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Monoclonal Antibodies To Detect Capsular Diversity among *Bacteroides fragilis* Isolates

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The capsular polysaccharide complex (CPC) of *Bacteroides fragilis* is composed of two distinct polysaccharides, designated PS A and PS B, and is a major virulence factor of this microorganism. In order to investigate the antigenic diversity of the CPCs of *B. fragilis* strains, we generated and characterized 10 monoclonal antibodies (MAbs) directed to the CPCs of three reference strains. The specificities of the MAbs were determined by enzyme-linked immunosorbent assay and dot-immunobinding assay. At least one MAb was specific for each PS A and PS B of the three strains. The MAbs were used to detect capsular antigens on the surface of 231 *B. fragilis* isolates from different geographical areas by a whole-cell dot-immunobinding assay. Over half of the strains, regardless of the country of origin, reacted with at least one MAb. Clinical extraintestinal infection isolates were significantly more reactive than fecal isolates, suggesting an association between capsular composition and the propensity to cause clinical infections. The patterns of reactivity of the isolates with the 10 MAbs were very different and sometimes extremely complex and indicated a sharing of epitopes among different capsular polysaccharides. The reactive strains could be grouped according to 32 different patterns; some patterns were relatively common, while others were rarer and were shown by only one or two strains. These results show that *B. fragilis* capsular polysaccharides are antigenically extremely diverse. This complexity and the large number of nonreactive strains indicate that a typing system based on *B. fragilis* capsular antigens will be difficult to establish.

Bacteroides fragilis is the predominating anaerobe isolated from clinical cases of intra-abdominal sepsis. We have demonstrated that the polysaccharide capsule of *B. fragilis* is a major virulence factor for this bacterium, as inferred from its ability to induce intra-abdominal abscess formation in a rat model of the disease process (15). The capsular polysaccharide complex (CPC) of *B. fragilis* is composed of two distinct polysaccharides with unique structural features (19). Each repeating unit possesses both positively and negatively charged groups that influence the ability of these saccharides to promote abscess formation in the host (21).

All *B. fragilis* capsular structures examined so far appear to comprise two polysaccharides with different migration properties in immunoelectrophoresis (IEP) at pH 7.3 (20): polysaccharide A (PS A) behaves as a neutral or slightly positively charged polysaccharide, while PS B is negatively charged. For the type strain, NCTC 9343, these two polysaccharides have been fully characterized and their fine structure has been elucidated (4).

Previously, polyclonal antiserum directed against the capsule of strain NCTC 9343 and monoclonal antibodies (MAbs) specific for PS A or PS B have been used to detect immunological similarities to the capsules of other *B. fragilis* strains. By using IEP, cross-reactions between the polysaccharides of strain NCTC 9343 and those of some other *B. fragilis* strains were found; the cross-reactions included either one or both of the polysaccharides (20). This previous study showed that *B. fragilis* capsules exhibit antigenic diversity, suggesting that capsular antigens could be used for serotyping *B. fragilis* strains.

Although *B. fragilis* is an important cause of infections in hospital settings (1), its nature as an endogenous pathogen has not encouraged epidemiological studies and the consequent development of a typing scheme. Attempts to develop serological typing for *B. fragilis* have been confined to individual laboratories (5, 10).

Because of the complexity of the *B. fragilis* capsule, it is apparent that serotyping by polyclonal antisera would require extensive cross-absorption and that the antigenic specificity would be difficult to establish. MAbs, although more time-consuming and expensive to produce, might recognize epitopes specific for PS A or PS B, potentially relevant for a typing scheme.

