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Murine Cytomegalovirus Infection: Hematological, Morphological, and Functional Study of Lymphoid Cells

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Mice were studied for 3 to 4 months after murine cytomegalovirus (MCMV) infection. Serial hematological parameters were evaluated. It was found that MCMV infection of mice was accompanied by the appearance of many atypical lymphocytes similar to those seen in association with the hematological features of mononucleosis associated with human CMV infection. Certain functions of splenocytes were studied in infected and uninfected animals during the 4 months after MCMV infection. Three periods were identifiable by the functional response of splenocytes during the course of MCMV infection. The initial phase was characterized by an elevated response of splenocytes to a T cell mitogen and a B cell mitogen (phytohemagglutinin and lipopolysaccharide, respectively). The intermediate phase was marked by productive virus replication in the salivary gland, injury to lymphoid elements, a depressed response of splenocytes to mitogens (phytohemagglutinin and lipopolysaccharide), and decreased humoral response to infection. During the final phase of MCMV infection, the response of splenocytes to phytohemagglutinin stimulation was again increased, and a nonproductive latent infection was established. Study by scanning electron microscopy of splenocytes during the course of infection revealed morphological changes which were correlated with functional alterations.

The syndrome of mononucleosis includes findings of fever, (mild) hepatitis, and evidence of lymphoid hyperplasia manifested by (inconsistent) lymph node enlargement, splenomegaly, and the presence of atypical (Downey-type) lymphocytes in peripheral blood. A significant proportion of mononucleosis syndromes have been associated with herpesvirus infections: Epstein-Barr virus with all heterophil-positive and some heterophil-negative cases (13) and cytomegalovirus (CMV) with many of the remaining heterophil-negative occurrences. The pathogenesis of mononucleosis syndromes remains obscure, although the late appearance of clinical symptoms (3 to 6 weeks after infection) and the evidence of lymphoproliferation suggest that these clinical presentations reflect immunopathological mechanisms. Murine CMV (MCMV) has been employed as a model for human infections (4, 7, 11), although it is not known whether the pathogenesis and clinical findings accompanying these infections in mice are comparable to those in humans. The purpose of the present investigation was threefold: (i) to determine whether MCMV infection is followed by the appearance of a mononucleosis syndrome in mice which is analogous to that in humans; (ii) to investigate the impact of MCMV infections upon some features of immune function; and (iii) to study the morphology of splenocytes by scanning electron microscopy (SEM) and to seek correlations of morphological and functional changes in these cells during the course of MCMV infection.

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MATERIALS AND METHODS

Specific pathogen-free CD-1 mice and BALB/c mice were obtained from Charles River Breeding Laboratories, Inc., and maintained in the animal facility at Duke University Medical Center. Young adult male mice aged 6 to 8 weeks were usually used for the experiments.

Virus. MCMV Smith strain was originally obtained from June Osborne of the University of Wisconsin and has been maintained in our laboratory by repeated passages in CD-1 mice. Salivary gland virus was prepared as previously described (3). Tissue culture virus was obtained by inoculating salivary gland virus into secondary mouse embryonic cell cultures and harvest-

ing the supernatant fluid when 95% of the monolayer exhibited cytopathic effects.

Blood counts. Animals were sacrificed by exsanguination via cardiac puncture. Total and differential leukocyte counts were performed by using standard hematological techniques. Packed erythrocyte volume (hematocrit) was determined for each animal.

Isolation of splenocytes. Spleen cells were obtained by stripping the content of a spleen into 5 ml of phosphate-buffered saline (pH 7). The erythrocytes, granulocytes, and nonviable cells were removed by centrifugation in Ficoll-Hypaque (2). Thereafter, the partially purified splenocytes were washed twice with RPMI medium containing 2% heat-inactivated (56°C for 30 min) human serum.

SEM. An aliquot of the Ficoll-hypaque-purified splenocytes was washed once with phosphate-buffered saline and immediately fixed with 1% glutaraldehyde in 0.135 M phosphate buffer (pH 7.4). Dehydration was performed by passing the specimen rapidly through an alcohol gradient, followed by a freon-alcohol gradient with nine changes of 100% freon (Trichlorotrifluoroethane, DuPont, Wilmington, Del.). The dehydrated specimen was then dried with a Bomar critical-point dryer (Bomar model SPC-gov/Ex) and coated with gold-palladium in a Mini-Coater (Film-Vac, Inc., Englewood, N.J.). Samples were examined in a Coates-Welter (CWIKSCAN-100) scanning electron microscope operating at 20 kV.

Lymphocyte stimulation. Splenocytes were cultured at a concentration of 2×10^6 cells per ml per tube in RPMI medium supplemented with glutamine and 2% heat-inactivated human serum. For the purpose of quality control, mitogens of the same lot number were employed, and the dosage for optimal response was determined. Lymphocytes were cultured with either phytohemagglutinin (PHA, a T cell mitogen) at 10 μl/ml per tube or *Escherichia coli* lipopolysaccharide (LPS, a B cell mitogen) at 100 μg/ml per tube added at the time of initiation of triplicate cultures. Tritiated thymidine (2 μCi per tube) was added on day 2, and the cultures were harvested 18 h thereafter. The cells of each sample were trapped on glass fiber filters, washed with 5% cold trichloroacetic acid, dried, and counted in toluene-based scintillation fluid. (RPMI medium, glutamine, and Phytohemagglutinin M-form were obtained from GIBCO Laboratories, Grand Island, N.Y.; [^{3}H]thymidine, specific activity 60 to 80 Ci/mmol was obtained from New England Nuclear Corp., Boston, Mass.)

