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Adherence of *Bacteroides fragilis* Group Species

ITZHAK BROOK†* AND M. LYNN MYHAL

Naval Medical Research Institute, Bethesda, Maryland 20814

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The ability of piliated and capsulated *Bacteroides fragilis* and *Bacteroides ovatus* to adhere to intestinal cells and mucus was investigated. The adherence of piliated and capsulated strains was at least five times greater than the adherence of their nonpiliated and noncapsulated or capsulate only counterparts. These data illustrate the importance of pili as promoters of adherence of *B. fragilis* group species to the gastrointestinal mucosa.

Bacterial attachment to mucous membranes is believed to be necessary for the process of colonization of human epithelium (1). *Bacteroides fragilis* group organisms are important pathogens in intra-abdominal and pelvic infections (7). Onderdonk et al. (12) demonstrated that the capsule is an important virulence factor in *B. fragilis* and that patients with *B. fragilis* disease develop a high titer of capsular antibodies. Although Onderdonk et al. (13) described the ability of capsulated *B. fragilis* to adhere to viable animal cells, they have not found this organism to possess pili. Pruzzo et al. (14) have shown the ability of piliated *B. fragilis* isolates to hemagglutinate erythrocytes and adhere to epithelial and human cells.

In a recent study, we have demonstrated the ability of noncapsulated *Bacteroides* strains to become capsulated and piliated following their passage in mice mixed with capsulated *Klebsiella pneumoniae* (3). The present study was designed to study the in vitro adherence to intestinal cells and intestinal mucus of the newly capsulated or piliated and capsulated *B. fragilis* group species compared with that of their nonpiliated and noncapsulated parent cells.

Organisms. All bacterial strains used in the experiments were recent clinical isolates. They included one strain each of *B. fragilis* (NMRI 13), *Bacteroides ovatus* (NMRI 22), *Escherichia coli* (NMRI 88), (piliated and nonpiliated forms), and *K. pneumoniae* (NMRI 2). The bacteria were kept frozen in skim milk at −70°C. They were identified by standard criteria (10, 16) and processed as previously described (4). Each *Bacteroides* species contained three forms: the first noncapsulate (<0.1% of the organisms had a capsule), the second capsulated only (>50% of the organisms had a capsule) and nonpiliated, and the third capsulate and piliated. The capsulate-only forms were induced by passage of each of the noncapsulate forms of *Bacteroides* spp. mixed with formalin-killed *K. pneumoniae* in a subcutaneous abscess in mice, and the capsulate and piliated form of each of the *Bacteroides* spp. was induced by passage of the noncapsulate form mixed with viable *K. pneumoniae* (4). The presence of a capsule was established by Hiss's staining method (10) and confirmed by electron microscopy after staining with ruthenium red (8). Ruthenium red staining demonstrated a homogeneous polysaccharide capsule external to the cell wall. The presence of pili was established by electron microscopy with negative staining (10).

Labeling of bacteria. *Bacteroides* spp. and *E. coli* strains were grown in brucella broth supplemented with 0.01 mCi of sodium [^{3}H]acetate per ml (specific activity, 90 mCi/mmol; New England Nuclear Corp., Boston, Mass.) (15). The inoculated tissue culture flasks were incubated anaerobically in anaerobic jars at 37°C for 18 to 20 h. Following incubation, the cells were harvested, washed twice in *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-Hanks (HH) buffer (pH 7.4), and suspended in the same buffer. The optical density of the bacterial suspension was then determined at 600 nm and adjusted to an absorbance of 0.95. A fraction of the suspension was removed, and the number of viable bacterial cells present was determined by plating dilutions on brucella agar. A second fraction was taken to determine the level of radioactivity present. Typically, the level of [^{3}H]acetate incorporation was 5×10^{-5} to 5×10^{-4} cpm per bacterium.

Adhesion assay. A previously described adhesion assay was used to study adherence (9). Briefly, INT 407 cells were seeded into 24-well polystyrene tissue culture plates and allowed to form monolayers overnight. The following day, the wells were washed twice with 0.5 ml of HH buffer (pH 7.4). Radiolabeled bacterial cells were also washed and suspended in HH buffer. Immediately after the INT 407 cells were washed, 0.25 ml of approximately 10^9 CFU of ^{3}H-labeled bacteria was added to each well. The tissue culture plates were then incubated at 37°C for 3 h. Subsequently, the wells were washed with HH buffer to remove nonadherent bacteria. Adherent bacteria were recovered by adding 0.5 ml of 0.5% sodium dodecyl sulfate to each well and reincubating the isolates for 2 h. Samples (0.25 ml) were then removed, and the level of radioactivity was determined.

Intestinal mucus adhesion assay. The ability of *Bacteroides* spp. strains to adhere to crude preparations of intestinal mucus was also examined. A previously described mucus adhesion assay was used (9). Briefly, mucus from the distal small intestines of 1-kg New Zealand rabbits (Dutchland Laboratories, Inc., Denver, Pa.) was prepared in HH buffer. Mucus (1.0 mg of protein per ml) was added to 24-well tissue culture plates (0.25 ml per well) and incubated overnight at 4°C. Following incubation, the wells were washed to remove unbound mucus and the adhesion assay was conducted as described above. Bovine serum albumin (BSA; 10 mg/ml) was added to control wells.

