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Adherence of *Shigella flexneri* to Guinea Pig Intestinal Cells Is Mediated by a Mucosal Adhesin

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Guinea pig colonic epithelial cells released by treating sections of the colon with solutions containing EDTA, dithiothreitol, and citrate avidly adhered *Shigella flexneri* bacteria. Separation of the intestinal cells from nonbound bacteria was achieved by differential sedimentation on a Percoll gradient. Adherence of *S. flexneri* to the colonic cells was Ca^{2+} (1 mM) and time dependent. The pH optimum was pH 6.2, and almost no attachment (<5%) was observed at low temperature (4°C). The average number of bacteria which bound to colonic cells was 70 bacteria per cell, whereas attachment to cells isolated from the ileum region was 6 bacteria per cell. Colonic cells obtained from the intestine of rabbits or rats did not adhere *Shigella*. Adherence to guinea pig colonic cells was inhibited (50%) by several carbohydrates, such as 0.1% fucose or 0.5% glucose, as well as by a lipopolysaccharide preparation (10 μg/ml) isolated from *S. flexneri*. Fixation of the bacteria with glutaraldehyde or preincubation of the bacteria with lectins or proteolytic enzymes did not affect their adherence. Proteolytic digestions or fixation of the epithelial cells, as well as pretreatments with lipopolysaccharide or fucose solutions, abolished their ability to adhere bacteria. These results indicate that a carbohydrate-binding substance on the surface of guinea pig colonic epithelial cells is responsible for the attachment of the *Shigella* bacilli.

The adherence of bacteria to epithelial cells is widely regarded as an important prerequisite for colonization and virulence manifestation of the microorganism (for recent reviews see 8 and 19). In most cases that were investigated, the attachment of bacteria to mammalian cells was mediated by bacterial cell surface appendages, such as fimbria, pili, or flagella (4). Some of these appendages have been found to possess specific carbohydrate-binding properties, sometimes named adhesins. One of the most commonly found mechanisms of bacterial adherence is mediated by cell surface appendages, such as type I pili and flagella, which enable bacteria to attach, in a mannose-sensitive manner, to erythrocytes, epithelial cells, or yeasts (17). Other sugar-specific adherence mechanisms are known. Certain uropathogenic *Escherichia coli* strains bind to Gal α(1-4) Gal components of globosides on uroepithelial cells (25), and *Vibrio cholerae* attaches to rabbit intestinal brush border membranes by a fucose-sensitive mechanism (11).

During this investigation we studied the adherence of nonpilated clinical isolates of *Shigella flexneri* to the intestinal mucosa of a number of animals. In addition to humans, another mammal that can be infected by this organism is the guinea pig (7). Our results show that the shigellae bound well to the colonic mucosa of guinea pigs and their attachment could be specifically blocked by fucose or glucose. In contrast to other bacterial adherence systems, the carbohydrate-sensitive binding component or adhesin that mediates the attachment was not detected on the *Shigella* cell surface but on the intestinal cell.

MATERIALS AND METHODS

Microorganisms. Clinical isolates of *S. flexneri* (1b), *Shigella sonnei*, *E. coli* strains 055, 0111, 0112, 0114, 0125, 0126, and 0147, were obtained from G. Altmann of the Microbiological Laboratories, Tel Hashomer Hospital, Israel. *S. flexneri* strain 2a M4243 was obtained from D. Kopecko, Washington, D.C.; *S. flexneri* 3a was obtained from E. Romanowska, Poland. *Shigella* strains were grown in Luria broth supplemented with 5 mM $CaCl_2$ and 0.2% glucose. Cells were stored at −70°C as described (10). Their virulence was repeatedly tested by the guinea pig eye inoculation technique (21). One of the *S. flexneri* (1b) strains was used throughout these experiments. *E. coli* strains were grown in 1% peptone (Difco Laboratories, Detroit, Mich.) 0.5% yeast extract, and 0.5% NaCl.

Preparation of radiolabeled bacteria. *S. flexneri* cells were inoculated in Luria broth supplemented with 0.5% glucose, 5 mM $CaCl_2$, and 0.1 mCi of [^{14}C]-glucose per ml (239 mCi/mmol; The Radiochemical Centre, Amersham, England) and incubated at 37°C overnight. The bacteria were harvested by centrifuga-

tion (9,000 × *g*, 10 min) and washed three times by suspension in saline and sedimentation as above. The specific activity of the bacteria obtained was between 1×10^3 and 2×10^3 bacteria per cpm. Other strains of *Shigella*, wherever tested, were similarly labeled. The *E. coli* cells were labeled in their respective media with the additon of [^{14}C]glucose, as described for *Shigella*. In all of these experiments more than 90% of the radiolabeled precursor was incorporated into macromolecular (precipitable in 5% trichloroacetic acid) components of the bacteria.

