JOURNAL OF VIROLOGY, Oct. 1984, p. 119–128
0022-538X/84/100119-10$02.00/0

Vol. 52, No. 1

Pathogenesis of Murine Cytomegalovirus Infection in Natural Killer Cell-Depleted Mice

JACK F. BUKOWSKI,[1,2] BRUCE A. WODA,[2] AND RAYMOND M. WELSH[1,2]*

Departments of Pathology[2] and of Molecular Genetics and Microbiology,[1] University of Massachusetts Medical Center, Worcester, Massachusetts 01605

Received 21 February 1984/Accepted 30 May 1984

The effect of natural killer (NK) cells on the course of acute and persistent murine cytomegalovirus (MCMV) infection was examined by selectively depleting NK cell activity by inoculation of mice with antibody to asialo GM1, a neutral glycosphingolipid present at high concentrations on NK cells. The dose of MCMV required to cause 50% mortality or morbidity in control C57BL/6 mice dropped 4- and >11-fold, respectively, in mice first treated with anti-asialo GM1. NK cell-depleted mice had higher (up to 1,000-fold) virus titers in their lungs, spleens, and livers at days 3, 5, 7, and 9 postinfection. Spleens and livers of control mice were virus-free by day 7 postinfection, and their lungs showed no signs of active infection at any time. In contrast, MCMV had disseminated to the lungs of NK cell-depleted mice by day 5, and these mice still had moderate levels of virus in their lungs, spleens, and livers at day 9. Markedly severe pathological changes were noted in the livers and spleens of NK cell-depleted, MCMV-infected mice. These included ballooning degeneration of hepatocytes and spleen necrosis. MCMV-infected, NK cell-depleted mice had severe spleen leukopenia, and their spleen leukocytes exhibited a significantly lower (up to 13-fold) response to the T cell mitogen concanavalin A when compared with those of uninfected and MCMV-infected controls. It appeared that NK cells exerted their most potent antiviral effect early in the infection, in a pattern correlating with interferon production and NK cell activation; treatment with anti-asialo GM1 later in infection had no effect on virus titers. The relative effect of NK cell depletion on MCMV pathogenesis depended on the injection route of the virus. NK cell depletion greatly augmented MCMV synthesis and pathogenesis in mice inoculated either intravenously or intraperitoneally but had no effect on the course of disease after intranasal inoculation, at any time point examined. One month after intraperitoneal inoculation of virus, NK cell depletion resulted in a six- to eightfold increase in salivary gland virus titers in persistently infected mice, suggesting that NK cells may be important in controlling virus synthesis in the salivary gland during persistent infection. This treatment did not, however, induce dissemination of virus to other organs. These data support the hypothesis that NK cells limit the severity, extent, and duration of acute MCMV infection and that they may also be involved in regulating the persistent infection.

Human cytomegalovirus (CMV) (reviewed in 35, 38, 45) is a ubiquitous herpesvirus capable of causing congenital birth defects (13), mononucleosis (25), hepatitis (14), and interstitial pneumonitis (15). It causes acute, persistent, and latent infections and can be reactivated from a latent state (reviewed in 45). CMV infection is particularly troublesome when patients are immunosuppressed during tumor therapy and transplantation procedures; patients frequently contract CMV pneumonitis, which has a high mortality rate (15). CMV itself is immunosuppressive (47), and the combination of that effect and the effects of chemotherapy and radiation can lead to opportunistic infections such as pneumocystosis caused by *Pneumocystis carinii* (44).

Because of its similarity to human CMV, murine cytomegalovirus (MCMV) (reviewed in 45) infection in mice has been used extensively as a model to study the pathogenesis of and the immune response to CMV. Such studies have shown a peak in natural killer (NK) cell activity 3 to 5 days postinfection (1, 43) and a virus-specific, *H-2*–restricted, cytotoxic-T lymphocyte (CTL) response 6 to 20 days postinfection (42). The CTL response is thought to be responsible for the clearance of the virus late in infection (19). However, there are marked differences in the susceptibility of various strains of mice to MCMV (as judged by death rate 3 to 5 days postinfection), and resistance correlates with the magnitude of the NK cell response rather than that of the CTL response (1, 8). No strain-related differences in macrophage function or macrophage susceptibility to MCMV infection have been found (4, 49).

NK cell-mediated cytotoxicity is augmented during acute (18, 32, 55, 56) and persistent (5) viral infections. This increase in NK cell-mediated cytotoxicity is a result of both proliferation and activation of the NK cell population (3). NK cells can be directly activated by interferon (IFN) (9, 10, 52, 54) or by viral glycoproteins (7). Indirect evidence has supported the concept that NK cells may mediate resistance to herpes simplex virus (31), Friend leukemia virus (27), and mouse hepatitis virus (30, 51). With regard to MCMV, Shellam and co-workers (1) showed that strains of mice having high NK cell activity were more resistant to MCMV infection than strains having low NK cell activity, and they also observed that NK cell-deficient homozygous beige mice were more susceptible to infection than their heterozygous NK-sufficient littermates (50). Using bone marrow chimeras, they demonstrated that bone marrow-derived cells were responsible for this resistance (50); however, since beige mice have other immune defects (53), the defect that was responsible for the lowered resistance was not known. There is some indication that a lymphocyte may control salivary gland MCMV titers during persistent infection (17) and that a lymphocyte resembling an NK cell, in concert with serum from mice persistently infected with MCMV, can mediate antibody-dependent, cell-mediated cytotoxicity against MCMV-infected targets (33).

* Corresponding author.

Recently, antibody to asialo GM1, a reagent which selectively depletes NK cell activity in vivo (12, 23, 24), has become available. Although this antibody almost totally depletes NK cell activity, it has no effect on cytotoxic macrophage (24), cytotoxic T (23, 24), or natural cytotoxic cell (unpublished data) functions. In addition, the concanavalin A (ConA) response (12) and the percentage of thy-1–positive (23) and surface immunoglobulin-positive (unpublished data) cells found in the spleens of in vivo-treated mice are unaffected. This antibody to asialo GM1 does not react with granulocytes (12) and, in the presence of complement, lyses less than 5% of the total number of spleen leukocytes (23, 26) while eliminating virtually all large granular lymphocytes, which contain the NK cells (26). We have recently shown that mice treated with this antibody synthesize more MCMV, mouse hepatitis virus, and vaccinia virus in their livers and spleens and have greater virus-induced liver damage at day 3 postinfection than do untreated mice (6). The antibody did not abrogate resistance to infection by inhibiting the IFN response, as antibody-treated, MCMV-infected mice had higher levels of plasma IFN than did untreated, MCMV-infected mice (6).

The results presented in this paper indicate that NK cell-depleted mice undergo a more severe and disseminated infection of longer duration than do control MCMV-infected mice and that NK cell depletion is most effective early in infection. The suppression of the T cell response (20) seen in control MCMV-infected mice is greatly enhanced in NK cell-depleted mice, correlating with higher virus titers and delayed viral clearance. Finally, we present some evidence suggesting that NK cells may play a role in controlling persistent MCMV infection.

MATERIALS AND METHODS

Animals. C57BL/6 (+/+, bg/+, and bg/bg) and BALB/c mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. Mice of either sex, 6 to 12 weeks old, were used in these experiments.

Cells. YAC-1 cells were derived from a Moloney leukemia virus-induced lymphoma in A/Sn mice and were maintained in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with antibiotics, glutamine, and 10% heat-inactivated fetal bovine serum (M.A. Bioproducts, Inc., Walkersville, Md.). Mouse embryo fibroblasts (MEF) were obtained as previously described (29, 48) from C57BL/6 mouse embryos and maintained in minimal essential medium (GIBCO Laboratories) with the same additives as those listed above. L-929 cells, a continuous liver cell line derived from C3H mice, were maintained in minimal essential medium as described above.