Hemolytic plaque assay. The ability of B-lymphocytes to transform into antibody-producing plasma cells after antigenic challenge was evaluated by a modification of the hemolytic plaque assay (9). Mice were inoculated intravenously with 0.1 ml of 50% washed sheep erythrocytes (SRBC). The mice were sacrificed 4 days after they had been inoculated with SRBC, and the spleen cells were purified in Ficoll-Hypaque. The cell concentration was adjusted to 2×10^6/ml. The assay was done on precleaned microscopic slides. Washed SBRC (0.05 ml of a 1:8 dilution of packed erythrocytes) were added to 0.2 ml of a spleen cell suspension. Complement, 0.05 ml of a 1:4 dilution of guinea pig serum (GIBCO Laboratories) was added to the cell mixture, which was then transferred to 0.6 ml of agarose at 45°C (0.5% agarose, L'industrie Biologique Francaise S.A.; with 0.05% diethylaminoethyldextran, Pharmacia Fine Chemicals, Uppsala, Sweden). The mixture was spread evenly on a prewarmed glass slide and allowed to set at room temperature. The slides were further incubated at 37°C in a humidified chamber for 3 h. Hemolytic plaques were counted with the aid of a modified microfilm projector.

Removal of adherent phagocytic cells. The depletion of phagocytic cells from splenocytes was achieved either by incubation of these cells on a glass surface for 3 h at 37°C or by the addition to the cells of carbonyl iron for 30 min with occassional agitation, after which the phagocytic cells were removed with the aid of a magnet.

Heterophil agglutinins. The Paul-Bunnell test was used to test for the presence of heterophil agglutinins in all serum samples.

Antibody response to antigenic challenge. Mice were inoculated intraperitoneally with washed SRBC (0.1 ml of 50% SRBC) at different time points after MCMV infection. The SRBC challenge was administered both to uninfected and MCMV-infected animals 1 week before sacrifice. The antibody response to SRBC was evaluated by hemagglutination and complement-dependent immune hemolysis.

RESULTS

Hematological changes during MCMV infection. Mice showed no sign of clinical illness after inoculation with 10^4 plaque-forming units of MCMV derived either from tissue culture supernatant or salivary gland homogenate. Despite the absence of recognizable illness, CD-1 mice responded to MCMV infection with a brief viremia lasting 1 to 3 days, similar to those observed in BALB/c mice (4), and a moderate lymphocytosis at 3 to 4 days postinoculation. Thereafter, the percentage of normal lymphocytes decreased accompanied by a significant increase in the percentage (>50% at week 4 postinfection) of atypical lymphocytes (Downey-type mononuclear cells) in the peripheral blood (Table 1). Normal peripheral blood was not restored until 8 weeks after initiation of the infection. When BALB/c mice were infected with MCMV, a similar decrease in the percentage of atypical lymphocytes with a concomitant 10-fold increase in the percentage of atypical lymphocytes was observed. The hematological changes after MCMV infection occurred much sooner (3 days after infection) and lasted for a shorter period of time (1 week) than those in CD-1 mice (Table 2). The rapid response to infection by the BALB/c mice may have obscured early lymphoproliferation. Since we expected less hematological variation among individuals of an inbred strain of mice, total and differential leukocyte counts were performed on samples from BALB/c mice. During week 1 after MCMV inoculation, a statistically significant in-

TABLE 1. *Differential cell counts of peripheral leukocytes of CD-1 mice*

Week postinfection	Experimental type	% Cell type[a]					
		Normal lymphocyte		Polymorphonuclear cells		Atypical lymphocyte	
0.5	Uninfected	49.7 ± 7.7	⊖	47.3 ± 9.8	↓	3.3 ± 3.2	⊖
	Infected	57.4 ± 13.2		31.9 ± 6.8		3.2 ± 4.5	
1	Uninfected	47.8 ± 10.0	↓	47.3 ± 9.8	↓	3.2 ± 3.3	↑
	Infected	28.5 ± 16.4		27.0 ± 8.5		37.3 ± 22.3	
2	Uninfected	45.1 ± 12.1	↓	47.0 ± 11.8	⊖	1.1 ± 2.3	↑
	Infected	15.8 ± 8.0		38.5 ± 13.9		38.2 ± 24.2	
4	Uninfected	54.3 ± 13.8	↓	37.3 ± 14.8	↓	<1	↑
	Infected	12.4 ± 8.1		22.0 ± 4.9		63.6 ± 12.3	
8	Uninfected	63.3 ± 8.2	⊖	27.2 ± 11.4	⊖	<1	⊖
	Infected	58.3 ± 13.0		33.0 ± 7.3		2.3 ± 1.7	
12	Uninfected	63.3 ± 8.2	⊖	27.2 ± 11.4	⊖	<1	⊖
	Infected	65.8 ± 8.3		35.5 ± 3.35		<1	

[a] Statistics were computed by the Student t test. Symbols: ⊖, not statistically significant at $P = 0.01$; ↓, statistically significant decrease at $P = 0.05$; ↑, statistically significant increase at $P = 0.05$.

crease in the total number of blood leukocytes was observed (Table 2). Although a decrease in the percentage of normal lymphocytes was observed, the absolute number of lymphocytes per microliter of blood was not found to be significantly different from that in controls. A significant increase in both the relative percentage and absolute number of polymorphonuclear leukocytes and of atypical lymphocytes was observed in the infected mice. After week 8 of infection, when MCMV latency was established in BALB/c mice, as evident by the absence of infectious virus in salivary gland tissue, leukocytosis was again observed. Although the relative percentages of lymphocytes and polymorphonuclear cells had returned to normal, an increase in the absolute numbers of both cell types was observed (Table 2). There was a significant increase in packed cell volume shortly after infection, followed by a drop in hematocrit lasting 4 weeks (Table 2).

