Disparate *In Vitro* Inhibition of Adhesion of Enteropathogenic *Escherichia coli* RDEC-1 by Mucins Isolated from Various Regions of the Intestinal Tract

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ABSTRACT

Escherichia coli RDEC-1 (serotype O15:H-) is a rabbit enteropathogen in which *in vivo* enteroadherence is both site specific and age related. To determine whether these differences could be related to mucins, we evaluated inhibition of binding of AF/R1 piliated RDEC-1 by mucins isolated from various segments of intestine of rabbits at different ages. Mucin was purified from intestinal crude mucus by cesium chloride serial ultracentrifugation. RDEC-1 was grown to promote the expression of hydrophobic mannose-resistant AF/R1 pili. Quantitation of *in vitro* bacterial binding was determined using a crystal violet colorimetric assay. In postweanling rabbits, inhibition of RDEC-1 binding by purified mucin derived from ileal segments ($45.1 \pm 2.6\%$, mean $\pm$ SEM) and proximal colonic segments ($46.0 \pm 5.5\%$) was less than purified mucins derived from jejunal segments ($70.0 \pm 2.0\%$) and distal colonic segments ($71.0 \pm$

3.7% , $p < 0.05$) of the intestinal tract. In all age groups, mucins derived from jejunal segments inhibited RDEC-1 binding to a greater level than mucins derived from ileal segments. In addition, inhibition of binding by mucin derived from proximal small intestine of postweanling rabbits ($70.0 \pm 2.0\%$) was greater than that of weanling rabbits ($55.2 \pm 3.5\%$, $p < 0.05$) with suckling rabbit inhibition ($62.1 \pm 3.5\%$) between these two levels. We conclude that mucin inhibition of RDEC-1 adhesion is both age and region related and therefore may contribute to both age-related and site localization of bacterial infections of the intestinal tract. (*Pediatr Res* 37: 75-80, 1995)

Abbreviations

EPEC, enteropathogenic *Escherichia coli*
ANOVA, analysis of variance

Mucin is a high-molecular-weight glycoprotein synthesized and secreted by specialized epithelial cells (*i.e.* mucous cells) present within a number of body organ systems including the gastrointestinal tract. Mucin is characterized by its large size (0.5×10^6 to 2.0×10^6 D), high content of carbohydrates [$>70\%$ (wt/wt)], and O-glycosidic bonds between N-acetylgalactosamine and either serine or threonine residues in the peptide backbone (1). Mucin is the major organic constituent of mucus, and its presence determines both the physical and gel-forming properties of mucus (1).

Mucus covers most of the surface of the gastrointestinal tract. Thus, it is interpositioned between the luminal contents of the intestine and the epithelial cells of the host. Mucus is proposed to have a number of functions including cytoprotection of the stomach, lubrication of intestinal contents, and protection against infection (1). Mucus may protect the under-

lying intestinal mucosa by a number of mechanisms, including prevention of enteric pathogen adhesion to epithelial cells. Adhesion of bacteria to epithelial cells is a complex process involving both specific and nonspecific interactions (2) that are crucial for the development of toxin delivery, colonization, and subsequent invasion (3).

The noninvasive, nonenterotoxigenic rabbit enteropathogen *Escherichia coli* RDEC-1 (serotype O15:H-) (4) has been shown to adhere to epithelial cells of the distal small intestine and proximal colon. Weanling rabbits are particularly susceptible to this enteropathogen, but postweanling animals may also develop enterocolitis. In addition to binding to mucosal cells, the organisms are also present in the mucous layer overlying surface epithelial cells of the intestinal tract (5). RDEC-1 expressing AF/R1 pili have been previously shown to bind to crude mucus and purified mucin of rabbit ileum that have been immobilized on polystyrene wells (6). Previously, we showed that purified mucins derived from colonic segments of rabbits inhibit *in vitro* adhesion of RDEC-1, whereas other components of crude mucus do not (7).