Virus. The Smith strain of MCMV was obtained from John Nedrud, Case Western Reserve University School of Medicine, Cleveland, Ohio (37). This virus was maintained by in vivo passage in weanling BALB/c mice. Salivary glands from mice inoculated 2 to 3 weeks previously with 10^4 PFU of MCMV were homogenized in a 10% suspension and cleared by centrifugation. Samples were stored at −70°C in 10% dimethyl sulfoxide. The Indiana strain of vesicular stomatitis virus was used in the IFN assays.

Anti-asialo GM1 antiserum. Rabbit antiserum to asialo GM1 was purchased from Wako Chemicals, USA, Inc., Dallas, Tex. This antiserum has previously been shown to deplete NK cell activity in vivo and in vitro (12, 23, 24) selectively. To deplete NK cell activity in vivo, anti-asialo GM1 was diluted 1:10 in RPMI medium and given intravenously (i.v.) in doses of 0.2 ml.

Cytotoxicity assay. Assay medium was RPMI medium supplemented with 0.1 M *N*-2-hydroxylethylpiperazine-*N'*-2-ethanesulfonic acid (Sigma Chemical Co., St. Louis, Mo.), 10% fetal bovine serum, glutamine, and antibiotics. The assay was performed as previously described (54). Briefly, YAC-1 target cells, labeled with 100 μCi of sodium [^{51}C]chromate (New England Nuclear Corp., Boston, Mass.) for 1 h at 37°C, were washed and mixed with various numbers of effector cells in round-bottomed microtiter wells at 10^4 cells per well. For spontaneous-release determination, medium was added to the wells, and 1% Nonidet P-40 was added for maximum-release determination. Plates were incubated 4 to 16 h at 37°C in a humidified atmosphere of 5% CO_2-95% air. At the end of the incubation, plates were centrifuged at 200 × *g* for 5 min, and 0.1 ml of the supernatant was collected and counted for radioactivity in a Beckman Gamma 5500 counter (Beckman Instruments, Inc., Palo Alto, Calif.). Data are expressed as percent specific release, calculated as follows:

$$100 \times \frac{\text{counts per minute (cpm) experimental} - \text{cpm spontaneous}}{\text{cpm maximum} - \text{cpm spontaneous}}$$

Spontaneous release was between 8 and 33%. Standard deviations of quadruplicate replica samples were less than 10% of the means and were not listed in the tables. Results of cytotoxicity assays against YAC-1 cells with individual mice are tabulated as the mean percent specific ^{51}CR release of four separate mice plus or minus the standard error of the mean (SEM).

Virus titration. MCMV titers were determined by using a 10% homogenate of tissue taken from individual mice. The number of PFU was determined by plaque assay with mouse embryo fibroblasts and 10-fold dilutions of tissue homogenate. Results are expressed as the geometric mean titers, i.e., the arithmetic averages of the logs for four separate animals titrated for virus individually plus or minus the SEM. Titers reported are $\log_{10}$ PFU per whole spleen, both lungs, both submaxillary salivary glands, and gram of liver. Where SEMs are omitted, one or more mice in that particular group had no detectable virus in that organ, and these mice were assigned a virus titer equivalent to the lowest amount of virus detectable by our assay method and averaged together with the other mice in that same group. In this instance, the resulting number was designated "less than." The *P* values represent the significance of the differences of the means between the designated sample and the normal, non-antibody-treated control and were calculated by the Student's *t* test. The mean lethal dose (LD_{50}) was calculated by the method of Reed and Meunch (46), using four mice per group and serial twofold dilutions of MCMV. The mean morbidity dose (morbidity $dose_{50}$) was calculated in the same manner, using ruffled fur and a hunched posture as criteria for clinical illness.

Mitogen stimulation. Spleen cells were dispensed in flat-bottomed microtiter wells at 3×10^5 cells per well in medium containing 2 μg of ConA (Sigma) per ml or in medium alone. These cells were incubated in the same manner as those used in cytotoxicity assays for 2 days after which 1 μCi of [^{3}H]thymidine (New England Nuclear Corp.) was added to each well. The next day, the cells were harvested with a microautomated sample harvester (MASH; Bellco Glass, Inc., Vineland, N.J.) and counted in a liquid scintillation counter (Beckman). Spleen cells from at least two mice were

TABLE 1. NK cell depletion results: increased viral dissemination and delayed clearance of virus[a]

Days postinfection	Anti-AGM1 treatment	% NK lysis	Virus (log_{10} PFU) per:			
			Spleen	Lung	Gram of liver	Salivary gland
3	−	20 ± 1.6	1.7 ± 0.2	<1.0[c]	3.1 ± 0.1	<2.0
	+	0.1 ± 0.9	4.6 ± 0.1[b]	1.6 ± 0.1	4.7 ± 0.1[b]	<2.0
5	−	12 ± 1.2	2.0 ± 0.1	1.5 ± 0.1	<2.8	<2.0
	+	8.5 ± 1.6	4.3 ± 0.1[b]	3.9 ± 0.0	4.7 ± 0.1[b]	<2.0
7	−	13 ± 2.0	<1.9	<1.1	<2.4	2.9 ± 0.2
	+	8.6 ± 0.4	3.7 ± 0.2[b]	2.9 ± 0.1	3.4 ± 0.4[b]	3.4 ± 0.2
9	−	8.5 ± 1.2	<0.7	1.5 ± 0.3	<2.5	5.3 ± 0.1
	+	6.5 ± 0.7	3.7 ± 0.3[b]	3.4 ± 0.1	3.1 ± 0.1[b]	5.5 ± 0.1

[a] Mice were either left untreated (−) or given anti-asialo GM1 (anti-AGM1) (+) 4 to 6 h before i.p. injection of 5×10^3 PFU of MCMV. In each treatment group, four C57BL/6 mice were sacrificed at various times postinfection; virus titrations and NK cell assays were performed. The assay length was 16 h, and the effector/target ratio was 11:1.

[b] $P < 0.001$.

[c] <, One or more mice had no detectable virus in that organ (see text).

pooled, and each number in Table 2 represents the mean cpm of four duplicate wells plus or minus the SEM.

IFN assay. Blood was collected in heparinized Natelson tubes from the retro-orbital sinus of mice anesthetized with ether, and plasma was obtained by centrifugation. Peritoneal washes were obtained by inoculating mice intraperitoneally (i.p.) with 3 ml of cold RPMI medium and collecting the fluid with a Pasteur pipette. This wash was centrifuged at $200 \times g$ for 5 min, and IFN assays were performed on the supernatants. Plasma or peritoneal wash samples were titrated by twofold serial dilutions in a 96-well, flat-bottomed microtiter plate to which L-929 cells were added at 3×10^4 cells per well. The wells were challenged 18 to 24 h later with 100 50% tissue culture infective dose U of vesicular stomatitis virus. IFN titers were expressed as the log_2 of the highest reciprocal dilution resulting in 50% reduction in cytopathic effect. Results are expressed as the geometric mean titers for four separate animals titrated for IFN individually plus or minus the SEM.

Histopathology. Tissue sections were fixed in buffered Formalin, embedded in paraffin, cut at 4 μm, and stained with hematoxylin-eosin. An American Optical Corp. (Buffalo, N.Y.) 110 microscope was used for examining tissue sections. A low-power field refers to the use of a ×10 objective and ×10-wide field oculars, yielding a field diameter of 1.7 mm. Tissue sections were examined in a blind manner, without knowing whether the source mouse was NK cell depleted.

