Modification of Acute Murine Cytomegalovirus Adrenal Gland Infection by Adoptive Spleen Cell Transfer

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Homozygous (*nu/nu*), athymic nude mice, infected with murine cytomegalovirus (MCMV), develop unremitting and ablative virus infection involving both the adrenal cortex and medulla. During acute infection, adoptive transfer of MCMV-immune, but not naive, spleen cells suppressed virus replication in the adrenal glands, but not the lungs or salivary gland. T lymphocytes, not macrophages or B cells, were responsible for limiting viral replication. The effect by donor cells was restricted by compatibility at the major histocompatibility locus. Restriction of MCMV replication in the adrenal gland was associated with T lymphocytes of the L3T4 phenotype. Thus, T-cell immunity is critical in regulating MCMV replication in the adrenal glands, and T lymphocytes restricted by class II major histocompatibility antigens mediate this effect.

It is now widely acknowledged that host cellular immune mechanisms play a pivotal role in the course and outcome of cytomegalovirus (CMV) infections (10). In humans, infections due to CMV are commonplace, while disseminated infection and diseases due to this agent occur primarily in the setting of abnormal host immunity. This fact, originally recognized with the advent of the transplantation era, has recently been emphasized by CMV infections occurring in the setting of defects of T-cell immunity seen with the acquired immune deficiency syndrome (11, 12). However, while the integrity of cellular immunity is critical in determining the outcome of CMV infection, the cellular mechanisms responsible for containing infection are largely unknown.

Studies in animals have significantly advanced our understanding of the roles of cellular and humoral immunity during CMV infection (16). These studies have also reaffirmed the preeminence of cellular immunity in determining the course of CMV infection (1, 33). Studies of murine CMV (MCMV) infection in athymic nude mice, deficient in T-cell immunity, exemplify this fact. During MCMV infection, athymic nude (*nu/nu*) mice develop virus replication in the adrenal gland (4, 28). In the absence of T-lymphocyte function, virus replication is unremitting, leading to ablation of the adrenal cortex and medulla (28). In contrast, MCMV infection of the adrenal gland does not occur in heterozygous littermates with intact T-cell function or in homozygous nude mice whose T-lymphocyte function has been restored before infection. Using this model, I further explored the role of host immunity in regulating acute CMV infection by reconstituting selected aspects of host immunity during acute infection. These studies demonstrate that MCMV replication in the adrenal gland during acute infection can be limited by the adoptive transfer of *H-2*-restricted, MCMV-immune T lymphocytes, but not naive T cells, B cells, or macrophages. This activity resided in the T lymphocytes possessing the L3T4 cell phenotype.

MATERIALS AND METHODS

Mice. For these experiments, 6- to 8-week-old female and male T-cell-deficient, homozygous (*nu/nu*), BALB/c AnN (*H-2D*) nude mice and their immunocompetent, heterozygous (*nu/+*) littermates were obtained from Frederick Cancer

Research Center, Frederick, Md. DBA/2N (*H-2D*) mice were purchased from Charles River Breeding Laboratories, Wilmington, Mass. BALB/B mice (*H-2B*) were obtained from G. M. Shearer, National Cancer Institute, Bethesda, Md. Animals were maintained in groups of five in isolator units in a laminar-flow hood and given free access to autoclaved Purina Laboratory Chow 500 and water. The colony was monitored for infections due to MCMV as well as other pathogens. Serum from all heterozygous *nu/+* study animals was consistently negative for antibody to MCMV unless experimentally infected.

Virus. The Smith strain of MCMV was originally obtained from M. C. Jordan (University of Minnesota, Minneapolis) as a 10% (wt/vol) homogenate of infected salivary gland tissue. For these experiments, the virus was passed once in tissue culture by using mouse embryo cells (MEC), and samples were prepared from clarified tissue culture supernatants (27). The virus pool used for these experiments contained 2.0×10^6 PFU of virus per ml. The inoculum for sham-infected controls was prepared from supernatants of uninfected MEC. Virus and control stocks were stored at -70°C . The virus stocks were screened by antibody generation assay (Microbiological Associates, Bethesda, Md.) and found to be free of other murine pathogens, including pneumonia virus of mice, reovirus type 3, K virus, polyomavirus, Sendai virus, minute virus of mice, murine adenovirus, lymphocytic choriomeningitis virus, murine hepatitis virus, ectromeliavirus, and lactic dehydrogenase virus.

