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Correlation of Survival from Murine Cytomegalovirus Infection with Spleen Cell Responsiveness to Concanavallin A[1] (38824)

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Subversion of host immune and nonimmune defense functions facilitates the establishment of virus infection (1). Murine cytomegalovirus (MCMV), a virus of the Herpes virus group, subverts several host-defense functions. It inhibits interferon production and is itself unaffected by interferon (2). MCMV also depresses the primary and secondary immune responses to sheep red blood cells (SRBC) in nonlethally infected weanling mice (3, 4). MCMV-infected weanling mice have depressed cell-mediated immune function as measured by increased skin graft survival, inhibition of *in vitro* phytohemagglutinin (PHA) stimulation, and depressed *in vitro* mixed leukocyte stimulation ratios (5). Thus far, however, none of these impaired host defense functions has been correlated with clinical status or death.

We have further dissected the effect of MCMV infection on host responses in an attempt to identify those responses that are crucial to survival. Adult, as well as weanling mice, were used to evaluate the possibility that defects of host defense were related to host immaturity. The response of spleen cells to Concanavallin A (Con A), a stimulator of T cells (6, 7), as well as the primary immune response to SRBC, a T-dependent antigen (8), were assayed. Our principal finding was that Con A unresponsiveness occurred only with spleen cells from lethally infected weanling and adult mice. Spleen cells from weanling and adult mice nonlethally infected with MCMV were capable of Con A stimulation.

Methods. Virus. MCMV was obtained from Dr. June E. Osborne as a 10% (w/v) infected salivary gland suspension, in 10% dimethylsulfoxide (DMSO). Stock virus was prepared by harvesting the submaxillary glands from weanling mice 3 wk after ip infection. A 10% w/v suspension was regarded as undilute. The virus seed was stored at −70°C in 10% DMSO. Stock virus prepared in this manner contained 2–4 $\times 10^6$ PFU/ml as assayed on mouse embryo tissue culture utilizing tragacanth in the overlay medium (9). This MCMV stock has been found to be free of Lactic dehydrogenase virus.

Mice. Inbred DBA/2 female mice were obtained from Jackson Laboratories, Bar Harbor, ME. In some experiments the ages of a population of weanling mice, in a single shipment, varied by several days. In others, they were exactly timed. Adult mice were used at 7–10 wk of age.

Animal inoculation. Animals were inoculated at an ip site with 0.2 ml of virus suspension. Test animals were sacrificed by exsanguination under ether anesthesia or by cervical dislocation.

Assay for anti-SRBC plaque-forming cells. Mice were immunized ip with 2×10^8 SRBC in normal saline 4 days after virus or diluent inoculation. Four days after SRBC injection, mice were sacrificed and their spleens removed. The spleens were examined for anti-SRBC plaque-forming cells by the method of Jerne and Nordin (10). Briefly, spleen cells were separated by gentle passage through a fine-mesh screen, washed by centrifugation in RPMI 1640, resuspended, and counted. Spleen cells were mixed with sheep erythrocytes in agar and poured onto a previously formed agar layer in a petri dish. After incubation of the petri dish for 1 hr, guinea pig complement was added for another hour of incubation. Plaques were counted with the aid of low-power magnification, and the results were calculated as plaques per spleen.

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In vitro Con A stimulation. Spleen cells were separated as noted above for the anti-SRBC plaque-forming cells assay. Viable cells (10×10^6) in 1 ml of RPMI 1640 with 2% normal murine serum were distributed in culture tubes. Five replicate samples for each experimental group were used. Con A was added to a final concentration of 5 μg/ml to each of half the total of culture tubes. After 2 days incubation of the culture tubes at 37°C under 5% CO_2, tritiated thymidine (^{3}H-TdR) was added to a final concentration of 1 μCi/ml for the final 20 hr of incubation. DNA was precipitated by trichloroacetic acid and processed on filter discs. Incorporated radioactivity was measured in scintillation vials utilizing solubilizer and primary and secondary fluors by a Packard Tri-Carb Liquid Scintillation Counter.

Results. Development of resistance to MCMV in weanling mice. Clinically ill MCMV-infected weanling mice exhibited a huddled posture, ruffled fur, and a delayed response to threat. These mice lost weight in comparison to noninfected age-matched controls which gained weight. No focal neurological signs were evident, and no virus was isolated from the brain.