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The composition of mucins varies from one region of the gut to another. For example, mucins derived from more distal regions of the gastrointestinal tract are larger or more aggregated and contain more protein with a unique amino acid profile compared with mucins purified from more proximal regions of the intestinal tract (1). Age-related variations in carbohydrate composition, amino acid composition and lectin-binding patterns of intestinal mucins have also been described (8, 9). Despite these histochemical and biochemical differences, there have not been studies to determine functional consequences. The aim of this study was therefore to examine the effects of mucins derived from various regions of the intestinal tract at different ages on bacterial attachment using an attaching and effacing rabbit diarrheal pathogen, *E. coli* strain RDEC-1.

METHODS

Preparation of soluble rabbit mucus. Soluble mucus was prepared from mucosa of various sections of the intestinal tract from eight postweanling male (weight 3 kg), 10 weanling (5-wk-old), and 10 suckling (2-wk-old) New Zealand White rabbits (Smith's Rabbitry, Harlan, IA). Crude mucus from corresponding intestinal segments and ages were pooled to provide enough material from which adequate amounts of mucin could be purified for subsequent experimentation. Weanling and suckling rabbits were litter mates. Mucosa from the proximal third of the small intestine (jejunum) and the distal third of the small intestine (ileum) were used to prepare mucus from the animals of various ages. In postweanling rabbits, the proximal half of the large intestine (proximal colon) and distal half of the large intestine (distal colon) were also harvested. Animals were killed using intracardiac T-61 (Hoechst-Roussel Agri-Vet Co., Somerville, NJ) after intramuscular anesthesia with ketamine (Aveco Co., Fort Dodge, IA) and xylazine (Rugby Laboratories, Rockville Centre, NY). A midline abdominal incision was made to open the abdominal cavity, and the various sections of the intestinal tract were removed. The excised sections were washed with normal saline at 4°C and opened along the longitudinal axis. Mucosal scrapings were obtained by gently scraping the luminal surface with a glass slide. The scrapings were immediately weighed and placed into a solution of proteinase inhibitors [100 mL/g (wet weight)] to minimize proteolytic degradation of native mucins (10). The proteinase inhibitor solution contained 5 mmol/L EDTA (Sigma Chemical Co., St. Louis, MO), 5 mmol/L N-ethylmaleimide (Sigma), 2 mmol/L phenylmethylsulfonyl fluoride (Sigma), and 0.01% sodium azide (Sigma). Scrapings in solution were then homogenized in a Waring blender (Waring Products Division, New Hartford, CT) for 30 s. The homogenate was then centrifuged at $30\,000 \times g$ min at 4°C to remove pelleted cellular and particulate debris. The soluble supernatant from each of the various intestinal segments, defined in this study as crude mucus, was stored at -70°C until use in subsequent purification.

Purification of goblet cell-derived mucin. Crude mucus was separated into its components including goblet cell-derived mucin by isopycnic ultracentrifugation, as described by Mantle

