Pathogenesis of Reactivated Latent Murine Cytomegalovirus Infection

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Sixteen weeks after inoculation, murine cytomegalovirus (MCMV) can no longer be detected in the tissues of mice. However, a 2-week course of immunosuppression with antilymphocyte serum and cortisone acetate results in reactivation and dissemination of the latent virus in all animals. In this study of reactivation, MCMV was first detected in the liver, usually during the first week of immunosuppression, and virus replication was shown to be restricted to hepatocytes. Subsequently, a viremia occurred, with spread of infection to other organs. The highest titers of virus were reached in salivary glands in which replication occurred in serous acinar cells. In the lung, virus-specific abnormalities were difficult to detect because of superimposed bacterial and fungal infections. However, interstitial pneumonitis could be produced when cortisone acetate was deleted from the immunosuppressive regimen. Although the site of virus latency has not been defined, this model system will be useful for study of reactivation of latent cytomegalovirus infection. (Am J Pathol 95:67–80, 1979)

IN THE PAST DECADE, cytomegalovirus has become appreciated as a major pathogen affecting the immunocompromised patient.[1] In this circumstance, the spectrum of infection ranges from asymptomatic shedding of virus in the urine or saliva to severe disseminated disease, with hepatic dysfunction and interstitial pneumonia being the predominant clinical manifestations.[2-4] Although the natural history of these infections is not well understood, clinical and epidemiologic observations indicate that, in many instances, the virus is reactivated within a latently infected host.[5-7]

Several experimental studies have shown that the pathogenesis of acute and chronic cytomegalovirus infection in mice is similar to that in humans,[8-10] and murine models of cytomegalovirus infection have become important sources of insight for human disease. As a pertinent example, we recently described a murine model of latent CMV infection in which virus which is no longer detectable in host tissues can be reactivated and disseminated during intense immunosuppressive treatment.[11] This report

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describes the sequence of virologic and histopathologic events which follow reactivation of the latent infection in that model.

Materials and Methods

Virus

Virus was the Smith strain of murine cytomegalovirus (MCMV) maintained by serial passage in mice, employing methods which have been described elsewhere.[11] Virus stocks were prepared as a 10% tissue homogenate of salivary gland in Eagle's minimal essential medium (MEM), 5% fetal calf serum, and 10% dimethylsulfoxide and stored at −70 C.

Establishment of Latent Infections

The method for establishment of latent MCMV infection has been previously described.[11] In brief, 6-week-old female C3H/St mice obtained from Strong Laboratories (La Jolla, Calif) were infected by subcutaneous administration of diluted salivary gland homogenate containing 10^3 plaque-forming units of MCMV. By 4 months after infection, 90% of the mice were found to be free of detectable virus by direct assay of organ homogenates; the remaining 10% had low-level productive infections limited to the salivary glands. Before immunosuppressive measures were initiated, all mice underwent surgical biopsy and virus assay of salivary gland tissue to detect and eliminate any mice persistently shedding MCMV.

Immunosuppression

The immunosuppression regimen consisted of a combination of antilymphocyte serum and cortisone acetate. Rabbit antiserum against murine lymphocytes (ALS) obtained from Microbiological Associates, Inc., was administered in a dose of 0.3 ml intraperitoneally twice weekly. Cortisone acetate (Merck Sharp & Dohme, West Point, Pa) was diluted in distilled water and 125 mg/kg was given intraperitoneally each day. Depending on the experimental design, this regimen was either continued or discontinued or the cortisone acetate was deleted after 14 days. To monitor the effects of the drugs, spleen/body weight ratios and peripheral blood white cell counts were obtained at the time mice were killed.

Assay of Tissues for Virus

At various intervals after initiation of immunosuppression, mice were killed by ether inhalation and exsanguination. At least 6 mice were examined at each sample interval. Whole blood was diluted 1 : 10 in Eagle's MEM with 5% calf serum and 10% DMSO, and 10% homogenates (w/v) of tissues were prepared in a Ten Broeck grinder using the same diluent. Virus in blood samples and tissue homogenates was quantitated by plaque assay in mouse embryo fibroblast monolayers under a tragacanth overlay.[12]

Histopathology

At the time of death, tissues were removed and placed in Bouin's fixative for 24 hours. For histologic examination of pulmonary tissue, the lungs and heart were removed *en bloc* and the lungs were perfused with Bouin's fixative via the pulmonary artery and the trachea.