RESULTS

Susceptibility to MCMV infection. When titrated in control C57BL/6 mice, 5×10^5 PFU of MCMV was equivalent to one LD_{50}, but when these mice were treated with anti-asialo GM1 4 to 6 h before infection, only 1.2×10^5 PFU was equivalent to one LD_{50}. At a dose of 1.5×10^5 PFU, 75% of antibody-treated mice and 0% of control mice died ($P < 0.01$). Whereas the dose of this stock required to cause morbidity $dose_{50}$ in control mice was 2×10^5 PFU, the morbidity $dose_{50}$ in anti-asialo GM1-treated mice was less than 1.8×10^4 PFU, the lowest dose used in the titration. Further, in other experiments reported throughout this paper, a dose of 5×10^3 PFU routinely rendered NK cell-deficient mice clinically ill but had no visible effect on control mice. Thus, there were 4- and at least 11-fold decreases in LD_{50} and morbidity $dose_{50}$, respectively, when NK cells were depleted by treatment with anti-asialo GM1.

Antibody treatment did not abrogate resistance to MCMV by inhibiting the IFN response, as both control and antibody-treated mice had similar plasma IFN titers 6 h after i.p. inoculation with 3×10^5 PFU of MCMV. Control and NK cell-depleted mice had 9.3 ± 0.2 and 9.5 ± 0.3 log_2 U of IFN per ml of plasma, respectively. With regard to the local IFN response, control and NK cell-depleted mice had 2.5 ± 0.3 and 3.0 ± 0.4 log_2 U of IFN per ml of peritoneal wash, respectively. These data, coupled with our previous observation at day 3 postinfection with 5×10^3 PFU (6), indicate that antibody treatment did not abrogate the IFN response 6 to 72 h postinfection.

Effect of NK cell depletion on viral synthesis, dissemination, and clearance. Control mice or those treated with anti-asialo GM1 were injected i.p. with 5×10^3 PFU of MCMV and sacrificed at various times postinfection. The results indicate that NK cell-depleted mice had significantly higher virus titers in the spleen, lung, and liver than did control mice at days 3, 5, 7, and 9 postinfection (Table 1). The magnitude of the differences in virus titer was most pronounced in the spleen, with NK cell-depleted mice having as much as 1,000 times more virus. Though dissemination of virus to the salivary gland was not accelerated in NK cell-depleted mice, an active infection of the lung was noted at days 5, 7, and 9 postinfection only in NK cell-depleted mice (Table 1) with control mice always having fewer than 100 PFU in their total lung tissue. These results indicate that NK cell depletion resulted in increased viral dissemination to the lung.

By day 9 postinfection, control mice had no detectable virus in their spleens and livers, whereas NK cell-depleted mice still had about 5,000 PFU per organ (Table 1), indicating that viral clearance from these organs was delayed.

As reported previously (6), NK cell-mediated lysis was almost totally eliminated by anti-asialo GM1 treatment when assayed at day 3 postinfection (Table 1). By day 5 postinfection, lytic activity was again detectable, and on day 9, NK cell activity on a cell-to-cell basis was comparable to that of the control mice.

Under these conditions of infection, control mice did not become clinically ill (i.e., hunched posture and ruffled fur), and their livers and spleens were completely virus-free by days 7 to 9 postinfection. In contrast, NK cell-depleted mice were clinically ill at days 4 to 9 postinfection, with moderately high virus titers in the lung, spleen, and liver at day 9 postinfection (Table 1), eventually falling to undetectable levels by days 12 to 15 postinfection (data not shown). In

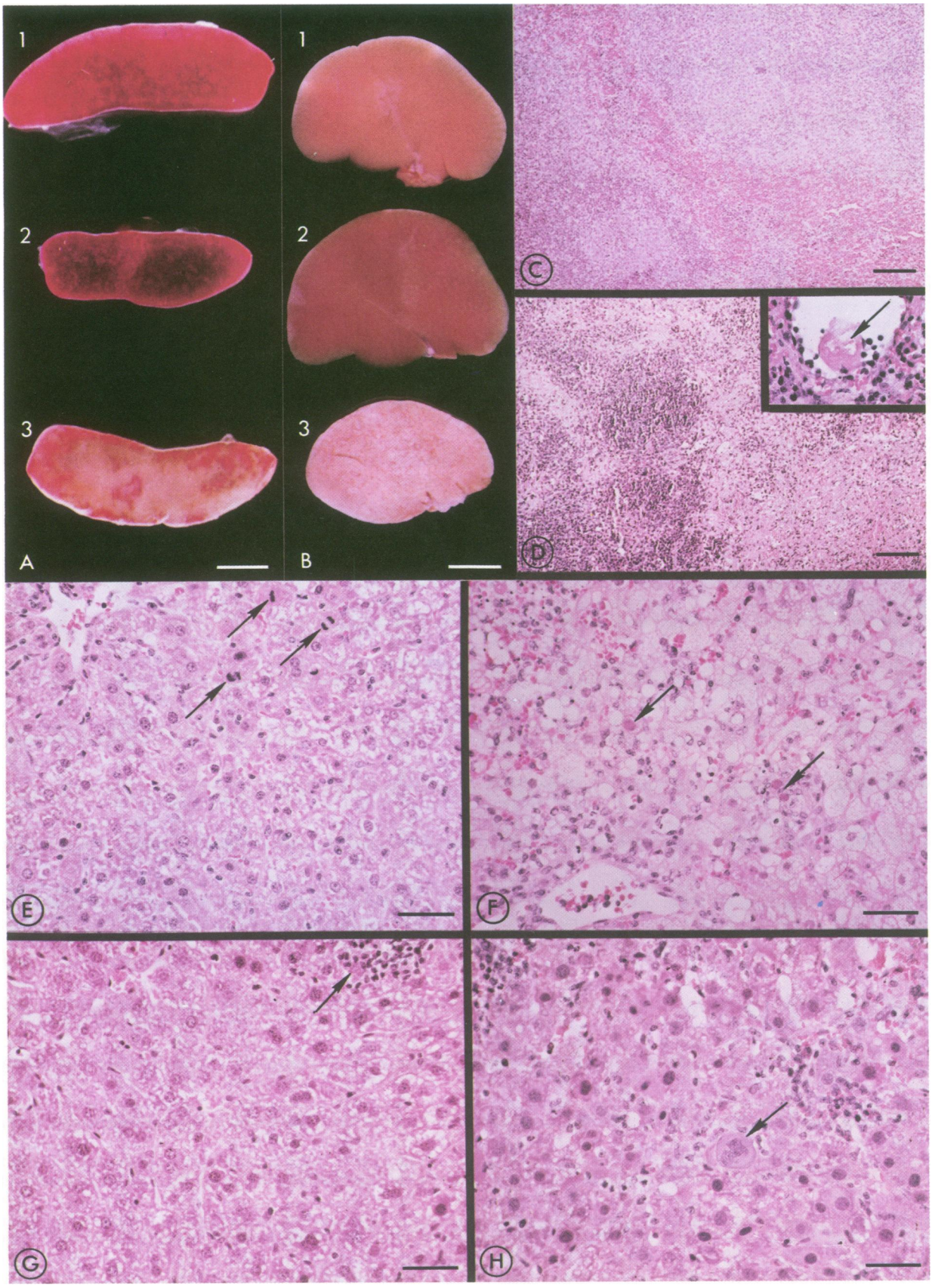
1
2
3
A
1
2
3
B
C
D
E
F
G
H

TABLE 2. NK cell depletion results: increased MCMV-induced suppression of the ConA response[a]

Treatment	10^2 cpm ± SEM at day postinfection:			
	3	5	7	9
Uninfected				
Unstimulated	5.3 ± 1.0	5.6 ± 1.1	9.4 ± 1.2	7.9 ± 0.7
ConA	1,200 ± 56	1,400 ± 14	690 ± 16	1,000 ± 22
Uninfected + anti-AGM1				
Unstimulated	8.6 ± 0.6	6.1 ± 0.4	ND[b]	7.4 ± 0.4
ConA	1,200 ± 94	1,400 ± 24	ND	1,100 ± 19
MCMV-infected				
Unstimulated	22 ± 2.5	2.7 ± 0.3	12 ± 2.0	9.7 ± 0.5
ConA	1,200 ± 79	490 ± 19	310 ± 14	1,000 ± 11
MCMV-infected + anti-AGM1				
Unstimulated	15 ± 2.6	41 ± 3.2	21 ± 2.9	15 ± 1.2
ConA	830 ± 25	36 ± 1.8	60 ± 3.3	540 ± 6.2

[a] Mice were left untreated or given anti-asialo GM1 (anti-AGM1). Half of each group was infected 4 to 6 h later i.p. with 5×10^3 PFU of MCMV, and the other half was left untreated. Mice were sacrificed at various days postinfection, and the spleen cells were assayed for response to ConA stimulation as described in the text. Each time point represents a separate assay with its own control from uninfected mice.