The KOS strain of herpes simplex virus type 1 was obtained from E. Shaskan and S. Weller (University of Connecticut, Farmington). The virus, which was plaque purified, was propagated in MEC culture. The virus stock had a titer of 2.4×10^6 PFU/ml.

Cell culture methods. MEC were prepared from embryos of late-term pregnant CD-1 mice (Charles River Breeding Laboratories, Kensington, N.Y.) by trypsin-EDTA disaggregation and maintained in tissue culture with Eagle minimum essential medium containing 10% fetal calf serum, 100 U of penicillin per ml, and 50 μg of gentamicin per ml. Quantitation of virus was performed by plaque assay in MEC monolayers in 24-well tissue culture clusters by using an overlay of minimum essential medium containing 20%

fetal calf serum, 0.02 M HEPES (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid) (pH 7.5), 1% methylcellulose, and antibiotics (23, 27).

Virus infection of mice. Infection was initiated by instilling 0.05 ml of stock virus into the nose during light ether anesthesia. Previous work has demonstrated that inoculation by this method produces lung infection in 100% of mice but produces less mortality than intraperitoneal inoculation (22, 27, 29). Sham-infected control animals were inoculated in parallel by using an equivalent volume of heat-inactivated virus. In one experiment, BALB/c mice were also inoculated with 10^4 PFU of herpes simplex virus type 1 (KOS) in 0.2 ml given intraperitoneally.

Serological methods. Immune serum to MCMV was produced as previously described (26). Briefly, *nu/+* mice were inoculated intranasally with 10^4 PFU of MCMV. Sham-inoculated mice served as controls. At 1 to 2 months after infection, serum was collected and heated at 56°C for 30 min. Titers of serum antibody to MCMV were determined by enzyme-linked immunosorbent assay as previously described (26).

Evaluation of virus infection. At various intervals after virus inoculation, mice were killed, and serum and tissues were obtained for study. To quantify virus, adrenal gland tissues were prepared as 2.5% (wt/vol) homogenates and other tissues as 10% (wt/vol) homogenates with minimum essential medium containing 10% newborn calf serum and 10% dimethyl sulfoxide and stored at -70°C until virus assay.

Collection of spleen cells. Spleen cells were collected from sham-infected or MCMV-infected *nu/+* littermates. Unless otherwise specified, MCMV-immune cells were collected 21 days after virus infection of the donor. Spleens were cut in half, and the cells were gently teased from the spleen by injecting 5 ml of phosphate-buffered normal saline (PBS). The erythrocytes were lysed by one cycle in 0.72% ammonium chloride-Tris hydrochloride (pH 7.2) and then washed twice in PBS. Cell number and viability were quantified by cell counting in a hemacytometer in the presence of 0.04% trypan blue.

Removal of cells. The spleen cells were depleted of adherent cells by incubating the suspension in 150-cm² plastic cell culture flasks for 1 h at 37°C. T lymphocytes were removed from the nonadherent fraction by treating the suspension at a density of 10^7 cells in 1.0 ml of PBS containing 0.2 M sodium azide with 1:20 final dilution of polyclonal rabbit anti-mouse theta serum (Litton Bionetics, Kensington, Md.) at 4°C for 1 h. The cells were washed in PBS and then incubated for 45 min at 37°C in PBS with 0.2 M sodium azide containing guinea pig complement at 1:4 dilution (Cooper Biomedical, Inc., West Chester, Pa.).

The B lymphocytes were similarly removed by incubating the cells with goat anti-mouse immunoglobulin G (heavy and light chains) at a dilution of 1:20 (Cooper Biomedical) followed by guinea pig complement. After the removal of the cell types, the cell number and viability were assessed.