and Allen (11). Briefly, crude mucus was suspended in a solution of cesium chloride (Fisher Scientific, Plano, TX) to a starting density of 1.41 g/mL. The suspension was placed into 35-mL polyallomer centrifuge tubes (DuPont Co., Wilmington, DE) and centrifuged in a Sorvall TFT 50.38 rotor (DuPont) at $105\,000 \times g$ for 48 h at 4°C. After ultracentrifugation, a needle was inserted to the bottom of centrifuge tubes, and eight fractions of equal volumes were collected using a peristaltic pump (Spectra/Chrom Macroflow, Spectrum Medical Industries, Houston, TX). The eight separate fractions were analyzed for buoyant density by refractometry (Abbe 3L, Milton Roy Co., Rochester, NY) and were found to range from 1.52 g/mL in the heaviest buoyant density fraction to 1.32 g/mL in the lightest fraction after both this first ultracentrifugation and a second ultracentrifugation. Buoyant density fractions were then placed into wetted cellulose dialysis tubing (SpectroPor 7, 50 000 M_r exclusion; Spectrum Medical Industries) and dialyzed against deionized water for 48 h at 4°C. Total protein concentration in each fraction was determined by the method of Lowry *et al.* (12) with BSA type V (Sigma) as standard. Glycoprotein content was determined using the periodic acid-Schiff assay with analysis by measuring OD 555 nm (13). Porcine stomach mucin type III (Sigma) was used as standard to quantitate relative mucin glycoprotein levels. Nucleic acid content was determined by measuring OD 260 nm (14). As previously described (1, 7, 15), fractions with a high content of glycoprotein *versus* total protein, a low nucleic acid content, and buoyant densities of approximately 1.45 g/mL were then pooled and ultracentrifuged a second time in cesium chloride. After the second ultracentrifugation, those fractions with a high glycoprotein-protein ratio, low OD 260 nm, and buoyant density between 1.47 and 1.41 g/mL were then pooled and used in this study as purified mucin. All steps in the preparation of crude mucus and purified mucin were performed at 4°C.

Compositional analysis of mucins. Amino acid analyses were performed by the Protein Core Facility at the University of Nebraska Medical Center using a Beckman 6300 amino acid analyzer (Beckman Instruments Inc., Palo Alto, CA). Briefly, the sample was hydrolyzed using 6 mol/L hydrochloric acid in 1% phenol at 110°C for 24 h. After hydrolysis, excess hydrochloric acid was removed under vacuum, and the sample was taken up in standard Beckman physiologic sample dilution buffer and chromatographed using Beckman physiologic buffers A, D, E, and F.

PAGE of mucins. SDS-PAGE of high-molecular-weight mucins was performed under nonreducing conditions according to the method of Laemmli (16) using a vertical slab system (0.75 mm $\times$ 8 cm $\times$ 7.3 cm) (Bio-Rad Laboratories, Richmond, CA). The separation gel contained 7.5% acrylamide, and the stacking gel contained 4% acrylamide. Mucin samples (7.5 μ g of protein) were mixed in an equal volume of 10% SDS buffer and electrophoresed through both the stacking and separating gels at a constant voltage (200 V) for 40 min. The minigel was then stained with silver (Bio-Rad silver stain kit) according to the method of Merrill *et al.* (17). Gels were calibrated with high-molecular-weight standards (Bio-Rad).

Bacteria and growth conditions. Stock cultures of RDEC-1 (serotype O15:H-) were held on Trypticase soy agar slants

(BBL Microbiology Systems, Cockeysville, MD) at 4°C. Organisms were grown in static, nonaerated Penassay broth (Difco Laboratories, Detroit, MI) to promote the expression of mannose-resistant pili, previously designated AF/R1 (18). Expression of the pili was confirmed by Western blotting of sonicated bacterial proteins against AF/R1 pilus-specific antiserum (data not shown) (19). The RDEC-1 were not serially passed, because serial passage promotes type 1 pilus expression (19). Strain M34, an isogenic mutant of RDEC-1 in which Tn5 insertions into the plasmid encoding for AF/R1 pili result in a loss of pilus expression (20), was used as a negative control for the adhesion assay. Outer membrane profiles of piliated RDEC-1 and M34 are similar after growth of the organisms in Penassay broth in both the presence and absence of an iron chelator (21). Therefore, in this study, M34 was grown in Penassay broth. Bacteria and antiserum were kindly provided by Dr. P. Sherman (The Hospital For Sick Children, Toronto, Canada).

Bacteria were harvested by centrifugation at $2500 \times g$ for 15 min. After two washes in sterile PBS (pH 7.4) at 25°C, bacteria were resuspended in PBS at a concentration of 10^{10} /mL as determined by measuring absorbance of bacteria at 600 nm.

