PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 127–132 (1987)

Gastroenteritis Caused By Human Rotaviruses (Serotype Three) In A Suckling Mouse Model*

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The pathogenic potential of human rotaviruses of serotypes 1 through 4 was evaluated in suckling mice. Oral inoculation of three different human rotaviruses of serotype 3 into 5-6 day old CD-1 mice caused disease characterized by diarrhea and dehydration. The mean 50% diarrhea inducing dose (DD_{50}) was $5x10^5$ pfu. Histopathological examination of small intestines revealed villus epithelial cell vacuolization localized to the distal one-third of the villus.

Only Serotype 3 rotaviruses exhibited a rapid phase of viral growth in the intestine between 7 and 12 hours post-inoculation. Larger inocula of rotavirus serotypes 1, 2, and 4 did not cause disease or typical histopathologic changes. However, immunoperoxidase staining for rotavirus antigen was positive in all serotypes tested indicating that infection can occur without apparent disease and is not serotype specific. This convenient in-vivo model can be used to evaluate attenuation of human origin vaccine candidates of serotype 3.

Introduction

Rotaviruses are the leading cause of viral gastroenteritis of infants in the developed as well as the developing world (1,2). The morbidity and mortality associated with diarrheal diseases in developing countries makes prevention of rotavirus gastroenteritis an important goal (3).

A convenient small animal model for the study of experimental infections initiated by human-origin rotaviruses (RV) would greatly enhance opportunities to develop a safe vaccine. We have previously demonstrated that the primate-origin rotavirus strain SA-11 causes classical symptoms of RV gastroenteritis in the suckling mouse while 50 fold greater titers were required to induce disease with a bovine rotavirus, NCDV (4). In this study we have determined that the mouse model system is similarly applicable to the study of disease induced by human rotaviruses of serotype 3.

Materials and Methods

Conventionally bred pregnant CD-1 mice were obtained from the Portage facility of the Charles River Breeding Laboratories. The mice were bled on

* Supported in part by grant T32AI07278 from the NIH and the Institute Merieux, Lyon, France.
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0037-9727/87 $1.50

arrival and housed in separate isolation units. Sera were tested by enzyme-linked immunoadsorbant assay (ELISA) and a plaque reduction neutralization assay (PRN) as previously described (5); only litters whose dams were seronegative were used in these studies.

Rotavirus serotype 3 strains WI77, WI78, CC3-17; WI79 (serotype 1) and $WISC_2$ (serotype 2) were isolated in the course of investigation of children with gastroenteritis in the winters of 1981-82 and 1982-83 (6). Adaptation to growth in cell culture was accomplished by the method of Sato et. al.(7). Each has been adapted to grow to a titer of $\geq 10^{5.5}$ pfu/ml in fetal green monkey kidney cells (MA-104). The Wa strain of human rotavirus was obtained from Richard Wyatt (Bethesda, MD). A seed stock of bovine rotavirus NCDV was obtained from Robert Yolken (Baltimore, MD). The S2 strain was obtained from Marie Riepenhoff-Talty (Buffalo, NY). The St. Thomas (ST) strain of human rotavirus was acquired from Jorge Flores (Bethesda, MD). The RRV strain of rotavirus was obtained from the American Type Culture Collection (Rockville, MD). The bovine rotavirus WC-3 was isolated from a cow in southeastern Pennsylvania in 1981 and adapted to growth in MA-104 cells. A viral infectivity plaque assay and PRN assay were used to determine viral concentrations and the serotype specificity of the different rotavirus strains.

Litters of 5-6 day old mice were orally inoculated with single doses of $10^{6.5}$ to $10^{7.8}$ pfu's of different rotavirus strains in equivalent volumes. Infant mice were inspected daily for diarrhea after gentle palpation of the abdomen. In those litters in which diarrhea developed, the rotavirus dose was titrated to determine the dose at which diarrhea was induced in 50% of the litter (DD_{50}).

