INFECTION AND IMMUNITY, Dec. 1980, p. 700-708
0019-9567/80/12-0700/09$02.00/0

Vol. 30, No. 3

Immediate Loss of Cell-Mediated Immunity to Murine Cytomegalovirus upon Treatment with Immunosuppressive Agents

DONALD M. MATTSSON,[1]† RICHARD J. HOWARD,[2] AND HENRY H. BALFOUR, JR.[1,3]*

Department of Laboratory Medicine and Pathology[1] and Department of Pediatrics,[3] University of Minnesota, Minneapolis, Minnesota, and Department of Surgery, University of Florida College of Medicine, Gainesville, Florida 32610[2]

Splenic lymphocytes from cytomegalovirus-infected mice lost their in vitro proliferative responses to cytomegalovirus antigen within 3 h after in vivo treatment with antilymphocyte globulin and prednisolone. The response was inhibited when the agents were administered separately or together, and inhibition persisted through a 2-week course of immunosuppression. Circulating specific antibodies were depressed by multiple injections of antilymphocyte globulin alone or with prednisolone, but not by prednisolone alone. Mitogen-induced blast transformation was immediately depressed by immunosuppression with both agents. Although the response to lipopolysaccharide returned briefly, it declined with continuing treatment. Cytomegalovirus infection augmented the depressive effect of immunosuppression on the lipopolysaccharide proliferative response. Prednisolone treatment of infected animals did not affect the concanavalin A response, and lipopolysaccharide stimulation decreased more slowly and to a lesser extent than it did in mice treated with antilymphocyte globulin or both agents. Loss of specific cell-mediated immunity and simultaneous depression of humoral immunity indicated that immunosuppression immediately created an inability to respond to an active cytomegalovirus infection.

Cytomegalovirus (CMV) is a major cause of morbidity and mortality for immunocompromised patients (6, 8, 18, 21, 22). In patients receiving immunosuppression after organ transplantation, CMV infection causes disease ranging from asymptomatic viruria to severe illness and death (21). Immunosuppression prevents these patients from responding immunologically to active viral infection, whether endogenously or exogenously acquired.

Both humoral immunity and cell-mediated immunity (CMI) are important in protecting animals from and ridding them of viral infections (4, 22). However, patients with intact humoral immunity and deficient CMI are more susceptible to viral infections than are patients with deficient humoral immunity alone (22). CMV, a member of the Herpesviridae family, is cell associated and is not normally available to attack by serum antibody; therefore, CMI may be a more important host defense against this virus.

Pollard et al. found a correlation between humoral immunity and CMI in subjects presumed to be naturally immune (18). Immunosuppressed allograft recipients, however, most of whom became infected with CMV, experienced defective CMV CMI for up to 3 years after cardiac and renal transplantation, whereas humoral immune responses returned much earlier (15, 18).

We recently developed a method for evaluating CMI to CMV in mice (12). Using this procedure, we reported that immunosuppression with prednisolone and horse anti-mouse antilymphocyte globulin (ALG) inhibited CMI to murine CMV (MCMV) but did not have much effect on circulating specific antibodies (10, 11). In this report, we extended our earlier observations to include the time period during which immunosuppressants were administered, and we tried to discern the separate effects of the drugs administered.

(This paper was presented in part at the combined meetings of the Western Section of the American Federation for Clinical Research, Western Society for Clinical Research, Western Society for Pediatric Research, and Western Region Society for Investigative Dermatology, Inc., 8 February 1980, Carmel, Calif.)

MATERIALS AND METHODS

Mice. Six-week-old C57BL/6 female mice were purchased from Charles River Breeding Laboratories, Bloomington, Mass. They were kept in clean, dry

† Address reprint requests to: Donald M. Mattsson, Box 437, Mayo Bldg., University of Minnesota, Minneapolis, MN 55455.

cages and fed Purina laboratory chow (Ralston Purina, St. Louis, Mo.) ad libitum.

Virus. MCMV was originally obtained from June Osborne, University of Wisconsin, Madison. A pool of MCMV was prepared from the homogenate of salivary glands taken from Swiss-Webster mice that had been infected with MCMV (13). The pool had a titer of 4×10^7 plaque-forming units per ml in primary mouse embryo fibroblast cells.