[b] ND, Not done.

both untreated and antibody-treated mice, MCMV remained in the salivary glands for at least 5 weeks.

Pathology. As reported earlier (6), livers from NK cell-depleted mice at day 3 postinfection had more foci of inflammation than did those from normal MCMV-infected mice, as well as large areas of hepatic necrosis (data not shown). At day 5 postinfection, livers from control MCMV-infected mice had resolving focal hepatitis and were undergoing active regeneration (Fig. 1E). There were many mitotic figures, and the hepatocytes had vesicular cytoplasm and pleomorphic nuclei. The inflammatory foci were small, numbering two per low-power field, and were composed predominantly of mononuclear cells with a few admixed neutrophils. Though a few giant cells were seen, cells with viral inclusions were not present. In contrast, livers from NK cell-depleted, MCMV-infected mice had severe and continuing disease and were yellow and atrophic in appearance (Fig. 1B). The hepatocytes had severe ballooning degeneration with multifocal areas of inflammation, and cells with viral intranuclear inclusions were present (Fig. 1F). No regenerative changes were evident.

Continuing resolution of hepatitis was evident in livers taken from control MCMV-infected mice on day 9 postinfection. Regenerative changes were nearing completion, as livers had fewer mitotic figures and diminished nuclear pleomorphism compared with those examined on day 5 postinfection (Fig. 1G). There was one inflammatory focus per five low-power fields, and small portal lymphocytic infiltrates were present. Livers taken from NK cell-depleted MCMV-infected mice at day 9 were undergoing active regeneration but had continuing focal hepatitis which appeared to be in the early stages of resolution (Fig. 1H). There were many mitotic figures, and the hepatocytes had vesicular cytoplasm and pleomorphic nuclei. There were five inflammatory foci per low-power field, and although there were no viral inclusions, some giant cells were present. In NK cell-depleted, MCMV-infected mice, hepatitis and liver damage at day 9 was more extensive than that seen in control MCMV-infected mice at day 5 postinfection.

Spleens from control MCMV-infected mice at day 9 postinfection were larger than those of uninfected mice (Fig. 1A). The spleens had hyperplasia of the white pulp and floridly reactive germinal centers with immunoblasts and plasma cells present in the marginal zones (Fig. 1C). The red pulp was relatively decreased in amount and contained occasional neutrophils. Cells with viral inclusions were not present. At day 9, spleens from NK cell-depleted, MCMV-infected mice exhibited multifocal necrosis (Fig. 1A). Microscopically, perifollicular necrosis and extensive destruction of the red pulp were seen (Fig. 1D). Multiple small and large thrombi were present in red pulp vessels (Fig. 1D). It is likely that necrosis of the red pulp eventuated in thrombosis of blood vessels with superimposed infarction of large areas of the spleen. Germinal centers were not present, but there were focal aggregates of plasma cells. Cells with viral inclusions were not present.

Although some lungs examined at days 5, 7, and 9 postinfection had mild focal interstitial pneumonitis, there

FIG. 1. (A) Spleens from control MCMV-infected (A1), control uninfected (A2), or anti-asialo GM1–treated, MCMV-infected (A3) mice. The spleen from the control infected mouse (A1) is hyperplastic. The spleen from the anti-asialo GM1–treated, MCMV-infected mouse (A3) shows extensive infarction. Bar, 0.38 cm. (B) Liver lobes from control MCMV-infected (B1), control uninfected (B2), or anti-asialo GM1–treated, MCMV-infected (B3) mice. The liver lobe from the anti-asialo GM1–treated, MCMV-infected mouse (B3) is smaller and pale in color as compared with that of the control MCMV-infected (B1) or control uninfected (B2) mouse. Bar, 0.40 cm. (C) Spleen from control MCMV-infected mouse showing hyperplastic white pulp and intact red pulp. Bar, 100 μm. (D) Spleen from anti-asialo GM1–treated, MCMV-infected mouse with inactive white pulp and necrotic red pulp. Inset shows thromboembolus (arrow) in a splenic vein. Bar, 100 μm. (E) Liver from control mouse at day 5 after MCMV infection, showing regeneration. The hepatocytes have pleomorphic nuclei, and mitoses are frequent (arrows). Few inflammatory cells are seen. Bar, 50 μm. (F) Liver from anti-asialo GM1–treated mouse at day 5 after MCMV infection. The hepatocytes show severe ballooning degeneration. Hepatocytes with viral intranuclear inclusions are present (arrows). Inflammatory cells are sprinkled throughout the liver, and regenerative changes are not yet apparent. Bar, 50 μm. (G) Liver from control mouse at day 9 after MCMV infection. Continuing resolution of the hepatitis is evident. The hepatocyte nuclei remain somewhat pleomorphic, and binucleate cells are frequent. A few inflammatory cells are present in a portal triad (arrow). Bar, 50 μm. (H) Liver from anti-asialo GM1–treated mouse at day 9 after MCMV infection showing resolving hepatitis. The hepatocyte nuclei are somewhat pleomorphic. The ballooning degeneration has resolved. Foci of inflammatory cells still remain. A liver giant cell is present (arrow). Bar, 50 μm.

TABLE 3. NK cell depletion results in spleen leukopenia in MCMV-infected mice[a]

Days post-infection	NK lysis (%)		Viable leukocytes/spleen ($\times 10^6$)	
	Control	NK-depleted	Control	NK-depleted
3	21 ± 3.1	0.0 ± 0.2	62 ± 3.8	52 ± 6.7
5	12 ± 1.2	8.5 ± 1.6	54 ± 1.0	14 ± 1.9
7	13 ± 2.0	8.6 ± 0.4	94 ± 3.3	33 ± 7.0
9	8.3 ± 0.6	6.5 ± 0.3	51 ± 3.8	10 ± 3.5

[a] Mice were challenged with 5×10^3 PFU of MCMV after anti-AGM1 treatment and sacrificed at various days postinfection. NK cell activity against YAC-1 cells was determined at an 11:1 effector/target ratio in a 16-h assay.

was no difference in lung pathology between NK cell-depleted and control mice.

Effect of NK cell depletion on MCMV-induced immunosuppression. Previous results have demonstrated that spleen cells from MCMV-infected mice have a diminished response to the T cell mitogen ConA (20). Table 2 data confirm these results and also show that NK cell depletion enhanced this MCMV-induced suppression but had no effect on the ConA response in uninfected mice. On days 5, 7, and 9 postinfection, mice treated with antibody had ConA responses 13-fold, 5-fold, and 2-fold lower than did control MCMV-infected mice, respectively. NK cell depletion also resulted in MCMV-induced leukopenia. Whereas antibody treatment alone had no detectable effect on spleen cell numbers (12, unpublished data), anti-asialo GM1-treated, MCMV-infected mice had up to fivefold fewer spleen leukocytes than did MCMV-infected controls (Table 3).

