Persistent Rotavirus Infection in Mice with Severe Combined Immunodeficiency

MARIE RIEPENHOFF-TALTY,^{1,2*} TARARAJ DHARAKUL,^{1,2} EDWARD KOWALSKI,^{1,2}
SUSAN MICHALAK,³ AND PEARAY L. OGRA^{1,2}

Departments of Pediatrics and Microbiology, The State University of New York at Buffalo, Buffalo, New York 14214¹; Division of Infectious Diseases and Microbiology Laboratories, The Children's Hospital of Buffalo, Buffalo, New York 14222²; and Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama, 35294³

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Rotaviruses are important pathogens of human infants and the infants of many animal species. The disease produced by these viruses can be described as an acute, self-limiting diarrheal disease, with virus replication localized to the differentiated epithelial enterocytes of the small intestine. Immunologically normal infants shed virus for approximately 5 to 12 days after the onset of infection. Recently, it has been shown that rotavirus can produce a chronic infection in severely immunocompromised children, with virus shedding and intermittent diarrhea lasting from 6 weeks to 2 years (G. A. Losonsky, J. P. Johnson, J. A. Winkelstein, and R. H. Yolken, *J. Clin. Invest.* 76:2362-2367, 1985; F. T. Saulsbury, J. A. Winkelstein, and R. H. Yolken, *J. Pediatr.* 97:61-65, 1980). These findings point to an important role for the immune system in recovery from the disease. The study described here examined the outcome of murine rotavirus infection in mice with severe combined B- and T-cell immunodeficiency (SCID) and in immunologically normal seronegative BALB/c mice. Persistent rotavirus infection was established in all mice with SCID which had been inoculated orally as pups. Low levels of virus replication and constant fecal virus shedding characterized the chronic infection. This is the first report of a persistent rotavirus infection in an animal model.

Rotavirus (RV) infections frequently produce an acute, self-limiting diarrheal disease in 6- to 18-month-old human infants. In addition, RV has been shown to produce chronic infection in immunocompromised children from 7 months to 18 years of age (15, 19). These children shed virus for as long as 6 weeks to 2 years. Immunologically normal infants generally stop shedding virus at 5 to 12 days after the onset of infection (19, 26).

Like other mammalian infants, suckling mice are exquisitely susceptible to RV infection. The pathogenesis of the infection in the mouse gut is very similar to that in humans. However, mouse RV (MRV) infection tends to have a more clear-cut age restriction than human RV infection. Only animals less than 15 to 16 days of age exhibit clinical disease after infection, and adult mice demonstrate minimal virus replication and no clinical disease (6, 14, 17, 20, 25). It is not clear whether recovery from acute infection in suckling animals is primarily a function of gut maturation or whether the immune system plays a critical role.

Little is known about the relative role of antibody or T-cell-mediated immunity in the mechanism of protection against or the pathogenesis of RV-induced disease. The present study was undertaken to examine the outcome of MRV infection in mice with severe combined B- and T-cell immunodeficiency (SCID) and control mice which were immunologically normal. This study used CB-17 mice with SCID and normal seronegative BALB/c mice.

Antibody-free young adult male and female CB-17 mice with SCID (original breeders were obtained from Fox Chase Cancer Center, Philadelphia, Pa.) were shipped in isolation cages from the University of Alabama animal laboratories to the Children's Hospital of Buffalo. Immediately on arrival, they were transferred in a biological safety cabinet to microisolator cages (Lab Products, Inc., Maywood, N.J.).

All water, food, and bedding for these animals were sterilized before being used. A total of 27 pups in five litters was used in the experiments.

Control mice were the immunocompetent BALB/c strain, the Igh congenic partner of the CB-17 strain with SCID. Eight litters of 1-day-old pups and their mothers were purchased from MRV-free colonies (Portage Facility, Charles River Breeding Laboratories, Inc., Boston, Mass.). Periodic testing for RV antibodies in the sera of these animals was negative. All animals were shipped in isolator cages and transferred to microisolator cages on arrival at Children's Hospital.

