JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1987, p. 1364–1369
0095-1137/87/081364-06$02.00/0

Vol. 25, No. 8

Comparison of Methods for Detection of Serum Antibody to Murine Rotavirus

WILLIAM T. FERNER,[1]† ROBIN L. MISKUFF,[1] ROBERT H. YOLKEN,[2] AND STEVEN L. VONDERFECHT[1,3]*

Division of Comparative Medicine,[1] Department of Pathology,[3] and Division of Pediatric Infectious Diseases,[2] The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Received 9 February 1987/Accepted 15 April 1987

Mice are frequently used as animal models for the study of rotaviral infections. Since natural infection is common in laboratory mice, it is important that rotaviral studies, as well as other studies utilizing suckling mice, employ animals of known immune status to murine rotavirus. A variety of homologous and heterologous enzyme immunoassay systems and an immunofluorescence technique were thus compared to determine the immunoassay that is most effective at detecting adult mice seropositive for rotaviral antibody. It was determined that a homologous enzyme immunoassay inhibition technique utilizing murine rotavirus-derived reagents was the most efficient serologic assay evaluated. A serologic response was consistently detected by this assay by 5 days after experimental rotaviral inoculation of adult mice. A homologous antibody-binding enzyme immunoassay, a heterologous inhibition enzyme immunoassay utilizing antigenically related simian rotavirus (SA-11) reagents, and an immunofluorescence technique utilizing Nebraska calf diarrhea virus antigens were found to be less sensitive for detecting serum antibody to murine rotavirus.

Rotaviruses are important causes of acute gastroenteritis in numerous species of animals, including humans (1, 3, 9). Murine rotavirus (MRV) infection is frequently used as an animal model to study the pathogenesis of and immune response to rotaviral infections (2, 8, 10, 13–15, 20). In addition to MRV, nonmurine strains of rotavirus are frequently experimentally inoculated into suckling mice (4, 11, 12). Since MRV is widespread and highly contagious, it is imperative that animals to be used in rotaviral research be carefully examined for evidence of MRV infection. Three strains of MRV, designated EW, EB, and EHP, have recently been reported (6). All three strains are pathogenic for suckling mice. The only consistent clinical sign of MRV infection is diarrhea within the first 10 to 14 days of life. Adult mice do not develop clinically apparent disease, but viral antigens can be detected in intestinal tissues and seroconversion occurs (2, 15). Of particular concern is the possibility that pregnant, seronegative mice could be exposed to MRV while being transported or housed awaiting the birth of their litters. In such a situation, subclinical infection and seroconversion in the adult mice could alter the susceptibility of newborn mice to rotaviral infection and thus have a deleterious effect on rotaviral research utilizing the mouse as a model. Additionally, the detection of MRV infection would be of importance in any type of research in which suckling mice are utilized. In this setting, the presence of diarrhea in experimental animals could invalidate the data obtained from these animals. We thus decided to evaluate a variety of immunoassays to determine which would most efficiently detect rotaviral antibody in mice experimentally infected as adults.

MATERIALS AND METHODS

Animals. Pregnant, specific-pathogen-free CD-1 mice were obtained from a commercial supplier (Charles River Breeding Laboratories, Inc., Portage, Mich.). Mice were tested upon arrival for antibodies to MRV utilizing the homologous antibody-binding and inhibition enzyme immunoassays described below. All incoming animals tested negative for rotaviral antibody. The dams were allowed to give birth naturally, and the pups remained with the dam until weaning at 21 days of age, at which time they were removed and grouped according to sex. All animals were housed, five to six animals per cage, in solid-bottom polycarbonate cages supplied with hardwood chip bedding and covered with a flexible filter bonnet. Mice were fed a commercial rodent diet and provided with water ad libitum. Male and female CD-1 mice ranging in age from 30 to 106 days were also purchased and served as sources of rotaviral antibody-free sera for standardization of the enzyme immunoassays.