Depletion of NK cells on various days postinfection. To determine the time interval during which NK cells were exerting their most potent antiviral effects, we treated groups of mice with anti-asialo GM1 on days 0, 1, and 2 postinfection and compared these groups with control MCMV-infected mice; all mice were sacrificed on day 3 postinfection. No significant differences in liver virus titers were seen between the groups whose NK cells were depleted at day 0, 1, or 2 postinfection, but all three groups had 25- to 50-fold–higher titers than did control MCMV-infected mice (Table 4). In slight contrast to the liver data, depletion of NK cells at day 2 postinfection failed to enhance spleen virus titers as effectively as did depletion at day 0 or 1, but nonetheless, significant enhancement of day 3 virus titers was noted in all NK cell-depleted mice. This indicates that the absence of NK cells between days 2 and 3 and between days 1 and 3 resulted in enhanced day 3 virus titers in the liver and spleen, respectively. This means that NK cells present between days 2 and 3 postinfection were required for the antiviral effect. This period of time corresponds to that necessary for IFN production and activation of NK cells (50). Note that MCMV infection induced higher day 3 plasma IFN titers in anti-asialo GM1-treated mice than in control mice, regardless of whether the antibody was given at day 0, 1, or 2 postinfection (Table 4).

We next tested whether NK cell depletion later in infection (day 6, when spleen virus was still detectable) would have any effect on viral titers in the spleen at day 9 postinfection. The dose of anti-asialo GM1 which totally depleted NK cell activity in our previous experiments (Table 1, day 3) reduced NK cell-mediated lysis by only about 30% when given on day 6 and assayed on day 9 postinfection (Table 5). It also had no effect on day 9 virus titers in the spleen, liver, or lung. This was in contrast to the data we had obtained when giving the antibody 4 to 6 h before infection. This was not an unexpected result, since activated NK cells are more resistant to the effects of anti-asialo GM1 treatment (H. Yang et al., submitted for publication). To address the possibility that day 9 virus titers were unaffected due to incomplete elimination of NK cells, we gave antibody on 3 separate days (days 6, 7, and 8) postinfection or gave none at all, and we sacrificed the mice on day 9. Most of the NK cell activity was removed by this treatment, but mice undergoing this treatment had no significant difference in virus titers as compared with control mice (Table 5). This indicates that NK cells are not necessary for the elimination of virus that occurs in the spleen between days 6 and 9 and that NK cell depletion at days 6 to 9 postinfection does not bring about dissemination to the lung or reappearance of virus in the liver.

Effect of inoculation route on NK cell-mediated resistance. NK cell depletion had similar effects on MCMV synthesis at day 3 postinfection whether the virus was given i.p. or i.v. Differences as great as 1,000-fold were noted in the spleen, and NK cell-depleted mice also had low but detectable virus titers in the lung (Table 6), which would increase by day 5 (Table 1). These data confirm and extend our previous results (6). NK cell-deficient beige mice yielded similar results (Table 6), in accord with the observation of Shellam et al. (50).

In contrast to the above observations, NK cell depletion had no effect on the course of intranasal infection at day 3 postinfection or at later times (Table 7). During the first 5 days after intranasal MCMV infection, virus synthesis occurs only in the lung (21). NK cell depletion had no effect on

TABLE 4. Results of NK cell depletion on day 0, 1, or 2 postinfection: enhanced MCMV titers on day 3 postinfection[a]

Day of anti-AGM1 treatment	% NK lysis	Virus ($\log_{10}$ PFU) per:		Log_2 IFN U per ml of plasma
		Spleen	Liver	
0	3.7 ± 0.4	3.6 ± 0.1	4.5 ± 0.1	11 ± 0.5
1	4.5 ± 0.6	3.3 ± 0.2	4.4 ± 0.1	11 ± 0.5
2	5.8 ± 0.6	1.9 ± 0.1	4.2 ± 0.1	10 ± 0.5
Control	58 ± 2.3	<1.0[b]	2.8 ± 0.1	6.8 ± 1.0

[a] Mice (16) were inoculated i.p. with 5×10^3 PFU of MCMV on day 0 and divided into groups of 4. One group was left untreated, and the other three groups were given anti-AGM1 at day 0, 1, or 2 postinfection. All mice were sacrificed on day 3 postinfection for determination of spleen NK cell activity, virus titers, and IFN titers.

[b] <, One or more mice had no detectable virus in this organ (see text).

TABLE 5. NK cell depletion at days 6 to 9 postinfection: no effect on day 9 MCMV titers

Expt	Anti-AGM1 treatment	% NK lysis	Virus ($\log_{10}$ PFU) per:			
			Spleen	Lung	Gram of liver	Salivary gland
1[a]	–	52 ± 0.4	<1.3[c]	<1.5	<2.5	5.1 ± 0.1
	+	35 ± 3.5	<1.3	<1.5	<2.5	5.2 ± 0.1
2[b]	–	39 ± 0.3	<1.3	1.5 ± 0.1	<2.5	4.8 ± 0.1
	+	5.0 ± 1.0	<1.3	1.5 ± 0.0	<2.5	4.6 ± 0.1

[a] Two groups of four mice were inoculated i.p. with 5×10^3 PFU of MCMV. On day 6 postinfection, when 200 to 500 PFU of virus were present in the spleen, one group (+) was given anti-asialo GM1 (anti-AGM1) i.v. The other group (–) did not receive the anti-AGM1. On day 9, the mice were sacrificed, and virus titrations and NK cell assays were performed.

[b] Same as experiment 1, except that mice were given anti-AGM1 daily on days 6 to 8.

[c] <, One or more mice had no detectable virus in this organ (see text).

TABLE 6. NK cell depletion results: enhancement of MCMV synthesis with i.p. or i.v. inoculation

Expt	Route of infection	Mouse	Anti-AGM1 treatment	% NK lysis	Virus (log_{10} PFU) per:	
					Lung	Spleen
1[a]	i.v.	C57BL/6 (bg/+)	−	61 ± 4.3	ND	2.3 ± 0.2
		(bg/bg)	−	47 ± 4.0	ND	4.4 ± 0.2[b]
2	i.v.	C57BL/6	−	ND	<1.0[d]	1.6 ± 0.3
			+	ND	1.7 ± 0.0	4.9 ± 0.0[b]
3[c]	i.p.	C57BL/6 (bg/+)	−	51 ± 6.1	ND	1.4 ± 0.1
			+	3.8 ± 1.1	ND	4.1 ± 0.1[b]
		(bg/bg)	−	9.5 ± 2.3	ND	3.9 ± 0.2[b]
4	i.p.	C57BL/6	−	30 ± 2.1	<1.0	1.7 ± 0.2
			+	0.2 ± 0.9	1.6 ± 0.1	4.6 ± 0.1[b]

[a] Groups of four mice each either untreated (−) or given anti-AGM1 (+) were inoculated either i.p. or i.v. with 5×10^3 PFU of MCMV 4 to 6 h later. All animals were sacrificed on day 3 postinfection, and virus titers were determined.
[b] $P < 0.001$.
[c] Data in this experiment were published previously (6) and are included in this table for comparison.
[d] <, One or more mice had no detectable virus in this organ (see text).

lung virus titers at any time postinfection, nor did it result in accelerated dissemination to the salivary gland (Table 7) or to any other organ tested (spleen, liver, kidney) (data not shown). In fact, dissemination to any organ except the salivary gland was rarely seen, and these rare instances did not correlate with the presence or absence of NK cells.

