALS AND METHODS

Virus and animals. The Smith strain of MCMV, obtained originally from Earl R. Kern, University of Utah, Salt Lake City, was prepared as a 10% (wt/vol) homogenate of salivary glands harvested from MCMV-infected Swiss Webster mice. Such pools regularly contained 10^7 PFU of MCMV when virus titers were measured on monolayers of mouse embryo fibroblasts (MEFs). BALB/c, C3H, and C57B/6 mice were purchased from Harlan Sprague Dawley, Inc., Indianapolis, Ind. Mice were 24 to 27 days old at the time of inoculation.

Virus assay. We assayed tissues and cell suspensions for MCMV by using confluent monolayers of MEFs grown in 24-well plates and an agarose overlay as described previously (2). Culture results were expressed as $\log_{10}$ PFU of MCMV per gram of tissue or $\log_{10}$ infectious foci per 10^6 cells.

In situ hybridization. Nucleic acid hybridization studies were performed by modifications of techniques described previously (3). The probe consisted of the MCMV DNA *Hin*dIII A fragment cloned into pACYC177 (courtesy of Ulrich Koszinowski, Institute of Animal Virus Diseases, Tübingen, Federal Republic of Germany) (7). This fragment does not code for immediate early MCMV RNAs (14).

In initial experiments, the whole plasmid containing the MCMV DNA *Hin*dIII A fragment was labeled by nick translation with [^{35}S]dCTP (Amersham Corp., Arlington Heights, Ill.) to specific activities of 4×10^8 to 6×10^8 cpm/μg of DNA. Controls for these experiments included (i) MCMV-infected and uninfected MEFs probed with the whole plasmid probe and (ii) MCMV-infected MEFs probed with ^{35}S-labeled pACYC177 cloning vector. The vector did not hybridize with MCMV-infected MEFs.

In subsequent experiments, the MCMV DNA *Hin*dIII A insert was gel purified and labeled either with [^{35}S]dCTP or with biotinylated dUTP (Enzo Biochem, Inc., New York, N.Y.) by nick translation methods. These probes were used to study leukocytes harvested during peak infection of BALB/c mice. Controls for these experiments included (i) uninfected and MCMV-infected MEFs and (ii) leukocytes harvested from BALB/c mice sham inoculated with normal salivary-gland homogenates. As an additional control, MCMV-infected MEFs were reacted with an HCMV probe consisting of the *Xba*I C fragment cloned in pACYC184 and labeled by nick translation with [^{35}S]dCTP. This probe did not hybridize with MCMV-infected cells.

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The sensitivity of ^{35}S-labeled MCMV DNA probes was determined by methods described by Haase et al. (11). MEFs were infected with MCMV at a multiplicity of infection of 0.1 and harvested sequentially postinfection. Aliquots of MCMV-infected cells were then studied by in situ hybridization and quantitative dot blot hybridization. The average number of autoradiographic grains per MCMV-infected cell was then compared with the number of MCMV genome copies per infected cell. Based on these methods, the sensitivity was determined to be 145 MCMV genome copies per cell at more than 10 grains above background.

Prior to hybridization with ^{35}S-labeled MCMV DNA probes, cell preparations were treated with 0.2 N HCl, proteinase K, and RNase and postfixed in 5% paraformaldehyde. The cells were incubated with 5 μl of probe mixture for 72 h at room temperature and then washed for 6 h with 50% formamide–0.6 M NaCl–1 mM EDTA–10 mM phosphate buffer (pH 6.0). Slides were coated with NTB 2 photoemulsion (Eastman Kodak Co., Rochester, N.Y.) and exposed for 48 to 72 h at 20°C. After development, the slides were counterstained with Hemacolor (Harleco, Gibbstown, N.J.). Cells containing more than 10 autoradiographic grains above background were considered positive for MCMV DNA.

For biotinylated probes, cells were pretreated with 0.2 N HCl and proteinase K and postfixed in 5% paraformaldehyde. The cells were then reacted with 5 μl of a hybridization mixture containing 5 ng of biotinylated MCMV DNA probe per μl, 50% formamide, 10% dextran sulfate, 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 250 μg of salmon sperm DNA per ml, denatured at 92°C for 3 min, and hybridized for 16 h at room temperature (20 to 22°C). Slides were then developed by using a Detek 1-hrp kit (Enzo Biochem), which has diaminobenzidine as the chromogen, and lightly counterstained with Harris hematoxylin. The presence of MCMV DNA was indicated by a brown precipitate. Results were expressed in terms of the number of virus-positive animals and the number of positive cells per 10^6 leukocytes.

Experimental protocol. Mice were inoculated intraperitoneally with 10^5 PFU of MCMV on day zero. At this inoculum, all BALB/c mice were infected, as determined by recovery of MCMV from spleen tissues. This inoculum produced 20% mortality in BALB/c mice, 30% mortality in C57BL/6 mice, and no mortality in C3H mice. Control mice received an equivalent dilution of salivary-gland homogenate prepared from uninfected Swiss Webster mice.

