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NOTES

A Murine Model for Oral Infection with a Primate Rotavirus (Simian SA11)

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Simian rotavirus SA11 was shown to replicate in the gastrointestinal tracts of infant mice after oral inoculation. Clinical symptoms, histopathological changes in the small intestinal mucosa, and the type-specific humoral immune response were all characteristic of rotavirus-induced gastroenteritis. The availability of this small animal model for the study of a primate rotavirus infection should expedite analysis of the immune response necessary for protection against challenge.

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 (1, 7). The worldwide impact of these viruses has excited interest in disease prevention via the development of a vaccine (8, 9). Rotaviruses have also been identified as a cause of infection and gastroenteritis in the young of most species of domestic and laboratory animals (5, 6, 10, 12, 17, 19, 25, 26). Numerous investigators have shown that convalescent antisera obtained from either animals or humans can neutralize rotaviruses isolated from heterologous host species (2, 3, 13, 18, 21, 27). This fact implies that heterologous rotaviruses may be useful as candidate vaccine strains.

The only animal models currently used for the study of orally acquired heterologous rotavirus infections are gnotobiotic calves, pigs, and lambs (4, 14, 20). Cross-protection has been demonstrated in gnotobiotic piglets and calves among selected simian, canine, porcine, bovine, and human rotaviruses (3, 22, 24). However, the expense, the need for specialized facilities, and the limited availability of gnotobiotic large animals have hampered animal model studies. In an effort to circumvent these problems, we have recently developed a model of oral infection with a primate rotavirus (simian SA11) using conventionally bred mice.

As mice are only susceptible to symptomatic infection with murine rotavirus between 3 and 14 days of age (23), we chose to evaluate the response of 7-day-old suckling mice to oral administration of nonmurine rotaviruses. Conventionally bred pregnant CD-1 mice obtained from Charles River Breeding Laboratories were bled on arrival and housed in separate isolation units. Sera were tested by radioimmunoassay and radioimmunoprecipitation, using rotavirus antigens as previously described (15); litters whose dams were seronegative were used in these studies. The Wa strain of human rotavirus was obtained from Richard Wyatt (Bethesda, Md.). A seed stock of simian rotavirus SA11 was obtained from H. H. Malherbe (San Antonio, Tex.). A seed stock of bovine rotavirus NCDV was obtained from Robert Yolken (Baltimore, Md.). The bovine rotavirus WC-3 was isolated from a cow in southeastern Pennsylvania in 1981 and adapted to growth in fetal rhesus monkey kidney cells (MA-104) in this laboratory. Virus was grown in MA-104 cells and purified as previously described (15).

Two groups of 31 7-day-old mice were orally inoculated with either 5×10^6 PFU of SA11 virus or a control preparation derived from uninfected MA-104 cells administered in an equivalent volume. Infants were inspected daily for diarrhea after gentle palpation of their abdomens. Forty-eight hours after infection, 28 of 31 animals inoculated with SA11 virus developed diarrhea and mild dehydration followed by a gradual resolution of symptoms over a period of 4 days. Diarrhea was characterized by bright yellow, opaque, liquid stools.

Sets of three mice inoculated with SA11 virus and pairs of two mock-infected mice were sacrificed 1 and 3 days after inoculation. Sections of the proximal and distal small intestine from all six simian virus-infected mice obtained 1 and 3 days after infection showed histopathological changes consisting of villus epithelial cell vacuolization localized to the distal one-third of the villus. There was no appreciable inflammation or necrosis; crypt epithelial cells and the lamina propria appeared normal. Typical histopathological

TABLE 1. Radioimmunoassay of sera from two groups of 31 CD-1 mice 6 weeks after oral inoculation with either SA11 (simian) rotavirus or a control preparation of uninfected MA-104 cells[a]

Inoculum	cpm at a serum dilution of:	
	10^{-2}	10^{-3}
SA11 (simian) rotavirus	4,994 ± 542	2,925 ± 328
Uninfected MA-104 cells	575 ± 43	354 ± 25

[a] Purified SA11 virus (100 ng) in sodium carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$) was added to individual wells of round-bottomed 96-well polyvinyl plates 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 ^{125}I-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 counter. Final values were obtained by subtracting the serum control values from the serum values obtained with immunoadsorbent (mean ± standard error).