NK cell depletion enhanced salivary gland MCMV titers during persistent infection. To examine the role of NK cells in controlling persistent MCMV infection, we treated mice at day 30 postinfection (infectious virus was found only in the salivary gland at this time) with thioglycolate and RPMI or thioglycolate and anti-asialo GM1. The thioglycolate technique was reported by Braughtigam et al. (4) to facilitate rescue of MCMV from latently infected mice. At day 5 after this treatment (day 35 postinfection), the mice were sacrificed; their peritoneal macrophages were cocultivated with mouse embryo fibroblasts, and their spleens, livers, kidneys, blood, lungs, and salivary glands were titrated for virus. MCMV was isolated from all macrophage cultures from both control and NK cell-depleted mice. NK cell depletion had no effect on the rapidity of virus isolations from cocultivated peritoneal macrophages, nor did it result in the reappearance of infectious virus in any of the organs tested (data not shown). However, NK cell depletion resulted in a six- to eightfold increase in salivary gland virus titers in three separate experiments (Table 8). Thus, NK cells may be involved in limiting viral synthesis in the salivary gland during the persistent phase of the infection.

DISCUSSION

The results presented in this paper suggest that NK cells may play a major role in limiting the mortality, morbidity, spleen necrosis, hepatitis, and immunosuppression associated with acute MCMV infection. It is likely that these cells limit the severity and duration of the infection by controlling viral synthesis and dissemination during the first few days of infection. Our experiments (Tables 4 and 5) suggest that NK cells may exert their most effective antiviral action at a time correlating with the onset of peak IFN production (50), NK cell activation (50), and NK cell division (3), i.e., between 24 and 72 h postinfection. Though mice depleted of NK cells 4 to 6 h preinfection had elevated virus titers by days 3 to 9 postinfection (Table 1), mice depleted of NK cells by day 6 postinfection had no detectable virus in their spleens or livers on day 9 (Table 5), suggesting that NK cells may not be necessary for viral clearance later in the course of acute infection, when MCMV-specific T cells are present. Clearance of virus later in infection has been shown by Ho (19) to be a function of *H-2*–restricted, MCMV-specific T cells. NK cell depletion before MCMV infection results in a 500- to 1,000-fold increase in spleen virus titers by day 3 postinfection, before CTL are detectable. The delayed clearance of virus seen at day 9 postinfection in NK cell-depleted mice may simply be due to the large viral antigen load resulting from NK cell depletion early in the infection, or it could also be due to increased virus-induced suppression of T cell function. It is well known that MCMV can suppress T cell function (20, 39) and that the degree of suppression correlates with virus titers (20). In our study, mice depleted of NK cells had higher virus titers and a greater degree of MCMV-induced suppression of the ConA response (Table 2) as compared with control mice. Since the ConA response is a measure of T cell-dependent function, it is likely that viral

TABLE 7. NK cell depletion: no effect on MCMV synthesis in the lung after intranasal inoculation[a]

Days postinfection	Mouse	Anti-AGM1 treatment	% NK lysis	Virus (log_{10} PFU) per:	
				Lung	Salivary gland
3	C57BL/6				
	(bg/+)	−	45 ± 4.0	3.1 ± 0.4	<2.0[b]
	(bg/bg)	−	7.2 ± 1.4	3.0 ± 0.1	<2.0
	C57BL/6	−	63 ± 1.4	3.1 ± 0.1	<2.0
		+	0.9 ± 0.4	3.4 ± 0.1	<2.0
5	C57BL/6				
	(bg/+)	−	37 ± 4.3	4.3 ± 0.1	<2.0
	(bg/bg)	−	4.1 ± 0.9	4.1 ± 0.2	<2.0
7	C57BL/6	−	68 ± 2.4	4.1 ± 0.1	2.8 ± 0.2
		+	37 ± 8.2	4.3 ± 0.2	2.9 ± 0.3
9	C57BL/6	−	26 ± 7.3	5.3 ± 0.2	5.1 ± 0.3
		+	14 ± 4.2	5.4 ± 0.1	5.3 ± 0.2

[a] Groups of four mice each either untreated (−) or given anti-AGM1 (+) were inoculated intranasally with 10^5 PFU of MCMV 4 to 6 h later. Mice were sacrificed at various times postinfection; virus titers and NK cell activities were determined.
[b] <2.0, One or more mice had no detectable virus in this organ (see text).

TABLE 8. NK cell depletion results: enhancement of MCMV titers in persistently infected mice[a]

Expt	Treatment	% NK lysis	Virus (log_{10} PFU) per salivary gland
1	RPMI	26 ± 2.3	4.1 ± 0.3
	Anti-AGM1	2.3 ± 0.2	5.0 ± 0.0[b]
2	RPMI	31 ± 2.8	2.9 ± 0.3
	Anti-AGM1	−1.6 ± 0.4	3.8 ± 0.4[c]
3	RPMI	37 ± 3.4	4.4 ± 0.2
	Anti-AGM1	14 ± 4.0	5.2 ± 0.1[b]

[a] Mice were inoculated i.p with 5×10^3 PFU of MCMV. Groups of 5 mice each were inoculated 30 days later with either RPMI medium or anti-asialo GM1 (Anti-AGM1), followed by 3 ml of 3% thioglycolate broth i.p. These mice were sacrificed 5 days later, and their organs were titrated for virus; spleen NK cell activity was also determined.
[b] $P < 0.001$.
[c] $P < 0.01$.

clearance, also a T cell-dependent function, could be inhibited, resulting in an infection and illness of longer duration.

The consequences of NK cell depletion on the pathogenesis of MCMV infection were profound. Severe ballooning degeneration of hepatocytes has previously been reported in irradiated, MCMV-infected mice (16), but due to the lack of specificity of irradiation, the immune deficit responsible for this degeneration was not determined. Mims and Gould (36) reported spleen necrosis (but not thrombosis and infarction) in MCMV-infected mice. However, even when high inoculating doses are given, mouse strains with genetically high NK cell activity (CBA, C57BL/6, and C57BL/10) (40) do not present with spleen necrosis, whereas strains with genetically low NK cell activity (A, BALB/c) (40) have necrotic spleens (36). Our experiments confirm and extend this correlation, showing that NK cell depletion of C57BL/6 mice followed by a low-dose (5×10^3 PFU) MCMV infection resulted in spleen necrosis.

The absence of NK cells also resulted in dissemination of the virus to the lung. This condition, coupled with immunosuppression, could set the stage for opportunistic infection in humans. Immunocompromised cancer and transplant patients, whose NK cell activity is low, are susceptible to CMV pneumonitis (41, 44). Almost all patients with acquired immunodeficiency syndrome have active CMV infection (28), and although the evidence for CMV as a cause for acquired immunodeficiency syndrome is not convincing, it is certainly possible that lowered NK cell activity could allow CMV to flourish and to exert its immunosuppressive effects, establishing optimal conditions for a putative acquired immunodeficiency syndrome-causing agent or other opportunistic agents to take over.

Whereas NK cell depletion had a dramatic effect on the course of i.p. or i.v. MCMV infection, it had no detectable effect on the course of intranasal infection. NK cells are present in the lung (43), and this laboratory has shown that antibody to asialo GM1 depletes lung NK cell activity and prevents the rejection of NK-sensitive YAC-1 cells from the lung (2). Quinnan et al. (43) have shown that cortisone treatment increases lung MCMV titers during the first 7 days of intranasal infection, and they suggested that this increase was due to the depletion of lung NK cells that they observed. Our present results indicate that NK cells are not mediating resistance during intranasal infection and that some other cortisone-sensitive effector system (not T cells) (43) is responsible for resistance against MCMV in the lung. In support of this concept, Biron et al. (2) have demonstrated that a cortisone-sensitive non-NK cell is responsible for preferential rejection from the lungs of virus-infected cells over uninfected cells in 4-h in vivo cytotoxicity assays.