Preparation of colonic epithelial cells. Dunkin-Hartley noninbred guinea pigs (2 months old, 400 to 500 g) were sacrificed by cervical dislocation. The colon was removed, thoroughly washed with a prewarmed (37°C) solution of 0.154 M NaCl and 1 mM dithiothreitol. The intestinal epithelial cells were released essentially as described by Weiser (26). The end of the colon was tied with surgical thread and filled with 50 mM phosphate buffer (pH 7.3) containing 27 mM sodium citrate and incubated for 15 min at 37°C. The solution was discarded, and the intestine was refilled with a prewarmed (37°C) solution containing phosphate-buffered saline (pH 7.2), 1.5 mM EDTA, and 0.5 mM dithiothreitol and incubated for 30 min at 37°C. The solution containing suspended mucosa cells was carefully removed, and the procedure, including the incubation, was repeated once more. The epithelial cell suspensions were combined, and the cells were sedimented by centrifugation (500 × *g*, 10 min), suspended, and washed with phosphate-buffered saline devoid of Ca^{2+} or Mg^{2+} and containing 1.5 mM EDTA. After the wash the cells were suspended in 20 mM morpholineethanesulfonic acid buffer (MES) (pH 6.2) containing 140 mM NaCl and 1 mM $CaCl_2$. Experiments in different pH solutions were done by suspending the cells in phosphate buffer of the desired pH. Intestinal epithelial cells from rabbits and rats were obtained by the incubation of the respective intestinal section by the above procedures. The viability of the epithelial cells was monitored by trypan blue exclusion.

Assay for adherence of bacteria to suspensions of epithelial cells. A suspension of epithelial cells (200 µl; 5×10^6 cells per ml) was incubated with radiolabeled bacteria (100 µl; 2×10^9 bacteria per ml) for 45 min at 37°C in a rotating rack (16 rpm). The interaction between the cells was terminated by adding saline (2.5 ml) and sedimentation of the epithelial cells (500 × *g*, 5 min). The supernatant containing mainly nonadhered bacteria was discarded. Separation of epithelial cells from the remaining nonadhered bacteria was achieved by a discontinuous density gradient centrifugation with Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden). A Percoll stock solution (100%) was prepared by the addition of 1 part of 10× concentrated physiological salts solution to 9 parts of Percoll (final osmolality, 230 mOs/kg of water). Further dilutions of the Percoll from stock solution were done with saline. The gradient was built on top of the sediment consisting of epithelial cells and bacteria by layering 0.5 ml of 100% Percoll followed by 1 ml of 60%, 1.5 ml of 40%, 2 ml of 30%, and 0.5 ml of 10% Percoll. After forming the discontinuous gradient, centrifugation was carried out at 5,000 × *g* for 15 min at room temperature. Under these conditions bacteria layered between 100 and 60% and were not observed in the epithelial cell layer that banded at 30%. The epithelial cell layer was collected with a Pasteur pipette and counted for radioactivity in a Triton X-100 toluene-based scintillation fluid. All of the experiments were done in triplicate.

Assay for adherence of bacteria to intact intestinal cell surfaces. Guinea pig intestinal sections containing the epithelial layer facing upwards were obtained after washing the intestine with saline and performing a longitudinal incision. The stretched tissue was placed on a flat Lucite surface with the mucosa facing upwards. A Lucite cover containing three round holes (radius = 0.35 mm, surface area = 0.38 cm^2) was screwed on top of the surface so that wells were formed and the exposed mucosa was accessible to incubation with radiolabeled bacteria added to the wells. Radiolabeled bacteria (2×10^8 in 100 µl of 20 mM MES buffer [pH 6.2] containing 140 mM NaCl and 1 mM $CaCl_2$) were added to the wells, and the instrument was incubated (37°C, 30 min) in a humid chamber. The reaction was terminated by washing the tissue three times with saline. The tissue was taken out of the instrument, the area of the intestinal mucosa that had been exposed to the labeled bacteria was cut, placed in a tube containing 1 ml of 2% sodium dodecyl sulfate, and heated at 100°C for 15 min. The radiolabeled bacteria were solubilized under these conditions (2), and their radioactivity was determined as described before.

