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Shigella dysenteriae type 1: The association of hemagglutination/adhesion with lipopolysaccharide

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ABSTRACT

In a previous study we demonstrated the ability of *Shigella dysenteriae* type 1 grown under stringent culture conditions to agglutinate mammalian erythrocytes. In this study we attributed the hemagglutinating activity (HA) to the polysaccharide fraction of bacterial cell lipopolysaccharides (LPS). LPS obtained from a rough mutant strain of *S. dysenteriae* type 1, lacking the O antigen polysaccharide side chain, failed to agglutinate erythrocytes, which clearly demonstrated the link between the O antigen polysaccharides and the HA. Smooth strains, even when not expressing HA, firmly adhered to cultured Henle Intestinal 407 cells, compared with poor binding by rough strains. Pretreatment of bacteria with lipopolysaccharide specific antisera inhibited both HA and binding to cultured human intestinal cells. The contribution of the polysaccharide side chains (and its relation to HA)--which appear to facilitate binding to cultured cells--to bacterial attachment to colonocytes and to the pathogenesis of shigellosis *in vivo*, needs to be confirmed in animal studies.

INTRODUCTION

Shigella dysenteriae type 1, the most pathogenic among the genus *Shigella*, is responsible for major epidemics of bacillary dysentery in developing countries (1,24). The ability to invade and multiply within host colonic epithelial cells is pathognomic of shigellae (21). Additional factors which may contribute to virulence include lipopolysaccharides (LPS), outer membrane proteins and Shiga toxin, although their precise relative role is far from clear.

Bacterial adhesion to epithelium of mucosal surfaces is an essential step in the pathogenesis of many diseases, including enteric infections (3). This process is either mediated by fimbriae, flagellae, capsule, glycocalyx, lipopolysaccharide (LPS), nonfimbrial adhesin or lectin (25). The attachment of *Shigella* to host cells has been reported; strains of *S. flexneri* type 1b adhere to guinea pig colonic cells (16) and *S. flexneri* type 5 to HeLa cells (31). We have recently shown that *S. dysenteriae* type 1 and *S. flexneri* strains agglutinate mammalian erythrocytes, and that the hemagglutination (HA) was resistant to D-mannose and sensitive to N-acetylneuraminic acid. N-acetylneuraminlactose, and α_1-glycoprotein. No fimbria was observed by electron microscopy in *S. dysenteriae* type 1 strains that were positive for HA (33). In the present study we have

characterized the hemagglutinin of *S. dysenteriae* type 1 and examined its relationship to adherence of bacteria to cells cultured *in vitro*.

MATERIALS AND METHOD

Bacterial strains. *Shigella* strains included in the study were obtained from diarrheal patients attending the Clinical Research Centre (CRC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka. *S. dysenteriae* type 1 strain 14731 was used for extraction of cell-surface components, and results were confirmed with three others (12588, 3351 and 26406). Non-pathogenic *E. coli* O8:K25 (strain 36000) and a rough mutant of *S. dysenteriae* type 1 (strain 60R) were obtained from the Centers for Disease Control, Georgia, Atlanta. Another rough mutant of *S. dysenteriae* type 1 (strain PSD-10) (15), was obtained from Khaleda Haider (ICCDR,B). Sereny's test (37) and Congo red binding assay (34) were used to confirm the virulence of strains. Bacterial cell surface hydrophobicity was measured using the salt aggregation test (SAT) (34). Bacteria were stained with India Ink to detect slime (11).

Media and storage. Bacteria stored at -70^{O}C were subcultured in trypticase soy (TS) agar and grown in Casamino acid-yeast extract (CYE) broth medium in the presence of 1 mM calcium chloride for 22 h at 37^{O}C to express HA properties (33). Bacteria were either

grown in screw cap test tubes containing 5 ml of medium for HA, or in 500 ml bottles (Gibco Diagnostics, Madison, Wis.) containing 150 ml of medium for extraction of bacterial components. Shigellae were also cultured in flasks. in TS agar or in TS broth, conditions that do not express hemagglutinating property (33).

