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Role of Macrophages in Resistance to Murine Cytomegalovirus

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The role of macrophages in protecting mice from murine cytomegalovirus (MCMV) was studied in Swiss, CBA/J, and C57BL/6J mice. CBA/J mice were more resistant to virus than were C57BL/6J mice at all ages tested. Prior treatment of adult Swiss mice with 60 mg of silica, a dose selectively toxic to macrophages, increased mortality due to MCMV infection. Transfer of syngeneic adult macrophages to suckling mice significantly increased their resistance to subsequent MCMV infection. Transfer of syngeneic, nonimmune adult lymphocytes to suckling mice also had a lesser but significant protective effect against subsequent MCMV challenge. In vitro infection of adult CBA/J and C57BL/6J macrophages with virulent and attenuated MCMV resulted in productive infection in only a small percentage of cells and recovery of very little virus from the extracellular fluid. Infection of CBA macrophages was no less productive than C57BL/6J nor was infection with virulent virus more productive than with attenuated virus. Histological examination of the livers of MCMV-infected CBA/J and C57BL/6J mice suggested that divergent cellular immune responses to infection might account for differences in susceptibility. It is postulated that the macrophage may facilitate the inductive phase of cellular immunity, one possible explanation for its demonstrated importance in host defenses against MCMV.

Numerous studies have demonstrated the importance of the "macrophage" in host defenses against a variety of viral infections (3, 5-7, 17, 23). (As in most of the literature, the term "macrophage" is used throughout this paper to refer to that population of mononuclear leukocytes which adhere to glass. This terminology is adopted for the sake of simplicity and is not intended to suggest that macrophages are the only cells present in this population, nor that they are solely responsible for the activities observed.) In the case of herpes simplex virus (HSV), a picture of macrophage function has developed based on studies of suckling mice, which develop lethal encephalitis due to infection, and of adult mice, which are resistant to infection. It appears that macrophage maturation is a contributing factor in this age-dependent resistance, since transfer of syngeneic adult macrophages to suckling mice protected them (6), whereas impairment of macrophage activity in adult mice with silica or antimacrophage serum allowed lethal infection to occur (23). Also, macrophages from suckling mice supported growth and spread of HSV in vitro whereas macrophages from adult mice did not (7), suggesting that the ability of HSV to multiply in and spread from suckling macrophages results in lethal infection and that changes occur during macrophage maturation that prevent HSV multiplication, thus protecting the animal from lethal infection.

Murine cytomegalovirus (MCMV), another member of the *Herpesvirus* family, is similar to HSV in that suckling mice are much more susceptible to infection than are adults (9). Since reticuloendothelial organs are the site of maximal involvement in the early stages of virulent, but not of attenuated, MCMV infection (15), and virulent MCMV has been shown to suppress clearance of Newcastle disease virus from the blood stream (12), it seemed reasonable to test the possibility that the macrophage is also an important host defense mechanism in MCMV infection. Therefore, in the present experiments, the influence of macrophages on the course of MCMV infection was studied in vivo, both in suckling mice pretreated with syngeneic adult macrophages and in adult mice pretreated with silica. In addition, in vitro infection of macrophages from two strains of mice—a resistant strain (CBA/J) and a susceptible strain (C57BL/6J)—was studied to deter-

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mine whether variations in the ability of macrophages to support viral replication might account for differences in the susceptibility of these two strains as well as the differences between virulent and attenuated MCMV.

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MATERIALS AND METHODS

Mice. Outbred Swiss mice were obtained from ARS Sprague-Dawley (Madison, Wis.). Swiss mice (free of known viral pathogens) used to generate virus pools were obtained from National Laboratory Animal Co. (Creve Coeur, Mo.). CBA/J and C57BL/6J (referred to subsequently as CBA and C57BL) inbred strains of mice and (CBA/J × C57BL/6J)F_1 hybrid mice were obtained from Jackson Laboratory (Bar Harbor, Me.).

Viruses. MCMV of the Smith strain maintained by mouse passage was used in these studies. Pools of virulent and attenuated MCMV (the latter passaged nine times in tissue culture) were prepared as previously described, with the exception that virus-containing materials were stored in medium which contained 10% dimethylsulfoxide (Me_2SO) as stabilizer instead of sorbitol (14).

