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Bacteroides vulgatus Outer Membrane Antigens Associated with Carrageenan-Induced Colitis in Guinea Pigs

JAMES L. BREELING,[1,2]* ANDREW B. ONDERDONK,[3] RONALD L. CISNEROS,[3] AND DENNIS L. KASPER[2,4]

Division of Infectious Diseases, Brigham and Women's Hospital,[1] Division of Infectious Diseases, Beth Israel Hospital,[4] and Channing Laboratory, Harvard Medical School,[2] Boston, Massachusetts 02115, and Department of Pathology and Infectious Disease Research Laboratory, Tufts University School of Veterinary Medicine, Boston, Massachusetts 02130[3]

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Previous experiments with the carrageenan model for ulcerative colitis demonstrated that the inflammatory response in guinea pigs can be enhanced by immunization with *Bacteroides vulgatus* and subsequent feeding of this organism to experimental animals. The studies reported here show that antigens extractable from the bacterial outer membrane by EDTA are responsible for this effect. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to analyze the outer membrane proteins from various strains as well as the lipopolysaccharides (LPS) extractable by the phenol-water method. Although the observed pattern of outer membrane proteins was complex, the strains could be divided into two electrophoretic types (phenons) on the basis of immunoblotting against a panel of antisera. Cross-absorbed antisera used in immunoblotting experiments identified four outer membrane proteins uniquely associated with the phenon capable of enhancing the colitis inflammatory response. These proteins had molecular weights of 100,000, 57,000, 34,000, and 21,000 when measured in 8% to 12% acrylamide gradient sodium dodecyl sulfate gels. Other antigens identified included at least one type of smooth LPS, three types of rough LPS, and a common antigen of 30,000 molecular weight among the strains of *B. vulgatus* tested. The outer membrane preparations were used in animal immunization and challenge experiments, and the severity of colitis was correlated with one electrophoresis type. The potential role of membrane proteins in the enhancement of colitis is discussed.

Ulcerative colitis is one of the major "idiopathic" diarrheal illnesses in humans. A microbiological cause for this illness has been extensively sought but has not yet been discovered. Clinical and experimental data suggest a major role for "autoimmunity" in the pathogenesis of ulcerative colitis (23). Investigations into the microbiological factors involved in an animal model of colitis that closely resembles the histopathologic features of the human condition have shown a special role for *Bacteroides vulgatus*. Prior studies with the carrageenan-fed guinea pig model of Watt and Marcus (27) have shown that *B. vulgatus* recolonization of gnotobiotic guinea pigs is uniquely sufficient to provoke colonic ulcerations in the presence or absence of carrageenan treatment (18). Metronidazole treatment of animals before and concurrent with carrageenan treatment prevented the development of cecal or large-intestinal ulceration (19). Immunization of guinea pigs with *B. vulgatus* before the administration of carrageenan and the feeding of viable *B. vulgatus* resulted in a more rapid development of ulcerations (20). Immunization with a phenotypically similar organism, *Bacteroides fragilis*, does not enhance pathology (20). Additional studies have shown that enhancement of the experimental disease can be adoptively transferred to naive recipients with spleen cells from actively immunized animals but not with immune serum (20). Because of the link between *B. vulgatus* and carrageenan-induced colitis, the present study was undertaken to determine whether factors present on the bacterial surface mediated the observed phenomenon. Bacterial outer membrane preparations were examined by polyacrylamide gel electrophoresis (PAGE) and immunoblotting and compared in the animal model for their ability to enhance experimental colitis.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. Strains from the Infectious Diseases Research Laboratory, Tufts University School of Veterinary Medicine, Boston, Mass., were identified as *B. vulgatus* by using the Anastat system (Scott Laboratories, Fiskeville, R.I.). Strains were streaked for purity, grown in pre-reduced anaerobically sterilized PY broth (Scott) for 48 h at 37°C, divided into portions, and frozen at –80°C until used. The sources of the strains are listed in Table 1.

