Comparison of Serological Tests for the Detection of Antibody to Natural and Experimental Murine Cytomegalovirus

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ABSTRACT

Three serological tests, i.e. complement fixation test, indirect immunofluorescent assay, and enzyme-linked immunosorbent assay (ELISA) were compared for sensitivity in the detection and titration of murine cytomegalovirus antibody. The three tests were compared using sera from experimentally inoculated and naturally infected mice bled at intervals from 3 to 140 days postinfection. In the acute infection, complement fixation and indirect immunofluorescent assay tests were of comparable sensitivity for early detection of antibody, whereas the ELISA was less sensitive. In persistent infection, higher titers were recorded with ELISA. Since murine cytomegalovirus has been shown to exert significant effects on the immune response of infected mice, this antigen should be included routinely in viral antibody screening programs.

Key words: Murine cytomegalovirus, ELISA, complement fixation test, indirect immunofluorescence test, viral diagnosis, enzyme-linked immunosorbent assay.

RÉSUMÉ

Cette expérience portait sur les trois méthodes sérologiques suivantes: la déviation du complément, l'immunofluorescence indirecte et l'épreuve immunoenzymatique ELISA; elle visait à comparer leur sensibilité pour la détection et le titrage des anticorps contre le virus cytomégalique murin. Les auteurs utilisèrent à cette fin du sérum prélevé chez des souris, de trois à 140 jours après une infection naturelle ou expérimentale. Dans la phase aiguë de l'infection, les deux premières méthodes affichèrent une sensibilité comparable, tandis que dans sa phase chronique, l'ELISA donna des titres plus élevés. Comme on a déjà démontré que le cytomégalovirus murin exerce une influence appréciable sur la réaction immunitaire des souris infectées, il faudrait toujours inclure cet antigène, lors de l'évaluation sérologique de l'état de santé d'une colonie de souris.

Mots clés: cytomégalovirus murin, déviation du complément, immunofluorescence indirecte, épreuve immunoenzymatique ELISA, diagnostic virologique.

INTRODUCTION

Murine cytomegalovirus (MCMV), a readily transmitted pathogen of mice, has provided important insights into the pathogenesis of cytomegalovirus infection in humans (1). However, persistent MCMV infection is among the numerous viral infections that might have detrimental effects on biomedical research. For instance, the infection can cause alterations of the respiratory function (2,3), it can potentiate bacterial, fungal, and viral infections (4,5), and it can affect the breeding efficacy of infected colonies (6). Most importantly, MCMV can alter the immune response (7-9).

For these reasons, it is essential that adequate tests be available for identification of the presence of the infection in mouse colonies. Serological assays have been described for the detection of MCMV antibody: the serum neutralization (SN) test (10), the complement fixation (CF) test (11,12), the indirect imunofluorescence (IFA) test and the enzyme-linked immunosorbent assay (ELISA) (12-15). However, somewhat conflicting results concerning the host response to MCMV have been recorded (16). This paper compares the relative sensitivity of three assay systems, CF, ELISA, and IFA tests for detection of MCMV antibody in experimentally and naturally infected mice.

MATERIALS AND METHODS

VIRUS

The Smith strain of MCMV was obtained from the American Type Culture Collection, Rockville, Maryland. The virus was passaged twice in mouse embryo fibroblasts to produce the virus stock. Virus was titered by standard plaque assay, aliquoted and frozen at -70°C. Infected cells showing 75 to 90% cytopathogenic effect were trypsinized, centrifuged at 50 × *g* for 15 min at 4°C and used in the three tests. The mouse antibody production (MAP) test (17), used for the detection of possible contamination of the strain with mycoplasma and murine viruses, proved to be negative.

