Comparison of Isolation in Cell Culture with Conventional and Modified Mouse Antibody Production Tests for Detection of Murine Viruses

MARK DE SOUZA AND ABIGAIL L. SMITH*

*Section of Comparative Medicine and Department of Epidemiology and Public Health,
Yale University School of Medicine, New Haven, Connecticut 06510*

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The sensitivity of the mouse antibody production test with intraperitoneal or intrasplenic inoculation of mice with reovirus type 3, minute virus of mice, lymphocytic choriomeningitis virus, or mouse hepatitis virus was compared with that of direct isolation in cultured cells. The mouse antibody production test for detection of mouse hepatitis virus was significantly more sensitive than virus isolation in permissive cells, but differences in sensitivity were less marked for the other three viruses. The intrasplenic route of inoculation did not yield seroconversion that occurred earlier or more consistently than that detected after intraperitoneal inoculation.

Rodent viruses are known to contaminate transplantable tumors and other biological materials (2, 4, 17, 19), and some are associated with human disease and death (2, 4-6, 11). The importance of rodent virus contamination of biological products has assumed a new dimension as a result of the preparation in mice of monoclonal antibodies destined for use in humans (3). Guidelines for safety testing are being developed (10) and include the use of the mouse antibody production (MAP) test to detect conventional rodent virus contamination. The MAP test is an indirect assay that relies on the immune response of the mouse as an indicator of virus contamination (12).

In this report, we present a comparison of the sensitivity of direct virus isolation in cell culture with that of the conventional MAP test with sera from mice injected intraperitoneally (i.p.) with any of four murine viruses. In addition, the sensitivity of a modified MAP test with sera from mice inoculated intrasplenically (i.s.) was assessed.

The agents chosen for this study were reovirus type 3, minute virus of mice (MVM), lymphocytic choriomeningitis virus (LCMV), and mouse hepatitis virus (MHV). The first three represent the murine viruses most commonly isolated in this laboratory from mouse-passaged biological materials, including transplantable tumors, hybridomas and their products, and cloned lymphoid cell lines (15, 16; unpublished observations). MHV was included because, although it has not been detected in biological materials submitted to this laboratory, it is the most prevalent of laboratory mouse viruses (8) and is, therefore, a good candidate to contaminate biological materials passaged in mice.

Four-week-old female Cr:ORL Sencar mice (Animal Genetics and Production Branch, National Cancer Institute, Bethesda, Md.) free of serum antibody to conventional murine viruses were used in all experiments. Mice were housed in microisolator cages (Lab Products, Maywood, N.J.) that were opened under a class II biological safety cabinet for servicing and animal manipulation. Virus (0.1 ml) was injected either i.p. or i.s., with i.s. inoculations performed as described by Spitz et al. (18), except that mice were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Crossing, N.J.) and incisions were

closed with wound clips rather than sutures. Four-week-old sentinel Sencar mice in open cages were placed on the bottom shelves of animal holding racks for serologic testing to ensure that virus transmission had not occurred.

The viruses used for these studies were the Dearing strain of reovirus type 3 (obtained from D. L. Knudson, Yale Arbovirus Research Unit), the immunosuppressive strain of MVM [designated MVM(i) and obtained from P. J. Tattersall, Department of Laboratory Medicine, Yale University], the 771422 strain of LCMV (isolated at Yale University from the L5178Y T-cell lymphoma), and the A59 strain of MHV (MHV-A59; obtained from American Type Culture Collection, Rockville, Md.).

17CL 1 cells (obtained from L. S. Sturman, N. Y. State Department of Health, Albany, N.Y.) and BHK-21 cells (American Type Culture Collection) were used for in vitro virus quantification. MVM(i) and LCMV were quantified in BHK-21 cells, and virus doses are expressed as fluorescent focus units (FFU). Cell culture infection with reovirus type 3 and MHV-A59 was assessed by microscopic observations of BHK-21 cell lysis 6 days after inoculation (reovirus) or 17CL 1 cell fusion 3 days after inoculation (MHV). Reovirus and MHV doses are expressed as median tissue culture infectious doses (TCID₅₀).

Sera diluted 1:10 in normal saline were screened for antibody to homologous antigen by indirect fluorescent-antibody staining of infected cells on Teflon-coated slides (13-15, 17). Tissues from selected moribund mice inoculated i.s. or i.p. with LCMV were examined after fixation in 10% neutral buffered Formalin, embedding in paraffin, sectioning at 5 µm, and staining with hematoxylin and eosin.

Cell cultures were exposed to a single series of 10-fold virus dilutions just prior to and immediately following mouse inoculations. Significant differences in virus titers were not noted for preinoculation and postinoculation virus suspensions. Mice were inoculated with alternating log₁₀ dilutions of virus. They were observed daily for clinical signs, and groups were killed with carbon dioxide on days 7, 14, 21, and 28 postinoculation for exsanguination by cardiac puncture.

Serology results for sera of mice exposed i.p. or i.s. to reovirus type 3 were similar (Table 1; i.s. data not shown). Some mice exposed to as little as 0.001 TCID₅₀ seroconverted.

* Corresponding author.