MRV (EDIM 5099; obtained from R. Wyatt, National Institutes of Health, Bethesda, Md.) was grown in suckling mice. The virus-rich intestinal homogenate was clarified by low-speed centrifugation and used as the inoculum. The 50% infectious dose (ID₅₀) was determined as follows. Groups of 10 litters of normal mice were fed serial dilutions of the virus in constant 5-μl volumes. The ID₅₀ was calculated as the amount of virus that produced moderate diarrhea in 50% of the animals within 72 h after virus inoculation. The titer of the preparation used in these experiments was 2×10^7 ID₅₀/ml. One- to three-day-old mice with SCID and normal suckling mice were fed 100 ID₅₀s in a 5-μl volume by using a micropipette and allowing the mouse sufficient time to suck and swallow the inoculum. Adult mice were fed 10^4 ID₅₀ in 40 μl.

Intestinal epithelial enterocytes were isolated by a procedure described previously (17). Briefly, the procedure involved removing the small intestine, opening it longitudinally, and washing it in a balanced salt solution. It was then placed in 5 ml of a buffered saline solution containing 0.5 mM EDTA and agitated gently for 5 min. A population of approximately 90% epithelial enterocytes was obtained after two or three periods of agitation. These cells were washed thoroughly, and smears were made on clean glass slides. The

* Corresponding author.

FIG. 1. Bar graph of MRV-positive enterocytes, as determined by RV-specific immunofluorescence assay. Each bar represents the percent positive (mean $\pm$ standard deviation) of enterocytes in at least three mice (approximately 2,000 enterocytes per slide and three slides per mouse). ■, mice with SCID; □, controls.

cells were fixed in cold acetone for 10 min and stained with porcine anti-RV serum made in gnotobiotic pigs (kindly supplied by L. Saif, Ohio Agricultural Research and Development Center, Department of Veterinary Science, Wooster, Ohio). The second stain was goat anti-porcine serum conjugated with fluorescein isothiocyanate (Miles Laboratories, Elkhart, Ind.) containing Evans blue as a counterstain. Approximately 2,000 enterocytes were counted in each preparation, and the number of MRV-positive cells was determined as a percentage of the total number of enterocytes.

At least twice daily, all animals were assessed for diarrhea and virus shedding. Diarrhea was monitored by applying gentle pressure on the abdomen and noting the color and consistency of the extruded feces. The feces were collected in 0.5 ml of phosphate-buffered saline, and a commercially available enzyme-linked immunosorbent assay was used for the detection of RV, using a monoclonal antibody (Kallestad Laboratories, Austin, Tex.).

Chi-square analysis was used to compare the level of clinical disease and the extent of virus replication (percentage of antigen-positive enterocytes) and fecal shedding in mice with SCID and normal control mice.

Oral inoculation of 27 infant mice with SCID and 32 immunologically normal control mice with 100 ID₅₀s of MRV resulted in 10 to 20% infected enterocytes during the first 5 days after infection (Fig. 1). However, by 12 days, less than 1% of the enterocytes in control mice was found to be positive for the virus, and no infected enterocytes could be detected after 18 days. On the other hand, in mice with SCID, as many as 25% of the enterocytes was positive for the virus at 7 days postinfection. The number of infected enterocytes increased to 35% by day 10 postinfection. The level of infected villous cells dropped to 10% on day 22 and remained at 1 to 3% between 56 and 80 days after infection.

The kinetics of fecal viral shedding in pups (Fig. 2) mirrored the pattern of virus replication depicted in Fig. 1. MRV shedding was positive in 100% of both mice with SCID and control animals from 2 to 11 days after inoculation. Between 12 and 17 days postinfection, about 50% of the control animals shed virus, and on days 18 and 19, only 2 of 21 fecal samples were positive for the virus (10%). Thereafter control animals were negative for the virus, while 100% of all samples from animals with SCID were positive for the virus.

