INFECTION AND IMMUNITY, Sept. 2006, p. 5419–5421 Vol. 74, No. 9
0019-9567/06/$08.00+0 doi:10.1128/IAI.00739-06

N-Acetyllactosamine Conjugated to Gold Nanoparticles Inhibits Enteropathogenic *Escherichia coli* Colonization of the Epithelium in Human Intestinal Biopsy Specimens

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Received 8 May 2006/Returned for modification 9 June 2006/Accepted 14 June 2006

We previously reported that the bundle-forming pilus-mediated localized adherence of enteropathogenic *Escherichia coli* to HEp-2, T84, and Caco-2 cells is inhibited by *N*-acetyllactosamine neoglycoconjugates. The results presented here extend this observation to the epithelium of biopsy specimens obtained from the human adult duodenum, terminal ileum, and colon.

Enteropathogenic *Escherichia coli* (EPEC) is a common cause of diarrhea in neonates in developing nations and in adults who travel to regions of the world where the organism is endemic (17). EPEC strains infect and colonize their hosts through a three-step interactive process that involves initial binding, signal transduction, and intimate adherence (4, 6, 8, 9, 14). In the first stage of this process, EPEC forms microcolonies on the host cell surface, a phenotype called localized adherence (7, 10) which is dependent on bundle-forming pili (BFP) expressed by the EPEC (7, 10). These BFP are also thought to be required for efficient colonization of the host during infection (3, 5).

We previously reported that *N*-acetyllactosamine (LacNAc) neoglycoconjugates inhibit EPEC localized adherence in laryngeal HEp-2 cells (21), as well as in the intestinal Caco-2 and T84 cell lines (13, 21). More recently, we demonstrated that BFP likely act as a LacNAc-specific adhesin in this processes (13). Here, we extended these tissue culture cell observations to human intestinal biopsy specimens to further explore the feasibility of using LacNAc-based glycoconjugate preparations as a potential treatment strategy in EPEC gastrointestinal infections.

After informed consent was obtained, tissue samples were obtained during colonoscopy procedures performed for colon cancer screening and/or during upper endoscopy procedures using standard large biopsy forceps. Biopsies from adjacent regions were used for pathological assessment. Only biopsy specimens with normal morphology as determined by histological review were used in this study. The method and process used for obtaining the specimens were reviewed and approved by the Ethics Review Panel of the University of Calgary. The ages and sexes of the subjects from whom biopsies were obtained are shown in Table 1. The mean age of the seven male and nine female subjects was 57 years (range, 51 years). Once removed, the biopsy samples were placed into ice-chilled phosphate-buffered physiological saline (pH 7.2) and used in the experiments within 1 h.

Polystyrene biopsy holders (Fig. 1) were custom designed so that EPEC binding could be physically restricted to the epithelial surface of the biopsy. The biopsy specimens were oriented and mounted with the luminal side up using a dissecting microscope. Thus, the mucosal biopsy involved a two-chamber system, with a bottom submucosal side and an upper luminal side. Both chambers were filled with Dulbecco modified Eagle medium during the experiments.

Prior to each experiment, BFP expression by the wild-type E2348/69 EPEC strain (13, 21) was induced by culturing the bacteria in Dulbecco modified Eagle medium as described previously (20, 21). The cultures were added to 48-well plates containing either 0.8 mg/ml LacNAc-Au or Pk-Au (13) and incubated for 30 min at 37°C in a CO_2 incubator. The method used for preparation of the LacNAc and Pk-Au compounds has been described previously (11). The medium was removed from the luminal upper compartment of a biopsy chamber, and 100 μl of the LacNAc-Au–E2348/69 or Pk-Au–E2348/69 mixture was added to the epithelial surface of a biopsy sample. After 30 min of incubation at 37°C in the CO_2 incubator, the biopsies were washed five times with ice-chilled phosphate-buffered saline to remove unbound bacteria.

To determine the number of adherent bacteria, whole genomic DNA was extracted from the intestinal biopsies using a QIAamp DNA Micro kit (QIAGEN, Mississauga, Ontario, Canada). Bacteria were quantified by performing real-time PCR of the intimin (*eaeA*) gene using the oligonucleotide primers described elsewhere (16). Real-time PCR was performed with a Rotor-Gene 3000 instrument (Corbett Research, Sydney, Australia) using a QuantiTect SYBR Green PCR kit (QIAGEN, Mississauga, Ontario, Canada) according to the manufacturer's instructions. The thermal cycling conditions used for amplifying the *eaeA* gene are described elsewhere (16). Five 10-fold dilutions of whole genomic DNA that was isolated from E2348/69 using a standard protocol (1) were used as an internal control for the PCR.

