Diarrhoea in Mice Infected with a Human Rotavirus

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SUMMARY

Oral inoculation of newborn mice with the MET strain of human rotavirus produced transient diarrhoeal disease. Light and scanning electron microscopy showed typical rotavirus-induced morphological lesions in the villous epithelium of the small intestine consisting of extensive cytoplasmic vacuolation, villous necrosis and atrophy. Virus recovered from intestinal suspensions of infected mice showed the typical electrophoretic profile of the genome of the inoculated strain. Rotavirus antibody appeared in infected mice 10 to 20 days after inoculation but not in controls or nursing dams. The availability of a small animal model for experimental infection with human rotaviruses should prove useful for virulence and protection studies.

The study of experimental infections by human rotaviruses has been limited by the need to use animals such as calves, piglets or lambs, preferably under gnotobiotic conditions (for review, see Estes et al., 1983). With the object of developing a simpler experimental model, we have attempted to infect newborn mice with the MET strain, classified as serotype 3 subgroup II, of human rotavirus. The virus was isolated by Dr M. Thouless and kindly supplied to us by Dr T. H. Flewett (Regional Virus Laboratory, East Birmingham Hospital, Birmingham, U.K.) at its 17th passage in monkey kidney cells. It was further passed twice in MA104 cells to provide the high titre stock preparation used for mouse inoculation. The MET strain was given preference over other cell-adapted human rotaviruses due to its fast growth and pronounced cytopathic effect in MA104 cultures.

Conventionally bred Swiss SW55 pregnant mice were kindly supplied by Dr S. Thales Torres (Instituto Biomédico, Universidade Federal Fluminense, Niterói, Brazil). Upon arrival the dams were tested for the presence of serum antibodies to rotavirus by an enzyme immunoassay (EIA) using simian rotavirus SAll as the viral antigen and peroxidase-labelled rabbit anti-mouse IgG for detection. All animals were found to be seronegative for rotavirus (titre < 1/50). Ten groups of 16 mice 5 to 6 days old were orally inoculated with 0.1 ml of serial 0-5 log~o dilutions of the viral preparation containing from 10⁶ to 10² tissue culture 50% infectious doses (TCD₅₀). One group was left as an uninfected control. At days 1, 2, 3, 6, 7, 10, 15 and 20 post-inoculation, one pair of mice at each dose level were bled and, on autopsy, duodenal sections were removed for histopathological and electron microscopical examinations. The remainder of the small and large intestines was homogenized in 1 ml phosphate-buffered saline. Intestinal suspensions were prepared by three cycles of freezing and thawing, clarified by centrifugation and inoculated into MA104 cells.

Mice inoculated with 10⁵ or higher TCD₅₀ developed profuse diarrhoea from 24 to 72 h after infection. Morphological alterations consisting of enlargement and vacuolation of cells, destruction of villus tips and dilation of stromal lymphatics could be observed in duodenal sections examined 24 h after inoculation (Fig. 1a). Infection progressed in the next 2 days with increased cytoplasmic vacuolation, nuclear pyknosis, disappearance of the brush border and rupture of severely damaged epithelial cells (Fig. 1b). Lesions were restricted to the columnar absorptive cells. Crypt cells were not affected. Regeneration of the intestinal villi began at 3 days, was well advanced by 6 days (Fig. 1c) and complete, leaving no signs of previous infection,
Fig. 1. Haematoxylin-eosin-stained duodenal sections obtained at (a) 1 day, (b) 3 days and (c) 6 days after oral infection with $10^5$ TCD$_{50}$ of the MET strain of human rotavirus. Magnification $\times 240$. 
Fig. 2. Scanning electron micrographs of duodenal segments from (a) a control and (b) an infected mouse 1 day after oral inoculation with $10^5$ TCD$_{50}$ of the MET strain of human rotavirus. Bar markers represent 100 μm.

Fig. 3. Polyacrylamide gel electrophoresis of dsRNA of (a) murine rotavirus ED1M, (b) simian rotavirus SA11, (c) human rotavirus MET strain and (d) rotavirus recovered from the intestine of infected mouse. Electrophoresis was in 7.5% polyacrylamide gel in Tris–glycine buffer. The gel was stained by silver impregnation (Pereira et al., 1983).

by 10 days. The extent of intestinal damage induced by the MET human rotavirus 24 h after infection was well demonstrated by scanning electron microscopy (Fig. 2b). Almost all villi tips were affected: some still full-length villi had greatly enlarged bulky extremities compared to the slender tips of normal villi (Fig. 2a), and others were severely damaged or destroyed with accumulation of desquamated cells and cellular debris within the intestinal lumen.

