Synergistic Rotavirus and *Escherichia coli* Diarrheal Infection of Mice

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Experimental or naturally acquired subclinical infections with rotavirus caused a significant increase in mortality of infant mice after challenge with enterotoxigenic *Escherichia coli* B44.

Acute infectious diarrhea is a leading cause of mortality both in children in developing countries and among domestic livestock throughout the world (1, 3, 10). Numerous human and veterinary surveys have determined that enterotoxigenic *Escherichia coli* and rotaviruses are two of the most frequently implicated enteropathogenic agents in infectious diarrhea (6, 12). Mixed infections are common, and it has been demonstrated that a simultaneous experimental infection of calves with *E. coli* and rotavirus causes diarrheal disease under circumstances in which neither pathogen alone causes diarrhea (1, 5, 11, 12).

For evaluation of novel antirotavirus agents, a small animal model of infectious neonatal diarrhea is an obvious prerequisite. We have recently shown that when 4-day-old mice are orally inoculated with enterotoxigenic *E. coli* possessing K99 or F41 pili, mortality results from a dehydrating diarrheal disease resembling that of calves (P. M. Newsome, M. Burgess, M. N. Burgess, K. A. Coney, M. E. Goddard, and J. A. Morris, submitted for publication). However, a mixed *E. coli*-rotavirus infection of neonatal mice may more closely resemble the naturally occurring disease state and allow the evaluation of a broader range of antidiarrheal agents. We therefore attempted to reproduce a mixed *E. coli*-rotavirus infection in mice.

Epizootic diarrhea of infant mice is a naturally occurring murine rotavirus infection which affects villous epithelial cells of the small intestine and has enterotoxigenic properties similar to those of calf rotavirus (7–9). As has been reported for bovine rotavirus, experimental infections with murine rotavirus tend to cause low mortality and are sometimes detected in apparently healthy animals (9, 11). The present paper describes experiments in which we infected mice simultaneously with rotavirus and enterotoxigenic *E. coli* B44.

Murine rotavirus (epizootic diarrhea of infant mice virus) was obtained from F. Brown, Animal Virus Research Centre, Pirbright, England, as a 10% clarified intestinal homogenate from infected mice. The virus was propagated in 4-day-old gnotobiotic BALB/c mice which were infected by oral administration of 50 μl of a 1:10 dilution of the virus stock. The infected mice were killed at 8 days of age, and their homogenized intestines were passed through a membrane filter (pore size, 0.22 μm) to produce a working virus stock which was stored at −70°C. The filtered virus gave a positive reaction by enzyme-linked immunosorbent assay (ELISA) at a maximum dilution of 1:1,000. *E. coli*, obtained from J. Morris, Central Veterinary Laboratories, Weybridge, England, was grown on tryptic soy agar (GIBCO Ltd., Paisley, Renfrewshire, Scotland), suspended in phosphate-buffered saline, and stored in liquid nitrogen. ELISAs for rotavirus were performed with Rotazyme tests kits (Abbott Laboratories, Isle of Sheppey, Kent, England). Intestinal homogenates (10%) of BALB/c mice (Olac Ltd., Blackthorne, Oxon, England) and MF-1 mice (D. Smith, Waltham, Surrey, England) gave negative ELISA results for rotavirus.

Mice were infected with 10⁶ CFU of *E. coli* or with rotavirus or both, essentially as described previously for *E. coli* (Newsome et al., in press). They were kept with their mothers throughout the experiment, and a daily record of mortality was kept for 10 days. Survival times were recorded with an imposed maximum of 10 days.

Mice inoculated with rotavirus alone became infected, as evidenced by the development of mild diarrhea, and showed a positive ELISA result 10 days later (Table 1). No mortality was observed in this group. *E. coli* caused severe diarrheal disease, as reported elsewhere (4, Newsome et al., in press), accompanied by 45% mortality (*P* < 0.01). However, mixed infection with *E. coli* and rotavirus caused a greater mortality than either disease agent alone. The increase in mortality was significant in comparison with all other groups (*P* < 0.01).

Since it was clear that rotavirus infection increased the susceptibility of mice to enterotoxigenic *E. coli* and antibodies to rotavirus antigen have been previously demonstrated in some strains of mice (9), we wondered whether apparent differences in susceptibility of mouse strains to *E. coli* infection (4; Newsome et al., in press) may be caused by subclinical rotavirus infection. We therefore tested two MF-1 mouse strains, which we had previously shown to differ in their susceptibilities to *E. coli* infection, for the presence of rotavirus. The ELISA results and the mortality resulting from challenge with *E. coli* B44 are shown in Fig. 1.

Our results show direct evidence (presence of rotavirus antigen) for subclinical rotavirus infection in one colony of MF-1 mice and show that this colony had greater sensitivity to *E. coli* B44 infection than did the rotavirus-free colony. The synergism, or mutual enhancement of pathogenicity, that we have observed between *E. coli* and rotavirus is predictable from the mechanisms by which these two agents are thought to cause diarrhea. Normal fluid conservation in the intestine is maintained by a fine balance of secretion and absorption. *E. coli* B44 secretes heat-stable enterotoxin A, which causes increased ileal secretion, probably from the intestinal crypts, whereas rotavirus damages the absorptive

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cells which are located at the villous tips (2, 8). In animals infected with both agents, a combination of increased secretion and impaired resorption should result in greater loss of intestinal fluid and subsequent mortality. This effect has been demonstrated previously in foals and calves (10, 12).

In humans, mixed E. coli and rotavirus infections have been noted (1, 5). For example, Guerrant et al. have reported that in Brazil one-quarter of the cases of rotavirus diarrhea surveyed were associated with simultaneous E. coli infection (5). These authors did not comment, however, on the severity of such mixed infections. The present study suggests that such infections may be particularly dangerous.

We believe this model of a mixed viral-bacterial diarrhea, together with the previously described mouse-E. coli diarrhea model (4; Newsome et al., in press), should facilitate the discovery of more effective antidiarrheal agents.

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**LITERATURE CITED**