On days 0, 1, 3, 5, 7, 14, and 21 after inoculation, mice were killed, and specimens of spleen and whole blood via cardiac puncture were obtained for study. Spleen tissues were prepared as 10% (wt/vol) homogenates in minimal essential medium containing 10% fetal calf serum and antibiotics and frozen at −70°C until assayed. Whole blood was diluted with an equal volume of media (minimal essential medium containing 10% fetal calf serum and antibiotics), underlayered with Ficoll-Hypaque (Histopaque; Sigma Chemical Co., St. Louis, Mo.), and centrifuged at 400 × *g* for 30 min. Leukocytes were then collected from the mononuclear cell-enriched interface and diluted in 1.0 ml of minimal essential medium containing 10% fetal calf serum and antibiotics. This interface was erythrocyte free and contained between 10 and 50% neutrophils.

Approximately 10^5 leukocytes were collected onto microscope slides by using a cytocentrifuge (Cytospin 2; Shandon Southern Instruments, Sewickley, Pa.). For in situ hybridization studies, slides were pretreated with Denhardt medium (11). Cell preparations were fixed with ethanol-acetic acid (3:1) and 95% ethanol and stored at 4°C until studied. For virus assay, aliquots (0.1 ml per well) of cell suspensions were plated in duplicate onto fresh MEF monolayers.

TABLE 1. Recovery of MCMV and detection of MCMV DNA in circulating leukocytes harvested from BALB/c, C57BL/6, and C3H mice

Day	No. (%) of BALB/c mice[a] with:		No. (%) of C57BL/6 mice with:		No. (%) of C3H mice with:	
	Virus[b]	DNA[c]	Virus	DNA	Virus	DNA
1	0/4 (0)	1/4 (25)	0/9 (0)	0/9 (0)	1/5 (20)	0/5 (0)
3	3/9 (33)	2/9 (22)	3/9 (33)	0/9 (0)	0/5 (0)	1/5 (20)
5	9/9 (100)	7/9 (78)	6/9 (66)	2/9 (22)	1/5 (20)	0/5 (0)
7	8/9 (89)	6/9 (66)	4/8 (50)	2/8 (25)	0/5 (0)	0/5 (0)
14	2/9 (22)	3/9 (33)	0/6 (0)	0/6 (0)	0/5 (0)	0/5 (0)
21	1/9 (11)	3/9 (33)	0/3 (0)	0/3 (0)	0/5 (0)	0/5 (0)

[a] Number of positive animals/number studied.
[b] Number of animals positive by plaque assay.
[c] Number of animals positive by in situ hybridization with a whole-plasmid MCMV DNA probe labeled with [^{35}S]dCTP as described in Materials and Methods.

Statistical methods. Results from virus assays or hybridization studies were compared by using two-tailed *t* tests ($P \leq 0.05$ was considered significant).

RESULTS

Detection of infectious virus. In initial experiments, we used virus plaque assay to characterize infection of leukocytes and spleen tissues in the various strains of mice. MCMV was detected in the leukocyte fractions of 33, 100, and 89% of BALB/c mice on days 3, 5, and 7, respectively (nine mice were studied per day) (Table 1; Fig. 1). Infection peaked on day 5, when there was a mean of 417 infectious foci of MCMV per 10^6 leukocytes. Fewer C57BL/6 mice were infected, and less virus was recovered. MCMV was detected in 33, 67, and 50% of C57BL/6 mice on days 3, 5, and 7, respectively (eight or nine mice were studied per day). Peak infection was also observed on day 5, when there was a mean of 148 infectious foci of MCMV per 10^6 leukocytes. In contrast with these results, MCMV was recovered from only 1 of 15 C3H mice (6.6%) studied on days 3 through 7 of infection.

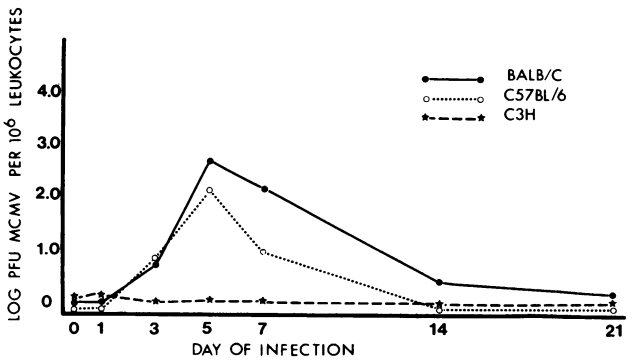

FIG. 1. Recovery of infectious MCMV from circulating leukocytes. Each point represents the mean of data from three to nine animals.

