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Effect of Murine Cytomegalovirus Infection on Mitogen Responses in Genetically Resistant and Susceptible Mice

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Suppression of the blastogenic response of spleen cells was found during murine cytomegalovirus infection of the genetically susceptible BALB/c and also the more resistant BALB.K strains of mice. These results were observed for both the T-cell mitogen concanavalin A and the B-cell mitogen lipopolysaccharide. As the viral inoculum was increased, there was greater immunosuppression within each strain, the time of maximum depression coinciding with peak virus titers in the spleen. Although both strains developed similar splenic virus titers and exhibited a similar decrease in the proportion of splenic T-lymphocytes, there was greater suppression of the mitogenic response during sublethal infection of the more susceptible BALB/c strain. The suppression could not be readily accounted for by the presence of suppressor cells or by a change in sensitivity to mitogen. The results suggest that the extent of immunosuppression induced by murine cytomegalovirus is determined in part by host genotype.

The role of virus-induced immunodepression in the pathogenesis of cytomegalovirus (CMV) infection has not been elucidated. Studies in the murine model show that both the humoral (8, 18) and cell-mediated immune (7, 24) responses are impaired, but the mechanism is not understood. Immunosuppression could result from direct lysis or change in function of infected lymphocytes, and small numbers of infected B-cells (15, 17), T-cells (29), and macrophages (16) have been found during acute infection. Alternatively, the virus could indirectly affect the immune response; these mechanisms fall into three categories (reviewed in reference 9): (i) alteration in lymphocyte traffic, (ii) alteration in macrophage function, and (iii) augmentation of suppressor cell activity. The effect of CMV on lymphocyte traffic has not been investigated, although a change in peripheral blood leukocyte number was noted in infected mice (19) and a decrease in the percentage of T-cells has been observed during congenital infection in humans (3, 23). It is clear that some macrophage functions are altered as phagocytosis is impaired during murine cytomegalovirus (MCMV) infection (27), but little evidence has been found for cells suppressing mitogen responses in MCMV-infected mice (24; this study), although suppressor cells have been reported during CMV-induced mononucleosis in humans (21).

Resistance of mice to a lethal dose of MCMV has been shown to be under genetic control and to be determined by both *H-2*- and non-*H-2*-associated genes (2, 4). It is not clear at present whether the *H-2*-linked control acts on the immune response or through the susceptibility of cells to infection. However, it has been postulated that death due to MCMV infection is a consequence of events following the depression of T-cell function (1). If this postulate proves correct, it would suggest that the extent of immunodepression induced by CMV may be genetically determined.

The studies presented in this paper examine the extent of immunodepression to mitogen stimulation in strains of mice genetically resistant or susceptible to MCMV. These data are correlated with the severity of the infection and are used in an examination of some of the mechanisms which may be responsible for immunodepression.

MATERIALS AND METHODS

Mice. Female outbred Canberra mice, highly inbred BALB/c (*H-2*^{d}), C3H/HeJ (*H-2*^{k}), and the BALB congenic strains carrying the *H-2*^{k} (BALB.K) or *H-2*^{b} (BALB.B) haplotype were bred at the University of Western Australia and used at 12 to 15 weeks of age.

Cell culture. Mouse embryo cultures were prepared from 14- to 15-day-old fetal Canberra mice by trypsin dispersion and were grown in Dulbecco essential modified medium (GIBCO, Grand Island, N.Y.) supplemented with penicillin, streptomycin, and 10% fetal calf serum (FCS; Flow Laboratories, Stanmore, N.S.W., Australia). Maintenance medium was Dulbecco essential modified medium containing 2% FCS.

Virus. The Smith strain of MCMV was maintained by passage in weanling BALB/c female mice. Salivary

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glands were harvested 17 days postinfection (p.i.) and stored at −70°C as a 50% homogenate in Dulbecco essential modified medium with 10% FCS.

Virus titrations. As described elsewhere (4), the minimum lethal dose of virus (LD_{50}) was calculated from the Kaerber equation, using five mice per serial twofold dilution, and plaque assays were carried out on secondary cultures of mouse embryo cultures with a 2% methylcellulose overlay (2). Virus titers in tissues were determined with 10% homogenates of tissue pooled from three mice, prepared in cold Dulbecco essential modified medium with 10% FCS, and stored at −70°C.

Inoculation schedules. Mice were inoculated intraperitoneally with 0.1 ml of virus suspension or normal salivary gland homogenate in mouse osmolarity phosphate-buffered saline. Inoculations were staggered so that all animals of one strain were harvested on the same day.