Collection of lymphocyte subsets. B cells and T-lymphocyte subsets were labeled by using monoclonal or polyclonal antisera labeled with fluorescein isothiocyanate (FITC) and collected by using the fluorescence-activated cell sorter (FACS) (Becton Dickinson & Co., Mountain View, Calif.). Briefly, nonadherent spleen cells were suspended in PBS with 0.2 M sodium azide at a density of 5×10^6 lymphocytes in 0.4 ml and reacted with FITC-labeled antibody. Lymphocytes were incubated for 1 h at 4°C in PBS with 0.2 M sodium azide containing FITC-goat anti-mouse immunoglobulin G

TABLE 1. Effect of adoptive spleen cell transfer on acute MCMV replication^a in the adrenal glands of T-cell-deficient nude mice

<i>nu/+</i> donor ^b spleen cells	MCMV titer ^c			Antibody titer ^d
	Salivary gland	Lung	Adrenal gland	
None	8.39	5.52	5.90	<10
Nonimmune	8.84	6.87	6.48	20
MCMV immune	8.55	6.36	<2.08	40

^a Athymic (*nu/nu*) mice were inoculated intranasally with 10^5 PFU of MCMV.

^b Spleen cells (20×10^6) given intravenously 7 days after virus inoculation of the *nu/nu* recipient.

^c Virus titers ($\log_{10}$ PFU per gram) in tissue homogenates pooled from five animals 14 days after virus inoculation.

^d Reciprocal of the enzyme-linked immunosorbent assay antibody titer.

(heavy and light chains) at a dilution of 1:20 to label B cells. Similarly, T lymphocytes were labeled by using FITC monoclonal antibody to Thy-1.2 (Becton Dickinson). To label T-lymphocyte subsets, FITC monoclonal anti-L3T4 (GK 1.5; courtesy of Sandy Via, National Cancer Institute, Bethesda, Md.) and FITC-labeled monoclonal antibody to Lyt-2 (Becton Dickinson) were used. After 45 min at 4°C, the cells were washed twice in PBS containing 0.2% bovine serum albumin and 0.02 M sodium azide. The cells were then sorted on the basis of size and fluorescence by using a FACS. After collection, the cells were washed twice in PBS, and the cell number and viability were reassessed.

Effect of cell preparations on MCMV infection. At 7 days after virus inoculation, the *nu/nu* mice were given spleen cell preparations from immunocompetent heterozygous *nu/+* littermates by intravenous tail vein injections. Unless otherwise specified, 2×10^7 mouse cells were given. For cells prepared with the FACS, 2×10^5 cells per mouse were given. After 7 and 14 days, the *nu/nu* recipients were killed, the serum was collected, and the virus content of the adrenal and salivary glands was quantified.

RESULTS

Reconstitution of immunity during acute infection. It has been previously demonstrated that restoration of T-cell-mediated immunity by transfer of splenic cells to *nu/nu* mice 21 days before infection prevents ablative CMV replication in the adrenal glands during subsequent viral infection (28). To better understand the immune mechanisms involved, I examined the ability of adoptive transfer of spleen cells during acute infection to modify the course of MCMV adrenal gland infection. At 7 days after MCMV infection, *nu/nu* mice received 2×10^7 spleen cells from normal or MCMV-immune *nu/+* littermates. After 7 days, the animals were examined for the extent of MCMV infection. The transfer of normal spleen cells did not significantly alter MCMV replication in the adrenal glands despite the production of antibody to MCMV (Table 1). In contrast, MCMV replication was suppressed in the adrenal glands of mice given spleen cells from immune animals. MCMV replication in the lungs and salivary glands was not significantly altered by either immune or nonimmune cells. Thus, acute adrenal gland MCMV replication was significantly altered by the passive transfer of spleen cells from *nu/+* mice immune to MCMV.