To obtain a pool of mouse-adapted HSV, an HF strain which had been passed an undetermined number of times in Vero cells was passed four times in primary mouse embryo cell culture (MECC). After each passage virus was harvested by freezing and thawing infected cells three times. The resultant pool of HSV was stored in small samples at −70 C.

Media and reagents. MECC were grown in medium 199 containing 10% calf serum, 5% lactoalbumin hydrolysate, and 0.06% sodium bicarbonate ($NaHCO_3$). Cells were maintained in medium containing 5% calf serum and 0.15% $NaHCO_3$.

Adherent peritoneal exudate cells (APEC) were harvested in Eagle minimal essential medium containing 20% fetal calf serum and 1 mmol of glutamine per liter. APEC were maintained in the same medium, to which 0.15% $NaHCO_3$ was added. Splenocytes were cultured in medium 199 containing 5% fetal calf serum and 0.06% $NaHCO_3$. Maintenance medium containing 0.8% tragacanth (10) was used for overlay. All media contained 200 U of penicillin and 200 μg of streptomycin per ml.

For fixation of cells, a solution of ethanol-acetic acid-formaldehyde (6:2:1) was used; crystal violet staining facilitated viral plaque visualization on fixed monolayers.

Silica was prepared by mixing 1.5 g of silica powder (325 mesh, Sargent-Welch Co.) in 400 ml of saline. The solution was allowed to settle for 15 min, and supernatant fluid was decanted and centrifuged for 15 min at 1500 rpm. The pellet was then resuspended in a small volume of saline, sterilized, put through a membrane filter (Millipore Corp.) of known weight, and adjusted to a concentration of 60 mg/ml with sterile saline. Particle size was approximately 2 μm by microscopic examination.

Neutral red staining of peritoneal exudate cells. Peritoneal exudate suspension was placed on slides coated with neutral red dye, covered with a cover glass, and incubated for 15 to 20 min at 37 C. Monocytes were distinguished microscopically from other cells with criteria described by Sabin (19); in particular, the cytoplasm of the macrophage was filled with fine, uniform particles that stained darker than neutrophilic granules, whereas lymphocytes in general had a clear cytoplasm.

Tissue culture. Primary and secondary MECC were prepared as previously described (14). To obtain APEC ("macrophages"), mice were sacrificed, the skin over the abdomen was removed, and 4 ml of medium was injected into the intact peritoneal cavity. The abdomen was massaged gently, and 3 ml of fluid was removed through a 20-gauge needle into a syringe. This fluid contained approximately 10^6 cells/ml, of which 50 to 60% were phagocytic as determined by neutral red staining. Fluids from four to five mice were pooled, 0.15% $NaHCO_3$ was added, and 3-ml samples were distributed into Falcon plastic tissue culture tubes (16 by 125 mm; for in vitro infection). After 12 to 18 h of incubation at 37 C in an atmosphere of 5% CO_2, nonadherent cells were removed by washing vigorously several times with 0.85% sterile saline. Cells were refed with maintenance medium. The remaining adherent cells were 95 to 99% phagocytic, as determined by neutral red staining.

"Stimulated" APEC were obtained by injecting 2 ml of thioglycolate medium by the intraperitoneal (i.p.) route 48 to 72 h prior to harvest of peritoneal cells; 70 to 80% of the 10^6 to 10^7 cells/ml obtained in this manner were phagocytic. Peritoneal fluids were processed as previously described, except 15-ml samples were dispensed in Falcon plastic tissue culture flasks (75 cm^2) along with 15 ml of maintenance medium (for transfer studies).

Splenocytes were suspended by forcing spleens and a small amount of medium through a wire mesh, diluted appropriately, and placed in plastic tissue culture flasks (75 cm^2). After 12 to 18 h of incubation, nonadherent cells were shaken into the medium which was then centrifuged lightly; pelleted cells were resuspended to desired volume. Adherent cells were washed several times and removed with a rubber policeman into saline, pelleted, and resuspended appropriately.