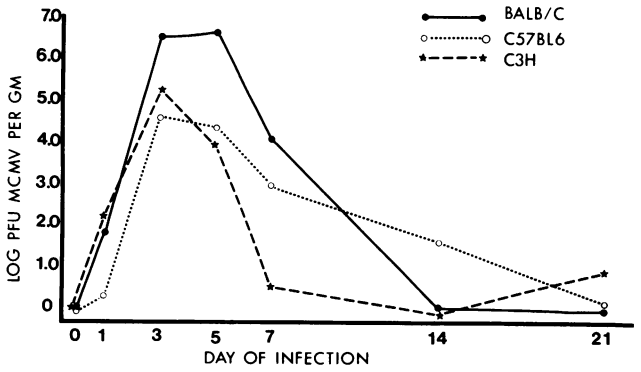

FIG. 2. Recovery of infectious MCMV from 10% (wt/vol) homogenates of spleen tissues. Each point represents the mean of culture results from three to nine animals.

Figure 2 summarizes the results of virus assays performed on spleen tissues harvested from BALB/c, C57BL/6, and C3H mice. In each strain, the peak infection occurred on day 3. Titers of MCMV were highest in BALB/c mice, with a mean peak of 6.56 ± 0.27 $\log_{10}$ PFU of MCMV per g of spleen tissue. Peak titers of MCMV in the spleens of C57BL/6 and C3H mice were lower, with mean values of 4.58 ± 2.10 and 5.29 ± 0.39 $\log_{10}$ PFU/g, respectively (four to nine mice were used per strain). In BALB/c mice, titers of MCMV in spleen tissues remained high through day 5 and were significantly higher than the corresponding day 5 titers in C57BL/6 ($P = 0.03$) and C3H ($P < 0.001$) mice.

Detection of MCMV DNA by in situ hybridization. To correlate virus assays with the presence of virus genomes, we next studied circulating leukocytes by in situ hybridization techniques. The quantitative results of these experiments (in terms of the numbers of MCMV DNA-positive animals and the numbers of MCMV DNA-positive cells per 10^6 leukocytes) are summarized in Tables 1 and 2.

MCMV DNA was detected in a single BALB/c mouse on day 1. Subsequently (on day 3), the leukocytes of two of nine animals (22%) were positive for MCMV DNA at levels of 10 and 13 MCMV DNA-positive cells per 10^6 leukocytes. Infection increased thereafter. On day 5, MCMV DNA was detected in the leukocytes of seven of nine (78%) BALB/c mice, with the numbers of MCMV DNA-positive cells per animal ranging from 10 to 126 MCMV DNA-positive cells per 10^6 leukocytes. On day 7, results were similar, and MCMV DNA was detected in the leukocytes of six of nine (66%) BALB/c mice. Thereafter, the number of MCMV DNA-positive animals and cells declined. On days 14 and 21, MCMV DNA was detected in 33% of the BALB/c mice studied. MCMV DNA was detected in C57BL/6 mice only on days 5 and 7, when it was found in the leukocytes of 22% (two of nine) and 25% (two of eight) of the mice, respectively. The mean number of MCMV DNA-positive leukocytes was generally smaller than in BALB/c mice, although results for day 5 were skewed by one animal with 1,000 MCMV DNA-positive cells per 10^6 leukocytes (Tables 1 and 2). In contrast with the results of BALB/c and C57BL/6 mice, MCMV DNA was detected in only 1 of the 30 C3H mice studied on days 1 through 21 of MCMV infection.

Detection of MCMV DNA in leukocytes harvested from BALB/c and C57BL/6 mice paralleled recovery of infectious virus from leukocyte fractions. As we had observed previously in studies of marrow cells (3), we found a strong positive correlation between detection of MCMV DNA in leukocytes and isolation of infectious virus ($r = 0.66$, df = 44, $P < 0.001$ for data from BALB/c mice).

To establish more completely the validity of our results, we studied a larger group of BALB/c mice (20 MCMV-infected and 20 sham-inoculated control mice) on day 5. Leukocytes from these animals were probed for MCMV nucleic acids by using the gel-purified, biotinylated *Hin*dIII A fragment. Slides were numbered and examined blindly. MCMV nucleic acids were detected in the leukocytes of 13 (65%) of 20 mice inoculated with MCMV versus none of the 20 control mice ($P < 0.001$ by the Fisher exact test). Pretreatment of positive specimens with RNase did not abolish staining, indicating the presence of MCMV DNA. Staining was observed only within nuclei, making it unlikely that our methods detected MCMV stuck to or phagocytized by leukocytes. Leukocytes from all 13 of the biotin-positive animals were also positive for MCMV by tissue culture assay. Conversely, culture-negative leukocytes from control or MCMV-inoculated animals were negative by in situ hybridization.