Virus. The MRV utilized in the studies was originally obtained from Michael Collins (Microbiological Associates, Bethesda, Md.). This isolate is believed to correspond to the EW strain (6). Preparations for mouse inoculation were made from intestinal tracts collected from MRV-infected suckling mice. Pooled intestines were ground in a hand-held Tenbroeck grinder containing sufficient phosphate-buffered saline with 0.01% $CaCl_2$ and $MgCl_2 \cdot H_2O$ to produce a 10% (wt/vol) suspension, briefly sonicated, and extracted twice with 0.5 volume of Freon 113. The aqueous phase was collected and centrifuged at 10,000 × *g* for 30 min at 5°C, and the resulting supernatant was passed through a membrane filter having a pore size of 0.2 μm. The filtrate, which constituted the inoculation stock, was divided into small aliquots and stored at −70°C. The 100% mouse infectious dose (MID_{100}) of the inoculum was determined by feeding serial 10-fold dilutions of the stock suspension to litters of 5- to 7-day-old suckling mice. The lowest dose that caused illness in all animals of the litter 3 days after inoculation was considered to be 1 MID_{100}.

The simian rotavirus (SA-11) and Nebraska calf diarrhea virus were originally obtained from H. Malherbe (Salt Lake City, Utah) and R. G. Wyatt (Bethesda, Md.), respectively and were propagated in MA-104 cells by standard procedures (17).

* Corresponding author.
† Present address: Animal Resources, Batelle Columbus Laboratories, Columbus, OH 43201.

Antigens. The MRV preparation used in the enzyme immunoassays was prepared from intestines collected from MRV-infected suckling mice. An intestinal homogenate was prepared and extracted with Freon 113 as described above. The aqueous phase collected after Freon extraction was centrifuged at 10,000 × *g* for 30 min at 5°C, and the resulting supernatant was placed on a discontinuous 20%/60% (wt/vol) sucrose cushion. After centrifugation at 100,000 × *g* for 2 h at 5°C, the material at the interface of the two sucrose solutions was collected, divided into small aliquots, and stored at −70°C. This material served as a semipurified MRV stock.

Semipurified SA-11 virus stock was prepared in a similar manner. Supernatant from SA-11 virus-infected cell cultures was briefly sonicated and centrifuged at 10,000 × *g* for 30 min at 5°C. The supernatant was collected, layered onto a discontinuous 20%/60% (wt/vol) sucrose cushion, and centrifuged as above. The material at the interface of the sucrose layers was collected and stored at −70°C. The viral suspension contained approximately 8×10^6 PFU.

Antisera. Chicken antisera to MRV and SA-11 virus were produced by the intramuscular inoculation of laying hens with 1 ml of the semipurified MRV or SA-11 viral preparation emulsified in an equal volume of Freund complete adjuvant. Fourteen days later, the chickens were given a booster injection of viral preparation emulsified in Freund incomplete adjuvant. Twenty-eight days after the initial injection, sera were collected for use in the enzyme immunoassays.

Guinea pig antisera to MRV and SA-11 viruses were produced in a similar manner after the subcutaneous inoculation of guinea pigs with the appropriate semipurified viral preparation.

Mouse antiserum to MRV was prepared by oral inoculation of 7- to 14-day-old suckling mice with MRV-containing intestinal homogenates. Twenty-eight days after inoculation, the mice were exsanguinated, and the sera were collected and pooled. This serum pool served as the positive serum control standard. Noninoculated weanling mice served as the source for pooled negative control standard serum.

Animal inoculation. Weanling mice were divided into two experimental study groups. The preliminary study group consisted of 52 28-day-old mice obtained from nine litters. Before inoculation of the mice with MRV, five randomly selected animals were exsanguinated to document the rotavirus-seronegative status of the group. The remaining mice were orally inoculated with 2×10^3 MID_{100} of MRV and divided into five groups. One randomly selected animal from each group was exsanguinated on selected days postinoculation. Serum was collected and stored at −70°C until used in the serologic assays.

The second study group consisted of 20 28-day-old mice which were inoculated intragastrically via an 18-gauge gastric gavage needle with approximately 2×10^4 MID_{100} of MRV. Each mouse was bled before virus inoculation and on selected days thereafter. The serum obtained was stored at −70°C.