Cell transfer studies. Stimulated APEC obtained from 7-week-old male C57BL mice were suspended in cold saline, 10^6 viable cells per 0.05 ml (as determined by counting cells which excluded 0.025% trypan blue). Adherent cells cultured from the spleens of the same mice, and nonadherent cells from those spleens, were processed as previously described and resuspended at concentrations of 10^6 and 10^7 cells per 0.05 ml, respectively. Litters of suckling mice 4 to 5 days old were randomized and divided into groups which received APEC, adherent spleen cells, nonadherent spleen cells, or diluent. Twenty-four hours later all mice were challenged with 2,000 plaque-forming units of MCMV. Some litters were observed for morbidity

and mortality. Others were sacrificed for assay of virus titers in liver and spleen.

Determination of virus titers in various organs. In all virus assay experiments, organs from two mice were pooled, ground with sterile sand with mortar and pestle, and resuspended to 10% (wt/vol) in maintenance medium containing 10% Me_2SO. Extracts were stored at −70 C for subsequent virus assay of 0.5-ml samples on duplicate MECC monolayers as previously described (15).

Silica pretreatment studies. Mice were divided into three treatment groups (all injections were by the i.p. route) as follows: group 1 received 60 mg of silica and 2 h later received MCMV; group 2 received saline and 2 h later received MCMV; and group 3 received 60 mg of silica and 2 h later received diluent. Mice were observed for illness and death; at 3 and 5 days after infection, pairs of animals were sacrificed for virus assay of liver and spleen.

Infection of macrophages in vitro and subsequent assays for MCMV. APEC from 6-week-old adult CBA and C57BL mice were cultured in Falcon tubes and infected 24 h after harvest with 10^5 PFU of virulent or attenuated MCMV in 0.2 ml of maintenance medium. Virus was allowed to adsorb for 2 h, and then maintenance medium was added. At 3-day intervals, medium was changed, and spent medium was assayed for virus. Infectious center assays were also carried out on cultures from both strains infected with both types of virus. At 3-day intervals, cells were scraped from duplicate tubes into 1 ml of cold saline; viable cells were counted in the presence of trypan blue, diluted, and placed on duplicate MECC monolayers in 0.5 ml of maintenance medium. After allowing 1 h for cells to adhere, overlay was added and cells were incubated for 5 days at 37 C and then washed, fixed, and stained for enumeration of plaques representing infectious centers.

RESULTS

Relative susceptibility of various strains of mice to MCMV. Before considering how alterations in the macrophage population might affect the course of MCMV infection in mice, the susceptibility of several strains of mice to MCMV was tested. Two inbred strains (CBA and C57BL), the F_1 hybrid of these two strains, and one outbred Swiss strain were chosen for this purpose. Mice at various ages were infected by the i.p. route with various doses of MCMV and subsequently observed for mortality. Figure 1 shows the relative susceptibility of mice of various strains and ages by comparing the doses required to obtain 50% mortality (mean lethal dose) in each group. The F_1 hybrid resistance pattern resembled the CBA parent, whereas the outbred Swiss strain, like the C57BL strain, was more susceptible to virus. It should be stated that the dose range between 0 and 100% mortality was very narrow; i.e., the dose capable of killing 100% of the mice in a group was never more than four times that which killed none of the mice. At all ages tested, the dose which killed 100% of C57BL mice failed to kill any CBA animals. Figure 1 also shows that within a given strain resistance increased with age.

Groups of 3-week-old CBA and C57BL mice were also inoculated by the i.p. route with various doses of HSV. The mean lethal dose for CBA and C57BL mice using this virus were not measurably different: 5 and 8 PFU, respectively. This suggests that the factor(s) determining susceptibility of the two strains to MCMV is not a general property applicable to other herpesviruses.

Effects of silica treatment of MCMV infection. It has been demonstrated that silica provides selective toxicity for one cell type —macrophages (1, 16). Therefore, an experiment was designed in which silica was used to decrease macrophage activity, on the premise that this might enhance MCMV infection. Adult Swiss mice were injected i.p. with either 60 mg of silica in 1 ml of saline or with diluent. Two hours later, mice were challenged i.p. with MCMV. At 3 and 5 days after infection, two mice in each group were assayed for MCMV in liver and spleen extracts; the remaining mice were observed for mortality.

