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Evaluation of Murine Cytomegalovirus Antibody Detection by Serological Techniques

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Naturally acquired murine cytomegalovirus (MCMV) infection in laboratory strains of mice induces antibody levels which are generally undetectable by standard techniques; therefore, MCMV has not been included routinely in mouse viral antibody screening programs. The relative sensitivity of three assay systems, the nuclear anticomplement immunofluorescence (NACIF), the enzyme-linked immunosorbent assay (ELISA), and the complement fixation (CF) test, was evaluated for the detection of MCMV antibodies. Sera were harvested from CD_1 male mice (33 days old) infected intraperitoneally with salivary gland-passaged MCMV (Smith strain). The sera were assayed separately at weeks 1 through 8, and at week 11, 16, and 25 post-inoculation; a total of 167 mice in 11 groups were tested. The animals tested at 1 week post-inoculation had low levels of antibodies to MCMV as measured by the NACIF test (1:10), whereas only 25% were positive by ELISA, and none was positive by CF until 5 weeks post-inoculation. A higher titer of MCMV antibodies was measured by CF (1:640) than by NACIF (1:40) at 6 months post-inoculation; yet, a titer of 1:3,200 was detected by ELISA from the same serum. The ELISA technique was more sensitive for detecting persistent infection with MCMV, and NACIF was more useful for detecting acute MCMV infection. Since MCMV can have significant long-term effects on the immune system, it is recommended that testing for antibodies to MCMV be included in mouse viral antibody screening protocols.

It is widely recognized that some strains of mice are more susceptible to murine cytomegalovirus (MCMV) than others (11, 14, 21) and that the virus can establish a chronic persistent infection in wild, as well as in laboratory, mouse strains (10, 24, 31). It is also possible for both latent and chronic persistent infection to coexist in different tissues in the same host (15). Experimental MCMV infection in mice has been shown to alter not only the respiratory system (7, 17, 32), but also hematological (9, 30) and biochemical homeostasis (16, 26). MCMV has also been found to enhance susceptibility to bacteria (12, 33), *Candida albicans* (13), and Newcastle disease virus (29). Most importantly, MCMV can suppress various parameters of the primary immune response (3–5, 15, 21).

It would be advantageous to monitor the incidence of MCMV infection and its effects on experimental protocols of, for example, immunological, carcinogenic, and toxicological studies. At present, due to the lack of a sufficiently sensitive assay system to detect the low levels of antibodies to MCMV which prevail during chronic persistent infection, neither rodent vendors nor investigators screen for MCMV. Histopathological evaluation is not an acceptable alternative because the absence of inclusion bodies in the submaxillary glands does not exclude MCMV infection (6).

The present investigation assesses the relative sensitivities of the nuclear anticomplement immunofluorescence (NACIF), enzyme-linked immunosorbent assay (ELISA), and complement fixation (CF) tests for the detection of antibodies to MCMV after experimental infection.

MATERIALS AND METHODS

Virus. A salivary gland (SG) homogenate of MCMV (Smith strain) was kindly supplied by Donald N. Medearis, Massachusetts General Hospital, Boston. Stock virus for animal inoculation was prepared by homogenizing and clarifying SGs from CD_1 mice which had been infected with virus ($10^{3.3}$ PFU/0.1 ml) 3 weeks earlier. Control SG homogenate was prepared similarly from mice which were not infected. Both MCMV and control SG homogenates were tested for MCMV and contamination with other viruses by inoculation onto cell monolayers of primary mouse embryo fibroblasts BHK-21, L929, and NCTC 1469 cells. Excluding the MCMV SG homogenate containing MCMV, both were negative for endogenous virus and mycoplasma.