Preparation of cell suspensions. Single-cell suspensions were prepared from minced and sieved spleens in cold RPMI 1640 (adjusted to mouse osmolarity and containing 10% FCS; GIBCO Laboratories, Grand Island, N.Y.). Erythrocytes and dead cells were removed as described elsewhere (25, 28). Cell suspensions from normal or infected mice were at least 95 or 90% viable, respectively, as determined by trypan blue exclusion. Thymocyte suspensions were prepared similarly except that erythrocyte removal was omitted.

Mitogen stimulation. Spleen cells (2×10^5 in 0.2 ml) were added to the wells of microtest trays (no. 3042; Falcon Plastics, Oxnard, Calif.) which contained 10 μl of a range of doses of mitogen (concanavalin A [ConA], 1.25 to 10.00 μg/ml; lipopolysaccharide [LPS], 100 to 500 μg/ml) per well. Four replicates were used, and control wells contained cells but no mitogen. The mitogens were ConA (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) and LPS (batch no. 3107-25, derived from *Shigella flexneri*, and also batch no. 3926-25 from *Escherichia coli*; Difco Laboratories, Detroit, Mich.). After 24 h at 37°C in a humid atmosphere of 10% CO_2, 0.2 μCi of ^{3}H-labeled 6T-thymidine (5 Ci/mmol; The Radiochemical Centre, Amersham, U.K.) was added to each well, and incubation was continued for a further 24 h. Cultures were harvested onto filter papers, using a multiple harvester (Skatron, Norway), which were transferred to 5 ml of 4% Permafluor-1 (Packard Instrument Co., Inc., Rockville, Md.) in toluene for liquid scintillation counting. Percent depression of the response was calculated from the number of disintegrations per minute (dpm) as follows: 100 × 1 − [(mean dpm of stimulated infected − mean dpm of unstimulated infected)/(mean dpm of stimulated uninfected − mean dpm unstimulated uninfected)].

Estimation of B-cells, T-cells, and macrophages. B-cell numbers were determined by immunofluorescence. To 1.5×10^6 spleen cells in 0.05 ml of mouse osmolarity phosphate-buffered saline containing 2% FCS was added 0.05 ml of an appropriate dilution of rabbit anti-mouse gamma globulin (Behringwerke, West Germany) for 1 h at 4°C. After three washes the cells were incubated for 30 min at 4°C in 0.05 ml of an appropriate dilution of fluorescein isothiocyanate-conjugated goat anti-rabbit globulin (GIBCO Laboratories). After three washes, the percentage of cells showing membrane fluorescence was determined by counting at least 400 cells. Sodium azide (0.02%) was present throughout the assay.

T-cell numbers were estimated by a ^{51}Cr release assay. Spleen cells (20×10^6) were incubated with 200 μCi of $Na_2{}^{51}CrO_4$ (The Radiochemical Centre) for 1 h at 37°C. After three washes, 2×10^5 labeled cells in 0.05 ml were added to each well of a microtiter tray followed by 0.05 ml of a 1/12 dilution of anti-Thy 1.2 antiserum (Searle, High Wycombe, U.K.) and 0.05 ml of rabbit complement, these dilutions being determined in preliminary experiments. It was found that the antiserum killed 100% of thymocytes, 37% of spleen cells from BALB/c nu/+ mice, and <1% from nu/nu mice. Six replicates were used with control wells containing cells and complement or medium only. In the absence of complement, anti-Thy 1.2 serum was not toxic. Total releasable counts were determined by the addition of 0.1 ml of 0.5% Triton X-100 to labeled cells. After 1 h at 37°C the trays were centrifuged at 200 × *g* for 5 min, and 0.1 ml of supernatant was harvested from each well for counting. The percent ^{51}Cr released was taken as a measure of the percent T-cells killed, as follows (counts per minute [cpm]): percent ^{51}Cr released = {[test (cpm) − complement control (cpm)]/[total release (cpm) − complement control (cpm)]} × (100%/1).

The number of macrophages present in spleen cell suspensions was estimated on smears stained for the presence of nonspecific esterase (26).

Statistics. The significance of differences between results obtained with animals inoculated with MCMV or normal salivary gland homogenate was calculated by using Student's *t* test.

