INFECTION AND IMMUNITY, Dec. 1990, p. 4030–4035
0019-9567/90/124030-06$02.00/0

Vol. 58, No. 12

Comparison of Receptors for 987P Pili of Enterotoxigenic *Escherichia coli* in the Small Intestines of Neonatal and Older Pigs

EVELYN A. DEAN

Physiopathology Research Unit, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

Received 25 June 1990/Accepted 29 August 1990

Enterotoxigenic *Escherichia coli* isolates that express 987P pili colonize the small intestine and cause diarrhea in neonatal (<6-day-old) but not in older (>3-week-old) pigs. However, 987P$^+$ *E. coli* isolates adhere in vitro to small-intestinal epithelial cells from pigs of both ages. This indicates that older pigs as well as neonatal pigs contain receptors for 987P pili and that resistance in older pigs is not due to a lack of intestinal receptors for 987P pili. In this study, we demonstrated that 3-week-old gnotobiotic pigs, like neonatal pigs, were colonized and developed diarrhea when challenged with 987P$^+$ *E. coli*. We compared 987P receptors in small-intestinal epithelial cell brush borders and in intestinal washes (luminal contents) from <1-day-old, 3-week-old gnotobiotic, and 3- to 4-week-old weaned pigs. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose filters, and 987P binding was demonstrated by immunoassay using purified 987P pili. Multiple 987P-binding components ranging from 33 to 40 kDa were found in brush borders from both 987P-susceptible (neonatal and gnotobiotic) and 987P-resistant (older) pigs; 987P binding to these receptors, which we called 987R, did not correlate with 987P susceptibility. A <17-kDa 987P receptor, 987M, was found in the mucus fraction of intestinal washes from 987P-resistant older pigs. Only trace amounts of 987M were detected in 987P-susceptible neonatal and gnotobiotic pigs. 987M comigrated with the 987P receptor previously isolated from adult rabbits. Receptors for 987P in the mucus of older pigs may inhibit 987P-mediated intestinal colonization by preventing the attachment of 987P$^+$ enterotoxigenic *E. coli* to intestinal epithelial receptors for 987P.

Enterotoxigenic *Escherichia coli* (ETEC) strains cause diarrhea in swine during the neonatal period and immediately after weaning. An important virulence attribute of ETEC strains is their ability to colonize the small intestine. 987P, K99, and K88 pili (fimbriae) are proteinaceous, filamentous bacterial appendages that promote adherence of ETEC to the villous epithelium and thus facilitate intestinal colonization. Host cell surface characteristics also play an important role in susceptibility to ETEC diarrhea. Pilus-specific receptors must be available on the villous epithelium for pilus-mediated adherence to occur. The best evidence for the existence of pilus-specific receptors was the demonstration that some pigs are genetically resistant to K88-mediated ETEC diarrhea because they lack intestinal receptors for K88 pili (14, 23).

Some pilus-mediated adherence is age specific. Strains of ETEC that produce K99 pili (K99$^+$ ETEC) or 987P pili (987P$^+$ ETEC) are commonly associated with diarrhea in neonatal pigs but not in older pigs (17). Age-related resistance to K99$^+$ ETEC in swine is associated with an age-dependent decrease of intestinal receptors for K99 pili (16, 21). The mechanism of age-related resistance to 987P-mediated diarrhea is not known.

Age-related resistance to 987P$^+$ ETEC diarrhea has been demonstrated experimentally. Following intragastric inoculation, 987P$^+$ ETEC strains colonize the small intestine and cause diarrhea in neonatal pigs but not in older postweaning pigs (6, 7). After inoculation into ligated ileal loops, 987P$^+$ ETEC strains are associated primarily with the villous epithelium in neonatal pigs but are associated with mucus and debris in the lumens in loops of older pigs (7). However, 987P$^+$ ETEC strains do adhere in vitro to small-intestinal brush borders isolated from both neonatal and older pigs, indicating that there are 987P-specific receptors on brush borders of both neonatal and older pigs (6, 7). Thus, age-related resistance to 987P$^+$ ETEC is not associated with an absence of 987P receptors in older pigs, as in the case of resistance to K99$^+$ ETEC.

Rabbits, like older pigs, are resistant to 987P-mediated adherence and colonization in vivo. However, 987P$^+$ bacteria do bind to intestinal epithelial cells from adult rabbits in vitro, indicating that there are 987P-specific receptors on these cells (3). A glycoprotein receptor for 987P has been identified and isolated from adult rabbit small-intestinal epithelial cell brush borders (4).