Enzyme immunoassays. Modifications of previously described antibody-binding (23) and inhibition (22) enzyme immunoassay systems were used to detect serum antibodies to MRV. Optimal dilutions for all reagents utilized in the immunoassays were determined by checkerboard titration (18). The isotype reactivity of the peroxidase-conjugated antisera was determined by reactions with mouse myeloma proteins (Litton Bionetics, Charleston, S.C.) bound to polystyrene microtiter plates.

The antibody-binding enzyme immunoassay for MRV antibody was conducted by first coating the wells of polystyrene microtiter plates (Immulon 2; Dynatech Laboratories, Inc., Alexandria, Va.) with either nonimmune chicken sera or chicken antiserum to MRV diluted in carbonate buffer (pH 9.6). After overnight incubation at 4°C, the wells were washed with phosphate-buffered saline containing 0.05% Tween 20 and covered with semipurified MRV stock for 2 h at 37°C. The wells were again washed, covered with the sera to be tested, and incubated overnight at 4°C. Affinity purified, peroxidase-labeled goat antibody to mouse immunoglobulin G (IgG) (γ chain specific) or to IgG and IgM (heavy and light chain specific) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) was added for 2 h at 37°C, followed by the addition of substrate. The substrate consisted of 0.4 mg of *O*-phenylenediamine and 0.4 μl of 30% H_2O_2 per ml of 0.01 M citrate buffer (pH 5.0). The reaction of enzyme on substrate was quantitated in a microplate colorimeter (Biotek Instruments, Burlington, Vt.) at a wavelength of 450 nm. For each sample tested, a net optical density (OD) was determined by subtracting the OD reading obtained from the wells coated with nonimmune chicken sera from the OD reading obtained from wells coated with chicken anti-MRV serum. Positive and negative control standards were assayed on each plate. To determine which test sera were positive or negative for rotaviral antibody by this enzyme immunoassay, 42 sera obtained from a group of rotavirus-seronegative mice were analyzed, and the standard deviation of the negative test sera around the standard, pooled negative control was determined. Any test sera with a net OD value at least 3 standard deviations above the net OD of the negative control standard was considered to be positive for rotaviral antibodies.

Both homologous and heterologous inhibition enzyme immunoassays were also performed. In the inhibition immunoassay, the presence of antibody in the test sera was detected by the ability of specific antibody to inhibit the binding of a standardized quantity of rotaviral antigen to the solid phase. In the homologous inhibition immunoassay, the serum to be tested was first mixed with a predetermined quantity of semi-purified MRV stock and incubated overnight at 4°C. The mixture was then transferred to the wells of polystyrene microtiter plates that had been coated with nonimmune or MRV hyperimmune chicken sera as above. After incubation for 30 min at 37°C, the wells were washed with phosphate-buffered saline-Tween 20, covered with guinea pig antiserum to MRV, and incubated for 30 min at 37°C. The wells were again washed and affinity purified, and peroxidase-labeled goat antibody to guinea pig IgG (Kirkegaard and Perry Laboratories, Inc.) was added for 2 h at 37°C, followed by the addition of the substrate. The heterologous inhibition immunoassay was conducted in a similar fashion but utilized SA-11 virus-derived reagents. Both positive and negative control standards were used on each plate, and a net OD value was determined as above. For each test serum, percent inhibition was calculated by the formula: % inhibition = [1 − (net OD of test serum/net OD of negative control serum)] × 100. The 42 individual rotavirus antibody-negative sera were again used to calculate the standard deviation of negative sera around the pooled negative control standard as described above. Any test serum producing a level of inhibition at least 3 standard deviations above the negative control standard was considered to be positive for rotaviral antibody.

Immunofluorescence. The indirect immunofluorescence technique was a modification of that described previously