Mice. A total of 167 33-day-old CD_1 specific-pathogen-free male mice (Charles River Breeding Labora-

tories, Inc., Kingston, N.Y.) were used in the study. Of these, 119 mice were injected intraperitoneally with $10^{4.6}$ PFU/0.1 ml of SG-passaged MCMV, and 48 mice were injected with control SG homogenate. The mice received were specified specific-pathogen free, and the sera from these mice were negative when screened for antibodies to the following mouse viruses before inoculation: MCMV, mouse hepatitis virus (MHV), Sendai virus, and mouse adenovirus by CF; pneumonia virus of mice by hemagglutination-inhibition; reovirus type 3 and mouse encephalomyelitis virus (GD VII) by neutralization. The mice were housed four per cage in solid-bottom cages with hardwood chips on racks without filter tops in a single cubicle (6 by 6 ft [ca. 1.8 m]) with approximately 30 unrecirculated air exchanges per h). The infected mice were housed on one side of the rack, and the uninfected mice were housed on the other. Food and water were given ad libitum. The cages were changed once per week; the control mice were handled first. Surgical gloves were worn, and all cages with bedding were autoclaved before the disposal of bedding and washing of cages.

Mouse embryo fibroblast cell cultures. Pregnant specific-pathogen-free CD_1 mice (Charles River Breeding Laboratories, Inc.) were exsanguinated by cardiac puncture under ether anesthesia to obtain 14- to 15-day-gestation embryos for dispersing with trypsin. The cells were grown in Eagle minimal essential medium with Hanks balanced salts and 10% fetal bovine serum (FBS) containing an antibiotic-antimycotic mixture (penicillin, 100 U/ml; streptomycin, 100 μg/ml; amphotericin B, 0.25 μg/ml [GIBCO Laboratories, Grand Island, N.Y.]). The cell monolayers were subcultured once for the plaque assay of virus titer and the preparation of virus and control antigens.

Schedule. Eight to ten infected and three to four control mice were tested post-inoculation (p.i.) at weekly intervals for 8 weeks and then at weeks 11, 16, and 25. The mice were anesthetized with ether and bled by cardiac puncture; the sera were separated and stored at −80°C until tested.

Virus preparation for antibody detection techniques. Virus and control antigens for all three serological tests were prepared simultaneously to minimize variability. First, tissue culture-passaged MCMV was adsorbed onto MEF monolayers (approximately 10 multiplicity of infection per cell) for 1 h. The monolayers were rinsed with Hanks balanced salt solution and incubated in Eagles minimal essential medium containing Earle salts with 5% FBS and antibiotic-antimycotic mixture.

The infected cells were trypsinized when viral cytopathic effect attained 80 to 90% of the monolayers. The cell suspension containing virus was sedimented by centrifugation at 500 × *g* for 15 min at 4°C. The pellet was suspended to one-tenth of the original culture volume with phosphate-buffered saline (PBS). Two-thirds of this suspension was transferred to another conical centrifuge tube for glycine extraction (GE) of antigen for the ELISA and CF tests; the remaining one-third was processed for the extraction of nuclei for the NACIF test. Uninoculated control cells were processed similarly.

(i) GE. A slight modification of the GE method described by Kettering and co-workers (19) was followed. The virus-containing suspension was centrifuged at 225 × *g* for 15 min at 4°C; the supernatant fluid was removed and replaced with 0.1 M glycine buffer (pH 9.5) at one-half of the volume transferred. This suspension was sonicated in an ice bath and centrifuged, and the supernatant (GE antigen) was removed and stored in 1-ml volumes at −80°C.

(ii) Nuclei extraction. The procedures for the preparation of naked nuclei for immunofluorescence as reported by Mintz and co-workers (25) were generally followed except that centrifugation was done at a slower speed for a longer period of time. Virus was centrifuged at 225 × *g* for 15 min at 4°C; the supernatant fluid was removed and replaced with an equal volume of sterile, distilled water containing 2% FBS. The suspension was pelleted again at 225 × *g* for 15 min at 4°C; this procedure was repeated twice. The final pellet was suspended in distilled water containing 2% FBS at one-half of the volume transferred. The slides for the NACIF test were prepared as described elsewhere (25), using glass slides with 10 7-mm-diameter etched wells per slide (Roboz Surgical Instruments Co. Inc., Washington, D.C.) and stored at −80°C.

