Response of Mice to Rotaviruses of Bovine or Primate Origin Assessed by Radioimmunoassay, Radioimmunoprecipitation, and Plaque Reduction Neutralization

PAUL A. OFFIT,* H. FRED CLARK, AND STANLEY A. PLOTKIN

The Children's Hospital of Philadelphia, Division of Infectious Diseases, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received 18 April 1983/Accepted 11 July 1983

Sera from (i) gnotobiotic BALB/c, CD-1, and CFW mice and (ii) conventional BALB/c mice were evaluated by radioimmunoassay, radioimmunoprecipitation, and plaque reduction neutralization, using the Wa, SA-11, and WC-3 (bovine) strains of rotavirus as the detecting antigens. The gnotobiotic mice had no antirotavirus antibody detectable by radioimmunoprecipitation and no neutralizing antibody at a dilution of 1:50 by plaque reduction neutralization. All sera from the conventional mice had rotavirus-specific antibodies detected by radioimmunoassay and by radioimmunoprecipitation at serum dilutions of 1:50 and 1:10,000, respectively. The antibodies were directed against viral proteins p116, p94, p88, and p84 of all three viruses, but had no neutralizing activity against heterologous rotaviruses at a dilution of 1:50. Conventional seropositive mice were parenterally immunized with the Wa, SA-11, or WC-3 strain of rotavirus. An approximate 100-fold increase in rotavirus-specific antibodies was detected by radioimmunoassay, and >20-fold selective neutralization of the immunizing strain of virus was observed. Sera from the mice immunized with Wa virus had antibodies directed against inner and outer capsid proteins of all three rotaviruses. The mouse can be a useful model for studying the immune response to heterologous rotavirus infection; preexisting antibodies presumably directed towards murine rotavirus do not prevent the development of a type-specific immune response to a nonmurine rotavirus.

Rotaviruses have been shown to be the single most important group of etiological agents of acute gastroenteritis requiring hospitalization of infants and young children, both in the United States and in developing countries (3, 4, 13, 29). The worldwide impact of these viruses has excited interest in disease prevention via the development of a vaccine (14, 15). The development of a successful vaccine will be facilitated by defining the immune response necessary for protection against challenge. Some light has been shed on the nature of the protein(s) responsible for evoking type-specific neutralizing antibodies by antisera prepared against purified viral antigens (1, 16, 24). However, numerous investigators have shown that convalescent antisera obtained from either animals or humans can neutralize rotaviruses isolated from heterologous species (5, 8, 25, 31, 37, 39, 43). This fact implies that a heterotypic rotavirus may be used as a candidate vaccine strain.

In both animals and humans, the prevalence of rotavirus-specific antibodies is extensive (10, 18, 20, 27, 30, 36, 41-43). Therefore, for the study of the immune response to rotaviruses, it has been difficult to identify an experimental animal model without preexisting rotavirus-specific antibodies. In the numerous studies of the immunization of animals with heterologous rotavirus antigens, the effect of these preexisting antibodies on the specificity of the immune response has not been elucidated. In this paper, we evaluate the polypeptide and neutralization specificities of sera obtained from (i) gnotobiotic BALB/c, CD-1, and CFW mice; (ii) conventional seropositive BALB/c mice obtained from commercial breeding laboratories; and (iii) conventional seropositive BALB/c mice parenterally immunized with heterologous rotaviruses. We show that (i) structural polypeptides on both the inner and outer capsids of the Wa, SA-11, and WC-3 rotavirus strains share antigenic determinants and (ii) preexisting antibodies presumably directed toward murine rotavirus do not prevent the development of a type-specific immune response to a nonmurine rotavirus.
MATERIALS AND METHODS

Animals and production of antisera. BALB/c mice (8 to 14 g) were obtained from Flow Laboratories, Inc. (McLean, Va.), Charles River Breeding Laboratories, Inc. (Wilmington, Pa.), and Harlan-Sprague-Dawley Breeding Laboratories (Madison, Wis.). Sera from adult, gnotobiotic CD-1 and BALB/c mice were obtained from Howard Blatt (Fox Chase, Pa.), and sera from adult, gnotobiotic CFW mice were obtained from Morris Pollard (Notre Dame, Ind.). Blood samples were obtained by retroorbital capillary puncture from all mice on the day of arrival. All animals were housed in separate units (isolators), with each unit containing its own air supply.