Preparation of CMV antigen. MCMV antigen for the in vitro humoral immunity and CMI assays was prepared as described previously (12). Briefly, secondary Swiss-Webster embryo fibroblasts were grown to confluence in 1,000-cm^2 roller bottles. Each bottle was then inoculated with MCMV (10^6 plaque-forming units per bottle). When the cytopathic effect involved 80 to 90% of the monolayer (approximately 5 days after inoculation), adherent cells were scraped off the glass into the medium, and the medium was harvested, frozen at −70°C, and thawed to release intracellular virus. One liter of tissue culture medium was clarified of cell debris by centrifugation for 20 min at $4,800 \times g$ in a Beckman J-21 ultracentrifuge. The supernatant was carefully decanted and centrifuged for 1 h at $32,296 \times g$ in a Beckman L5-50 ultracentrifuge. The resulting pellet was suspended in 40 ml of Hanks balanced salt solution and centrifuged in an SW27 rotor for 1 h at $131,453 \times g$ and then was resuspended in a final volume of 2 ml (a 500-fold concentration). The pellet suspension was titrated for infectious virus as described previously (12) and contained 10^7 plaque-forming units per ml. Protein content was 2,000 μg/ml as measured by the Lowry method (16). The virus suspension was heat inactivated for 1 h at 56°C, after which no infectious virus was detectable in the cell cultures, and then stored at −70°C until use. A control antigen was also prepared and processed as described above from fibroblasts not infected with MCMV.

Cell proliferation assay. The cell culture technique that we utilized has been described previously (20). Mice were sacrificed, and their spleens were removed aseptically. Splenic lymphocytes were suspended in RPMI 1640 medium containing 10% fetal calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Erythrocytes were removed from the cell suspension by the Ficoll-Hypaque technique of Boyum (5). The mononuclear spleen cell fraction was washed two times in RPMI 1640 medium. Then, 0.05 mM 2-mercaptoethanol was added to the medium, and 2.5×10^5 spleen cells in 0.1 ml of medium were seeded in individual wells of microtissue culture plates (Linbro lot 76-003-05; Flow Laboratories, Inc., Rockville, Md.). One microgram of MCMV antigen or control antigen in 0.1 ml of medium was added to each of six wells for each group of animals assayed, and the plates were incubated for 6 days at 37°C in a 5% CO_2 atmosphere. Blast transformation experiments were conducted as described previously (11). A 1-μg amount of concanavalin A (ConA; Sigma Chemical Co., St. Louis, Mo.) or 2 μg of *Escherichia coli* O26:B6 endotoxin lipopolysaccharide (LPS; Difco Laboratories, Detroit, Mich.), was added in 0.1 ml of medium to triplicate wells that contained 2.5×10^5 spleen cells. The cultures were incubated for 48 h at 35°C in a 5% CO_2 atmosphere. For the last 6 h of incubation of the proliferation assays, 1 μCi of [*methyl*-^{3}H]thymidine (specific activity, 6.7 Ci/mmol) was added to each well. Plates were frozen at −20°C at the end of incubation and then thawed before individual wells were harvested on glass fiber filter paper with a Skatron cell harvester (Flow Laboratories, Inc.). The radioactivity of the individual wells was determined as counts per minute by liquid scintillation counting with a Beckman LS-3133T scintillation counter. Results were expressed as the mean and standard error of replicate cultures.

MCMV CF antibody. Mice were bled serially either from the retroorbital venous plexus, with blood being collected in heparinized microcapillary tubes, or from the axillary artery and vein, with blood being transferred from Pasteur pipettes into glass tubes. Sera were separated, heat inactivated at 56°C for 30 min, and stored at −70°C until all samples for each experiment had been collected. Complement-fixing (CF) antibody titers were measured in a microtiter system by the standard method of our diagnostic virology laboratory (3). The assay was done by using 4 U of the MCMV antigen, 1% sensitized sheep erythrocytes, and 2 U of guinea pig complement. Results were expressed as reciprocal titers.