Serological techniques. (i) CF test. The sera were evaluated by the standard Laboratory Branch CF test (Microbiological Associates, Bethesda, Md.), diluted 1:10, and heat inactivated, and serial twofold dilutions were made. The proper dilution of antigen was determined with MCMV immune and control sera from CD_1 mice.

(ii) NACIF test. The slides were thawed and placed in moist chambers. Serial twofold dilutions of heat-inactivated sera in PBS were prepared beginning at 1:10 through 1:640; then 0.025 ml of each serum dilution was added to each well and allowed to react for 30 min at 37°C. After three 5-min rinses in PBS, the slides were drained, and 0.025 ml of guinea pig complement (Flow Laboratories, Inc., McLean, Va.), containing 8 U, was added to each well. After a 30-min incubation period at room temperature in a moist chamber, the slides were rinsed and drained, and 0.025 ml of fluorescein-conjugated goat anti-guinea pig C3 (B1C/B1A) (Cappell Laboratories, Inc., Cochranville, Pa.) diluted 1:12 in PBS containing 0.01% Evans blue was added to each well. The slides were maintained at room temperature in a moist chamber for 30 min, rinsed again in PBS, air dried, and evaluated under oil immersion on an A/O fluorescent microscope (exciter filter 450-490, barrier filter 520, 50-W mercury vapor lamp).

(iii) ELISA. This test was developed from the method of Voller and co-workers (34). The addition of 10% FBS with 1% control GE antigen during antigen adsorption to the plates was found to reduce the problem of nonspecific reactivity significantly. ELISA was done in microtiter plates (96 wells per plate; Dynatech Laboratories, Inc., Alexandria, Va.); alkaline phoshatase was used as the enzyme conjugate and was prepared in goats against mouse immunoglobulin G (IgG) (heavy and light chains) (Bionetics, Inc., Kensington, Md.). MCMV antigen and control-coated plates were stored at −80°C before use. The block titration of positive and negative reference sera with MCMV and control antigen was done to standardize the test system before the assay of the test sera. Serial twofold dilutions of test sera were prepared from a 1:100 dilution in PBS with 0.5% Tween 20. Absorbance was measured on an Abbott Bichromatic Ana-

lyzer-100 (Abbott Laboratories, North Chicago, Ill.) with filter 450-415 (nm). The ELISA value for each serum dilution was determined by subtracting the mean absorbance with control GE antigen from the mean absorbance with MCMV GE antigen. A value greater than 0.125 was considered to be positive.

MCMV antigenic specificity. The specificities of the three serological tests were evaluated to confirm that no cross-reactivity occurred. Virus-specific mouse antisera were obtained from commercial (M. A. Bioproducts, Inc., Walkersville, Md.) and private (W. Shek, Charles River Breeding Laboratories, Inc., Wilmington, Mass.) sources.

Monitoring for infection with other murine viruses. Antibody titers to MHV, Sendai virus, mouse adenovirus, and pneumonia virus of mice were determined as described previously; those to reovirus type 3 and GD VII were determined by anticomplement immunofluorescence on infected L929 and BHK-21 cell monolayers, respectively. Samples found positive for MHV by CF were tested by ELISA, using MHV polyvalent antigen (M. A. Bioproducts) in an alkaline phosphatase conjugate system as described for MCMV.

RESULTS

Specificity of the serological tests. Mouse antisera to the following rodent viruses did not react in any of the MCMV tests: MHV, reovirus type 3, mouse adenovirus, and GD VII; anti-complementary activity was noted to Sendai and pneumonia virus of mice in the CF test only. The MCMV ELISA test was standardized by block titration of MCMV and control antigens against positive and negative MCMV sera (Table 1). The 1:100 dilution of positive serum against MCMV and control antigen at 1:100 resulted in an ELISA value closest to 1.0 (0.861), and the negative serum at the same dilution had an ELISA value of 0.091. This dilution of antigen was used in all subsequent tests to distinguish positive sera from negative sera.