Conventional BALB/c mice were primed intraperitoneally with 250 µl of a clarified virus stock (Wa [4 × 10^6 PFU/ml], SA-11 [5.1 × 10^6 PFU/ml], or WC-3 [1.5 × 10^7 PFU/ml]) and boosted 3 and 5 weeks later by the intravenous inoculation of 5 µg of purified virus. Sera were obtained 4 days after the second booster inoculation.

Cells and viruses. African green monkey kidney cells (CV-1) were grown in BHK cell medium (19), supplemented with 10% fetal bovine serum and 25 µg of gentamicin per ml. Fetal rhesus monkey cells (MA-104) were grown in BHK cell medium supplemented with 10% fetal bovine serum, 100 U of penicillin per ml, and 100 µg of streptomycin per ml.

The Wa strain of human rotavirus was obtained from Richard Wyatt (Bethesda, Md.). A seed stock of simian rotavirus SA-11 was obtained from H. H. Malherbe (San Antonio, Tex.). The bovine rotavirus WC-3 was isolated from a cow in southeastern Pennsylvania in 1981 and adapted to growth in CV-1 cells in this laboratory.

Plaque-purified stocks of Wa, SA-11, and WC-3 viruses for use in these studies were prepared in CV-1 cells. Confluent monolayers of CV-1 cells grown in 150-cm² flasks were washed twice with phosphate-buffered saline (PBS) and infected at a multiplicity of infection of 0.1 PFU/cell. After an adsorption period of 30 min at 37°C, BHK medium containing 40 µg of trypsin (Flow) per ml without serum was added. The cells and culture fluid were harvested when a cytopathic effect including ≥90% of the cell monolayer was evident. The tissue culture fluids were clarified by centrifugation for 10 min at 600 × g.

Purification of virus. Infected CV-1 cells were disrupted by three cycles of freezing and thawing, and the resultant suspension was clarified by centrifugation for 25 min at 15,300 × g. The cell pellet was suspended in TNC buffer (0.05 M Tris–HCl [pH 7.5], 0.15 M sodium chloride, 0.01 M calcium chloride) and extracted twice with 1,1,3-trifluorochloroethane. The aqueous phase from this extraction was combined with the supernatant fluid from the initial clarification and centrifuged at 5°C for 2 h at 125,000 × g. The pellets were suspended in TNC buffer, mixed with CsCl at an initial density of 1.36 g/cm³, and subjected to isopycnic centrifugation at 5°C for 18 h at 200,000 × g, using a type 50.1 rotor (Beckman). After centrifugation, the visible top and bottom viral bands were collected after bottom puncture of the tubes, suspended in TNC buffer, and pelleted at 5°C for 2 h at 250,000 × g. The pellets were suspended in TNC buffer, divided into 10-µl portions, and stored at −70°C. To estimate the quantity of rotavirus purified, the absorbance of the final suspension was measured. It was assumed that the specific absorbance was equal to that of reovirus (5.1 absorbance U at 260 nm/ml of virus) (35).

Viral infectivity plaque assay. Viral infectivity was assayed by plaque formation in six-well plastic tissue culture plates (Flow) containing confluent monolayers of MA-104 cells. After the growth medium was removed, each well was washed twice with PBS. Each well was inoculated with 0.1 ml of a serial 10-fold dilution of the virus. After an adsorption period of 30 min at 37°C, 2.5 ml of overlay medium, consisting of 0.5% purified agar (Agarose; Seakem) and 13 µg of trypsin per ml in Eagle minimal essential medium, was added. The cultures were placed in a humidified incubator for 4 days at 37°C in 5% CO₂. A second overlay medium, containing 0.5% purified agar and 0.05% neutral red in Earle balanced salt solution, was then added, and the plaques were counted approximately 5 h later.