Since naive donor cells did not alter MCMV infection and MCMV-immune cells did, I assessed the appearance of the

TABLE 2. Capacity of donor spleen cells, harvested at times after MCMV infection of the donor, to modify acute MCMV replication^a in the adrenal glands of athymic nude mice

<i>nu/+</i> donor ^b lymphocytes	No. of days after donor infection ^c	MCMV titer ^d in adrenal gland ^e
None		6.27
Nonimmune		6.78 (0)
Immune	7	<2.08 (99.9)
Immune	14	3.08 (99.9)
Immune	21	2.73 (99.9)
Immune	28	<2.08 (99.9)
Immune	48	<2.08 (99.9)
Immune	60	2.90 (99.9)

^a As in Table 1, footnote a.^b As in Table 1, footnote b.^c *nu/+* donors inoculated intranasally with 10⁵ PFU of MCMV.^d As in Table 1, footnote c.^e Numbers in parentheses are the percent reduction in viral titer.

protective effect in spleen cells at various times after virus administration to the donor. The antiviral activity of splenic lymphocytes harvested from *nu/+* donors at 0, 7, 14, 21, and >21 days after virus inoculation was assessed (Table 2). While naive cells were ineffective in altering MCMV replication, cells derived from mice as early as 7 days after virus infection were able to suppress MCMV replication after passive transfer of cells. This effect persisted up to 60 days after donor inoculation.

To determine which splenic cells were responsible for modifying MCMV infection, I depleted the spleen cell preparations of cell populations. Elimination of either adherent cells or the B lymphocytes did not alter the inhibitory effect (Table 3). In contrast, depletion of T lymphocytes completely ablated the ability of immune splenic cells to inhibit MCMV replication in the adrenal gland.

H-2 restriction of transferred cells. I examined the influence of H-2 compatibility between the donor and recipient on MCMV replication in the adrenal glands after passive transfer of immune spleen lymphocytes (Table 4). At 7 days after MCMV infection, homozygous nude mice received either no cells or 2×10^7 spleen lymphocytes from MCMV-immune donors of different genetic backgrounds. Nude mice

TABLE 3. Effect of normal, MCMV-immune, or nonadherent immune spleen cells or immune splenic T or B lymphocytes on acute MCMV replication^a in the adrenal glands of T-cell-deficient nude mice

<i>nu/+</i> donor ^b spleen cells	MCMV titer ^c		Antibody titer ^d
	Salivary gland	Adrenal gland ^e	
None	7.78	5.81	<10
Nonimmune	7.88	5.80 (0)	10
MCMV immune	7.60	2.60 (99)	20
Nonadherent immune cells ^f	7.87	3.49 (99)	40
Nonadherent T-depleted cells ^g	8.27	5.71 (25)	<10
Nonadherent B-depleted cells ^g	7.88	<2.08 (99)	10

^a As in Table 1, footnote a.^b As in Table 1, footnote b.^c As in Table 1, footnote c.^d As in Table 1, footnote d.^e As in Table 2, footnote e.^f Adherent cells removed by plastic adherence.^g T or B cells depleted by polyclonal immune serum and complement.TABLE 4. Influence of donor-recipient H-2 compatibility on the antiviral effect of spleen lymphocytes during acute MCMV replication^a in the adrenal and salivary glands of T-cell-deficient BALB/c (H-2D) nude mice

Donor spleen ^b lymphocytes	Donor H-2 locus	MCMV titer ^c		Antibody titer ^d
		Salivary gland	Adrenal gland ^e	
None		8.17	6.63	<10
BALB/c (MCMV immune)	<i>d</i>	7.64	<2.08 (100)	40
DBA/2N (MCMV immune)	<i>d</i>	8.12	4.67 (99)	80
BALB/B (MCMV immune)	<i>b</i>	8.15	6.66 (0)	<10
BALB/c (HSV immune)	<i>d</i>	7.49	5.72 (86)	<10

^a As in Table 1, footnote a.^b As in Table 1, footnote b.^c As in Table 1, footnote c.^d As in Table 1, footnote d.^e As in Table 2, footnote e.