HA assays. HA was carried out on slides (33), or in U-bottom microtitre plates (Cooke, Alexandria, Va.) using 0.5% (vol/vol) erythrocytes. Thirty µl of 2-fold dilutions of bacterial components were mixed with an equal volume of guinea pig erythrocytes (0.5%, vol/vol). The plates were sealed and incubated for 1 h at room temperature with shaking (160 rpm on a rotary shaker). Results were recorded after 30 min and scored as positive when the erythrocyte suspension was distributed over the bottom of the wells, and negative when a red button was clearly visible. Titers were recorded as the arithmetic mean of the minimum concentration of material at which HA was clearly visible. HA was tested with guinea pig, human (types A,B,O), monkey, rabbit and sheep erythrocytes (33). In most assays however, guinea pig and human (type O) erythrocytes were used.

Inhibition of HA. Inhibition of HA was tested with 1% N-acetylneuraminic acid (Sigma), 1% fetuin (from fetal calf serum, Sigma) and other carbohydrates (33). Inhibition by LPS specific rabbit antibody was tested after incubation with test material for 1 h at 37°C.

Extraction and purification of bacterial components. To determine whether in *S. dysenteriae* type 1 HA was mediated by protein, extraction methods used for *E. coli* were utilized (14,35). For isolation of the hemagglutinin, outer membrane proteins were also prepared by either a lysozyme and EDTA extraction procedure (17), or a simple buffer extraction technique (29). LPS was extracted with hot phenol water (43), and purified by ultracentrifugation at 100,000 X *g* for 4 h. Nucleic acid contaminants in LPS (5-10 mg/ml) were removed by treatment with deoxyribonuclease and ribonuclease (Sigma) (20 µg/ml), and protein contaminants, by treatment with Proteinase K (Sigma) (100 µg/ml). LPS was collected by centrifugation at 100,000 X *g* for 4 h.

Delipidation of LPS. LPS from *S. dysenteriae* type 1 strains was delipidated (27) by refluxing in 1% (vol/vol) acetic acid. The aqueous layer was lyophilized, resuspended in water and centrifuged at 100,000 X *g* for 4 h to remove unhydrolysed LPS. After lyophilization, the material was dialyzed against PBS and used for HA assays.

Cell debris from bacteria grown in CYE medium were removed by centrifugation at 8000 X *g* for 30 min. The clear culture supernatant was collected, passed through a membrane filter (0.45 µm, Millipore), dialyzed for 24 h against deionized water and lyophilyzed. Culture supernatants prepared in this manner

are referred to as "CS" in the rest of the text. Uninoculated culture medium, treated as above, was used as control.

Components used for HA assays described below were freeze dried and reconstituted in PBS. Protein estimation was done by the dye binding method (2), and carbohydrate content in LPS was determined by the phenol-sulfuric acid method (10). Purified LPS and other LPS containing fractions were quantitated on the basis of carbohydrate content rather than dry weight. To further confirm the presence of LPS, the content of 2-keto-3-deoxy-octulonate, a core sugar in LPS, was estimated (42). LPS was also detected in fractions by the Chromogenic Limulus Amoebocyte Lysate method (LAL) (Whittaker Bioproducts, Inc. Walkersville, MD.) using LPS from *E. coli* 055-B5 as standard (32). Sialic acid content in materials was assayed (38) using N-acetyl neuraminic acid as standard.

Gel filtration. Culture supernatants were fractionated on Sepharose 4B (74 x 2 cm) (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). The elution volume was 108 ml for Blue dextran (Pharmacia), and 118 ml for LPS, from *S. dysenteriae* type 1 strains. Fractions were assayed for protein (at 280 nm) and for carbohydrate content (10). Fractions were pooled, lyophilized and tested for HA activity.

Analyses of LPS and proteins. Fractions were analyzed after electrophoresis on polyacrylamide gels (13.5%) in the presence of sodium dodecyl sulfate (20). Gels were stained with 0.2% Coomassie blue to detect proteins and silver nitrate for LPS (39).