We hypothesized that age-related resistance to 987P-mediated adherence and diarrhea in swine is due to age-related differences in small-intestinal receptors for the 987P pili. The objectives of the experiments described in this article were (i) to determine whether gnotobiotic pigs, like conventionally reared pigs, develop resistance to 987P$^+$ ETEC-mediated diarrhea by 3 weeks of age and (ii) to compare intestinal receptors for 987P pili in neonatal, gnotobiotic, and older pigs and adult rabbits.

(This work was presented in part at the 14th International Symposium on Microbial Ecology and Disease, San Antonio, Tex., 21 to 23 September 1989, and at the 90th Annual Meeting of the American Society for Microbiology, Anaheim, Calif., 13 to 17 May 1990.)

MATERIALS AND METHODS

Bacteria. Porcine ETEC 987 (987P$^+$, O9:K103:NM [18]) and I36, a 987P$^-$ mutant of strain 987 (12), were grown and maintained under conditions that promote 987P pilus expression (3, 11, 13). All cultures were tested for 987P piliation by slide agglutination with anti-987P serum before use (19).

Pigs. Neonatal pigs, <1 day old, were obtained by cesarean section from four crossbred swine, deprived of colos-

trum, and not fed (1). Gnotobiotic pigs, 3 weeks old, were obtained from a crossbred sow by cesarean section and maintained in isolators (25). The diet consisted of autoclaved SPF-LAC (Borden, Elgin, Ill.) given three times daily for the first week and twice daily thereafter. The pigs were started at 270 ml of SPF-LAC per day and gradually increased to 620 ml per day by 3 weeks. Older pigs, 3 to 4 weeks old, were naturally farrowed by three crossbred swine (gilts) at the National Animal Disease Center, weaned at 3 weeks of age, and fed a postweaning diet (22).

Inoculations. Gnotobiotic pigs were inoculated intragastrically at 3 weeks of age with 12 ml of half-strength tryptic soy broth containing 10^{10} CFU of *E. coli* 987 or I36, as described previously (18). Animals were sacrificed 18 h postinoculation, and sections of ileum were collected for counts of viable bacteria and for immunofluorescence and morphology studies, as previously described (18). Pigs were considered colonized if there were $>10^8$ bacteria per 5-cm segment and if bacterial layers were seen microscopically.

Purified 987P pili. Purified 987P pili were prepared from strain 987, as previously described (13). Pilus concentration was determined by A_{280} by using a molar extinction coefficient of 0.337 (unpublished data).

Anti-987P serum. Anti-987P serum and the immunoglobulin G (IgG) fraction were prepared as previously described (7, 19).

Brush borders. Small-intestinal epithelial cell brush borders were isolated from neonatal, gnotobiotic, and older pigs and from adult (>4-month-old) female New Zealand White rabbits, as previously described (3, 22, 23). The brush borders from each group, except those of the gnotobiotic pigs, were pooled before being tested for 987P receptors. The three samples from gnotobiotic pigs were tested individually. Brush borders were stored at −80°C until used.

Small-intestinal washes. Small-intestinal washes containing luminal contents were obtained from neonatal pigs, gnotobiotic pigs, older pigs, and adult rabbits by rinsing small-intestinal segments with a buffer that contained protease inhibitors (10). This method is used in our laboratory for collecting intestinal secretions for antibody determinations. At necropsy, the ends of a 1- to 2-m segment from the lower half of the small intestine were ligated, and the segment was filled with 10 ml (neonatal pigs) or 50 ml (older pigs and rabbits) of a solution containing 0.05 mg of soybean trypsin inhibitor (Sigma, St. Louis, Mo.) per ml in 25 mM EDTA (10) and placed on ice for transport to the laboratory. Upon receipt in the laboratory, the segments were placed at room temperature for 20 min, and then intestinal contents were collected and centrifuged at 650 × *g* at 4°C for 10 min. Phenylmethylsulfonyl fluoride (100 mM in 95% ethanol, prepared fresh) was added to the supernatant (10 μl of phenylmethylsulfonyl fluoride per ml of supernatant), and the supernatant was further clarified by centrifugation at 27,000 × *g* at 4°C for 20 min. The supernatant was removed, and an additional 10 μl of phenylmethylsulfonyl fluoride and 10 μl of 1% sodium azide per ml were added. This mixture was incubated for 15 min at room temperature, and then bovine serum albumin was added to 0.1% to provide an alternate substrate for any remaining proteases. All samples were stored at −80°C until used.