Figures 3 through 5 illustrate the results of the in situ hybridization studies. Figures 3A through D indicate control hybridizations. With the radiolabeled probes, autoradiographic grains often obscured the morphology of MCMV DNA-containing cells. However, when MCMV DNA-positive cells could be identified, they were usually mononuclear cells (Fig. 4A and B), possibly monocytes. MCMV DNA was infrequently observed in cells that could be identified as neutrophils. When biotinylated probes were used, MCMV DNA was observed only in mononuclear cells (Fig. 5).

TABLE 2. Detection of MCMV DNA in circulating leukocytes harvested from BALB/c, C57BL/6, and C3H mice

Day	BALB/c		C57BL/6		C3H	
	Mean[a] (range) no. of cells	No. of mice[b]	Mean (range) no. of cells	No. of mice	Mean (range) no. of cells	No. of mice
1	25 ± 50 (0–100)	4	0	9	0	5
3	3 ± 5 (0–13)	9	0	5	20 ± 40 (0–100)	5
5	54 ± 45[c] (0–125)	9	129 ± 330 (0–1,000)	9	0	5
7	108 ± 195[d] (0–501)	9	25 ± 53 (0–151)	8	0	5
14	29 ± 47 (0–112)	9	0	6	0	5
21	6 ± 11 (0–25)	9	0	3	0	5

[a] Mean ± standard deviation of MCMV DNA-positive cells per 10^6 murine leukocytes. Cells were studied by using a whole-plasmid MCMV DNA probe as described in Materials and Methods.
[b] The number of mice studied per day. A total of 119 mice were studied.
[c] $P = 0.0038$ when compared with results for C3H mice.
[d] $P = 0.033$ when compared with results for C3H mice.