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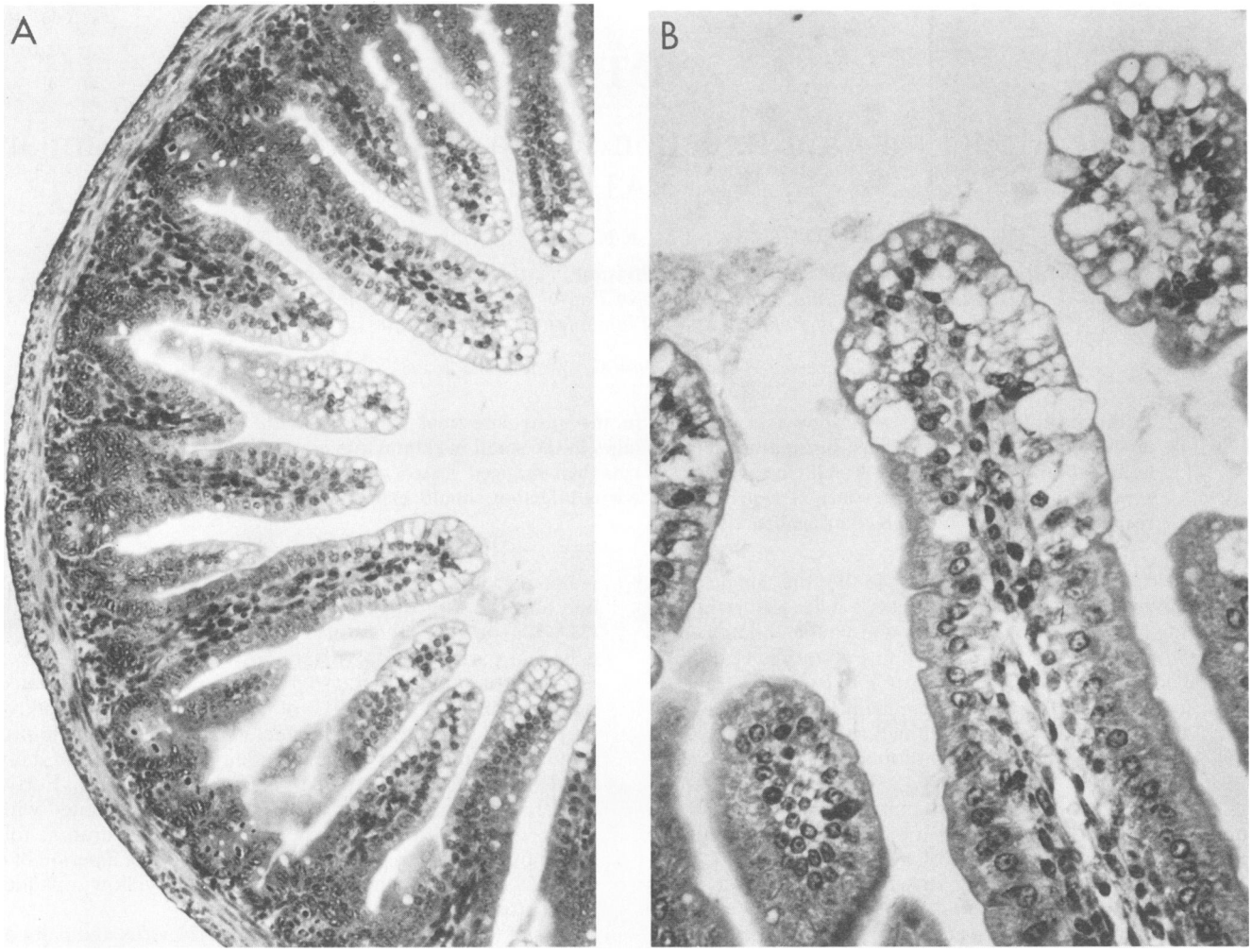

