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Surveillance of Mice for Antibodies to Murine Cytomegalovirus

CHRISTINE A. ANDERSON, JAMES C. MURPHY, AND JAMES G. FOX*

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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The sera of 256 mice from nine commercial sources were screened for antibodies to murine cytomegalovirus (MCMV) because a surveillance of this virus has not been reported in the literature for over a decade. Although no evidence of antibodies to MCMV were detected by complement fixation or nuclear anticomplement immunofluorescence, 54.7% of these sera did have antibodies that were detected by enzyme-linked immunosorbent assay. These data emphasize the need for proper containment of laboratory mice to prevent the potential outbreak of acute MCMV infection. Including MCMV antibody surveillance by enzyme-linked immunosorbent assay in routine health monitoring of mice and imparting these findings in an analysis of the role of MCMV on interpretation of experimental results is advised.

A method to control naturally acquired murine cytomegalovirus (MCMV) infection in laboratory mouse colonies has not been developed because sensitive serologic techniques to detect the presence of antibody have not been available. Although the most specific method of screening for the prevalence of infection with MCMV is by virus isolation from saliva or throat swab (14), it is very time-consuming (up to 2 months) and consequently expensive. Our laboratory reported previously (1) that whereas a nuclear anticomplement immunofluorescence (NACIF) test may be more sensitive in the detection of antibodies to MCMV during an acute infection, an enzyme-linked immunosorbent assay (ELISA) may be more suitable in screening for the presence of chronic persistent infection. MCMV can have profound effects on host immune mechanisms (2, 3), especially after immunosuppression (9, 13); therefore, MCMV can potentially compound or interfere with experimental data being derived from MCMV-infected mice. The present investigation was therefore undertaken, with techniques developed in our laboratory to determine the prevalence of MCMV antibody in various strains of commonly used mice from different commercial vendors.

Virus and control antigens for all three serological tests were prepared as described previously (1). Briefly, a glycine extract of MCMV and control mouse embryo fibroblast antigen were used for the complement fixation (CF) test and ELISA. For the NACIF test, MCMV-infected and uninfected mouse embryo fibroblast nuclei were extracted by washing the cell cultures in distilled water containing 2% fetal bovine serum.

Mice and sera were obtained from nine different vendors. Some animals were maintained behind barriers, whereas others were housed in conventional facilities. The animals were exsanguinated by cardiac puncture while under ether anesthesia. The serum was separated from the clot, removed, and stored at −20°C. All samples were coded before testing. After completion of all tests, it was ascertained that the following stocks and strains had been tested: Swiss, CD_1, C57BL/6 (*H-2*^{b}), 129 (*H-2*^{b}), A (*H-2*^{a}), BALB/c (*H-2*^{d}), DBA/2 (*H-2*^{d}), C3H (*H-2*^{k}), CBA (*H-2*^{k}), B6C3F_1 (*H-2*^{b,k}), BCF_1 (*H-2*^{b,d}), and BDF_1 (*H-2*^{b,d}).

All three serological techniques were performed as reported previously (1). The standard laboratory branch CF test (Microbiological Associates, Bethesda, Md.) was performed with immune and control sera from CD_1 mice that were included as controls.

In the NACIF test, heat-inactivated sera, beginning at a 1:5 dilution, were added to individual wells on glass slides (Roboz Surgical Instrument Co., Inc., Washington, D.C.) containing fixed preparations of virus-infected or control nuclei. Guinea pig complement (Flow Laboratories, Inc., McLean, Va.) and fluorescein-conjugated goat anti-guinea pig C3 (B1C/B1A) (Cooper Biomedical, Inc., West Chester, Pa.) containing Evans blue were used in this assay. The slides were evaluated on a Zeiss fluorescent microscope with a 50-W mercury vapor lamp using the Zeiss BP450-490 excitation filter and LP520 barrier filter.

In the ELISA, a 1:100 dilution of test serum was prepared in phosphate-buffered saline with 0.5% Tween 20 and added to antigen-fixed wells (96 wells per plate; Dynatech Laboratories, Inc., Alexandria, Va.). An alkaline phosphatase conjugate was used which was prepared in goats against mouse immunoglobulin G (IgG) (heavy and light chains) (Bionetics, Inc., Kensington, Md.). Absorbance was measured on an Abbott Bichromatic Analyzer-100 (Abbott Laboratories, North Chicago, Ill.) with a filter size of 450 to 415 nm. An ELISA value greater than 0.125 was considered positive.