Preparation of LPS. Lipopolysaccharide (LPS) was prepared from *S. flexneri* by the phenol-water extraction procedure described by Westphal and Jann (27). A portion of the LPS (20 mg) was also treated with 1 ml of alkali (5% KOH in methanol) for 60 min at 60°C. After this treatment, 1 ml of water was added, and the solution was acidified with HCl to pH 5. Extractions of the fatty acids were done with hexane (three times), and the remaining water phase was extensively dialyzed against water and lyophilized.

Another portion of the phenol-extracted LPS was oxidized with 10 mM sodium metaperiodate in 0.2 M sodium acetate buffer (pH 4.5) for 1 h in the cold. The oxidation was terminated by adding excess ethylene glycol and extensive dialysis. Carbohydrate composition of the LPS was determined by gas chromatography (3).

Polysaccharide from *E. coli* strain O1:K1 (polyneuraminic acid) and from *E. coli* strain 08 containing Man α(1-2) Man structures were kind gifts from K. Jann of the Max Planck Institute, Freiburg, West Germany. Human lactotransferrin and a glycopeptide fraction isolated from lactotransferrin containing 14 mol% fucose were kind gifts from J. Montreuil, Lille, France.

Scanning electron microscopy. Colonic epithelial cells were incubated with *S. flexneri*, as described before, and separated on a Percoll gradient. The epithelial cells were washed in saline and fixed with 1.5% glutaraldhehyde for 1 h at room temperature. After washing, the cells were incubated in 0.1 M sodium cacodylate buffer (pH 7.2) containing 1% osmium tetroxide for 30 min. The cells with adhered bacteria were fixed to planchets coated with gold and examined with a Jeol JSM-35C scanning electron microscope.

Pretreatments of cells and bacteria. Glutaraldehyde fixation was done in 0.25% glutaraldehyde for 30 min at room temperature. Proteolytic digestions included trypsin (2 mg/ml, 15 min) or pronase (2 mg/ml, 15 min) or both. Heat inactivation was done for 15 min in a

water bath at 100°C. Digestion with 1 U of neuraminidase (*V. cholerae*; Behring Institute) was done at 37°C in sodium acetate buffer (pH 5.5) for 15 min. Interaction of cells with lectins, concanavalin A, wheat germ agglutinin, and fucose-binding protein (Miles Yeda, Rehovoth, Israel) was done by incubating the cells with the lectin (0.2 mg/ml) at 37°C for 15 min.

RESULTS

Elecron micrographs of the clinical strain of *S. flexneri* 1b cells used in these studies did not reveal any cell surface appendages, such as pili or flagella. The bacteria did not agglutinate erythrocytes of human types (A, B, O), rabbit, or guinea pig, and they also did not agglutinate yeast cells (17).

The adherence of *Shigella* to guinea pig colonic epithelial cells is shown in Fig. 1. The micrographs did not clearly show whether the bacteria were bound directly to the cell surface or to a thin mucous layer on the epithelial cells.

Radioactively labeled *S. flexneri* cells adhered well to guinea pig colonic epithelial cells. (Fig. 2). The attachment reaction required 1 mM Ca^{2+} ions, and these could not be replaced by Mg^{2+} or Mn^{2+}. The optimal pH for adherence was pH 6.2, and the attachment was temperature and time dependent (Fig. 3). Adherence of bacteria began to decrease after 60 min of incubation.

Epithelial cells released from various regions of the guinea pig intestinal tract markedly differed in their ability to bind *Shigella* (Table 1). The epithelial cells which most avidly bound the bacteria were those from the colon, especially from the descending section of the colon. The average number of bacteria that adhered to the colonic cells was 70 bacteria per cell, whereas binding to the epithelial cells from the ileum was only 6 bacteria per cell. Only the colonic cells of the guinea pig displayed attachment capability. Colonic cells taken from rats, hamsters, or rabbits did not have any significant attachment capability (Table 2).