This study describes the first attempt to differentiate *B. fragilis* strains on the basis of capsular epitopes. Ten MAbs directed to the CPCs of three reference strains cross-reacted with over 50% of *B. fragilis* strains from different sources and different geographical areas. The diversity of the reactivity patterns suggests a wide heterogeneity of capsular antigens in the species *B. fragilis.*

MATERIALS AND METHODS

Bacterial strains. Reference strains *B. fragilis* NCTC 9343, ATCC 23745, and NCTC 2429 were used for the production of MAbs. For the study, 231 *B. fragilis* isolates were examined. These included 172 strains originating from clinical extraintestinal infections, of which 102 were from Italy (some from the collection of the Istituto Superiore di Sanità, Rome, and others kindly provided by R. Di Rosa, University of Rome, and by M. G. Menozzi, University of Parma), 39 were from the United Kingdom (kindly provided by S. Tabaqchali, St. Bartholomew's Hospital, London, and by B. Duerden, Public Health Laboratory Service, Cardiff, Wales), and 31 were from the United States (from the collection of Channing Laboratory, Brigham and Women's Hospital, Boston, Mass.). Reference strains

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NCTC 11295 and 11870 were also included; these strains, originally isolated in the United Kingdom, are included among the British strains below. Fifty-seven isolates of *B. fragilis* were obtained from the feces of adults and children in Italy. Additional clinical isolates included as controls were 20 strains of *Bacteroides* species other than *B. fragilis*: *B. thetaiotaomicron* (12 strains), *B. vulgatus* (4 strains), and *B. distasonis* (4 strains).

For all strains, identification was confirmed by a commercially available enzyme method (ID 32A; bioMérieux, Marcy-l'Etoile, France) or by conventional methods (9) using Cystine Tryptic Agar (Difco Laboratories, Detroit, Mich.) plates for sugar fermentation (13). The strains were kept frozen at −80°C or stored at room temperature in cooked-meat medium (Oxoid, Basingstoke, United Kingdom) until used. When required, *Bacteroides* strains were plated onto Columbia agar (Oxoid) plates containing 5% sheep blood, 0.5% yeast extract, 5 mg of hemin per liter, and 0.5 mg of vitamin K per liter and incubated in an anaerobic cabinet (Microflow Anaerobic System; MDH, Andover, United Kingdom) at 37°C for 48 h. Purity and anaerobic requirement checks were carried out routinely.

Purification of CPC. Preparation and purification of the CPC of *B. fragilis* were accomplished by previously published methods (19). The absence of contaminating lipopolysaccharide (LPS) in capsular preparations was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (20).

MAb production. MAbs to the CPCs of three reference strains (NCTC 9343, ATCC 23745, and NCTC 2429) were produced. These strains were chosen because previous studies (reference 24 and unpublished observations) showed that polyclonal antisera to the capsules of these strains cross-reacted with a large number of *B. fragilis* isolates.

BALB/c mice were immunized by intraperitoneal injection of whole bacterial cells (approximately 10^9 cells) once a week for 3 weeks and were then given two booster injections at 3-week intervals before being sacrificed. The splenocytes of the immune mice were fused with cells of the P3X63-AG8.653 nonsecreting mouse myeloma cell line by the method of Oi and Herzenberg (14) with 50% polyethylene glycol at 37°C. When hybridoma clones were visible, the supernatants were directly tested by immunological assays.