Growth conditions. One standard loopful of thawed organisms was transferred to a blood agar plate and grown overnight in an anaerobic jar (Gas-Pak; BBL Microbiology Systems, Baltimore, Md.) at 37°C. The next day, one loopful of bacteria was suspended in 1 ml of sterile 0.15 M NaCl and inoculated into PY broth (Scott) for overnight incubation. On the following day, 50 ml of this medium was inoculated into 1,500 ml of medium in an anaerobically sterilized 2-liter stoppered flask (proteose peptone-yeast extract medium [Difco Laboratories, Detroit, Mich.] supplemented with 0.5% NaCl, 1% dextrose, 5% fetal calf serum, and 0.05% cysteine; pH adjusted to 7.00 with potassium phosphate). After incubation for 36 h, the organisms were centrifuged at 10,000 × *g* at 4°C for 20 min and washed twice with 50 mM phosphate buffer. All cultures were checked for contamination at the end of the growth cycle.

Outer membrane preparation. A modification of the method of Kasper and Seiler (9) was used for the isolation of *B. vulgatus* outer membranes. After washing, organisms were suspended at 24°C in buffer containing 0.05 M sodium phosphate, 0.15 M NaCl, and 0.01 M EDTA (pH of final buffer, 7.4), followed by incubation at 60°C for 30 min. The bacteria were subjected to mild shearing by passage through a 25-gauge hypodermic needle by manual pressure. Organisms were pelleted by centrifugation at 10,000 × *g* at 4°C for

TABLE 1. Identity of *B. vulgatus* strains

Strain	Source
BV435	Abdominal abscess; human
BV20-15	Ulcerative colitis; human
BV26-21	Ulcerative colitis; human
ATCC 8482	American Type Culture Collection
40G2-33	Carrageenan-induced colitis; guinea pig
BV16-4	Antibiotic-associated colitis; human
BV10-9	Healthy human stool
40G1-38	Carrageenan-induced colitis; guinea pig

20 min, followed by centrifugation of the supernatant at 80,000 × *g* at 4°C for 2 h to pellet the outer membranes. The pellet was then suspended in the EDTA buffer with 0.5% *N*-lauroyl sarcosine added and kept at 24°C for 30 min to remove non-outer membrane components (3). Both centrifugations were repeated, and the gel-like pellet was suspended in water and lyophilized.

LPS extraction. The phenol-water method of Westphal and Jann (29) was used to extract lipopolysaccharide (LPS). Pelleted organisms were suspended in 45% aqueous phenol at 68°C for 30 min. After separation of phases at 4°C, the aqueous layer was removed, residual phenol was extracted with ether, and the LPS-containing aqueous phase was dialyzed. LPS was purified by repeated ultracentrifugation at 80,000 × *g* at 4°C for 4 h, redissolved in water, and lyophilized. The material obtained was checked for protein and nucleic acids by UV absorption at 260 and 280 nm.

Animal immunizations. Male Hartley strain guinea pigs weighing 250 to 300 g were used for all experiments (Charles River Breeding Laboratories, Wilmington, Mass.). Animals were housed in groups of six within stainless steel cages and were given food and sterile water, supplemented with B and C vitamins, ad libitum. Before the experimental protocol was begun, a blood sample was obtained from each guinea pig by percutaneous transthoracic cardiac puncture following an injection of ketamine as a short-duration anesthetic.

Animals (eight groups of six animals each) were immunized with outer membrane preparations of the eight *B. vulgatus* strains shown in Table 1. Each animal received three injections of the outer membrane preparation per week for 3 weeks, at a dose of 10 μg per animal. One injection per week was given in Freund incomplete adjuvant via the footpad, and the other two injections were administered by subcutaneous injection of the antigen in buffer. Animals were rested for 1 week and then given a subcutaneous booster injection of 10 μg of the appropriate antigen per animal. Additional serum samples were obtained from immunized animals on days 14 and 21 of the immunization schedule for determination of antibody levels (by microtiter agglutination assay of the whole homologous organisms). All animals achieved an agglutination titer of 1:128 or greater to the homologous immunizing strain. For immunoblotting experiments, rabbit antisera were also raised using the same protocol.

Animal challenge. After the booster injections and after serum antibody levels were measured, 5% degraded carrageenan was administered to all groups via the drinking water for a period of 23 days, along with daily oral feedings of 10^8 CFU of viable *B. vulgatus* 40G2-33, a strain known from previous experiments to produce colitis.