CELL CULTURES

Primary cell cultures of whole mouse embryos were established by conventional methods from trypsinized cell suspensions and were grown in equal parts of medium 199 (Hanks' base) and minimum essential medium (Earle's base) (Gibco Laboratories, Grand Island, New York) supplemented with 10% fetal calf serum (FCS), 2 μg/mL of glutamine and 10 μg/mL of gentamicin sulfate (Gibco Laboratories, Grand Island, New York). Cell cultures were maintained in Earle's minimum essential medium containing 2% FCS, glutamine, and gentamicin.

MICE

Random-bred CD-1 mice were used (Charles River Canada, Inc., St-Constant, Québec). All mice were

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Submitted September 12, 1986.

housed under conventional conditions in plastic solid-bottom cages with filter bonnets and kept in separate rooms to prevent cross-contamination among experimental groups. The animals were determined free of antibodies to Sendai virus, polyoma virus, K virus, Theiler's mouse encephalomyelitis virus, ectromelia, reovirus 3, lymphocytic choriomeningitis virus, minute virus of mice, pneumonia virus of mice, mouse hepatitis, mouse adenovirus and murine cytomegalovirus by complement fixation or hemagglutination inhibition assays (18).

EXPERIMENTAL MOUSE INFECTIONS

To study the temporal antibody response, 119 weanling mice were divided into five groups. In group 1, 31 mice were inoculated intraperitoneally with 10^4 plaque forming units (PFU) of the virus given intraperitoneally in (0.25 mL). In group 2, 20 mice were inoculated intranasally with approximately $10^{3.5}$ PFU of the virus (in 0.05 mL). Groups 3 and 4 consisted respectively of 31 and 19 mice placed in the same cages with animals that had been experimentally infected 7 and 35 days previously. Control mouse sera were obtained from 18 mice of group 5 inoculated intraperitoneally with uninfected tissue culture fluid. At each test interval two to five mice were randomly selected. The mice were anesthetized with ether and bled by cardiac puncture.

IFA ASSAY

As described above, mouse embryo fibroblasts were infected with MCMV. Infected cells and uninfected control cells were trypsinized, washed three times in sterile distilled water and suspended in distilled water containing 2% FCS at a concentration of 10^6 cells per mL. Drops of cell suspension were placed in each of twelve 5-mm circles on glass slides (Flow Laboratories, Mississauga, Ontario) which were air dried, fixed in cold acetone and stored at -70°C. To detect virus-specific antibody, circles containing either virus or control antigen were reacted with 25 μL of diluted serum (1:10 through 1:320) for 30 min at 37°C. Normal mouse serum was used as control. After three washes in PBS, 25 μL of fluorescein-conjugated rabbit anti-mouse IgG (Miles Laboratories, Rexdale, Ontario) were applied to each spot. Slides received three further washes in PBS, were air dried and examined under ultra-violet illumination. The highest serum dilution giving fluorescent staining was recorded as the IFA titer.

ANTIGEN PREPARATION FOR CF AND ELISA TECHNIQUES

Virus and control antigens were prepared as previously described (12,19). Briefly, a glycine extract of MCMV-infected or control mouse embryo fibroblasts was used. The cell suspension was centrifuged at 225 × g for 15 min at 4°C. The supernatant fluid was removed, replaced with 0.1 M glycine buffer (pH 9.5) at one-half of the original volume, and the suspension was sonicated in an ice bath and centrifuged at 500 × g for 15 min at 4°C. The supernatant was stored at -70°C prior to use as antigen.

CF TEST

The sera were evaluated by standard methods (20,21) in microtiter plates (Nunc, Roskilde, Denmark). Sera to be tested were diluted 1:8 and heat inactivated at 56°C. Serial twofold dilutions were made and tested against the optimal dilution of the antigen and two hemolytic units of complement. Appropriate controls were included for each serum tested, as well as other reagents.

