Adverse Effects of Cytomegalovirus Vaccination in Mice

M. COLIN JORDAN, *Division of Infectious Diseases, Department of Medicine, and Reed Neurological Research Center, University of California School of Medicine, Los Angeles, California 90024*

ABSTRACT Studies of live attenuated cytomegalovirus (CMV) vaccine have recently been initiated in man. The possibilities of latent infection and disease resulting from reactivation of vaccine virus are major concerns. Because markers for attenuation of tissue culture-passaged mouse CMV (MCMV) exist, studies of potential adverse effects of vaccination were initiated in mice. Plaque-purified MCMV was passed 12 times in cell culture ("vaccine virus") and shown to be attenuated by virtue of loss of lethality and diminished replication in reticuloendothelial organs of normal mice. Although subcutaneous inoculation of 10^5 plaque-forming units of wild virus was lethal for mice immunosuppressed with antilymphocyte serum (18/18 died), "vaccine MCMV" killed only 3/18 ($P < 0.05$) and was thus shown to be highly attenuated even in immunosuppressed animals. 4 mo after subcutaneous inoculation of vaccine MCMV, no infectious virus was detectable in the tissues of normal C_3H mice. However, immunosuppression with anti-lymphocyte serum and cortisone caused MCMV reactivation, dissemination, and widespread cytomegalic inclusion disease in 19 of 20 animals. Characterization of the reactivating virus recovered from salivary glands indicated that reversion to virulence had occurred. Thus, vaccine MCMV, although markedly attenuated initially, established latent infection, reactivated after immunosuppression, and reverted to virulence, at least in salivary gland tissue. These data from the murine model substantiate the need for careful surveillance and virologic study of patients given experimental CMV vaccine.

INTRODUCTION

Infections caused by cytomegalovirus are common and represent a particular problem among immunosuppressed patients and pregnant women (1–6). These considerations have prompted investigators to develop and study live attenuated cytomegalovirus vaccines. Although preliminary trials have been initiated in normal volunteers (7–10) and in renal transplant patients (11), several concerns have arisen regarding the widespread use of such vaccines (12). First, there are no means of showing that tissue culture-passaged human cytomegalovirus (CMV)[1] strains are in fact attenuated because the virus does not infect experimental animals (1). Similar concerns would apply to the possibility of reversion of vaccine CMV to full virulence and subsequent person-to-person spread after passage through the human host. Second, CMV is a member of the herpes group of viruses and, as such, appears to be capable of establishing latent infection in man that may later reactivate and produce disease (1). The behavior of a "vaccine CMV" strain in this regard is obviously an important consideration.

For the past several years, studies of the pathogenesis of experimental and natural CMV infection in mice have yielded important findings that closely mimic human infection (13–18). Of relevance to vaccination, Osborne and Walker (19) showed in 1970 that murine CMV (MCMV) was attenuated by serial passage in tissue culture. In addition, loss of lethality for suckling mice and diminished viral replication in the liver and spleen of weanling mice were found to be valuable markers of viral attenuation. Subsequently, vaccination of mice with this attenuated virus has been shown to prevent the morbidity and mortality induced by challenge with wild MCMV (19, 20). Moreover, models of latency and subsequent reactivation of MCMV have also been developed and studied profitably in various laboratories (21–25). Thus, many of the issues of concern regarding vaccination of humans with CMV can now be studied experimentally in the murine system. In this report, investigations of the potential

This work was presented in part at the Fourth Herpesvirus Workshop, Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 30 September 1979.

Dr. Jordan's present address is Medical Service, Veterans Administration Medical Center, Martinez, Calif. 94553.

Received for publication 22 October 1979 and in revised form 3 December 1979.

[1] *Abbreviations used in this paper*: ALS, antilymphocyte serum; CMV, cytomegalovirus; MCMV, murine cytomegalovirus; MEC, mouse embryo cell; PFU, plaque-forming unit.

adverse effects of vaccination with attenuated MCMV in mice are described with particular emphasis on the development of vaccine virus latency, subsequent reactivation, and reversion to virulence.

METHODS

Mice. Studies of lethality of MCMV in newborn mice and replication of the virus in the liver and spleen of weanling mice were performed in outbred Swiss strains obtained from Timco Breeding Laboratories, Houston, Tex. Experiments relating to latency and reactivation of MCMV were done in C_3H/J (The Jackson Laboratory, Bar Harbor, Maine) or C_3H/ST (L. C. Strong Research Foundation, San Diego, Calif.) inbred female mice. During immunosuppressive therapy, individual mice were housed in separate cages to prevent spread of viral infection.