Table 1 shows data from such an experiment; the greatest difference in mortality between silica-treated mice and those receiving only virus occurred at a dose of 4×10^5 PFU of

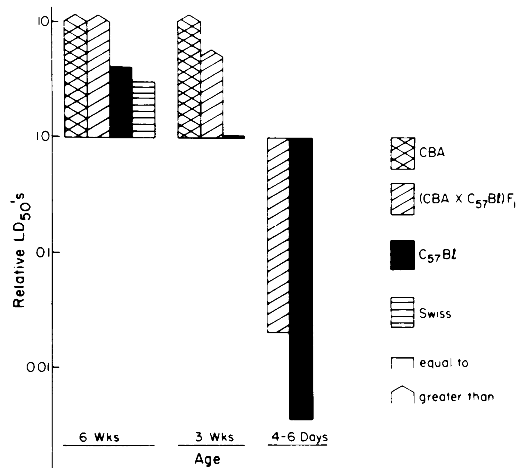

FIG. 1. *Relative susceptibility of various strains and ages of mice to MCMV infection. Mean lethal dose (LD_{50}) values are expressed as factors of a standard LD_{50} (2×10^5 PFU) which was obtained for C57BL weanling mice. Pointed tops indicate an LD_{50} greater than that indicated for instances in which the highest dose available did not kill 50% of the mice.*

TABLE 1. *Effects of silica treatment on the percent mortality in adult Swiss mice infected with MCMV*[a]

Dose (PFU) of virus	Mortality of 6-week-old mice (%)	
	Treated with silica, and virus	Treated with virus only
6×10^5	100	90
4×10^5	75	12
3×10^5	0	0

[a] Eight adult Swiss mice were injected by the i.p. route with 55 to 60 mg of silica 2 h prior to infection with MCMV. An equal number of controls received diluent 2 h prior to infection. Mice were then observed for mortality.

MCMV; the narrow range between lethal and nonlethal doses was again evident. At all doses of virus, titers of MCMV in liver extracts at 3 days after infection were 4- to 20-fold higher in mice that received silica than in animals that did not. Titers of virus in the liver dropped sharply by 5 days in mice that received virus only, whereas mice that had received silica still had levels of virus as high or higher than those observed 3 days after infection. In general, treatment with silica had little effect on the amount of virus detected in spleens of mice at either interval. In most cases, deaths occurred slightly earlier in silica-treated mice, although the difference was usually not greater than 24 h. Mice that received silica without virus remained healthy.

It was postulated that if the difference in susceptibility of CBA and C57BL mice were due to some difference in macrophage activity, treatment of CBA mice with silica might make these animals as susceptible to MCMV as C57BL mice. Therefore, 3-week-old CBA mice were inoculated i.p. with 60 mg of silica. Two hours later, these mice, as well as groups of untreated CBA and C57BL mice, received various doses of MCMV i.p. Although treatment with silica lowered the dose of virus necessary to produce 100, 50, and 0% mortality in CBA mice, these mice were still considerably more resistant than untreated C57BL mice (Table 2).

MCMV infection in suckling mice previously treated with syngeneic adult macrophages. The observed effects of silica suggested that decreasing macrophage activity had an enhancing effect on the course of MCMV multiplication and lethality. The greater lethality of MCMV for suckling mice relative to adults was also previously demonstrated (Fig. 1). To determine the effects of enhanced macrophage activity on the course of MCMV infection, 4- to 5-day-old C57BL mice were inoculated with approximately 10^6 adult, syngeneic macrophages 24 h prior to infection with MCMV. One group of control mice was inoculated with 10^7 to 10^8 adult, syngeneic lymphocytes (nonadherent cells) 24 h before infection; a second control group was not treated before infection. Mice were then observed for mortality. At 2, 4, 6, and 8 days after infection, two mice from each group were sacrificed for virus assay of spleen and liver.

Pretreatment of suckling mice with macrophages before MCMV infection resulted in a significant decrease in the number of deaths which occurred compared to mice which received only MCMV ($P < 0.025$) (Fig. 2); P values were calculated by the binomial test. Also, the time at which deaths occurred was slightly delayed in mice pretreated with macrophages. Pretreatment with lymphocytes conferred a significant degree of protection ($P < 0.05$); however, macrophages conferred a greater degree of protection than lymphocytes ($P < 0.05$) (Fig. 2). Unlike the silica experiments, there was no significant difference in titers of virus in either spleen or liver extracts, despite the significant differences in mortality.