Evaluation of MCMV antibody detection techniques. Table 2 summarizes the results of the study. The NACIF test detected a maximum antibody titer of 1:10 as early as 1 week p.i. in 100% of the mice tested, whereas only 25% of the same infected mice were positive by ELISA with a maximum antibody titer of 1:100. No titers were detected by CF until 5 weeks. The range of antibody titers measured by CF at this time (1:40 to 1:640) was similar to that determined by NACIF. Antibody titers to MCMV measured by ELISA increased significantly between 4 weeks (1:100 to 1:400) and 5 weeks (1:1,600 to 1:12,800) and peaked at 8 weeks, with a mean titer of 1:9,600 (Fig. 1). All infected mice seroconverted at 25 weeks by all three tests with the exception of one by the NACIF test. At 25 weeks p.i., the titers measured by immunofluorescence were generally threefold lower than those determined by CF. The mean antibody titer at 25 weeks was 1:363 by CF, 1:154 by NACIF, and 1:2,400 by ELISA.

One control mouse was found to be positive by ELISA at 5 weeks, by NACIF at 6 weeks, and by CF at 11 weeks. At 25 weeks, the control animals were positive more frequently by the CF test (four of five) and ELISA (five of five) than by the NACIF test (one of five).

Results of tests for antibodies to other murine viruses. No antibodies were detected in the sera of animals obtained at the beginning of the study. At 6 and 7 weeks, two control mice had low levels of CF antibodies (1:10) to MHV. At 16 and 25 weeks, the sera of three mice previously inoculated with MCMV were found to have MHV CF titers of 1:10. Only two of the five mice were positive by MHV ELISA.

DISCUSSION

The data from the evaluation of MCMV antibody detection techniques (Table 2) have raised several important points regarding the nature of MCMV infection in mice and carry some impli-

TABLE 1. Block titration to determine reagent dilutions for ELISA[a] absorbance as measured at 450 to 415 nm

Antigen dilution	ELISA value at dilution of positive reference serum of:					ELISA value at dilution of negative reference serum of:				
	50	100	200	400	800	50	100	200	400	800
MCMV										
1:50	2.856	1.849	1.158	0.791	0.453	0.160	0.153	0.046	0.056	0.044
1:100	2.288	1.400	1.005	0.777	0.416	0.159	0.105	0.052	0.081	0.038
1:200	1.990	1.381	0.925	0.681	0.451	0.162	0.188	0.115	ND[b]	ND
Control										
1:50	0.653	0.524	0.529	0.455	0.303	0.253	0.054	0.064	0.049	0.034
1:100	0.464	0.539	0.431	0.333	0.300	0.128	0.014	0.040	0.036	0.024
1:200	0.533	0.785	0.482	0.326	0.266	0.150	0.023	0.019	0.015	ND

[a] The highest dilution of MCMV antigen and of positive serum giving an ELISA value closest to 1.0 was used in all tests. In the example shown, MCMV antigen was used at 1:100, and the sera were diluted beginning at 1:100 in all subsequent tests.

[b] ND, Not done.

TABLE 2. Evaluation of antibody detection techniques for evidence of infection with MCMV