Strains of MCMV. As previously described (24), the virulent Smith strain of MCMV was maintained by serial passage in mice and harvested as a 10% (wt/vol) homogenate of salivary gland tissue in Eagle's minimal essential medium supplemented with 5% fetal calf serum and antibiotics. The virus pool had a titer of 3.0×10^7 plaque-forming units (PFU)/ml in secondary mouse embryo cell (MEC) culture under overlay with 0.8% gum tragacanth.

To prepare attenuated MCMV for use as a "vaccine virus," single plaques were aspirated wih Pasteur pipettes from terminal virus dilutions in tissue culture plastic trays (Costar, Data Packaging, Cambridge, Mass.). The virus from these isolated microplaques was then reinoculated onto new MEC monolayers, and the tissue culture fluids were harvested when cytopathic effects involved 75% of the monolayer. Supernatant virus was then titrated under overlay; the end dilution plaques were isolated a second time. This MCMV clone was used to infect MEC at a multiplicity of infection of 1.0, and the resulting virus was repassaged 12 consecutive times in MEC; one additional plaque purification was done after the eighth passage. The final vaccine virus pool had a titer of 6.4×10^6 PFU/ml. As will be presented subsequently, this virus was shown to be attenuated by virtue of loss of lethality for suckling mice and diminished replication in the livers and spleens of weanling mice.

Establishment of latent infection. The techniques and criteria for the establishment of latent infection with wild-type virulent MCMV have been described in detail elsewhere (24, 25). Briefly, 4–6-wk-old C_3H mice are inoculated subcutaneously with 10^3 PFU of MCMV, which produces transient replication of virus in the salivary glands. However, by 4 mo after inoculation, no infectious virus can be detected in homogenates of any organ in 90% of these mice. The remaining 10% have low-level salivary gland infection; these virus shedders are excluded from experiments by surgical excision biopsy and direct assay of salivary gland tissue for infectious virus. Animals classified as latently infected have no detectable MCMV in any organ by direct assay of homogenates, cocultivation on indicator MEC for several weeks, centrifugal force inoculation of homogenates, or establishment and serial passage of fibroblast explant cultures from pooled salivary glands (25). More recently, studies using immunofluorescence to detect viral antigens or nucleic acid cytohybridization to detect MCMV DNA have also been negative.[2] Nevertheless, undetectable MCMV persists in a latent state in these mice because 2 wk of immunosuppression with antilymphocyte serum (ALS) and cortisone acetate results in reactivation and dissemination of virus in all animals. Presently, the exact tissue and cellular site(s) responsible for the maintenance of the latent MCMV in this system is not known in contrast to the models of Olding et al. (21) and Mayo et al. (26) where latent virus can be activated from splenic lymphocytes in vitro.

Measurement of attenuation and virulence of MCMV. To define MCMV strains as attenuated or virulent for mice, two criteria developed by Osborne and Walker (19) were used. First, lethality of the MCMV strain to be tested for virulence was assessed in 48–72-h-old suckling mice by intraperitoneal inoculation of 1,000 PFU of virus. As will be shown, within 14 d of infection mortality rates of 90–100% were found with virulent strains, whereas mortality rates ranged from 0–15% for attenuated strains within this time period. Second, some MCMV isolates were selected at random for determination of their ability to replicate in the reticuloendothelial system of mice. Here, 10^5 PFU of the virus to be tested was inoculated intraperitoneally into 4-wk-old Swiss mice. 7 d later, 10% homogenates of liver and spleen were prepared and titrated for virus content. As noted by Osborne and Walker (19), virulent MCMV virtually always replicates to titers at least 100-fold higher than attenuated MCMV in these organs. Because the two methods of determination of virulence correlated very closely, lethality in suckling mice was considered the primary criterion although reticuloendothelial replication was used as a confirmatory test for randomly selected virus isolates.

Measurement of antibody responses in mice. To determine the titer of circulating antibody against MCMV in control and infected animals, an indirect immunofluorescence technique was used as described in detail elsewhere (27).