given no cells developed substantial virus replication in the adrenal glands, while MCMV replication in mice given isogenic cells from MCMV-immune BALB/c donors was suppressed. Immune lymphocytes from DBA/2N mice, which are H-2 compatible with BALB/c but mismatched at non-H-2 loci also suppressed adrenal infection, although somewhat less than isogenic cells. In contrast, adrenal replication was unaffected by the transfer of cells from BALB/B mice, a congenic strain of BALB/c, which differs from BALB/c mice only at the H-2 locus. As a control for the effects of nonspecific activation of donor cells, lymphocytes from BALB/c mice previously infected with herpes simplex virus type 1 (KOS) were transferred. These cells were more efficient than the BALB/B cells but less efficient than the MCMV-immune, H-2-matched cells. In general, MCMV replication in the salivary gland was unaffected by passive spleen cell transfer from any of the MCMV-immune donors.

Influence of the quantity of spleen cells on MCMV adrenal gland infection. Because subsequent studies with cells prepared by the FACS would require the administration of substantially less than 2×10^7 cells per mouse, the number of immune spleen lymphocytes required to effect a decrease in MCMV adrenal replication was determined. At 7 days after virus inoculation of *nu/nu* mice, graded doses of MCMV-immune spleen *nu/+* lymphocytes were passively transferred (Table 5). As few as 10⁵ immune spleen lymphocytes resulted in a significant decline in MCMV replication in

TABLE 5. Effect of graded doses of nonadherent, immune spleen cells on acute MCMV replication^a in the adrenal glands of T-cell-deficient nude mice^b

<i>nu/+</i> donor ^b spleen cells	MCMV titer ^c		Antibody titer ^d
	Salivary gland	Adrenal gland ^e	
None	7.78	6.47	<10
10 ⁷	7.88	3.02 (99)	160
10 ⁶	7.60	5.56 (88)	10
10 ⁵	7.87	5.80 (78)	20
10 ⁴	8.27	6.45 (0)	<10

^a As in Table 1, footnote a.^b Number of immune spleen cells given intravenously 7 days after virus inoculation of the *nu/nu* recipients.^c As in Table 1, footnote c.^d As in Table 1, footnote d.^e As in Table 2, footnote e.

TABLE 6. Effects of specific subsets of MCMV-immune spleen lymphocytes on acute MCMV replication^a in the adrenal and salivary gland of athymic nude mice

Donor spleen ^b cell phenotype	MCMV titer ^c in adrenal gland ^d	MCMV antibody ^e
None	6.04	<10
Immune lymphocytes	3.03 (99)	10
L3T4 cells	4.06 (99)	10
Lyt-2 cells	6.13 (0)	10

^a As in Table 1, footnote *a*.^b Spleen cells from MCMV-immune, *nu/nu* donors were stained with FITC-labeled monoclonal antibodies to L3T4 or Lyt-2 and prepared with the FACS. Immune spleen cells (20×10^6) or T-cell subsets (2×10^5) were administered intravenously 7 days after virus inoculation of the recipient.^c Virus titer ($\log_{10}$ PFU per gram) in tissue homogenates pooled from a minimum of five recipients 14 days after virus inoculation.^d As in Table 2, footnote *e*.^e As in Table 1, footnote *d*.

the adrenal gland. The ability of this number of cells to effect a reduction in virus replication suggested that reconstruction experiments with lymphocyte populations prepared by FACS separation would be feasible.

Restoration of immunity by using splenic lymphocyte subpopulations. To define the specific T-lymphocyte populations that are capable of modulating MCMV replication in the adrenal glands, immune spleen T lymphocytes were sorted into the L3T4 and Lyt-2 phenotypes and administered to MCMV-infected *nu/nu* mice (Table 6). B lymphocytes were also prepared by this method as an additional control. At 7 days after virus inoculation of *nu/nu* mice, 2×10^5 cells of the specific subtypes derived from MCMV-immune donors were administered by tail vein injection. Both the B lymphocytes and T cells of the Lyt-2 phenotype had a negligible effect on MCMV replication in the adrenal gland. In contrast, administration of cells with the L3T4 phenotype suppressed viral replication in the adrenal glands.