Immunofluorescence Techniques

Immunofluorescence techniques were employed for measurement of antibody in MCMV-infected mice and for detection of viral antigens in tissues. In the antibody assay,

infected and uninfected mouse embryo cells were used as antigen. C3H mouse embryo fibroblasts infected with MCMV were collected after the development of confluent cytopathic effects, with uninfected fibroblasts serving as negative controls. Cells were centrifuged into a pellet, and small aliquots were placed on clear premarked microscope slides and air dried. After fixation in cold acetone for 15 minutes, the slides were stored at 4 C until use. Two-fold dilutions of serums to be tested for antibody were applied in duplicate to slides containing infected and uninfected fibroblasts and were reacted for 45 minutes at 27 C. Slides were then washed for 15 minutes in phosphate-buffered saline at pH 7.4 and stained for 45 minutes at 27 C using fluorescein isothiocyanate (FITC)-labeled rabbit antimouse γ-globulin (N. L. Cappel Laboratories Inc., Cochranville, Pa). The endpoint was the highest dilution of serum giving distinct immunofluorescence when the slides were examined microscopically.

An antiserum for detection of MCMV antigens in tissues was prepared by intraperitoneal inoculation of 6-week-old female C3H mice with 10^5 PFU of MCMV. After 3 weeks, serum was collected, pooled, and frozen. Serums prepared by this method had a titer of 1:160 or 1:320 against MCMV when measured by the indirect immunofluorescence test described above. An ammonium-sulfate–precipitated globulin fraction of the mouse anti-MCMV serum was coupled with FITC in a ratio of 0.0125 mg FITC/mg globulin using the method of Cherry et al.[13] The conjugated serum was dialyzed against PBS and passed over a Sephadex G-50 column; the fractions containing the conjugated serum were pooled. This conjugate gave a positive immunofluorescent reaction at dilutions up to 1:40 when tested against CMV-infected mouse embryo fibroblasts, but no reaction was discernible with uninfected mouse embryo fibroblasts.

Results

Effects of Drug Administration

The spleen/body weight ratios, white blood cell counts, and anti-MCMV antibody titers of latently infected and uninfected control mice were followed to monitor the effects of the immunosuppressive regimen. As shown in Table 1, these measurements did not differ from the two groups of animals prior to the initiation of treatment. After 14 days of

Table 1—Spleen/Body Weight Ratio, Total White Blood Cell Counts, and Antibody Titers Against MCMV of Uninfected Mice and Mice With Latent MCMV Infection During ALS and Cortisone Treatment

Day of drug treatment	Uninfected mice			Mice with latent MCMV infection		
	Spleen/body weight	Total WBC count	Antibody* titer	Spleen/body weight	Total WBC count	Antibody* titer
0	0.0045	5125	<20	0.0055	5555	40
14	0.0010	2644	<20	0.0025	2047	20
21	—	—	<20	0.0027	—	40
28	—	—	—	0.0026	—	80
35	—	—	—	—	—	80
42	—	—	—	—	—	40

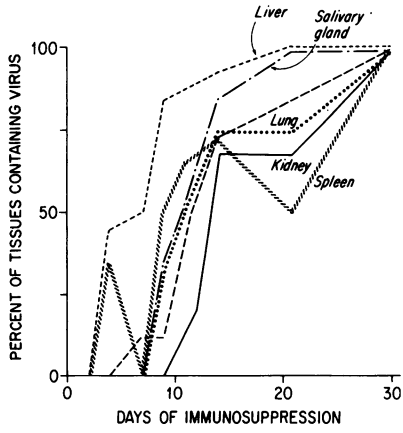
* Indirect fluorescent antibody titer expressed as reciprocal of highest dilution showing specific fluorescence

immunosuppression, the spleen/body weight ratios and white blood counts had significantly declined in both groups of animals, and continuation of the regimen for 4 weeks in infected mice resulted in a persistently low spleen/body weight ratio. Two related findings are also of interest: First, 1 week after deletion of cortisone acetate from the regimen (at 14 days), the spleen/body weight ratio reverted to 0.0078, indicating that splenic hypertrophy had occurred. Second, discontinuation of all drug administration (also at 14 days) resulted in return of spleen/body weight ratio to normal in 7 days.

Data presented in Table 1 also show that all latently infected mice processed significant serum antibody titers against MCMV and that these did not change significantly during ALS and cortisone acetate administration. None of the uninfected control animals had detectable antibody against the virus.

Virus Isolations

Reactivation and dissemination of latent virus occurred in 100% of animals after 21 days of ALS and cortisone acetate administration. The sequence and frequency of virus isolation from various tissues during immunosuppression are illustrated in Text-figure 1. Liver was the earliest tissue consistently yielding virus, usually on Day 4. Although MCMV was also found in spleen homogenates of 3 of 9 animals on Day 4, isolation from this tissue was inconsistent until disseminated infection had occurred. After the early appearance of virus in liver, infection became generalized and was detected with increasing frequency in all tissues examined. By Day 21, virtually all mice had generalized infection.