Immunosuppression. Mice were immunosuppressed with (i) 0.5 mg (25 mg/kg) of prednisolone, (ii) 5 mg (250 mg/kg) of ALG given intraperitoneally, and (iii) both prednisolone and ALG. The mouse ALG was prepared in horses by Richard Condie of the Minnesota ALG Program.

Experimental design. Animals that received virus were injected intraperitoneally with 2×10^5 plaque-forming units of wild-type MCMV on day 0. Uninfected and infected animals were tested for humoral immunity and CMI on day 18, when peak MCMV immune responses are reached (12). Immunosuppression of uninfected and infected groups was then initiated; other groups did not receive immunosuppression. Prednisolone was administered daily, and ALG was administered every other day from days 18 or 19 to 30 or 31. Two to three animals from each group were sacrificed at each time point and tested for MCMV immunity and mitogen-induced blast transformation starting 3 h after the first injection of immunosuppressants and continuing throughout the course of immunosuppression. In a separate study of humoral immunity to MCMV, individual animals were bled serially from the retroorbital venous plexus at points before and after infection and before, during, and after treatment with both ALG and prednisolone. Changes in the reciprocal titers of infected, immunosuppressed mice were compared with those of infected animals that were not immunosuppressed.

Analysis of data. Serological data were converted to the $\log_2$ of the reciprocal titer, and a t statistic was generated from the differences of paired observations. Radioisotope incorporation data were converted to the $\log_{10}$ (7). A mean and standard deviation were determined from replicate cultures, and means were compared by deriving a t statistic based on the pooled estimate of variance.

RESULTS

Effect of immunosuppression on MCMV CF antibody. Infected, immunosuppressed

mice showed a marked depression of circulating specific antibody at the end of immunosuppression when compared with preimmunosuppression titers. Nonimmunosuppressed controls had no significant change in titer. Figure 1 shows that although CF titers were high in all animals before immunosuppression (each line represents the arithmetic mean of values from four animals), CF antibody was depressed in the immunosuppressed mice by day 25 ($P < 0.05$ when compared with the results from day 18). By day 35, the arithmetic mean titer of treated animals had fallen to 8, which was significantly different from the results from day 18 ($P < 0.005$). Titers did not change in nonimmunosuppressed animals during the period that the other group was immunosuppressed. Upon cessation of immunosuppression, the CF titers of immunosuppressed mice returned to approximately the levels of day 18 ($P < 0.02$ when day 35 is compared with day 67); no change in titer occurred in untreated animals after the period of immunosuppression.

When the immunosuppressants were administered separately, titers from treated and untreated infected animals were compared by pairing observations obtained at three time points during immunosuppression. Titers of infected mice immunosuppressed with prednisolone were not different from those of infected, untreated mice. However, ALG alone or in conjunction with prednisolone elicited a significant depression of CF titers ($P < 0.001$ and $P < 0.001$, respectively).

Effect of immunosuppression on blast transformation induced by MCMV antigen. One day before immunosuppression, MCMV-infected and uninfected animals were assayed to assure that MCMV CMI had developed (24,127 ± 4,198 versus 3,294 ± 568 cpm; $P < 0.001$). Within 3 h of the initial injection of immunosuppressants, cells of infected animals receiving treatment did not incorporate [^{3}H]thymidine in vitro in the presence of MCMV antigen (402 ± 60 cpm) when compared with cells from infected, nonimmunosuppressed mice (11,044 ± 2,541 cpm; $P < 0.001$) (Table 1). Cells from uninfected, untreated mice also failed to respond in vitro (1,428 ± 223 cpm; $P < 0.001$).

When ALG and prednisolone were administered separately to infected animals (Table 2), either treatment effectively abrogated the proliferative response. Whereas infected animals responded to MCMV in vitro before immunosuppression (19,996 ± 2,242 cpm; $P < 0.001$ when compared with uninfected control) and after initiation of treatment (30,509 ± 3,375; $P < 0.001$ when compared with uninfected control), ALG-treated animals immediately lost their response (507 ± 74 cpm; $P < 0.001$) as did prenisolone-treated animals (3,818 ± 1,960 cpm; $P < 0.001$). Regardless of whether the immunosuppressants were used separately or together, defective blastogenesis persisted with continuing immunosuppression.