Enzyme treatment. Suspensions of *S. dysenteriae* type 1 (1×10^{10} CFU) in 0.5 ml of PBS were treated with 50 µg of trypsin (Flow Laboratories Herts, England) or 100 µg of proteinase K and incubated for 4 h at $37^{o}C$. Culture supernatants and LPS were also treated similarly.

Guinea pig erythrocytes (50% vol/vol) were treated with 100 to 1000 milliunits of neuraminidase obtained from *Clostridium perfringens* (Sigma) and incubated as described above before testing for HA assays.

Antibodies. New Zealand white rabbits were immunized twice subcutaneously with CS (100 µg) mixed with Freund's complete adjuvant, at ten days interval, followed by a third dose (150 µg), fifteen days later. Serum was collected one week after the last injection and stored at $-20^{o}C$. The rabbit antibody titer to *S. dysenteriae* type 1 LPS (anti-CS) was determined by ELISA (24) using LPS purified from *S. dysenteriae* type 1 (25 µg/ml) as source of antigen; it was found to be 2.56×10^{4}. For absorption studies, serum (1 ml) was incubated with 3 mg of *S. dysenteriae* type 1 LPS for 1 h at $37^{o}C$ and then

overnight at 4°C. After incubation, the absorbed serum was centrifuged at 35,000 X *g*. Convalescent sera collected 21 days after onset of infection in 3 adult patients with *S. dysenteriae* type 1 were also included in the study.

Crossed immunoelectrophoresis and double immunodiffusion. Crossed immunoelectrophoresis (41) was performed on glass plates (5 x 5 cm) with agarose (1%) in Tricine buffer (pH 8.7). Double immunodiffusion was carried out according to Ouchterlony (30) on microscopic glass slides.

Bacterial adhesion to cultured epithelial cells. Henle intestinal 407 cells (Int.407) (Flow laboratories, Inc., Herts. England) were maintained in Basal Eagle Medium (Flow) supplemented with 15% newborn calf serum (Flow) and 2 mM glutamine. Cells were seeded with 1×10^5 in culture vials (Kimble, Toledo, Ohio) containing cover slips (diameter 12 mM). After 18 h cells were inoculated with 100 µl of bacterial suspensions containing 1×10^9 CFU and centrifuged at 500 X *g* for 10 min at 4°C. The vials were incubated for a further 20 min at 37°C in 5% CO_2 and then washed 10-times in PBS to remove non-adherent bacteria. Cover slips were fixed with methanol, stained with Giemsa and examined under a microscope (X1000). Both HA positive and HA negative strains of *S. dysenteriae* type 1 were included. In addition smooth Congo red HA negative and HA positive strains (34), and rough mutants of *S. dysenteriae*

type 1 strains were also examined in the adhesion assays (Table 2). The effect of serum, bacterial components, carbohydrates, proteins, and enzymes on the adhesion of bacteria to Int.407 was determined (Table 3). For each assay bacteria adhering to 10 infected cells were counted and the number of bacteria adhering per cell was calculated. The mean and ± standard error of bacteria adhering per cell from five separate experiments were calculated. Extent of adhesion under different conditions was expressed as the percentage of binding compared to that of the HA positive control strain 14731. Student's t test was used to calculate differences in adhesion due to the effect of various pretreatments. P values exceeding 0.05 were considered not significant.

Bacterial adhesion to immobilized mucin. Human mucin (extracted from a human colon at post-mortem) was fractionated (20 mg/ml) on a column of Sepharose 4B (2.2 x 36 cm). Fractions eluting in the region of Blue dextran (60 ml) were dialyzed against PBS and lyophilized. For immobilization purposes, 0.5 ml of human mucin (1.5 mg/ml) was immobilized (8) on culture vials containing cover slips. Adhesion assays with bacteria were carried out as described above and adhesion was expressed as the number of adherent bacteria per sq mm.