TABLE 1. Seroconversion of mice inoculated intraperitoneally with reovirus type 3, MVM, LCMV, or MHV-A59

Virus	Dose	No. of positive sera/no. of sera tested at the following day postinoculation:			
		7	14	21	28
Reovirus type 3	1,000 TCID ₅₀	3/3	3/3		
	10 TCID ₅₀	0/3	3/3	3/3	
	0.1 TCID ₅₀		1/3	1/3	2/3
	0.001 TCID ₅₀			1/3	2/3
MVM	650 FFU	3/3	3/3		
	6.5 FFU	0/3	2/3	2/3	
	0.065 FFU		0/3	0/3	1/3
	0.00065 FFU			0/3	0/3
LCMV	10 FFU	2/3	1/1		
	0.1 FFU	3/3	No survivors	No survivors	
	0.001 FFU		0/3	1/3	0/2
	0.00001 FFU			0/3	0/3
MHV-A59	0.05 TCID ₅₀	1/5	3/3		
	0.005 TCID ₅₀	0/5	2/4	0/4	1/3
	0.0005 TCID ₅₀		0/4	0/2	1/3

Isolation in cell culture and the MAP test were approximately equal in sensitivity for MVM detection (Table 1), and there was a tendency for mice inoculated i.s. to develop antibody earlier than mice inoculated i.p. (data not shown). For instance, five of six mice that were seropositive at 21 or 28 days after inoculation with 0.065 FFU of MVM had been exposed by the i.s. route.

The MAP test was slightly more sensitive than isolation in BHK-21 cells for detection of LCMV (Table 1). LCMV antibody was detected in sera of only two mice (one i.s. inoculated, one i.p. inoculated) that received doses of virus lower than those associated with mortality. Mortality among mice given 10 FFU of LCMV was two of six mice after i.p. injection (average day of death [ADD] = 9.0 ± 0.0) and three of six mice after i.s. injection (ADD = 7.3 ± 0.6). Among mice given a 100-fold-lower dose of LCMV, six of nine i.p.-inoculated mice died (ADD = 9.5 ± 1.1) and three of nine i.s.-inoculated mice died (ADD = 10.3 ± 0.6). Clinically affected mice were lethargic and hunched without evidence of neurologic signs, and their livers were pale on gross examination. Lesions in tissues of moribund mice that had been inoculated i.s. or i.p. with 0.1 FFU of LCMV were similar for the two groups and included necrotizing inflammation of all lymphoid tissues, parotid salivary glands, pancreas, splenic red pulp, liver, intestine, and mesentery. Mild focal choriomeningitis was also observed.

Very high mortality was sustained by mice exposed i.p. to doses of MHV-A59 ranging from 1,000 to 0.1 TCID₅₀ (Table 2), rendering impossible a systematic comparison of anti-

body development by i.s.- and i.p.-inoculated mice. The experiment was therefore repeated with larger numbers of mice and threshold concentrations of virus (Table 1). In this instance, mice were inoculated with serial 10-fold concentrations of virus ranging from 0.05 to 0.0005 TCID₅₀. The mortality rate for i.p.-inoculated mice remained high, but there were no clear-cut differences in antibody development between the i.p.- and i.s.-inoculated groups (data not shown).

Sentinel mice did not seroconvert to any of the agents used for these studies.

The results of the current study suggest wide variation in the relative sensitivity of the MAP test and cell culture isolation. The MAP test was somewhat more sensitive than cell culture isolation for detection of reovirus type 3 and LCMV, and it was about 1,000 times more sensitive than 17CL 1 cells for detection of MHV. For MVM, the two methods were approximately equal in sensitivity. While it could be argued that more sensitive cell lines may exist, we used cells commonly cited for cultivation of the viruses tested.

The mortality observed after inoculation of Sencar mice with LCMV or MHV-A59 cannot be relied on as a consistent indicator of the presence of either virus. Stocks of Swiss mice vary in their responses to murine virus infections, and the virus strain can also affect the outcome of infection. The histopathology associated with infection by the LCMV strain used in the current studies is consistent with that described for viscerotropic LCMV disease (7). Similarly, MHV strains vary widely in their pathogenicity for adult mice, and stocks of the same virus strains prepared in different laboratories or different host systems may have different tissue tropisms (1). One unexplained observation in the current studies was the irregular dose-response curve for deaths after i.p. inoculation of MHV-A59 (Table 2). The existence of defective interfering virus particles has been reported for MHV (9), but the irregular death pattern cannot be immediately attributed to that mechanism. An additional point of interest is the basis for high MHV-A59-associated mortality among i.p.-inoculated mice relative to that observed for i.s.-inoculated mice. Studies designed to explain this observation are in progress.

TABLE 2. Comparative mortality among mice inoculated i.s. or i.p. with MHV-A59

Virus dose (TCID ₅₀)	i.s.		i.p.	
	Deaths ^a	ADD $\pm$ SD	Deaths ^a	ADD $\pm$ SD
1,000	2/6	6.5 ± 0.7	6/6	4.3 ± 1.2
10	4/8	5.8 ± 0.5	3/9	6.0 ± 1.0
0.1	0/8		9/9	5.8 ± 0.8
0.001	0/8		1/6	6.0

^a Results are given as the number of mice that died/number of mice inoculated.

Our hypothesis that i.s. inoculation might result in earlier seroconversion than that seen after i.p. inoculation was not consistently supported by this study. The i.s. injection with cell fusion 4 days after antigen inoculation was used successfully to elicit monoclonal antibodies when very small amounts of antigen were available (18). It was conjectured that i.s. inoculation of antigen maximizes the number of specific B-cell blasts by direct local antigen stimulation and minimizes uptake and elimination of antigen in other locations (18). Based on the labor-intensive nature of the method, it does not seem reasonable to suggest that i.s. inoculation be used instead of or in parallel with i.p. inoculation for MAP testing. However, rodent virus diagnostic laboratories might effectively reduce the time, labor, and biohazard risk associated with testing if cell culture inoculation was adopted as a prescreening method. This approach could detect high concentrations of virus, if present, and might eliminate the need to test some samples in mice. Given the substantial cost of MAP testing, any measures that reduce the time required for testing or eliminate some phases of testing are of great benefit.

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