RESULTS

Mitogen responsiveness during mild sublethal infection. The blastogenic response of genetically susceptible BALB/c (*H-2*^{d}; relative LD_{50} = 1.0) mice was compared with the resistant congenic BALB.K (*H-2*^{k}; relative LD_{50} = 10.2) strain during a mild sublethal MCMV infection ($10^{4.0}$ PFU per mouse). Figure 1 shows the spleen cell responses to the T-cell mitogen ConA and the B-cell mitogen LPS. The peak response depicted occurred with the same mitogen concentration (ConA, 2.5 to 5.0 μg/ml; LPS, 200 μg/ml) in both strains on each day after infection.

In BALB/c mice the response to ConA was 70% depressed compared with controls on day 2, maximally depressed (84%; $P < 0.001$) on day 4, and 43% depressed on day 8 (Fig. 1A). The LPS response was also lower than that of control cells on days 2 (93%) and 4 (91%) but was recovering by day 8 (23% depression; Fig. 1C). In contrast, whereas BALB.K mice exhibited a depressed mitogenic response with kinetics similar to that of BALB/c mice (Fig. 1B and D), the extent was much less marked. Thus, compared with controls the maximum depression to ConA which occurred on day 4 was 45% ($P < 0.001$) and the response had almost recovered by day 6 (Fig. 1B). With LPS there was 56% depression

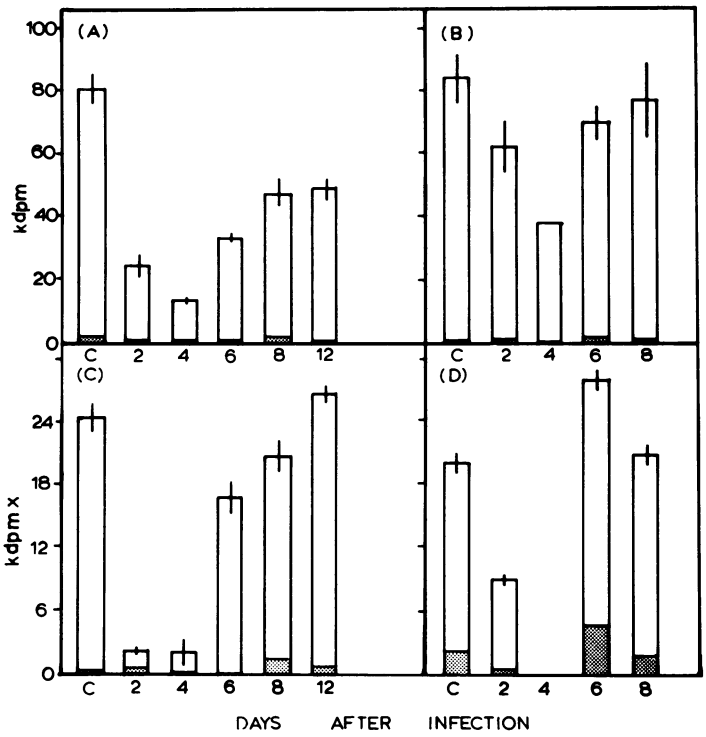

FIG. 1. Mitogenic response, after mild sublethal infection with $10^{4.0}$ PFU per mouse, to stimulation by ConA (A, B) and LPS (C, D) of BALB/c mice (A, C) and BALB.K mice (B, D). Stippled areas represent the response of unstimulated cells, bars represent standard errors, and C is the result from control mice inoculated with normal salivary gland homogenate. Four replicate samples were assayed from three pooled spleens at each time point.

by day 2 ($P < 0.001$) and recovery by day 6 (Fig. 1D).

LPS responsiveness during severe acute infection. Animals were inoculated with $10^{5.2}$ PFU per mouse, equivalent to 2 LD_{50} in BALB/c mice, a dose which kills all BALB/c mice on days 4 to 6 p.i. The same virus concentration caused a severe, nonlethal infection in the BALB.K strain.

There was an almost total abrogation of the response of BALB/c spleen cells to LPS (96% depression; $P < 0.005$) on day 2, which continued on days 4 and 5, the days of greatest mortality (Fig. 2A). In contrast, depression of the BALB.K response was less marked (Fig. 2B), although it was significantly reduced compared with control values by day 2 ($P < 0.001$). Greatest depression was observed on day 4, with a rapid recovery and greater than normal response by day 12 p.i. Again, the peak response was obtained at the same mitogen concentration on each day after infection.

When the dose of virus was increased to $10^{6.3}$ PFU per mouse, all BALB/c mice died on days 3 to 4 p.i. and all BALB.K mice died on days 3 to 5 p.i. Severe depression of the response to LPS was observed on days 1 and 2 in both strains (Fig. 2C and D) over a range of mitogen concentrations.