SEM. Three morphological types of lymphocytes were observed: smooth, villous, and ruffled forms representing 40 to 45, 50 to 55, and 5 to 10% of cells in uninfected CD-1 mice, respectively (Fig. 1 and 2). In an attempt to identify the type of cells associated with these morphological forms, thymocytes of a Swiss white mouse (T cells) and splenocytes of an NIH nude mouse (primarily B cells) were studied by SEM. Cells were stripped from the specific organs into phosphate-buffered saline, fixed with 1% glutaraldehyde, and processed for SEM. When precautions were taken to minimize any experimental or environmental disturbance to these cells, the predominant morphological form of either T or B cells was the smooth cell. However, when the cells were cultured in vitro with either specific mitogens or tissue culture-grown MCMV, they produced microvilli and transformed into villous forms (Fig. 3). Nonstimulated cells showed signs of deterioration in culture (Fig. 3C and D). Lymphocytes cultured in the presence of fetal calf serum also manifested a tendency to acquire microvilli. Villous forms probably represented activated lymphocytes, and it was not possible to distinguish T and B cells based upon surface morphology.

During week 1 postinfection, both the smooth and villous forms decreased in number appreciably, with a concomitant increase in damaged cells (broken, small, and shrunken) and ruffled forms with numerous membrane folds (Fig. 1). Studies of the lymphocytes isolated from infected animals showed a striking tendency for clumping, most evident at 2 to 3 weeks postinoculation with virus (Fig. 4). An increased number of enlarged cells, especially those with a smooth morphology, were observed after week 2 of infection. Thereafter, damaged cells were observed less frequently, and villous forms became more prevalent, although the percentage of cells with ruffled morphology remained higher in infected than in uninfected mice (Fig. 5).

Samples of splenocytes depleted of surface adherent and phagocytic cells were processed for SEM examination. Although after the depletion of adherent cells a general decrease in the number of ruffled cells was observed 1 to 2 weeks postinfection in both the infected and control groups, splenocytes from infected animals still showed a higher (20%) percentage of ruffled cells than did the controls (5%). The percentage of large transformed lymphoblasts was much higher in the samples from infected animals (30 to 35%) than in the controls (4 to 6%).

Lymphocyte response to mitogens. Figure 6 illustrates the ratio of the mitogen stimulation

TABLE 2. *Relative percentage and absolute number of peripheral blood polymorphonuclear cells, lymphocytes, and atypical lymphocytes in experimentally infected and control BALB/c mice*[a]

Weeks postinfection	Experimental type	Total leukocytes per mm^3		Normal lymphocytes (%)		Normal lymphocytes (no./mm^3)		Polymorphonuclear cells (%)		No. of polymorphonuclear cells per mm^3		Atypical lymphocytes (%)		Atypical lymphocytes (no./mm^3)		Packed cell volume (%)	
0.5	Uninfected	6,215 ± 2,450	↓	88.5 ± 76	↓	5,408 ± 1,893	↓	6.0 ± 4.0	↑	218 ± 213	↑	4.0 ± 2.0	↑	118 ± 120	↑	46.0 ± 2.0	↑
	Infected	3,553 ± 550		39.8 ± 21.4		1,434 ± 867		43.0 ± 12.2		1,540 ± 525		14.6 ± 12.7		565 ± 511		53.0 ± 3.0	
1	Uninfected	3,520 ± 797	↑	85.0 ± 9.5	↓	2,599 ± 659	Θ	13.8 ± 10.4	↑	526 ± 310	↑	1.3 ± 0.9	↑	38 ± 38	↑	53.0 ± 3.0	↓
	Infected	7,950 ± 2,462		46.0 ± 19.2		3,414 ± 1,504		36.3 ± 15.6		2,966 ± 165		11.2 ± 9.4		862 ± 469		42.0 ± 2.0	
2	Uninfected	4,427 ± 1,154	Θ	62.5 ± 10.3	Θ	2,856 ± 1,197	Θ	33.5 ± 2.5	Θ	1,493 ± 395	Θ	(0–11)[b]	Θ	0–278[c]	Θ	46.4 ± 2.0	↓
	Infected	4,950 ± 1,303		59.2 ± 10.3		3,236 ± 630		36.3 ± 6.3		2,087 ± 876		(0–4)[b]		0–220[c]		38.6 ± 2.0	
4	Uninfected	4,070 ± 2,104	Θ	68.0 ± 0.6	Θ§	3,029 ± 184	Θ	21.5 ± 0.5	↑	869 ± 309	↑	(0–0)[b]	Θ	0–0	Θ	45.3 ± 0.1	↓
	Infected	5,647 ± 2,449		52.8 ± 10.2		3,555 ± 1,739		36.5 ± 4.9		1,986 ± 776		(0–3)[b]		0–90[c]		38.3 ± 3.0	
8	Uninfected	4,098 ± 1,296	↑	77.7 ± 5.8	Θ	2,870 ± 1,349	↑	18.3 ± 4.9	Θ	928 ± 196	↑	(0–9)[b]	Θ	0–524[c]	Θ	45.3 ± 3.0	Θ
	Infected	11,312 ± 2,907		73.2 ± 10.3		7,961 ± 2,977		21.2 ± 3.8		2,360 ± 678		(0–4)[b]		0–400[c]		44.6 ± 3.0	
12	Uninfected	3,226 ± 960	↑	69.2 ± 8.4	Θ	1,674 ± 198	↑	27.4 ± 8.5	↓	916 ± 131	↑	(0–6)[b]	Θ	0–193[c]	Θ	45.8 ± 1.0	Θ
	Infected	13,493 ± 2,208		77.8 ± 1.9		10,458 ± 1,893		14.5 ± 8.7		2,337 ± 408		(0–1)[b]		0–135[c]		43.8 ± 1.0	

[a] Symbols: ↑, significant increase at $P = 0.05$; ↓, significant decrease at $P = 0.05$; Θ, not statistically significant at $P = 0.05$; Θ§, significant at $P = 0.01$.