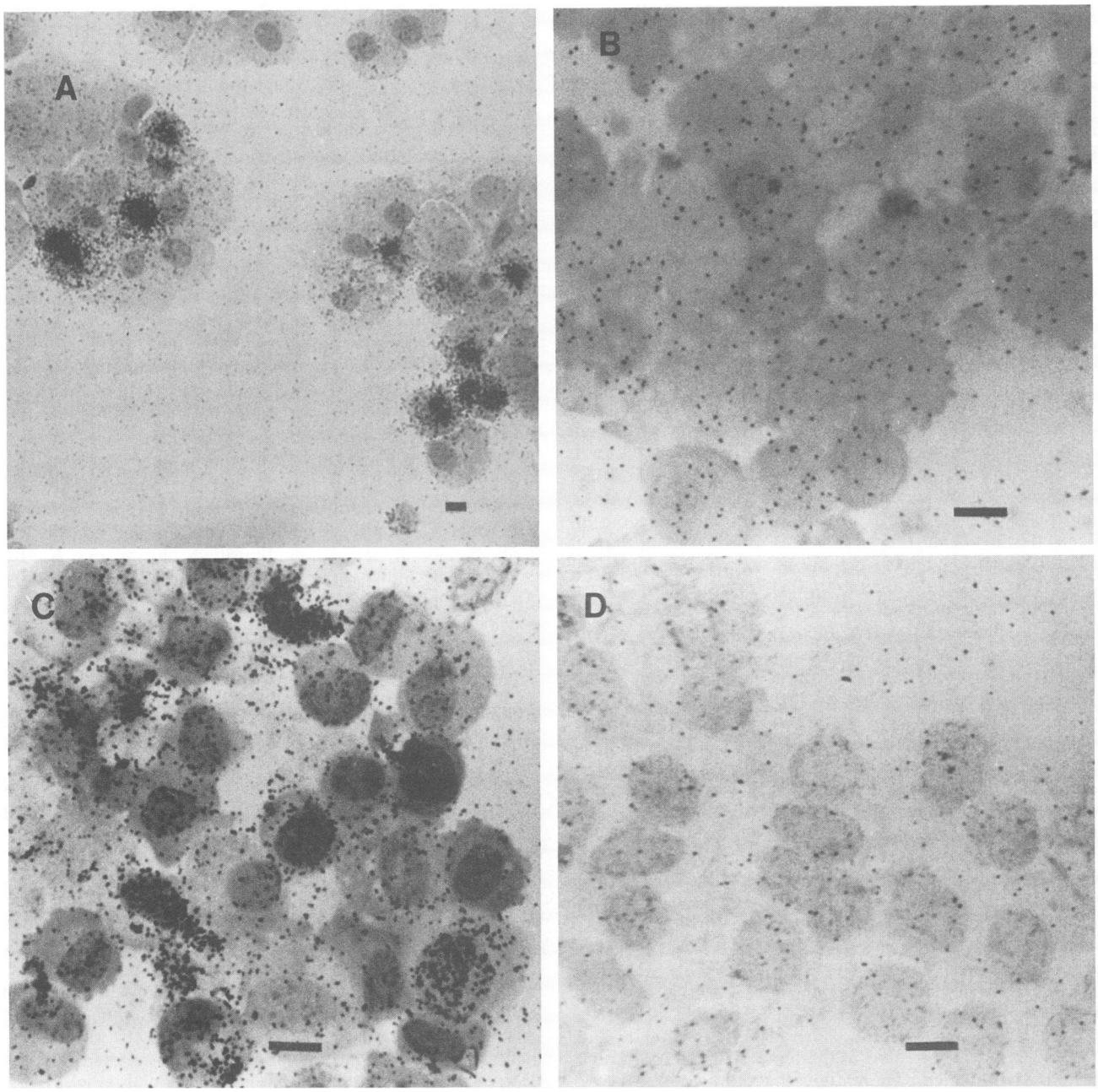

FIG. 3. Examples of control hybridizations for MCMV DNA by using ^{35}S-labeled probes. (A) Photomicrograph of MCMV-infected MEFs infected at a multiplicity of infection of <0.1, harvested on day 5, and hybridized with the ^{35}S-labeled whole-plasmid MCMV DNA probe. Several cells show numerous autoradiographic grains. Bar, 10 μm. (B) Photomicrograph of MCMV-infected MEFs incubated with ^{35}S-labeled cloning vector pACYC177. Cells show only background autoradiographic grains. Bar, 10 μm. (C) Photomicrograph of MCMV-infected MEFs probed with a ^{35}S-labeled, gel-purified MCMV DNA *Hin*dIII A fragment. Several cells contain numerous autoradiographic grains. Bar, 10 μm. (D) Photomicrograph of uninfected MEFs reacted with the ^{35}S-labeled, gel-purified MCMV DNA *Hin*dIII A fragment. Cells show only background autoradiographic grains. Bar, 10 μm.

DISCUSSION

In the current studies, we used in situ hybridization methods to detect MCMV DNA in the circulating leukocytes of 3-week-old BALB/c, C57BL/6, and C3H mice infected with MCMV intraperitoneally. In susceptible BALB/c and C57BL/6 mice, infectious virus and/or MCMV DNA were frequently detected during acute MCMV infection. By contrast, we found MCMV DNA in the circulating leukocytes of only one C3H mouse. Detection of MCMV DNA in leukocytes harvested from BALB/c mice correlated strongly with isolation of infectious virus from leukocyte fractions, an observation which supports the conclusion that MCMV replicated in circulating leukocytes. However, the total number of cells which contained MCMV DNA was small. At peak infection, MCMV DNA was detected in 0.001 to 0.1% of the circulating leukocytes of BALB/c or C57BL/6 mice.

The results of in situ hybridization experiments for BALB/c and C57BL/6 mice extend previous studies of MCMV