Effect of immunosuppression on mitogen-induced blast transformation. As in the MCMV CMI studies, one injection of both im-

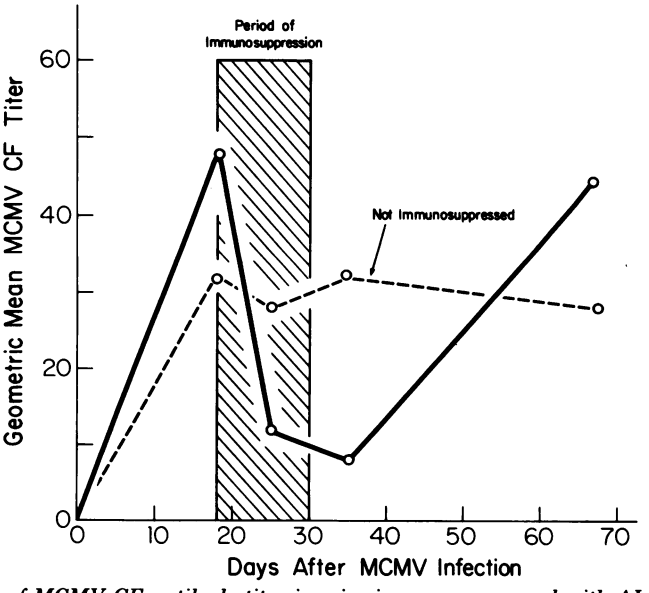

FIG. 1. *Depression of MCMV CF antibody titer in mice immunosuppressed with ALG and prednisolone after virus infection. Each line represents the arithmetic mean of the reciprocal titers from four serially bled animals.*

TABLE 1. *CMI to MCMV after immunosuppression with ALG and prednisolone*[a]

Group	cpm ± standard error							
	Before immunosuppression		At following time after initiation of immmunosuppression					
			3 h		5 days		9 days	
	MCMV antigen	Control antigen	MCMV antigen	Control antigen	MCMV antigen	Control antigen	MCMV antigen	Control antigen
1. Uninfected, not immunosuppressed	3,294 ± 568	1,466 ± 174	1,428 ± 223	1,208 ± 140	1,333 ± 254	786 ± 93	1,271 ± 155	6,908 ± 1,089
2. Infected, not immunosuppressed	24,127 ± 4,198[b]	1,075 ± 204	11,044 ± 2,541[b]	798 ± 133	27,742 ± 8,927[b]	904 ± 180	14,101 ± 2,052[b]	1,990 ± 549
3. Uninfected, immunosuppressed			324 ± 67[c]	255 ± 33	619 ± 61[c]	648 ± 65	888 ± 86[c]	3,218 ± 439
4. Infected, immunosuppressed			402 ± 60[c]	308 ± 46	729 ± 108[c]	583 ± 69	1,893 ± 433[c]	5,297 ± 1,426

[a] Mice were immunosuppressed with ALG and prednisolone at 18 to 30 days after virus infection.
[b] $P < 0.001$ compared with group 1.
[c] $P < 0.001$ compared with group 2.

TABLE 2. *CMI to MCMV after immmunosuppression with either ALG or prednisolone*[a]

Group	cpm ± standard error							
	Before immunosuppression		At following time after initiation of immunosuppression					
			1 day		4 days		8 days	
	MCMV antigen	Control antigen	MCMV antigen	Control antigen	MCMV antigen	Control antigen	MCMV antigen	Control antigen
1. Uninfected, not immunosuppressed	1,948 ± 459	989 ± 130	2,460 ± 370	1,461 ± 162	2,136 ± 245	928 ± 45	2,537 ± 121	1,373 ± 180
2. Infected, not immunosuppressed	19,996 ± 2,242[b]	753 ± 59	30,509 ± 3,375[b]	1,129 ± 235	27,407 ± 3,295[b]	655 ± 79	52,213 ± 8,623[b]	1,903 ± 648
3. Infected, ALG immunosuppressed			507 ± 74[c]	215 ± 28	4,011 ± 507[c]	840 ± 59	5,979 ± 1,617[c]	938 ± 104
4. Infected, prednisolone immunosuppressed			3,818 ± 1,960[c]	614 ± 116	3,212 ± 1,052[c]	705 ± 125	1,326 ± 121[c]	640 ± 54
5. Infected, ALG and prednisolone immunosuppressed			407 ± 116[c]	311 ± 41	708 ± 78[c]	290 ± 36	575 ± 35[c]	408 ± 32