ELISA TEST

Details were essentially as described previously (22). Wells of polystyrene plates (Cooke Engineering Co., Alexandria, Virginia) were coated with 100 μL of MCMV or control antigen optimally diluted in PBS and incubated overnight at room temperature. The optimum dilution of antigen as shown by block titration of positive and negative reference mouse sera with MCMV and control glycine-extract antigens had been determined to be 1:600. Test sera were diluted in PBS with 0.2% Tween 20 and 10% FCS, and 100 μL of each serum dilution were added to both a test antigen and control antigen well. The plates were incubated for 2 h at room temperature and rinsed with PBS-Tween 20. The enzyme conjugate (100 μL) prepared in rabbits against mouse IgG labeled with peroxidase (Mandel Scientific, Ville-St-Pierre, Québec) was used. Wells were washed with PBS-Tween 20. Orthophenyldiamine substrate (100 μL) (Sigma Chemical, Co., St-Louis, Missouri) was added to each well for 30 min at room temperature and the reaction was stopped by the addition of 100 μL of 1N HCl. The absorbance was read using a Titertek Multiskan Photometer (Flow Laboratories Inc., Mississauga, Ontario) at a 492-nm wavelength. The virus-specific end-point antibody titer was determined as the reciprocal of the dilution of serum which gave an optical density of 1.0, a value determined as two standard deviations above the mean density obtained with a series of MCMV seronegative serum (22).

RESULTS

Following intraperitoneal inoculation (Table I), the CF test detected antibody titers in two of four mice, and the IFA detected antibody in one of four mice as early as seven days post-inoculation. No titers were measured by ELISA until three weeks post-inoculation. At that time, antibody was detected in two of four mice. By day 35, antibody was detected in all animals by all three assays. At day 140

TABLE I. Prevalence and Titers of MCMV Antibody after Intraperitoneal Inoculation of CD-1 Mice

Day postinoculation	No.of sera tested	Positive			Titer[a]		
		CF	IFA	ELISA	CF	IFA	ELISA
3	4	0	0	0	0	0	0
7	4	2	1	0	11	20	0
14	4	4	3	0	19	16	0
21	4	4	4	2	16	29	140
35	4	4	4	4	32	95	640
56	4	4	4	4	54	80	1280
101	3	3	3	3	40	40	1015
140	4	4	4	4	20	40	128

[a]Mean geometric titers of positives

postinoculation, the antibody response as detected by the three techniques had declined.

Following intranasal inoculation (Table II), the CF test detected antibody titers in three of four mice 14 days postinoculation, i.e. one week later than following intraperitoneal inoculation. The IFA test detected antibody at day 21, whereas titers were measured by ELISA on day 35 in only two out of three mice.

In mice placed in contact with animals infected seven days previously, antibody titers were first detected with the CF method 35 days postcontact in three out of four mice (Table III). Indirect immunofluorescent assay titers appeared on day 56 in two of four mice. Minimal titers were recorded by ELISA at day 77 in two of three mice.

In mice placed in contact with animals infected 35 days previously, antibody titers were detected with CF and IFA methods 14 days postcontact in two out of three mice and with ELISA in one of three mice (Table IV).

TABLE II. Prevalence and Titers of MCMV Antibody after Intranasal Inoculation of CD-1 Mice

Day postinoculation	No.of sera tested	Positive			Titer[a]		
		CF	IFA	ELISA	CF	IFA	ELISA
3	3	0	0	0	0	0	0
7	3	0	0	0	0	0	0
14	4	3	0	0	20	0	0
21	3	3	3	0	32	13	0
35	3	3	3	2	102	80	335
56	2	4	4	4	54	80	1280
101	2	2	2	2	32	40	1280

[a]Mean geometric titers of positives

TABLE III. Prevalence and Titers of MCMV Antibody in Mice Placed in Contact with Infected Animals[a]

Day postinoculation	No.of sera tested	Positive			Titer[b]		
		CF	IFA	ELISA	CF	IFA	ELISA
7	4	0	0	0	0	0	0
14	4	0	0	0	0	0	0
21	4	0	0	0	0	0	0
35	4	3	0	0	50	0	0
56	4	2	2	0	63	28	0
77	3	3	3	2	102	50	158
134	5	5	5	3	13	46	165