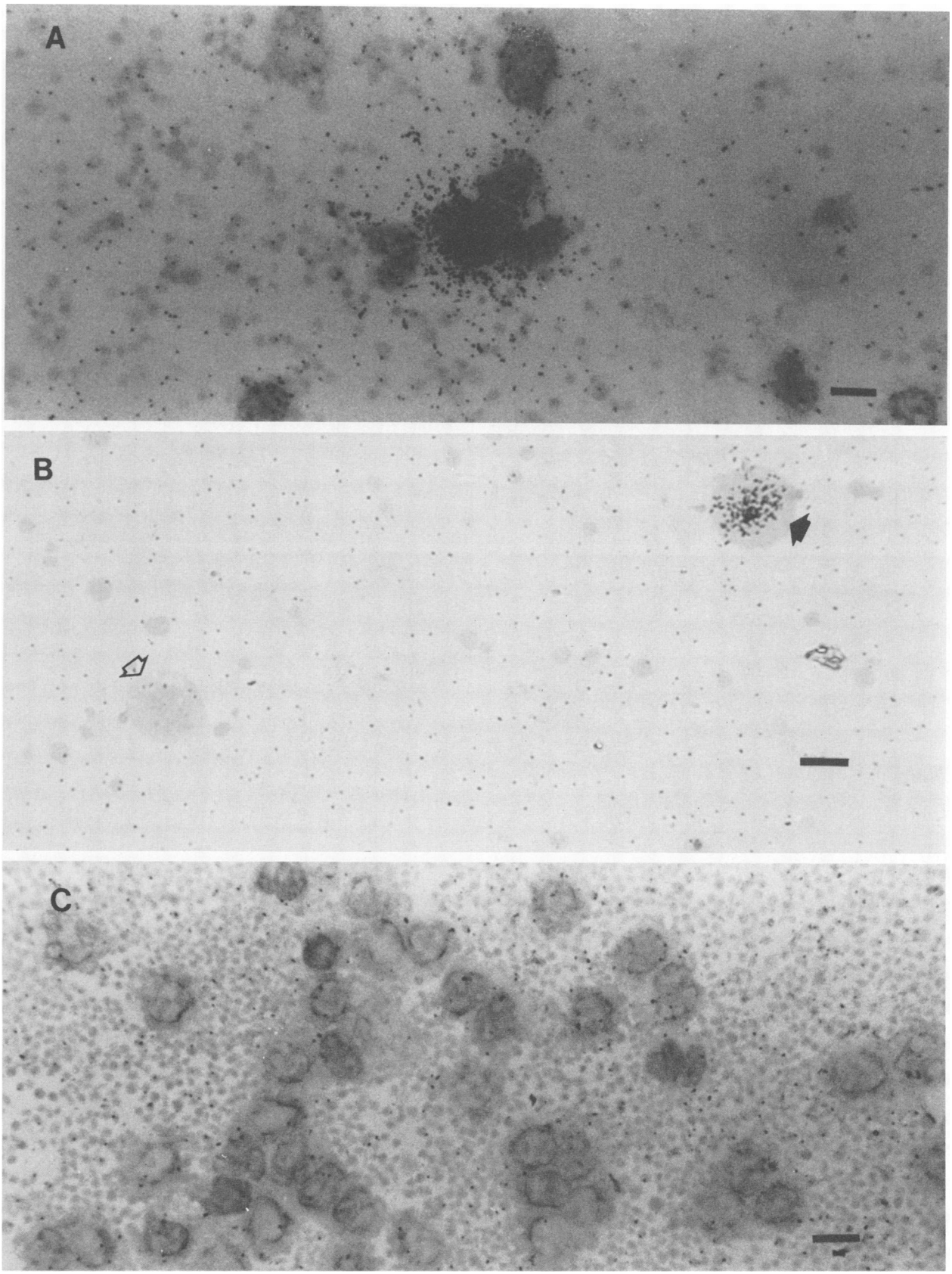

FIG. 4. Leukocytes probed for MCMV DNA. (A) Leukocytes harvested from a BALB/c mouse on day 7 of infection and hybridized with the ^{35}S-labeled whole-plasmid MCMV DNA probe. A single cell shows numerous autoradiographic grains, whereas adjacent cells show only background grains. Bar, 10 μm. (B) Leukocytes harvested from a C57BL/6 mouse on day 5 of infection and hybridized with the ^{35}S-labeled whole-plasmid MCMV DNA probe. One cell (⧫) contains numerous autoradiographic grains. Another cell (◊) contains only background grains. Bar, 10 μm. (C) Leukocytes harvested from a sham-inoculated, control BALB/c mouse on day 5 and reacted with the ^{35}S-labeled whole-plasmid MCMV DNA probe. Cells show only background grains. Bar, 10 μm.