PRN assay. The plaque reduction neutralization (PRN) assay was a modification of the technique described previously by Matsuno et al. (25). A virus suspension containing 500 PFU of Wa, SA-11, or WC-3 rotavirus per ml was mixed with an equal volume of serial fivefold dilutions of serum (heat treated at 56°C for 30 min). The serum-virus mixture was incubated in a water bath at 37°C for 30 min. The serum-virus mixture (0.2 ml) was then inoculated onto confluent monolayers of MA-104 cells in six-well plates and incubated for 30 min at 37°C. The plates were then washed twice with PBS, and the addition of the overlay medium and counting of viral plaques were performed as described above.

RIA. A 100-ng amount of purified Wa virus in sodium carbonate buffer (15 mM Na₂CO₃, 35 mM NaHCO₃) was added to individual wells of round-bottomed 96-well polyvinyl plates (Costar) and kept at room temperature overnight. Each well was then treated with 1% bovine serum albumin in PBS. Serum samples were diluted in 0.1% bovine serum albumin in PBS and incubated with viral immunoadsorbent for 90 min at room temperature. Serum controls were performed by adding each serum dilution to wells without immunoadsorbent. The wells were then washed with PBS and incubated for 90 min with 125I-labeled rabbit anti-mouse F(ab')₂. After a final washing procedure, the wells were separated from the plate and assayed for radioactivity in a gamma radiation counter. Final values were obtained by subtracting the serum control values from the serum values obtained with immunoadsorbent.

Preparation of lysates of [35S]methionine-labeled rotavirus-infected cells. Monolayers of MA-104 cells in 35-mm plastic petri dishes (Flow) were washed twice with PBS 12 h before infection, after which BHK medium without serum was added. The cells were then mock infected with BHK medium or infected at a multiplicity of infection of 10 with Wa, SA-11, or WC-3 rotavirus. After a 30-min adsorption period at 37°C, BHK cell medium supplemented with 3 µg of actinomycin D per ml and 5 µg of trypsin per ml was added. At 12 h postinfection, the cells were washed once with PBS, and then the medium was changed to methionine-free BHK medium supplemented with 3 µg of actinomycin D per ml and 5 µg of trypsin per ml. At 13 h postinfection, the cells were labeled for 20 min
MURINE IMMUNE RESPONSE TO ROTAVIRUS

VOL. 42, 1983

FIG. 1. [35S]methionine-labeled rotavirus polypeptides synthesized in vitro were separated by electrophoresis in 10% SDS-polyacrylamide gels, and the bands were visualized by fluorography. Lane A, WC-3 virus-infected cells; lane B, SA-11 virus-infected cells; lane C, Wa virus-infected cells; and lane D, uninfected cells.

with 500 μCi of [35S]methionine per ml (New England Nuclear Corp., Boston, Mass.). Cell lysates were prepared by adding 200 μl of buffer containing 0.8 M KCl, 10 mM Tris-hydrochloride (pH 7.8), 1 mM phenylmethylsulfonyl fluoride and 1% Triton X-100 followed by 800 μl of 10 mM Tris-hydrochloride (pH 7.8), 1 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100. The lysates were then centrifuged for 45 min at 5°C for 250,000 × g. The supernatant fluids were removed and used as a source of labeled rotavirus proteins for radioimmunoprecipitation (RIP) (see below).

RIP of rotavirus proteins. Cell lysates were preadsorbed with staphylococcus protein A Cowan strain I (SAC I) by adding 500 μl of a 10% SAC I suspension to 1.0 ml of lysate. After incubation for 15 min at 0°C, the bacteria were removed by centrifugation for 2 min at 12,800 × g. The preadsorbed supernatants were then divided into 5-μl portions, to which 5 μl of undiluted serum was added. After 18 h at 4°C, 75 μl of 10% SAC I was added to each serum-lysate mixture and allowed to incubate at 0°C for 1 h. The bacteria were pelleted and washed three times with PBS containing 2% fetal bovine serum and 0.5% Triton X-100 and three times with PBS containing 0.1% sodium dodecyl sulfate (SDS) and 0.5% Triton X-100. The adsorbed labeled proteins were recovered by suspending the bacterial pellets in 20 μl of sample buffer containing 0.25 M Tris-hydrochloride (pH 6.8), 20% glycerol, 1% SDS, 2% 2-mercaptoethanol, and 0.003% phenol red and boiling the suspension for 3 min. The bacteria were pelleted, and the supernatants were applied to SDS-polyacrylamide gels.