Detection of relevant-MAb-producing clones. For each CPC, an enzyme-linked immunosorbent assay (ELISA) was developed to detect relevant-MAb-producing clones. Wells of polystyrene microtiter plates (Immulon A; Dynatech Laboratories, Chantilly, Va.) were coated with 100 μl of poly-L-lysine-coupled CPC (6) (2.5 μg/ml in 50 mM phosphate buffer, pH 7.0) at 37°C for 2 h. The plates were then blocked with 5% skim milk in phosphate buffer for 2 h and used immediately or kept in sealed boxes at −20°C. Supernatants of hybridoma cultures were directly transferred to the coated ELISA plates (100 μl per well) and incubated for 1 h at 37°C. After a washing with phosphate buffer–0.05% Tween 20, alkaline phosphatase-conjugated anti-mouse polyvalent immunoglobulin (Sigma Company, St. Louis, Mo.) was added to each well, and the plates were incubated for 1 h. The reaction was developed by adding the phosphatase substrate *p*-nitrophenyl phosphate (Sigma), and the A_{405} was read with an automated ELISA plate reader (Bio-Rad Laboratories, Richmond, Calif.). For each fusion, only reactivity to the CPC of the strain used for immunization (homologous CPC) was tested. Supernatants which gave a positive ELISA reaction were further screened by a dot-immunobinding assay (DIA) with the CPC as the antigen (see below). Subsequently, tests were performed to determine whether MAbs were directed to PS A or PS B. In the case of strain NCTC 9343, both PS A and PS B were available in purified form so that a DIA with the purified polysaccharides could be performed to determine the specificity of the MAbs as previously described (19). In the case of strain ATCC 23745, only PS A was available in purified form (unpublished data); therefore, a DIA was performed only with PS A. The reactivity of a MAb to PS B of strain ATCC 23745 was indirectly inferred by a positive reaction with the CPC and a negative reaction with PS A. In the case of strain NCTC 2429, neither polysaccharide was available in purified form; consequently, several clones reactive with the CPC in the DIA had to be subcloned and expanded so that they could be injected into mice to produce ascitic fluid. This was used in IEP experiments to define the antigenic specificities of the MAbs (see below).

All the hybridoma lines selected were subcloned twice by limiting-dilution cloning.

Characterization of the MAbs. The culture supernatants of each hybridoma clone were pooled, aliquoted, and kept frozen at −20°C until used in the DIA. The classes and subclasses of the MAbs were determined by an enzyme immunoassay (mouse hybridoma subtyping kit; Boehringer Mannheim Italia SpA, Milano, Italy) according to the recommendations of the manufacturer. The concentration of antibodies in the supernatants was evaluated by a double-antibody sandwich ELISA. Doubling dilutions of the supernatants were added to wells coated with a polyvalent anti-mouse immunoglobulin (Boehringer Mannheim). Then, a phosphatase-labelled class-specific anti-mouse immunoglobulin G (IgG) or IgM (Sigma Chemicals) was added in order to bind to the captured MAbs. The detection method was the same as above. Purified irrelevant mouse MAbs of known concentrations belonging to the same class and subclass as the MAbs to be quantified (IgG2a, IgG3, and IgM) were used to determine reference curves. Both samples and standards were examined in duplicate. A computer program based on a reference-line method (12) was used to determine the concentrations of MAbs in the supernatants. The antigenic specificity of each MAb for either PS A or PS B was confirmed by IEP using ascitic fluids obtained from pristane-primed BALB/c mice inoculated with MAb-secreting hybridoma cells.

DIA. A loopful of *Bacteroides* culture from a Columbia blood agar plate was inoculated into 10 ml of brain heart infusion medium containing yeast extract (0.5%) and hemin (5 mg/liter), and the culture was incubated in the anaerobic cabinet for 18 h. A volume of 1 ml of this culture was centrifuged in an Eppendorf centrifuge (5,000 × *g*), and the bacterial cells were washed twice in phosphate-buffered saline (PBS) (0.1 M; pH 7.4) and then resuspended in PBS to the original volume. Two microliters of this suspension were spotted onto nitrocellulose (NC) sheets. Several replicas of the spots were prepared. Together with the strains to be tested, each NC sheet contained spots of the reference strains (NCTC 9343, ATCC 23745, and NCTC 2429). The NC was dried at 37°C for 1 h and was used immediately or kept refrigerated at +4°C in a sealed container. For the immunobinding assay, the NC was blocked at room temperature with 50 mM Tris-HCl–200 mM NaCl (pH 7.4) (Tris buffer) containing 5% skim milk (Difco Laboratories) and 0.3% sodium azide (blocking buffer) and then allowed to react with each MAb (hybridoma culture supernatant diluted 1:4 in blocking buffer) at room temperature for 2 h. After three washes (5 min per wash) in Tris buffer containing 0.05% Tween 20, the NC was probed with alkaline phosphatase-conjugated anti-mouse IgG or IgM (according to the class of the MAb) (Sigma) diluted 1:500. After three more washes, the NC was developed with BCIP-NBT (5-bromo-4-chloro-3-indolylphosphate toluidinium–nitroblue tetrazolium) substrate (Sigma). The development of a purple color was taken as a positive reaction, while a barely visible faint color was considered a negative reaction. All the DIA experiments were performed in duplicate with different broth cultures of the test strains.