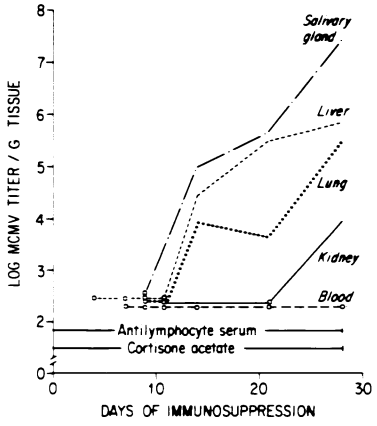
TEXT-FIGURE 1—Sequence and frequency of cytomegalovirus isolation from mice during immunosuppression with antilymphocyte serum and cortisone acetate. Each point represents pooled tissue from at least 6 animals.

Quantitative assays for virus content of various tissues during immunosuppression are shown in Text-figure 2. It can be seen that, although MCMV was detected as early as Day 4 in the liver and Day 9 in the salivary glands, titers in all tissues remained less than 300 PFU/g until Day 11. Subsequently, virus titers increased rapidly in lung, liver, and salivary glands. It should also be noted that significant amounts of virus did not appear in the kidneys until 28 days of immunosuppression.

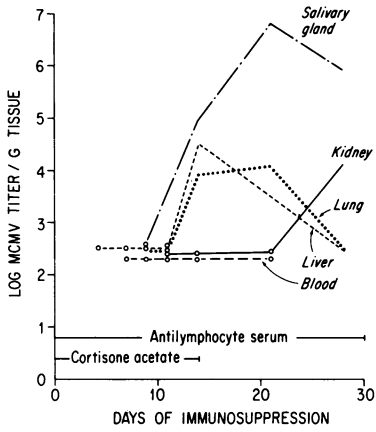
Finally, as illustrated in Text-figure 3, discontinuation of cortisone acetate after 14 days of administration resulted in rapid decline in virus titer in the lung and liver and, to a lesser extent, in the salivary glands. Discontinuation of both ALS and cortisone at that time resulted in rapid disappearance of virus in all tissues except the salivary glands (data not shown).

Histopathology

Histologic changes closely paralleled the virologic findings and were first detected in the liver. Although virus could be recovered as early as the fourth day of immunosuppression, no specific histopathologic alterations were noted prior to the 14th day. At this time, 7 of 15 animals showed hepatic changes indicating CMV infection. The earliest specific finding was the presence of isolated hepatocytes containing MCMV intranuclear inclusions. Foci of inflammatory cells and infected hepatocytes scattered randomly throughout the liver parenchyma (Figure 1) represented more advanced alterations. Hepatocytes in these lesions were in various stages of degeneration, which progressed from intranuclear inclusions with margination of nuclear chromatin to cytoplasmic basophilia and cytomegaly. Ultimately, cellular degeneration occurred, leaving only



TEXT-FIGURE 2—Amount of cytomegalovirus in various murine tissues during the administration of antilymphocyte serum and cortisone acetate. *Open circles* indicate the lower limits of sensitivity for the plaque assay.



TEXT-FIGURE 3—Amount of cytomegalovirus in various murine tissues during immunosuppression with antilymphocyte serum and cortisone acetate. Note that cortisone acetate was discontinued after Day 14. *Open circles* indicate the lower limits of sensitivity of the plaque assay.

an acidophilic residue. Inflammatory cell infiltrates, comprised mainly of mononuclear cells and some polymorphonuclear leukocytes, began to appear and surround the hepatocytes as they became cytomegalic. The intensity of this inflammatory infiltrate progressed with continued degeneration of hepatocytes, and the number and size of inflammatory foci increased progressively as immunosuppression was continued. Finally, it is important to note that no virus-specific changes were noted in cells other than hepatocytes.

Salivary glands showed no virus-specific changes prior to Day 14, when acinar cells with intranuclear inclusions were detected, primarily in the serous portion of the gland (Figure 2). Although inclusion-bearing cells were occasionally seen in mucinous areas of the gland, they were always located between mucinous alveoli, appearing to involve the serous demilunes, and evidence of infection was never detected in ductal cells, connective tissues, blood vessels, or other structures. In addition, lesions in all parts of the gland were highly focal, bearing no consistent relationship to other structures. There was no accompanying inflammatory cell infiltrate, and only minimal disruption of histologic architecture was apparent.

As was predicted by the decline in spleen/body weight ratio, the administration of ALS and cortisone acetate resulted in marked splenic atrophy. Although white follicles were still recognizable, they were very small and comprised solely of large lymphocytes. The red pulp was uniformly devoid of small lymphocytes and contained primarily red blood cells, reticuloendothelial cells, megakaryocytes, and other blood elements. Occasionally, an inclusion-bearing cell of unknown identity was found in the red pulp area. Cessation of cortisone administration resulted in rapid

reversal of these changes, and within 1 week spleens and other lymphoid structures demonstrated active lymphocyte division and replication.