Tissue examination. Animals were sacrificed at the completion of carrageenan administration, and the entire gastrointestinal tract was examined grossly for the presence of ulcerations and other abnormalities. Tissue was obtained from the cecum, transverse colon, and rectum, placed into 10% neutral buffered Formalin, and processed for preparation of hematoxylin- and eosin-stained sections. Slides were coded and examined by a blinded reviewer. Tissues from each animal were scored for the presence of gross ulcerations, polymorphonuclear leukocytes in the lamina propria, crypt abscesses, epithelial thinning, edema, and distortion of normal crypt architecture, as described previously by Onderdonk et al. (20). The scoring system used a scale of 0 (no abnormalities) to 12 (severe abnormalities in all tissues). Scores for each group were averaged, and group comparisons were made using a two-sample *t* test and assuming unequal variances.

Polyacrylamide electrophoresis. Sodium dodecyl sulfate (SDS)-PAGE was performed with the Laemmli buffer system (11) on 10-μl samples containing 4 to 6 μg of protein. Lyophilized membranes were solubilized in sample buffer (0.1 M Tris, pH 8, 5 mM dithiothreitol, 1% SDS, and 4 M urea to aid in disaggregation of LPS) for 1 h at 37°C, followed by the addition of 5 mM iodoacetamide for 30 min at 37°C. Equal amounts of outer membrane protein were applied to wells in a 3% acrylamide stacking gel–8 to 10% acrylamide gradient separating gel (slab size, 10 by 7 by 0.75 cm) for analysis of proteins by Coomassie staining, or to a 4% acrylamide stacking gel–13% acrylamide separating gel for analysis of LPS by silver staining (Bio-Rad Laboratories, Richmond, Calif.). Electrophoresis (equipment from Hoeffer Scientific Instruments, San Francisco, Calif.) was done at 10 mA through the stacking gel and 20 mA through the separating gel with constant current and tap-water cooling until the bromophenol blue dye front was 0.5 cm from the end of the gel. Protein standards (molecular weights: bovine albumin, 67,000; ovalbumin, 43,000; soybean trypsin inhibitor, 23,000) were used.

Immunoblotting. After SDS-PAGE, proteins were electrophoretically transferred from the gel onto nitrocellulose sheets (Transphor Unit; Hoeffer Scientific Instruments) in the presence of 25 mM sodium phosphate (pH 6.5) for 18 h at 60 mA in a reservoir cooled with flowing tap water. After electrotransfer, the nitrocellulose sheets were immersed in a blocking buffer (5% skim milk, 0.5% Tween 20, and 0.01 M phosphate-buffered saline, pH 7.2) for 2 h at 37°C and then in immune serum diluted 1:500 in blocking buffer for 2 h at room temperature. The sheets were washed three times for 5 min (0.01 M phosphate-buffered saline, pH 7.2) and probed with a 1:500 dilution (in blocking buffer) of a goat anti-guinea pig immunoglobulin G alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, Mo.) for 2 h at room temperature. After three 5-min washes with phosphate-buffered saline, substrate solution consisting of 270 μl of indoxylphosphate (as a 5-mg/ml solution in dimethylformamide [DMF]), 36 μl of 3 M $MgCl_2$, 2.7 ml of a 0.1% solution

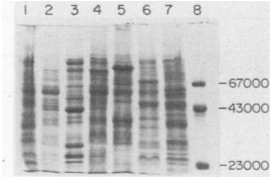

FIG. 1. Gradient (8 to 12% acrylamide) SDS-PAGE of outer membranes from *B. vulgatus*. Each lane contains 4 to 6 μg of protein. Stained by Coomassie stain. Strains: Lane 1, 40G1-38; lane 2, 40G2-33; lane 3, BV26-21; lane 4, BV20-15; lane 5, BV435; lane 6, BV16-4; lane 7, BV10-9. Lane 8, Molecular weight markers.

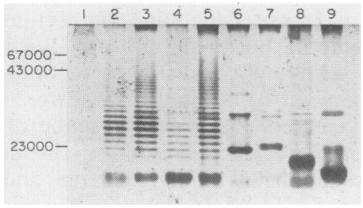

FIG. 2. SDS-PAGE (13% acrylamide) of LPS preparations from *B vulgatus*. Each lane contains 2 to 4 μg of carbohydrate. Stained by silver method. Lane 1, Molecular weight markers. Strains: Lane 2, 40G2-33; lane 3, BV20-15; lane 4, BV435; lane 5, ATCC 8482; lane 6, BV16-4; lane 7, 40G1-38; lane 8, BV10-9; lane 9, BV26-21. Lanes 2 through 5, Smooth LPS; lanes 6 through 9, rough LPS with common antigen seen at 30,000 molecular weight.