Dose-response assay. In order to determine the detection limits of the MAbs for homologous purified polysaccharide or homologous whole bacterial cells in the DIA, a dose-response assay was performed. Fivefold dilutions of purified CPCs (starting from 1 mg/ml) of reference strains NCTC 9343, ATCC 23745, and NCTC 2429 were made, and 2 μl of each was applied to NC strips. Similarly, 2-μl samples of fivefold dilutions of whole bacterial cells from an overnight broth culture (approximately 2×10^9 to 3×10^9 CFU/ml) were applied to NC. The NC strips were probed with the homologous MAbs by the procedure described above. Two independent sets of experiments were carried out. The lowest dilution of purified CPC or of bacterial cells which was clearly stained positive by a MAb was considered the detection limit for that MAb.

IEP. IEP was performed at pH 7.3, as previously described (19). The antigens were the purified CPCs (2 to 3 μg) of the three reference strains. The source of antibodies was homologous polyclonal rabbit antisera directed to whole bacteria (19) or mouse ascitic fluids. After incubation, the slides were washed and stained with Coomassie blue.

RESULTS

MAb specificity. For the generation of MAbs against the CPC of *B. fragilis*, whole bacterial cells were used as immunogens, and the hybridomas obtained were screened by ELISA for the secretion of antibodies specific for the *B. fragilis* CPC. Positive clones were subsequently tested in a DIA for reactivity with homologous CPC. Eighty percent of the supernatants reactive in the ELISA were also positive in the DIA. This discrepancy between the reactivity of MAbs in the ELISA and DIA could be due either to nonspecific binding of the MAbs in the ELISA or to conformational differences in epitope presentations on the two different surfaces. Positive supernatants were further screened for reactivity with purified PS A or PS B from the homologous strain, if available, by DIA. The specificity was confirmed in all cases by IEP results (see below). None of the MAbs obtained were found to react with both PS A and PS B. In some cases, MAbs reacting to the same polysaccharide were found to be of different isotypes, and they were chosen for further study.

Altogether, 10 hybridoma cell lines were established. The characteristics of these MAbs are detailed in Table 1. Four MAbs were IgM and six MAbs were IgG, of which five belonged to the IgG3 subclass. Three MAbs recognized the CPC of strain NCTC 9343 (two directed to PS AI and one to PS BI), four MAbs recognized the CPC of strain ATCC 23745 (two each directed to PS AII and PS BII), and three MAbs recognized the CPC of strain NCTC 2429 (two MAbs to PS AIII and one to PS BIII).

The MAbs directed to PS AI, BI, AII, BII, and BIII were specific; however, both MAbs directed to PS AIII (NCTC

TABLE 1. Characteristics of MAbs

MAb	Isotype	Concn[a] (μg/ml)	Reactivity with:		Sensitivity[c] to:	
			Strain	PS[b]	CPC (ng)[d]	Whole cells (CFU)[d]
CE3	IgG3	11	NCTC 9343	AI	16	6×10^4–3×10^5
IC5	IgM	51	NCTC 9343	AI	80	3×10^5
G9	IgG3	30	NCTC 9343	BI	16–80	3×10^5
F4	IgG2a	24	ATCC 23745	AII	2,000–400	5×10^5–2×10^6
2G5	IgM	44	ATCC 23745	AII	3–16	1×10^5–5×10^5
F11	IgG3	12	ATCC 23745	BII	80	5×10^5
IE8	IgM	39	ATCC 23745	BII	80	5×10^5
3C3	IgM	37	NCTC 2429	AIII	80	2×10^5
4D5	IgG3	20	NCTC 2429	AIII	3–16	2×10^5
4C4	IgG3	19	NCTC 2429	BIII	80	1×10^6