[a] Mice were immunosuppressed with either ALG, prednisolone, or both at 18 to 30 days after virus infection.
[b] $P < 0.001$ compared with group 1.
[c] $P < 0.001$ compared with group 2.

munosuppressants immediately and significantly inhibited the in vitro transformation induced by the mitogen ConA (Table 3) when compared with nonimmunosuppressed animals. The response of cells from immunosuppressed animals to ConA, whether or not the animals had been infected with MCMV, remained significantly depressed ($P < 0.001$) throughout the course of immunosuppression.

Like the results from ConA and MCMV antigen stimulation, LPS blastogenesis of treated mice (Table 4) was depressed by 3 h after the initiation of immunosuppression ($P < 0.005$) but was not different from untreated animals by 24 h. Thereafter, animals were assayed about 24 h after each injection of immunosuppressants. Inhibition of the LPS response of cells from infected, immunosuppressed animals occurred again on day 8 of immunosuppression ($P < 0.005$ when compared with both nonimmunosuppressed groups) and thereafter for both immunosuppressed groups ($P < 0.005$). However, when this indirect measure of immune function was used, the depression appeared greater for infected (group 4) than uninfected (group 3) immunosuppressed animals.

When prednisolone was administered by itself (Table 5) to previously infected animals, ConA blast transformation was not different from that of nonimmunosuppressed animals ($P > 0.7$ after 1 day of immunosuppression, and $P > 0.8$ after 12 days). ALG treatment alone, however, created a significant depression of the ConA response ($P < 0.05$ when compared with both nonimmunosuppressed groups), as did immunosuppression of infected animals with both agents ($P < 0.05$ when compared with both nonimmunosuppressed groups).

ALG given alone to infected animals depressed LPS lymphocyte stimulation, as did ALG and prednisolone given together (Table 6). A significant difference between infected animals immunosuppressed with ALG and both nonimmunosuppressed groups appeared on day 1 after immunosuppression began ($P < 0.001$), not on day 2, and again on days 4 and 12 ($P < 0.001$). This pattern was similar to that of the infected, immunosuppressed group (Table 4). Although LPS blastogenesis (Table 6) was inhibited at all time points during immunosuppression ($P < 0.001$) in infected animals treated with both agents, a depression due to prednisolone first occurred after 4 days of immunosuppression ($P < 0.001$) and thereafter.

TABLE 3. *ConA-induced spleen cell blastogenesis in mice immunosuppressed after MCMV infection*[a]

Group	cpm ± standard error				
	Before immunosuppression	At following time after initiation of immunosuppression			
		3 h	4 days	8 days	14 days
1. Uninfected, not immunosuppressed	73,453 ± 5,214	42,574 ± 176	116,832 ± 10,046	93,849 ± 2,647	69,534 ± 10,546
2. Infected, not immunosuppressed	30,265 ± 1,475	27,926 ± 1,063	160,989 ± 7,660	110,468 ± 998	50,797 ± 390
3. Uninfected, immunosuppressed	24,811 ± 1,648	414 ± 19[b]	2,823 ± 1,329[b]	2,212 ± 921[b]	9,228 ± 1,354[b]
4. Infected, immunosuppressed	35,886 ± 2,790	555 ± 91[c]	844 ± 13[c]	1,716 ± 239[c]	1,026 ± 173[c]

[a] Mice were immunosuppressed with ALG and prednisolone at 18 to 30 days after virus infection.
[b] Significantly less than group 1; $P < 0.001$.
[c] Significantly less than groups 1 and 2; $P < 0.001$.