Incubation of guinea pig colonic cells with *Shigella*, under our experimental conditions, did not lead to any phagocytosis of the bacteria. Electron micrographs of thin sections of epithe-

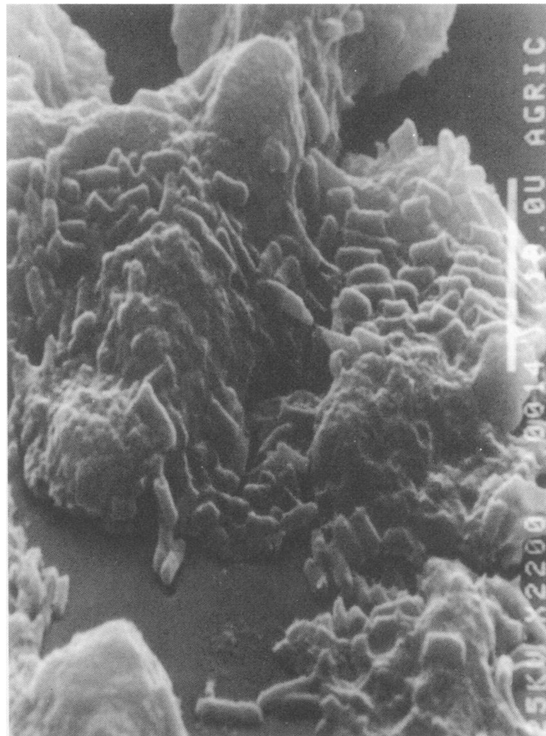

FIG. 1. Adherence of *S. flexneri* 1b bacilli to guinea pig colonic epithelial cells as seen by scanning electron microscopy. Colonic epithelial cells were incubated with bacteria as described in the text and separated on a Percoll gradient. The epithelial cells were washed in saline and fixed with (1.5%) glutaraldehyde for 1 h at room temperature. After being washed, the cells were incubated in 0.1 M sodium cacodylate buffer (pH 7.2) containing 1% OsO_4 for 30 min. The cells with the adhered bacteria were fixed to cover slips coated with gold and examined with a Jeol JSM-35 scanning electron microscope. ×1,320

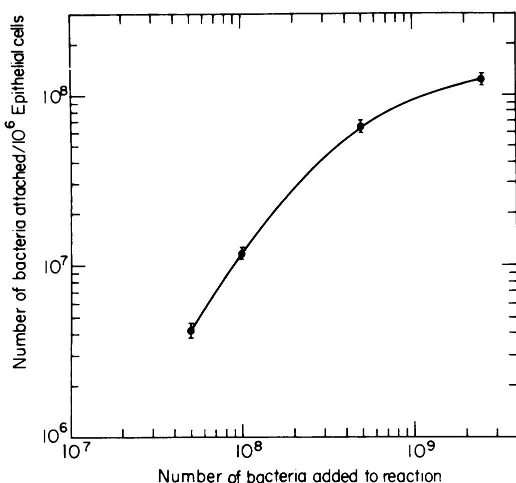

FIG. 2. Adherence of *S. flexneri* 1b bacilli to colonic epithelial cells. Incubations were carried out at 37°C for 45 min with radiolabeled bacteria, as described in the text. Nonadhered bacteria were separated by a Percoll gradient.

lial cells did not reveal any bacteria inside the cells (data not shown). A number of other *S. flexneri* 1b isolates, as well as strain M4243 (serotype 2a), were tested for their adherence to epithelial cells, and all were found to adhere at levels very comparable (±15%) to those of *S. flexneri* 1b, with which most experiments were done.

Inhibition of Shigella adherence. The adherence of *Shigella* to guinea pig colonic cells was inhibited by fucose and glucose (Fig. 4). Fifty percent inhibition of attachment was obtained at 0.8 mg of fucose per ml (either L or D) or 2.7 mg of D-glucose per ml. Other monosaccharides, such as mannose, had some inhibitory activity, but higher concentrations (10 mg/ml) were required to obtain a similar effect (Table 3).

The adherence of *Shigella* to the epithelial cells appeared to be reversible. The addition of 1% fucose to cells that had been incubated with *Shigella* for 40 min removed over 90% of the attached bacteria. The small amount of *Shigella* that adhered to intestinal cells isolated from the ileum, cecum, or from the ascending colon could not be inhibited by the addition of fucose (Table 1).

A large number of high-molecular-weight glycoconjugates were tested for their ability to inhibit the adherence reaction. The only glycopeptides which had an inhibitory effect were the fucose-containing ones from lactotransferrin (16). Addition of this glycopeptide (1 mg/ml), which contains 14 mol% fucose, inhibited the adherence of *Shigella* by >80%.