Discontinuous SDS-polyacrylamide gel electrophoresis. Discontinuous SDS-polyacrylamide gel electrophoresis was performed on vertical slabs (1.5 by 100 mm), using 10-mm-wide sample wells formed in the stacking gel. The stacking gel consisted of the acrylamide monomer (5% [wt/vol]), N,N'-methylenebisacrylamide (0.13% [wt/vol]), 0.125 M Tris base (pH 6.8), N,N',N'-tetratemethylenediamine (0.05%) [vol/vol]), ammonium persulfate (0.04% [wt/vol]), and SDS (0.1% [wt/vol]). The resolving gel consisted of the acrylamide monomer (10% [wt/vol]), N,N'-methylenebisacrylamide (0.26% [wt/vol]), 0.375 M Tris base (pH 8.8), tetramethylenediamine (0.05% [vol/vol]), ammonium persulfate (0.06% [wt/vol]), and SDS (0.1% [wt/vol]).

Electrophoresis was performed at 30 mA per gel. Molecular weight standards prepared as described above (Bio-Rad Laboratories, Richmond, Calif.) were detected by staining with silver nitrate as described by Merril et al. (28). Fluorograms were prepared as described by Laskey and Mills (17).

RESULTS

35S-labeling of rotavirus polypeptides for RIP. 35S-labeled Wa, SA-11, and WC-3 polypeptides were produced in MA-104 cells. Figure 1 shows the SDS-polyacrylamide gel electrophoresis patterns of the virus-specified proteins as determined by comparison with a mock-infected control lysate. To determine whether virus-specified proteins were nonstructural or were located on the inner capsid or outer capsid of the virion, we compared our protein electropherograms with those of previous investigators (6, 22). The molecular weights of virus-specified proteins were determined by comparison with proteins of known molecular weight (Bio-Rad) (Table 1). Slight differences in molecular weights are found among homologous viral proteins of rotavirus strains.

Analysis of sera from conventional and gnotobiotic mice. (i) RIA. Rotavirus-binding antibody

TABLE 1. Molecular weights of Wa, SA-11, and WC-3 rotavirus proteins

<table>
<thead>
<tr>
<th>Designation</th>
<th>Wa (×10²)</th>
<th>SA-11 (×10²)</th>
<th>WC-3 (×10²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>116</td>
<td>116</td>
<td>116</td>
</tr>
<tr>
<td>(p88, p84)</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>(p61, p29)</td>
<td>88</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>O</td>
<td>84</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>60</td>
<td>61</td>
</tr>
<tr>
<td>NS</td>
<td>55</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>I</td>
<td>41</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>O</td>
<td>38</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>NS</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>NS</td>
<td>34</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>O</td>
<td>29</td>
<td>29.5</td>
<td>30</td>
</tr>
<tr>
<td>O</td>
<td>27</td>
<td>27.5</td>
<td>27.5</td>
</tr>
</tbody>
</table>

* I, Inner capsid protein; O, outer capsid protein; NS, nonstructural protein. The proteins in parentheses are proteolytic cleavage products (6, 22).
was detected in the sera of 25 conventional BALB/c mice at a dilution of 1:50 and was greater than twice the value shown in the sera of 16 gnotobiotic BALB/c, CD-1, and CFW mice tested at the same dilution (Fig. 2A). No significant differences were found in the levels of apparently low nonspecific radioimmunoassay (RIA) reactivity in different strains of gnotobiotic mice.

(ii) RIP. All conventional BALB/c mice obtained from commercial laboratories were found by RIP to have rotavirus-specific antibodies directed against structural proteins p94, p88, p84, and p41 at dilutions of 1:100 and 1:10,000, using 35S-labeled Wa, SA-11, or WC-3 viral polypeptides (Fig. 3). (The bands detected at a molecular weight of approximately 150,000 probably represent antibodies directed against either [i] cellular proteins or [ii] poorly solubilized viral proteins.) No rotavirus-specific antibodies were detected in sera from gnotobiotic BALB/c, CD-1, and CFW mice by RIP.