No CMV-specific lesions were found in kidneys until 23 days of immunosuppression, when inclusion-bearing cells were found in the proximal tubular epithelium and in the cells of the glomerular tuft. After more prolonged immunosuppression, cytomegalic inclusion-bearing cells were found in additional tissues such as the acini of the pancreas, the fibrous capsule of the ovaries, the mesentery adjacent to the pancreas, and the adrenals. Little or no inflammatory reaction surrounded infected cells in these sites. No lesions were ever noted in the myocardium, esophagus, trachea, or thymus.

In the lung, histopathologic changes induced by cytomegalovirus were difficult to assess because of intercurrent pulmonary processes. Localized bronchopneumonia was present in 1 of 8 latently infected mice which were not immunosuppressed and in 6 of 19 latently infected animals early in the course of immunosuppression (before CMV could be isolated from lung tissue). Sporadic bronchopneumonia was also found throughout the course of immunosuppression, occuring in both CMV-infected and uninfected control mice. In all cases, the pneumonia involved both airways and alveoli, with secondary interstitial infiltration of inflammatory cells. Generally, the cellular infiltrates were polymorphonuclear, although foamy mononuclear cells and giant multinucleated syncytial cells were occasionally found. Alveolar and airway debris was common, and special strains frequently revealed bacteria or fungi in both areas. Occasionally, an intranuclear inclusion-bearing cell was found among the other inflammatory cells.

Despite the obvious problem of concurrent bronchopneumonia due to other agents, certain observations concerning the effect of reactivated MCMV on the lung can be made. Although treatment with ALS and cortisone for up to 42 days did not result in significant virus-specific effects, interstitial changes clearly due to MCMV were produced when the immunosuppressive regimen was altered. Latent MCMV was reactivated using the standard immunosuppressive procedure, but cortisone was discontinued after 14 days. Eight of 10 mice inspected 7 days after cessation of cortisone treatment had interstitial hypercellularity and 2 had frank interstitial pneumonitis. None of the uninfected control animals showed these alterations. As shown in Figure 3, the pulmonary abnormalities consisted of scattered focal interstitial accumulations of mononuclear cells with intranuclear inclusions. In more advanced cases, the septums were markedly thickened and hypercellular. These abnormalities were similar to early changes described by Brody and Craighead in the interstitial

pneumonia of acutely infected mice treated with ALS.[14] Finally, mice inspected 14 days after cessation of cortisone treatment showed no pulmonary abnormalities.

Immunofluorescence Studies

Immunofluorescence studies corroborated the other analyses. Thus, CMV-specific antigens were first detected in the liver. After 14 days of immunosuppression, small numbers of hepatocytes bearing virus-specific cytoplasmic and nuclear antigens were scattered throughout liver parenchyma. With continued immunosuppression, infection progressed to produce larger areas of parenchymal disruption containing multiple fluorescent cells. These foci coincided with the areas of inflammatory cell infiltration seen on light microscopy. Viral antigens appeared to be restricted to hepatocytes and were not detected in inflammatory cells. No specific fluorescence was observed in any other hepatic cell.

After 18 days of immunosuppression, numerous cells bearing viral antigens were found in the serous portion of the salivary gland. As was noted by light microscopy, these cells were scattered throughout the parenchyma (Figure 4) and appeared to be acinar cells. Viral antigens were only rarely detected in the mucinous portion of the gland, and here they seemed to be nonmucinous acinar cells. No specific fluorescence was noted in ductal cells, blood vessels, connective tissue, or lymphoid aggregates.

Discussion

The importance of disseminated cytomegalovirus infections among immunocompromised patients has been increasingly recognized in recent years (as many as 90% of renal allograft recipients develop active infection[3]). Among bone marrow transplant patients, the problem is even more critical in that interstitial pneumonia, frequently associated with CMV infection, has severely limited the potential success of the transplantation procedure.[15] As previously mentioned, the source of the viral infection in these patients is often unclear, although many infections are thought to result from reactivation of latent virus previously dormant in the host. Since systematic study of latent CMV infection and the events involved in reactivation and disease is not possible in humans, the experiments described here were conducted in a murine model.

In the mice used in our experiments, MCMV could no longer be detected in the tissues 16 weeks after infection. However, immunosuppression with antilymphocyte serum and cortisone acetate caused reactivation and widespread dissemination of virus in all animals. Analysis

of the temporal course of events during reactivation revealed that MCMV was usually detected earliest in the liver, frequently by the fourth day of immunosuppression. Subsequently, viremia occurred and MCMV disseminated to involve all organs assayed. The highest viral titers were reached in salivary gland, which is the most permissive tissue for replication of MCMV.[9]

As indicated grossly by splenic atrophy and histologically by disappearance of splenic lymphocyte follicles, the degree of immunosuppression achieved with ALS and cortisone acetate in these animals was severe. A marked depression in numbers of circulating white blood cells, predominantly lymphocytes, was also documented. It is clear that the cortisone acetate played a critical immunosuppressive role, since discontinuation of this agent after 14 days was accompanied by regeneration of lymphoid structures in the spleen and cessation of MCMV replication in the liver and lung. In this regard, the ability of corticosteroids to perpetuate replication of MCMV in the salivary glands of mice during the acute phase of infection, presumably as a result of an anti-inflammatory effect, has previously been noted by Henson et al.[16]