Intestinal mucus preparation. Intestinal washes were separated at room temperature by chromatography in 0.1 M phosphate buffer (pH 7.0) on a Sepharose CL-4B column, as described by Sherman and Boedeker (24). The column-purified void volume was subsequently referred to as the intestinal mucus preparation.

Filter blot assay for 987P receptors. Brush border and intestinal wash preparations were adjusted for protein content, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and electroblotted onto nitrocellulose, and 987P receptors were identified by an immunoperoxidase filter blot assay similar to that previously described (4), with the following exceptions. Tris-buffered saline (0.01 M Tris–154 mM NaCl, pH 7.4) was substituted for phosphate-buffered saline, avidin-conjugated anti-rabbit IgG and biotinylated horseradish peroxidase (Vectastain, Vector Laboratories, Burlingame, Calif.) replaced horseradish peroxidase-conjugated protein A, and 4-chloro-1-naphthol (0.5 mg of 4-chloro-1-naphthol per ml, 0.015% H_2O_2, and 16.7% methanol in Tris-buffered saline) replaced *o*-dianisidine as a substrate.

SDS-PAGE. Electrophoretic separations were performed by the method of Laemmli (15) with precast SDS–10 to 20% polyacrylamide minigels (Enprotech, Hyde Park, Mass.). β-Mercaptoethanol was omitted from the sample treatment buffers because it affected 987P binding to some receptors (unpublished observation).

Protein and carbohydrate estimations. Protein concentrations were estimated by the modified Lowry procedure described by Peterson (20), with bovine serum albumin as the standard. Total carbohydrate concentration was estimated by the phenol-sulfuric acid method (9) with glucose as the standard.

RESULTS

Intragastric inoculation of gnotobiotic pigs with 987P$^+$ ETEC. To determine whether 3-week-old gnotobiotic pigs, like conventionally reared pigs, are resistant to 987P$^+$ ETEC-mediated diarrhea, we challenged six gnotobiotic pigs via intragastric inoculation with ETEC 987 (987P$^+$ ETEC). Eighteen hours postinoculation, three of these pigs were dead. The three survivors had severe diarrhea and were severely dehydrated. All of these pigs were considered colonized, as there were 1.5×10^9 bacteria per 5-cm segment of ileum (geometric mean) and bacterial layers were seen on the villous epithelium (Fig. 1A). In contrast, none of three control gnotobiotic pigs challenged with ETEC I36, a 987P$^-$ mutant of strain 987, had diarrhea or were colonized. These pigs contained a mean of 3.6×10^8 bacteria per segment, but no bacterial layers were seen on the villous surfaces (Fig. 1B). These 3-week-old gnotobiotic pigs resembled neonatal pigs in their susceptibility to 987P-mediated ETEC diarrhea.

987P receptors in small-intestinal epithelial cell brush borders. Small-intestinal epithelial cell brush borders from neonatal, gnotobiotic, and older pigs were tested for 987P receptors by the filter blot assay. Brush borders from all three groups contained multiple 987P-binding components with molecular masses ranging from 33 to 40 kDa (Fig. 2). We called this complex 987R. The 987P receptor activity (i.e., the intensity of the immunoperoxidase reaction when similar amounts of protein were loaded onto the gel) of 987R appeared to be greater in brush borders from gnotobiotic and older pigs than in brush borders from neonatal pigs. Trace amounts of <17-kDa 987P receptors that were similar to 987M in intestinal washes from older pigs (Fig. 3) were detected in some, but not all, brush border preparations from pigs in all three groups. In the example shown, only the gnotobiotic pig brush borders showed <17-kDa receptor activity (Fig. 2, lane B).