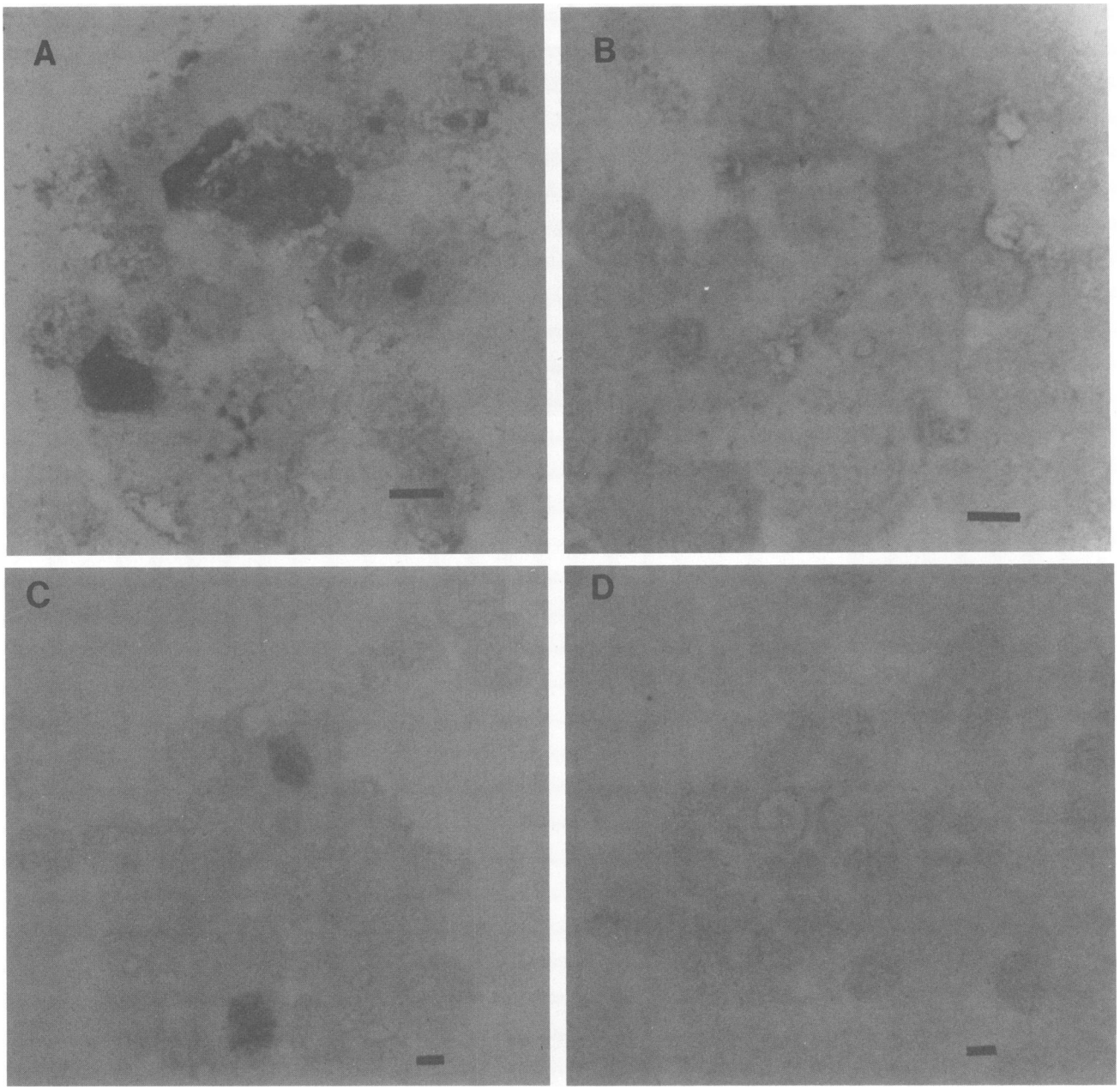

FIG. 5. Examples of in situ hybridizations with biotinylated MCMV DNA probes. (A) Photomicrograph of MCMV-infected MEFs hybridized with the gel-purified, biotinylated MCMV DNA *Hin*dIII A fragment. Several cells contain intranuclear precipitate, indicating the presence of MCMV nucleic acids. Bar, 10 μm. (B) Photomicrograph of uninfected MEFs reacted with the biotinylated probe as in panel A. Cells show only background staining. Bar, 10 μm. (C) Leukocytes harvested from a BALB/c mouse on day 5 of infection and hybridized with the gel-purified, biotinylated MCMV DNA *Hin*dIII A fragment. Two cells show nuclear precipitates, indicating the presence of MCMV nucleic acids. Bar, 10 μm. (D) Leukocytes harvested from a sham-inoculated, control BALB/c mouse and probed for MCMV DNA as in panel C. Cells show only background staining. Bar, 10 μm.

infection by conventional virologic methods (4, 5, 22). Wu and Ho, studying MCMV infection of 8-week-old (adult) BALB/c mice, observed that MCMV viremia began 3 days after intravenous inoculation of MCMV and continued for 2 weeks (22). At peak infection (day 6), Wu and Ho detected MCMV in low titer from cell lysates of sonicated lymphocytes (22). This observation implied that MCMV replicated in blood lymphocytes, either at low titer or in small numbers of cells. The current experiments confirmed that small numbers of circulating leukocytes appear to support MCMV replication.

Our data regarding C3H mice suggest that the leukocytes of such mice differed intrinsically in their susceptibility to MCMV infection. Previous studies have established that C3H mice are relatively resistant to MCMV infection and that such resistance is associated, at least in part, with the $H\text{-}2^k$ haplotype (1, 10, 19). Recently, Price et al. observed that MCMV infection of peritoneal macrophages in vitro was

strongly influenced by the H-2 phenotype and attributed such differences to H-2-mediated effects on the MCMV-cell interaction (18). Because we studied acute infection, we do not know whether C3H leukocytes might support latent MCMV infection.

The current results also extend the observations of Mercer and Spector (16). These investigators used in situ hybridization methods to study spleen sections from MCMV-infected BALB/c and C3H mice and found major differences in the quantities of MCMV DNA in such tissues. After 24 h of infection, BALB/c mice developed disseminated infection in spleen tissues, whereas infection in C3H mice did not progress. In the present studies, the prolonged peak of virus replication in the spleens of susceptible mice corresponded to detection of infectious virus and MCMV DNA in the circulating leukocytes.

On the basis of these observations, we conclude that MCMV infects and replicates in the circulating leukocytes of susceptible mouse strains. We speculate that viremia in resistant mice may be plasma associated and that productive infection rarely occurs in the leukocytes of such animals. In the current experiments, leukocyte infection was most frequent in BALB/c mice, intermediate in C57BL/6 mice, and rare in C3H mice. These variations in leukocyte infection appear to be another manifestation of the genetically determined differences in susceptibility to MCMV. Replication of CMV in circulating leukocytes may be an important factor in the dissemination of CMV infection in susceptible hosts.