Histopathologic studies of the reactivated MCMV infection demonstrated significant abnormalities in several organs, and, as in humans, the liver was severely involved. Typical MCMV intranuclear inclusions were found in hepatocytes scattered widely throughout the parenchyma. A mononuclear inflammatory cell infiltrate usually surrounded the infected cells and became progressively more severe as immunosuppression continued. Immunofluorescence studies demonstrated that the MCMV replication was restricted to the hepatocytes. In the salivary glands MCMV intranuclear inclusions and specific viral antigens were found almost exclusively in acinar cells of serous epithelium and only rarely in the mucinous cells. Thus, the histopathologic changes in the liver and salivary glands resulting from reactivation of latent MCMV were similar to those seen in acute infection [10,16,17] and in wild mice with chronic MCMV infection which disseminated following treatment with anti-thymocyte serum.[18]

Because of the high incidence of interstitial pneumonitis in patients with disseminated CMV infection, the pulmonary alterations in the murine model merit specific discussion. Replication of MCMV to high titer in the lungs occurred uniformly among mice with reactivating viral infection. In some of these animals, pneumonitis with characteristic cytomegalic inclusion-bearing cells in the interstitial areas did develop. However, interstitial pneumonia also occurred among uninfected control animals during immunosuppression and was also seen at times in infected

mice before MCMV could be recovered from lung tissue. Finally, superimposed bacterial and fungal infection in the lungs of immunosuppressed mice made interpretation of the pulmonary histologic changes difficult. Thus, the profound immunosuppression achieved with ALS and cortisone predisposed animals to develop intercurrent pulmonary infections which made it impossible to assess concisely the role of MCMV in pneumonitis. Since characteristic CMV pneumonitis could be produced when corticosteroid administration was discontinued after 14 days, alternative regimens which cause less severe immunosuppression are under investigation. Possibly, germfree animals will also assist in such a delineation.

Finally, a brief consideration of the site(s) in which the latent virus is maintained should be presented. The studies of reactivation and dissemination described here do not establish sites in which the virus is harbored. In contrast to other models of latent MCMV infection in which different virus strains and routes of infection were employed,[19,20] we have not been able to activate latent MCMV *in vitro* by co-cultivation or explantation of tissues or *in vivo* by transplantation experiments. The early appearance of MCMV in the liver and, less often, in the spleen during immunosuppression does not necessarily implicate these organs as sites of latent virus infection, since either might have been seeded as a result of viremia arising elsewhere. Reactivation and dissemination of virus occur consistently following immunosuppression of splenectomized mice,[21] indicating that in this model MCMV cannot be harbored exclusively in the spleen. Our application of *in situ* molecular hybridization methods may resolve this issue, which is basic to understanding the pathogenesis of cytomegalovirus infections.

References

1. Craighead JE: Cytomegalovirus pulmonary disease. Pathobiol Annu 5:197–220, 1975
2. Bodey GP, Werklake PT, Douglas G, Levin RH: Cytomegalic inclusion disease in patients with acute leukemia. Ann Intern Med 62:899–906, 1965
3. Rifkind D, Goodman N, Hill RB: The clinical significance of cytomegalovirus infection in renal transplant recipients. Ann Intern Med 66:1116–1128, 1967
4. Henson D, Siegel SE, Foccillo DA, Matthew E, Levine AS: Cytomegalovirus infections during acute childhood leukemia. J Infect Dis 126:469–481, 1972
5. Weller TH: The cytomegaloviruses: Ubiquitous agents with protean clinical manifestations. N Engl J Med 285:203–214, 267–274, 1971
6. Lang DJ: Cytomegalovirus infections in organ transplantation and post transfusion: An hypothesis. Arch Gesamte Virusforsch 37:365–377, 1972
7. Lang DJ, Chung KS, Schwartz JN, Daniels CA, Harwood SE: Cytomegalovirus replication and the host immune response. Yale J Biol Med 49:45–48, 1976
8. Brodsky I, Rowe WP: Chronic subclinical infection with mouse salivary gland virus. Proc Soc Exp Biol Med 99:654–655, 1958