[a]Mice placed in contact seven days postinfection (I.P.)
[b]Geometric mean titer of positives

TABLE IV. Prevalence and Titers of MCMV Antibody in Mice Placed in Contact with Infected Animals[a]

Day postinoculation	No.of sera tested	Positive			Titer[b]		
		CF	IFA	ELISA	CF	IFA	ELISA
7	3	0	0	0	0	0	0
14	3	2	2	1	45	16	40
21	3	3	3	2	81	32	25
35	3	2	3	3	32	25	160
56	2	2	2	2	91	113	640
111	2	2	2	2	16	40	160

[a]Mice placed in contact 35 days postinfection (I.P.)
[b]Geometric mean titer of positives

DISCUSSION

The CF and IFA tests described in these experiments were comparable to each other in sensitivity and were slightly more sensitive than ELISA for detection of antibody at an earlier stage. Thus, in experimental infection the CF and IFA tests detected antibody in more mice at the early stage postinfection than ELISA. In natural infection, the CF and IFA tests were also found to be useful diagnostic tools. The CF test has the advantage over the IFA test in lack of subjectivity in the results produced. The CF test could detect antibody as early as one week postinfection. The IFA and ELISA tests, however, remain useful tests for detection of antibody in chronically infected colonies.

There are somewhat conflicting reports concerning the host response to MCMV. The SN and CF responses been shown to be delayed in several studies. However, as pointed out by Osborn (16), this may be the result of technical rather than biological variations. Mannini and Medearis (10) have studied the appearance of neutralizing antibody during the course of MCMV infection and have concluded that the virus was a weak antigen, since relatively low titers of SN antibody were recorded during acute infection and antibody was often not detectable during chronic infection. Medearis (11) has tested sera of chronically infected mice for both SN and CF antibodies and found very little antibody during the first month of infection, with subsequent appearance of CF titers during the ensuing six months; SN antibody titers remaining low throughout the study. Anderson *et al* (12) have reported a late detection of MCMV antibody by the CF test at five weeks postinfection.

Conflicting results have also been recorded relative to the serological response of mice kept in contact with infected animals. Medearis (11) has not detected antibody in mice housed in the same cage with infected animals, whereas Anderson *et al* (12) have found seroconversion in control mice housed in the same room with experimentally infected animals.

Enzyme-linked immunosorbent assay and nuclear anticomplement immunofluorescence (NACIF) tests have been used recently for detection of

MCMV antibody (12). The NACIF test was found more sensitive than ELISA during acute infection. Shanley *et al* (14) have reported that the antibody titers measured by ELISA were comparable to those of the IFA assay in both specificity and sensitivity. These results, however, were not substantiated (15). The CF technique used in the present work has been shown to be very sensitive for the detection of antibody to a number of antigens (21). Our results demonstrate that the test is also sensitive for detecting low levels of antibody to MCMV; all CF negative sera were also negative by ELISA.

Although ELISA has been shown to be a sensitive serological procedure used in the diagnosis of several laboratory animal viral infections (24-27), in the present investigation this assay was not found to be a more sensitive diagnostic tool than CF or IFA for the detection of early antibody to MCMV in experimental and natural infections. This observation is similar to an earlier report (12). The CF test appears to be a valuable test for detecting early levels of antibody to MCMV; it detected more positive sera than ELISA.

Experimentally infected mice were found to transmit infectious virus. Mice inoculated with MCMV, such as for the preparation of reference immune reagents or for experimental research should be adequately isolated from other experimental or colony animals.

The CF and IFA tests for MCMV anti body detection have been shown to be useful diagnostic tools because of their greater sensitivity than ELISA for detection of early antibody. However, all three tests can be used for detection of chronic infection. In view of the high sensitivity of available serological assays, it is recommended that testing of antibody to MCMV be included in mouse viral screening programs.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (F.C.A.R. 0S-006) and the Natural Sciences and Engineering Research Council of Canada (N.S.E.R.C. A3314).

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