Wk p.i.	CF[a]		NACIF[a]		ELISA[a]	
	Infected mice	Control mice	Infected mice	Control mice	Infected mice	Control mice
1	0/8 (<10)	0/4 (<10)	8/8 (10)	0/4 (<10)	2/8 (100–200)	0/4 (<100)
2	0/10 (<10)	0/3 (<10)	10/10 (40)	0/3 (<10)	4/9 (100–200)	0/4 (<100)
3	0/9 (<10)	0/4 (<10)	8/9 (20–160)	0/4 (<10)	4/9 (100–200)	0/4 (<100)
4	0/9 (<10)	0/4 (<10)	9/9 (10–40)	0/4 (<10)	5/9 (100–400)	0/4 (<100)
5	8/9 (40–640)	0/4 (<10)	9/9 (80–640)	0/4 (<10)	9/9 (1,600–12,800)	1/4 (100)
6	10/10 (160–640)	0/4 (<10)	10/10 (160–640)	1/4 (10)	10/10 (1,600–12,800)	2/4 (100–200)
7	9/9 (80–640)	0/4 (AC)[b]	9/9 (20–160)	1/2 (10)	9/9 (1,600–12,800)	3/4 (100–400)
8	8/8 (40–640)	0/4 (AC/TAC)[b]	8/8 (40–320)	0/4 (<10)	8/8 (6,400–12,800)	2/4 (100)
11	8/8 (160–640)	1/4 (10)	8/8 (10–160)	0/4 (<10)	8/8 (3,200–25,600)	4/4 (100–400)
16	6/6 (80–640)	1/3 (160)	6/6 (20–320)	1/3 (160)	6/6 (160–12,800)	3/3 (200–6,400)
25	16/16 (10–640)	4/5 (10–80)	15/16 (10–80)	1/5 (10)	16/16 (1,600–12,800)	5/5 (100–400)

[a] Number positive/number tested (range of antibody titer as reciprocal of highest dilution of MCMV antigen and of positive serum giving an ELISA value closest to 1).

[b] AC, Anticomplementary; TAC, tissue anticomplementary.

cations for cytomegalovirus infection in humans. An interesting finding in this study was the delay in the detection of MCMV antibody titers by ELISA. Although the conjugate was of the IgG class with heavy and light chains, it was not anticipated that at 4 weeks only 55% of the animals tested would be positive by ELISA, whereas 100% of the same infected animals had

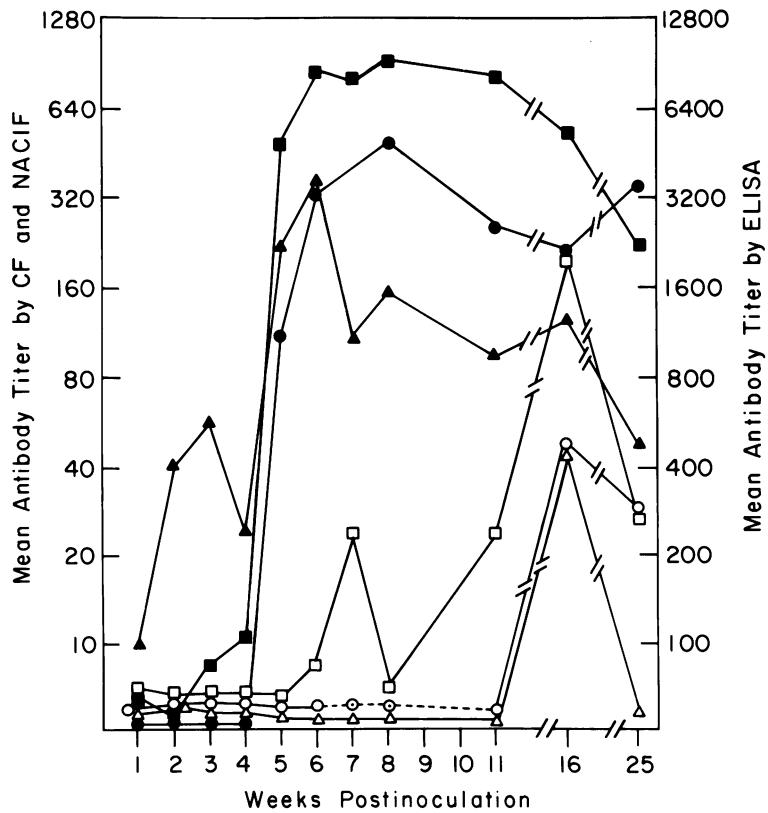

FIG. 1. Mean antibody titers to MCMV. Symbols: ●, CF (infected mice); ▲, NACIF (infected mice); ■, ELISA (infected mice); ○, CF (control mice); --⊙--, CF—anticomplementary (control mice); △, NACIF (control mice); □, ELISA (control mice).

seroconverted by the NACIF test. Since two of eight animals were positive by ELISA at 1 week, this test appears to be able to detect early IgG antibodies; however, it is apparently not as sensitive as the NACIF test for diagnosing acute infection. Since only 50% of the animals tested by ELISA were positive during the first 4 weeks p.i., and significant levels of antibodies could be detected at the close of the study, the expression of some fractions of IgG may have been suppressed during the acute phase and not during the chronic phase of infection. This agrees with an earlier proposal offered by Osborn and coworkers (28).