9. Mannini A, Medearis DN: Mouse salivary gland virus infections. Am J Hyg 73:329–343, 1961
10. Henson D, Smith RD, Gehrke J: Nonfatal mouse cytomegalovirus hepatitis. Am J Pathol 49:871–888, 1966
11. Jordan MC, Shanley JD, Stevens JG: Immunosuppression reactivates and disseminates latent murine cytomegalovirus. J Gen Virol 27:419–423, 1977
12. Selgrade MK, Osborn JE: Role of macrophages in resistance to murine cytomegalovirus. Infect Immun 10:1383–1390, 1974
13. Cherry WB, Goldman M, Carski TR: Fluorescent antibody techniques in the diagnosis of communicable diseases. Public Health Service Publication No. 729, 1960, pp 38–39
14. Brody AR, Craighead JE: Pathogenesis of pulmonary cytomegalovirus infection in immunosuppressed mice. J Infect Dis 129:677–689, 1974
15. Meyers JD, Spencer HC Jr, Watts JC, Gregg MB, Stewart JA, Troupin RH, Thomas ED: Cytomegalovirus pneumonia after human marrow transplantation. Ann Intern Med 82:181–188, 1975
16. Henson D, Smith RD, Gehrke J, Neapolitan C: Effects of cortisone on nonfatal mouse cytomegalovirus infection. Am J Pathol 51:1001–1011, 1967
17. McCordock HA, Smith MG: The visceral lesions produced in mice by the salivary gland virus of mice. J Exp Med 63:303–310, 1936
18. Gardner MB, Officer JE, Parker J, Estes JD, Rongey RW: Induction of disseminated virulent cytomegalovirus infection by immunosuppression of naturally chronically infected wild mice. Infect Immun 10:966–969, 1974
19. Olding LB, Jensen FC, Oldstone MBA: Pathogenesis of cytomegalovirus infection. I. Activation of virus from bone marrow-derived lymphocytes by *in vitro* allogenic reaction. J Exp Med 141:561–572, 1975
20. Mayo DR, Armstrong JA, Ho M: Reactivation of murine cytomegalovirus by cyclophosphamide. Nature 267:721–723, 1977
21. Jordan MC: Unpublished data

Acknowledgments

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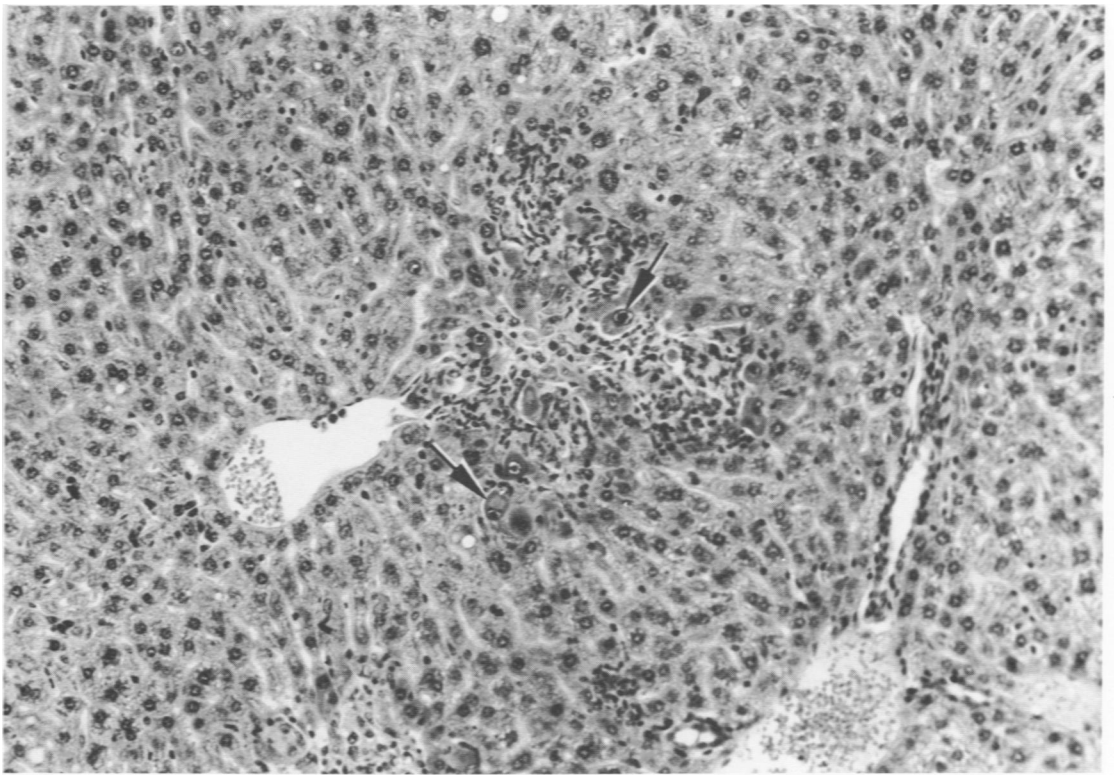
[*Illustrations follow*]