Carbohydrate analysis of the phenol-extracted LPS of *S. flexneri* cells revealed that it contained rhamnose, glucose, and *N*-acetylglycosamine in molar ratios of approximately (1:2:0.5), with only traces of protein (<2%) (24). Apparently due to their glucose content, the *Shigella* cells were well agglutinated by concanavalin A. The *Shigella* LPS was a very potent inhibitor of the adherence of intact *Shigella* to the guinea pig colonic cells. Fifty percent inhibition of adherence was achieved by 7 μg of *Shigella* LPS per ml (Fig. 5). Other bacterial cell surface polysaccharides were also tested for their inhibitory activity. The lipopolysaccharide from *E. coli* strain 08 containing the oligosaccharide of mannose [Man α(1-2)Man] (13) inhibited 90% of the *Shigella* adherence of 5 mg/ml. The polysaccha-

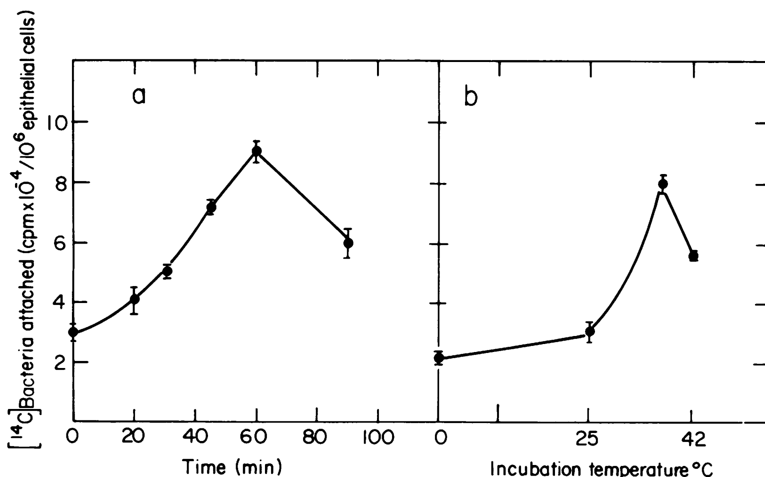

FIG. 3. Adherence of *S. flexneri* 1b bacilli to colonic epithelial cells. (a) Time dependence; (b) temperature dependence. In these experiments the ratio between added bacteria and colonic cells was 200:1.

TABLE 1. Affinity of adherence of *S. flexneri* to guinea pig intestinal tract sections[a]

Region in the intestinal tract	Bacteria per cell	% Inhibition by 1% fucose
Duodenum	3 ± 1	<3%
Jejunum	3 ± 1	<3%
Ileum	6 ± 2	<3%
Ascending colon	17 ± 3	20 ± 3
Transverse colon	52 ± 5	64 ± 2
Descending colon	78 ± 7	85 ± 5

[a] The guinea pig intestinal tract was washed and dissected into regions. Each region was ligated separately, and the epithelial cells were collected (see the text) and suspended at the same concentration in buffered saline-MES (pH 6.2) with 1 mM $CaCl_2$. The epithelial cells (10^6) were incubated with ^{14}C-labeled *Shigella* (2×10^8, 1.3×10^3 bacteria per cpm) at 37°C for 45 min in the absence or presence of 1% fucose. The percent inhibition was calculated from the difference between the attachment of *S. flexneri* in the presence or absence of fucose. Results represent standard error of the mean of three determinations.

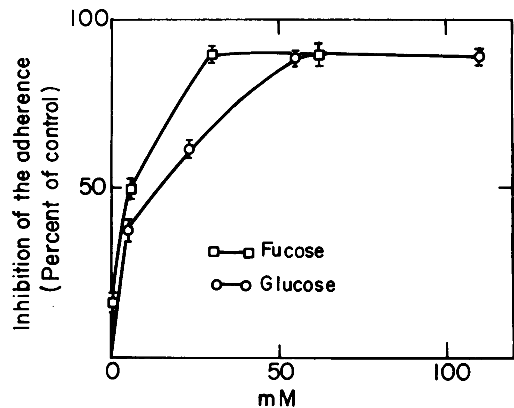

FIG. 4. Inhibition of adherence of *S. flexneri* to colonic epithelial cells by the additions of fucose or glucose. The carbohydrate was added and mixed with the colonic cells before the addition of the bacteria. Incubations were done under standard conditions.

ride from *E. coli* strain O1:K1, which contains polyneuraminic acid (colominic acid), did not inhibit the adherence. Another, as yet unidentified, LPS from a clinical isolate of *E. coli* (7343) was a relatively poor inhibitor, and 5 mg/ml were required to attain 50% inhibition of adherence.