The time course of clinical illness paralleled the pathological findings. Abdominal distension and lethargy were observed 15 to 20 h after inoculation, followed by overt diarrhoea at 24 to 72 h, when maximum histological lesions were observed. The severity of diarrhoea decreased on the following day, but infected mice were significantly smaller than age-matched control mice. Recovery, however, was fast and complete as most mice regained the lost weight in about a week.

Rotavirus was recovered from intestinal suspensions obtained at 1 and 2, but not at 3 days or later. The rotavirus recovered was identified as the MET strain by the characteristic electrophoretic pattern of its RNA genome which clearly distinguishes it from the rotavirus of epizootic diarrhoea of infant mice (EDIM) or other rotaviruses currently used in our laboratory (Fig. 3).
Serum antibodies specific for rotavirus were detected at a low level (1/200) on the 10th day and attained a titre of 1/1800 on the 15th and 20th day after infection. Uninoculated controls and dams, including the ones nursing infected mice, remained free of rotavirus antibody.

Inoculation of $10^{4.5}$ TCD$_{50}$ of the same virus preparation produced a delayed and shortened period of diarrhoeal illness whereas mice inoculated with $10^4$ TCD$_{50}$ did not develop overt disease, although inspection of their intestines showed loosened yellow stools on the third and fourth days after infection as opposed to the bright orange, solid stool observed in uninoculated controls. Histopathological changes, virus recovery and immune response in these mice were the same as in those infected with the higher, disease-producing, doses. Virus doses lower than $10^3$ TCD$_{50}$ seemed not to infect mice since no pathological lesions could be observed in their intestines and no serological response could be detected up to 20 days after inoculation, when the experiment was terminated.

In order to investigate whether the human rotavirus replicates in the murine gastrointestinal tract, four animals were sacrificed at short time intervals after infection of 48 mice with $10^5$ TCD$_{50}$ of the MET virus. Suspensions made from their whole intestines were titrated in MA104 cells grown in microtitre plates. Virus growth was assessed by cytopathic effect observed after staining with crystal violet and by testing fluids from individual wells by an EIA as described by Pereira et al. (1983). After a sharp drop during the first 8 h, a short exponential phase of viral growth occurred during the next 4 h followed by a gradual decline (Fig. 4). The results of the current study with a human rotavirus are similar to those obtained by us (unpublished results) and by Offit et al. (1984) after oral infection of mice with the simian rotavirus SA11. In both cases, virus infectivity in intestinal tissue never exceeded the dose inoculated but the sharp rise between 8 and 12 h is indicative of virus replication. This is supported by the extensive intestinal lesions as well as by the antibody responses unlikely to have arisen in the absence of active infection. However, the possibility of a toxic viral action in the animals inoculated with the higher doses cannot be excluded.

Similar studies now underway revealed that the Wa strain, serotype 1, subgroup II of human rotavirus (Wyatt et al., 1980) and the human–bovine reassortant virus DSI made by H. B.
Greenberg (Greenberg et al., 1981) and classified as serotype 2, subgroup I (Gerna et al., 1984) are also capable of inducing intestinal lesions in newborn mice. To our knowledge, there are no published reports describing the susceptibility of infant mice to human rotaviruses. Given the paramount importance of rotaviruses in the aetiology of gastroenteritis in children, great effort has been devoted lately to the development of an appropriate vaccine and towards a better understanding of the nature and degree of protective immunity against this group of viruses. The murine rotavirus (EDIM) has provided an excellent model for studies on the pathogenesis of rotavirus infection (Little & Shadduck, 1982), on the factors predisposing neonates and young animals to infection and diarrhoeal disease (Wolf et al., 1981; Riepenhoff Talty et al., 1982) and on the role of local and systemic antibodies on disease prevention and resolution (Sheridan et al., 1983; Eydeloth et al., 1984). The availability of a murine model for infection with human rotaviruses opens the way for the evaluation of the cross-protection afforded by a rotavirus against challenge with different human rotavirus serotypes, as well as providing a simple laboratory model for testing candidate vaccine strains.

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REFERENCES


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