RESULTS

Extraction and purification of the hemagglutinin. The outer membrane proteins and cell surface proteins of *S. dysenteriae* type 1 strain 14731 prepared by different methods did not agglutinate erythrocytes in protein concentrations of up to 10 mg/ml, either on slides or in microtitre plates. Treatment of hemagglutinating bacteria with trypsin or proteinase K had no effect on the HA nor did pretreatment of erythrocytes with neuraminidase.

Culture supernatants of *S. dysenteriae* type 1 strain 14731 grown in the CYE medium caused HA at concentrations of 52.6 µg/ml and above (Table 1). Heating CS at 100^{o}C for up to 30 min or treatment with trypsin or proteinase K had no effect on the HA. When CS was fractionated through a column of Sepharose 4B, HA (30.6 µg/ml) was detected in materials eluting (Pool-1, fractions 90-115 ml) at a volume similar to the region at which LPS (118 ml) from *S. dysenteriae* type 1 was eluted. Subsequent fractions (230-380) did not contain HA activity. Pool-1 contained high concentration of carbohydrates (1.05 mg/ml) but relatively low concentration of protein (0.126 mg/ml). Pool-1 showed a reaction of identity with LPS when analyzed by double immunodiffusion by the Ouchterlony's method against rabbit (anti-CS) serum. CS, Pool-1 and LPS; showed similar types of precipitin lines when analyzed against rabbit (anti-CS) serum by crossed immunoelectrophoresis. Pool-1 gave a positive reaction

with thiobarbitauric acid confirming the presence of KDO and was positive in Limulas Amoebocyte Lysate assay indicating the presence of endotoxin (detected up to a concentration of 50 pg/ml). Heat treatment or treatment with proteolytic enzymes did not affect HA activity. These results confirmed that Pool-1 contained LPS.

Purified LPS obtained from *S. dysenteriae* type 1 strain 14731 agglutinated erythrocytes (Table 1). Treatment with heat or enzymes did not decrease activity. The HA activity of CS, Pool-1 and LPS (up to 80 µg/ml) was completely inhibited by rabbit (anti-CS) serum (1:100 dilution in PBS). Serum absorbed with LPS however did not inhibit the HA of these preparations. These results confirmed that the HA in cell-free cultures of *S. dysenteriae* type 1 was associated with LPS.

Similar results were obtained with CS and LPS derived from HA positive *S. dysenteriae* type 1 strains 12588, 3351 and 26406.

Pre-treatment of LPS, CS and Pool-1 with N-acetyl neuraminic acid or fetuin resulted in loss of HA activity. Polysaccharides obtained by delipidation of LPS showed the highest HA activity (Table 1). LPS obtained from *S. sonnei*, *S. boydii*, rough mutant of *S. dysenteriae* type 1 PSD-10, *E. coli* 055-B5 or non-pathogenic *E. coli* 08-K25 failed to show any HA activity (Table 1). LPS obtained from *S. dysenteriae* type 1 strains cultivated under non-

HA conditions were nevertheless positive for HA in mictrotiter plates. LPS from HA positive and HA negative strain of 14731 of *S. dysenteriae* type 1 showed the same pattern on silver-stained polyacrylamide gel. Culture supernatant appeared similar but with more bands staining in the high molecular mass region (94-43 KDal).

Strains of *S. dysenteriae* type 1 grown in the CYE broth medium contained slime as detected microscopically with the India ink stain. This was more apparent in bacteria which had been cultured longer than 18 h. Hemagglutinating strains always contained high amounts of slime compared to non-hemagglutinating cultures. The SAT value or *S. dysenteriae* type 1 strains in the CYE medium was found to be 2.0 M, whereas in TSA or TS broth, it was 1.5 M. This showed that there was a decrease in surface hydrophobicity. The presence of slime, however, did not affect agglutination of the bacteria in O-antigen specific sera. Agglutination of erythrocytes by strains of *S. dysenteriae* type 1 was also visualized under the microscope (X10). It was found that slimy material present in cultures agglutinated erythrocytes, forming large aggregates (300 µm to >500 µm). Small aggregates of erythrocytes were also present but these were only visible microscopically. The small aggregates were also observed with LPS and CS when examined microscopically. When cultures of HA positive strains were shaken in PBS for 1 h, centrifuged and retested, HA could not be detected either by slide agglutination or under the microscope.