Subjection of the *Shigella* LPS preparation to a number of chemical treatments, such as periodate oxidation or saponification with methanolic KOH, significantly affected its inhibitory capacity (Table 3). Saponification also markedly affected the solubility properties of the remaining polysaccharide. The hydrophobic nature of the LPS seems not to be the important factor in inhibition since a mixture of thymus glycolipids (acetone fraction at 50 μg/ml) did not inhibit adherence. Furthermore, lactoceramide and free fatty acids, such as lauric or myristic, did not cause any inhibition of adherence (Table 3). These results indicate that (i) only a certain carbohydrate structure on the LPS is recognized by the agglutinin and (ii) the inhibition by the *Shigella* LPS is not due to a amphoteric or toxic effect on the colonic cells.

Numerous pretreatments of the *Shigella* cells, such as glutaraldehyde fixation, proteolytic digestions, or heat inactivation, did not affect their adherence to the colonic epithelial cells (Table 4). Pretreatments of the colonic cells by proteolytic digestion, fixation with glutaraldehyde (or formaldehyde), or heat inactivation completely abolished the subsequent adherence of *Shigella* to the colonic cells (Table 5). Moreover, preincubation of the colonic cells with solutions of fucose (5 mg/ml), glucose, or *Shigella* LPS followed by washing of the cells was sufficient to inhibit any subsequent adherence of *Shigella* (Table 5).

TABLE 2. Adherence of *S. flexneri* to colonic epithelial cells of different animals

Animal	Adherence[a] (bacteria per cell)	% Inhibition[a] by 1% fucose
Guinea pig	70 ± 5	90 ± 5.3
Rat	22 ± 3.4	<3
Rabbit	10 ± 2.2	<3
Hamster	<10	<3

[a] Results represent standard error of the mean of three determinations. For experimental conditions, see the text, as well as Table 1, footnote *a*.

TABLE 3. Effect of carbohydrates, as well as *Shigella* LPS and related compounds, on the adherence of *Shigella* to colonic cells

Compound	Amt (mg/ml)	Adherence[a] (% of control)
Mannose	10	52 ± 4.5
Glucose	10	26 ± 4
Fucose	10	14 ± 3
Rhamnose	10	86 ± 5
Lactose	10	96 ± 2
LPS	5	5 ± 2.1
Periodate-treated LPS	5	20 ± 3.3
KOH-methanol-treated LPS	5	66 ± 3.5
Lactoceramide	0.2	100 ± 1.3
Lauric acid	0.05	100 ± 1.5
Myristic acid	0.05	100 ± 2.1

[a] Results represent standard error of the mean of three determinations. For experimental conditions of phenol extraction of *Shigella* LPS, as well as treatments of such LPS, see the text.

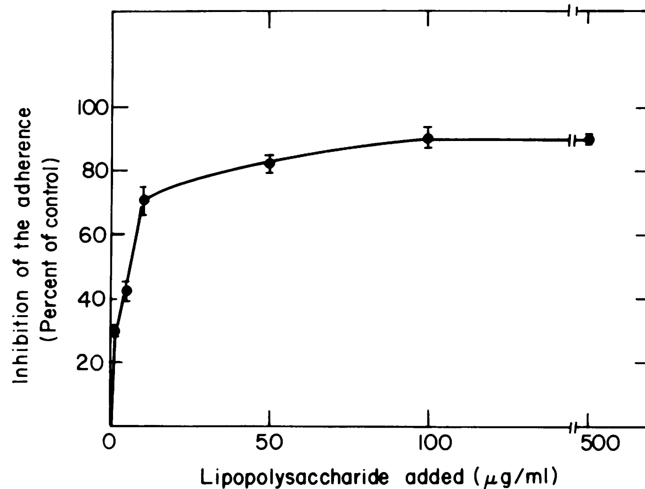

FIG. 5. Inhibition of adherence of *S. flexneri* to colonic epithelial cells by the addition of a phenol-extracted preparation of *S. flexneri* LPS.

