

Human Rotavirus in Lambs: Infection and Passive Protection

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A human stool filtrate containing rotavirus which was administered orally to gnotobiotic lambs caused diarrhea, virus excretion, development of antibodies to rotavirus, and pathological changes in the villi of the small intestine. Thus, lambs may serve as experimental animals for the study of human rotavirus infections. This model system was used to study passive protection. Human immunoglobulin G (IgG) containing antibody to rotavirus was fed to lambs 24 to 78 h after birth, and the lambs were infected with lamb-passaged human rotavirus when 30 h old. The lambs treated with IgG did not develop diarrhea, and virus excretion was delayed in onset and shortened in duration. It may be possible to make similar use of IgG to protect children at risk in a rotavirus outbreak. The treatment did not prevent the lambs developing antibody to rotavirus.

Rotaviruses have been identified as a cause of neonatal diarrhea in several species of animals, including humans (2) and sheep (8). Neonatal piglets and rhesus monkeys have been infected successfully with human rotavirus (1, 10, 11), although attempts to infect mice, calves, and a Bonnet monkey with human rotavirus failed (1, 4, 5). In this report we describe the successful infection of gnotobiotic lambs with human rotavirus.

No prophylaxis is available for rotavirus infections in humans, although a vaccine is used to prevent calf rotavirus infections (6). However, since successful control of lamb rotavirus infections by oral administration of serum or colostrum containing antibody to rotavirus has been described (9), we investigated the possibility of controlling infection of lambs with the human rotavirus by using human immunoglobulin G (IgG) containing antibodies to rotavirus.

MATERIALS AND METHODS

Transmission of human rotavirus to lambs. Four gnotobiotic lambs were used. A stool was obtained from a 9-month-old diarrheic baby admitted to Ruchill Hospital. This child lived in an urban environment and had no known contact with any animal species in which rotavirus has been identified. Rotaviruses were observed in large numbers in the stool specimen by direct electron microscopy (EM). One part of stool was mixed with four parts of phosphate-buffered saline, and the mixture was clarified by centrifugation at $1,400 \times g$ and filtered through a membrane filter (0.45- μm pore size) to give a bacteria-free filtrate, which was given orally in 5-ml amounts to lambs 1 and 2. For subsequent passage, feces collected from lamb 1 on days 2 through 6 after

infection were pooled, extracted similarly, and administered orally in 1-ml amounts to lambs 3 and 4. All four lambs were infected when 2 days old.

The lambs were observed daily, with particular attention being paid to the consistency of the feces, samples of which were taken for EM examination. A 20% extract of the stool was made in phosphate-buffered saline, and the mixture was then clarified and centrifuged at $114,000 \times g$ for 1 h. The pellet was suspended in 2 drops of EM diluent and mixed with an equal volume of 3% potassium phosphotungstate (pH 7), and the mixture was applied to a carbon-Formvar-coated grid for EM examination. Serum was collected from lambs 1 and 2 on day 14 after infection and was tested for antibody to rotavirus in an immunofluorescence (IF) test with calf rotavirus grown in tissue culture as the antigen (7).

Portions of small intestine were removed from lambs 3 and 4 under deep sodium pentobarbitone anesthesia on days 2 and 4 after infection, respectively, the lambs were then killed, and the intestinal contents were collected. The tissues were fixed in 1% glutaraldehyde in phosphate buffer (pH 7.4). Pieces of mucosa selected for EM examination were postfixed in osmium tetroxide and embedded in Araldite. Suitable areas for ultrathin sectioning were selected by examination of 1- μm Giemsa-stained Araldite sections. The remaining glutaraldehyde-fixed tissues were transferred to 10% buffered formalin-saline and processed for light microscopy.

Treatment of lambs with IgG. Fractions from seven batches of human normal IgG were donated by the Protein Fractionation Centre of the Scottish National Blood Transfusion Service. All were tested for rotavirus antibody by the IF test. The batch selected for subsequent use had a titer of 1:160, although all batches had a titer of at least 1:40.

Five gnotobiotic lambs (5 through 9) were fed on diluted condensed cows' milk on day 1 of life. When 24 h old, lambs 5 through 7 were each given 750 mg

of human IgG orally prior to milk feeding. This treatment was given at each feed during days 2, 3, and 4 of life, with four feeds of IgG and milk being given to each lamb per day. Each lamb therefore received a total of 9 g of IgG over the 3 days. Lambs 8 and 9 were fed only diluted condensed cows' milk. When 30 h old, all five lambs were challenged orally with 3 ml of a filtered extract of a mixture of one part intestinal content from lamb 3 with four parts phosphate-buffered saline.

The lambs were examined daily, and samples of feces were taken by rectal swabs for examination by EM. Serum samples were collected at intervals and tested for rotavirus antibody by the IF test. To check whether absorption of IgG from the gut occurred, the sera were also assayed for human IgG by using Tri-Partigen-IgG immunodiffusion plates (Behringwerke AG) with appropriate standards. In routine examination of feces, no bacteria were isolated until the 7th day of life.

RESULTS

Transmission of human rotavirus to lambs.

Two days after infection with human stool filtrate, lambs 1 and 2 developed a liquid diarrhea that persisted for 3 days. No other clinical signs were apparent. Onset of diarrhea coincided with the start of rotavirus excretion, which was continuous for 6 days and sporadic for 3 additional days in both lambs. Serum collected 14

days after infection had an antirotavirus titer of $>1:40$.