[b] Number represents the highest percentage of atypical lymphocytes found in this group (three or four of six animals in this group had no atypical lymphocytes).

[c] Number represents the highest number of atypical lymphocytes per mm^3 of blood found in this group (three or four of six animals of this group had no atypical lymphocytes in the blood smears).

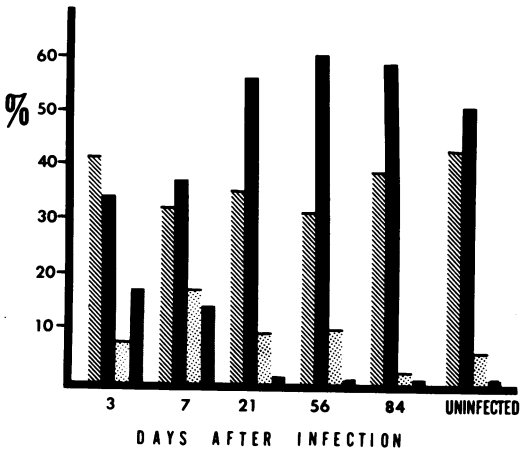

FIG. 1. *Effect of MCMV infection on the percentages of four major morphological types of splenocytes distinguishable by SEM. Percentages were determined by counting >500 cells from four or more randomly selected microscopic fields. Symbols: ▨, smooth form; ■, villous form; ▩, ruffled form; ▤, damaged form.*

indices of cultures of lymphocytes from infected and uninfected animals. Stimulation index was calculated by the formula: [^{3}H]thymidine incorporation (cpm) by mitogen-stimulated cells/[^{3}H]thymidine incorporation (cpm) in nonstimulated control cells. In this figure, the base-line index ratio 1 reflected no difference between uninfected and infected animals. Any value greater than 1 reflected stimulation, and any value below 1 reflected a depression. A triphasic lymphocyte response to PHA was observed in the course of MCMV infection of CD-1 mice (Fig. 6). There was a brief elevation in lymphocyte response to PHA which occurred before or coincident with virus recovery from the salivary gland. This initial elevation of the ratio of the mitogen stimulation indices was followed by a depressed response to PHA about week 1 postinfection, when virus could be readily isolated from the salivary gland and when SEM showed a large number of ruffled and damaged cells. Thereafter, lymphocyte response to PHA was gradually restored to normal, cellular damage was no longer demonstrable by SEM, although active salivary gland infection was still present, and the presence of atypical lymphocytes was prominent. At this time, the villous form of lymphocytes continued to dominate the SEM picture, indicating that recovery from the MCMV infection was a gradual process. Finally, 3 to 4 months postinfection, an elevated lymphocyte response to PHA was again observed. At this point, SEM studies of peripheral cells showed that the ratio of smooth and villous forms approximated that seen in uninfected animals. This late response corresponded to a time when latent MCMV infection in mice is usually established (2).

The response of lymphocytes to LPS was depressed during weeks 1 and 2 after MCMV infection. Unlike the T cell response to PHA, no secondary elevation of B cell response was observed (Fig. 6). A similar response to B cell mitogens was observed in splenocytes from BALB/c mice. Shortly after infection (3 to 4 days), some mice responded to PHA and LPS with a significant increase in [^{3}H]thymidine incorporation, whereas others were already depressed in their response to those mitogens. When small numbers of animals were employed (six animals per group, two separate experiments), the overall stimulatory response between infected and control groups of animals was not statistically significant. However, when the mitogen responses were assayed the day after infection (in three separate experiments), a consistently elevated mitogen response was observed (with the ratio of stimulation index ranging from 1.5 to 2.9).

Humoral response to MCMV infection and B cell function. Complement-fixing antibody to MCMV was first detectable at week 1 postinfection, and its titer gradually increased to a peak after week 4. The complement-fixing titer was maintained at a high level throughout the study (Table 3). Heterophil antibody to SRBC was not detected during the course of the infection. However, when the infected animals were challenged with SRBC, they were able to respond by producing hemagglutinating antibody as well as a hemolysin (Table 3). Although the humoral response of the animals to antigenic challenge was temporarily depressed coincident with the acute phase of MCMV infection, the capacity to produce antibody returned to normal within 2 weeks postinfection.

B cell function, as evaluated by the hemolytic plaque assay, decreased during the initial phase of infection but returned rapidly to normal (Table 4).