987P receptors in small-intestinal washes. To determine whether there are different 987P receptors in the lumens of

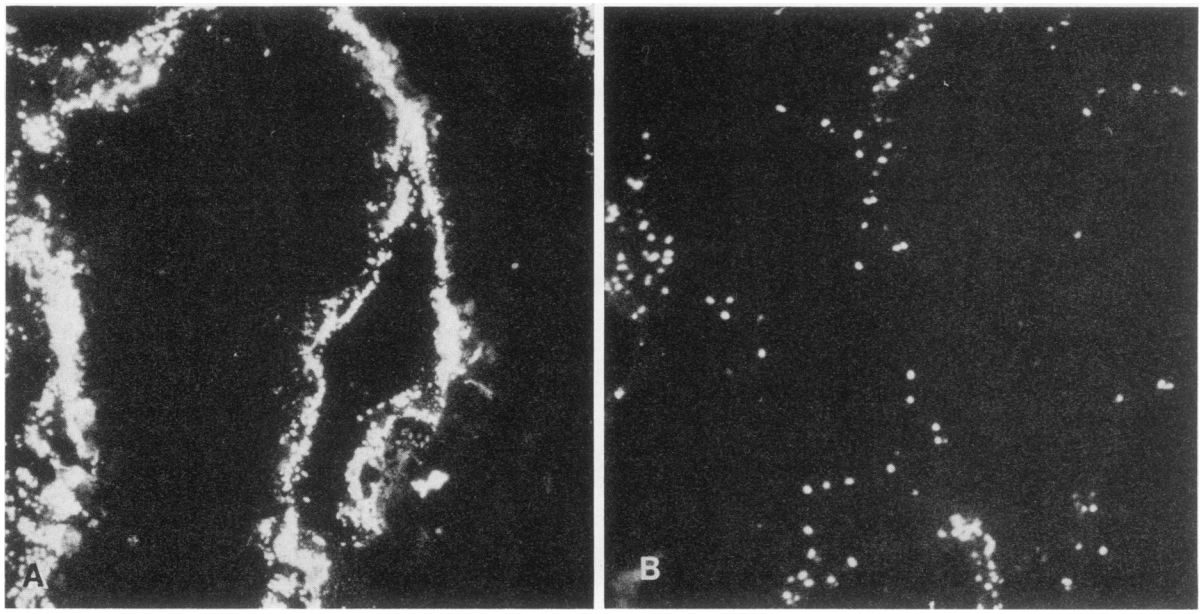

FIG. 1. Association of *E. coli* 987 with small-intestinal epithelium in gnotobiotic pigs. Frozen sections of ileum from 3-week-old gnotobiotic pigs 18 h after challenge with strain 987 (A) or I36 (B) are shown. Sections were stained with fluorescein-conjugated anti-O9:K103 serum. Most of the strain 987 bacteria were in layers adherent to villi. Most of the strain I36 bacteria were not adherent to villous epithelium but were observed in the intestinal lumen.

the small intestines of older pigs that are resistant to 987P, washes were collected from the small intestines of neonatal, gnotobiotic, and older pigs, and 987P receptors were identified by the filter blot assay. A low-molecular-mass (<17-kDa) 987P-binding component, called 987M, was seen in intestinal washes from older pigs (Fig. 3, lane C). 987M was found in the intestinal mucus preparation (void volume) after intestinal washes from older pigs were separated by Sepharose CL-4B chromatography (Fig. 3, lane D). Similar receptors were seen in neonatal and gnotobiotic pigs (Fig. 3, lanes A and B, respectively), but the 987P receptor activity in these was less than that in washes from older pigs and is not evident in the photomicrograph. Intestinal washes from older pigs contained more carbohydrate relative to the amount of protein than did washes from neonatal and gnotobiotic pigs. However, when intestinal washes were adjusted for carbohydrate concentration rather than for protein concentration, the amount of 987M in the older pigs still appeared to be greater (results not shown). None of the intestinal washes contained any 987P receptors comparable to 987R in porcine brush borders (Fig. 2).

Comparison with the 987P receptor isolated from rabbits.

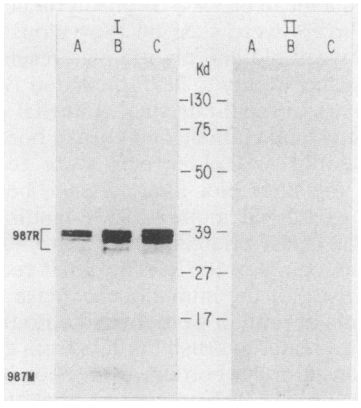

FIG. 2. Immunoperoxidase assay demonstrating 33- to 40-kDa 987P receptors (I, 987R) in small-intestinal epithelial cell brush borders isolated from neonatal (lane A), gnotobiotic (lane B), and older (lane C) pigs and <17-kDa (987M) receptors in brush borders from gnotobiotic (lane B) pigs. 987P receptors were not detected when incubation with 987P pili was omitted in the control assay (II). Assays were performed as described in the text. Each well contained 8 μg of total protein.