Examination for the presence of suppressor cells. The possibility that virus-induced suppression of blastogenesis was mediated by suppressor cells was examined with mixtures of 10:0 to 0:10 spleen cells from normal and virus-infected ($10^{4.0}$ PFU per mouse) mice taken on the day of maximum depression of blastogenesis. Each assay was carried out separately, and therefore the results of cells from uninfected mice differ. The mixing of cells from infected and uninfected mice of the susceptible BALB/c or BALB.B ($H\text{-}2^b$) strain of mice (relative LD_{50} = 1.3) showed no evidence for the action of suppressor cells on the response to ConA (Fig. 3) or LPS (data not shown). Cells were also taken at 20 days p.i. from the more resistant BALB.K strain, which had by then recovered from the infection, and were mixed with cells from BALB.B mice at the time of maximum depression of the BALB.B cellular response to mitogen (day 4 p.i.). Again, no evidence was obtained for the presence of suppressor cells in either population in the response to either ConA (Fig. 3) or LPS (data not shown).

Titer of MCMV. With inocula of 10^4 or $10^{5.6}$

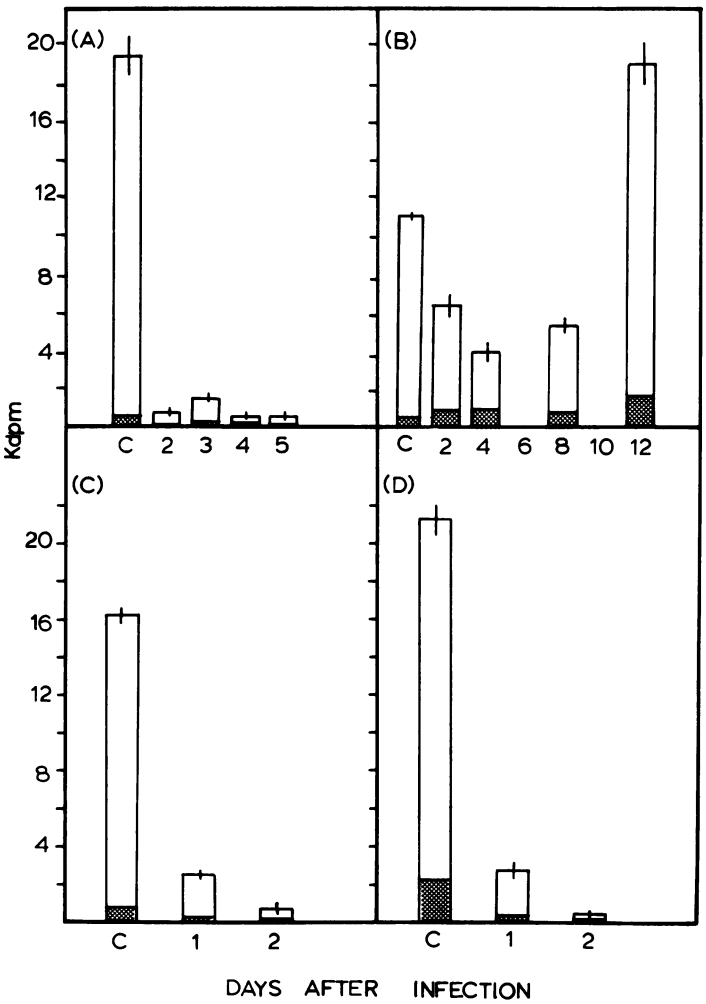

FIG. 2. LPS response after severe acute infection with $10^{5.2}$ PFU per mouse (A, B) or lethal infection with $10^{6.3}$ PFU per mouse (C, D) of BALB/c (A, C) and BALB.K (B, D) mice. Stippled areas represent standard errors, and C is the result from control mice inoculated with normal salivary gland homogenate. Four replicate samples were assayed from four to six pooled spleens.