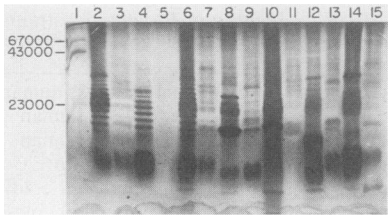

FIG. 3. SDS-PAGE (13% acrylamide) of crude phenol-water extraction comparing the aqueous-phase LPS content with the phenol-phase content. Each lane contains 5 μl of sample. Stained by silver method. Lane 1, molecular weight markers. Further lanes contain aqueous and phenol phases, respectively, of indicated strains: lanes 2 and 3, BV20-15; lanes 4 and 5, BV435; lanes 8 and 9, BV16-4; lanes 6 and 7, ATCC 8482; lanes 10 and 11, 40G1-38; lanes 12 and 13, BV10-9; lanes 14 and 15, BV26-21. The majority of LPS is extracted into the aqueous phases by the method of Westphal and Jann (29).

of Nitro Blue tetrazolium in 50 mM sodium carbonate–1 mM $MgCl_2$ (pH 9.8), and 24 ml of 50 mM sodium carbonate–1 mM $MgCl_2$ (pH 9.8) was added. Once bands were fully developed, the reaction was stopped with water.

RESULTS

SDS-PAGE of the outer membrane preparations allowed the comparison of potential antigenic factors in several *B. vulgatus* strains. Figure 1 shows the profile of outer membrane proteins as revealed by Coomassie staining. The protein patterns are complex and reflect 20 to 30 membrane-associated proteins within the 15,000 to 100,000-molecular-weight range. Simultaneous silver and Coomassie staining of gels run without urea in the solubilization buffer resulted in broad and indistinct banding (results not shown), possibly due to incomplete disaggregation of the LPS-membrane protein complexes extracted by the EDTA method. The presence of 4 M urea in the membrane solubilization buffer was found to be helpful in resolving some of the low-molecular-weight bands. There was considerable strain-to-strain variation in outer membrane band patterns.

LPS preparations made by the phenol-water method of Westphal and Jann (29) were also examined by silver-staining SDS-PAGE gels (Fig. 2). Smooth LPS types were defined as more than three regularly-spaced silver-stained bands extending from the dense lipid A band migrating with the dye front. The usual "ladder"-like silver staining pattern of smooth LPS types, reflecting the multiple repeating units of the O side chain, was found on four of the *B. vulgatus* strains, including ATCC 8482. Rough LPS patterns were found on the remaining four strains. The rough LPS displayed nonuniformity as well, with the lipid A bands migrating to three different molecular weight regions in 13% acrylamide gels, suggesting at least three different variants of the lipid A-core region in *B. vulgatus* LPS. LPS found in water phases and phenol phases was compared (Fig. 3) to determine whether the rough LPS types were more soluble in the phenol phase, as has been determined for other bacteria (4). In all cases, most of the LPS was found in the water phase. Therefore, all subsequent work was done on the LPS from the water phase. Another antigen of molecular weight around 30,000 was found in the water-phase extracts of all strains. This band was visualized by silver staining, but not by Coomassie staining, and may represent the common antigen described by others (21); the staining characteristics suggest this could be a membrane-associated lipoprotein or carbohydrate.

Immunoblotting of outer membrane preparations against the guinea pig sera raised to each of the eight *B. vulgatus* strains was done to detect major antigenic similarities and differences between strains. Not surprisingly, each antiserum reacted most strongly with its homologous outer membrane proteins. Eight to 10 major proteins per strain were seen to be serologically active. In addition, antisera to certain strains demonstrated selectively strong cross-reactivity to membrane proteins of other strains. These data are summarized in Table 2, and an example of the immunoblotting is shown in Fig. 4. The *B. vulgatus* strain tested divided into two "phenons" (closely related protein electrophoresis type) of cross-reactivity, indicating antigenic similarity as has been found for other bacterial species (25).