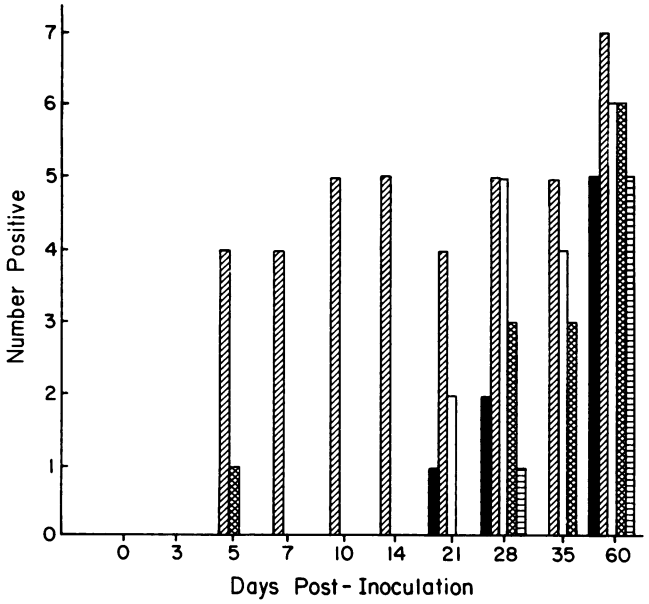

FIG. 1. Presence of MRV antibodies in sera of experimentally inoculated mice. Five animals were tested at each time point on days 0 to 35 postinoculation. Seven animals were tested on day 60. ▨, MRV inhibition enzyme immunoassay; ▩, anti-IgM-IgG antibody-binding enzyme immunoassay; □, anti-IgG antibody-binding enzyme immunoassay; ▣, SA-11 inhibition enzyme immunoassay; ■, indirect immunofluorescence.

(19). Briefly, confluent monolayers of MA-104 cells in 96-well tissue culture plates were infected with Nebraska calf diarrhea virus. Twenty-four hours later, cells were fixed with an acetone–95% ethanol mixture (6:4, vol/vol). Antibodies to rotavirus were detected by first incubating a 1/10 dilution of test sera with the wells, followed by fluorescein-labeled goat antibody to mouse IgG (heavy and light chain specific) (Antibodies Inc., Davis, Calif.). Plates were inverted and examined with an epifluorescence microscope. Wells containing fluorescent cells were scored as positive for rotaviral antibody. Positive and negative control sera were assayed on each plate.

RESULTS

A preliminary study was initially performed in which serum collected from five animals at selected intervals was analyzed for the presence of rotaviral antibodies by each of the immunoassays described above. Serum rotaviral antibodies were detected in some animals by all methods utilized; however, the number of samples tested positive at each time point varied depending on the test methodology (Fig. 1). Serum rotaviral antibody was first detected by the homologous inhibition enzyme immunoassay on day 5 postinoculation and was detected in at least four of the five animals tested at each interval throughout the study. In contrast, rotaviral antibody was first detected by the heterologous inhibition immunoassay utilizing SA-11-derived reagents on day 28 postinoculation when only one of five animals tested positive. Not until day 60 post inoculation was antibody to rotavirus detected in more than half of the animals tested by this method. Serum rotaviral antibody was detected by the antibody-binding enzyme immunossay utilizing peroxidase-conjugated anti-mouse IgG on day 21 postinoculation and was detected in at least four of the five animals tested at each interval through the remainder of the study. Antibody to rotavirus was detected by the binding immunoassay utilizing the anti-IgG-IgM conjugate in one animal on day 5 postinoculation, but it was not until 28 days after inoculation that more than half of the animals tested at each interval were consistently seropositive. The indirect immunofluorescence technique first detected serum rotaviral antibody on day 21 postinoculation, but it was not until day 60 postinoculation that antibody to rotavirus was detected in more than half of the animals tested by this method.

After evaluating the data obtained from the preliminary study, we determined that the homologous inhibition immunoassay and the binding assay utilizing an anti-IgG conjugate were of most value in detecting mice that had converted to a rotavirus-seropositive status after infection as adults. These two enzyme immunoassays were then used in conjunction with indirect immunofluorescence to analyze the serum samples obtained from the serially bled mice for the presence of rotaviral antibodies (Fig. 2).

In agreement with the preliminary study, antibodies to MRV were first detected by the homologous inhibition enzyme immunoassay on day 5 postinoculation when 18 of the 20 animals tested positive. The number of seropositive samples detected remained high through day 64 of the study. In comparison, rotavirus-seropositive samples were first identified by the antibody-binding enzyme immunoassay utilizing the peroxidase labeled anti-mouse IgG 10 days after inoculation, but it was not until day 36 postinoculation that more than half of the animals tested as rotavirus seropositive. Of the 20 serum samples, 8 were tested as rotavirus antibody positive by indirect immunofluorescence 10 days after viral inoculation. At least 9 of the 20 samples were tested as seropositive by this methodology through day 36 postinoculation; however, the number of positive samples had decreased to 7 of 20 at the termination of the study.