PFU per mouse, the titer of infectious virus was determined on days 1, 2, 3, 5, 7, and 9 p.i., and the peak titer occurred on days 2 to 3 in both liver and spleen, coinciding with the time of greatest immunodepression. During mild sublethal infection (inoculation of 10^4 PFU per mouse) similar titers (Table 1) were found on day 3 in the spleens of BALB.K mice (1.7×10^4 PFU per spleen) and BALB/c mice (2.5×10^4 PFU per spleen), although much greater depression of the mitogen response was found in the latter strain (Fig. 1). Mice of both strains were also given a severe acute infection, using a dose of $10^{5.6}$ PFU per mouse, which kills all BALB/c mice by days 4 to 5 but at which all BALB.K mice survive. There was an increase in the total amount of virus found in the spleens and livers in each strain (Table 1). Although the titer of virus in the spleen was similar in both strains (BALB/c, 4.6×10^5 PFU per spleen; BALB.K, 7.4×10^5 PFU per spleen), there was complete suppression of the mitogen response in BALB/c but not BALB.K mice with this virus dose (Fig. 2). The difference in resistance between the two strains to the severe acute infection is reflected in the virus titers of the liver. There was 15-fold less virus in the livers of BALB.K compared with BALB/c mice on day 3. However, liver titers were similar in both strains during the mild sublethal infection.

Spleen weight and density. During mild sublethal infection (10^4 PFU per mouse) spleens from BALB/c and BALB.K mice increased in weight (data not shown). Although in the BALB/c strain

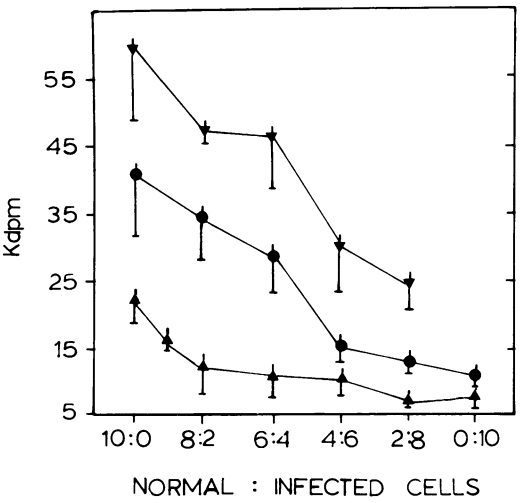

FIG. 3. Mitogenic response (± standard deviation) after stimulation by ConA spleen cells from: ▼, BALB/c mice inoculated with normal salivary gland homogenate and mixed with cells from CMV-infected BALB/c mice (taken 4 days p.i.); ▲, BALB.B cells treated and mixed as before, with BALB.B cells; ●, BALB.K mice (taken 20 days p.i.) mixed with cells from CMV-infected BALB.B mice (taken 4 days p.i.). The total spleen cell number was 2×10^5 cells per culture throughout.

the increase was not statistically significant due to variations in weight caused by the onset of splenic necrosis in some animals, the increase was significant in BALB.K mice on days 4 to 12 p.i. ($P < 0.025$ to $P < 0.005$), with no macroscopic evidence of splenic necrosis in this strain. Spleen density decreased in both strains and was lower than that of controls at 12 days p.i. During an infection which was lethal to BALB/c and BALB.K mice ($10^{6.3}$ PFU per mouse), the mean spleen weight and density decreased in both strains (data not shown).

Proportion of B-cells, T-cells, and macrophages. B-cells, T-cells, and macrophages were quantified in the spleen, as a change in their proportions could account for the decrease in mitogen responsiveness. There was no significant change in the percentage of macrophages in either BALB/c or BALB.K mice during severe acute infection ($10^{5.2}$ PFU per mouse). It should be noted, however, that the proportion of macrophages varied from 5 to 10% even in preparations from control animals. The number and percentage of B-cells were normal on each day in BALB.K mice (controls, 31%; day 3 p.i., 30%), and although there was a fourfold decrease in the total number of B-cells in the BALB/c strain by day 3 p.i., their percentage was almost normal at this time (controls, 27%; day 3 p.i., 20%).

In contrast, the proportion of T-cells in the spleen did alter during MCMV infection (Table 2). In BALB/c mice it decreased from 42 to 17% by day 3 of a severe acute infection, whereas in BALB.K there were only 19% T-cells on day 1, with a return to normal levels by day 3. The proportion of T-cells was examined further during a mild sublethal infection (10^4 PFU per mouse) in two resistant strains of mice, BALB.K and C3H/HeJ. The proportion of T-cells was again found to have fallen dramatically. There were only 11% T-cells in the spleens of BALB.K mice (Table 2) and 19% T-cells in C3H/HeJ mice on day 2 p.i. By day 4 these percentages had increased to 35 and 21% in the BALB.K and C3H/HeJ strains, respectively.