Bacterial adhesion assay. Adhesion of bacteria was examined using a modification of an *in vitro* method described by Shea and Williamson (22). Various amounts of bacteria suspended in 0.3 mL of PBS were added to wells of a 96-well polystyrene tissue culture plate (Immulon 4; Dynatech Laboratories, Chantilly, VA). After incubation for 1 h at 37°C, nonadherent bacteria were removed by two washings with sterile PBS. To stain adherent bacteria, 300 μ L of 0.01% wt/vol aqueous crystal violet dye (Sigma) were added to the wells and incubated at room temperature for 5 min. To remove nonincorporated dye, wells were washed twice with PBS. Adherent bacteria that had incorporated the crystal violet were then lysed with a 2% wt/vol sodium deoxycholate (Sigma) detergent solution to release the dye from cell membranes into solution. Plates were then incubated while being shaken at room temperature for 1 h to ensure even distribution of the dye. Microtiter plates were then read on an EAR 340 AT microplate reader (SLT Instruments, Salzburg, Austria) at 570 nm. Values were blanked against OD 570 nm readings of wells containing only lysed bacteria, sodium deoxycholate, and PBS. All samples were run in triplicate.

To examine the inhibitory effects of mucins purified from various regions of the intestinal tract at varying ages on bacterial binding to microtiter wells, various amounts of mucin were mixed with 10^9 bacteria before their addition to microtiter wells. The total volume that was added to each well was 0.3 mL. The adhesion assay proceeded exactly as described above with the 1-h incubation and subsequent steps.

Statistics. Results are expressed as mean $\pm$ 1 SEM unless otherwise indicated. Graphs represent 95% confidence intervals. Comparisons between two groups were determined using two-tailed unpaired *t* test (23). Comparisons between multiple groups were done by using the Fisher protected least-significant-difference one-factor ANOVA at 95% confidence intervals (24).

RESULTS

Characterization of mucins. To evaluate both relative sizes of samples collected as purified mucins from various regions of the intestinal tract and purity of samples, 7.5 μ g of mucin protein were subjected to SDS-PAGE and staining with silver. Material collected as purified mucin from the proximal and distal colonic segments of postweanling rabbits did not enter the separating gels but was found at the stacking/separating gel interface. Mucin prepared from jejunal and ileal segments entered only the upper separating gels also to a level representing molecular weights of greater than 200 kD. In addition, no low-molecular-weight contaminants were visualized in these purified mucin preparations. These findings are consistent with previous published reports that higher molecular weight mucins are derived from colon than from other intestinal segments (1).

The major peptide backbone of intestinal mucins contains an enriched content of residues of the amino acids serine, threonine, and proline (1). Two minor regions of the mucin peptide core have relatively increased amounts of either aspartic acid and glutamic acid or glycine, alanine, and valine (1) residues. As shown in Table 1, the amino acid composition of both major and minor peptide regions of mucins from both large-intestinal and small-intestinal segments was comparable with previous results (1, 7, 15).

Mucin inhibition of bacterial binding. The binding of RDEC-1 to brush borders, crude mucus, purified mucins, and polystyrene wells has been previously shown to be dependent on expression of hydrophobic AF/R1 pili (7, 21). Increasing numbers of AF/R1 piliated RDEC-1 and M34 in the inoculum as estimated by measuring the absorbance at OD 600 nm correlated directly with the number of live viable bacteria determined to be present by colony forming unit determinations (established in laboratory, data not shown), and OD 600 nm measurements were used subsequently to determine inoculum size. Increased adherence of bacteria to polystyrene as measured by absorbance at OD 570 nm in the crystal violet assay directly correlated with AF/R1 piliated RDEC-1 inoculum size (Fig. 1). In contrast, increased numbers of M34 bacteria in the inoculum did not result in greater binding of M34 to the polystyrene wells (Fig. 1), thus confirming the importance of the presence of the AF/R1 pili binding to polystyrene wells that was used for our *in vitro* adhesion assay.

