INFECTION AND IMMUNITY, Feb. 1985, p. 573–574
0019-9567/85/020573-02$02.00/0

Vol. 47, No. 2

Synergistic Rotavirus and *Escherichia coli* Diarrheal Infection of Mice

P. M. NEWSOME†* AND K. A. CONEY

Research Division, Beecham Pharmaceuticals, Walton Oaks, Tadworth, Surrey KT20 7NT, United Kingdom

Received 20 August 1984/Accepted 31 October 1984

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 etiological 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 antidiarrheal 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 epitheliotropic 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, Warlingham, Surrey, England) gave negative ELISA results for rotavirus.

Mice were infected with 10^6 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

* Corresponding author.
† Present address: Biosciences Research Centre, Beecham Pharmaceuticals, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ, United Kingdom.

TABLE 1. Effect of *E. coli* and rotavirus, alone or in combination, on mortality in mice[a]

Group	Treatment	No. of mice in group	Diarrhea	Mean ± SEM time (days)	Mortality					Rotavirus detected at end of expt[c]
					No. (%)	Significance (*P*) compared with group[b]				
						A	B	C	D	
A	Control	23	None	—[d]	0 (0)	—	NS	0.01	0.01	No
B	Rotavirus alone	11	Slight	—	0 (0)	NS	—	0.01	0.01	Yes
C	*E. coli* alone	22	Pronounced	6.5 ± 0.8	10 (45)	0.01	0.01	—	0.01	No
D	*E. coli* + rotavirus	36	Pronounced	3.0 ± 0.5[e]	30 (84)	0.01	0.01	0.01	—	Yes

[a] Smith MF1 mice received 10^6 CFU of *E. coli* B44 or a 1:10 dilution of working rotavirus stock or both in 50 μl of phosphate-buffered saline on day 4 of life.
[b] A 2 × 2 contingency was used. NS, Not significant.
[c] Determined by ELISA.
[d] —, No mortality during 10-day observation period.
[e] Significantly shorter than survival times of mice infected with *E. coli* alone ($P < 0.01$).

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.

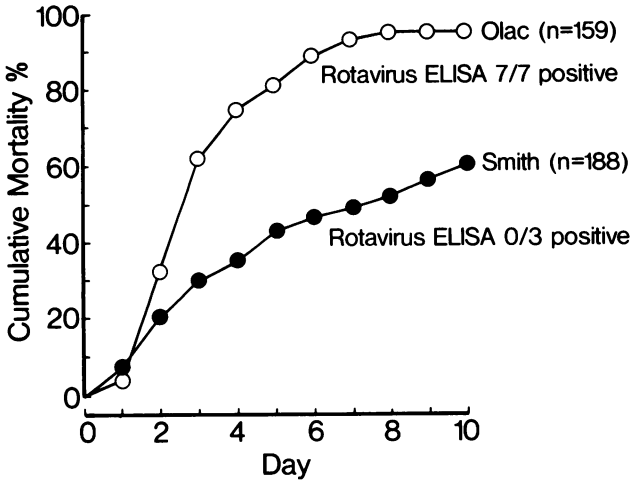

FIG. 1. Cumulative mortality of MF-1 mice from colonies with and without subclinical rotavirus infection after oral inoculation with 10^6 CFU of *E. coli* B44. Statistically significant differences were observed between ELISA results ($P < 0.05$) and between numbers of animals which died after 3 days ($P < 0.01$). Mean (± standard error of the mean) survival time was significantly shorter in mice harboring rotavirus (3.7 ± 0.2 days) than in mice which were free of rotavirus (6.4 ± 0.3 days; $P < 0.001$). Each curve represents the composite result of six separate experiments. The ELISA-positive mice showed a positive response at gut homogenate dilutions of 10^{-4} to 10^{-5}.

We thank M. Boyd and F. Brown for the gift of epizootic diarrhea of infant mice virus and L. Bannister and P. Diprose for their capable technical assistance.

LITERATURE CITED

1. **Black, R. E., M. H. Merson, A. S. M. M. Rahman, M. Yunus, A. R. M. A. Alim, I. Huq, R. H. Yolken, and G. T. Curlin.** 1980. A two year study of bacterial, viral and parasitic agents associated with diarrhoea in rural Bangladesh. J. Infect. Dis. **142**:660–664.
2. **Burgess, M. N., R. J. Bywater, C. M. Cowley, N. A. Mullan, and P. M. Newsome.** 1978. Biological evaluation of a methanol-soluble, heat-stable *Escherichia coli* enterotoxin in infant mice, pigs, rabbits, and calves. Infect. Immun. **21**:526–531.
3. **Commonwealth Agricultural Bureaux.** 1981–1984. Animal Disease Occurrence, vol. 1–4. Commission of the European Communities, Farnham.
4. **Duchet-Suchaux, M.** 1980. Le souriceau, modele d'etude de la diarrhee calibacillare. Ann. Microbiol. (Inst. Pasteur) **131B**: 239–250.
5. **Guerrant, R. L., L. V. Kirchoff, D. S. Shields, M. K. Nations, J. Leslie, M. A. de Sousa, J. G. Araujo, L. L. Correia, K. T. Souer, K. E. McClelland, F. L. Trowbridge, and J. M. Hughes.** 1983. Prospective study of diarrhoeal illness in Northeast Brazil: patterns of disease, nutritional impact, etiologies and risk factors. J. Infect. Dis. **148**:986–997.
6. **House, J. A.** 1978. Economic impact of rotavirus and other neonatal diseases agents of animals. J. Am. Vet. Med. Assoc. **173**:573–576.
7. **Kraft, L. M.** 1957. Studies of the etiology and transmission of epidemic diarrhoea of infant mice. J. Exp. Med. **106**:743–755.
8. **Moon, H. W.** 1978. Mechanism in the pathogenesis of diarrhoea: a review. J. Am. Med. Assoc. **172**:443–448.
9. **Smith, A. L., D. L. Knudson, J. F. Sheridan, and F. X. Paturzo.** 1983. Detection of antibody to epizootic diarrhoea in infant mice (EDIM) virus. Lab. Anim. Sci. **33**:442–445.
10. **Snyder, J. D., and M. H. Merson.** 1982. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. Bull. W.H.O. **60**:605–613.
11. **Tzipori, S. R., T. J. Makin, M. L. Smith, and F. L. Krautil.** 1981. Clinical manifestations of diarrhea in calves infected with rotavirus and enterotoxigenic *Escherichia coli*. J. Clin. Microbiol. **13**:1011–1016.
12. **Tzipori, S., R. Makin, M. L. Smith, and F. Krautil.** 1982. Enteritis in foals induced by rotavirus and enterotoxigenic *Escherichia coli*. Aust. Vet. J. **58**:20–23.