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LITERATURE CITED

1. **Allan, J. E., and G. R. Shellam.** 1984. Genetic control of murine cytomegalovirus infection: virus titers in resistant and susceptible strains of mice. Arch. Virol. **81**:139–150.
2. **Bale, J. F., Jr., E. R. Kern, J. C. Overall, Jr., and J. R. Baringer.** 1983. Impaired migratory and chemotactic activity of neutrophils during murine cytomegalovirus infection. J. Infect. Dis. **148**:518–525.
3. **Bale, J. F., Jr., M. E. O'Neil, R. Giller, S. Perlman, and U. Koszinowski.** 1987. Murine cytomegalovirus genomic material in marrow cells: relation to altered leukocyte counts during sublethal infection of mice. J. Infect. Dis. **155**:207–212.
4. **Booss, J., and E. F. Wheelock.** 1977. Role of viremia in the suppression of T-cell function during murine cytomegalovirus infection. Infect. Immun. **17**:378–381.
5. **Cheung, K. S., and D. J. Lang.** 1977. Transmission and activation of cytomegalovirus with blood transfusion: a mouse model. J. Infect. Dis. **135**:841–845.
6. **Cheung, K. S., J. K. Li, J. M. Falletta, J. L. Wagner, and D. J. Lang.** 1981. Murine cytomegalovirus infection: hematological, morphological, and functional study of lymphoid cells. Infect. Immun. **33**:239–249.
7. **Ebeling, A., G. M. Keil, E. Knust, and U. H. Koszinowski.** 1983. Molecular cloning and physical mapping of murine cytomegalovirus DNA. J. Virol. **47**:421–433.
8. **Fiala, M. L., and S. Chatterjee.** 1982. The role of lymphocytes in infections due to Epstein-Barr virus and cytomegalovirus. J. Infect. Dis. **146**:300.
9. **Fiala, M., J. E. Payne, T. V. Berne, T. C. Moore, W. Henle, J. Z. Montgomerie, S. N. Chatterjee, and L. B. Guze.** 1975. Epidemiology of cytomegalovirus infection after transplantation and immunosuppression. J. Infect. Dis. **132**:421–433.
10. **Grundy (Chalmer), J. E., J. S. Mackenzie, and N. F. Stanley.** 1981. Influence of *H-2* and non-*H-2* genes on resistance to murine cytomegalovirus infection. Infect. Immun. **32**:277–286.
11. **Haase, A., M. Brahic, L. Stowring, and H. Blum.** 1984. Detection of viral nucleic acids by *in situ* hybridization. Methods Virol. **7**:189–226.
12. **Ho, M.** 1981. The lymphocyte in infections with Epstein-Barr virus and cytomegalovirus. J. Infect. Dis. **143**:857–862.
13. **Hudson, J. B.** 1979. The murine cytomegalovirus as a model for the study of viral pathogenesis and persistent infections. Arch. Virol. **62**:1–29.
14. **Keil, G. M., A. Ebeling-Keil, and U. H. Koszinowski.** 1984. Temporal regulation of murine cytomegalovirus transcription and mapping of viral RNA synthesized at immediate early times after infection. J. Virol. **50**:784–795.
15. **Martin, D. C., D. A. Katzenstein, G. S. M. Yu, and M. C. Jordan.** 1984. Cytomegalovirus viremia detected by molecular hybridization and electron microscopy. Ann. Intern. Med. **100**: 222–225.
16. **Mercer, J. A., and D. H. Spector.** 1986. Pathogenesis of acute murine cytomegalovirus infection in resistant and susceptible strains of mice. J. Virol. **57**:497–504.
17. **Osborn, J. E., and N. T. Shahidi.** 1973. Thrombocytopenia in murine cytomegalovirus infection. J. Lab. Clin. Med. **81**:53–63.
18. **Price, P., J. G. Winter, S. Nikoletti, J. B. Hudson, and G. R. Shellam.** 1987. Functional changes in murine macrophages infected with cytomegalovirus relate to H-2-determined sensitivity in infection. J. Virol. **61**:3602–3606.
19. **Quinnan, G. V., Jr., and J. F. Manischewitz.** 1987. Genetically determined resistance to lethal murine cytomegalovirus infection is mediated by interferon-dependent and -independent restriction of virus replication. J. Virol. **61**:1875–1881.
20. **Rinaldo, C. R., Jr., P. H. Black, and M. S. Hirsch.** 1977. Interaction of cytomegalovirus with leukocytes from patients with mononucleosis due to cytomegalovirus. J. Infect. Dis. **136**:667–678.
21. **Schrier, R. D., J. A. Nelson, and M. B. A. Oldstone.** 1985. Detection of human cytomegalovirus in peripheral blood lymphocytes in a natural infection. Science **230**:1048–1051.
22. **Wu, B. C., and M. Ho.** 1979. Characteristics of B and T lymphocytes from mice after inoculation with cytomegalovirus. Infect. Immun. **24**:856–864.
23. **Zaia, J. A., S. J. Forman, M. T. Gallagher, E. Vanderwal-Urbina, and K. G. Blume.** 1984. Prolonged human cytomegalovirus viremia following bone marrow transplantation. Transplantation **37**:315–317.