[a] Concentration of antibodies in supernatants (see text for method).
[b] Roman numerals indicate the origin of PS A or B (I from NCTC 9343, II from ATCC 23745, and III from NCTC 2429).
[c] Detection limit obtained by using unprocessed hybridoma culture supernatants.
[d] Minimum quantity of purified homologous CPC or homologous whole cells per spot detected in the DIA (results of two separate experiments; discrepancies, when present, represent 1 fivefold dilution).

2429) also reacted with strain ATCC 23745 in the whole-cell DIA but not with the purified CPC in the DIA or IEP (data not shown).

The concentrations of antibodies in the hybridoma supernatants used for the study ranged from 11 to 51 μg/ml. In the DIA, each MAb exhibited a reproducible detection limit for homologous CPC and homologous whole bacterial cells. The lower limit of detection of MAbs for homologous antigens, particularly for purified CPC, varied by up to 2 orders of magnitude. In subsequent studies, the number of bacterial cells of the test strains that were applied to NC for DIA was approximately 6×10^7.

Figure 1 demonstrates the IEP precipitin patterns of MAbs specific for PS A and PS B of the three reference strains. Rabbit polyclonal antiserum, used as a control, yielded the typical precipitin pattern seen previously with homologous CPC. This precipitin pattern consists of lines for PS A and PS B and a line of intermediate electrophoretic mobility presumably corresponding to precipitin c (19), which has been previously shown to be an aggregate of the component polysaccharides (23). A precipitin line characteristic of either PS A or B was seen with all MAbs. Several of the MAbs also gave a second precipitin arc with a mobility characteristic of precipitin c.

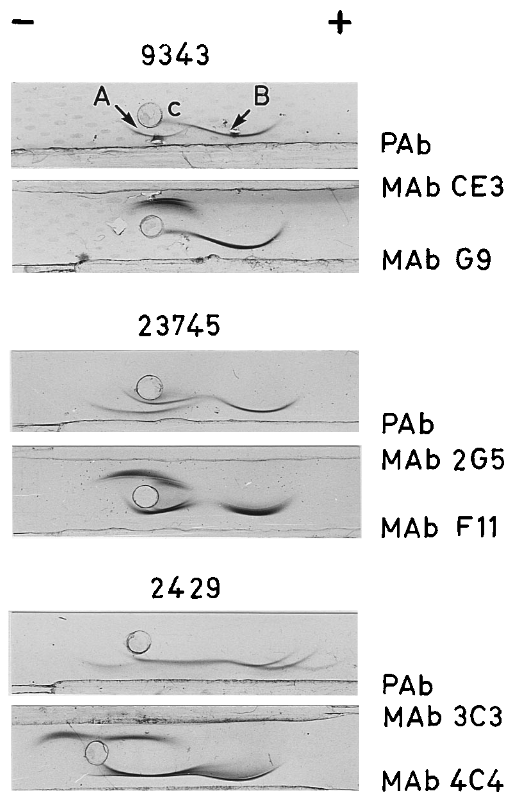

FIG. 1. IEP precipitin patterns of the purified CPCs of *B. fragilis* reference strains NCTC 9343, ATCC 23745, and NCTC 2429. MAbs were obtained from mouse ascitic fluid. For the specificities of the MAbs, see Table 1. Complete IEP profiles are obtained by precipitation with homologous rabbit polyclonal antiserum (PAb), while with the MAbs profiles corresponding to only PS A (neutral or slightly positive) or PS B (negative) are obtained. In some cases, precipitin c, a line with intermediate mobility, is also revealed. For strain NCTC 9343, PS A (A), PS B (B), and precipitin c (c) are indicated.

