

Use of the K88 Antigen for In Vivo Bacterial Competition with Porcine Strains of Enteropathogenic *Escherichia coli*

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Received for publication 27 February 1975

Infant mice were used to measure the amount of fluid accumulation (enterosorption) in the intestinal tract after oral inoculation of a porcine strain of enteropathogenic *Escherichia coli* (K88⁺, Ent⁺). Significant reduction in the amount of fluid found in the intestinal tract was observed if the mice were first inoculated with a K88-possessing, non-enterotoxigenic strain of *E. coli*. The protection provided is thought to be due to specific competition for attachment sites on cells of the small intestine.

In swine, production of neonatal diarrhea has been shown to be due to the ingestion of *Escherichia coli* possessing the ability to produce enterotoxin (8, 11-12, 14). Enterotoxin, by virtue of increasing the activity of adenylcyclase in the cells of the small intestine, ultimately results in severe fluid loss (7). Cells lining other parts of the intestine, especially the colon, have been shown to be relatively refractive to the action of the toxin (12). Thus, it can be said that enterotoxigenic *E. coli* to be effective producers of disease must remain in the small intestine, preferably close to the epithelial cells most sensitive to the action of the toxin. One recognized mechanism by which this association occurs is through the attachment via fimbrial antigens (K88 antigens) to sites on the epithelial surface of the small intestine (1, 2, 4-6, 9, 13, 15, 16, 19).

This has suggested to us that prior attachment of a nontoxigenic strain to the same attachment site as that of a K88-possessing, enterotoxigenic strain would prevent disease. To test this hypothesis, a model using infant mice was developed whereby significant fluid loss into the intestinal tract resulted after oral inoculation of an enterotoxigenic, K88-possessing *E. coli* (K88⁺, Ent⁺). Experiments were designed to determine whether prior inoculation of infant mice with a non-enterotoxigenic, K88-possessing *E. coli* (K88⁺, Ent⁻) would prevent fluid loss mediated by the toxigenic strain.

MATERIALS AND METHODS

Animals. CF-1 mice (Carworth Farms) of either sex, 36 to 72 h old, were used for all experiments. The infant mice remained with their mothers at all times.

Bacterial strains. Strain P-233 is Ent⁺, K88⁺ and of serotype 0138, K81, 88ac. This strain was nalidixic

acid resistant. Strain P-66 is Ent⁻, K88⁺ and of serotype 0141, K85ab, 88ab. Strain P-66a is Ent⁻, K88⁻ and of serotype 0141, K85ab. All of the strains used were of porcine origin. Prior to inoculation of these strains, the presence or absence of K88 antigen was determined by a slide agglutination test with K88-specific antiserum.

Mode of infection. All strains of bacteria were inoculated orally. Bacterial suspensions, grown in tryptose broth, were placed in the oral cavity of the infant mice with a 0.001-ml calibrated loop.

Measurement of disease production in infant mice. Individual litters of mice were divided into two groups; one group received 10⁸ cells of strain P-233, and the other received 10⁷ cells of strain P-66. Twenty-four hours later all the mice were killed, and the ratio of the weight of the small and large intestine to total body weight was determined (gut ratio) (3).

In addition to the determination of the ratios described above, spleen, heart, and lungs were ground in Ten-Broeck tissue grinders and cultured on MacConkey agar.

Competition experiments. Individual litters of mice were divided into two groups. One group was given between 10⁶ and 10⁷ cells of strain P-66; the other group was given 10⁶ to 10⁷ cells of strain P-66a or nothing. Four hours later 1 × 10⁶ to 5 × 10⁶ cells of strain P-233 were inoculated. All of the mice were killed 20 to 24 h later and gut ratios were determined.

Kinetic experiments. Two groups of mice were used for the kinetic experiments; one group was inoculated with 10⁸ to 10⁷ cells of strain P-66, and the other was inoculated with 10⁶ to 10⁷ cells of strain P-66a. Four hours later both groups were inoculated with 10⁶ cells of strain P-233. At different time intervals after receiving strain P-233, a mouse from each group was killed, and 12-mm sections of jejunum and terminal colon were placed in sterile Ten-Broeck tissue grinders, ground, and then quantitatively placed on MacConkey agar containing 100 μg of nalidixic acid per ml.

Statistical analysis. A paired variant test was used to analyze the data.

RESULTS

The gut ratios of infant mice fed strain P-233 compared to P-66 are shown in Table 1. In all cases, those mice that received strain P-233 had a mean gut ratio >0.090, whereas those that received P-66 were <0.070. Only on rare occasions were bacteria cultured from organs, indicating that these bacteria were not invasive.

The effect of the preinoculation of strain P-66 on the gut ratios of mice that subsequently received P-233 is shown in Table 2. The gut ratios of those mice preinoculated with strain P-66 were significantly ($P < 0.001$) less than the gut ratios of mice not preinoculated. On the other hand, the gut ratios of mice preinoculated with strain P-66a were not significantly ($P > 0.05$) different than those of mice receiving P-233 alone (Table 3).

To determine the effect of preinoculation of strain P-66 on the rate P-233 moved through the intestinal tract, the ratios of the number of P-233 cells found in the jejunum to the number found in the colon were compared to the ratios obtained when mice were preinoculated with P-66a. During the first 14 h, the median ratio (number of organisms per 12-mm section of jejunum/number of organisms per 12-mm section of colon) was 0.03 if the mice were first inoculated with strain P-66 and 167 if P-66a were given first. During the period 23 to 46 h after inoculation, the median ratio was 0.07 if the mice were first inoculated with strain P-66 and 0.7 if P-66a was given first.