Immunosuppression of mice. For immunosuppression of animals, rabbit antiserum against murine lymphocytes (ALS), obtained from Microbiological Associates, Walkersville, Md., was given intraperitoneally in a dose of 0.3 ml twice weekly. In some experiments, cortisone acetate (Merck Sharpe and Dohme Div., Merck & Co., Inc., West Point, Pa.) was added at a dose of 125 mg/kg i.p. daily. The effects of these regimens on peripheral lymphocyte counts, MCMV antibody titers, spleen size, and dissemination of the viral infection have been described (27).

RESULTS

Attenuation of vaccine MCMV. Initial experiments to determine the degree of attenuation of the tissue culture-passaged "vaccine" MCMV are summarized in Table I. The mortality rates caused by challenge of suckling mice with 1,000 PFU of the vaccine strain were far less than those induced by wild virus. Because similar homogenates from uninfected animals were not lethal for suckling mice, this difference was not the result of a toxic effect of salivary gland homogenate in the case of wild virus. It can also be seen that replication of virus in the liver and spleen of weanling mice was much greater for the wild MCMV strain, correlating closely with the suckling mouse mortality findings.

Serum samples were collected from weanling animals 14 d after vaccination with attenuated MCMV. Using the immunofluorescence technique, antibody titers of 1:80 to 1:320 were found in all 12 animals tested. Similar antibody responses were noted in animals inoculated with wild virus.

[2] Gerdes, J., J. D. Shanley, M. C. Jordan, and J. G. Stevens. Unpublished data.

TABLE I

Virulence Characteristics of Vaccine and Wild-Type MCMV Strains in Suckling and Weanling Mice

Virus strain	Suckling mouse mortality*	MCMV Titers in tissues of weanling mice‡	
		Spleen	Liver
	%	*PFU/gm*	
Vaccine MCMV	1/32 (3)	$<1.0 \times 10^2$	4.4×10^2
Wild-type MCMV	28/30 (93)	6.1×10^6	2.3×10^5
Salivary gland homogenate without MCMV	0/15 (0)	—	—

* Number of deaths/number inoculated within 14 d after intraperitoneal inoculation of virus.

‡ MCMV titer obtained in spleen and liver homogenates 7 d after intraperitoneal challenge with 10^5 PFU of virus.

TABLE II

*Effect of Immunosuppression on Reactivation and Dissemination of Latent Murine CMV**

Mice	Salivary gland	Liver	Spleen	Kidney	Lungs
Uninfected	0/10‡	0/10	0/10	0/10	0/10
Infected with virulent MCMV	12/12	12/12	11/12	12/12	12/12
Infected with vaccine MCMV					
Experiment 1	8/8	8/8	6/8	7/8	8/8
Experiment 2	7/8	6/8	5/8	5/8	6/8
Experiment 3	4/4	4/4	4/4	4/4	4/4
Total	19/20	18/20	15/20	16/20	18/20

* Organ homogenates (10% wt/vol) were assayed for infectious MCMV after immunosuppression with ALS and cortisone acetate for 14 d.

‡ Number infected/number tested.

Because human CMV vaccine might be considered for immunocompromised patients, the degree of attenuation of the vaccine MCMV strain was also assessed in immunosuppressed weanling mice. Here, beginning 5 d before inoculation of 10^5 PFU of virulent or attenuated MCMV and continuing for 14 d after infection, ALS was administered twice weekly at a dose of 0.3 ml i.p. This dose of wild virus was uniformly lethal (18/18 mice died), whereas most animals survived a similar challenge with the vaccine virus despite the immunosuppression (3/18 died; $P < 0.01$). Thus, the vaccine MCMV strain used in these and subsequent experiments was shown to be highly attenuated, even in severely immunosuppressed animals.

Establishment of latent infections with MCMV vaccine. As previously noted, wild MCMV establishes an undetectable latent infection that reactivates and disseminates after immunosuppression of mice with ALS and cortisone acetate (24). To determine whether vaccine MCMV would establish a similar latent infection, groups of C_3H mice were inoculated subcutaneously with 10^3 PFU of attenuated virus. 16 wk after inoculation, salivary gland tissue obtained by surgical biopsy was assayed for the presence of detectable MCMV; none was found. However, as shown in Table II, a 2-wk regimen of immunosuppression with ALS and cortisone acetate induced reactivation in 19 of the 20 animals, and most had widespread dissemination of infection. This frequency of reactivation of latent MCMV for the attenuated virus was very similar to that found for virulent MCMV (Table II). In addition, histologic examination of tissues from animals with reactivating MCMV vaccine infection indicated that virus-specific lesions were present. Characteristic intranuclear inclusions were seen within cytomegalic cells in the liver, lung, spleen, pancreas, kidneys, and salivary glands that were very similar, if not identical, to those seen after reactivation and dissemination of virulent MCMV (27). In control mice that had never been infected with MCMV, no virus or intranuclear inclusions were noted after immunosuppression. This finding indicates that the C_3H mouse strain used in these experiments was not naturally infected with latent MCMV before arrival in the laboratory.