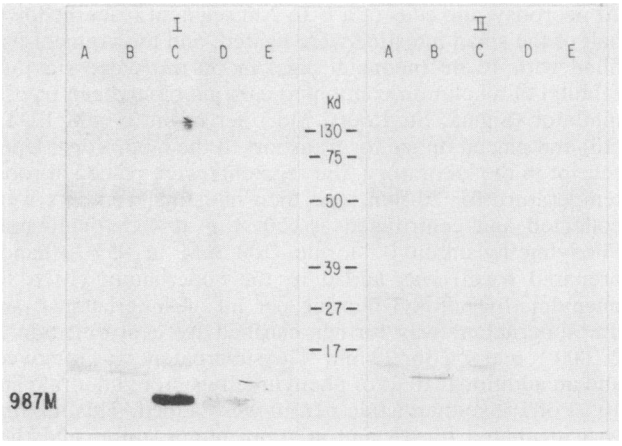

FIG. 3. Immunoperoxidase assay demonstrating <17-kDa 987P receptors (I, 987M) in small-intestinal washes obtained from neonatal (lane A), gnotobiotic (lane B), and older (lane C) pigs. 987M was present in the void volume (small-intestinal mucus fraction [lane D]) but not in the included volume (lane E) of intestinal washes from older pigs after separation by Sepharose CL-4B chromatography. 987P receptors were not detected when incubation with 987P pili was omitted in the control assay (II). Assays were performed as described in the text. Wells contained 40 (lanes A through C), 6 (lane D), or 202 (lane E) μg of total protein.

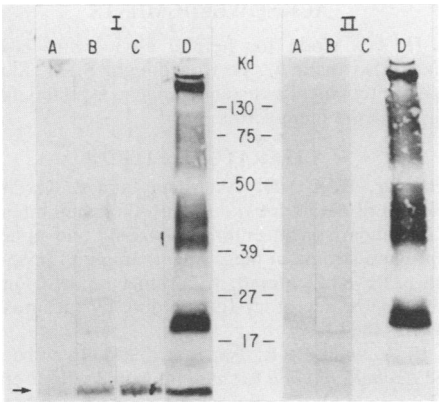

FIG. 4. Immunoperoxidase assay demonstrating 987P receptors (I, arrow) in the small-intestinal washes obtained from an older pig (lane B) and in small-intestinal brush borders (lane C) and washes (lane D) collected from adult rabbits. The 987P receptor that was previously isolated from adult rabbits is shown in lane A. 987P receptors were not detected when the 987P pili were omitted in the control assay (II). Assays were performed as described in the text. Wells contained 1 (lane A), 40 (lane B), 8 (lane C), and 50 (lane D) μg of total protein.

As shown in Fig. 4, 987M in intestinal washes from older pigs (lane B) comigrated with the 987P receptor previously isolated from rabbit brush borders (lane A) (4) and with similar 987P receptors in brush borders (lane C) and intestinal washes (lane D) collected from rabbit small intestines. There were no 987P receptors comparable to 987R in porcine brush borders (Fig. 2) in rabbit brush borders or intestinal washes.

Effects of various treatments on 987P receptor activity. Both the 987R and 987M in brush borders (Fig. 5, lane B) and 987M in intestinal washes (Fig. 5, lane G) from older pigs lost their ability to bind 987P pili after they were boiled for 1 h. Three freeze-thaw cycles, incubation for 4 h at 37°C, and incubation for 24 h at 4°C (Fig. 5, lanes C through E, respectively) did not appear to affect 987P binding by either 987R or 987M.

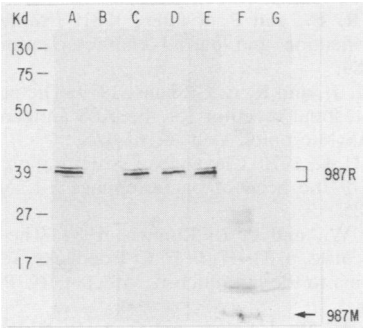

FIG. 5. Immunoperoxidase assay demonstrating the effects of various treatments on the 987P receptors (987R and 987M) in small-intestinal epithelial brush borders (lanes A through E) and intestinal washes (lanes F and G) from older pigs. Brush borders that had been stored at −80°C (lane A) were boiled for 1 h (lane B), subjected to three freeze-thaw cycles (lane C), incubated at 37°C for 4 h (lane D), or stored at 4°C overnight (lane E). The intestinal wash sample that had been stored at −80°C (lane F) was boiled for 1 h (lane G). The assay was performed as described in the text.