Adherence of *Shigella* to colonic mucosal surfaces. To find out whether there was any correlation between the attachment of *Shigella* to suspensions of colonic cells that were released from the intestinal surface by EDTA and reducing agents, the direct adherence of bacteria to colonic mucosal surfaces was determined with the aid of an instrument (see above). The adherence was Ca^{2+}, time, and temperature dependent, similar to what was found with suspended cells. The amount of *Shigella* adhered per mucosal surface area was $5.4 \times 10^6 \pm 5 \times 10^5$ bacteria per 0.38 cm^2 of exposed colonic mucosa. The average number of mucosal cells that can be released and recovered from an equivalent area of the colon is $8.4 \times 10^4 \pm 8 \times 10^3$ cells. Thus, upon calculation and comparison of the number of bacteria that adhered to the surface of the mucosa with the equivalent number of isolated mucosal cells, a very similar value was obtained (ca. 64 bacteria per cell). This finding, as well as the scanning electron micrographs, suggests that only the surface (which faces the lumen) of the mucosal cells binds the bacteria. Thus, the data on adherence of bacteria to isolated mucosal cells in suspension can be interpreted as binding to the surface of the intestinal mucosa.

TABLE 4. Effect of pretreatments of bacteria on adhesion

Pretreatment with:	Amt, concn, or condition	Adherence[a] (% of control)
Trypsin	2.5 mg/ml	95 ± 3.4
Pronase	2.5 mg/ml	81 ± 2.5
Glutaraldehyde	0.25%	79 ± 2.9
Formaldehyde	0.25%	40 ± 3.7
LPS	15 μg/ml	98 ± 1.5
Fucose	10 mg/ml	107 ± 2.6
Heated	100°C, 10 min	80 ± 2.7

[a] Results represent standard error of the mean of three determinations. Bacteria were pretreated with the above-mentioned agents and then extensively washed (three times) with buffered saline (MES [pH 6.2]) containing 1 mM CaCl and only then interacted with the colonic cells. For treatment conditions see the text.

Both fucose (10 mg/ml) and *Shigella* LPS (5 mg/ml) solutions were very effective inhibitors of bacterial adherence to the colonic surface (Table 6). Glucose, which was a good inhibitor of adherence to suspended cells, was a poor inhibitor to the colonic surface. Furthermore, the addition of fucose or LPS either before (followed by washing) or after (30 min) the addition of the radiolabeled *Shigella* reduced almost totally the number of bacteria adhered to the mucosal surface. In addition, pretreatment of the colon with EDTA and dithiothreitol followed by washing of the mucosal surface also prevented (less than 10%) the subsequent adherence of *Shigella* to the intestinal cells (Table 6).

DISCUSSION

Shigellosis involves two distinct regions of the intestine, the proximal small bowel and the colon, resulting in two distinct intestinal disease syndromes, watery diarrhea and dysentery, respectively (G. T. Keusch, R. A. Donohue, and M. Jacewicz, *in* F. Domer, ed., *Encyclopedia of Pharmacology*, in press). *Shigella* bacilli are also known to secrete an enterotoxin that has cytotoxic activity (9, 12, 15). One of the key events in

TABLE 5. Effect of pretreatments of epithelial cells on adhesion of *Shigella*

Pretreatment with:	Amt or concn	Adherence[a] (% of control)
Trypsin	2.5 mg/ml	21 ± 1.7
Pronase	2.5 mg/ml	12 ± 2.1
Glutaraldehyde	0.1%	1.2 ± 0.9
Formaldehyde	0.25%	4 ± 0.6
LPS	15 μg/ml	1.5 ± 0.6
Fucose	10 mg/ml	26 ± 0.8

[a] Freshly prepared guinea pig colonic cells were pretreated with the above agents for not longer than 15 min, after which the cells were washed once with buffered saline (MES [pH 6.2]) containing 1 mM $CaCl_2$. Results represent standard error of the mean of three determinations.

the pathogenesis of shigellosis, which apparently occurs several hours after initial infection, is the penetration of the bacteria into the mucosal cells of the colon (9). Ascertaining the mechanism by which the *Shigella* bacilli adhere to the surface of the host cells before their entry is of primary importance and provided the rationale for initiating these studies.

The mechanism of adherence between bacteria and mammalian epithelial cells has been elucidated in a variety of systems. In most cases investigated, the ability of certain bacteria to adhere has been ascribed to heat-labile, proteinous substances with carbohydrate-binding affinities (adhesins or lectins) that were associated with one of the bacterial cell surface appendages (pili, fimbriae, or flagella) (5, 6, 20, 22). Many of these appendages have been shown to promote bacterial adherence to epithelial cells. A number of different carbohydrate-binding specificities were found in various bacterial species, and several of these adhesins have been isolated and characterized (20, 25). One of the best known adhesins, the mannose-binding substance present in large numbers of gram-negative bacteria (17), has been found to reside in type I pili of many *E. coli* isolates, as well as in flagellum-like structures of other *E. coli* (6), *Serratia marcescens* (5a) and even in certain *Shigella* strains (4).