The phenon associated with cases of human ulcerative colitis was common to five strains (40G2-33, BV26-21, BV20-15, ATCC8482, and BV435; designated phenon alpha). The other three strains were weakly cross-reactive with alpha-type strains by immunoblotting, but showed

TABLE 2. Outer membrane proteins cross-reactive to antisera by immunoblotting

Strain	Reaction of antiserum against strain:							
	BV435	BV20-15	BV26-21	ATCC 8482	40G2-33	BV16-4	BV10-9	40G1-38
BV435	+	+	+	+	+	−	−	−
BV20-15	+	+	+	+	+	−	−	−
BV26-21	+	+	+	+	+	−	−	−
ATCC 8482	+	+	+	+	+	−	−	−
40G2-33	+	+	+	+	+	−	−	−
BV16-4	−	−	−	−	−	+	+	+
BV10-9	−	−	−	−	−	+	+	+
40G1-38	−	−	−	−	−	+	+	+

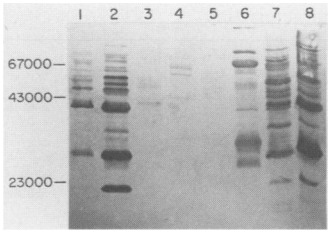

FIG. 4. SDS-PAGE (10% acrylamide). Immunoblotting of outer membrane antigens onto nitrocellulose blot developed with antiserum to strain 40G2-33. Each lane contains 4 to 6 μg of protein. Strains: Lane 1, *B. vulgatus* 299601A; lane 2, BV26-21; lane 3, BV10-9; lane 4, 40G1-38; lane 5, BV16-4; lane 6, BV435; lane 7, BV20-15; lane 8, 40G2-33. Lanes 2, 6, 7, and 8 are typical of the alpha phenon.

some similarity toward each other (strains BV16-4, BV10-9, and 40G1-38; designated phenon beta). Hyperimmune rabbit antisera raised to the prototypical alpha-type phenon strain (*B. vulgatus* 40G2-33) was used in cross-absorption experiments to further clarify the protein electrophoresis types. Samples of strain 40G2-33 immune rabbit antisera were absorbed with whole bacteria from the beta phenon group and used in immunoblotting experiments against the outer membrane proteins resolved by SDS-PAGE. Figure 5 shows an example of antisera to strain 40G2-33 cross-absorbed with strain BV16-4. The beta phenon organisms selectively absorbed reactivity to the homologous strains as well as to some of the protein bands in the alpha phenon set. However, four major protein bands remained in the alpha phenon set (molecular weights, 100,000, 57,000, 34,000, and 21,000). These four proteins were unique to the alpha phenon type and are immunologically reactive outer membrane antigens. The significance of this was tested in the animal model of ulcerative colitis.

The colitis scores for the strains tested are shown in Table 3. After the 23-day period of carrageenan challenge, there were distinct differences in the severity of colitis in animals immunized with *B. vulgatus* 40G2-33 outer membranes versus control animals. However, there were variations between groups of animals immunized with the outer membranes of different *B. vulgatus* strains; generally, alpha-type phenon strains were found to produce the more severe disease. Interestingly, the ATCC 8482 strain, which is the type reference strain for *B. vulgatus* classification was also capable of colitis enhancement. Animals immunized with two *B. vulgatus* beta strains not associated with colitis (BV10-9 from healthy human stool and BV16-4 from a case of antibiotic-associated colitis mediated by *Clostridium difficile*) did not demonstrate any enhancement of carrageenan-induced colitis when compared with control animals.

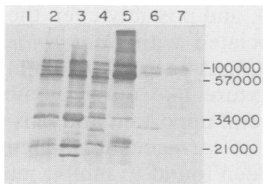

FIG. 5. Gradient (8 to 12% acrylamide) SDS-PAGE. Immunoblot developed with hyperimmune rabbit antiserum to strain 40G2-33, cross-absorbed with strain 16-4. Each lane contains 4 to 6 μg of protein. Strains: Lane 1, 40G1-38; lane 2, 40G2-33, lane 3, BV26-21; lane 4, BV20-15; lane 5, BV435; lane 6, BV16-4; lane 7, BV10-9.

TABLE 3. Results of immunization and challenge experiments[a]

Strain	Mean colitis score ± ED	*P* value[b]	% Enhancement over nonimmune controls
BV435	6.5 ± 1.5	<0.09	30
BV20-15	6.5 ± 1.7	<0.12	30
BV26-21	8.5 ± 1.4	<0.002	70
ATCC 8482	7.0 ± 1.2	<0.02	40
40G2-33	6.5 ± 1.3	<0.06	30
BV16-4	5.3 ± 1.6	<0.70	6
BV10-9	6.2 ± 1.5	<0.20	24
40G1-38	7.0 ± 1.1	<0.01	40
Nonimmune control	5.0 ± 0.7		

[a] Six animals in each group were immunized with outer membrane preparations according to the protocol given in the text and challenged with 5% carrageenan in their drinking water plus 10^8 CFU of viable strain 40G2-33 daily.