Of the 256 mouse serum samples which were tested for the presence antibodies to MCMV by CF, NACIF, and ELISA, none had antibodies that were detected by CF or NACIF. In contrast, 54.7% (140 of 256) of the same sera, tested by ELISA, were positive. The prevalence of antibodies to MCMV from the various vendors is shown in Table 1. Although the number of mice tested from each age group and vendor varied, there appeared to be a general increase with age in the number of mice positive for MCMV. Mice from vendors 4 and 7 showed an increase of 40 and 60%, respectively, between the 3- to 4-week age group and the 6-week age group. However, vendor 5 had an increase of only 23% (54 to 77%) between the 8-week age group and retired breeders. The 6-week age group demonstrated the greatest variation in antibody detection, since vendors 2 and 3 both housed mice in barrier specific-pathogen-free facilities (all of their mouse serum samples were negative), and vendor 4 housed mice in conventional facilities (100% of their mice were positive). There was no significant difference in prevalence between sexes.

* Corresponding author.

TABLE 1. Prevalence of antibodies to MCMV detected by ELISA

Vendor no.	No. of positive strains/total no. of strains tested	No. of positive mouse serum samples/total no. of mice tested (%) at age (wk):			Total % positive for each vendor
		3–4	6	RB[a]	
1	3/3	5/15 (33)	9/20 (45)	ND[b]	40
2	1/2	2/5 (40)	0/10 (0)	ND	13
3	0/1	ND	0/5 (2)	ND	0
4	2/2	3/5 (60)	10/10 (100)	ND	87
5	5/6	ND	30/56 (54)[c]	44/57 (77)	65
6	1/1	3/5 (60)	ND	ND	60
7	1/1	4/10 (40)	4/4 (100)	ND	53
8	6/7	8/20 (53)	12/29 (41)	ND	41
9	1/1	ND	3/5 (60)	ND	60
Total	12/12	58/116 (50)	38/83 (46)	44/57 (77)	46[d]

[a] RB, Retired breeders.
[b] ND, Not done.
[c] Mice were 8 weeks old.
[d] Mean value for all vendors.

To determine the relationship of strain to age, the data were compared with results from retired breeders from a single source (Fig. 1). Although C57BL/6, BALB/c, DBA/2, and CBA strains did show an increase in prevalence when compared with retired breeders, other strains (129, A) did not. The CBA strain showed the greatest increase, since no antibodies to MCMV were detected in 8-week-old mice, yet all of the mice tested as retired breeders had positive serum samples. It should be emphasized that both age groups of these strains were from the same vendor.

Since some of the same strains of mice tested were from more than one source or were from both 3- to 4-week and 6-week age groups, findings from these mice were evaluated to determine whether the prevalence of antibodies to MCMV was influenced more by source than by strain or age (Table 2). The source of the animals may have been a factor. For example, commonly used outbred CD_1 mice at 6 weeks of age had a range of 10 to 100% prevalence, depending on the vendor. Two strains at 3 to 4 weeks of age, one known as high-sensitivity BALB/c ($H\text{-}2^d$) and one known as high-resistance C3H ($H\text{-}2^k$) (17), demonstrated a variable prevalence of antibodies to MCMV which may also be attributed to vendor source.

Since 54.7% of the 256 sera tested were positive by ELISA but negative by CF and NACIF, the mode of stimulation which elicited a specific response to MCMV remains unclear. The route of introduction (inhalation or ingestion), age and strain of mice, virulence of the virus, and immune status of the host must all be considered.

The method of animal reproduction and subsequent maintenance may be important factors in maintaining mice which are free of infection with MCMV since no antibodies to MCMV were detected in mice at 6 weeks of age from vendors 2 and 3 (Table 1). Jordan (11) and others (5, 17) have proposed aerosol transmission of MCMV, and Mannini and Medearis (13) have demonstrated that experimental infection with MCMV can spread to cagemates, probably via saliva and contaminated bedding. In our earlier experimental study, control animals also seroconverted; this probably occurred because of aerosol exposure (1).