Bacterial cultures, PBS extracts, CS and LPS did not contain sialic acids when tested by the resorcinol-HCl method (38).

Adhesion to Int.407 cells. When incubated with Int.407 cells for 20 min at 37°C the HA positive strains of *S. dysenteriae* type 1 showed firm adherence (Table 2) (Fig. 1B). When incubation of bacteria with Int.407 cells was allowed to progress longer, cell invasion occurred (Fig. 1C). Adhesion was also temperature-dependent; bacteria cultured at 37°C adhered better than those grown at 30°C. Adhesion to Int.407 cells was not uniform: more bacteria adhered to some cells than to others. Int.407 cells grown for 18 h on coverslips were better suited for this assay than cells grown for 40 h and longer. Smooth, Congo red positive and negative strains, grown under hemagglutinating or non-hemagglutinating conditions, equally adhered to Henle 407 cells. In contrast, rough mutants of *S. dysenteriae* type 1 showed poor binding (Table 2).

Binding of strains of *S. dysenteriae* type 1 to Int.407 was completely inhibited by pretreatment of cells with homologous LPS (Table 3) at a concentration of 0.18 mg/ml. CS at a similar concentration inhibited approximately 60% of adherence. Human convalescent sera and rabbit (anti-CS) serum also inhibited to some extent bacterial adhesion. When Int.407 cells were treated with wheatgerm agglutinin, binding of bacteria was reduced to 16%

of control. Protease and neuraminidase treatment of the intestinal cells also reduced considerably the numbers of binding bacteria. However, the presence of human mucin increased the binding of the bacteria to Int.407 cells (174%). Thus, various pre-treatments (Table 2) significantly affected the adhesion of *S. dysenteriae* type 1 to Int.407 cells (p values ranging from 0.01 to <0.001).

Binding to mucin. Strains of *S. dysenteriae* type 1 also bound to immobilized human mucin (average of 1.2×10^4/sq.mm) (Fig. 1D) Binding of bacteria to Int.407 cells in the presence of mucin could not be inhibited by any of the above conditions.

DISCUSSION

The results show that HA activity of *S. dysenteriae* type 1 is associated with lipopolysaccharide present in culture supernatants and with LPS purified from the bacteria. No proteins were linked with LPS in this interaction since heat or proteases did not affect the HA ability of purified LPS or components separated from the culture supernatant. The observation that rabbit serum raised against CS inhibited this activity, and absorption of the LPS antibodies removed this inhibition, further confirmed the role of LPS in the agglutination of erythrocytes.

LPS in general are known to be cytotoxic to macrophages, polymorphonuclear leukocytes, platelets, B-lymphocytes and fibroblasts (4). LPS from *S. sonnei* stimulates human mononuclear leucocytes to produce a strong procoagulant activity (26). In addition LPS has been suggested to play a role in intravascular coagulation in septicemia due to gram-negative bacteria and to be involved in complications arising from severe shigellosis (18). Studies have suggested that LPS released from bacteria in the culture medium is more potent than LPS extracted from cell surfaces using chemical means (35). In this study, however, the extracted LPS from bacterial cell surface had higher activity, which possibly is because protein components complexed with LPS were not required for the HA activity.

The agglutination of erythrocytes by LPS from *S. dysenteriae* was a highly specific process and possibly involved the O-antigenic carbohydrate side chains. Polysaccharides separated from the Lipid A component of LPS also agglutinated erythrocytes. It was not possible to test Lipid A for HA because of its insolubility in aqueous solutions although this component is known to have affinity for cell membranes (4). LPS extracted from the rough mutant of *S. dysenteriae* type 1 PSD-10 which is deficient in polysaccharide side chains (15) showed no agglutinating property. The rough strains also exhibited poor adherence to Int.407 cells.