The NACIF test offers a simple and rapid method for detecting the presence of MCMV antibodies as early as 1 week p.i. Since NACIF was positive early, it was the best of the three assays to detect IgM antibodies. Another advantage of the NACIF test is that it is not affected by anticomplementary sera (19).

The late detection of MCMV antibodies by the CF test (5 weeks) generally agrees with an earlier report by Mannini and Medearis (22), but some differences were noted. In their study, Swiss-Webster mice seroconverted at 4 weeks p.i. and CF antibody titers which were detected with a freeze-thawed (FT) antigen were greater than and persisted longer than neutralizing antibody titers. A FT antigen consists mostly of enveloped particles and intact dense bodies with few nucleocapsids, whereas GE antigen results in a population of virus nucleocapsids with few enveloped particles and disintegrating dense bodies (18). A distinct rise and fall of antibody titer were noted with FT antigen, whereas no significant trend of CF antibody reduction was evident in this study, for the mean antibody titer at 25 weeks p.i. was 1:363 (Fig. 1). Betts and coworkers (2) found similar differences with FT and GE antigen preparations to human CMV.

The virus strain used for detecting antibodies is an important consideration when evaluating techniques since it has been reported that the passage of the Smith strain through various tissue culture systems has created biological differences (27). It has been found that human CMV strains can differ widely in requirements for complement for detection of neutralizing antibodies (1), and this may be true for MCMV antibody detection as well. In addition, genetic factors in susceptibility to MCMV infection, as noted previously, and age may influence the production of those antibodies requiring complement. A search for MCMV serum antibodies in various strains of mice being conducted currently in our laboratory may provide evidence for this hypothesis.

The detection of MCMV antibodies in control mice late in the experiment may be due to (i) a nonspecific reaction, (ii) the emergence of an active infection in the control mice which was previously latent, (iii) undetectable MCMV in the control SG inoculum which was expressed upon passage into a susceptible host, (iv) transmission of virus by aerosol to control mice from the MCMV-inoculated mice. A nonspecific reaction is not likely since the sera did not contain detectable antibodies to the other viruses tested. Since the sera were negative for MCMV antibodies by all three serological tests at the beginning of the study, the reactivation of latent infection may not be likely; however, virus isolation studies were not done to eliminate this possibility. Cheung and co-workers (8) detected MCMV-specific DNA in SG extracts from uninoculated mice, using nucleic acid hybridization. Therefore, it is possible that there was activation of a latent infection in the mouse or that MCMV was in the control SG inoculum. The transmission of the virus from infected to control mice by aerosol during animal handling or the proximity of control and infected mice is also a likely explanation and emphasizes the need for strict isolation measures where the control of potential MCMV infection is desired. In view of reports that experimental intranasal infection with MCMV can induce subclinical interstitial pneumonitis (7, 17, 32) and that MCMV has been isolated from mouth swabs of mice for up to 1 year when inoculated as weanlings (6), proper containment is essential.

The detection of MHV by CF during the study cannot be attributed to the immunosuppressive properties of MCMV since antibody titers were detected first in control mice. This may reflect the exposure of the colony to a human coronavirus. Two human strains of coronavirus have been found to be antigenically related to several strains of MHV, as well as to experimentally induce asymptomatic infection in mice (23). Since the detection of MHV antibodies by CF is reported to give a low rate of false-positive results (20), and two sera were positive for MHV by ELISA, it must be accepted that infection with a coronavirus did occur. The most probable source is cross-contamination from MHV-infected mice housed in adjacent cubicles.