Using this approach, we observed significant variability in

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TABLE 1. Patient characteristics and number of adherent EPEC E2348/69 cells for intestinal biopsies in the presence of 0.8 mg/ml Pk-Au[a]

Patient	Age (yr)	Sex	Biopsy region	No. of adherent E2348/69 cells
00	62	M	TC	2.3×10^6
14	46	M	TC	1.4×10^7
17	74	M	TC	1.2×10^6
20	32	F	TC	2.3×10^4
22	52	M	TI	7.6×10^6
			TC	2.7×10^6
31	80	F	TC	7.3×10^6
34	47	F	Duodenum	1.2×10^3
			TI	1.5×10^5
			TC	1.2×10^4
35	67	M	TC	480
42	83	M	TC	2.7×10^6
53	44	F	TC	4.5×10^6
59	60	M	Duodenum	400
			TC	2.2×10^3
66	80	F	TC	4.4×10^3
68	38	M	Duodenum	3.4×10^5
			TI	1.4×10^6
			TC	2.0×10^5
70	73	F	Duodenum	150
			TC	970
71	48	F	Duodenum	260
			TI	1.2×10^3
84	65	F	TC	3.8×10^5
94	67	F	TC	1.2×10^7

[a] M, male; F, female; TC, transverse colon; TI, terminal ileum.

the number of EPEC cells attached to the individual biopsy specimens (Table 1). In the transverse colon biopsies alone, we observed a >5-log range in EPEC adherence; the values ranged from 480 adherent bacteria for patient 35 to 1.2×10^7 adherent bacteria for patient 94 (Table 1). For two patients, no EPEC bound to the biopsy samples (data not shown). There was no correlation between subject age or sex and the number of adherent bacteria ($P = 0.655$ and $P = 0.952$, respectively, as determined by the Pearson product-moment correlation test).

Regardless of the variability in EPEC binding, LacNAc-Au at a concentration of 0.8 mg/ml inhibited (compared to the negative Pk-Au control) EPEC adherence to the epithelial surface in all biopsies obtained from all subjects (Fig. 2) (13). The inhibitory effect was significant in the duodenal ($P = 0.002$), the terminal ileum ($P = 0.002$), and the transverse colon ($P < 0.001$) biopsy specimens (as determined by the Wilcoxon signed-rank test). The residual EPEC binding to the intestinal biopsy specimens when LacNAc-Au was present may be attributed to other potential EPEC adhesins, including a phosphatidylethanolamine-specific BFP-associated adhesin (2, 22), flagella (11), EspA (15), and intimin (18, 19).

This study allowed us to extend our previous observation that EPEC may exploit host cell LacNAc or LacNAc-related glycan receptor sequences during colonization of HEp-2, Caco-2, and T84 cells in culture to the adult human intestinal epithelium. This suggests that a BFP-associated lectin-like adhesin is likely involved in the initial attachment of EPEC to LacNAc or LacNAc-related receptors on intestinal epithelial cells in