FIG. 1. Hematoxylin and eosin-stained section of the distal ileum from a CD-1 mouse 3 days after oral inoculation with SA11 (simian) rotavirus; (A) ×100. (B) ×1,000.

changes are shown in Fig. 1. Rotavirus antigen was detected by immunoperoxidase staining in ca. 5 to 10% of villus epithelial cells of the ileum 3 days after infection; infected cells showed discreet brush border and occasional apical intracytoplasmic staining. The majority of the specifically stained cells were proximal in the villus to the vacuolated cells; there was no staining of crypt epithelial cells. The rotavirus-specific staining was ablated when the detecting antiserum was first absorbed with purified SA11 virus. None of the mock-infected animals showed diarrhea, histopathological changes of the small intestine, or rotavirus-specific staining of intestinal cells by the immunoperoxidase technique.

Antibodies directed against purified SA11 virus were detected by radioimmunoassay of sera obtained from mice 6 weeks after virus inoculation; no anti-SA11 virus antibodies were detected in mock-infected animals (Table 1). The specificity of this immune response was confirmed by plaque reduction neutralization assay which showed selective neutralization of SA11 virus in comparison with the Wa (human) and WC-3 (bovine) rotaviruses (Table 2). (Sera from dams of inoculated animals tested by plaque reduction neutralization assay were found to have no neutralizing activity against the SA11, Wa, or WC-3 strains of rotaviruses at a serum dilution of 1:50).

To examine the relationship between viral dose and the pathological-immunological response, sets of 10 7-day-old CD-1 mice were orally inoculated with serial 10-fold dilutions of an SA11 virus stock; sera were tested by radio-

TABLE 2. Plaque reduction neutralization assay of sera from CD-1 mice 6 weeks after oral infection with SA11 (simian) rotavirus[a]

Animal	Antibody titer[b] with:		
	Wa	SA11	WC-3
1	250	≥1,250	<50
2	<50	≥1,250	<50
3	<50	≥1,250	<50
4	250	≥1,250	<50

[a] The plaque reduction neutralization assay has been previously described (15). A virus suspension containing 500 PFU of Wa, SA11, 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 again washed twice with PBS, and addition of the overlay medium and counting of viral plaques were performed as described previously.

[b] Antibody titer is expressed as the reciprocal of the serum dilution showing a 50% reduction in the mean plaque count for each virus.