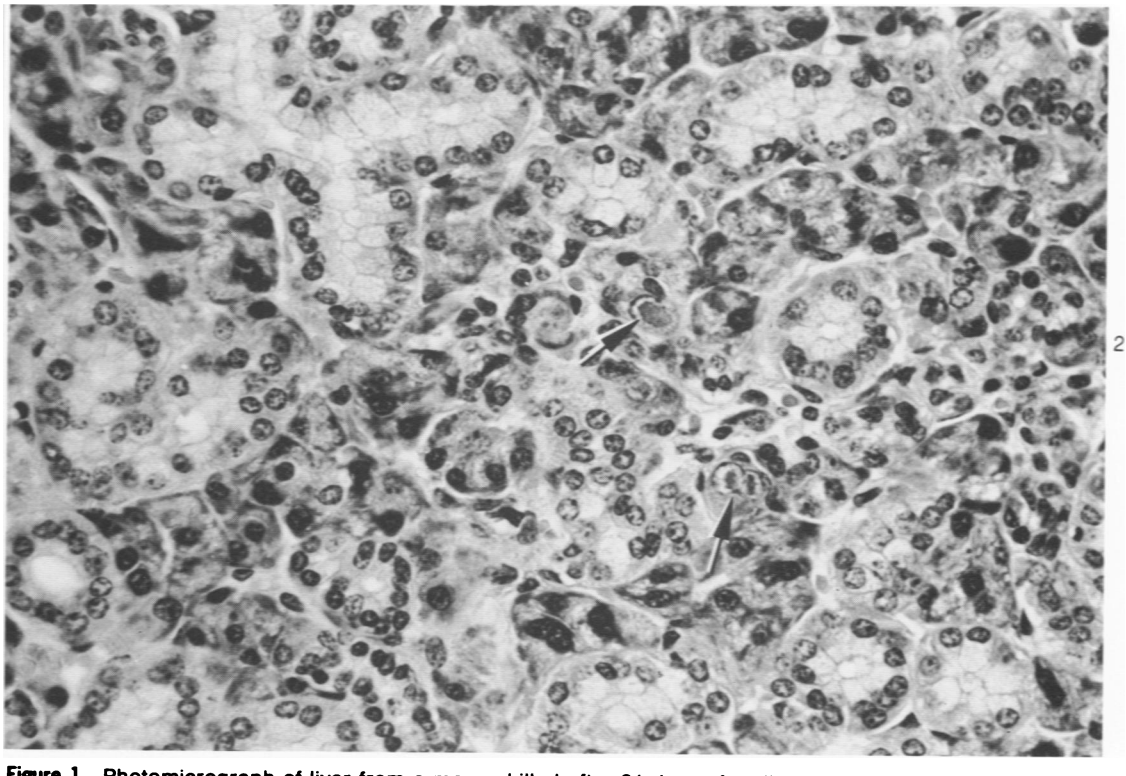


Figure 1—Photomicrograph of liver from a mouse killed after 21 days of antilymphocyte serum and cortisone treatment. A focal area of necrosis with mononuclear cell infiltration and inclusion-bearing hepatocytes (*arrows*) in various stages of degeneration is shown. (× 125) (with photographic reduction of 10%) **Figure 2**—Photomicrograph of salivary gland from a mouse killed after 21 days of antilymphocyte serum and cortisone treatment. Cytomegalic serous acinar cells containing intranuclear inclusions (*arrows*) can be seen. (× 125) (with photographic reduction of 10%)

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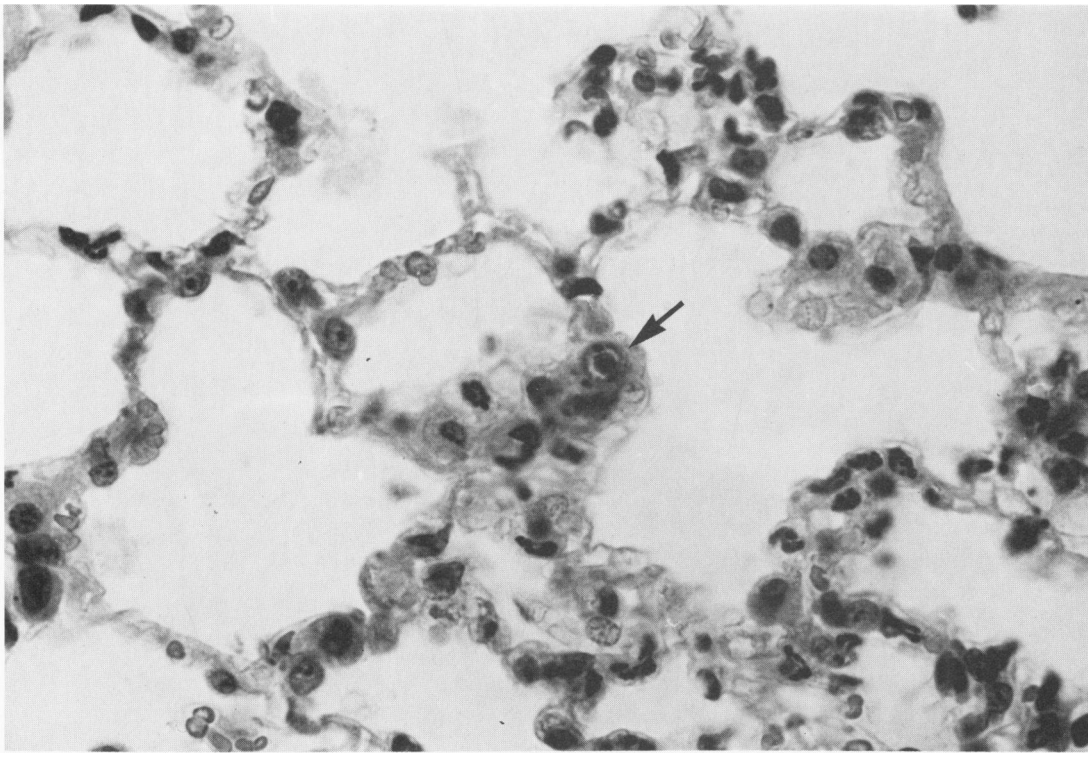