Replication of human rotaviruses in the mouse model was studied by orally inoculating litters of 6 day old mice with 10^6 to 10^7 pfu of different strains of rotavirus representing serotypes 1 through 4. Mice were sacrificed at timed intervals post-infection and their intestines excised and homogenized as

TABLE I: Diarrhea and Histopathologic Changes in Mice Following Oral Infection with Rotaviruses

Serotype	Strain	Maximum Incidence of Diarrhea Induced (%)	DD_{50}* (PFU/dose)	Histopathology IP	Histopathology H&E
One	WI79	14	$>10^{6.6}$	+	-
	WA	0	$>10^{6.6}$	+	-
Two	$WI\text{-}SC_2$	0	$>10^{5.5}$	ND	ND
	S2	0	$>10^{7.0}$	+	-
Three	WI77	100	$10^{5.0}$	+	+
	WI78	100	$10^{6.0}$	+	+
	CC3-17	100	$10^{5.8}$	+	+
	SA-11 (simian)	100	$10^{5.0}$	+	+
	RRV (simian)	100	$10^{4.3}$	ND	ND
Four	ST	0	$>10^{7.3}$	+	-
Bovine	NCDV	100	$10^{6.7}$	+	+
	WC3	12	$10^{7.8}$	ND	ND

* DD_{50} = Dose at which diarrhea is induced in 50% of mice
IP = Immunoperoxidase
H&E = Hematoxylin-Eosin
ND = Not Done

described previously (8). In addition, sections of distal small intestine were obtained at 72 hours post inoculation, fixed in Bouin's solution, and embedded in paraffin for later histopathological study.

Immunoperoxidase staining was performed on the intestinal sections using hyperimmune rabbit sera against SA-11 rotavirus and a biotinylated goat anti-rabbit IgG (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA) (9).

Results

Three different human rotavirus isolates, all serotype 3, caused disease in 5-6 day old CD-1 mice with DD_{50} requirements ranging from $10^{4.3}$ to $10^{6.0}$ (table 1). Diarrhea was rarely or never induced by human-origin rotaviruses of serotypes 1, 2 or 4. Rotavirus infection in mice was characterized by watery-yellow diarrhea with mild

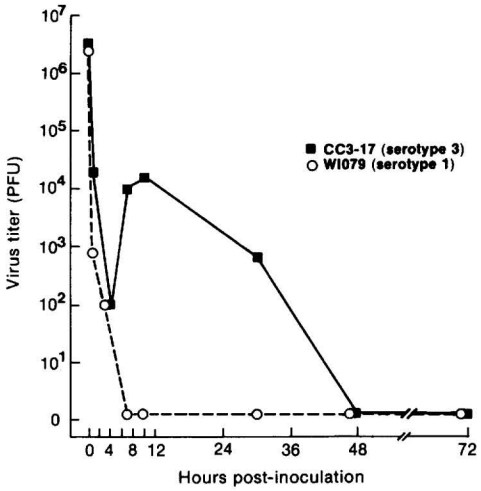

Figure 1. Viral infectivity of intestinal homogenates (pfu per total intestinal tract) from CD-1 mice orally infected with either CC3-17 (serotype 3) or WI079 (serotype 1) rotavirus. The dose of virus used to inoculate animals is shown as the viral titer 0 hours after infection.