LPS derived from smooth strains inhibited adhesion of *S. dysenteriae* type 1 to Int.407 cells. Izhar *et al.* (16) have shown that LPS from *S. flexneri* 1b inhibited the binding of the homologous strain to guinea pig colonic cells, which suggested that the LPS is the component responsible for adhesion. But, since treatment of bacteria with proteases did not result in loss of binding whereas treatment of the epithelial cells led to a loss of affinity, these workers concluded that the adhesin was present on the surface of the host cells. Our results however, indicated that the heat- and protease-resistant adhesin for both erythrocytes and for the cultured epithelial cells was associated with the LPS. It has been shown before that only smooth strains of *Shigella* (21,40) are virulent although an exact function was not assigned to LPS. In *Salmonella typhi* however, it was shown that smooth LPS is necessary for adhesion and invasion (28). LPS obtained from strains of *S. sonnei* form 1, *S. boydii* type (1-6) and from *E. coli* (O8:K25 or O55:B5) under the same culture conditions did not agglutinate erythrocytes, which possibly points out to the non-hemagglutinating nature of these organisms (33).

HA activity of *S. dysenteriae* type 1 is sensitive to sialic acids (33) and the hemagglutination associated with the LPS was also sensitive to sialic acid or to fetuin obtained from fetal calf serum, a sialic acid (5.7% NANA) containing protein. This suggests that the LPS binds to sialic acid containing receptors

on erythrocytes. The binding of protein adhesins to erythrocytes is mediated via receptors (22,23) containing specific carbohydrates including sialic acids, which may or may not be sensitive to sialinidases such as neuraminidase (13,14.22,23). We have demonstrated that treatment of erythrocytes with neuraminidase from *Clostridium perfringens* did not decrease the affinity of the hemagglutinin to erythrocytes, while similar treatment of the cultured intestinal cells resulted in a marked decrease in binding of the bacteria as has been reported in other interactions. The reason for the resistance of erythrocytes to treatment with neuraminidase is not clear, but indicates that, possibly, the enzyme has no effect on the LPS binding site. Other proteolytic and hydrolytic enzymes have to be tested to further characterize the hemagglutinin binding site on the surface of erythrocytes.

Adhesion to Int.407 cells of *S. dysenteriae* type 1 requires less stringent conditions than hemagglutination. Strains cultured in trypticase soy medium or in casamino acid-yeast extract medium showed almost similar degree of adhesion to Int.407 cells. Izhar *et al.* (16) have also shown that non-hemagglutinating and non-piliated strains of *S. flexneri* 1b bind to guinea pig colonic cells suggesting that the occurrence of HA activity in these strains is not a prerequisite for bacterial adhesion. We have found that both virulent and avirulent smooth strains showed similar binding to cells. Adherence of a virulent

strain of *S. flexneri* type 5 to HeLa cells, however, was 10-fold higher than that of the smooth avirulent isogenic mutant (31), a characteristic which was protein dependent. We have earlier shown that strains of *Shigella flexneri* (types 1a. 1b, 2a, 2b) have HA properties when grown under similar conditions as that of *S. dysenteriae* type 1 (33). It is possible that *Shigella* spp. may express a number of adhesins under different cultural conditions. The association of adhesins with LPS is less common than with protein; other bacteria in which adhesins were shown to be associated with LPS include *Campylobacter jejuni* (25), *V. cholerae* (6), *S. typhi* (28), and *E. coli* F-18 (7). Bacterial glycocalyx were also shown to contribute to the binding of some *E. coli* to host surfaces (5,14,19). We suggest that the CYE medium stimulates production of slime which is responsible for agglutination of erythrocytes. The presence of these components, possibly polysaccharides also result in a decrease of the surface hydrophobicity measured by SAT. Bacterial slimes can be removed by heat and by extraction in buffers. Heat treatment of the bacteria or extraction in PBS result in complete loss of HA activity (33), which suggests that the slime is degraded by such treatment. Electron microscopic examination of bacteria may help demonstrate the presence of these substances as has been shown with some *E. coli* strains (5,14). It has been suggested previously that shigellae also produce such extracellular polysaccharides (12), however, the chemical nature of the polysaccharide was not determined. A further chemical analysis of these components is required.