Reactivities of *B. fragilis* strains. A total of 231 *B. fragilis* strains were tested. Of these, 125 (54%) reacted with at least one MAb. All results were verified in duplicate experiments, and no discrepancies were found. There were some differences in the reactivities of strains with specific MAbs according to the source of isolation (Table 2). Between 58 and 65% of clinical isolates from each of the three countries of isolation were reactive with at least one MAb. Italian strains isolated from clinical extraintestinal infection samples were more frequently reactive than Italian strains isolated from feces, and this difference was statistically significant. None of the clinical isolates of *Bacteroides* tested, including 12 *B. thetaiotaomicron*, 4 *B. vulgatus*, and 4 *B. distasonis* isolates, reacted with MAbs to *B. fragilis.*

Patterns of reactivity of *B. fragilis* strains. The patterns of reactivity of the *B. fragilis* strains with the MAbs were very complex. With the panel of MAbs used, 33 different patterns of reactivity were obtained for 231 strains tested (Table 3). Some of these patterns (especially patterns 3, 4, 17, and 18) comprise quite a large number of strains. The majority of the reactive strains reacted only with MAbs directed to one polysaccharide. When two MAbs directed to the same reference polysaccharide were available, most test strains reacted with only one of the two MAbs. For example, MAbs CE3 and IC5 both were produced by immunizing mice with PS AI (strain NCTC 9343). However, they demonstrate different patterns of reactivity with *B. fragilis* strains. We must assume that if two MAbs reacting to a single reference polysaccharide give different patterns of reactivity with *B. fragilis* strains, this represents differences in the epitope specificity of the MAbs. Moreover, this suggests

TABLE 2. Reactivities of *B. fragilis* isolates with MAbs according to origin

Country and type of sample[a]	No. of isolates	No. (%) reactive
Italy		
Clinical	102	59[b] (58)
Fecal	57	22[b] (38.5)
United Kingdom, clinical	41	24 (59)
United States, clinical	31	20 (65)
Total	231	125 (54)

[a] Clinical samples were from extraintestinal infections.
[b] $P = 0.03$ (χ^2 test).

TABLE 3. Patterns of reactivity obtained with 231 *B. fragilis* strains and a panel of 10 MAbs

Pattern	No. of strains	Reactivity with the indicated MAb[a] to strain:									
		NCTC 9343			ATCC 23745				NCTC 2429		
		CE3 (AI)	IC5 (AI)	G9 (BI)	F4 (AII)	2G5 (AII)	F11 (BII)	1E8 (BII)	3C3 (AIII)	4D5 (AIII)	4C4 (BIII)
1	8	+	−	−	−	−	−	−	−	−	−
2	8	−	+	−	−	−	−	−	−	−	−
3	15	−	−	+	−	−	−	−	−	−	−
4	12	+	−	+	−	−	−	−	−	−	−
5	2	+	+	+	−	−	−	−	−	−	−
6	4	−	−	−	+	−	−	−	−	−	−
7	1	−	−	−	−	+	−	−	−	−	−
8	2	−	−	−	−	−	+	−	−	−	−
9	1	−	−	−	−	−	−	+	−	−	−
10	3	−	−	−	+	+	−	−	−	−	−
11	1	−	−	−	+	−	+	−	−	−	−
12	4	−	−	−	+	−	−	+	−	−	−
13	1	−	−	−	−	+	+	−	−	−	−
14	1	−	−	−	−	+	−	+	−	−	−
15	2	−	−	−	−	−	+	+	−	−	−
16	2	−	−	−	+	+	−	+	−	−	−
17	16	−	−	−	−	−	−	−	+	−	−
18	19	−	−	−	−	−	−	−	−	+	−
19	5	−	−	−	−	−	−	−	+	+	−
20	1	−	−	−	−	−	−	−	+	−	+
21	1	−	−	−	−	−	−	−	−	+	+
22	2	−	−	−	−	−	−	−	+	+	+
23	2	+	−	−	+	−	−	+	−	−	−
24	1	−	−	+	−	−	−	+	−	−	−
25	1	+	+	+	−	−	+	−	−	−	−
26	1	+	−	+	−	−	−	−	+	−	−
27	1	+	−	+	−	−	−	−	−	+	−
28	1	−	+	+	−	−	−	−	−	+	+
29	3	−	−	+	−	−	−	−	−	+	−
30	1	−	−	−	+	−	−	−	−	+	−
31	2	−	−	−	+	+	−	−	−	−	+
32	1	−	−	−	−	−	+	+	+	−	−
33	106	−	−	−	−	−	−	−	−	−	−