DISCUSSION

The K88 antigen is thought to be an important factor in enabling porcine strains of enterotoxigenic *E. coli* to attach to the epithelial surface of the small intestine (2, 10, 17, 18). In

TABLE 1. Gut ratios of infant mice 24 h after oral inoculation of either an enterotoxigenic (strain P-233) or a non-enterotoxigenic (strain P-66) strain of *E. coli*^a

Litter no.	Mean gut ratio	
	P-233 ^b	P-66 ^c
1	0.091 (6) ^d ± 0.013 ^e	0.068 (6) ± 0.007
2	0.093 (5) ± 0.015	0.058 (4) ± 0.006
3	0.101 (5) ± 0.010	0.062 (4) ± 0.005
4	0.099 (5) ± 0.007	0.069 (5) ± 0.007

^a Gut ratio = weight of intestinal tract/total body weight.

^b Inoculated with 10⁷ cells of strain P-233.

^c Inoculated with 10⁷ cells of strain P-66.

^d Number in parenthesis represents the number of mice tested.

^e Standard deviation.

TABLE 2. Gut ratios of mice preinoculated either with no bacteria or with strain P-66 (K88⁺, Ent⁻) and then orally inoculated with strain P-233 (K88⁺, Ent⁺)^a

Litter no.	Mean gut ratio	
	P-66 ^b + P-233 ^c	P-233 ^c
5	0.085 (4) ^d ± 0.010 ^e	0.122 (6) ± 0.015
6	0.071 (5) ± 0.015	0.110 (5) ± 0.020
7	0.057 (5) ± 0.004	0.085 (4) ± 0.005
8	0.062 (5) ± 0.005	0.110 (5) ± 0.012
9	0.062 (5) ± 0.008	0.094 (6) ± 0.010
10	0.061 (4) ± 0.008	0.065 (4) ± 0.006
11	0.089 (5) ± 0.010	0.144 (5) ± 0.024
12	0.057 (5) ± 0.007	0.092 (6) ± 0.013
13	0.071 (5) ± 0.020	0.104 (5) ± 0.012
14	0.049 (5) ± 0.005	0.059 (5) ± 0.008

^a Gut ratio = weight of intestinal tract/total body weight.

^b Preinoculated with 10⁶ to 10⁷ cells of strain P-66.

^c Inoculated with 1 × 10⁸ to 5 × 10⁸ cells of strain P-233.

^d Number in parenthesis represents the number of mice tested.

^e Standard deviation.

TABLE 3. Gut ratios of mice preinoculated either with no bacteria or with strain P-66a (K88⁻, Ent⁻) and then orally inoculated with strain P-233 (K88⁺, Ent⁺)^a

Litter no.	Mean gut ratio	
	P-66a ^b + P-233 ^c	P-233 ^c
15	0.105 (6) ^d ± 0.026 ^e	0.104 (5) ± 0.022
16	0.087 (5) ± 0.020	0.092 (4) ± 0.010
17	0.089 (5) ± 0.013	0.076 (4) ± 0.008
18	0.080 (4) ± 0.009	0.079 (6) ± 0.011
19	0.109 (4) ± 0.007	0.100 (6) ± 0.004
20	0.098 (5) ± 0.014	0.092 (5) ± 0.009
21	0.103 (4) ± 0.010	0.110 (5) ± 0.007
22	0.115 (3) ± 0.015	0.095 (5) ± 0.019

^a Gut ratio = weight of intestinal tract/total body weight.

^b Preinoculated with 10⁶ to 10⁷ cells of strain P-66a.

^c Inoculated with 1 × 10⁸ to 5 × 10⁸ cells of strain P-233.

^d Number in parenthesis represents the number of mice tested.

^e Standard deviation.

so doing, enterotoxigenic strains are brought in close approximation to a cell that has been shown to be most sensitive to the action of the toxin (12). The function of the K88 antigen, therefore, appears to assure that those strains that possess it will remain in the small intestine.

We have shown that mice inoculated with a

K88⁺, Ent⁺ strain will result in a significant amount of fluid accumulation, even though on occasion the amount of fluid was slightly less than established criteria (3). These variations may be explained either by genetic differences in susceptibility or by the mode of inoculation. By using litter-mates, these differences were minimized. Infant mice preinoculated with a K88⁺, Ent⁻ strain of *E. coli*, will have a significant reduction in fluid accumulation subsequent to the inoculation of a K88⁺, Ent⁺ strain. This protection does not occur with a K88⁻, Ent⁻ strain. This would imply that the K88⁺, Ent⁺ strain would have fewer sites on which to attach and, therefore, would traverse the intestinal tract quicker when compared to a situation in which the sites were not already occupied. Our findings suggest this, although the number of organisms found in the small intestine compared to the number found in the large intestine was extremely variable.

The loss of neonatal pigs and calves to colibacillosis is economically significant. Control of this disease with antimicrobial agents, husbandry procedures, or various immunological approaches has been generally unsuccessful. Although it is risky to extrapolate between different species of animals, we feel that using bacterial competition for specific attachment sites generates some exciting possibilities for prevention of this enteric disease and possibly many others.

ACKNOWLEDGMENTS

We thank Nina Wiger for technical assistance. We also wish to thank H. Williams Smith for providing strains P-233, P-66, and P-66a, and Carlton Gyles for providing anti-K88 antiserum.

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