A great deal of variability among individual animals was seen when serially obtained samples were evaluated. Of the 20 animals, 7 were found to be rotaviral antibody positive by all three test methodologies. These animals were tested positive by all three assays throughout the 92-day course of the study (Table 1). A second group consisting of 10 animals tested positive for MRV antibody by at least one of the methods at some time during the study but reverted to rotavirus-seronegative status on one or more of these assays before termination of the study (Table 2). Two of these

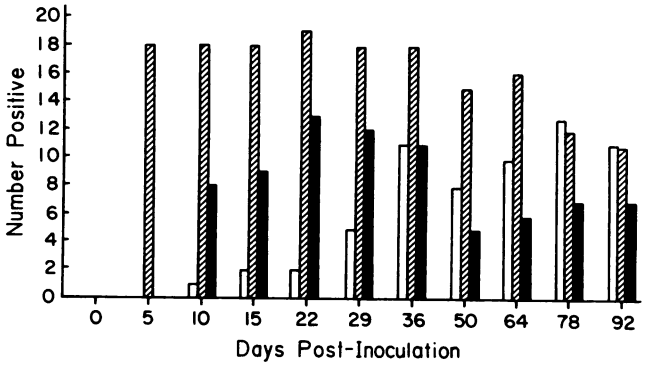

FIG. 2. Presence of MRV antibodies in sera of experimentally inoculated, serially bled mice. Twenty samples were tested by each assay at each time point. ▨, MRV inhibition enzyme immunoassay; □, anti-IgG antibody-binding enzyme immunoassay; ■, indirect immunofluorescence.

TABLE 1. Rotavirus antibody in serially bled mice representative of group 1

Animal no.	Assay system	Rotavirus antibody at the following day postinoculation[a]:										
		0	5	10	15	22	29	36	50	64	78	92
1	Inhibition[b]	−	+	+	+	+	+	+	+	+	+	+
	Binding[c]	−	−	−	−	−	+	+	+	+	+	+
	IFA[d]	−	−	+	+	+	+	+	+	+	+	+
2	Inhibition	−	+	+	+	+	+	+	+	+	+	+
	Binding	−	−	−	−	−	−	−	−	+	+	+
	IFA	−	−	−	+	+	+	+	−	+	+	+
3	Inhibition	−	+	+	+	+	+	+	+	+	+	+
	Binding	−	−	−	+	−	+	+	+	+	+	+
	IFA	−	−	+	+	+	+	+	−	+	+	+

[a] +, Tested rotavirus seropositive; −, tested rotavirus seronegative.
[b] Homologous inhibition enzyme immunoassay.
[c] Antibody-binding enzyme immunoassay with enzyme-labeled, anti-mouse IgG.
[d] Immunofluorescence.

TABLE 2. Rotavirus antibody in serially bled mice representative of group 2

Animal no.	Assay system	Rotavirus antibody at the following day postinoculation[a]:										
		0	5	10	15	22	29	36	50	64	78	92
4	Inhibition[b]	−	+	+	+	+	+	+	+	+	+	+
	Binding[c]	−	−	−	−	−	−	−	+	+	+	+
	IFA[d]	−	−	+	+	+	−	−	−	−	−	−
5	Inhibition	−	+	+	+	+	+	+	+	+	+	+
	Binding	−	−	−	−	−	−	+	−	+	+	−
	IFA	−	−	−	−	+	+	−	−	−	−	−
6	Inhibition	−	+	+	+	+	+	+	+	+	+	−
	Binding	−	−	−	−	−	−	+	−	−	−	−
	IFA	−	−	−	+	+	+	−	−	−	−	−

[a] +, Tested rotavirus seropositive; −, tested rotavirus seronegative.
[b] Homologous inhibition enzyme immunoassay.
[c] Antibody-binding enzyme immunoassay with enzyme-labeled, anti-mouse IgG.
[d] Immunofluorescence.