[b] Using two-sample *t* test assuming unequal variances for each group versus control.

Since the nonimmune control animals fed carrageenan and *B. vulgatus* 40G2-33 also developed colitis, the significance of the score is obscured by the control value of 5.0 (on a 0-to-12-point histological severity scale). By a nonparametric two-sample *t* test (assuming unequal variances) comparing group means, while accounting for standard deviation, small differences in histological score (such as between values of 5.0 and 6.5) were shown to be statistically significant. Normalizing the score to show percent enhancement in the severity of colitis reflected differences between groups more clearly (Table 3).

DISCUSSION

The virulence factors of a variety of enteric pathogens such as *Salmonella* and *Shigella* spp. have been well studied. In many cases, outer membrane components play an important role in mucosal adherence, invasion, complement activation, resistance to serum bactericidal activity and phagocytosis, etc. In the guinea pig model of carrageenan-induced colitis, a normal colonic inhabitant, *B. vulgatus*, is implicated in the pathogenesis of the colitis. This study defines some of the outer membrane components of this organism and correlates some of these components with immune enhancement of colitis.

The EDTA extraction method of Kasper and Seiler (9) has been shown to produce preparations of intact outer membranes in a number of *Bacteroides species, including B. fragilis* (9) and *B. melaninogenicus* (13). When outer membrane protein patterns have been examined, similarity in patterns has been observed for most strains of these species (10). Because of this, the outer membrane protein profile has been suggested as a taxonomic criterion for clinical laboratories (22). Our data indicate significant variation of outer membrane proteins between isolates of *B. vulgatus* despite the similarity of other phenotypic characteristics used in classification (for example, the fermentation of glucose and rhamnose but not trehalose, growth in bile, lack of indole production, antibiotic resistance patterns, and the production of volatile fatty acids as measured by gas chromatography). This complexity in outer membrane protein pattern has been observed in *Bacteroides thetaiotaomicron* by Kotarski and Salyers (10). For *B. vulgatus*, the variation may represent taxonomic uncertainty created by the phenotypic characteristics chosen to define the species (5, 6).

We have used two phenotypic traits to differentiate *B. vulgatus* strains: outer membrane proteins and LPS. SDS-

TABLE 4. Summary of outer membrane protein characteristics

Strain	Source	LPS type	Alpha phenon type	Immune enhancement[a]
BV435	Abdominal abscess	Smooth	Yes	Yes
BV20-15	Ulcerative colitis patient	Smooth	Yes	Yes[b]
BV26-21	Ulcerative colitis patient	Rough	Yes	Yes
ATCC 8482	American Type Culture Collection	Smooth	Yes	Yes
40G2-33	Carrageenan-induced colitis	Smooth	Yes	Yes
BV16-4	Antibiotic-associated colitis	Rough	No	No
BV10-9	Healthy human	Rough	No	No
40G1-38	Carrageenan-induced colitis	Rough	No	Yes[b]

[a] Challenge with strain 40G2-33.
[b] Incapable of immune enhancement when the homologous strain was used in challenge (17).

PAGE and Western blotting (immunoblotting) of proteins against a panel of guinea pig antisera showed variation in outer membrane proteins of *B. vulgatus* among the eight strains tested. We prefer the term "phenon" to reflect the strain variation of outer membrane proteins visualized by this technique (25). Variations in outer membrane proteins have been observed for single species under different growth conditions, but our preparations were made using identical growth and nutrient conditions and therefore must reflect other differences between strains. This form of protein profiling offers considerable advantages for typing bacterial strains of clinical interest, especially when no other typing method exists (25). The protein patterns have been found to be consistent with DNA-DNA hybridization data for *Bacteroides ureolyticus* (26) and other bacteria. In the case of *B. vulgatus*, the alpha phenon type was found to correlate with the enhancement of colitis and the presence of four major outer membrane proteins. The functions of these proteins and their role in the induction of immunity (local or systemic) remain to be defined. The genetic nature behind the expression of these proteins unique to the "pathogenic" phenon is also unknown. For example, are the differences reflective of different DNA homology groups, different species, different plasmids, or different phage infections?