Table 1. Amino acid composition of mucins

Age	Mucin source	Amino acid content (mol/100 mol)					
		Ser + Thr	Pro	Asp + Glu	Gly + Ala	Val	
Suckling	J	25		27.3		19.2	
	I	31.3		23.2		15.2	
Weanling	J	27.9		25.3		15.3	
	I	31.0		26.6		15.6	
Postweanling	J	37.5		20.2		12.8	
	I	30.4		23.7		15.2	
	PC	39.6		19.1		11.8	
	DC	34.1		19.9		16.3	

J, jejunum; I, ileum; PC, proximal colon; DC, distal colon; Ser, serine; Thr, threonine; Pro, proline; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Ala, alanine; Val, valine.

Figure 1. Characterization of crystal violet colorimetric assay. Differential binding of bacterial strains for the *in vitro* adhesion assay is shown. Significantly more AF/R1-piliated RDEC-1 (○) bound to the wells than when a non-AF/R1-piliated Tn5 mutant, strain M34 (●), was used in the adhesion assay. Results are expressed as mean $\pm$ SEM of five experiments. All experiments were run in triplicate.

in these experiments. These data are similar to those data previously reported using ³H-thymidine to radiolabel bacteria to quantitate bacterial adhesion to polystyrene microtiter wells (7, 15).

Concentration-dependent inhibition of RDEC-1 binding was observed using purified mucin derived from all segments of the intestinal tract from all age groups. For example, using mucin derived from ileal segments of suckling rabbits, there was 14% inhibition of RDEC-1 binding with 1 μ g/mL mucin per well, 62% inhibition with 2.5 μ g/mL mucin per well, and complete inhibition with 5 μ g/mL mucin per well. As with suckling ileal mucin, there was virtually complete inhibition of bacterial adhesion with mucin protein concentrations of 5 μ g/mL for mucins derived from other segments at the various ages of rabbits used. To evaluate relative differences of mucin inhibition of bacterial binding, comparisons between mucins derived from the various intestinal segments were therefore based on the concentration of mucin that would allow for relative differences to be evaluated, namely 2.5 μ g/mL mucin protein, in adhesion assays.

When equal amounts of mucin (based on mucin protein determinations) were mixed with piliated RDEC-1, there were differences noted in the levels of inhibition along the intestinal tract in postweanling animals. As shown in Figure 2, inhibition of bacterial binding by mucins derived from ileal ($45.1 \pm 2.6\%$, mean $\pm$ SEM) and proximal colonic segments ($46.0 \pm 5.5\%$) of post weanling rabbits were significantly less than mucins derived from jejunal ($70.0 \pm 2.0\%$) and distal colonic segments ($71.0 \pm 3.7\%$, $p < 0.05$).

The extent of AF/R1 piliated RDEC-1 inhibition by equal amounts of mucin was greater for mucins derived from the proximal small intestine compared with mucins derived from the distal small intestine in suckling ($62.1 \pm 3.5\%$ versus $37.3 \pm 3.0\%$, $p < 0.01$), weanling ($55.2 \pm 3.5\%$ versus $35.7 \pm 4.6\%$, $p < 0.01$), and postweanling rabbits ($70.0 \pm 2.0\%$

Figure 2. Inhibition of bacterial binding by mucins from postweanling rabbits. Compared with inhibition of bacterial binding by mucins derived from jejunal and distal colonic segments, RDEC-1 binding in the presence of mucin (2.5 μ g/mL protein) derived from ileal and proximal colon segments of intestinal tract of postweanling rabbits was significantly lower (ANOVA, $p < 0.05$). Results are graphed as means with 95% confidence intervals and are based on at least 14 determinations.

versus $45.1 \pm 2.6\%$, $p < 0.01$) as shown in Figure 3. There were no differences in the degree of inhibition when comparing the three age groups for mucins derived from the distal small intestine (ANOVA, $p > 0.05$), but there was greater inhibition afforded by mucin derived from proximal small intestine of postweanling compared with weanling animals, with suckling values falling between these two age groups (ANOVA, $p < 0.05$).