TABLE 4. *LPS-induced spleen cell blastogenesis in mice immunosuppressed after MCMV infection*[a]

Group	cpm ± standard error				
	Before immunosuppression	At following time after initiation of immunosuppression			
		3 h	1 day	8 days	14 days
1. Uninfected, not immunosuppressed	128,608 ± 3,195	194,854 ± 27,016	182,668 ± 4,895	109,177 ± 3,758	77,587 ± 3,664
2. Infected, not immunosuppressed	153,038 ± 4,582	223,915 ± 18,660	208,026 ± 12,288	193,033 ± 7,071	96,576 ± 12,377
3. Uninfected, immunosuppressed	179,405 ± 18,597	57,366 ± 5,094[b]	161,038 ± 11,628	37,713 ± 8,579	32,263 ± 2,569[b]
4. Infected, immunosuppressed	155,959 ± 1,800	53,658 ± 4,270[c]	192,956 ± 26,882	7,339 ± 314[c]	5,055 ± 339[c]

[a] Mice were immunosuppressed with ALG and prednisolone at 18 to 30 days after virus infection.
[b] Significantly less than group 1; $P < 0.005$.
[c] Significantly less than groups 1 and 2; $P < 0.005$.

Administration of both immunosuppressants seemed to inhibit LPS transformation to a greater extent than did prednisolone by itself and in an initially less transient manner than did ALG by itself.

DISCUSSION

One injection of ALG and prednisolone immediately and profoundly inhibited lymphocyte proliferation to MCMV antigen in previously infected animals. Immunosuppression also created a significant transient depression of circulating specific antibody. These data extend our earlier studies on the relationship of MCMV immunity and immunosuppression.

Accumulating observations indicate that CMI may be a more important host defense than humoral immunity in combating an established herpesvirus infection (4, 22). The cell-associated nature of CMV is demonstrated in vivo. When CMV is present in the blood, it is best isolated from buffy coat cells but not from plasma, where the virus would be subject to serum antibody (19). Compelling evidence for the importance of CMI is found in the occurrence of morbidity and mortality in CMV-infected transplant recipients despite demonstrable circulating specific antibodies (2, 15). In addition, Notkins showed that immune lymphocytes prevented the spread of herpes simplex virus in a fibroblast monolayer, whereas antibody did not (17). More recently, Ho demonstrated in mice the protective effect of passively transferred CMV-specific cytotoxic lymphocytes and the abrogation of protection by treatment of the cells with anti-θ serum (9).

In a study of normal subjects, none of the participants seronegative to CMV and 90% of those seropositive to CMV demonstrated significant in vitro antigen-specific lymphocyte transformation (18). In contrast, CMV CMI was absent for up to 3 years after cardiac transplantation and immunosuppression (18). For renal

TABLE 5. *ConA-induced spleen cell blastogenesis in mice immunosuppressed with either ALG or prednisolone after MCMV infection*[a]

Group	cpm ± standard error		
	Before immunosuppression	At following time after initiation of immunosuppression	
		1 day	12 days
1. Uninfected, not immunosuppressed	227,654 ± 2,057	171,287 ± 2,768	119,032 ± 1,201
2. Infected, not immunosuppressed	137,533 ± 5,933	113,471 ± 11,247	157,749 ± 10,858
3. Infected, ALG immunosuppressed	207,738 ± 6,210	19,450 ± 15,364[b]	10,784 ± 6,142[b]
4. Infected, prednisolone immunosuppressed	273,051 ± 4,783	115,633 ± 3,370	118,175 ± 5,469
5. Infected, ALG and prednisolone immunosuppressed	254,396 ± 6,280	1,963 ± 26[b]	5,528 ± 2,578[b]

[a] Mice were immunosuppressed with either ALG, prednisolone, or both at 18 to 30 days after virus infection
[b] Significantly less than groups 1 and 2; $P < 0.05$.

TABLE 6. *LPS-induced spleen cell blastogenesis in mice immunosuppressed with either ALG or prednisolone after MCMV infection*[a]

Group	cpm ± standard error				
	Before immunosuppression	At following time after initiation of immunosuppression			
		1 day	2 days	4 days	12 days
1. Uninfected, not immunosuppressed	54,661 ± 790	57,304 ± 161	49,216 ± 2,544	41,665 ± 1,581	37,779 ± 1,086
2. Infected, not immunosuppressed	56,689 ± 1,677	82,904 ± 2,768	25,142 ± 3,096	32,178 ± 1,078	40,136 ± 1,931
3. Infected, ALG immunosuppressed	81,845 ± 2,704	9,885 ± 419[b]	30,818 ± 795	6,685 ± 179[b]	5,281 ± 628[b]
4. Infected, prednisolone immunosuppressed	97,594 ± 730	64,892 ± 1,427	40,498 ± 1,970	21,513 ± 619[b]	12,756 ± 517[b]
5. Infected, ALG and prednisolone immunosuppressed	88,462 ± 3,187	5,414 ± 353[b]	8,828 ± 183[b]	3,562 ± 153[b]	2,332 ± 221[b]