Both the NACIF test and ELISA are suitable for detecting antibodies to MCMV; however, the NACIF test appears to be more reliable for detecting acute infection since antibody titers had decreased significantly by the end of the study. ELISA appears to be more suitable for detecting persistent infection because antibody titers remained high in MCMV-infected mice. The data presented support the advisability of monitoring laboratory mice for MCMV infection and document the sensitivity of the ELISA test for detecting chronic infections.

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LITERATURE CITED

1. **Andersen, H. K.** 1972. The influence of complement on cytomegalovirus neutralization by antibodies. Arch. Gesamte Virusforsch. **36**:133–140.
2. **Betts, R. F., S. D. George, B. B. Rundell, R. B. Freeman, and R. G. Douglas, Jr.** 1976. Comparative activity of immunofluorescent antibody and complement-fixing antibody in cytomegalovirus infection. J. Clin. Microbiol. **4**:151–156.
3. **Bixler, G. S., Jr., and J. Booss.** 1980. Establishment of immunologic memory concurrent with suppression of the primary immune response during acute cytomegalovirus infection of mice. J. Immunol. **125**:893–896.
4. **Booss, J., and E. F. Wheelock.** 1977. Role of viremia in the suppression of T-cell function during murine cytomegalovirus infection. Infect. Immun. **17**:378–381.
5. **Booss, J., and E. F. Wheelock.** 1977. Progressive inhibition of T-cell function preceding clinical signs of cytomegalovirus infection in mice. J. Infect. Dis. **135**:478–481.
6. **Brodsky, I., and W. P. Rowe.** 1958. Chronic subclinical infection with mouse salivary gland virus. Proc. Soc. Exp. Biol. Med. **99**:654–655.
7. **Brody, A. R., and J. F. Craighead.** 1974. Pathogenesis of pulmonary cytomegalovirus infection in immunosuppressed mice. J. Infect. Dis. **129**:677–689.
8. **Cheung, K.-S., E.-S. Huang, and D. J. Lang.** 1980. Murine cytomegalovirus: detection of latent infection by nucleic acid hybridization technique. Infect. Immun. **27**:851–854.
9. **Cheung, K.-S., J. K. K. Li, J. M. Falletta, J. L. Wagner, and D. J. Lang.** 1981. Murine cytomegalovirus infection: hematological, morphological, and functional study of lymphoid cells. Infect. Immun. **33**:239–249.
10. **Gardner-Murray, B., J. E. Officer, J. Parker, J. D. Ester, and R. W. Rongey.** 1974. Induction of disseminated virulent cytomegalovirus infection by immunosuppression of naturally chronically infected wild mice. Infect. Immun. **10**:966–969.
11. **Grundy (Chalmer), J. E., and C. J. M. Melief.** 1982. Effect of nu/nu gene on genetically determined resistance to murine cytomegalovirus. J. Gen. Virol. **61**:133–136.
12. **Hamilton, J. R., J. C. Overall, and L. A. Glasgow.** 1976. Synergistic effect on mortality in mice with murine cytomegalovirus and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Candida albicans* infections. Infect. Immun. **14**:982–989.
13. **Hamilton, J. R., J. C. Overall, and L. A. Glasgow.** 1977. Synergistic infection with murine cytomegalovirus and *Candida albicans* in mice. J. Infect. Dis. **135**:918–924.
14. **Hanshaw, J. B.** 1968. Cytomegaloviruses, p. 1–23. *In* S. Gard, C. Hallaver, and K. F. Meyer (ed.), Virology monographs, vol. 3. Springer-Verlag, New York.
15. **Hudson, J. B.** 1979. The murine cytomegalovirus as a model for the study of viral pathogenesis and persistent infections. Arch. Virol. **62**:1–29.