Bacterial adhesins with other carbohydrate specificities, such as Gal α(1-4)Gal of several *E. coli* uropathogens (25) or the fucose binding of *V. cholerae* (11), have been described. In this study we chose to investigate first the adherence mechanism of clinical isolates of *S. flexneri* that were nonpilated and did not agglutinate erythrocytes or yeasts. One of the most noteworthy findings of this investigation was that the nonpilated *Shigella* avidly adhered to colonic epithelial cells from guinea pigs (mainly from the transverse and descending sections of the colon) and had very little binding affinity to cells isolated from the small intestine or from the intestines of rabbits, rats, or hamsters. This fact is interesting because guinea pigs (7), as well as humans and Rhesus monkeys (18), are among the few mammals known that can be infected by *Shigella*. The other important finding of this study was that the attachment of *Shigella* to the guinea pig colonic cells was a Ca^{2+}-dependent, fucose- (and glucose-) sensitive process in which the host cell was the one that provided the adhesive substance for binding of the bacteria.

The complex nature of the interaction of bacteria with the mucosal surfaces of animal intestines may be a reflection of the complexity of these surfaces. In its simplest form the mucosa has two main features, the epithelial cell surface and the mucous gel, which provides a mantle of varying thickness over the epithelium. From our results it is still unclear what are the nature and the location of the colonic cell component which is active in binding the *Shigella*. Our preliminary experiments indicate that the adhesin can be partially washed off from the colonic cells by saline solutions. A soluble substance was obtained which was heat and protease labile and caused (upon addition of Ca^{2+} ions) a fucose- or glucose-sensitive agglutination of several *S. flexneri* isolates, as well as some *S. sonnei* strains. Moreover, the soluble colonic substance (adhesin) was also found to agglutinate a number of other enteropathogens, mainly of the invasive type (*E. coli* 0114, 0126, 0164), but not others, such as *E. coli* 055, 0111, 0112, or 0125 (Izhar et al., Israel J. Med. Sci., in press). Experiments are in progress to try to isolate and characterize this colonic cell adhesin. We hope that these investigations will also shed more

TABLE 6. Inhibition of adherence of *S. flexneri* to guinea pig colonic mucosal cell surfaces

Addition or treatment	Amt (mg/ml)	Adherence[a] (% of control)
Control		–
LPS	0.5	26 ± 3.2
Fucose	10	57 ± 2.6
Glucose	10	85 ± 3.1
Prewashing of the mucosal surface with (1 mM) EDTA and 1.5 mM dithiothreitol		10 ± 3.7

[a] The reactions were done in the wells system under standard conditions wih ^{14}C-radiolabeled *Shigella* in the absence or presence of inhibitor. At the end of the reaction the mucosal surface was washed three times. The well area was cut and boiled in 2% sodium dodecyl sulfate. The sodium dodecyl sulfate solutions were collected and counted for radioactivity. Results represent standard error of the mean of three determinations.

light on the old unexplained observations of Duguid and Gillies (4) of clumping of nonfimbriated *Shigella* bacilli by colonic mucin. The fact that the LPS of *S. flexneri* has a very high affinity for the mammalian adhesin indicates that it may serve as the bacterial receptor for this agglutinin.

The attachment of *Shigella* to the colonic epithelial cells is somewhat reminiscent in its requirement for Ca^{2+} and fucose sensitivity of the attachment of *V. cholerae* to brush-border surfaces of the intestine (11). In view of what was shown in this study with *Shigella*, it would be interesting to reexamine the attachment of *V. cholerae* and see whether the host intestinal cells display any binding activity.

Mammalian adhesins with Ca^{2+}-dependent and both fucose- and mannose-binding properties have been found. These carbohydrate-binding proteins, which are apparently involved in glycoprotein attachment and clearance mechanisms, have been isolated and characterized from rat livers and macrophages (14, 23). Furthermore, a lectin-like substance present in chicken small intestines and specific for galactose has been partially characterized (1). Our finding that colonic cells may be coated by a substance containing adhesin with fucose-binding properties poses a number of interesting, yet still unanswered, questions. Nothing is known, for example, about the exact location and physiological role of this substance in the colon. Considerable work is still needed to clarify this question, as well as to find out whether colon pathogens, such as *Shigella*, do indeed use the interaction with the adhesin for the infectious process in vivo.

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