In vitro infection of macrophages from adult CBA and C57BL mice with virulent and attenuated MCMV. Since previous experiments demonstrated that macrophage activity in vivo is an important component of the host defense mechanism against MCMV, attempts were made to assess differences in the ability of various macrophages to support the replication of virus. Macrophages from adult CBA and C57BL mice were infected with 10^5 PFU of virulent or attenuated MCMV in vitro. At 3-day intervals thereafter, extracellular fluids were assayed for virus, and cells were assayed for infectious centers. At 3 days after infection, in all groups only a small percent of infected

TABLE 2. *Effects of silica treatment of the percent mortality in CBA weanling mice*[a]

Mouse strain	Silica treatment	Doses (PFU) yielding:		
		90-100% mortality	50-60% mortality	0-10% mortality
CBA	0	8×10^5	4×10^5	2×10^5
CBA	55 to 60 mg	4×10^5	2×10^5	1×10^5
C57BL	0	8×10^4	5×10^4	3×10^4

[a] Three-week-old CBA mice were injected by the i.p. route with 55 to 60 mg of silica 2 h prior to infection with MCMV. Equal numbers of CBA and C57BL mice received diluent 2 h prior to infection. Mice were then observed for mortality.

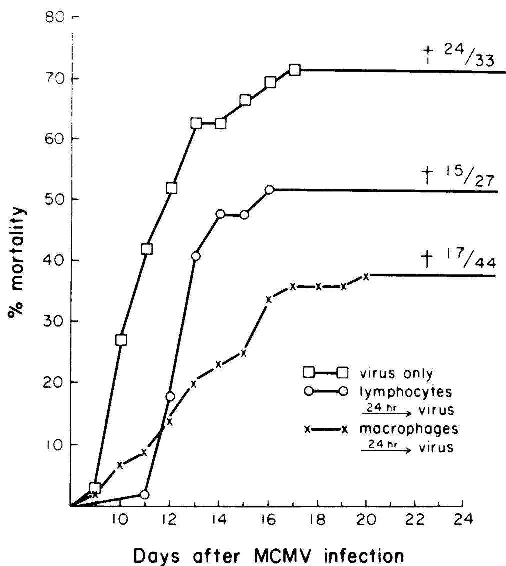

FIG. 2. *Effects of pretreatment with syngeneic adult macrophages or lymphocytes on the percent and distribution of mortality after MCMV infection in C57BL suckling mice. Four- to six-day-old mice were inoculated with 10^6 adult syngeneic macrophages 24 h prior to infection with 2,000 PFU of MCMV. Controls received either 10^7 to 10^8 adult, syngeneic lymphocytes, or were not treated prior to infection. Mice were observed for mortality. The data graphed is a composite of several experiments. Total number of deaths per total number of animals is represented for each group. The percentage of mortality in the macrophage-treated group is significantly different from that in the group which received virus only ($P < 0.025$). The percentage of mortality in the lymphocyte-treated group is significantly different from that in the other two groups ($P < 0.05$).*

macrophages could be detected (Fig. 3). The proportion of infected cells increased with time in culture so that by 9 days as many as 50% of the cells were infected. Results between experiments were quite variable, and there was no indication of more widespread infection with virulent virus or in C57BL cells, as one might have expected if pathogenicity were directly related to the ability of the host macrophage to support viral multiplication. Very little virus was detected in the extracellular fluid of any of the cultures studied; the yield was generally at least 10-fold lower than the initial inoculum.

Detection of histological changes in organs from MCMV infected CBA and C57BL mice. To further compare CBA and C57BL mice, 3-week-old animals from each strain were inoculated with a dose of virus calculated to produce 50% mortality in C57BL mice. Two animals from each group were sacrificed at daily intervals 1 to 6 days after infection; the spleens, livers, brains, lungs, and thymuses were removed, fixed in formalin, sectioned, and stained with hematoxylin and eosin. No pathological changes were observed in thymus or brain sections. In both strains, comparable interstitial inflammation was observed in sections of lung, and changes similar to those previously described (13, 18) were observed in spleen.