The question remains as to how natural infection occurs. Studies on wild mice have shown that MCMV can be isolated from salivary glands or saliva from most adult wild mice but not from young mice (8). Colostrum and breast milk has been found to be protective (14); thus, it has been widely accepted that MCMV is probably acquired early in life and persists (15). By analogy, Cook (7) reported that 70 to 80% of guinea pigs 6 months of age and older shed guinea pig cytomegalovirus in saliva, and Hartley et al. (10) found that most commercial guinea pig complement contains high antibody titers to guinea pig cytomegalovirus.

The mice in this surveillance were probably not experiencing an acute infection. More likely, they had an infection of the latent type. Although virus isolation was not performed, there was no clinical evidence of disease and there were no complement-requiring antibodies detected by CF or NACIF tests. This mimics other indigenous murine viruses, such as mouse hepatitis virus and Theiler's mouse enceph-

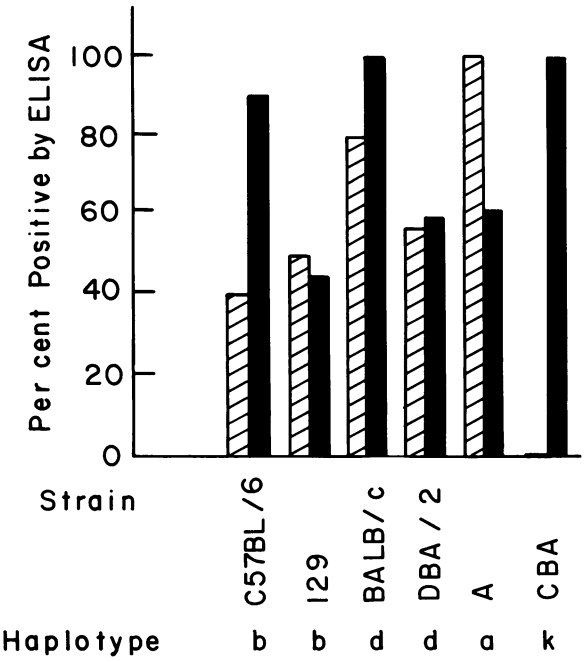

FIG. 1. Comparison of antibodies to MCMV in different strains of mice by age from a single vendor. CBA mice, 8 weeks old, demonstrated 0% positivity by ELISA. ▨, 8-week-old mice; ■, retired breeders.

TABLE 2. Comparison of age with prevalence of antibodies to MCMV

Strain (vendor no.)	No. of positive mouse serum samples/total no. of mice tested by ELISA at age (wk):		% Change between age groups
	3–4	6	
CD_1 (1)	0/5	1/10	10
BALB/c (1)	3/5	8/10	20
C3H (2)	2/5	0/5	−40
C3H (4)	3/5	5/5	40
CD_1 (7)	4/10	4/4	60
BALB/c (8)	0/5	1/2	50
DBA/2 (8)	0/5	2/4	50

alomyelitis virus. The prevalence of antibodies to MCMV detected by ELISA in our study does not differ from that which has been reported for mouse hepatitis virus from mouse sera tested by ELISA (16). Although mouse thymic agent, another herpesvirus, was not included in this study, cross-reactivity has been reported not to occur (15).

Testing mouse colonies by ELISA appears to be a valuable tool for diagnosing latent infections, since certain serologic tests may be deceptively negative for antibodies to MCMV and histology has been found to be a poor indicator (4). Studies of natural MCMV infection in wild mice (8) emphasize that the virus can maintain itself and can spread readily in a population. Since murine tumors, particularly myelomas and hybridomas, commonly harbor indigenous murine viruses, this assay should be included when screening certain tumors by the mouse antibody production test. Proper derivation, maintenance, containment, and serologic surveillance of all strains of laboratory mice are important because MCMV has been shown to cause serious immunosuppression of the host (15). In summary, this serological survey with a sensitive ELISA suggests that natural infection of laboratory mice by MCMV may be more prevalent than has been recognized previously; however, this study did not establish the biological importance of the ELISA reactivity. Immunosuppressive regimens to elicit active MCMV infections in latently infected (i.e., MCMV-ELISA antibody positive) mice coupled with virus isolation attempts are needed to definitively determine the significance of positive ELISA titers to natural MCMV infection in laboratory strains of mice.

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