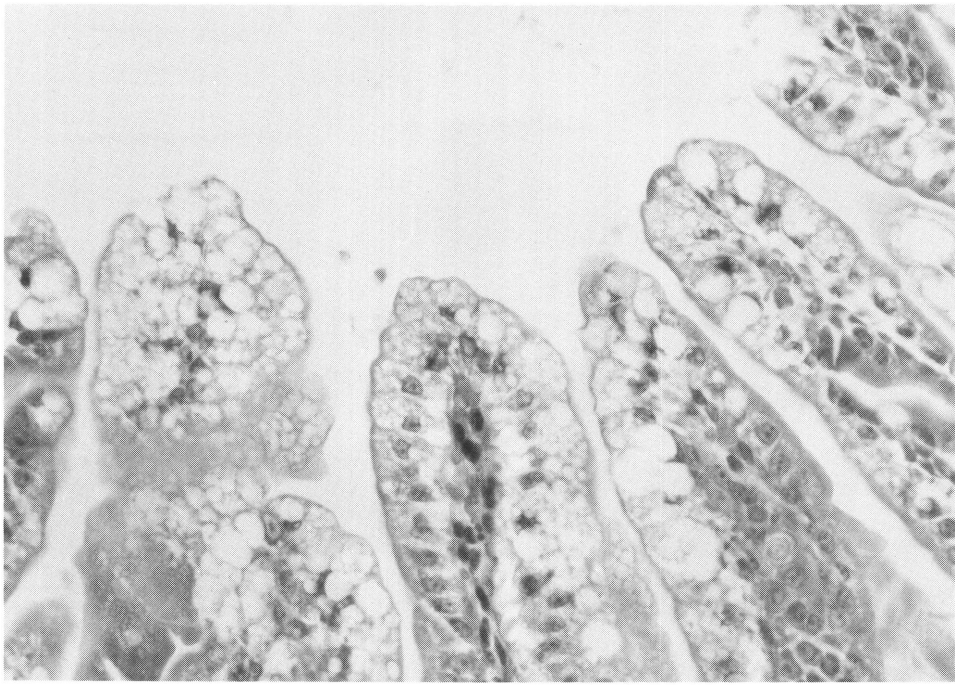

Figure 2. Hematoxylin and eosin stained section (X 400) of distal ileum from a CD-1 mouse 3 days after oral inoculation with CC3-17 (human serotype 3) rotavirus.

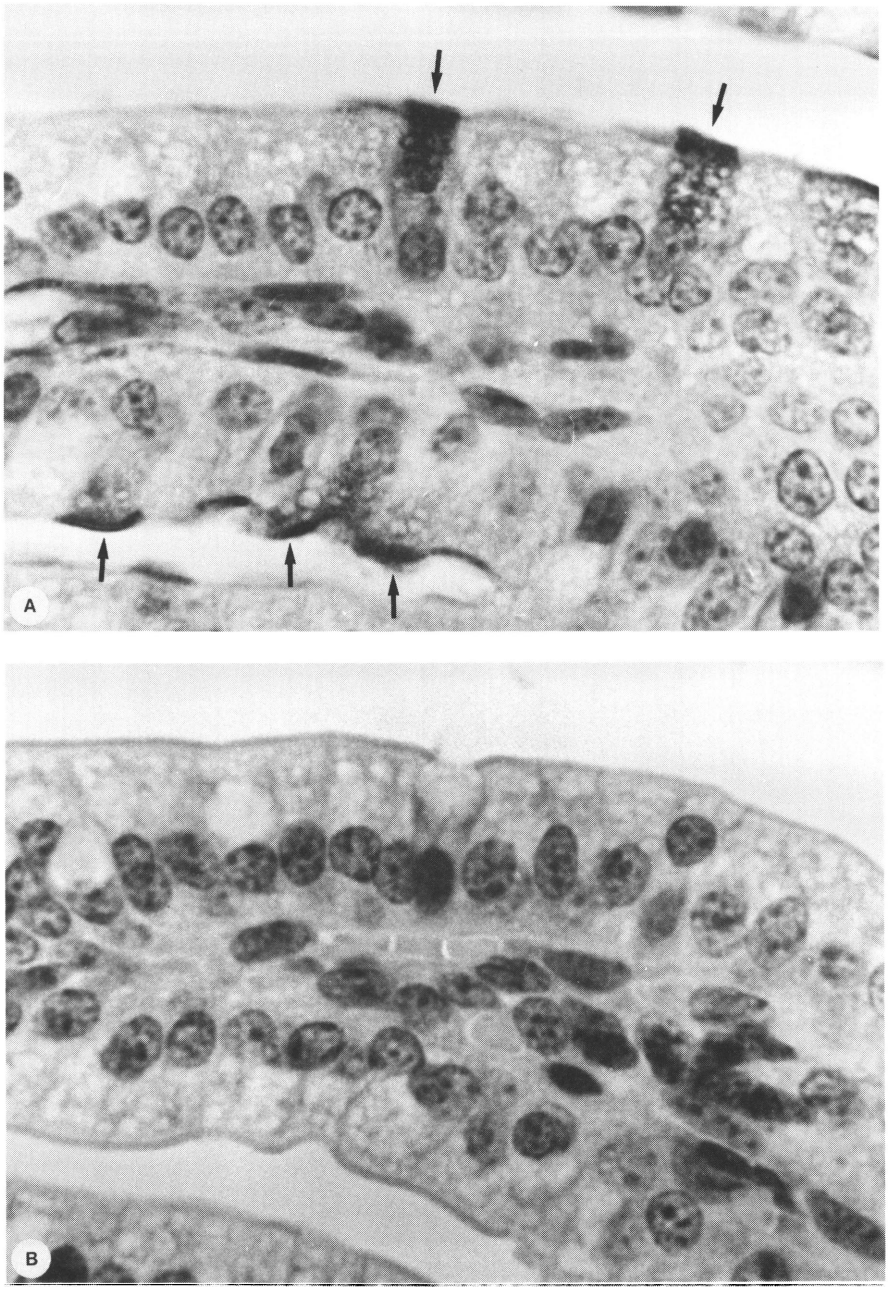

Figure 3 A). Immunoperoxidase staining (X 1000) of distal ilium from a CD-1 mouse 3 days after oral inoculation with WI-79 (human serotype 1) rotavirus showing (arrows) discreet brush border and/or diffuse cytoplasmic staining.