Clinical disease was evident in mice with SCID and in control suckling mice (Table 1). Diarrhea began between 46

and 54 h after oral inoculation of the virus. By day 3 after infection, approximately 70 to 80% of the animals produced liquid yellow feces after gentle abdominal pressure or there was obvious fecal staining of the anus. Thus, the course of the clinical disease, like virus replication and shedding, was similar in the experimental and control groups during the acute phase of infection. The similarity between groups also was observed in the extended period of diarrhea. Passage of liquid feces continued for nearly twice as long in mice with SCID as in the controls. Also, although liquid stools were no longer evident in mice with SCID after about 16 days postinfection, feces remained softer and lighter colored in these mice than in control mice. Clinical disease appeared more severe in mice with SCID, as evidenced by a lethargic appearance, reduced suckling, and the inability of the mice with SCID to pass feces on day 5 postinfection. However, the increased severity did not result in mortality. Only one

FIG. 2. Bar graph comparing RV shedding in mice with SCID and control mice. The percentage of animals with virus in feces was determined by RV-specific enzyme-linked immunosorbent assay of multiple daily fecal samples. Animals were infected at 2 days of age. Numbers at the tops of the bars indicate the number of samples tested. ■, mice with SCID; □, controls.

TABLE 1. Description of clinical disease

Mouse strain	Onset of diarrhea (h pi ^a)	Frequency of diarrhea at day 3 pi (no. of samples/no. of mice)	Duration of liquid stools (days)	Effect on feeding at days 4-6 pi	Mortality/no. of mice
CB-17 with SCID	48-54	18/27	15.5	Reduced suckling	1/19
BALB/c control	46-48	31/38	8.4	None	0/38

^a pi, Postinfection.

animal died, and that was 24 h after inoculation, at which time the feces were still negative for RV.

CB-17 mice with SCID, described first in 1983 (4), have been characterized as lacking functional T cells as well as pre-B and B cells (7). Cells of the myeloid lineage are normal, and natural killer cells are present (9, 10). The mice are unable to eliminate *Listeria monocytogenes* after experimental infection, in contrast to normal CB-17 mice which eliminate all viable bacteria and become resistant to secondary infection (2). In the present study, 2-day-old mice with SCID were infected with RV. Virus replication, shedding, and clinical disease were assessed and compared with that in age-matched seronegative immunocompetent controls. It was found that RV infection in suckling mice with SCID was particularly severe and, unlike infection in normal mice, continued through weaning and into adult life. In mice with SCID, 1 to 3% of the enterocytes appeared to be infected at any time. Fecal virus shedding was a consistent characteristic of the chronic disease. Persistent infection also could be initiated in adult mice with SCID which had been inoculated orally, but only acute infection was seen in normal mice after oral virus inoculation.

The persistent infection in the severely immunocompromised animals suggests that the immune system plays a role in recovery from RV infection. From the information presently available on the role of the immune system in RV infection, it is known that specific neutralizing antibody present locally in sufficient amounts can modify and even prevent the infection in lambs, calves, mice, and humans (3, 18, 21, 22). Other reports describe protection as a result of the immune response to heterologous virus infection in human infants (1, 5, 23). However, it is not clear what aspect of the immune response is the basis for this protection. The role of the immune system in recovery from infection is even less well defined than it is in protection. It is evident that there is a systemic antibody response to RV infection (12, 13, 16, 27). It is not known whether this is a factor in recovery. Reports have also described the local antibody response in the gut but have not been able to show definitely that it plays a role in recovery (8, 16, 20, 24).

This is the first report of a persistent RV infection in an animal model. Since these animals lacked both functional T and B cells, we were unable to identify which of these is more important in recovery. In two reports of chronic RV infection in immunodeficient children, the diagnosis included X-linked agammaglobulinemia, SCID, and common variable immunodeficiency disease (15, 19). The presence of chronic disease in the agammaglobulinemic patient suggests the importance of specific antibody for complete recovery from RV infection. Further support for this comes from the work done on nude mice in our laboratory (M. Riepenhoff-Talty, T. Dharakul, E. Kowalski, D. Sterman, and P. L. Ogra, in J. McGhee, ed., *Recent developments in mucosal immunology*, in press) and elsewhere (11). Eiden and colleagues (11) found no virus shedding or clinical disease by day 13 postinfection in athymic nude mice. Lack of T-cell compe-

tence did not impede complete recovery in the mice described (11). These data suggest the need for specific antibody to completely obliterate virus replication. Studies using mice with SCID to define the comparative importance of antibody or T-cell factors are in progress in our laboratory. This model should be extremely useful for gaining information about immunity to RV infection.

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