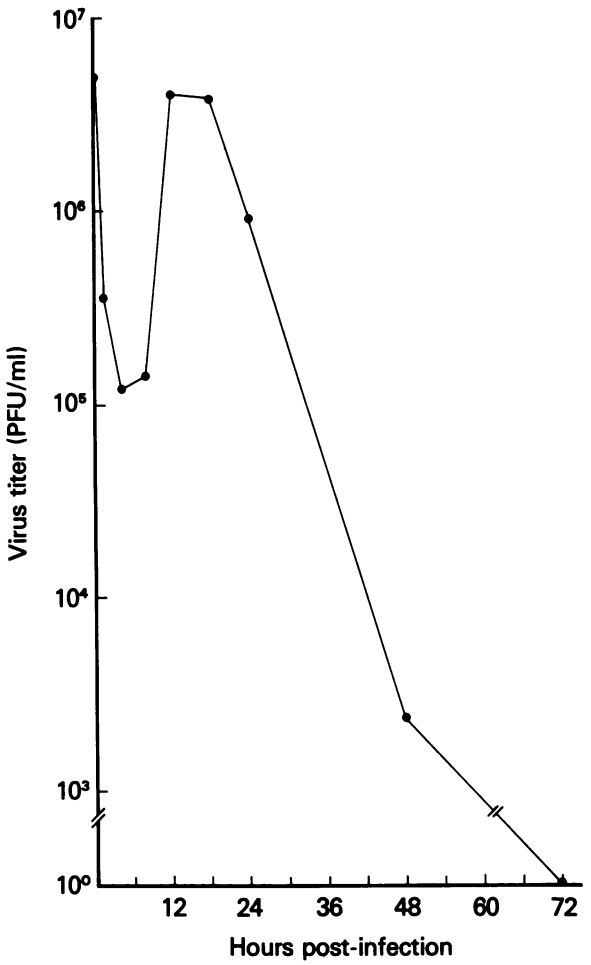

FIG. 2. Viral infectivity of intestinal homogenates from CD-1 mice orally infected with SA11 (simian) rotavirus. Viral infectivity was assayed by plaque formation in six-well plastic tissue culture plates containing confluent monolayers of MA-104 cells (15). After the growth medium was removed, each well was washed twice with PBS. Intestinal homogenates were clarified at 12,800 × *g* for 2 min, and supernatants were passed through a 0.22-μm filter (Amicon). Each well was inoculated with 0.1 ml of a serial 10-fold dilution of the gut homogenate. 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% CO_2. A second overlay medium containing 0.5% purified agar and 0.03% neutral red in Earle balanced salt solution was then added, and plaques were counted 5 h later. The dose of virus used to inoculate animals in this experiment (5×10^6 PFU) is shown as the viral titer 0 h after infection.

immunoassay 6 weeks post-inoculation. None of the animals inoculated with less than 5×10^6 PFU of SA11 virus developed diarrhea or dehydration. Mice inoculated with 5×10^5, 5×10^4, or 5×10^3 PFU of SA11 virus showed a rotavirus-specific immune response similar in magnitude to that observed in mice inoculated with 5×10^6 PFU of virus. Mice inoculated with less than 5×10^3 PFU of SA11 virus did not develop a rotavirus-specific immune response.

An experiment was performed to determine whether a complete cycle of SA11 virus replication occurred in the murine gastrointestinal tract. Seven-day-old CD-1 mice were orally inoculated with 5×10^6 PFU of SA11 virus. Pairs of mice were sacrificed at intervals after infection; the small and large intestines were excised together and homogenized in 1 ml of phosphate-buffered saline (PBS). Viral infectivity was assayed by plaque formation in MA-104 cells (15). There was a logarithmic phase of viral growth 8 to 12 h after infection, followed by a rapid decline in the titer of viral infectivity (Fig. 2). Although the maximal amount of infectious virus was produced 12 to 18 h after infection, mice showed diarrhea and mild dehydration from 24 through 96 h postinfection. These observations have been repeated in subsequent experiments with similar results. Recovered virus was indistinguishable from SA11 virus on the basis of the viral RNA electropherogram (16). No infectious rotavirus was recovered from the gastrointestinal tracts of mice inoculated with 5×10^6 PFU of bovine rotavirus (strain NCDV) when tested 4 to 72 h post-inoculation.

There are no published reports demonstrating that newborn mice orally inoculated with nonmurine rotaviruses develop the clinical symptoms, small intestinal cell histopathological changes, or humoral immune response characteristic of a rotavirus-induced gastroenteritis. The pathogenesis of murine rotaviruses in infant mice has been previously studied (11, 23). Seronegative 4-day-old BALB/c mice orally inoculated with murine rotavirus developed diarrhea 2 days after inoculation followed by a gradual resolution of symptoms over the succeeding 8 days (11). Infectious virus was shed in the feces of infected animals from 1 through 8 days after inoculation. Unlike murine rotaviruses, SA11 rotavirus does not undergo an extended cycle of replication in the murine gastrointestinal tract.

To our knowledge, the described replication of SA11 rotavirus in the gastrointestinal tracts of infant mice represents the first demonstration of the successful use of a common laboratory animal for induction of infection and gastroenteritis with a rotavirus of heterologous host origin. Consistently positive results require the use of a high-titered rotavirus inoculum and vigorous testing of mouse dams for the absence of preexisting rotavirus-specific antibodies. Determination of maximum levels of viral replication requires assay of infected gut tissues before the onset of diarrhea. Preliminary results indicate that we may, under similar conditions, induce gastroenteritis in mice with a human rotavirus isolated from a child in Philadelphia in 1983 and adapted to growth in MA-104 cells in this laboratory. Other parameters of this infection are currently being investigated. The use of a small animal model for the study of heterologous rotavirus infections should expedite analysis of cross-protection against challenge among selected rotavirus strains.

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