animals converted to a seronegative status by all test methodologies. Data obtained from the remaining three animals are shown in Table 3. These animals were tested as positive for MRV antibodies by the inhibition enzyme immunoassay; however, the response was transient or sporadic, and all three animals returned to seronegative status during the course of the study. With the exception of one sample on day 78 postinoculation, these three animals were consistently tested as rotavirus seronegative by the antibody-binding enzyme immunoassay and the immunofluorescence assay. The only assay system that detected all 20 animals as being seropositive at some point in the study was the inhibition immunoassay. The antibody-binding and the immunofluorescent techniques detected 16 of 20 and 15 of 20 animals, respectively, as being rotavirus seropositive.

Further evaluation of the data showed that in only 4 of the 220 serum samples evaluated over the 92-day study period did the homologous inhibition enzyme immunoassay fail to detect antibodies to MRV when the antibodies were detected by another assay method. In all four instances these samples were tested positive by the antibody-binding assay utilizing the anti-mouse IgG-peroxidase conjugate. In addition, if samples tested as rotavirus antibody positive by any one of the immunoassays were declared to be rotavirus seropositive, 166 of the 200 serum samples collected after MRV inoculation would be judged to contain rotaviral antibodies. Of these 166 samples, 162 were tested positive by the homologous inhibition assay, while 63 and 78 samples were tested positive by the antibody-binding and immunofluorescence tests, respectively.

DISCUSSION

The group A rotaviruses possess common antigens that are located largely on the inner capsid protein, VP6, as well as virus neutralization or serotype-specific antigens located on outer capsid proteins VP3 and VP7 (5, 7). Owing to the presence of the common inner capsid determinants, heterologous rotaviruses are frequently used as sources of antigens to be utilized in assays for the detection of rotaviral antibody. Viral antigens derived from SA-11 virus have thus been previously reported to be an efficient substitute for MRV antigens in the detection of serum antibody to MRV (15). In the present study, an inhibition enzyme immunoassay utilizing MRV-derived reagents was superior to the other serologic assays evaluated in that antibody was detected earlier and more consistently in experimentally inoculated adult animals. Assays using antigenically related rotaviruses were also evaluated. These assay systems were found to be less sensitive methodologies for detecting serum antibody to MRV. This discrepancy between these results and those of previous studies might be accounted for by differences in test methodologies or experimental protocols. In the previous study, solid-phase-bound SA-11 virus was used as the antigen in an antibody-binding immunoassay, while in the present study SA-11 virus in solution was used as the antigen in an inhibition enzyme immunoassay format. Additionally, the serum samples evaluated previously were pooled samples obtained from suckling mice that were experimentally inoculated with MRV at 1 to 7 days of age. At this age, mice suffer an extensive intestinal infection that leads to symptomatic gastroenteritis. In contrast, in the present study, mice were inoculated at 28 days of age, a time when only limited viral replication occurs and clinical disease does not result (2, 15). Thus, the characteristics of the immune response in these two populations in terms of levels and subclasses of antibody might be expected to differ. The specific purpose of the present study was to identify an efficient and sensitive

TABLE 3. Rotavirus antibody in serially bled mice representative of group 3

Animal no.	Assay system	Rotavirus antibody at the following day postinoculation[a]:										
		0	5	10	15	22	29	36	50	64	78	92
7	Inhibition[b]	−	+	−	−	+	−	−	−	+	−	−
	Binding[c]	−	−	−	−	−	−	−	−	−	−	−
	IFA[d]	−	−	−	−	−	−	−	−	−	−	−
8	Inhibition	−	+	−	−	−	−	−	−	−	−	−
	Binding	−	−	−	−	−	−	−	−	−	+	−
	IFA	−	−	−	−	−	−	−	−	−	−	−
9	Inhibition	−	+	−	−	−	−	−	−	−	−	−
	Binding	−	−	−	−	−	−	−	−	−	−	−
	IFA	−	−	−	−	−	−	−	−	−	−	−

[a] +, Tested rotavirus seropositive; −, tested rotavirus seronegative.
[b] Homologous inhibition enzyme immunoassay.
[c] Antibody-binding enzyme immunoassay with enzyme-labeled, anti-mouse IgG.
[d] Immunofluorescence.

method for the detection of animals initially exposed to MRV upon shipping or upon introduction into our colonies. For this purpose, the inhibition enzyme immunoassay utilizing MRV-derived reagents was clearly the most efficient.