DISCUSSION

These experiments explored the ability of mucins to inhibit bacterial adhesion. We showed regional differences in the capacity of mucin to inhibit AF/R1-piliated RDEC-1 binding using an *in vitro* adhesion assay and showed that these differences were also age related. The abilities of the various mucins to inhibit *in vitro* adhesion corresponded to the *in vivo* findings for the propensity of the rabbit EPEC RDEC-1 to cause infection of the ileum and proximal colon in weanling animals (5, 25).

RDEC-1 (serotype O15:H-) is an EPEC originally isolated in postweanling rabbits. RDEC-1 adhere to the ileum, cecum, and colon of infected animals (25). Under transmission electron microscopy, RDEC-1 adheres to rabbit epithelial cells *in vivo* (26) in a manner identical with the intimate attachment of both EPEC and enterohemorrhagic *E. coli* to enterocytes in humans (27). The ability of microorganisms to adhere to epithelial cells *in vitro* is directly correlated with their infectivity *in vivo* (3). This relationship holds true for the adherence of RDEC-1 to epithelial cells *in vitro* and infectivity *in vivo* (28). Infection of rabbits with AF/R1-piliated RDEC-1 results in greater morbidity and mortality than infection with nonpiliated mutants (20). The AF/R1 pilus is a hydrophobic adhesin ligand expressed on the surface of RDEC-1 (21, 29). AF/R1 pili mediate adhesion of organisms to luminal glycoproteins (5), ileal mucins (6), M cells (30), and enterocytes (18, 29) of rabbit intestine. The importance of the AF/R1 pilus in adhesion

Figure 3. Age-related inhibition of bacterial binding. Inhibition of RDEC-1 binding to polystyrene wells in the presence of mucins ($2.5 \mu\text{g/mL}$ protein) derived from jejunal segments of suckling (solid black bars), weanling (hatched bars), and postweanling (gray dotted bars) rabbits was greater in all age groups than inhibition by mucins derived from ileal segments ($a, b, c; p < 0.05$). Results are expressed as a percentage of RDEC-1 adhesion in the presence of PBS alone. Results are graphed as means with 95% confidence intervals and are based on at least 12 determinations.

was evident in the *in vitro* adhesion assay model system used in these experiments. Whereas increasing the size of the inoculum of AF/R1-piliated RDEC-1 led to greater binding as measured by absorbance measurements, the nonpiliated mutant strain M34 showed minimal adhesion to the polystyrene wells regardless of inoculum size. Thus, the RDEC-1 infection in rabbits has not only been proposed to represent an animal model of human EPEC disease but the *in vitro* assay, which takes advantage of the hydrophobic nature of the AF/R1 pilus in binding to polystyrene, allows for evaluation of the effects of mucin on adhesion.

In vivo, adhesion of intestinal pathogens to mammalian cells is a complex process. Enteroadhesion may be mediated by both specific stereochemical interactions (*i.e.* receptor-ligand) and nonspecific physicochemical interactions (*i.e.* hydrophobicity, surface charge) (2, 3, 31). AF/R1-piliated RDEC-1 is more hydrophobic than the nonpiliated mutant strain M34 (11). Increased hydrophobicity of mucosal surfaces in the colon could be a factor contributing to the proliferation and colonization of the bacterial microflora (32). We have previously shown that there are regional differences in surface hydrophobicity of intestinal and colonic mucosa in postweanling rabbits. Specifically, increased surface hydrophobicity of proximal and

