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Development of Resistance with Host Age to Adhesion of K99$^+$ *Escherichia coli* to Isolated Intestinal Epithelial Cells

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When isolated intestinal epithelial cells from neonatal and older pigs, calves, and mice were tested for adhesion by K99$^+$ enterotoxigenic *Escherichia coli*, cells from older animals were resistant to adhesion.

Neonatal pigs, calves, and lambs are very susceptible to diarrheal disease caused by enterotoxigenic *Escherichia coli* (ETEC), but clinical infections caused by ETEC have not been reported in the adults. Colonization of the small intestine of these species is facilitated by pili (K88, K99, or 987P) which effect adherence to intestinal epithelium (5). K99$^+$ ETEC have been associated with diarrheal disease of newborn pigs, calves, and lambs. Calves and lambs become resistant to experimental challenge with K99$^+$ ETEC by 2 days of age (7). The K99$^+$ ETEC have not been reported from the diarrheal disease in pigs after weaning at 3 to 8 weeks old. In contrast, K88$^+$ ETEC are associated with diarrheal disease of pigs during both the neonatal and postweaning periods (8). Additionally, neonatal mice are susceptible to K88$^+$ and K99$^+$ ETEC but develop resistance to these strains as adults (4).

The purpose of this study was to determine the adhesion of K99$^+$ ETEC to isolated intestinal epithelial cells taken from neonatal and older pigs, calves, and mice.

Epithelial cells were isolated from the entire small intestine (3, 9) of pigs 1 day (neonatal), 3 weeks, or 6 weeks old (Fig. 1). Six of nine neonatal pigs and one of three 6-week-old pigs were colostrum deprived and reared on artificial diets in isolation. The remainder was naturally born and reared. Epithelial cells were isolated and pooled from 1-m segments of the upper, middle, and lower thirds of the small intestine of calves 12 h, 4 days, or 2 weeks old (Fig. 2). The calves were separated from the dams at birth, fed colostrum from a bottle for the first 12 h, and reared in isolation. Epithelial cells were isolated from the entire small intestine of 2-day-old, 2-week-old, or postparturient CF1 mice (ARS, Sprague Dawley, Madison, Wis.) (Fig. 3). All epithelial cells were suspended in Mg^{2+}, Ca^{2+} free phosphate-buffered saline at about 2×10^6 epithelial cells per ml and stored at 4°C until used. The *E. coli* are listed in Table 1. Strains 431 and B41 were grown on Minca agar (minus glucose plus IsoVitaleX), which enhances expression of K99 (2). Strains 263 and 123 were grown on sheep blood agar. Strain 263, which carries the K88 pilus, was used as a strongly adherent control; strain 123, which has neither K99 nor K88 pili but which may express the type 1 pilus, was used as a nonadherent control. After overnight incubation at 37°C, the bacterial cultures were suspended in 0.85% NaCl so the optical density at 430 nm was 2.2 (about 10^9 *E. coli* per ml). This suspension or a fivefold concentration was used in the adhesion tests.

The in vitro adhesion assay was conducted as previously reported (3). A trial consisted of cells from a neonate paired with cells from one or two older animals. Each *E. coli* suspension was individually mixed with an equal volume of each epithelial cell preparation so the ratio was roughly 500 or 2,500 *E. coli* per epithelial cell, depending on the concentration of *E. coli* used. Two microscopists counted the number of *E. coli* adhering (up to 70 *E. coli* per cell) to each of 20 epithelial cells in a mixture (total of 40 cells per mixture). The host age of the isolated epithelial cells and strain of *E. coli* in a mixture was unknown to the microscopists. Each trial was repeated at least three times, each on a different day with freshly grown cultures of *E. coli*. An analysis of variance was used and, when necessary, the sum of the squares was partitioned to make the desired comparisons in each species.

The results exemplified (Fig. 1) were obtained by using neonatal and 6-week-old pigs from three different herds (total of four herds tested). Results with colostrum-deprived and conventional pigs were similar and were combined for statistical analysis. From 8.8 to 14.5 (95% confidence interval) more K99$^+$ ETEC adhered to epithelial cells from the neonates than adhered to the cells from the 6-week-old pigs. Both K99$^+$ strains adhered to epithelial cells from neonatal pigs in significantly greater numbers ($P \leq 0.001$)

than strain 123 but in lower numbers than strain 263. The resistance to adhesion by K99+ ETEC, characteristic of cells from 6-week-old pigs, was not demonstrable with cells from 3-week-old pigs. The K99+ ETEC adhered to cells from the 12-h-old calf in significantly greater numbers ($P \leq 0.001$) than to cells from the 2-week-old calf (Fig. 2). Both K99+ strains adhered in significantly greater numbers ($P \leq 0.001$) to cells from the neonatal mice than to cells from the adult mice (Fig. 3). Strain 263 consistently adhered to

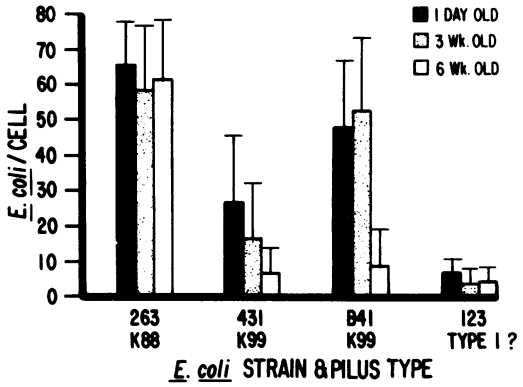

FIG. 1. *Number of E. coli adherent to intestinal epithelial cells isolated from pigs of different ages. Results with epithelial cells isolated from four pigs (1 day old), two pigs (3 weeks old), and three pigs (6 weeks old) are presented. Bars represent the mean ± one standard deviation of the number of adherent bacteria per epithelial cell. Bacteria were used at 5 × 10⁹/ml and epithelial cells at 2 × 10⁶/ml.*