(iii) PRN. Pre-immunization sera from nine conventional BALB/c mice tested by PRN did not neutralize Wa, SA-11, or WC-3 virus at a dilution of 1:50 despite the presence of rotavirus-specific antibodies detected by RIA and RIP. Sera from 16 gnotobiotic BALB/c, CD-1, and CFW mice tested by PRN were found to have no neutralizing activity against Wa, SA-11, or WC-3 virus at a dilution of 1:50.

FIG. 2. (A) RIA of sera from 25 conventional unimmunized BALB/c (●–●) and 16 gnotobiotic BALB/c, CD-1, and CFW (○–○) mice. The titers are represented as the mean plus or minus the standard error. (B) RIA of sera from 18 conventional BALB/c mice immunized with either Wa, SA-11, or WC-3 virus as described in the text. The titers are represented as the mean plus or minus the standard error of pre- (●–●) and post- (○–○) immunization sera.

FIG. 3. (1) RIP analysis of undiluted serum from an unimmunized conventional BALB/c mouse using 35S-labeled WC-3 (lane A), SA-11 (lane B), and Wa (lane C) viral polypeptides. (2) RIP of serum from an unimmunized conventional BALB/c mouse at dilutions of 1:100 (lane A) and 1:10,000 (lane B), using 35S-labeled Wa viral polypeptides.
TABLE 2. PRN assay of sera from conventional BALB/c mice parenterally immunized with either Wa, SA-11, or WC-3 rotavirus

<table>
<thead>
<tr>
<th>Immunizing antigen</th>
<th>Animal</th>
<th>Neutralization by sera (reciprocal of serum dilution) from mice immunized with:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wa</td>
<td>SA-11</td>
</tr>
<tr>
<td>Wa</td>
<td>WA R₂</td>
<td>9,375</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>WA NM</td>
<td>3,000</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>WA R₁</td>
<td>&gt;12,500</td>
<td>300</td>
</tr>
<tr>
<td>SA-11</td>
<td>SA NM</td>
<td>&lt;100</td>
<td>&gt;12,500</td>
</tr>
<tr>
<td></td>
<td>SA R₂</td>
<td>&lt;100</td>
<td>11,125</td>
</tr>
<tr>
<td></td>
<td>SA R₁</td>
<td>&lt;100</td>
<td>&gt;12,500</td>
</tr>
<tr>
<td>WC-3</td>
<td>WC RL</td>
<td>&lt;100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>WC R₁</td>
<td>&lt;100</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>WC L₁</td>
<td>&lt;100</td>
<td>380</td>
</tr>
</tbody>
</table>

Analysis of sera from parenterally immunized conventional mice. (i) RIA. A total of 18 BALB/c mice were immunized with either Wa, SA-11, or WC-3 rotavirus. Pre- and post-immunization sera were tested by RIA (Fig. 2B). Post-immunization an approximately 100-fold increase in rotavirus-specific antibody titers above preimmunization levels was found. No significant differences in antibody titers among mice immunized with Wa, SA-11, or WC-3 rotavirus were found.

(ii) RIP. Sera from mice immunized with Wa rotavirus were tested by RIP, using ^35^S-labeled Wa, SA-11, or WC-3 viral polypeptides. Typical results are shown in Fig. 4. Sera from animals immunized with Wa virus had antibodies directed against proteins which comprise the inner and outer capsid layers of Wa, SA-11, and WC-3 viruses. Our difficulty in precipitating the polypeptide with a molecular weight of 116,000 (I₁) by RIP using sera from animals parenterally immunized with rotavirus has been experienced by other investigators (6, 21).

(iii) PRN. Three sera from each group of six mice immunized with either Wa, SA-11, or WC-3 virus were selected at random for testing by PRN (Table 2). Neutralization of the immunizing virus at serum dilutions of 1:3,000 or greater was shown by all of the sera tested, but neutralization of heterologous viruses was also sometimes found at dilutions approximately 20-fold lower.

**DISCUSSION**

All conventional 3- to 4-week old BALB/c mice tested from three commercial laboratories were shown to have detectable rotavirus-specific antibodies by both RIA and RIP. The RIP test was more sensitive than the RIA, with antibodies detected at a dilution of 1:10,000 by RIP as compared with a dilution of 1:50 by RIA. Rotavirus-specific antibodies were clearly absent in gnotobiotic murine sera tested by the same assays. The gnotobiotic BALB/c, CD-1, and CFW mice provide us with a consistently seronegative population of adult animals, a situation apparently difficult to maintain with BALB/c mice in commercial breeding laboratories.