A strikingly increased resistance to lethal infection with MCMV occurred during the fourth week of life. Exactly aged DBA/2 mice were distributed by weight in equal proportions to each experimental group of 10 mice each. At 22, 25, and 28 days of age, the mice were inoculated ip with 0.2 ml virus suspension containing 3.1×10^4 PFU. As shown in Fig. 1, 90% of the animals inoculated at 22 days of age died; only 30% of the animals inoculated at 25 days, and none of the animals inoculated at 28 days died. Experiments utilizing the same virus dose with other lots of mice aged 22 days revealed mortalities of 40%–100%, indicating that the time of maturation of resistance varied among groups of mice.

Response to SRBC of infected weanling and adult mice. Weanling and adult mice infected with doses of MCMV insufficient to cause clinical illness were studied for their immune response to SRBC. Four days after inoculation ip with a nonlethal dose of MCMV, mice were immunized with 2×10^8

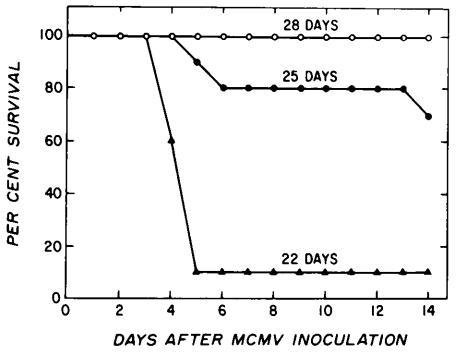

FIG. 1. Survival of mice infected with 3.1×10^4 PFU of MCMV at 22, 25, and 28 days of age.

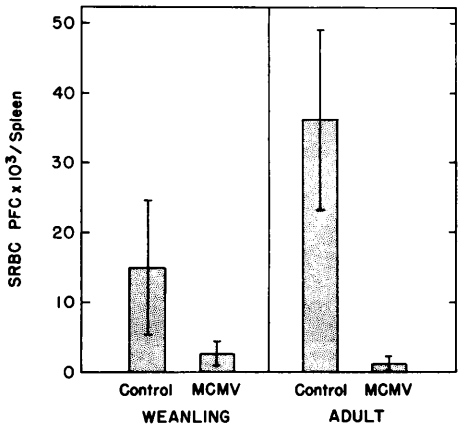

FIG. 2. Immune response to SRBC of weanling and adult mice infected with nonlethal doses of MCMV. Weanlings infected with 6×10^1 PFU of MCMV; adults infected with 6×10^3 PFU MCMV.

SRBC in normal saline. They were sacrificed 4 days after SRBC immunization and spleens removed for assay of anti-SRBC plaque-forming cells. As shown in Fig. 2, spleens from infected weanling and adult mice contained fewer anti-SRBC plaque-forming cells than did spleens from uninfected age-matched control mice.

In vitro response to Con A by spleen cells from MCMV-infected weanling and adult mice. Weanling or adult mice were inoculated with either a lethal or a nonlethal dose of virus. Four days after MCMV, mice were sacrificed and spleen cells processed for *in vitro* Con A stimulation.

The amount of ^{3}H-TdR incorporated spontaneously by control spleen cells varied from experiment to experiment (Tables I and II). However, spontaneous incorporation by

TABLE I. CON A RESPONSE OF SPLEEN CELLS FROM NONLETHALLY MCMV-INFECTED WEANLING AND ADULT MICE.[a]

Experimental groups	*In vitro* ^{3}H-TdR Incorporation[b]		
	Spontaneous	Con A exposed	Stimulation index[c]
Weanling			
Control	7.8 ± 1.0	61.9 ± 8.4	7.9
MCMV	13.2 ± 1.4	55.3 ± 4.2	4.2
Adult			
Control	9.2 ± 0.9	634.9 ± 29.5	69
MCMV	21.6 ± 3.7	407.0 ± 29.1	19

[a] Weanlings infected with 6×10^2 PFU MCMV; adults infected with 6×10^3 PFU MCMV.
[b] Expressed as counts/minute $\times 10^{-3} \pm$ SD.
[c] Con A exposed/spontaneous.