DISCUSSION

Hematological changes in mice in response to MCMV infection were found to have some resemblance to those associated with mononucleosis syndromes in humans. When CD-1 mice were infected with MCMV, a brief period of lymphoproliferation was indicated by an initial increase in the percentage of peripheral blood lymphocytes, followed by a decline after week 1 of infection. The decline in the relative percent-

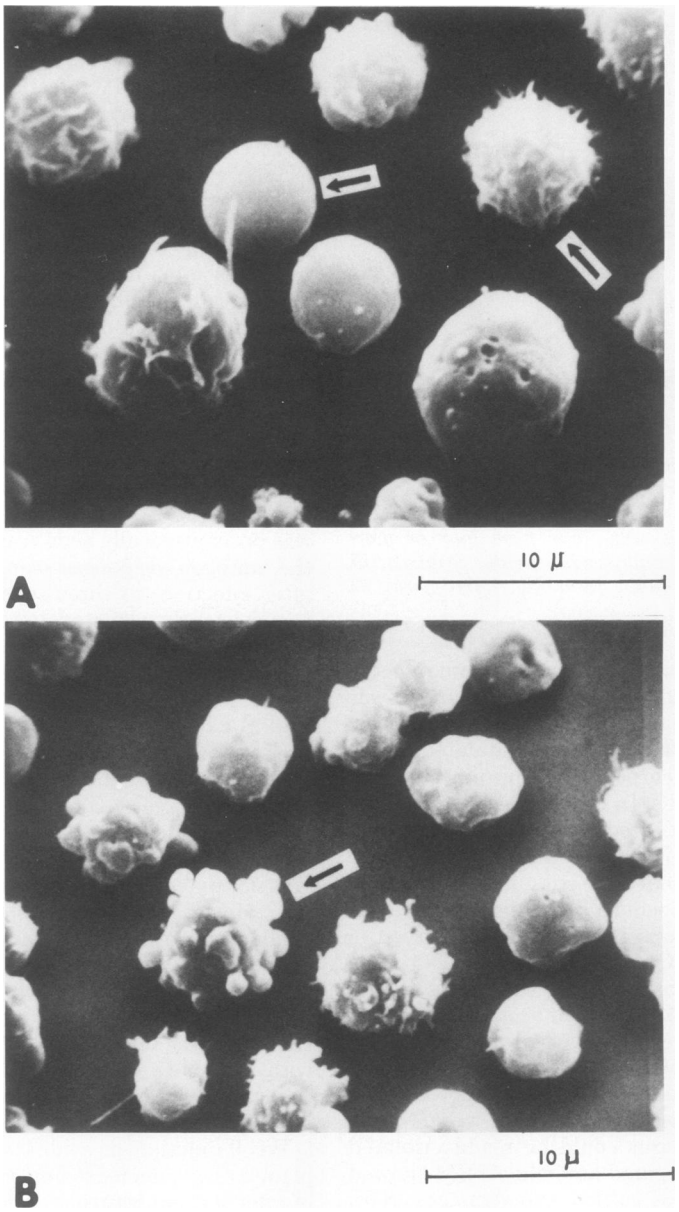

FIG. 2. *Micrograph by SEM of Ficoll-Hypaque-purified splenocytes showing the three major morphological forms. (A) Smooth and villous forms; (B) ruffled form.*

age of peripheral blood lymphocytes was a direct result of a sharp increase in the percentage of atypical Downey-type mononuclear cells. To minimize the individual variation, an inbred strain of mice, BALB/c, was used to study the relative and absolute changes in the number of peripheral blood lymphocytes. In this system, although a decline in the percentage of lymphocytes similar to that observed in CD-1 mice also occurred after week 1 of MCMV infection, no significant changes in the absolute number of lymphocytes per cubic millimeter was evident in the peripheral blood. However, both the relative percentage and absolute number of atypical lymphocytes increased dramatically in response to MCMV infection. After week 2 of infection, both