In conclusion, strains of *S. dysenteriae* type 1 have adhesive property which is associated with smooth LPS and the hemagglutinating ability is only an amplification or expression of its ability to bind to host surfaces. This study and those of Izhar *et al.* (16) and Pal and Hale (31) together highlight the role of adhesins in the interaction of strains of *S. flexneri* and *S. dysenteriae* type 1 with host cell surfaces. The significance of bacterial adhesion in the pathogenesis of shigellosis needs to be clarified *in vivo*; it is possible that serospecific LPS antibody which is often demonstrated in patients recovering from *S. dysenteriae* type 1 and *S. flexneri* infections (24), play a major role in protection against species and serotype-specific reinfection by preventing bacterial adhesion.

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TABLE 1 : Hemagglutinating activity (HA) of bacterial components of *S. dysenteriae* type 1 14731 and LPS of other strains

Bacterial component	HA	Minimum concentration required for HA (µg/ml)#
Culture supernatant	+	52.6 (± 3)
Sepharose 4B Eluate (Pool-1)	+	30.6 (± 1.8)
LPS (from *S. dysenteriae* type 1)	+	18.58 (± 1.01)
Polysaccharides (from delipidation of LPS)	+	3.98 (± .152)
LPS (from *E. coli* 08:K25) strain 36000	-	-
LPS (from *E. coli* 055:B5)	-	-
LPS (from *S. sonnei* Form 1) strain 6577	-	-
LPS {*S. boydii* (1-6)} strain 33744	-	-
LPS (Rough mutant PSDIO)	-	-

#All materials were quantified on the basis of carbohydrate content and results shown are the mean and standard error of 3 experiments. Components negative for HA were tested up to a concentration of 10 mg/ml

TABLE 2 : Ahesion to Int.407 cells of hemagglutinating (HA) and non-HA variants of strain 14731, compared with 2 rough mutant strains of *S. dysenteriae* type 1, expressed as mean and standard error (±SE) of 5 experiments

*Strain	Adhesion No. of bacteria/cell (±SE)	Adhesion (% of control)	P value
1. Strain 14731, HA positive	75.8 (7.05)	100%	
*2. Strain 14731 HA negative virulent	70.0 (5.69)	92.3	NS#
3. Strain 14731 HA positive avirulent	76.0 (5.09)	100.24	NS
4. Rough mutant PSD-10	12.8 (2.59)	15.5	<.001
5. Rough mutant 60-R	15.2 (3.84)	20.0	<.003

*Except (2) all strains were grown in CYE medium (33)

#Not significant (P>0.05)

TABLE 3 : Effect of various pre-treatment regimes on adhesion of *S. dysenteriae* type 1 strain 14731 to Int. 407

Pre-treatment	Conc (/ml)	Adhesion (% of control)*	Difference from control (P value)
Neuraminidase treatment	100 units	20.0	0.002
Wheat germ agglutinin	0.05 µg	16.1	0.001
Mucin (human)	0.05 µg	174.6	0.003
LPS (S.dyst.type 1)	0.18 µg	0.000	0.000
Culture supernatant	0.18 µg	40.89	0.01
#Rabbit (anti CS)serum		33.4	0.006
#Human convalescent serum (1)		18.46	0.001
" " " (2)		25.1	0.000
" " " (3)		37.9	0.003

*Compared with binding of bacteria not treated before adhesion as shown in Table 2

#All sera were tested at 1:50 dilution in PBS

FQ:mh/B7:ADHESIN

LEGEND TO FIGURE

Fig. 1 : Adhesion of *S. dysenteriae* type 1 (strain 14731) to Int.407 after an incubation of 20 min (B) and invasion (C) after 3 h compared with uninoculated Int.407 cells (A). The binding to immunobilized mucin is shown in D.