A recent report (11) had suggested that early IFN production by 6 h postinfection mediated resistance to MCMV infection either directly or via NK cell activation. Our present data show that NK cell depletion with anti-asialo GM1 did not abrogate the early IFN response. Therefore, lack of IFN cannot account for the lowered resistance in the antibody-treated mice.

When administered in vivo, anti-asialo GM1 antibody has no detectable effect on any immune functions tested other than NK cell activity (12, 23, 24). However, it does bind to some monocytes and thymocytes (12), and it could possibly have unknown effects on non-lymphoid tissue. Recent work in our laboratory (unpublished data) provides further indication that it is indeed NK cells which are mediating resistance. Athymic nude mice treated with anti-asialo GM1 have enhanced MCMV titers. Newborn mice (which are NK cell deficient) that received adoptively transferred, control adult spleen cells resisted lethal MCMV infection. The adult cells still protected the mice if the cells were immunochemically depleted of T cells or depleted of macrophages and granulocytes by size separations. Adult cells did not protect if NK cells were removed by antibody to asialo GM1. Selgrade and Osborn (49), using the same adoptive transfer system, also demonstrated that macrophage-depleted adult spleen cells could mediate protection. A potential mechanism for NK cells to control MCMV infection is also suggested by our results (unpublished data) showing that in the presence of IFN, which protects uninfected targets, MCMV-infected cells are much more susceptible to NK cell-mediated lysis.

MCMV has the capacity to establish persistent and latent infections, which can be reactivated under conditions of immunosuppression (22, 34), even in the face of moderately high antibody titers. Although we have so far not demonstrated a role for NK cells in controlling reactivation of latent infection, we have shown that NK cell depletion during the persistent phase of the infection resulted in significant increases in salivary gland virus titers (Table 8) but not in dissemination of the virus from that organ. To our knowledge, this is the first body of evidence in support of a role for NK cells in limiting viral synthesis in a persistent viral infection.

It thus appears that NK cells may play a role in limiting viral synthesis and virus-induced pathology in the early stages of some acute viral infections, but not others, such as lymphocytic choriomeningitis virus (6). The present data suggest that NK cells act early in acute MCMV infection to reduce the viral load at a time period corresponding to that of IFN production and activation of NK cell-mediated cytotoxicity, but before virus-specific antibody and cytotoxic T cells are detectable. An increased viral load in NK cell-depleted mice could lead to dissemination of virus to the lung. More virus leads to enhanced suppression of the T cell response which could likely result in delayed clearance of virus and a prolongation of hepatitis. It thus appears likely that NK cells acting early in the infection can limit the severity, extent, and duration of acute MCMV infection and that they may play a role in controlling persistent infection.

ACKNOWLEDGMENTS

This research was supported by Public Health Research Grants AI 17672 and CA 34461 from the National Institutes of Health and by grant IN-129 from the American Cancer Society. J.F.B. is a recipient of a Massachusetts State Fellowship, and R.M.W. is a

recipient of National Institutes of Health Research Career Development Award AI 00432.

We thank Eva Moring for preparation of the tissue sections and Chris Hebert for photographing the specimens and processing the color photographs.

LITERATURE CITED

1. **Bancroft, G. J., G. R. Shellam, and J. E. Chalmer.** 1981. Genetic influences on the augmentation of natural killer (NK) cells during murine cytomegalovirus infection: correlation with patterns of resistance. J. Immunol. **126**:988–994.
2. **Biron, C. A., S. Habu, K. Okumura, and R. M. Welsh.** 1984. Lysis of uninfected and virus-infected cells in vivo: a rejection mechanism in addition to that mediated by natural killer cells. J. Virol. **50**:698–707.
3. **Biron, C. A., L. R. Turgiss, and R. M. Welsh.** 1983. Increase in NK cell number and turnover rate during acute viral infection. J. Immunol. **131**:1539–1545.
4. **Braughtigam, A. R., F. J. Dutko, L. B. Olding, and M. B. A. Oldstone.** 1979. Pathogenesis of murine cytomegalovirus infection: the macrophage as a permissive cell for cytomegalovirus infection, replication, and latency. J. Gen. Virol. **44**:349–359.
5. **Bukowski, J. F., C. A. Biron, and R. M. Welsh.** 1983. Elevated natural killer cell-mediated cytotoxicity, plasma interferon, and tumor cell rejection in mice persistently infected with lymphocytic choriomeningitis virus. J. Immunol. **131**:991–996.
6. **Bukowski, J. F., B. A. Woda, S. Habu, K. Okumura, and R. M. Welsh.** 1983. Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis *in vivo*. J. Immunol. **131**:1531–1537.
7. **Casali, P., J. G. P. Sissons, M. J. Buchmeier, and M. B. A. Oldstone.** 1981. *In vitro* generation of human cytotoxic lymphocytes by virus. Viral glycoproteins induce non-specific cell-mediated cytotoxicity without release of interferon. J. Exp. Med. **154**:840–855.
8. **Chalmer, J. E., J. S. MacKenzie, and N. F. Stanley.** 1977. Resistance to murine cytomegalovirus linked to the major histocompatability complex of the mouse. J. Gen. Virol. **37**:107–114.
9. **Einhorn, S., H. Blomgrem, and H. Strander.** 1978. Interferon and spontaneous cytotoxicity in man. I. Enhancement of the spontaneous cytotoxicity of peripheral lymphocytes by human leukocyte interferon. Int. J. Cancer. **22**:405–412.
10. **Gidlund, M., H. Orn, H. Wigzell, A. Senik, and I. Gresser.** 1978. Enhanced NK activity in mice injected with interferon and interferon inducers. Nature (London) **273**:759–761.
11. **Grundy (Chalmer), J. E., J. Trapman, J. E. Allan, G. R. Shellam, and C. J. M. Melief.** 1982. Evidence for a protective role of interferon in resistance to murine cytomegalovirus and its control by non-*H-2*-linked genes. Infect. Immun. **37**:143–150.
12. **Habu, S., H. Fukui, K. Shimamura, K. Okumura, and N. Tamaoki.** 1981. *In vivo* effects of anti-asialo GM1. Reduction of NK activity and enhancement of transplanted tumor growth in mice. J. Immunol. **127**:34–38.
13. **Hanshaw, J. B.** 1971. Congenital cytomegalovirus infection: a fifteen year perspective. J. Infect. Dis. **123**:555–561.
14. **Hanshaw, J. B., R. F. Betts, G. Simon, and R. C. Boynton.** 1965. Acquired cytomegalovirus infection: association with hepatosplenomegaly and abnormal liver function tests. N. Engl. J. Med. **272**:602–609.
15. **Hedley-White, E. T., and J. E. Craighead.** 1965. Generalized cytomegalovirus inclusion disease after renal homotransplantation. N. Engl. J. Med. **272**:473–475.
16. **Henson, D., R. D. Smith, and J. Gehrke.** 1966. Non-fatal mouse cytomegalovirus hepatitis. Combined morphologic, virologic, and immunologic observations. Am. J. Pathol. **49**:871–888.
17. **Henson, D., and A. J. Strano.** 1972. Mouse cytomegalovirus: necrosis of infected and morphologically normal submaxillary gland acinar cells during termination of chronic infection. Am. J. Pathol. **68**:183–202.
18. **Herberman, R. B., M. E. Nunn, H. T. Holden, S. Staal, and J. Y. Djeu.** 1977. Augmentation of natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic targets. Int. J. Cancer. **19**:555–564.
19. **Ho, M.** 1980. Role of specific cytotoxic lymphocytes in cellular immunity against murine cytomegalovirus. Infect. Immun. **27**:767–776.
20. **Howard, R. J., and J. S. Najarian.** 1974. Cytomegalovirus-induced immune suppression. II. Cell-mediated immunity. Clin. Exp. Immunol. **18**:119–126.
21. **Jordan, M. C.** 1978. Interstitial pneumonia and subclinical infection after intranasal inoculation of murine cytomegalovirus. Infect. Immun. **21**:275–280.
22. **Jordan, M. C., J. D. Shanley, and J. G. Stevens.** 1977. Immunosuppression reactivates and disseminates latent murine cytomegalovirus. J. Gen. Virol. **37**:419–423.
23. **Kasai, M., M. Iwamosi, Y. Nagai, K. Okumura, and T. Tada.** 1980. A glycoprotein on the surface of mouse natural killer cells. Eur. J. Immunol. **10**:175–180.
24. **Kawase, I., D. L. Urdal, C. G. Brooks, and C. S. Henney.** 1982. Selective depletion of NK cell activity in vivo and its effect on the growth of NK sensitive and NK resistant tumor cell variants. Int. J. Cancer **29**:567–574.
25. **Klemola, F., and L. Kaariainen.** 1965. Cytomegalovirus as possible cause of disease resembling infectious monucleosis. Br. J. Med. **2**:1099–1104.
26. **Kumagai, K., K. Itoh, R. Suzuki, S. Hinuma, and F. Saitoh.** 1982. Studies of murine large granular lymphocytes. I. Identification as effector cells in NK and K cytotoxicities. J. Immunol. **129**:388–394.
27. **Kumar, V., and M. Bennett.** 1981. Genetic resistance to Friend virus-induced erythroleukemia and immunosuppression. Curr. Top. Microbiol. Immunol. **92**:65–82.
28. **Lane, H. C., H. Masur, L. C. Edgar, G. Whalen, A. H. Rook, and A. S. Fauci.** 1983. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. **309**:453–458.
29. **Lee, G. D., and R. Keller.** 1982. Natural cytotoxicity to murine cytomegalovirus-infected cells mediated by mouse lymphoid cells: role of interferon in the endogenous natural cytotoxicity reaction. Infect. Immun. **35**:5–12.
30. **Levy-Leblond, E., and J. M. Dupuy.** 1978. Neonatal susceptibility to MHV infection in mice. I. Transfer of resistance. J. Immunol. **118**:1219–1222.
31. **Lopez, C.** 1981. Resistance to herpes simplex virus type I. Curr. Top. Microbiol. Immunol. **92**:15–24.
32. **MacFarlan, R. I., W. H. Burns, and D. O. White.** 1977. Two cytotoxic cells in peritoneal cavity of virus-infected mice: antibody-dependent macrophages and non-specific killer cells. J. Immunol. **119**:1569–1574.
33. **Manischewitz, J. E., and G. V. Quinnan, Jr.** 1980. Antivirus antibody-dependent cell-mediated cytotoxicity during murine cytomegalovirus infection. Infect. Immun. **29**:1050–1054.
34. **Mayo, D. R., J. A. Armstrong, and M. Ho.** 1977. Reactivation of murine cytomegalovirus by cyclophosphamide. Nature (London) **267**:721–723.
35. **Michelson-Fiske, S.** 1977. Human cytomegalovirus. A review of developments between 1970 and 1976. Biomedicine **26**:16–22.
36. **Mims, C. A., and J. Gould.** 1978. Splenic necrosis in mice infected with cytomegalovirus. J. Infect. Dis. **137**:587–591.
37. **Nedrud, J. G., A. M. Collier, and J. S. Pagano.** 1979. Cellular basis of susceptibility to murine cytomegalovirus: evidence from tracheal organ culture. J. Gen. Virol. **45**:737–744.
38. **Osborn, J. E.** 1975. Diseases caused by human cytomegaloviruses, p. 1–10. *In* Practice of pediatrics. Harper & Row, Publishers, Inc., New York.
39. **Osborn, J. E., A. A. Blazkovec, and D. I. Walker.** 1968. Immunosuppression during acute murine cytomegalovirus infection. J. Immunol. **100**:835–844.
40. **Petranyi, G., R. Kiessling, and G. Klein.** 1975. Genetic control of "natural" killer lymphocytes in the mouse. Immunogenetics **2**:53–61.
41. **Quinnan, G. V., N. Kirmani, A. H. Rook, J. F. Manischewitz, L.**