16. **Iqbal, M., and N. G. Grand.** 1973. Biochemical events in murine cytomegalovirus-infected mouse liver. Proc. Soc. Exp. Biol. Med. **142**:567–571.
17. **Jordan, M. C.** 1978. Interstitial pneumonia and subclinical infection after intranasal inoculation of murine cytomegalovirus. Infect. Immun. **21**:275–280.
18. **Kettering, J. D., N. J. Schmidt, D. Gallo, and E. H. Lennette.** 1977. Anticomplement immunofluorescence test for antibodies to human cytomegalovirus. J. Clin. Microbiol. **6**:647–649.
19. **Kettering, J. D., N. J. Schmidt, and E. H. Lennette.** 1977. Improved glycine-extracted complement-fixing antigen for human cytomegalovirus. J. Clin. Microbiol. **6**:647–649.
20. **Leow, F. M., and J. G. Fox.** 1983. Animal health surveillance and health delivery systems, p. 69–82. *In* H. L. Foster, J. D. Small, and J. G. Fox (ed.), The mouse in biomedical research, vol. III. Normative biology, immunology and husbandry. Academic Press, Inc., New York.
21. **Lussier, G.** 1975. Murine cytomegalovirus (MCMV), p. 223–247. *In* C. A. Brandly and C. E. Cornelius (ed.), Advances in veterinary medicine, vol. 19. Academic Press, Inc., New York.
22. **Mannini, A., and D. N. Medearis, Jr.** 1961. Mouse salivary gland virus infections. Am. J. Hyg. **73**:329–343.
23. **McIntosh, K., A. Z. Kapikian, K. A. Hardison, J. W. Hartley, and R. M. Chanock.** 1969. Antigenic relationship among coronaviruses of man and between human and animal coronaviruses. J. Immunol. **102**:1109–1118.
24. **Medearis, D. N., Jr.** 1964. Mouse cytomegalovirus infection. II. Observations during prolonged infections. Am. J. Hyg. **80**:103–120.
25. **Mintz, L., R. C. Miner, and A. S. Yeager.** 1980. Anticomplement immunofluorescence test that uses isolated fibroblast nuclei for detection of antibodies to human cytomegalovirus. J. Clin. Microbiol. **12**:562–566.
26. **Mohamed, A. H.** 1974. Murine cytomegalovirus-infected liver: depletion of acid phosphatase with concomitant increase of alkaline phosphatase. Microbiology **10**:103–113.
27. **Osborn, J. E.** 1982. Cytomegalovirus and other herpesviruses, p. 267–292. *In* H. L. Foster, J. D. Small, and J. G. Fox (ed.), The mouse in biomedical research, vol. II. Diseases. Academic Press, Inc., New York.
28. **Osborn, J. E., A. A. Blazkovec, and D. L. Walker.** 1968. Immunosuppression during acute murine cytomegalovirus infection. J. Immunol. **100**:835–844.
29. **Osborn, J. E., and D. N. Medearis, Jr.** 1967. Suppression of interferon and antibody and multiplication of Newcastle disease virus in cytomegalovirus infected mice. Proc. Soc. Exp. Biol. Med. **124**:347–353.
30. **Osborn, J. E., and N. T. Shahidi.** 1973. Thrombocytopenia in murine cytomegalovirus infection. J. Lab. Clin. Med. **81**:53–63.
31. **Rowe, W. P., J. W. Hartley, and R. J. Huebner.** 1962. Polyoma and other indigenous mouse viruses, p. 131–142. *In* R. C. J. Harris (ed.), The problems of laboratory animal disease. Academic Press, Inc., New York.
32. **Shanley, J. D., E. L. Pesanti, and K. M. Nugent.** 1982. The pathogenesis of pneumonitis due to murine cytomegalovirus. J. Infect. Dis. **146**:388–396.
33. **Stanley, J. D., and E. L. Pesanti.** 1980. Replication of murine cytomegalovirus in lung macroprophages: effect on phagocytosis of bacteria. Infect. Immun. **29**:1152–1159.
34. **Voller, A., D. Bidwell, and A. Bartlett.** 1979. Microplate enzyme immunoassays for the immunodiagnosis of virus infections, p. 506–512. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology. American Society for Microbiology, Washington, D.C.