In contrast, livers from C57BL mice exhibited histological changes different from those seen in CBA livers. Intranuclear inclusions characteristic of cytomegalovirus were detectable in liver sections from both strains 1 day after infection (Fig. 4); but although inclusion-bearing cells seen in C57BL liver were surrounded by inflammatory cells, the same cytomegalic cells in CBA liver sections were not. Inclusion-bearing cells in the absence of inflammatory foci were ob-

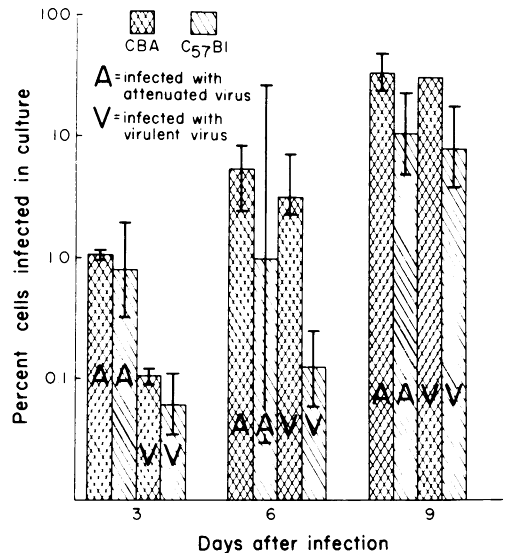

FIG. 3. *In vitro infection of macrophages from adult CBA and C57BL mice with virulent and attenuated MCMV: infectious center assay. Macrophage cultures were infected with 10^5 PFU of either 9th-passage attenuated MCMV or virulent MCMV. At 3, 6, and 9 days after infection, macrophages from two cultures were removed from their tubes, counted, diluted, and placed on duplicate MECC monolayers. Macrophage-monolayer preparations were incubated at 37 C for 1 h before overlay was added. Plaques were counted after 5 days of incubation at 37 C. Each plaque represented an infected macrophage in the initial culture. The average number of plaques, times the dilution factor, was divided by the total number of cells in the original culture to obtain the percent of cells infected (infectious centers). Averages and ranges of the number of infectious centers obtained from various experiments are plotted.*