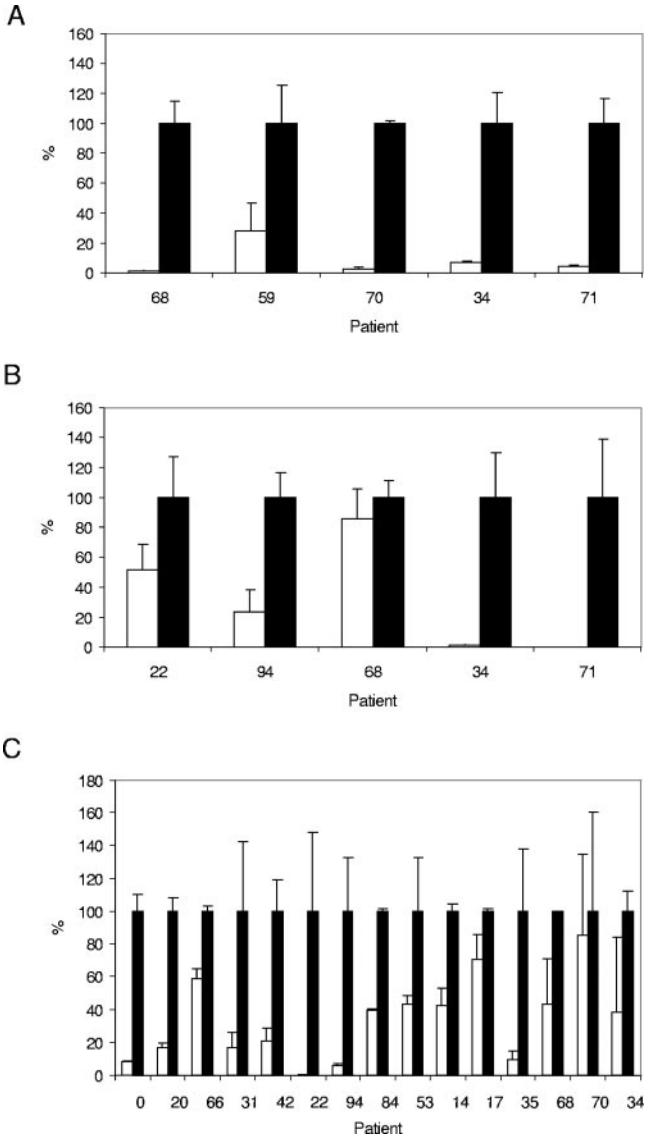

FIG. 2. E2348/69 adherence to duodenal (A), terminal ileum (B), and transverse colon (C) biopsies. The results are expressed as percentages of adherence in the presence of LacNAc-Au (open bars) relative to the adherence observed in the presence of Pk-Au (solid bars). Experiments were performed in duplicate, and the error bars indicate ranges.

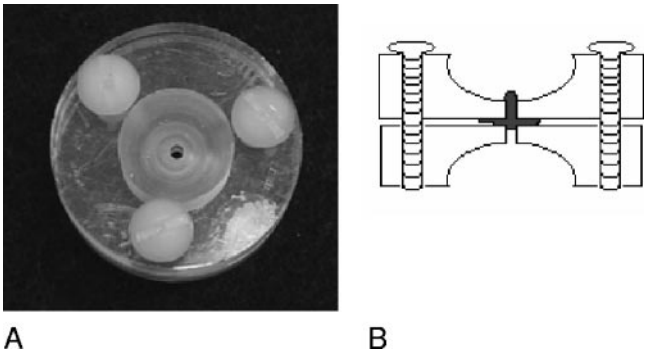

FIG. 1. Front (A) and side (B) views of the apparatus used to mount biopsy specimens so that the epithelial surface could be physically isolated from the nonepithelial cells and tissues.

adults at least. This suggestion is further supported by studies which showed that mutant EPEC strains that lack the capacity to express BFP were less virulent in human adult volunteer challenge studies (3). In contrast to these results, Hicks and colleagues (12) reported that BFP were not important mediators of EPEC early adherence to pediatric intestinal biopsy specimens when the in vitro organ culture system was used. The discrepancy between our results and those of Hicks and colleagues may be attributed to differences between EPEC attachment to adult tissues and EPEC attachment to pediatric tissues. However, in a more recent paper, Cleary and colleagues (5) reported that BFP are required for efficient colonization of pediatric intestinal biopsy tissue at early times, a conclusion that is consistent with the results reported here.

Since LacNAc-Au reduced EPEC binding to the epithelial surface of all the biopsy specimens examined, such a glycoconjugate preparation may offer a new therapeutic modality for managing patients with EPEC infections and also may have prophylactic value for subjects traveling to regions where the organism is endemic. Our results also support investigation of the therapeutic or prophylactic potential of Au-based glycoconjugates bearing specific glycan sequences implicated in the colonization strategies of other important microbial pathogens.

Financial support was provided by the Canadian Bacterial Diseases Network (G.D.A.), the Alberta Ingenuity Centre for Carbohydrate Science (G.D.A. and P.I.K.), and the Canadian Institutes for Health Research (G.D.A.). R.M.H. was the recipient of a studentship from NSERC.

G.D.A. is a coinventor on U.S. patent 6,291,435 for the treatment of diarrhea caused by enteropathogenic *E. coli*.

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Editor: A. D. O'Brien