The LPS type visualized by SDS-PAGE was another phenotypic characteristic of *B. vulgatus* studied. The closely related LPS of *B. fragilis* has been extensively evaluated. It differs from aerobic bacterial LPS in the absence of 2-keto-3-deoxyoctulosonic acid and heptose and the lack of pyrogenicity. However, other properties such as B-cell mitogenicity and the Shwartzman reaction are preserved (7, 28). Most workers have defined only rough LPS in *B. fragilis*, although some contend that a few clinical isolates appear smooth (21). The *B. vulgatus* LPS demonstrated in Fig. 2 show at least one type of smooth LPS and three types of rough LPS; all were extractable into the aqueous phase by the phenol-water method (similar to those of *B. fragilis*). The smooth-type LPS was associated with the alpha-type phenon and the immune enhancement of colitis for all strains studied, except for BV26-21 (Table 4).

Other antigenic molecules exist on the outer membrane of gram-negative bacteria. Well-described encapsulated organisms are found among *B. fragilis* strains, but evidence for capsules in other *Bacteroides* species is variable (8). Using conditions which in our laboratory visualize *B. fragilis* capsular polysaccharide by SDS-PAGE and silver staining, we have bene unable to visualize capsular-type high-molecular-weight material on any of the *B. vulgatus* strains tested (data not shown). Although suggestive, this does not represent proof that *B. vulgatus* isolates lack capsular polysaccharides.

Onderdonk et al. (20) have shown that *B. vulgatus* is a sufficient cause of ulcerative lesions in gnotobiotic guinea pigs. Prior immunization with whole, viable organisms enhanced the severity of colitis on subsequent challenge with homologous strains and carrageenan as an adjuvant. The EDTA-extracted outer membrane antigens prepared in these experiments duplicated this phenomenon when used as immunizing antigens in the carrageenan-induced colitis model. Since the severity of illness and histological score for the inflammatory lesions were similar, it is likely that outer membrane antigen(s) is a significant factor in the pathogenesis of colitis in this model.

The method of membrane purification using EDTA and sarcosyl makes it unlikely that the animals were immunized with either extracellular bacterial products (e.g., exotoxins or metabolic products such as fatty acids or peptides) or intracellular or cytoplasmic contents. Of the two components of the outer membranes (proteins and LPS) studied by the methods reported here, the outer membrane alpha phenon correlated best with in vivo activity. LPS type did not correlate as well since smooth and rough isolates were capable of enhancing colitis (e.g., BV26-21, Table 4). Two nonpathogenic isolates (BV10-9 from healthy human stool and BV16-4 from antibiotic-associated colitis due to *C. difficile*) were relatively impotent immune enhancers of colitis. Both had rough LPS types and beta phenons as determined by immunoblotting. Two pathogenic isolates from human ulcerative colitis patients (BV20-15 and BV26-21) had similar phenons and demonstrated immune enhancement in experimental animals. Strain 40G1-38 belonged to a nonpathogenic phenon, but was capable of immune enhancement when challenged with strain 40G2-33. In previous experiments, when animals immunized against whole 40G1-38 were given homologous challenge with 40G1-38, enhancement of colitis was not observed. This indicates a different potency for strains depending on the challenging organism.

Besides outer membrane proteins and LPS, other membrane-associated antigens have immune-stimulating and immune-modulating effects. These include lipoproteins (14), peptidoglycan (12), and possibly even the sphingolipids that are uniquely associated with *Bacteroides* sp. outer membranes (15). The effects seen after immunizing with a complex antigenic mixture like outer membrane preparations may be due to several important antigens presented at once. The adjuvant role of carrageenan challenge is also unclear; carrageenan has been found to inhibit macrophage-lymphocyte interactions (2), induce changes in gut epithelial glycoproteins (1), and accelerate the immunoglobulin M-immunoglobulin G switch after immunization with *Salmonella* sp. O antigen (16).

Several theories of the pathogenesis of human ulcerative

colitis exist. One mechanism suggests production of autoantibody against a colon-specific antigen (24) and subsequent inflammation due to antibody-dependent cell-mediated cytotoxicity. The possibility of a bacterial antigen triggering autoantibody production has been suggested. The isolation and purification of bacterial antigens involved in an experimental model of colitis will allow testing of this hypothesis.

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