From these experiments, it is clear that the vaccine MCMV strain established a latent infection after vaccination, and that reactivation of the vaccine virus occurred during immunosuppression. In addition, tissue lesions consistent with disseminated cytomegalic inclusion disease were found in all animals examined.

Characteristics of reactivating vaccine virus. The vaccine MCMV strains recovered from salivary gland tissue of several animals were characterized in terms of their ability to kill suckling mice and to multiply in the reticuloendothelial system of weanling mice. These data are shown in Table III. It can be seen that both the virulent control MCMV and the attenuated vaccine virus behaved as expected in both suckling and weanling mice. However, the vaccine MCMV strains recovered from immunosuppressed animals with reactivating latent infection were clearly virulent in suckling mice. In addition, those MCMV strains tested in weanling mice also replicated in the reticuloendothelial system in a manner similar to wild MCMV. Thus, the vaccine MCMV strains recovered from salivary gland tissue of mice after reactivation of latent infection had reverted to full virulence.

These data do not allow determination as to whether vaccine MCMV reverted to virulence before or after establishment of latent infection. To answer this question, virus recovered from salivary gland homogenates prepared 2 wk after initial inoculation of vaccine MCMV into mice was tested for virulence markers.

TABLE III

Virulence Characteristics of Eight Vaccine MCMV Strains Recovered from Salivary Glands after Establishment and Reactivation of Latent Infection

Source of MCMV	Suckling mouse mortality	MCMV titers in tissues of weanling mice	
		Spleen	Liver
	%	*PFU/gm*	
Virulent MCMV control	15/15 (100)	1.2×10^6	6.3×10^4
Attenuated MCMV control	2/16 (11)	$<1.0 \times 10^2$	$<1.0 \times 10^2$
Reactivating vaccine MCMV			
1	15/16 (94)	2.1×10^5	3.1×10^4
2	13/15 (87)	8.8×10^5	1.3×10^4
3	14/14 (100)	1.1×10^6	5.1×10^4
4	9/9 (100)	—	—
5	12/12 (100)	2.1×10^5	3.1×10^4
6	13/13 (100)	—	—
7	11/12 (92)	1.6×10^6	8.2×10^4
8	9/10 (90)	3.6×10^5	2.5×10^5

Data presented in Table IV show that MCMV obtained from animals during this acute stage of the infection had reverted to virulence as measured both in suckling and weanling mice before development of latency. Thus, establishment of latent infection was clearly not required for reversion of vaccine virus to virulence.

DISCUSSION

From these experiments in the murine model, it is apparent that the vaccine MCMV strain was highly attenuated at the time of inoculation of animals. As expected (19), this virus was not lethal for suckling mice and replicated poorly in the reticuloendothelial system of weanling mice. In addition, a relatively large challenge dose (10^5 PFU) of the vaccine virus failed to kill weanling animals immunosuppressed with ALS. The magnitude of these differences from wild-type control virus was significant and consistently demonstrable.

TABLE IV

Virulence Characteristics of Vaccine MCMV Recovered from Salivary Glands of Mice 14 d after Inoculation

Virus strain	Suckling mouse mortality	MCMV titers in tissues of weanling mice	
		Spleen	Liver
	%	*PFU/gm*	
Vaccine MCMV	0/12 (0)	1.2×10^2	$<1.0 \times 10^2$
Wild MCMV control	10/10 (100)	5.8×10^6	4.8×10^5
Vaccine MCMV in salivary glands 14 d after inoculation			
1	13/13 (100)	3.6×10^5	1.6×10^5
2	15/17 (88)	—	—
3	11/12 (92)	6.0×10^6	7.4×10^4
4	13/14 (93)	—	—

4 mo after vaccination, no infectious virus was detectable in the tissues of normal mice. However, immunosuppression produced reactivation and dissemination of the vaccine virus in virtually all animals, and histologic evidence of widespread cytomegalic inclusion disease was found. In this respect, the vaccine MCMV behaved in a fashion identical to wild-type virulent MCMV, which can be consistently activated from a latent state by immunosuppression (22, 24–27). Indeed, characterization of the reactivating vaccine virus recovered from salivary glands clearly indicated that reversion to virulence had occurred.