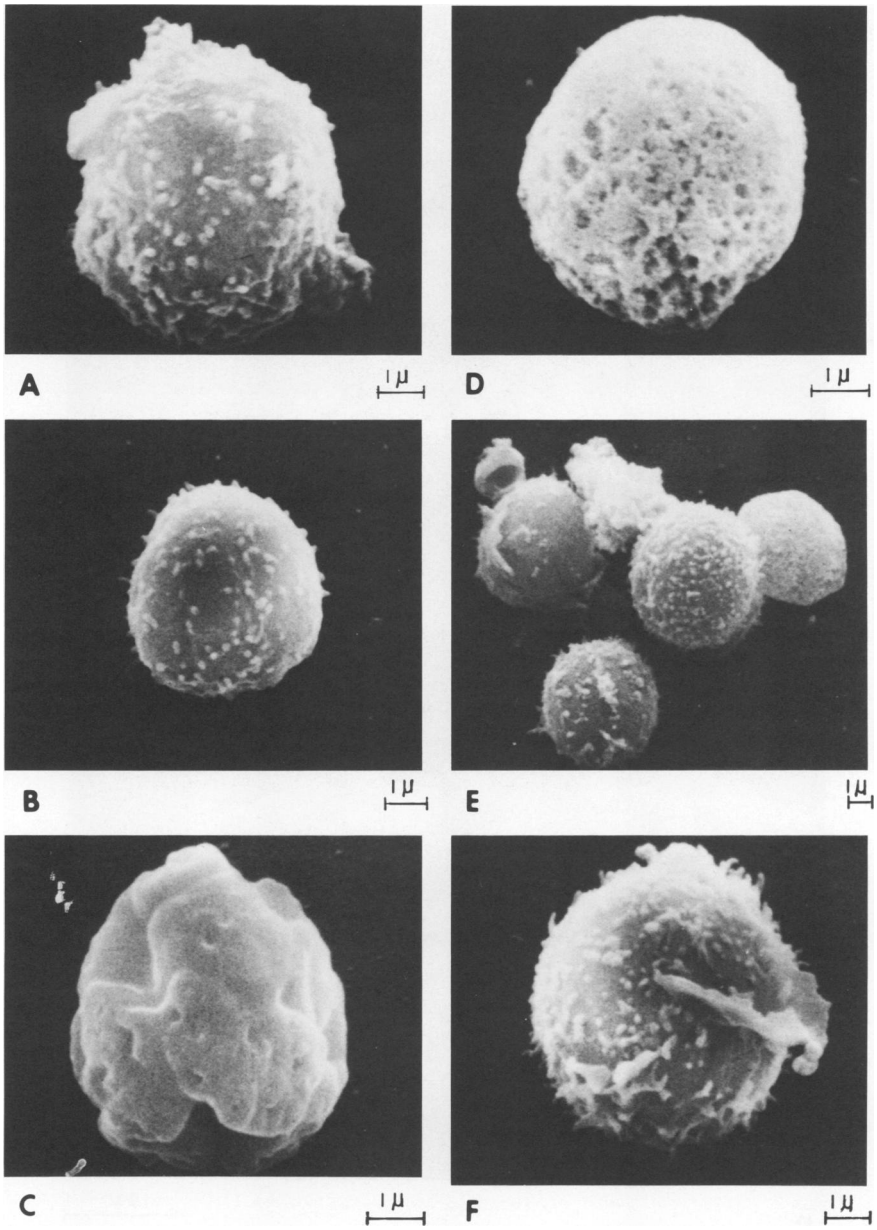

FIG. 3. *Thymocytes of a CD-1 male mouse (T cells) and splenocytes from an NIH nude mouse (B cell) cultured in vitro for 2 days with mitogens or MCMV in the absence of fetal calf serum. (A) T cell cultured with PHA; (B) T cell cultured with MCMV; (C) T cell cultured with E. coli LPS; (D) B cell cultured with PHA; (E) B cell cultured with MCMV; (F) B cell cultured with E. coli LPS.*

the relative percentage and absolute number of lymphocytes returned to the normal range. A late leukocytosis was evident at week 8 postinfection, corresponding to the time when latent infection in these mice was established. During this time, the number of peripheral blood lymphocytes per microliter of blood was significantly increased, although the relative percentage of these cells remained in the normal range. These data suggest that MCMV infection may have a stimulatory effect on the lymphoid system, resulting in a lymphoproliferative response in the host, as evident in the initial and late phases of infection. During the acute phase of

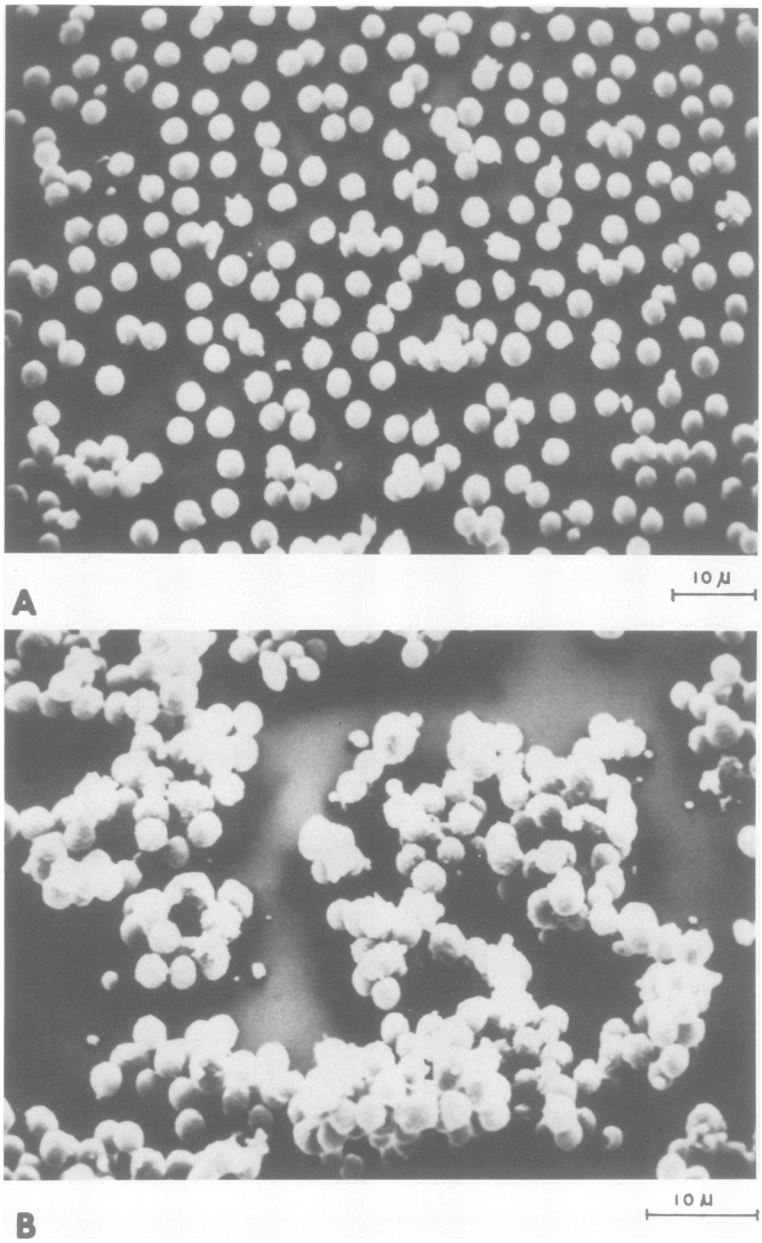

FIG. 4. *Micrograph by SEM of Ficoll-Hypaque-purified splenocytes from: (A) a healthy uninfected CD-1 male mouse and (B) a CD-1 male mouse infected with MCMV 2 weeks previously.*

infection when virus production is at its peak, a certain subpopulation of the lymphoid cells susceptible to MCMV injury may be selectively destroyed while other lymphocyte subpopulations are stimulated to proliferate. The decrease in the relative and absolute numbers of lymphocytes was only briefly apparent after MCMV infection since the effect of cell destruction was countered by the process of cell proliferation.