Calculations. All assays were run in triplicate. Since similar data were obtained from each experiment, the results from only one experiment are presented. Results are expressed as the mean of triplicate sets of wells. Student's *t* test was used to determine significance. *P* values exceeding 0.05 were considered not significant. Numbers of adherent

* Corresponding author.

† Address for correspondence: Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

TABLE 1. Adherence of *E. coli* and *B. fragilis* group strains to INT 407 cells and intestinal mucus

Bacterial strains	CFU (10^6/well ± SE)[a]		
	INT 407 cells	Mucus	BSA[b]
E. coli (noncapsulated, nonpiliated)	5.2 ± 0.2	4.8 ± 0.2	4.6 ± 0.1
E. coli (noncapsulated, piliated)	26.8 ± 0.6	24.5 ± 0.4	6.0 ± 0.4
B. fragilis (noncapsulated, nonpiliated)	3.6 ± 0.1	6.8 ± 0.4	8.3 ± 0.6
B. fragilis (capsulated, nonpiliated)	5.4 ± 0.2	9.1 ± 0.5	7.9 ± 0.4
B. fragilis (capsulated, piliated)	18.5 ± 0.3	29.6 ± 0.6	10.4 ± 0.8
B. ovatus (noncapsulated, nonpiliated	6.2 ± 0.3	7.5 ± 0.8	7.6 ± 0.6
B. ovatus (capsulated, nonpiliated	8.0 ± 0.3	9.2 ± 0.5	7.1 ± 0.6
B. ovatus (capsulated, piliated)	32.3 ± 0.4	28.8 ± 0.6	9.5 ± 0.3

[a] Mean CFU per well was determined from triplicate sets of wells.
[b] 10 mg/ml; 0.25 ml per well.

bacteria were calculated from the following formula: (cpm/well)/[(cpm/ml) × (ml/CFU)] = CFU/well.

Adhesion of piliated *E. coli* and piliated and capsulated *B. fragilis* and *B. ovatus* to INT 407 cells as well as to mucus was significantly greater ($P < 0.005$) than the adhesion of the similar bacterial strains that were nonpiliated (*E. coli*), capsulate only (*Bacteroides* spp.), or noncapsulate and nonpiliated (*Bacteroides* spp.) (Table 1). Adherence of all organisms to BSA was low.

This study illustrates the ability of the piliated *B. fragilis* group species to adhere to intestinal epithelium as well as to intestinal mucus. Piliated strains showed about five times more adherence than nonpiliated strains. The presence of capsule did not affect the rate of adherence. We were not, however, able to produce piliated *Bacteroides* spp. without the presence of a capsule (4). Further work is warranted to investigate the adherence of noncapsulated, piliated *Bacteroides* spp. We were, however, able to produce encapsulation and pili formation in *Bacteroides* spp. using bacterial strains other than *K. pneumoniae* (3, 4). This suggests that the capsule and pili are of *Bacteroides* origin. The pili may be responsible for the ability of *B. fragilis* group species to adhere to the intestinal epithelium cells as well as to the mucous layer. Since the pili were produced by the organisms within an abscess, they may enable single bacterial cells to adhere to each other, forming a net of bacteria that resists phagocytosis. Pili and capsule may interact synergistically to prevent phagocytosis and enable the formation of an abscess.

B. fragilis group species interactions with the mucus component of the mucosal surface have not been studied. The intestinal mucous layer is a substantial structure, often 30 to 50 μm thick, and is continuous with the glycocalyx of the mucus (15). This viscous glycoprotein gel that covers the epithelial cells of the intestinal tract is likely to be the initial point of interaction between the host and *B. fragilis* group species. The increased adherence of piliated *B. fragilis* group species to the mucous surface may promote colonization with these organisms.

Pruzzo et al. (14) observed the increased adherence of piliated *B. fragilis* group strains compared with nonpiliated strains. However, all the strains investigated by Pruzzo et al. were noncapsulated. Onderdonk et al. (13) found increased adherence of encapsulated *B. fragilis* to peritoneal mesothelium. However, pili were not found in the *B. fragilis* isolates used in that study. In contrast to Onderdonk et al. (13), we were unable to show that the presence of a capsule by itself is sufficient to enhance adherence of *B. fragilis* group strains to intestinal epithelium and mucus. However, the presence of pili enhanced that capacity.

We have recently observed an increased recovery rate of pili-forming *B. fragilis* group species in the gastrointestinal tract as well as from abdominal abscesses (2). However, pili were generally absent in *Bacteroides* isolates recovered from blood. In contrast, encapsulated *B. fragilis* group species were found more often in abdominal abscesses and blood and rarely in the gastrointestinal tract normal flora. *B. fragilis*, like some *E. coli* strains recently described, might be able to modulate formation of pili and capsule in relation to environmental conditions (6, 11). *B. fragilis* group isolates undergo morphological changes as they emerge from the gastrointestinal tract into the peritoneal cavity and form abscesses and induce bacteremia. When colonizing the gut, the presence of pili enables the organisms to adhere to the mucous membranes, and since they do not encounter neutrophils, the presence of a capsule provides them no advantage. However, in abscesses, the capsule provides protection from phagocytosis. In contrast, when in the blood, the pili may interfere with the systemic spread of the organisms, as piliated bacteria may be more easily phagocytized (14).

More studies are warranted to further investigate the mechanism of the emergence of pili and their role in pathogenesis of *B. fragilis* group species infection.

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