Previous studies of acute infections in mice by Osborne and Walker (19) showed that attenuation of MCMV was an unstable characteristic. A single back passage of attenuated virus in mice resulted in return of virulence, at least as measured by virus multiplication in weanling animals. The data presented here confirm and extend these findings to include corroborative lethality studies in suckling mice. In addition, characterization of the vaccine virus shortly after inoculation indicated that reversion to virulence occurred early, during the acute stage of infection. Thus, the virus that established latent infection was already virulent and, not surprisingly, remained so during the reactivation process. This observation sheds light on the events involved in the establishment of latency by vaccine virus and indicates that reversion was not

directly related to viral latency. However, from the standpoint of potential adverse effects of a CMV vaccine, the implications of those findings are the same whether reversion occurred before or after latent infection was established.

The relevance of these results to human CMV vaccination is not yet known. Because the murine model can be manipulated experimentally, important fundamental features of the pathogenesis of acute, chronic, and, more recently, latent infection have been elucidated. In all important respects, the findings have substantiated our concepts of the virus-host interactions that are thought to occur in man. Because distinct markers for attenuation of the mouse virus exist (19), and protection against wild-virus challenge has been demonstrated (19, 20), the system is suitable for investigations of both the beneficial and potential adverse effects of CMV vaccination. However, the fact remains that MCMV is a different virus from the human CMV strains. It is conceivable that repeated cell-culture passage serves to attenuate human CMV in a more stable manner than with MCMV, and that reversion to virulence may not occur as readily in man after vaccination. In this regard, the lack of a suitable marker to determine the virulence status of the human CMV vaccine strains is a major drawback.

To date, significant systemic adverse reactions to vaccination with cell-culture passaged CMV have not been noted in man (7–10). Both humoral and cell-mediated virus-specific immune responses have been demonstrated in vaccinated patients (11). Whether the vaccine will be effective in preventing or modifying CMV infections, particularly in immunocompromised patients, remains to be determined. In preliminary trials of CMV vaccination in renal transplant recipients, six of nine vaccinated patients subsequently excreted CMV in the urine, but restriction endonuclease analyses of the DNA of recovered viruses were different from the vaccine strain (11). Studies with the murine model indicate that the cautious selection of vaccinated patients and the rigorous clinical and virologic surveillance employed to date are clearly warranted in studies of the safety and efficacy of human CMV vaccine.

ACKNOWLEDGMENTS

I thank Dr. Jack G. Stevens for helpful discussion and comments, and acknowledge the expert technical assistance of JoAnne L. Takagi.

This work was supported by U. S. Public Health Service grant AI-06246 from the National Institute of Allergy and Infectious Diseases, and by program project CA-23175 from the National Cancer Institute.