Cellular injury was evident in the spleen, although it was not apparent in the peripheral blood. We have previously reported the depletion of cellular elements in the spleen, resulting in a reduction in the size of the spleen in infected

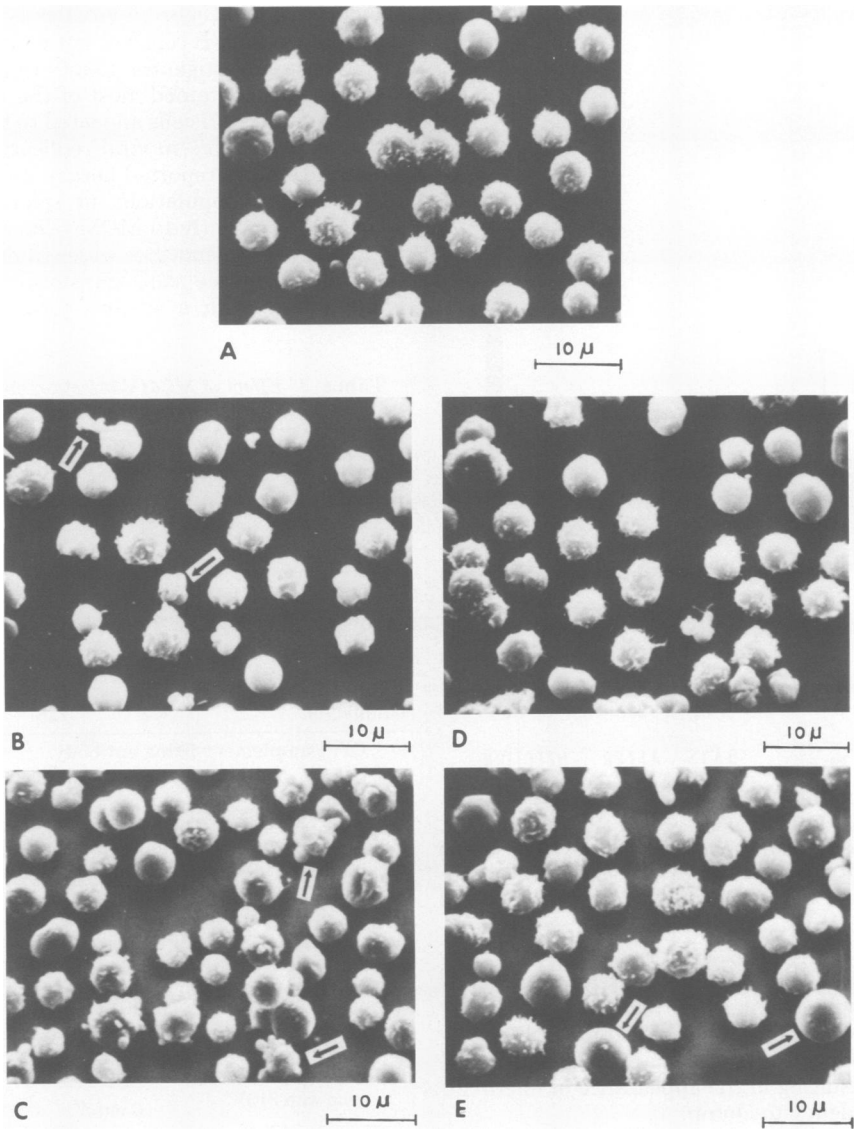

FIG. 5. *Micrograph by SEM of Ficoll-Hypaque-purified splenocytes taken from CD-1 males at various times after MCMV infection. (A) Uninfected cells showing two major morphological types, the smooth and villous cells normally found in the spleen; (b) cells at 3 days postinfection showing an increased number of damaged cells (arrows); (C) cells at 1 week postinfection showing increased number of ruffled cells (arrows) and a tendency for cell agglutination; (D) cells at 4 weeks postinfection showing a gradual return to normal; (E) cells at 8 weeks postinfection showing the appearance of many large smooth blast cells.*

mice (6). In the present studies of splenocytes by SEM, cell injury was evident. Four distinct types of cell were identifiable by SEM. Besides the villous and smooth cells found in a typical splenocyte preparation from mice, a significant number of ruffled and injured cells were observed in preparations from mice at week 1 after MCMV infection. Polliak and associates believe that T cells possess a smooth surface, in contrast to the villous morphology of B cells (12). Other investigators have found that it is impossible to distinguish T and B cells on the basis of surface morphology (1, 5). The studies reported herein indicate that cells with a smooth surface may change into cells with numerous surface villi after MCMV infection or mitogenic stimulation.