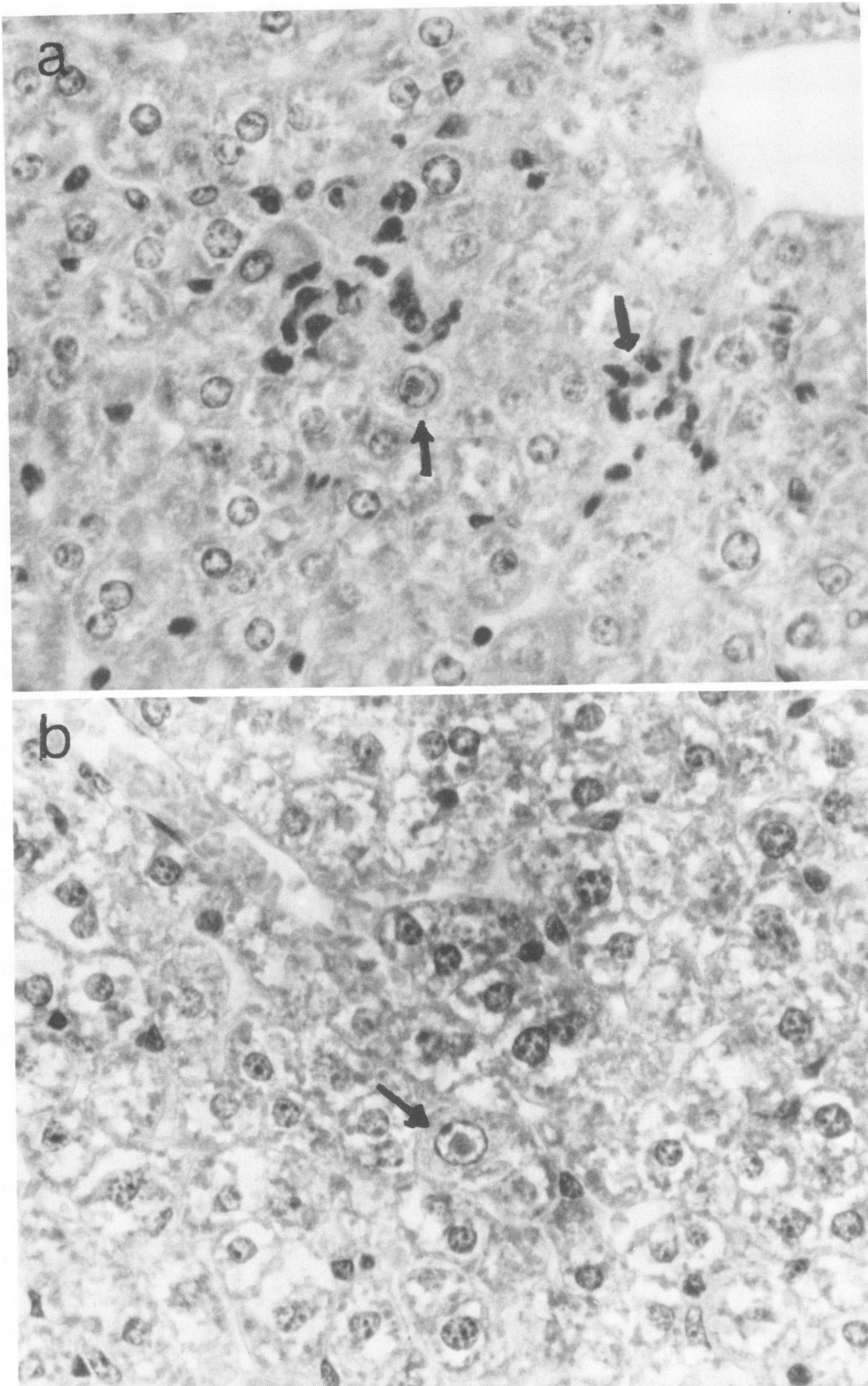

FIG. 4. *Hematoxylin and eosin stained sections of C57BL (a) and CBA (b) liver taken 1 day after MCMV infection. Three-week-old CBA and C57BL mice were inoculated with MCMV, and livers were removed from two mice in each group at 1 day post-infection, fixed in formalin, sectioned, and stained with hematoxylin and eosin. (a) C57BL liver section with an intranuclear inclusion characteristic of cytomegalovirus; and the inclusion-bearing cell is surrounded by inflammatory cells; (b) a similar inclusion in a section of CBA liver; however, there are no signs of inflammation (×250).*

served in liver sections from CBA mice throughout the 6-day period examined, whereas inclusions were always accompanied by inflammation in C57BL liver sections. Patterns of hepatic infiltration also differed, in that inflammatory foci tended to be scattered throughout the liver in both portal and parenchymal foci in C57BL mice; by contrast, inflammation in CBA mice was notably confined to portal areas and even in those areas was not as extensive as that seen in C57BL mice.

DISCUSSION

The data presented here demonstrated that CBA mice were more resistant to MCMV at all ages than were C57BL mice of comparable age, and that, as previously shown (9), susceptibility to MCMV is inversely proportional to age. Studies of resistant and susceptible strains of mice to both mouse hepatitis virus and several togaviruses—formerly group B arboviruses—demonstrated that susceptibility to these viruses is inherited by an autosomal dominant trait (8). In contrast, the present study of MCMV infections showed that the response of (CBA × C57BL)F_1 hybrid mice to MCMV resembled that of the resistant CBA parent, suggesting that the mechanisms responsible for resistance to mouse hepatitis virus and group B arboviruses are not the same as those responsible for resistance to MCMV infection. This finding contrasts with data presented by Diosi et al. (4), in which susceptibility to a wild strain of MCMV inoculated by the intracerebral route seemed to be inherited as an autosomal dominant trait. Probably the mechanisms of resistance necessary for prevention of infection initiated intracranially are different from those which mediate i.p. infection. The present data also show that there is no difference in the susceptibility of CBA and C57BL mice to HSV, suggesting that the defense mechanisms against HSV also differ from those against MCMV even though these two viruses are closely related.