The total number of thymocytes was counted in BALB/c mice during mild sublethal infection. Their number decreased by day 1 and continued to fall until day 4, the last day of assay. There were 83, 33, 23, and 9×10^6 leukocytes per thymus in control and day 1, 2, and 4 animals, respectively. The thymus weight did not change during this period. The number of leukocytes in

TABLE 1. Virus titer in the liver and spleen during mild sublethal[a] and severe acute[b] infection

Organ/mouse strain	PFU/organ[c] during:			
	Mild sublethal infection		Severe acute infection	
	Day 2 p.i.	Day 3 p.i.	Day 2 p.i.	Day 3 p.i.
Spleen				
BALB/c	1.3×10^4	2.5×10^4	6.6×10^5	4.6×10^5
BALB.K	3.5×10^3	1.7×10^4	5.4×10^5	7.4×10^5
Liver				
BALB/c	2.1×10^4	3.3×10^4	4.3×10^6	3.2×10^6
BALB.K	1.3×10^4	1.1×10^4	1.8×10^5	1.8×10^5

[a] Mice infected with 10^4 PFU of MCMV.
[b] Mice infected with $10^{5.6}$ PFU of MCMV.
[c] From three pooled organs.

the peripheral blood of these animals also changed, with means of 9.2×10^6 leukocytes per ml of blood in control animals and 9.4×10^6, 2.2×10^6, and 4.9×10^6 on days 1, 2, and 4, respectively, in infected BALB/c mice.

DISCUSSION

Although mice of a variety of strains and of different ages have been used in separate studies (1, 7, 10, 24) the effect of MCMV on the mitogenic response has not been compared in mice of resistant and susceptible phenotypes. The *k* haplotype of the *H-2* complex, and undefined non-*H-2* genes present in C57BL and C3H mice confer relative resistance to MCMV (4). We have examined the effect of the *H-2* haplotype on virus-induced suppression of mitogenic responses, using adult BALB/c ($H\text{-}2^d$; relative LD_{50} = 1.0) mice and the more resistant congenic BALB.K ($H\text{-}2^k$; relative LD_{50} = 10.2) strain.

It was shown that at a given virus concentration there was greater suppression of the response of spleen cells from BALB/c mice than from the more resistant BALB.K strain, that the period of maximum depression coincided with peak virus titers in the spleen in contrast to a previous suggestion (9), and that within each strain there was greater suppression of blastogenesis as the viral inoculum was increased. This last observation confirms a previous report that used DBA/2 mice (1) and an in vitro model of MCMV infection (12). In addition to these effects, which appear to be directly related to the virus, we also observed the apparent influence of host genes in modulating the depression of the blastogenic response during MCMV infection. Thus, with both a mild sublethal and a severe acute infection there was a greater depression of blastogenesis in BALB/c than in BALB.K spleen cells despite the fact that both strains developed similar virus titers in the spleen and exhibited a similar decrease in the proportion of splenic T-lymphocytes. These results suggest that immunosuppression by MCMV is determined in part by the host genotype. A range of mitogen concentrations was used to exclude the possibility that virus infection merely altered the mitogen dose requirement for optimal lymphocyte stimulation.

It has been suggested that the extent of immunodepression does not correlate with the severity of spleen lesions and that immunosuppression is observed before the time of peak virus synthesis in the spleen (9). Our data do not allow a firm conclusion to be drawn about the relationship between splenic necrosis and the extent of immunodepression. However, it was noted that some of the BALB/c but none of the BALB.K mice developed splenic necrosis during the sublethal infection. During lethal infection both strains exhibited a decrease in spleen weight and complete suppression of blastogenesis. The time of greatest suppression also coincided with peak virus titers in the spleen.

The mechanism of virus-induced immunosuppression is unclear. In vitro studies have shown that suppression can be induced by the interaction of MCMV and spleen leukocytes alone (12), and since less than 1% of the cells were infected, direct cellular damage was considered to be an unlikely explanation of immunosuppression in this model (11). We observed that BALB/c and BALB.K mice which exhibit different degrees of

TABLE 2. T-cell number during MCMV infection

Day p.i.	Strain	Severe acute infection[a]			Strain	Mild sublethal infection[b]		
		Leukocytes ($\times 10^6$)[c]	T-cells ($\times 10^6$)[c]	% T-cells		Leukocytes ($\times 10^6$)[c]	T-cells ($\times 10^6$)[c]	% T-cells
Control	BALB/c	75	31	42	BALB.K	51	23	46
	BALB.K	76	31	40	C3H	25	9	36
1	BALB/c	52	18	34				
	BALB.K	93	18	19				
2					BALB.K	48	5	11
					C3H	13	3	19
3	BALB/c	25	5	17				
	BALB.K	68	29	43				
4					BALB.K	58	20	35
					C3H	27	6	21

[a] $10^{5.2}$ PFU per mouse.
[b] $10^{4.0}$ PFU per mouse.
[c] Per spleen. Mean number from a pool of three to six spleens.

immunosuppression had similar titers of MCMV in the spleen, suggesting that in vivo the splenic titer of MCMV is not directly related to the extent of immunosuppression.