DISCUSSION

Both clinical and experimental observations have indicated that T-cell-mediated immunity is critical in determining the outcome of CMV infections in both humans and animals (10). Defects in T-cell-mediated immunity or immunosuppressive measures affecting the T-cell immune system results in disseminated CMV infection. The importance of cell-mediated immunity in containing CMV is emphasized by the observations of Starr and Allison that MCMV infection, even initiated with a relatively small dose of virus, was invariably lethal in congenitally athymic nude mice (33). These authors also demonstrated that the passive transfer of primed, but not naive, T lymphocytes prevented mortality in BALB/c mice challenged with a lethal dose of MCMV. However, the mechanisms of protection or the T cells responsible were not defined.

While MCMV infection of T-cell-deficient mice results in unrelenting virus replication involving multiple tissues, involvement of the adrenal gland results in extensive destruction of both cortex and medulla (28). These observations are similar to the adrenal necrosis associated with CMV infection seen in individuals with abnormalities of T-cell function due to the acquired immune deficiency syndrome (38). The current experiments were intended to define the cell populations responsible for altering the course of MCMV repli-

cation by examining the effects of adoptive transfer of selected cell populations during acute MCMV infection involving the adrenal gland. Administration of spleen cells from MCMV-immune donors resulted in a dramatic reduction of virus replication in the adrenal gland within 7 days of transfer. In contrast, naive spleen cells did not alter the progression of MCMV replication, even 14 days after cell transfer. The ability to significantly alter MCMV replication appeared in the donor spleen cells as early as 7 days after MCMV infection and persisted for up to 60 days. Protection was restricted by compatibility of the donor and the recipient at the *H-2* locus. Depletion experiments indicated that T lymphocytes, but not B lymphocytes or adherent cells, were responsible for this effect. Analysis of the *in vivo* effects of T lymphocytes separated on the basis of L3T4 and Lyt-2 phenotypes indicated the antiviral activity resided primarily in cells with L3T4 markers. T lymphocytes of the Lyt-2 subset exerted only a slight effect on MCMV replication. Thus, T-cell-mediated immunity played a pivotal role in modulating the extent of MCMV replication in the adrenal gland of T-cell-deficient mice during acute infection.

Cell-mediated-immunity mechanisms are known to play a significant role in host defenses against many viral infections, although the relative contributions of the various aspects of cellular immunity are only now being defined. Since the original observations of Zinkernagel and Doherty, attention has been focused primarily on antiviral cytotoxic T-lymphocyte (CTL) activity as a major mechanism of host defense (13, 32, 40). *In vitro*, effective killing of cells infected with virus requires that CTL recognize viral antigens on the cell surface in conjunction with their own class I major histocompatibility antigens (5, 6, 13, 41, 42). These lymphocytes are predominantly of the Lyt-2 phenotype (3, 13, 32).

The generation of CTL activity during acute MCMV infection has been reported by a number of investigators, although detection of CTL activity has not been uniform (9, 17–20, 24, 25, 30, 31). Quinnan and co-workers reported that *H-2*-restricted CTL activity developed in mice after intranasal and intraperitoneal infections (18, 19). This activity, mediated by Thy-1.2, Lyt-2 cells, peaked 6 to 10 days after inoculation and then became undetectable late in infection. Similarly, Sinickas and co-workers detected CTL activity in the popliteal lymph nodes after subcutaneous MCMV inoculation (30, 31). This CTL activity was not detected in cells taken directly from the nodes but required a period of *in vitro* incubation to become manifest.