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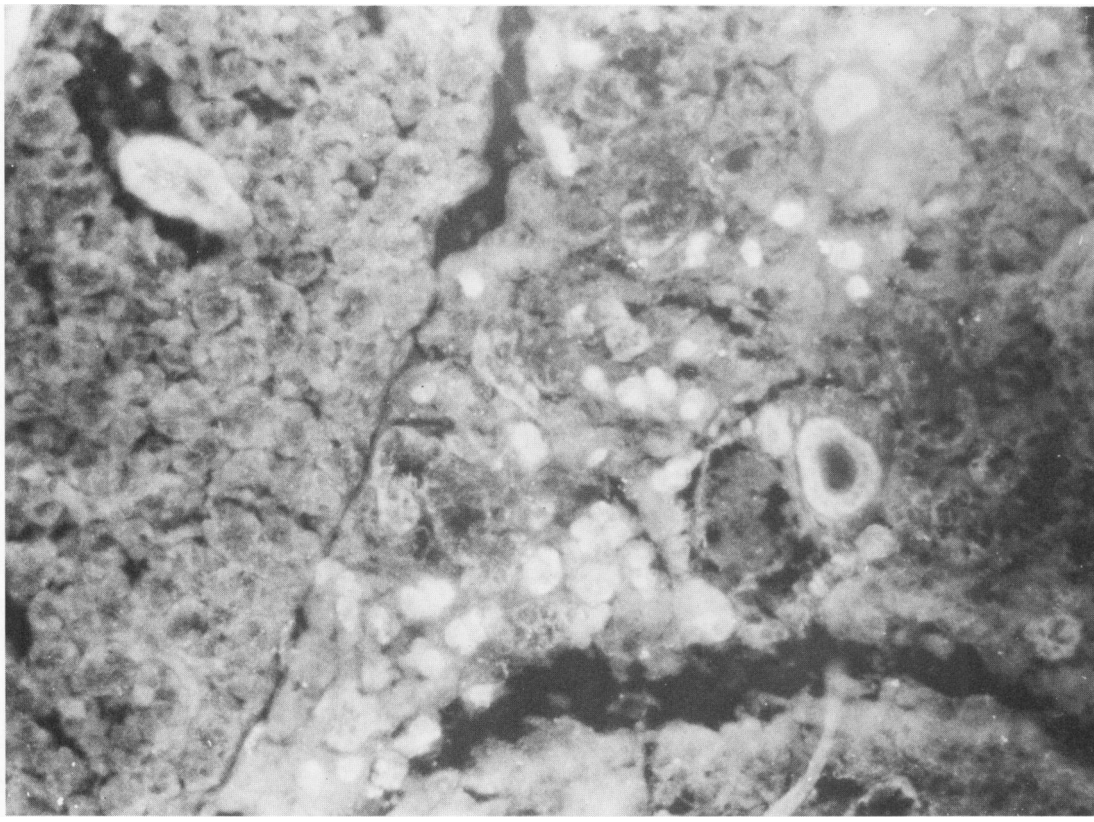

Figure 3—Photomicrograph of lung from a mouse killed after 21 days of antilymphocyte serum and cortisone treatment. A cytomegalic intraseptal cell containing an intranuclear inclusion (*arrow*) is present. (× 200) (with photographic reduction of 10%) **Figure 4**—Photomicrograph of salivary gland from a mouse killed after 21 days of antilymphocyte serum and cortisone treatment. The section was stained by immunofluorescent methods for cytomegalovirus antigens. Cells in the serous portion of the gland (*right*) stain selectively for viral antigens. The positive reaction in ducts of the mucinous portion represents nonspecific autofluorescence. (× 240) (with photographic reduction of 10%)