Selective infection of leukocyte subpopulations might cause depression, and MCMV has been found in splenic macrophages (16), B-cells (15, 17), and circulating T-cells (29). In the present study, there was no significant change in the percentage of splenic macrophages or B-cells but there was a decrease in the proportion of T-lymphocytes. A change in T-cell number has also been observed during human CMV infection (3, 23) and could explain the depressed blastogenesis to ConA stimulation. However, the more resistant BALB.K and C3H mice showed a similar change, a phenomenon also observed in resistant and susceptible strains of mice shortly after infection with *Listeria monocytogenes* (14). Impairment of macrophage functions which are necessary for optimal blastogenesis to mitogens (5, 22) would also cause suppression of mitogenesis, but the effect of MCMV-infected macrophages on the response to mitogens has yet to be resolved (12, 24). A correlation has been drawn recently between the peak number of adherent cells infected in vivo and the time of maximum immunosuppression (13).

The possible role of suppressor cells in immunosuppression during CMV infection is also controversial. Depression of mitogen responsiveness mediated by a plastic-adherent mononuclear cell has been reported in cultures on peripheral blood leukocytes from patients with CMV mononucleosis (21). In the present study, resistant and susceptible mice were examined for the action of suppressor cells by a method which had been used to detect suppressor cells in trypanosome-infected mice (20). No evidence was found for cell-mediated inhibition of blastogenesis, and similar results have been reported previously for MCMV-infected BALB/c mice (24).

Although the effect of MCMV-induced immunosuppression on survival of the infection is not clear, it has been postulated that death due to MCMV is a consequence of events after the depression of T-cell function (1). However, we observed greater depression of both T- and B-cell blastogenesis in BALB/c than in the more resistant BALB.K mice. Furthermore, the contribution of specific immunity to resistance to MCMV is hard to evaluate because deaths occur 3 to 6 days p.i., before specific immunity is well established (6).

As the degree of impairment of response to mitogens, in mice of the BALB/c background, appears to reflect the resistance status of the animal, then the suggestion (10) that immunosuppression during MCMV infection is a normal component of the regulation of immune responsiveness may prove to be incorrect. The use of strains of mice which differ in resistance, in the titer of infectious virus in selected organs, and in the immune response to MCMV will be a useful means of dissecting the relative roles of the factors contributing to immunosuppression.

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LITERATURE CITED

1. **Booss, J., and E. F. Wheelock.** 1977. Progressive inhibition of T-cell function preceding clinical signs of cytomegalovirus infection in mice. J. Infect. Dis. **135**:478–481.
2. **Chalmer, J. E., J. S. Mackenzie, and N. F. Stanley.** 1977. Resistance to murine cytomegalovirus linked to the major histocompatibility complex of the mouse. J. Gen. Virol. **37**:107–114.
3. **Fiorilli, M., M. C. Sirianni, A. Spaziani, F. Aluti, and A. Pana.** 1978. Immune responses in cytomegalovirus infection. Lancet **i**:780–781.
4. **Grundy, J. E., J. S. Mackenzie, and N. F. Stanley.** 1980. Genetic control of murine cytomegalovirus infection. I. Influence of *H-2* and non-*H-2* genes of resistance. Infect. Immun. **32**:277–286.
5. **Habu, S., and M. C. Raff.** 1977. Accessory cell dependence of lectin-induced proliferation of mouse T lymphocytes. Eur. J. Immunol. **7**:451–457.
6. **Ho, M.** 1980. Role of specific cytotoxic lymphocytes in cellular immunity against murine cytomegalovirus. Infect. Immun. **27**:767–776.
7. **Howard, R. J., J. Miller, and J. S. Najarian.** 1974. Cytomegalovirus-induced immune suppression. II. Cell-mediated immunity. Clin. Exp. Immunol. **18**:119–126.
8. **Howard, R. J., and J. S. Najarian.** 1974. Cytomegalovirus-induced immune suppression. I. Humoral immunity. Clin. Exp. Immunol. **18**:109–118.
9. **Hudson, J. B.** 1979. The murine cytomegalovirus as a model for the study of viral pathogenesis and persistent infections. Arch. Virol. **62**:1–29.
10. **Kelsey, D. K., G. A. Olsen, J. C. Overall, Jr., and L. A. Glasgow.** 1977. Alteration of host defense mechanisms by murine cytomegalovirus infection. Infect. Immun. **18**:754–760.
11. **Loh, L., and J. B. Hudson.** 1979. Interaction of murine cytomegalovirus with separated populations of spleen cells. Infect. Immun. **26**:853–860.
12. **Loh, L., and J. B. Hudson.** 1980. Immunosuppressive effect of murine cytomegalovirus. Infect. Immun. **27**:54–60.
13. **Loh, L., and J. B. Hudson.** 1981. Murine cytomegalovirus infection in the spleen and its relationship to immunosuppression. Infect. Immun. **32**:1067–1972.
14. **Mandel, T. E., and C. Cheers.** 1980. Resistance and susceptibility of mice to bacterial infection. III. Histopathology of listeriosis in resistant and susceptible strains. Infect. Immun. **30**:851–861.
15. **Mims, C. A., and J. Gould.** 1978. Splenic necrosis in mice infected with cytomegalovirus. J. Infect. Dis. **137**:587–591.
16. **Mims, C. A., and J. Gould.** 1978. The role of the macrophage in mice infected with cytomegalovirus. J. Gen. Virol. **41**:143–153.
17. **Olding, L. B., F. C. Jensen, and M. B. A. Oldstone.** 1975. Pathogenesis of cytomegalovirus infection. I. Activation of virus from bone-marrow derived lymphocytes by *in*