The rotavirus-specific antibodies in the conventional mice presumably represent either previous exposure to murine rotavirus (epizootic diarrhea of infant mice) or antibodies passively acquired from a dam infected with this virus. Sheridan et al. (34), using C57BL, CD-1, and CF-1 mice, have presented preliminary data, indicating that there is a positive correlation between the titers of circulating rotavirus-specific immunoglobulin G (IgG) in the dam and those in her litter as detected by enzyme-linked immunoadsorbent assay. The IgG appeared to be of colostral milk origin as decay of antibody occurred after weaning. Furthermore, colostrally deprived animals derived by cesarean section from an IgG-seropositive dam were seronegative. In their study, a neonate of a dam with a serum IgG titer of approximately 1:8,000 had titers of 1:512 and 1:32 at 21 and 42 days of age, respectively. A titer of 1:50 as detected by RIA in our study of 3- to 4-week-old conventional mice does not allow a distinction between the passive or active acquisition of rotavirus-specific antibodies.

Rotaviruses have been found to share one or more antigens by various techniques, including complement fixation, immunofluorescence, gel diffusion, immune electron microscopy, and enzyme-linked immunoadsorbent assay (39, 43, 45). By immune electron microscopy, heterologous convalescent antiserum has been shown to
Sera from conventionally bred mice immunized with Wa virus had antibodies directed against the inner and outer capsid proteins of Wa virus, as well as against those of SA-11 and WC-3 virus by RIP. Studies of convalescent and random sera from humans, as well as from various animal species, have consistently shown neutralizing activity against heterologous rotaviruses (5, 8, 25, 31, 37, 39, 43). However, to date, studies using polyclonal, monospecific, and monoclonal antibody preparations have not provided a clear-cut explanation of this phenomenon. One possible explanation for the interspecies neutralizing activity of convalescent antisera is that some degree of homology of the outer capsid protein(s) of rotaviruses responsible for evoking neutralizing antibodies exists. Kalica et al. (12), in analyzing reassortants between the bovine and human strains of rotavirus, found that neutralizing specificity regularly segregated with the ninth RNA gene segment of the human rotavirus. Investigations using monospecific sera have shown that a high titer of type-specific neutralizing antibodies is evoked by immunizing guinea pigs with the major outer capsid glycoprotein (~p38) (1, 17, 24). Using sera from immunized conventional mice, we have demonstrated some degree of homology among the outer capsid proteins (including outer capsid protein p38) of these three rotavirus strains. The evaluation of sera from gnotobiotic mice hyperimmunized with nonmurine rotavirus strains will determine whether these heterologous neutralizing antibodies are due to inherent cross-reactive domains on the 38-kilodalton protein among rotavirus strains or are secondary to previous exposure of the immunized mouse to murine rotavirus. The titration of the RIP activity of sera from mice hyperimmunized with different rotaviruses may clarify the relationship between cross-neutralization by PRN and RIP-detected shared determinants among homologous outer capsid proteins. These studies are now in progress in our laboratory.

Using RIA, RIP and PRN, we have defined a seropositive population of conventional mice, as well as a seronegative population of gnotobiotic mice. Our studies of parenterally immunized conventional mice show that (i) structural polypeptides of the inner and outer capsids of the Wa, SA-11, and WC-3 rotavirus strains share antigenic determinants; and (ii) a type-specific immune response to a nonmurine rotavirus can be obtained despite the presence of preexisting antibodies presumably directed against murine rotavirus. The mouse can be a useful model for evaluating the immune response to rotaviruses from other species.
ACKNOWLEDGMENTS

We thank Dianna Hannigan and Kathy Dolan for their technical assistance. We also thank Walter Gerhard, William Stroop, Charles Hackett, Mark Frankel, and Jan Tuttleman for their helpful discussions and careful reading of the manuscript.

This work was supported by Public Health Service grant F 32 AI-06733 from the National Institutes of Health to P.A.O. and, in part, by the Hassel Foundation.

LITERATURE CITED