[a] The polysaccharides recognized by the MAbs are indicated in parentheses.

that although specific epitopes are shared, they are not part of the same polysaccharide.

In some cases, the patterns of reactivity were extremely complex, with single strains reacting with several MAbs directed to different polysaccharides. For example, strain 17905 (Table 3, pattern 28) reacts with MAbs to PS AI, AIII, BI, and BIII.

DISCUSSION

The goal of this work was to study the serologic relatedness of capsular polysaccharides within the *B. fragilis* species. Understanding the relatedness of these polysaccharides could lead to serologic classification, which might be a useful epidemiological tool. The basis of this hypothesis was the observation that *B. fragilis* strains express at least two distinct polysaccharides on their surface (20). In at least one strain (NCTC 9343), the polysaccharides exist in an ionically linked complex (the CPC) which makes separation of these antigens technically difficult (23). Each polysaccharide of strain NCTC 9343 consists of oligosaccharide repeating units with free amino, carboxyl, and/or phosphate groups giving the CPC its highly charged characteristics (4). It has also been shown that the polysaccharides of the *B. fragilis* strains examined so far are antigenically diverse but that several show cross-reactivity with the NCTC 9343 CPC (20).

In this study, we have attempted to further characterize the antigenic diversity of the polysaccharides. We chose to utilize MAbs because production of polyclonal antisera required immunization with purified component polysaccharides which were technically difficult to obtain. Moreover, MAbs do not exhibit variability from batch to batch, as is the case with rabbit polyclonal antisera, and they target defined epitopes and might be useful for studies of the immunochemical structure of the capsules. Ten MAbs reactive to PS A and PS B of three reference strains were selected; the antibodies represented different isotype and epitope specificities. Some MAbs to the same strain, especially those produced against strain ATCC 23745, exhibited ample differences in the lower limit of detection of homologous polysaccharide. This might represent differences in avidity between the MAbs but could also represent differences in the availability of the single epitopes, especially in the purified CPC.

Using the panel of MAbs, we examined strains from three different countries. The strains from the United Kingdom and United States were all clinical isolates, while the strains from Italy represented both clinical extraintestinal infection and fecal isolates. The strains were examined by a DIA for sharing of surface epitopes to which the MAbs were directed. The method gave reproducible results, and the patterns of reactivity of the strains appeared stable. This was observed in partic-

ular with the reference strains, which were examined several times upon different subcultures.

Fifty-four percent of strains tested reacted with at least one MAb. Reactions were seen more commonly for the clinical extraintestinal infection isolates from the three countries (between 58 and 65%), while only 37% of fecal isolates from Italy were recognized. This prevalence of shared polysaccharide epitopes in clinical extraintestinal infection isolates compared with fecal isolates may be due to the choice of the reference strains used to generate the MAbs, which were originally clinical isolates, and suggests that there may be an association of certain capsular types with a propensity to cause clinical infections.

We believe that the lack of reactivity of a large number of isolates with the MAbs does not indicate that the strains do not possess a capsule, since the presence of capsules in all *B. fragilis* strains examined, both from feces and from clinical infection samples, has been found previously (20). Rather, it appears that there exists a large antigenic variety of capsules and that only a portion of them are composed of epitopes recognized by the panel of MAbs.