Lambs 3 and 4 also developed diarrhea and excreted rotavirus. No macroscopic pathological changes were seen in these lambs. Under light microscopic examination, some villi in the ileum were shortened, spatulate, and infiltrated by macrophages. Ultrastructural examination showed that many epithelial cells of the small intestinal villi had shortened, fused, or deformed microvilli and contained cytoplasmic vesicles bounded by single membranes. Rotavirus-like particles were found in these cells and in subepithelial phagocytic cells (Fig. 1).

Treatment of lambs with IgG. The feces of the three treated lambs (5 through 7) were normal in color and consistency. Virus excretion was not detected until 48 h after infection and then continued for a mean of 4.6 days (range, 4 to 6 days).

At 24 h after infection, the two untreated lambs (8 and 9) appeared dull and had diarrhea. Excretion of rotavirus was detected in the feces of both lambs at this time and continued for a total of 7 days in each lamb.

Human IgG was not detected in the serum of any lamb when sampled on days 1 to 12 after infection. At the time of infection, none of the

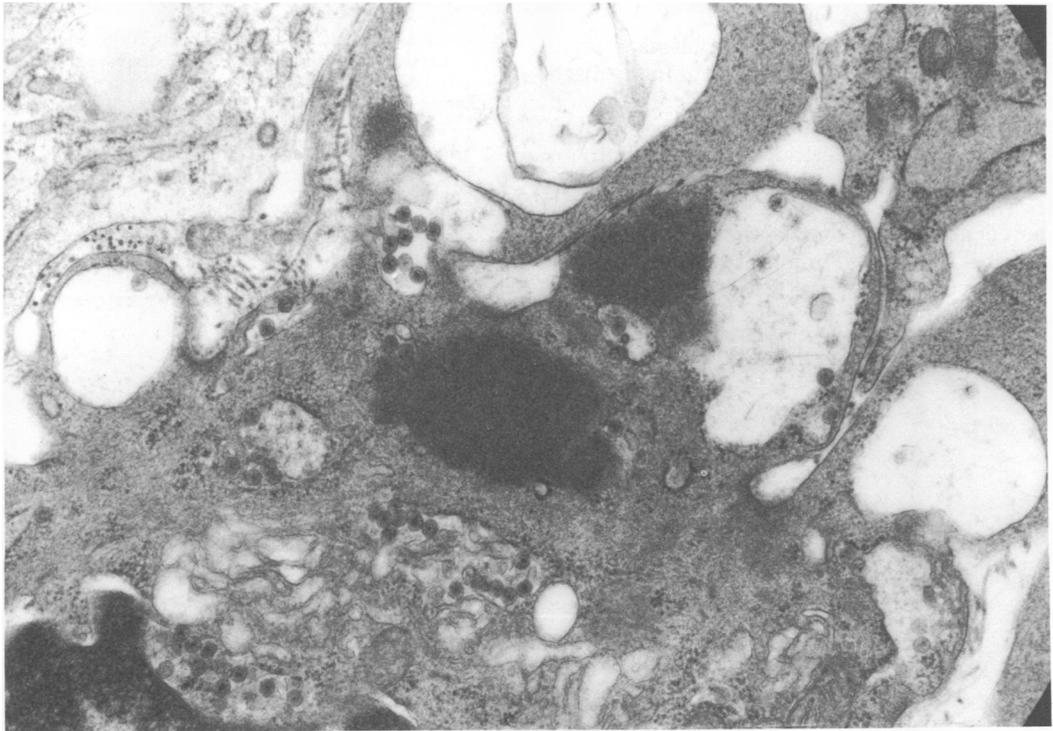


FIG. 1. Rotavirus-like particles in phagocytic subepithelial cell in ileum from lamb 3 killed 2 days after infection with human rotavirus. Lead citrate and uranyl acetate; $\times 26,450$.

five lambs had serum antibody of human or ovine origin to rotavirus by the IF test. By 12 days after infection, all lambs other than no. 5 had developed IF antibody to rotavirus.

DISCUSSION

Human rotavirus infected lambs. The production of clinical disease, the prolonged period of virus excretion, the development of antibodies, and the pathological changes detected by light microscopy and EM all suggest an active infection. Thus, gnotobiotic lambs may serve as experimental animals in the study of human rotavirus infections.

By this model system, the protective effect of human IgG given orally was assessed clinically and virologically. Clinical signs were absent from three treated lambs, whereas diarrhea occurred in all six untreated lambs. Virus excretion was delayed in onset and shortened in duration in the treated lambs, but was not reduced to the marked extent achieved in lamb rotavirus protection experiments (9). It is suggested that the presence of antibody to rotavirus in the gut partially neutralized both the initial challenge virus and virus subsequently released from infected cells. This may have reduced the level of virus infection and associated dysfunctions of absorption to the extent that no clinical disease occurred.

Human IgG is a product of plasma protein fractionation and is, at present, underutilized (J. G. Watt, personal communication). It would appear to have potential for protecting children against rotavirus infections, particularly in an outbreak in hospitalized children such as that described by Flewett et al. (3). The development of antibody in most of the protected lambs indicates that active immunity to rotavirus infections occurred by day 12. This enhances the value of the treatment, since exposure to rota-

virus during the treatment may result in subsequent immunity.

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