DISCUSSION

Strains of ETEC that produce 987P pili colonize the small intestines and cause diarrhea in neonatal pigs but not in older pigs. In this study, we found that 3-week-old gnotobiotic pigs, like neonatal pigs, were colonized and developed severe diarrhea when challenged with 987P$^+$ ETEC. Thus, unlike conventionally raised pigs, gnotobiotic pigs did not develop resistance to 987P$^+$ ETEC by 3 weeks of age.

987P$^+$ bacteria bind in vitro to brush borders isolated from both neonatal and older pigs (6, 7). This indicates that pigs of both ages have receptors for 987P pili. We hypothesized that 987P receptors in the small intestines of neonatal and older pigs are similar but that there are more 987P receptors in the small-intestinal lumens of older pigs than in neonatal pigs (7). Adherence of 987P$^+$ bacteria to receptors in the intestinal lumens of older pigs might prevent 987P-mediated intestinal colonization by preventing the attachment or facilitating the clearance of 987P$^+$ bacteria.

Consistent with this hypothesis, brush borders from pigs of both ages contained similar 987P receptors (987R) (Fig. 1) and 987P binding to 987R appeared to be greater in brush borders from 987P-resistant older pigs. However, since 987R activity in brush borders from 987P-susceptible 3-week-old gnotobiotic pigs resembled that of older (resistant) pigs rather than that of neonatal (susceptible) pigs, these age-related quantitative differences in 987R did not correlate with age-related resistance to 987P-mediated colonization.

After inoculation into ligated ileal loops, 987P$^+$ bacteria are associated primarily with mucus and debris in the lumens in loops in older pigs (7) rather than with the villous epithelium as in neonatal and gnotobiotic pigs. In this study, we found that intestinal washes from older pigs contained a low-molecular-mass 987P receptor that we called 987M. Trace amounts of 987M were also detected in neonatal and gnotobiotic pigs. The intensity of 987P binding to 987M in intestinal washes correlated with resistance to 987P-mediated ETEC diarrhea; i.e., there appeared to be more 987M in samples from 987P-resistant older pigs than in samples from 987P-susceptible neonatal and gnotobiotic pigs.

987M was found in the mucus fraction (void volume) after intestinal washes from older pigs were separated by Sepharose CL-4B column chromatography (Fig. 3, lane D). Although components in this fraction should have molecular masses in the millions, 987M had an apparent molecular mass of <17 kDa by SDS-PAGE. This suggests that 987M may be part of a larger complex in intestinal mucus or that it is a degradation product. The 987P receptor isolated from rabbits aggregates in aqueous solutions and also appears larger by size exclusion chromatography than by SDS-PAGE (4).

None of the intestinal washes examined contained any of the larger 987R that was found on brush borders. This may contradict our earlier hypothesis that 987P receptors that are released from the brush borders of older pigs are responsible for age-related resistance to 987P-mediated colonization. However, it is possible that the smaller 987M in intestinal washes represents a cleavage product of the larger 987R in brush borders. The experiments described here can neither support nor refute this hypothesis. However, there was no apparent decrease in the amount of 987R or increase in the amount of 987M after brush borders from older pigs were subjected to repeated freezing and thawing or to incubations at 4 or 37°C, conditions that might have facilitated degradation of the large receptor.

It is unlikely that 987P-specific secretory IgA antibody in

the intestinal washes of older pigs was responsible for the observed 987P binding because (i) secretory IgA does not coelute with mucus on Sepharose CL-4B column chromatography (2); (ii) the intestinal washes were collected from pigs that had not been exposed to 987P$^+$ bacteria and had not received maternal milk for at least 6 days prior to collection of intestinal washes; (iii) 987P receptors were detected in intestinal washes from gnotobiotic pigs; and (iv) anti-porcine IgA serum did not bind to the 987P receptors on Western blots (immunoblots) (unpublished observation).