B). Control picture of the same villus with oblation of staining with prior absorption of the detecting anti-serum with purified SA11 virus.

TABLE II: Serum Neutralization* Titers In Mice Following Single Oral Inoculation With Human Rotaviruses

Serotype	Strain	Inoculating Dose (Pfu/ml)	Mice#	Reciprocal Titers
1	WI079	$1.3x10^7$	1	<50
			2	170
2	S_2	$1.2x10^7$	1	<50
			2	<50
3	CC3-17	$2.6x10^6$	1	940
		$5x10^5$	1	270
			2	490
4	ST3	$2.8x10^7$	1	90
			2	<50

* Plague reduction neutralization performed against the homologous virus.

\# Mice designated as 1 or 2 were given the same inoculating dose of virus.

dehydration developing 24 to 48 hours post-inoculation. The infection usually resolved by the 5th to 6th day post-inoculation without mortality. Each of the serotype 3 strains tested (WI77, WI78 and CC3-17) exhibited a single rapid phase of viral growth in the intestine between 7 and 12 hours post-inoculation followed by a rapid decline over the next 48 hours. Figure 1 demonstrates a characteristic replication curve following infection with serotype 3 rotaviruses. No replication was seen with WI79 (serotype 1) S_2 (serotype 2) or ST. (serotype 4). All viruses recovered from the intestinal suspensions, were re-cultivated in tissue culture and shown to have the same electrophoretic pattern as the inoculated strain.

Hematoxylin-eosin staining of the distal small intestine revealed villus epithelial cell vacuolization localized to the distal one-third of the villus in only those mice infected with serotype 3 strains of rotavirus (Figure 2). The histological appearance of the intestines of mice inoculated with other serotypes was similar to that of control litters inoculated with cell-free medium from uninfected MA104 cells.

Although mice inoculated with larger doses ($10^{6.6}$ to $10^{7.0}$) of rotaviruses of serotypes 1, 2 and 4 did not show diarrhea, typical histopathologic changes or intestinal replication; rotavirus antigen was detected by immunoperoxidase staining in all serotypes tested (table 1). Figure 3A demonstrates the staining seen in mice following oral inoculation with WI-79, human serotype 1. Although the mouse was asymptomatic approximately 10% of cells demonstrated discreet brush border or diffuse cytoplasmic staining. The rotavirus specific staining was oblated when the detecting antiserum was first absorbed with purified SA11 virus (Figure 3B). Although the immunoperoxidase staining of intestinal sections did not allow an exact assessment of the number of infected cells, there appeared to be a higher incidence of stained cells in the serotype 3-infected mice.

A neutralizing antibody response was detected in mice with symptoms of rotavirus diarrhea (table 2). Serotype 3 rotavirus strain CC3-17 induced neutralizing antibody detectable at 4 weeks post-infection. Other serotypes occasionally induced neutralizing antibody in lower titers.

Discussion

We have previously determined that adult mice could be useful for preparation of specific hyperimmune reference anti-sera to heterologous rotaviruses (5). Subsequently (8), it was determined that typical rotavirus disease can be induced in newborn mice inoculated with the serotype 3 simian rotavirus SA-11. In this study, we have determined that diarrhea is consistently induced with all serotype 3 rotaviruses tested, whether of human or lower primate origin.

Immunoperoxidase staining was positive for all human rotavirus strains tested indicating transcription and translation of the viral genome and production of viral proteins. However, intestinal cells of mice infected with serotype three strains demonstrated more intense staining and disease ensued. The human serotype 3 strains of rotavirus appear to be serotypically similar to at least one strain (EB) of murine rotavirus, as shown by Harry Greenberg in 1986 (10). This similarity demonstrated by a neutralization assay indicates the possibility of shared epitopes on VP3 and/or VP7 and may account for the virulence of human serotype 3 strains in mice (11).

The availability of this small animal model may help in evaluating both attenuation or immunogenicity of serotype 3 rotavirus. Such information is desirable both because serotype 3 rotavirus is a common cause of human disease and because principles of attenuation ascertained for one serotype may be applicable to others.

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Received September 2, 1986.
P.S.E.B.M. 1987, Vol. 184.
Accepted November 7, 1986.