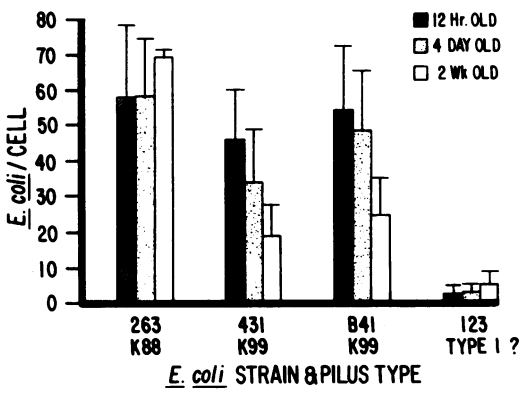

FIG. 2. *Number of E. coli adherent to intestinal epithelial cells isolated from calves of different ages. Results with epithelial cells isolated from one calf (12 h old), two calves (4 days old), and one calf (2 weeks old) are presented. Bars represent the mean ± one standard deviation of the number of adherent bacteria per epithelial cell. Bacteria were used at 5 × 10⁹/ml and epithelial cells at 2 × 10⁶/ml.*

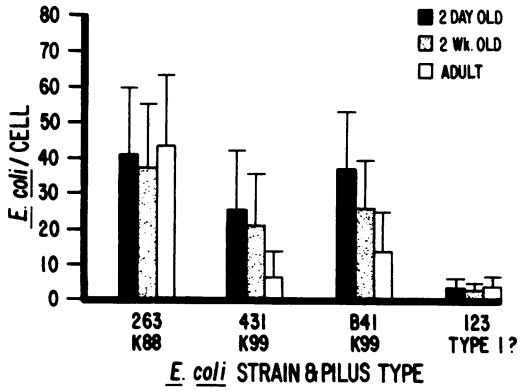

FIG. 3. *Number of E. coli adherent to intestinal epithelial cells isolated from mice of different ages. Results with epithelial cells isolated and pooled from 50 mice (2 days old), 35 mice (2 weeks old), and 10 mice (adult). Bars represent the mean ± one standard deviation of the number of adherent bacteria per epithelial cell. Bacteria were used at 1 × 10⁹/ml and cells at 2 × 10⁶/ml.*

TABLE 1. *E. coli strains with serotype, known pilus type, origin, and enterotoxigenicity*

Strain	Serotype	Pilus	Animal of origin	Enterotoxin[a]
B41	O101:K99:NM	K99	Calf	ST
431	O101:K30,99:NM	K99	Pig	ST
263	O8:K87,88ab:H19	K88	Pig	LT, ST
123	O43:K-:H28	None	Pig	None

[a] ST, Heat-stable enterotoxin; LT, heat-labile enterotoxin.

epithelial cells of the host species in much greater numbers than did strain 123. Epithelial cells from animals of different ages did not vary in their susceptibility to adhesion by these two strains.

The data support the hypothesis that epithelium in the small intestine develops resistance to K99-mediated adhesion with increasing host age. The resistance demonstrated seemed to be innate rather than antibody-mediated because: (i) cells of 6-week-old pigs from three herds resisted adhesion by K99+ ETEC but were susceptible to adhesion by K88+ ETEC even though K88 antigen occurs more commonly than K99 antigen among pig ETEC (1, 5a); (ii) cells from a 6-week-old colostrum-deprived pig were resistant to K99 mediated adhesion; (iii) a similar pattern occurred in the three species tested even though K99+ ETEC are not reported from natural diarrheal disease in mice.

ETEC from both neonatal and weanling pigs

have been tested for K99 antigen (5, 5a). To date, $K99^+$ isolates have been confined to ETEC from neonates. The resistance to adhesion reported here may occur in vivo and contribute to this age distribution in pigs. However, cells from 4-day-old calves and 2-week-old mice were susceptible to adhesion by $K99^+$ ETEC. It seems unlikely that the marked resistance to $K99^+$ ETEC which calves develop by 2 days of age and which mice develop before adulthood can be explained by resistance to adhesion as tested in this system.

Effects attributable to the *E. coli* were also observed. The acapsular strain B41 consistently adhered in greater numbers than the capsular strain 431 which may reflect masking of the K99 pilus by capsular material (6). Even with neonatal cells the $K88^+$ strains adhered in greater numbers than the $K99^+$ strains. This could reflect more avid adhesive forces, more pili, or more host cell receptors for the $K88^+$ strain than for the $K99^+$ ETEC. Paradoxically, the $K88^+$ strain adhered in very high numbers to the calf cells even though such strains have not been shown to colonize calf intestine in vivo. However, as previously reported for $K88^+$ ETEC adherent to calf intestinal epithelial cells in vitro (8), the observed adhesion occurred predominantly on basolateral membranes rather than on brush borders. Adhesion of the $K88^+$ and $K99^+$ ETEC to other host cells and the $K99^+$ to calf cells tended to be random. There was also extensive adhesion between the $K88^+$ ETEC.

In conclusion, the variation among ETEC in their host species specificity is generally recognized. Apparently, there is also variation among ETEC in their host age specificity.

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