vitro allogeneic reaction. J. Exp. Med. **141**:561–572.
18. **Osborn, J. E., A. A. Blazkovec, and D. L. Walker.** 1968. Immunosuppression during acute murine cytomegalovirus infection. J. Immunol. **100**:835–844.
19. **Osborn, J. E., and N. T. Shahidi.** 1973. Thrombocytopenia in murine cytomegalovirus infection. J. Lab. Clin. Med. **81**:53–63.
20. **Pearson, T. W., G. E. Roelants, M. Pinder, L. B. Lundin, and K. S. Mayor-Withey.** 1979. Immune depression in trypanosome infected mice. III. Suppressor cells. Eur. J. Immunol. **9**:200–204.
21. **Rinaldo, C. R., W. P. Carney, B. S. Richter, P. H. Black, and M. S. Hirsch.** 1980. Mechanisms of immunosuppression in cytomegaloviral mononucleosis. J. Infect. Dis. **141**:488–485.
22. **Rosenstreich, D. L., J. J. Farrar, and S. Dougherty.** 1976. Absolute macrophage dependency of T lymphocyte activation by mitogens. J. Immunol. **116**:131–139.
23. **Schauf, V., A. J. Strelkauskas, and A. Deveikis.** 1976. Alteration of lymphocyte subpopulations with cytomegalovirus infection in infancy. Clin. Exp. Immunol. **26**:478–483.
24. **Selgrade, M. K., A. Ahmed, K. W. Sell, M. E. Gershwin, and A. D. Steinberg.** 1976. Effect of murine cytomegalovirus on the *in vitro* response of T and B cells to mitogens. J. Immunol. **116**:1459–1465.
25. **Shortman, K., N. Williams, and P. Adams.** 1972. The separation of different cell classes from lymphoid organs. V. Simple procedures for the removal of cell debris, damaged cells and erythroid cells from lymphoid cells. J. Immunol. Methods **1**:273–287.
26. **Stuart, A. E., J. A. Habeshaw, and A. E. Davidson.** 1978. Phagocytes *in vitro*. *In* D. M. Weir (ed.), Handbook of experimental immunology, 3rd ed. Blackwell Scientific Publications, London.
27. **Tegtmeyer, P. J., and J. E. Craighead.** 1968. Infection of adult mouse macrophage *in vitro* with mouse cytomegalovirus. Proc. Soc. Exp. Biol. Med. **129**:690–694.
28. **Von Boehmer, H., and K. Shortman.** 1973. The separation of different cell classes from lymphoid organs. IX. A simple and rapid method for removal of damaged cells from lymphoid cell suspensions. J. Immunol. Methods **2**:293–301.
29. **Wu, B. C., and M. Ho.** 1979. Characteristics of infection of B and T lymphocytes from mice after inoculation with cytomegalovirus. Infect. Immun. **24**:856–864.