[a] Mice were immunosuppressed with either ALG, prednisolone, or both at 18 to 30 days after virus infection
[b] Significantly less than groups 1 and 2; $P < 0.001$.

transplant recipients there is little relationship between CF antibody titer and protection from CMV infection (2), and a dissociation posttransplant between CMV humoral immunity and CMI has been noted (15).

In the investigations presented here there is a correlation between an immediate loss of MCMV CMI in immunosuppressed, infected animals and a slightly later depression of CF antibodies. Whereas we had previously reported no depression of MCMV antibody during immunosuppression (10, 11), those results were based on sera from different animals sacrificed at each time point. Although the animals used were genetically uniform, variation in antibody production among animals may have obscured the depression observed when individual animals were serially bled throughout the course of immunosuppression.

Several weeks after cessation of immunosuppression, humoral immunity returned to and even exceeded levels seen in nonimmunosuppressed, infected controls. Mitogen-induced lymphocyte proliferation, also depressed by immunosuppression, returned after immunosuppression was stopped; MCMV-specific transformation, once inhibited by immunosuppression, remained depressed throughout the duration of our observations (11).

Previous investigations of the in vivo effects of immunosuppressive agents on immune function have shown that ALG inhibits cell-mediated immune reactions and that antibody production can be depressed when ALG is administered before the eliciting antigen (1). The effect of corticosteroids has led to the differentiation of steroid-sensitive and -resistant T cells. Spleen cells from mice given one injection of hydrocortisone showed a slight increase or no change in ConA and LPS responsiveness. Antigen- and mitogen-induced transformations have not been studied in long-term, steroid-treated animals, however. Like ALG, steroid administration at the time of antigenic challenge depressed subsequent antibody formation (1).

In an earlier study we showed that nonspecific immunosuppression at the time of MCMV infection prevented the development of CMI but did not depress humoral immunity (10, 11). The data presented here reveal an abrogation of established lymphocyte proliferative responses to MCMV antigen after a single injection of either prednisolone or ALG and depression of an established primary antibody response by multiple injections of ALG but not by prednisolone. As may be expected, mitogen and antigen blastogenes were not present in ALG-immunosuppressed mice, and the response to ConA was not affected by prednisolone. Depression of the thymus-independent LPS response by extended prednisolone treatment and the apparent synergy between ALG and prednisolone in depressing this and the ConA response in infected animals are of interest, as is the highly steroid-sensitive nature of the response to viral antigen of sensitized lymphocytes. Kelsey et al. reported that the subpopulation of lymphocytes induced to proliferate by MCMV antigen were θ-bearing (14). MCMV antigen-induced lymphocyte proliferation was reduced markedly by anti-θ serum but only slightly by anti-gamma globulin. Clearly, there is a need for further studies to expand our understanding of the cell populations involved in these responses.

Immediate and prolonged defective MCMV CMI and transient depression of specific CF antibodies resulted when previously infected animals received a course of immunosuppression with ALG and prednisolone. Alteration of both of these immune responses in immunosuppressed renal transplant recipients may explain why these patients are susceptible to reactivation and dissemination of CMV.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AM13083 and AM18883 from the National Institutes of Health.