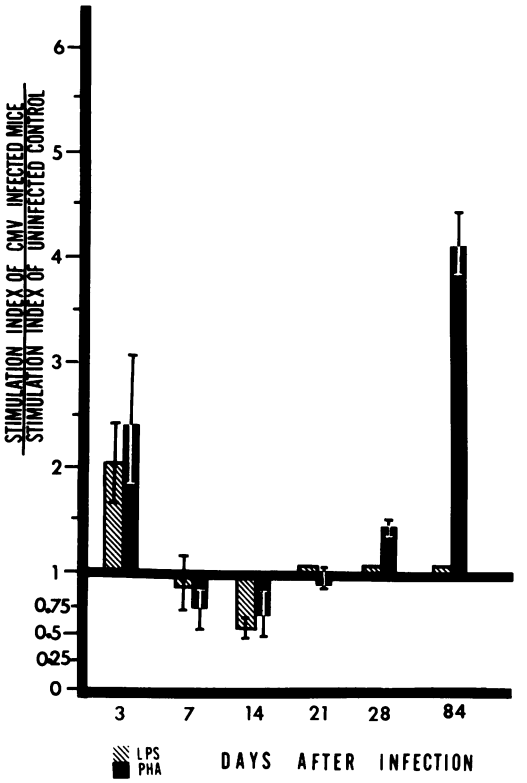

FIG. 6. *Splenocyte response to mitogens in the course of MCMV infection. Symbols: ▨, LPS; ■, PHA. Cultures were in triplicate. Stimulation index was computed as the ratio of [^{3}H]thymidine incorporation (cpm) by mitogen-stimulated and unstimulated cultures.*

It seems likely that villous cells represent activated lymphocytes rather than the typical morphology of B cells. MCMV infection of lymphocytes produced changes in the surface membranes, resulting in the appearance of microvilli and a tendency to clump.

Besides the smooth and villous forms, a third major cell type was represented by ruffled surface morphology. These cells could be removed by allowing them to attach to plastic surfaces and by the carbonyl iron procedure, suggesting that they might be adherent cells possessing phagocytic properties. A significant increase in the percentage of ruffled cells was also observed 1 week after MCMV infection. These ruffled cells apparently lost their surface adherent properties on glass surfaces or plastic surfaces. On the other hand, these cells may represent a nonadherent subpopulation of lymphocytes responding with membrane transformation after MCMV infection. Hudson and his associates, in studying MCMV infection of subpopulations of splenocytes, had reported virus associated with macrophages and B cells but not with T cells (8, 10). These investigators found that whereas macrophages contained most of the virus present in the spleen, B cells appeared to be the only cell type permissive to viral replication (8, 10). From the studies reported herein, it is apparent that different populations of splenocytes respond independently to MCMV infection. Subpopulations of splenocytes were either activated to produce surface villi, transformed to large lymphoblasts with a smooth surface, injured,

TABLE 3. *Effect of MCMV infection on humoral responses of the host*

Day post-infection	CF[a]	HetAb[b]	HL/HA[c]	Virus titer (PFU/ml)[d]
3	<2	<2	128	0
7	4	<2	32	10^2
14	4	<2	4	10^6
21	32	<2	128	10^6
28	64	<2	64	10^6
42	32	<2	128	10^5
56	32	<2	64	10^3
84	32	<2	64	0
Uninfected	<2	<2	128	0

[a] CF, Complement-fixing antibody.
[b] HetAb, Heterophil antibody induced by MCMV infection.
[c] HL/HA, Hemolysin and hemagglutinating-antibody titer in response to antigenic challenge of 0.1 ml of 50% SRBC inoculated intraperitoneally 1 week before the day of sacrifice.
[d] Virus titer of the salivary gland was expressed in plaque-forming units (PFU) per milliliter.

TABLE 4. *B cell function as measured by hemolytic plaque assay of splenocytes from BALB/c mice*

Time pre- or postinoculation with SRBC	PFU[a]/10^6 splenocytes	
	Control[b]	Infected[c]
1 day preinoculation	393 ± 107[d]	496 ± 134
At time of infection	329 ± 43	2,096 ± 1,404
3 days postinoculation	360 ± 92	18 ± 8
7 days postinoculation	274 ± 158	8 ± 6
14 days postinoculation	330 ± 51	6 ± 17
21 days postinoculation	240 ± 2	262 ± 57
140 days postinoculation	300[e]	307 ± 7

[a] PFU, Plaque-forming units.
[b] Control mice were inoculated intraperitoneally with 0.1 ml of an appropriate dilution of heat-inactivated normal salivary gland tissue homogenate.
[c] Infected mice were inoculated intraperitoneally with 0.1 ml of salivary gland homogenate containing 10^4 PFU of MCMV.
[d] The mean value ± the standard deviation of each group of three to six animals.
[e] Value was determined from one animal.

resulting in cell death, or modified in their surface membrane properties.

The triphasic response to PHA of splenocytes from infected mice was unexpected. The cellular injury observed during week 1 postinfection was also reflected in the maximal depression of cellular response to mitogen. The results suggested that the depression of cellular response to mitogen was a result of cell injury and the depletion of mitogen-responsive cells. On the other hand, an impairment in macrophage functions due to MCMV infection in these cells could conceivably result in a depressed response of T cells to mitogens (14). The early and late phases of response to PHA might be associated with a stimulatory effect of CMV and a proliferative response to lymphoid cells. SEM evidence that an increasing number of splenocytes underwent blast transformation during recovery from the period of cell injury also lends some support to this speculation.

MCMV infection did not seem to have a prolonged adverse effect on the humoral immune function of the host. The infected mice were capable of mounting an effective humoral response to foreign antigen once the acute phase of infection was over. The depression of humoral response during acute MCMV infection could be a direct result of cell injury and the selective depletion of the B cells and their precursors after MCMV infection.

Antibodies against MCMV appeared 1 week postinfection. The presence of specific antibody in the circulation is not effective in eliminating the virus from affected organ systems. However, antibody may be essential to protect the lymphoid cells (especially the B cells) from further injury by virus, leading to the eventual establishment of latent viral infection in appropriate target cells of the host.

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