Adoptive transfer studies have proven useful in studying the cell-mediated-immunity mechanisms which influence the *in vivo* outcome of infection for a number of viruses. These studies have demonstrated that factors such as host, route of infection, and time after infection strongly influence the cell-mediated-immunity responses elicited (14). Passive transfer of class I restricted cells influences the course of infection for influenza A, lymphocytic choriomeningitis virus, herpes simplex virus, and MCMV (2, 9, 14, 20, 24, 39). In the case of MCMV, studies by Ho demonstrated that the transfer of class I-restricted immune lymphocytes suppressed virus replication in the recipient spleens after intraperitoneal inoculation (9). This protective effect could be induced *in vitro* by cocultivation of responder spleen cells with UV-irradiated, MCMV-infected syngeneic cells, or *in vivo*, by MCMV infection of donors. In the latter, the protective effect appeared in donor spleen cells 7 days after infection and appeared to wane by 30 days. These observations, in conjunction with the recent report that adoptive transfer of CTL of the Lyt-2 phenotype could suppress

MCMV replication in the lungs of lethally irradiated BALB/c mice (21), indicate the role of class I-restricted CTL during acute MCMV infection. This does not appear to be the case for MCMV infection of the adrenal gland in nude mice. There are several potential explanations of the failure of Lyt-2 lymphocytes to affect MCMV infection in the adrenal glands in this model. The cell preparation procedure, while not damaging the L3T4 cells, may alter the function of the Lyt-2 cells. Similarly, the number of Lyt-2 cells available by this method may be below the threshold needed to alter virus replication, although the cell titrations would suggest that this is not likely. In addition, in a recent depletion experiment, a substantially larger number of Lyt-2 cells were unable to alter MCMV adrenal infection (unpublished data). It is more likely that class I-restricted cells alone are not able to trigger the cell-mediated-immunity functions needed in the nude mouse to alter MCMV infection of the adrenal gland.

The mechanisms by which L3T4 lymphocytes influence MCMV replication in the adrenal glands are also not clear. Although lymphocytes of the L3T4 phenotype have been linked primarily to helper cell functions or delayed-type hypersensitivity, this view is probably incomplete (36). Recent studies have indicated that the correlation of lymphocyte phenotype and function is not absolute (34, 35). It may be more accurate to view cells of the L3T4 phenotype as ones whose activities are restricted by the recognition of class II major histocompatibility antigens. Most commonly, these cells function in a helper capacity, although other class II-restricted activities, including CTL, have been described previously (7, 15, 36). Thus, these cells could be acting by any of a number of mechanisms including direct CTL, delayed-type hypersensitivity, and antibody-dependent cellular cytotoxicity. Alternatively, they may be providing helper function for either maturation of T-cell precursors or for B-cell antibody production in the *nu/nu* recipient. Finally, the cells may be acting by the release of lymphokines such as gamma interferon or interleukin II, which might influence the susceptibility of the adrenal cells to MCMV infection. Clearly, prior experience with MCMV is necessary to prime the cells, since naive cells were ineffective. However, the mechanism by which these cells alter virus infection will require further study.

It is apparent from these experiments that the effects of adoptive immunity differed in different host tissues. Transfer of immune T cells significantly altered the course of acute MCMV replication in the adrenal gland. In contrast, there was little effect on virus propagation in the lungs or salivary gland. Although the mechanisms responsible for these differences are not known, they suggest that, within a given host, different aspects of the host immune system can influence the course of MCMV replication in different tissues. One explanation may lie in the specialized functions of the host tissue. The adrenal glands are hormone-responsive tissues, active in glucocorticoid and mineralocorticoid, as well as adrenergic synthesis. In vitro, glucocorticoids have been shown to influence MCMV replication (37). In addition, adrenocorticotropin stimulation has been shown to influence the ability of another microorganism, *Besnoitia jellisoni*, to cause adrenal necrosis (8). It is possible that such factors may stimulate or limit MCMV replication in these hormone-responsive tissues.

These studies demonstrate that MCMV replication in the adrenal gland is directly affected by cellular immunity. In the absence of T-cell-mediated immunity, MCMV replicates and destroys the adrenal gland, while restoration of T-cell func-

tion suppresses replication. The mechanisms responsible are currently being investigated.

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