The lack of correlation between in vitro adherence and in vivo intestinal colonization and diarrhea production by 987P$^+$ ETEC in older pigs is similar to that observed for adult rabbits (3). Also, antibodies against the glycoprotein receptor for 987P isolated from rabbits (4) bind to goblet cells in neonatal piglet ileum (5). We hypothesized that the small intestines of older pigs contain 987P receptors that are similar to the 987P receptors in rabbits. Consistent with this hypothesis, 987M in the intestinal washes from pigs comigrated on SDS-PAGE with the 987P receptor isolated from small-intestinal brush borders of adult rabbits (Fig. 4) (4) and with similar receptors in intestinal washes from rabbits. The 987P receptors isolated from rabbit brush borders may have originated in intestinal mucus. Like the rabbit 987P receptor, 987M appeared to contain carbohydrate; i.e., a band with a similar molecular mass band was stained with periodic acid-Schiff stain (results not shown).

Pilus-specific interactions with intestinal mucus have been reported to either enhance or inhibit intestinal colonization by enteric pathogens. Sherman and Boedeker (24) showed that RDEC-1-piliated *E. coli* strains are associated with luminal glycoproteins during in vivo infection of rabbits and coaggregate with rabbit glycoprotein preparations in vitro. They suggest that luminal glycoproteins within the mucus layer in rabbit small intestines promote intestinal colonization by serving as a site for the replication and colonization of RDEC-1-piliated bacteria. In contrast, Drumm et al. (8) demonstrated that mucus glycoproteins inhibited rather than promoted the binding of RDEC-1 *E. coli* to rabbit ileal microvillus membranes in vitro. Similarly, Laux and Cohen (D. Laux and P. S. Cohen, 14th Int. Symp. Microbial Ecol. Dis., 1989) reported that *E. coli* expressing K88 or K99 pili bind to luminal glycoproteins from mouse small intestine and colon. They suggest that specific receptors in mucus can interfere with bacterial attachment to epithelial receptors and may allow more efficient clearing of K88$^+$ and K99$^+$ bacteria.

In this study, we showed a correlation between age-related differences in 987P receptors in porcine intestinal mucus and susceptibility to 987P-mediated ETEC diarrhea. We hypothesize that 987M in the intestinal mucus protects older pigs from 987P-mediated diarrhea by preventing contact between 987P pili and 987R on the brush borders. Consistent with this hypothesis, intestinal washes from older but not from neonatal or gnotobiotic pigs agglutinated 987P$^+$ETEC in vitro (unpublished observation). This agglutination precluded the use of in vitro inhibition studies using isolated brush borders to determine whether intestinal mucus from resistant pigs can inhibit the adherence of 987P$^+$ bacteria to brush borders. Further in vivo studies are needed to determine whether intestinal mucus from older pigs can prevent 987P-mediated adherence and colonization in neonatal pigs.

ACKNOWLEDGMENTS

I thank H. W. Moon for helpful advice and histopathologic evaluations. I also thank L. E. Rohrbough, S. M. Skartvedt, and N. J. Tappe for technical assistance and A. L. Bates for assistance in preparation of the manuscript.