distal colonic mucosa was found compared with duodenal, jejunal, and ileal small intestinal mucosal segments. Surface tension measurements of mucus derived from ileal mucosal segments was significantly greater than surface tension measurements of mucus derived from distal colonic mucosal segments (33). Because surface tension is inversely correlated with hydrophobicity, the findings suggested that mucus could be responsible for the differences noted in the mucosal surface hydrophobicity. However, as seen in Figure 2, regions of intestinal tract in postweanling rabbits with high surface hydrophobicity (*i.e.* proximal colon and distal colon) (33) show both low inhibition ($45.9 \pm 5.5\%$) with mucin from the proximal colon and higher inhibition of bacterial binding ($71.0 \pm 3.7\%$) with mucin from the distal colon. Also, jejunal and ileal intestinal segments were shown to be similar to each other with low surface hydrophobicity relative to surface hydrophobicity measurements of colonic segments (33) but showed high ($70.0 \pm 2.0\%$) and lower ($45.1 \pm 2.6\%$) inhibition of bacterial binding for mucin derived from jejunal and ileal segments in the present set of experiments. Thus, the hydrophobic character of surface mucosa does not appear to be the only reason for varying capacities of mucins from various regions of the intestinal tract to inhibit AF/R1-piliated RDEC-1 binding, but further confirmation will require the use of agents to block hydrophobic domains on soluble mucins or, alternatively, block hydrophobic domains on bacteria.

Specific stereochemical interactions also are important factors in *in vivo* adhesion of bacteria to epithelial cells. Adherence studies using RDEC-1 indicate that this strain attaches to receptors located on host epithelial cells (34). These receptors are first detected at 21 d of age and by d 35 (weanling animals) have reached adult levels (35). Adhesion of EPEC to receptors on epithelial cells facilitates the ability to elicit diarrhea. Variation in expression of eukaryotic receptors has traditionally been considered to account for these age-related differences in lapine susceptibility to RDEC-1 infection. RDEC-1 has been previously shown to adhere to rabbit jejunal brush-border membranes *in vitro*, verifying the presence of RDEC-1 receptors in rabbit jejunum (28). However, adhesion of RDEC-1 to epithelial cells in the jejunum is not observed during *in vivo* infection of rabbits (28). One possible explanation for the lack of jejunal adhesion by RDEC-1 during experimental infection of rabbits is that the organisms do not have access to mucosal receptors because they are rapidly cleared from the upper intestine. Self-filling blind loops created in the jejunum to mimic stasis were inoculated with RDEC-1 and were not found to promote increased RDEC-1 mucosal attachment (25). Another possibility may be the strategic position of mucin between the intestinal luminal contents and the underlying epithelial cells. Interference with access to mucosal receptors may be one of the mechanisms by which mucins protect the underlying epithelial cells against bacterial infection. As shown in Figure 3, mucin derived from jejunal segments in all age groups showed significantly greater inhibition of bacterial binding than did mucin derived from ileal segments, thus supporting the concept of the importance of mucin in prevention of infection.

Although this study was not designed specifically to identify the factor(s) such as apomucin isoform expression, carbohydrate composition, or posttranslational modifications such as sulfation that are responsible for mucin inhibition of RDEC-1 adhesion, some inferences may be made. It may be possible that mucin binds to an epithelial receptor used by bacteria or alternatively to specific bacterial outer membrane proteins thereby preventing the approximation of bacteria with the epithelial cell bacterial receptor. We used a polystyrene *in vitro* adhesion assay system that had a hydrophobic polystyrene target for AF/R1-piliated RDEC-1 bacteria. Because mucin inhibited this binding, the results suggests that mucin bound to specific bacterial proteins may be playing a more prominent role than mucin blocking epithelial receptors. The presence of Gal-GalNAc in both rat and human mucins that mimic cellular receptors to which *Entamoeba histolytica* lectin binds (36) suggests specific molecular mimicry of epithelial receptors within mucins. To date, a number of human mucin genes have been defined (37). Whether there is varying capacity of distinct mucin species to inhibit bacterial adhesion and whether there may be disparate regional localization of the various mucin species remain to be defined. However, the findings that the efficiency of mucins in preventing attachment of RDEC-1 varies along the intestinal tract and with age further support a specific role for mucin in mucosal host defense.

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