Jackson, G. Moreschi, G. W. Santos, R. Saral, and W. H. Burns. 1983. Cytotoxic T cells in cytomegalovirus infection. HLA-restricted T lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone marrow transplant recipients. N. Engl. J. Med. **307**:7–13.

42. **Quinnan, G. V., J. F. Manischewitz, and F. A. Ennis.** 1978. Cytotoxic T lymphocyte response to murine cytomegalovirus infection. Nature (London) **273**:541–543.
43. **Quinnan, G. V., Jr., J. E. Manischewitz, and N. Kirmani.** 1982. Involvement of natural killer cells in the pathogenesis of murine cytomegalovirus interstitial pneumonitis and the immmune response to infection. J. Gen. Virol. **58**:173–180.
44. **Rand, K. H., R. B. Pollard, and T. C. Merigan.** 1978. Increased pulmonary superinfection in cardiac-transplant patients undergoing primary cytomegalovirus infection. N. Engl. J. Med. **258**:951–953.
45. **Rapp, F.** 1983. The biology of cytomegaloviruses, p. 1–66. *In* B. Roizman (ed.), The herpesviruses, vol. 2. Plenum Publishing Corp., New York.
46. **Reed, L. J., and H. Meunch.** 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. **39**:493–497.
47. **Rinaldo, C. R., Jr., W. D. Carney, B. S. Richter, P. H. Black, and M. S. Hirsch.** 1980. Mechanisms of immunosuppression in cytomegaloviral mononucleosis. J. Infect. Dis. **141**:488–495.
48. **Selgrade, M. J., and J. E. Osborn.** 1973. Divergence of mouse brain interferon response following virulent or avirulent Newcastle disease virus inoculation. Proc. Soc. Exp. Biol. Med. **143**:12–18.
49. **Selgrade, M. K., and J. E. Osborn.** 1974. Role of macrophages in resistance to murine cytomegalovirus. Infect. Immun. **10**:1383–1390.
50. **Shellam, G. R., J. E. Allan, J. M. Papadimitriou, and G. J. Bancroft.** 1981. Increased susceptibility to cytomegalovirus infection in beige mutant mice. Proc. Natl. Acad. Sci. U.S.A. **78**:5104–5108.
51. **Tardieu, M., C. Hery, and J. M. Dupuy.** 1980. Neonatal susceptibility to MHV_3 infection in mice. II. Role of natural resistance. J. Immunol. **124**:418–423.
52. **Trinchieri, G., and D. Santoli.** 1978. Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. J. Exp. Med. **147**:1314–1333.
53. **Vassali, J. D., A. Granelli-Paperno, C. Griscelli, and E. Reich.** 1978. Specific protease deficiency in polymorphonuclear leukocytes of Chediak-Hagashi syndrome and beige mice. J. Exp. Med. **147**:1285–1290.
54. **Welsh, R. M.** 1978. Cytotoxic cells induced during lymphocytic choriomeningitis virus infection of mice. I. Characterization of natural killer cell induction. J. Exp. Med. **148**:163–181.
55. **Welsh, R. M., and R. M. Zinkernagel.** 1977. Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice. Nature (London) **268**:646–648.
56. **Wong, C. Y., J. J. Woodruff, and J. F. Woodruff.** 1977. Generation of cytotoxic T lymphocytes during Coxsackie B-3 infection. III. Role of sex. J. Immunol. **119**:591–597.