LITERATURE CITED

1. **Bach, J. P.** 1975. Corticosteroids; antilymphocyte sera, p. 21-70; 227-303. *In* E. L. Tatum and A. Neuberger (ed.), The mode of action of immunosuppressive agents. North Holland Publishing Co., Amsterdam.
2. **Balfour, H. H., Jr., M. S. Slade, J. M. Kalis, R. J. Howard, R. L. Simmons, and J. S. Najarian.** 1977. Viral infections in renal transplant donors and their recipients: a prospective study. Surgery **81**:487-492.
3. **Benyesh-Melnick, M.** 1969. Cytomegaloviruses, p. 701-732. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections, 4th ed. American Public Health Association, Inc., New York.
4. **Bloom, B. R., and B. Rager-Zisman.** 1975. Cell-mediated immunity in viral infections, p. 113-116. *In* A. L. Notkins (ed.), Viral immunology and immunopathology. Academic Press, Inc., New York.
5. **Boyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J. Clin. Lab. Invest. Suppl. 97 **21**:77-89.
6. **David, D. S., S. J. Millian, J. C. Whitsell, G. H. Schwartz, R. R. Riggio, K. H. Stenzel, and A. L. Rubin.** 1972. Viral syndromes and renal homograft rejection. Ann. Surg. **175**:257-259.
7. **Dixon, W. J., and F. J. Massey, Jr.** 1969. Introduction to statistical analysis, 3rd ed., p. 324. McGraw-Hill Book Co., New York.
8. **Fiala, M., J. E. Payne, T. V. Berne, T. C. Moore, W. Henle, J. Z. Montgomerie, S. N. Chatterjee, and L. B. Guze.** 1975. Epidemiology of cytomegalovirus infec-

tion after transplantation and immunosuppression. J. Infect. Dis. **132**:421–433.
9. **Ho, M.** 1980. Role of specific cytotoxic lymphocytes in cellular immunity against murine cytomegalovirus. Infect. Immun. **27**:767–776.
10. **Howard, R. J., D. M. Mattsson, and H. H. Balfour, Jr.** 1979. Cell-mediated immunity to cytomegalovirus in mice and in renal transplant recipients. Transplant. Proc. **11**:75–78.
11. **Howard, R. J., D. M. Mattsson, and H. H. Balfour, Jr.** 1979. Effect of immunosuppression on humoral and cell-mediated immunity to murine cytomegalovirus. Proc. Soc. Exp. Biol. Med. **161**:341–346.
12. **Howard, R. J., D. M. Mattsson, M. V. Seidel, and H. H. Balfour, Jr.** 1978. Cell-mediated immunity to murine cytomegalovirus. J. Infect. Dis. **138**:597–603.
13. **Howard, R. J., and J. S. Najarian.** 1974. Cytomegalovirus-induced immune suppression. I. Humoral immunity. Clin. Exp. Immunol. **18**:109–118.
14. **Kelsey, D. K., J. C. Overall, Jr., and L. A. Glasgow.** 1978. Correlation of the suppression of mitogen responsiveness and the mixed lymphocyte reaction with the proliferative response to viral antigen of splenic lymphocytes from cytomegalovirus-infected mice. J. Immunol. **121**:464–470.
15. **Linnemann, C. C., Jr., C. A. Kauffman, M. R. First, G. M. Schiff, and J. P. Phair.** 1978. Cellular immune response to cytomegalovirus after renal transplantation. Infect. Immun. **22**:176–180.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **Notkins, A. L.** 1974. Immune mechanisms by which the spread of viral infections is stopped. Cell. Immunol. **11**: 478–483.
18. **Pollard, R. B., K. H. Rand, A. M. Arvin, and T. C. Merigan.** 1978. Cell-mediated immunity to cytomegalovirus infection in normal subjects and cardiac transplant patients. J. Infect. Dis. **5**:541–549.
19. **Rinaldo, C. R., Jr., P. H. Black, and M. S. Hirsch.** 1977. Interaction of cytomegalovirus with leukocytes from patients with mononucleosis due to cytomegalovirus. J. Infect. Dis. **136**:667–678.
20. **Rytel, M. W.** 1976. Humoral and cell-mediated immunity to cytomegaloviruses. Yale J. Biol. Med. **49**:63.
21. **Simmons, R. L., C. Lopez, H. H. Balfour, Jr., J. Kalis, L. C. Rattazzi, and J. S. Najarian.** 1974. Cytomegalovirus: clinical virological correlations in renal transplant recipients. Ann. Surg. **180**:623–634.
22. **Wheelock, E. F., and R. S. Toy.** 1973. Participation of lymphocytes in viral infections. Adv. Immunol. **16**:123–184.