Previous studies have shown that rotaviruses derived from different host species can be distinguished by postinfection serum virus-blocking activity in an enzyme-linked immunosorbent assay (21). The present data demonstrated that an inhibition enzyme immunoassay utilizing MRV-derived reagents was more effective at detecting MRV antibody than was an assay utilizing SA-11 virus-derived reagents. The data are thus consistent with previous observations and indicate that postinfection serum having antibody to both type- and group-specific antigenic determinants more efficiently blocks the binding of homologous virus, as opposed to heterologous virus, to the solid-phase-adsorbed capture antibody in the inhibition enzyme immunoassay format.

A homologous inhibition enzyme immunoassay similar to that reported here has been used previously in a study of the immune response to experimental MRV infection (2). While a postinoculation period of only 10 days was evaluated in that study, by 5 days after inoculation antibody was detected in animals inoculated either 1 or 28 days of age. Earlier studies in suckling mice indicate that this early antibody is most likely IgM (15); however, we have been unable to confirm this when either goat anti-mouse IgM-IgG (Fig. 1) or goat anti-mouse IgM (unpublished observation) antibody conjugates were used in an antibody-binding enzyme immunoassay format. The reason for the increased sensitivity of the inhibition assay versus the antibody-binding assay is not clear but may relate to the relative stability of virus-antibody binding in solution (as in the inhibition assay) as compared with antibody binding to solid-phase-bound antigen (as in the antibody-binding assay). It may also relate to variations in immunoglobulin isotypes or subclasses recognized by the various enzyme-labeled antiglobulins utilized in the studies. Regardless of the antibody isotype present in the early phase of the antirotaviral immune response, the homologous inhibition assay was consistently effective at detecting this response; thus, similar assays might also be considered for use in the early, serologic diagnosis of infection with other viral agents.

Other investigators have compared an antibody-binding enzyme immunoassay with indirect immunofluorescence to determine which methodology could most effectively detect previous exposure to rotavirus in laboratory mice (16). These workers suggest that the enzyme immunoassay is more sensitive than immunofluorescence. However, single serum samples from only 23 mice were evaluated in this study. Our data showed that the immunofluorescence test may be slightly more sensitive than the antibody-binding enzyme immunoassay, although the two assay systems were roughly comparable.

The variability seen in the sequentially obtained serum samples serves to point out that even when a combination of immunoassays is utilized, an animal may be tested as immunologically naive to rotavirus when, in fact, it has been previously infected with MRV and has generated an immune response. Whether these animals are more likely to subsequently excrete virus and thereby serve as a source of colony infection is not known. Patterns of viral shedding in adult animals and the relationship between viral shedding and persistence of a detectable immune response should be examined in future studies. Additionally, the natural course of rotaviral infection in offspring of truly immunologically naive dams versus those from dams exposed to virus but either lacking or possessing detectable immune responses should be further examined.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants K01 RR00020, RR00130, RR07002, and 1 R01 AM 33089 from the National Institutes of Health.