Whereas the mechanisms of resistance of MCMV and HSV appear to be different, the macrophage seems to be important to both. Data from the present study yielded results similar to in vivo experiments with HSV (6, 23), in that prior treatment with silica—which is selectively toxic for macrophages (1, 16)—increased the susceptibility of adult Swiss mice to MCMV and increased titers of virus in the livers of these mice; transfer of syngeneic adult macrophages to C57BL suckling mice increased their resistance to MCMV infection.

Nachkov et al. (11) demonstrated that C57BL macrophages phagocytized a synthetic polypeptide more strongly than did CBA macrophages, suggesting that differences do exist between macrophages from these two strains. However, although treatment of weanling CBA mice with silica resulted in increased susceptibility to MCMV, even these treated mice were more resistant than untreated C57BL weanling animals. Therefore, it appears that, although differences in macrophage activity may be partially responsible for the difference in susceptibility of CBA and C57BL mice to MCMV, other factors must also be important.

A number of studies have demonstrated that macrophages from suckling mice are less active than adult macrophages in several respects, including (i) their ability to produce interferon in response to HSV (6) and antibody in response to sheep erythrocytes (2; W. Braun and L. H. Lasky, Fed. Proc. **26**:642, 1967); (ii) their ability to prevent growth and spread of several viruses (7, 23); and (iii) their ability to respond to proteose peptone stimulation (6). The increased resistance to MCMV of suckling mice pretreated with adult macrophages suggests that some activities of the mature macrophage are vital to host defenses against MCMV. The delay observed in the onset of deaths which did occur in macrophage-treated suckling mice relative to untreated animals resembles the delay in onset of deaths seen as a result of tissue-culture attenuation of virus relative to virulent virus (15). It is possible that transfer of macrophages curtails viral infection in some organs but diverts it to others in which lethal damage may eventually occur.

It is notable that transfer of nonimmune adult lymphocytes also provided some degree of protection to suckling mice, although not to the extent observed after transfer of macrophages. This is in contrast to experiments with HSV where adult lymphocytes had no protective effect for suckling mice (6), and suggests another difference in host response to these two closely related viruses.

Although results from the in vivo experiments in this study corresponded, for the most part, with studies using HSV instead of MCMV, results of in vitro experiments differed from Johnson's studies (7) which showed that HSV could replicate in and spread from macrophages derived from susceptible suckling mice, but not from resistant adult mice. In vitro infection of macrophages with MCMV demonstrated that macrophages from resistant CBA mice were at least as susceptible to infection as those from susceptible C57BL animals and that infection with attenuated virus was not less productive

than with virulent MCMV. In general, only a very small percentage of macrophages appeared to be productively infected initially. This was also the result of several attempts to infect suckling macrophages (unpublished data), and contrasts with results reported by Tegtmeyer and Craighead (20) in which extracellular MCMV was recovered from mouse macrophages infected in vitro at concentrations 10- to 100-fold higher than the inoculum. Reasons for this difference in findings are unclear.

The in vitro studies do not support the concept of a pivotal role for the macrophage per se in controlling viral replication and dissemination. Instead, comparison of the histological changes in livers of MCMV-infected CBA and C57BL mice suggests that the difference in susceptibility may be related to the type of cellular immune response which occurs, since the inflammatory response in C57BL livers was much greater than that observed in CBA livers. The importance of the macrophage in resistance to MCMV may be related to its role in the inductive phase of cellular immunity. In this regard, the macrophage may act to present and/or process antigen for recognition by thymus-derived lymphocytes (T cells) (21, 22). In the case of MCMV infection, a rapid response to the antigen might result in resolution of infection before enough antigen is produced to make a cellular immune response lethally destructive. Macrophages may also act to remove excess antigen which might be capable of eliminating isolated T cells, thus tolerizing the animal (21).

The results of this study show that, although the macrophage is important to host defenses against MCMV, this does not correlate with inability of virus to replicate and spread from these cells as is the case with HSV infection. Some other functions of the macrophage—possibly related to the inductive phase of cellular immunity—must be responsible for the importance of this cell to host defenses against MCMV.

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