LITERATURE CITED

1. **Bertschinger, H. U., H. W. Moon, and S. C. Whipp.** 1972. Association of *Escherichia coli* with the small intestinal epithelium. I. comparison of enteropathogenic and nonenteropathogenic porcine strains in pigs. Infect. Immun. **5**:595–605.
2. **Crowther, R., S. Lichtman, J. Forstner, and G. Forstner.** 1985. Failure to show secretory IgA binding by rat intestinal mucin. Fed. Proc. **44**:691.
3. **Dean, E. A., and R. E. Isaacson.** 1982. In vitro adhesion of piliated *Escherichia coli* to small intestinal villous epithelial cells from rabbits and the identification of a soluble 987P pilus receptor-containing fraction. Infect. Immun. **36**:1192–1198.
4. **Dean, E. A., and R. E. Isaacson.** 1985. Purification and characterization of a receptor for the 987P pilus of *Escherichia coli*. Infect. Immun. **47**:98–105.
5. **Dean, E. A., and R. E. Isaacson.** 1985. Location and distribution of a receptor for the 987P pilus of *Escherichia coli* in small intestines. Infect. Immun. **47**:345–348.
6. **Dean, E. A., S. C. Whipp, and H. W. Moon.** 1987. The role of epithelial receptors for 987P pili in age-specific intestinal colonization by enterotoxigenic *Escherichia coli*. *In* K. Oshida (ed.), Proceedings of U.S.-Japan Cholera Conference. KTK Scientific Publishers, Tokyo, Japan.
7. **Dean, E. A., S. C. Whipp, and H. W. Moon.** 1989. Age-specific colonization of porcine intestinal epithelium by 987P-piliated enterotoxigenic *Escherichia coli*. Infect. Immun. **57**:82–87.
8. **Drumm, B., A. M. Robertson, and P. M. Sherman.** 1988. Inhibition of attachment of *Escherichia coli* RDEC-1 to intestinal microvillus membranes by rabbit ileal mucus and mucin in vitro. Infect. Immun. **56**:2437–2442.
9. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
10. **Elson, C. O., W. Ealding, and J. Lefkowitz.** 1984. A lavage technique allowing repeated measurement of IgA antibody in mouse intestinal secretions. J. Immunol. Methods **67**:101–108.
11. **Isaacson, R. E.** 1981. Pili of enterotoxigenic *Escherichia coli*, p. 213–231. *In* S. D. Acres, A. J. Forman, and H. Fast (ed.), Proceedings of the Third International Symposium on Neonatal Diarrhea. VIDO, Saskatoon, Canada.
12. **Isaacson, R. E., B. Nagy, and H. W. Moon.** 1977. Colonization of porcine small intestine by *Escherichia coli*: colonization and adhesion factors of pig enteropathogens that lack K88. J. Infect. Dis. **135**:531–539.
13. **Isaacson, R. E., and P. Richter.** 1981. *Escherichia coli* 987P pilus: purification and partial characterization. J. Bacteriol. **146**:784–789.
14. **Kearns, M. J., and R. A. Gibbons.** 1979. The possible nature of the pig intestinal receptor for the K88 antigen of *Escherichia coli*. FEMS Microbiol. Lett. **6**:165–168.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Moon, H. W., and P. L. Runnels.** 1984. The K99 adherence system in cattle, p. 31–37. *In* E. C. Boedecker (ed.), Attachment of organisms to the gut mucosa, vol. 1. CRC Press, Inc., Boca Raton, Fla.
17. **Morris, J. A., W. J. Sojka, and G. A. H. Wells.** 1982. K99 and 987P adhesins on *Escherichia coli* enteropathogenic for piglets. Vet. Rec. **111**:165–166.
18. **Nagy, B., H. W. Moon, and R. E. Isaacson.** 1976. Colonization of porcine small intestine by *Escherichia coli*: ileal colonization and adhesion by pig enteropathogens that lack K88 antigen and by some acapsular mutants. Infect. Immun. **13**:1214–1220.
19. **Nagy, B., H. W. Moon, and R. E. Isaacson.** 1977. Colonization of porcine intestine by enterotoxigenic *Escherichia coli*: selection of piliated forms in vivo, adhesion of piliated forms to epithelial

cells in vitro, and incidence of a pilus antigen among porcine enteropathogenic *E. coli*. Infect. Immun. **16**:344–352.

20. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. **83**:346–356.
21. **Runnels, P. L., H. W. Moon, and R. A. Schneider.** 1980. Development of resistance with host age to adhesion of $K99^+$ *Escherichia coli* to isolated intestinal epithelial cells. Infect. Immun. **28**:298–300.
22. **Sarmiento, J. I., T. A. Casey, and H. W. Moon.** 1988. Postweaning diarrhea in swine: experimental model of enterotoxigenic *Escherichia coli* infection. Am. J. Vet. Res. **49**:1154–1159.
23. **Sellwood, R., R. A. Gibbons, G. W. Jones, and J. M. Rutter.** 1975. Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes. J. Med. Microbiol. **8**:405–411.
24. **Sherman, P. M., and E. C. Boedeker.** 1987. Pilus-mediated interactions of the *Escherichia coli* strain RDEC-1 with mucosal glycoproteins in the small intestine of rabbits. Gastroenterology **93**:734–743.
25. **Whipp, S. C., I. M. Robinson, D. L. Harris, R. D. Glock, P. J. Matthews, and T. J. L. Alexander.** 1979. Pathogenic synergism between *Treponema hyodysenteriae* and other selected anaerobes in gnotobiotic pigs. Infect. Immun. **26**:1042–1047.