LITERATURE CITED

1. **Estes, M. K., E. L. Palmer, and J. F. Obijeski.** 1983. Rotaviruses. A review. Curr. Top. Microbiol. Immunol. **105**:123–184.
2. **Eydelloth, R. S., S. L. Vonderfecht, J. F. Sheridan, L. D. Enders, and R. H. Yolken.** 1984. Kinetics of viral replication and local and systemic immune responses in experimental rotavirus infection. J. Virol. **50**:947–950.
3. **Flewett, T. H., and G. N. Woode.** 1978. The rotaviruses. Arch. Virol. **57**:1–23.
4. **Gombold, J. L., and R. F. Ramig.** 1986. Analysis of reassortment of genome segments in mice mixedly infected with rotaviruses SA11 and RRV. J. Virol. **57**:110–116.
5. **Greenberg, H., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh.** 1983. Serological analysis of the subgroup protein of rotavirus using monoclonal antibodies. Infect. Immun. **39**:91–99.
6. **Greenberg, H. B., T. V. Phuoc, and R. Jones.** 1986. Cultivation and characterization of three strains of murine rotavirus. J. Virol. **57**:585–590.
7. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82**:8701–8704.
8. **Little, L. M., and J. A. Shadduck.** 1982. Pathogenesis of rotavirus infection in mice. Infect. Immun. **38**:755–763.
9. **McNutty, M. S.** 1978. Rotaviruses. J. Gen. Virol. **40**:1–18.
10. **Noble, R. L., R. W. Sidwell, A. W. Mahoney, B. B. Barnett, and R. S. Spendlove.** 1983. Influence of malnutrition and alterations in dietary protein on murine rotaviral disease. Proc. Soc. Exp. Biol. Med. **173**:417–426.
11. **Offit, P. A., H. F. Clark, and S. A. Plotkin.** 1983. Response of mice to rotaviruses of bovine or primate origin assessed by radioimmunoassay, radioimmunoprecipitation, and plaque reduction neutralization. Infect. Immun. **42**:293–300.
12. **Offit, P. A., R. D. Shaw, and H. B. Greenberg.** 1986. Passive protection against rotavirus-induced diarrhea by monoclonal antibodies to surface proteins vp3 and vp7. J. Virol. **58**:700–703.
13. **Riepenhoff-Talty, M., P. C. Lee, R. J. Carmody, H. J. Barrett, and P. L. Ogra.** 1982. Age-dependent rotavirus-enterocyte interactions. Proc. Soc. Exp. Biol. Med. **170**:146–154.
14. **Riepenhoff-Talty, M., E. Offor, K. Klossner, E. Kowalski, J. Carmody, and P. L. Ogra.** 1985. Effect of age and malnutrition on rotavirus infection in mice. Pediatr. Res. **19**:1250–1253.
15. **Sheridan, J. F., R. S. Eydelloth, S. L. Vonderfecht, and L. Aurelian.** 1983. Virus specific immunity in neonatal and adult mouse rotavirus infection. Infect. Immun. **39**:917–927.
16. **Smith, A. L., D. L. Knudson, J. F. Sheridan, and R. X. Paturzo.** 1983. Detection of antibody to epizootic diarrhea of infant mice (EDIM) virus. Lab. Anim. Sci. **33**:442–445.
17. **Urasawa, T., S. Urasawa, and K. Taniguchi.** 1981. Sequential passages of rotavirus in MA-104 cells. Microbiol. Immunol. **25**:1025–1035.
18. **Voller, A., D. Bidwell, and A. Bartlett.** 1980. Enzyme-linked immunosorbent assay, p. 359–370. In N. R. Rose and H. Friedman (ed.), Manual of clinical immunology, 2nd ed. American Society for Microbiology, Washington, D.C.
19. **Vonderffecht, S. L., and B. I. Osburn.** 1982. Detection of antirotavirus antibody-producing cells in paraffin-embedded tissue sections. J. Am. Vet. Med. Assoc. **43**:356–359.
20. **Wolf, J. L., G. Cukor, N. R. Blacklow, R. Dambrauskas, and J. S. Trier.** 1981. Susceptibility of mice to rotavirus infection: effects of age and administration of corticosteroids. Infect. Immun. **33**:565–574.

21. **Yolken, R. H., B. Barbour, R. G. Wyatt, A. R. Kalica, A. Z. Kapikian, and R. M. Chanock.** 1978. Enzyme-linked immunosorbent assay for identification of rotaviruses from different animal species. Science **201**:259–262.
22. **Yolken, R. H., R. G. Wyatt, B. A. Barbour, H. W. Kim, A. Z. Kapikian, and R. M. Chanock.** 1978. Measurements of rotavirus antibody by an enzyme-linked immunosorbent assay blocking assay. J. Clin. Microbiol. **8**:283–287.
23. **Yolken, R. H., R. G. Wyatt, G. Zissis, C. D. Brandt, W. J. Rodriquez, H. W. Kim, R. A. Parrott, J. J. Urrutia, L. Mata, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1978. Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. **299**: 1156–1164.