REFERENCES

1. Weller, T. H. 1971. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. *N. Engl. J. Med.* **285:** 203–214, 267–274.
2. Hanshaw, J. B. 1971. Congenital cytomegalovirus infections: a fifteen year perspective. *J. Infect. Dis.* **123:** 555–561.
3. Abdallah, P. S., J. B. D. Mark, and T. C. Merigan. 1976. Diagnosis of cytomegalovirus pneumonia in compromised hosts. *Am. J. Med.* **61:** 326–332.
4. Niemann, P. E., W. Reeves, G. Ray, N. Fluornoy, K. G. Lerner, G. E. Sale, and E. D. Thomas. 1977. A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogeneic bone marrow grafts. *J. Infect. Dis.* **136:** 754–767.
5. Reynolds, D. W., S. Stagno, K. G. Stubbs, A. J. Dahle, M. M. Livingston, S. S. Saxon, and C. A. Alford. 1974. Inapparent congenital cytomegalovirus infection with elevated cord IgM levels. *N. Engl. J. Med.* **290:** 291–296.
6. Hanshaw, J. B. 1976. School failure and deafness after "silent" cytomegalovirus infection. *N. Engl. J. Med.* **295:** 468–472.
7. Elek, D. S., and H. Stern. 1974. Development of a vaccine against mental retardation caused by cytomegalovirus infection *in utero*. *Lancet.* **I:** 1–5.
8. Just, M., A. Buergin-Wolff, G. Emoedi, and R. Hernandez. 1975. Immunization trials with live attenuated cytomegalovirus Towne 125. *Infection.* **3:** 111–114.
9. Plotkin, S. A., J. Farquhar, and E. Hornberger. 1976. Clinical trials of immunization with the Towne 125 strain of human cytomegalovirus. *J. Infect. Dis.* **134:** 470–475.
10. Neff, B. J., R. E. Weibel, E. B. Buynak, A. A. McLean, and M. R. Hilleman. 1979. Clinical and laboratory studies of live cytomegalovirus vaccine Ad-169. *Proc. Soc. Exp. Biol. Med.* **160:** 32–37.
11. Glazer, J. P., H. M. Friedman, R. A. Grossman, S. E. Starr, C. F. Barker, L. J. Perloff, E. S. Huang, and S. A. Plotkin. 1979. Live cytomegalovirus vaccination of renal transplant candidates: a preliminary trial. *Ann. Intern. Med.* **91:** 676–683.
12. Pagano, J. S., and E. S. Huang. 1974. Vaccination against cytomegalovirus? (letter). *Lancet.* **I:** 866–867.
13. Mannini, A., and D. N. Medearis. 1961. Mouse salivary gland virus infections. *Am. J. Hyg.* **73:** 329-343.
14. Henson, D., and C. Neapolitan. 1970. Pathogenesis of chronic cytomegalovirus infection in submaxillary glands of mice. *Am. J. Pathol.* **58:** 255–267.
15. Selgrade, M. K., and J. E. Osborne. 1974. The role of macrophages in resistance to murine cytomegalovirus. *Infect. Immun.* **10:** 1383–1390.
16. Brody, A. R., and J. E. Craighead. 1974. Pathogenesis of pulmonary cytomegalovirus infection in immunosuppressed mice. *J. Infect. Dis.* **129:** 677–689.
17. Jordan, M. C. 1978. Interstitial pneumonia and subclinical infection after intranasal inoculation of murine cytomegalovirus. *Infect. Immun.* **21:** 275–280.
18. Wu, B. C., J. N. Dowling, J. A. Armstrong, and M. Ho. 1975. Enhancement of mouse cytomegalovirus infection during host-versus-graft reaction. *Science (Wash. D. C.).* **190:** 56–58.
19. Osborne, J. E., and D. L. Walker. 1970. Virulence and attenuation of murine cytomegalovirus. *Infect. Immun.* **3:** 228–236.
20. Howard, R. J., and H. H. Balfour. 1977. Prevention of morbidity and mortality of wild murine cytomegalovirus by vaccination with attenuated cytomegalovirus. *Proc. Soc. Exp. Biol. Med.* **156:** 365–368.
21. Olding, L. B., F. C. Jensen, and M. B. A. Oldstone. 1975. Pathogenesis of cytomegalovirus infection. I. Activation of virus from bone marrow-derived lymphocytes by in vitro allogenic reaction. *J. Exp. Med.* **141:** 561–572.

22. Mayo, D. R., J. A. Armstrong, and M. Ho. 1977. Reactivation of murine cytomegalovirus by cyclophosphamide. *Nature (Lond.).* **267:** 721–723.
23. Cheung, K. S., and D. J. Lang. 1977. Detection of latent cytomegalovirus in murine salivary and prostate explant cultures and cells. *Infect. Immun.* **15:** 563–575.
24. Jordan, M. C., J. D. Shanley, and J. G. Stevens. 1977. Immunosuppression reactivates and disseminates latent murine cytomegalovirus. *J. Gen. Virol.* **37:** 419–425.
25. Jordan, M. C. 1978. Murine models of cytomegalovirus latency. *In* Persistent Virus Infections. J. G. Stevens, G. J. Todaro, and C. F. Fox, editors. Academic Press, Inc., New York. 805–810.
26. Mayo, D., J. A. Armstrong, and M. Ho. 1978. Activation of latent murine cytomegalovirus infection: co-cultivation, cell transfer, and the effect of immunosuppression. *J. Infect. Dis.* **138:** 890–898.
27. Shanley, J. D., M. C. Jordan, M. L. Cook, and J. G. Stevens. 1979. Pathogenesis of reactivated latent murine cytomegalovirus infection. *Am. J. Pathol.* **95:** 67–80.