TABLE II. CON A RESPONSE OF SPLEEN CELLS FROM LETHALLY MCMV-INFECTED WEANLING AND ADULT MICE[a]

	In vitro ^{3}H-TdR incorporation[b]		
	Spontaneous	Con A exposed	Stimulation index[c]
Weanling			
Control	5.4 ± 0.3	286.1 ± 46.1	52.9
MCMV	27.0 ± 2.1	34.2 ± 3.3	1.3
Adult			
Control	32.3 ± 0.8	1072.2 ± 31.2	33.2
MCMV	93.7 ± 6.6	142.3 ± 6.3	1.5

[a] Weanlings infected with 1.3×10^4 PFU MCMV; adults infected with 2×10^5 PFU MCMV.
[b] Counts/minute $\times 10^{-3} \pm$ one SD.
[c] Con A exposed/spontaneous.

spleen cells from infected age-matched mice was substantially above that of spleen cells from uninfected mice (Tables I and II).

Spleen cells from nonlethally infected mice were capable of significantly increased ^{3}H-TdR incorporation on exposure to Con A in comparison to spontaneous incorporation (Table I). In marked contrast, spleen cells from mice infected with lethal amounts of virus failed to double the incorporation of ^{3}H-TdR on exposure to Con A (Table II). Differences in spleen cell viability as determined by exclusion of erythrocin B did not account for this failure of Con A stimulation.

Discussion. Four-week-old weanling mice are frequently used in experiments with MCMV (3–5, 14). Our studies have demonstrated a striking increase in resistance to the lethal effects of MCMV during the fourth week of life of DBA/2 mice. The rapid development of such resistance during this period necessitates the use of exactly aged mice in experiments with MCMV.

Although extensive pathological changes in the liver, spleen, adrenals, and lymph nodes have been documented (13), the cause of death in lethally MCMV infected mice is not known. Depression of the platelet count and hematocrit in nonlethal MCMV infections of weanlings (14) raises the possibility of a hematologic cause of death. Since Herpes simplex virus can cause a terminal encephalitis in mice (15), we observed mice lethally infected with MCMV for signs of focal neurological disease. None was seen and we were unable to isolate virus from the brain. Since, in addition, others have not found pathological lesions in the brain (12), it seems unlikely that the cause of death is infection of the central nervous system.

MCMV infection depresses the primary humoral immune response to SRBC in the weanling mouse (3). Our finding that this primary immune response to SRBC is also depressed in acutely infected adults indicates that this effect can occur in immunologically mature mice. Furthermore, the virus-depressed immune response to SRBC does not correlate with mortality since it can occur in adult and weanling mice without associated or subsequent clinical illness. These studies of humoral immunity must be regarded as indirect since they involve the response to nonviral rather than MCMV antigens.

MCMV infection has been shown by others to interfere with weanling spleen cells response to PHA *in vitro* (5). Four-week-old C57BL/6 murine spleen cells, removed 5 days after murine infection with 2×10^5 PFU MCMV, revealed an elevated resting incorporation of ^{3}H-TdR compared with control cells. No significant stimulation above resting incorporation followed exposure to PHA. No mortality studies were included to establish a correlation between survival and PHA responsiveness. In our

studies, utilizing weanling and adult mice, we also demonstrated elevated spontaneous incorporation of ^{3}H-TdR in resting spleen cells from infected mice. We found, however, that spleen cells from nonlethally infected mice were capable of diminished but significant stimulation by another mitogen, Con A. Complete suppression to Con A stimulation occurred only during lethal infections. Thus, the response to Con A correlated well with the clinical severity of MCMV infection.

Since the B cell response to SRBC is T-cell dependent (8), it is not possible with this response to localize the defect in MCMV-infected mice to T cell or B cell function. The defect may also be in macrophage antigen processing. However, the predominant response to soluble Con A is by T cells (6, 7). Our data, therefore, strongly suggest that DBA/2 splenic T cells play a crucial role in host defense against MCMV since the capacity to respond to Con A is retained except in lethal infections.

Summary. Spleen cells from nonlethally MCMV-infected weanling and adult DBA/2 mice had diminished responses to Con A stimulation. In contrast, only lethal MCMV infections were associated with a complete suppression of the Con A response. The immune response to SRBC was depressed even in asymptomatic infections of weanling and adult mice. A marked maturation of resistance to the lethal effects of MCMV infection was found to occur during the fourth week of life.

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