The majority of isolates shared at least one epitope with the CPC of one of the three reference strains. Selected MAbs such as CE3, G9, 3C3, and 4D5 recognized altogether 75% of reactive strains, while other epitopes appear to be rarer. The patterns of reactivity obtained with the panel of MAbs were numerous and in some cases extremely complex. Certain patterns of reactivity, such as that of CE3 and G9 (both MAbs produced against the CPC of strain NCTC 9343), were relatively common, whereas others occurred with only one or two strains. Since we tested a limited number of MAbs, representing a limited number of polysaccharide epitopes, strains showing the same pattern of reactivity with the MAbs do not necessarily have the same CPC; in fact, it is probable that they have different capsular structures with one or more shared epitopes.

It is intriguing that MAbs 3C3 and 4D5, produced against strain NCTC 2429, cross-reacted with whole cells of strain ATCC 23745 but not with its purified CPC. By SDS-PAGE and immunoblotting of ATCC 23745 whole-cell extracts, we were able to determine that these MAbs recognize a slowly migrating broad band corresponding to the CPC (data not shown). This indicates that the epitopes recognized by the MAbs are located on the native CPC and that they are lost upon the standard purification procedures. Only chemical definition of the purified CPC and of the epitope specificity of the MAbs could shed light on this point. In our study, the use of MAbs circumvented the problems connected with purification of CPC and of its polysaccharide components, as using these reagents we were able to directly detect the epitopes of the native CPC on the surface of the bacterial cells.

The sharing of polysaccharide epitopes among *B. fragilis* strains is not surprising, since polysaccharide epitopes often represent narrow portions of the polysaccharide molecule, such as a di- or trisaccharide. Such structures can be shared also among capsules of unrelated bacterial species and represent the basis for serologic cross-reactions between different bacterial groups (16). By using polyclonal antiserum, cross-reaction between *B. fragilis* NCTC 9343 PS B and *Vibrio vulnificus* capsular polysaccharide has been found. The cross-reaction is probably due to a moiety containing quinovosamine (7). A MAb directed to the immunodominant epitope of *B. fragilis* LPS (β-1-6-linked digalactose) cross-reacted with the polysaccharide of group B *Streptococcus* type II (11).

Using our panel of MAbs, we did not find cross-reactions with other *Bacteroides* species. However, only a limited number of strains were tested; it is possible that some cross-reactions could occur, especially with strains belonging to species for which encapsulation has been described (2).

The complex patterns of reactivity and the large number of nonreactive strains imply that *B. fragilis* polysaccharides will be difficult to use as the basis for a serologic classification system. Previous studies to define *B. fragilis* serology were based on the use of polyclonal antisera directed to uncharacterized antigens present on the surface of *B. fragilis* cells. Lambe and Moroz devised a serological scheme based on cross-absorbed antisera produced against heat-resistant antigenic components of seven *B. fragilis* strains, obtaining 22 serogroups (10). Using 20 type-specific antisera to heat-stable somatic antigen preparation, Elhag and Tabaqchali were able to distinguish 60% of their strains by agglutination reactions (5). Babb and Cummins performed various serological tests for *B. fragilis* strains. These investigators noted that heat-stable extracts of several *B. fragilis* strains gave one or more precipitin lines when reacted with heterologous antisera in double immunodiffusion and concluded that there existed a complex interrelationship of antigenic components within the species *B. fragilis* (3). Our results, using highly specific immunologic probes, such as MAbs, have confirmed and extended the previous findings. We have found that *B. fragilis* exhibits a wide antigenic heterogeneity. Many pathogenic bacteria display a variety of capsular serotypes. For *Escherichia coli*, 74 capsular polysaccharide K antigens have been described (18); these K antigens are found in many different combinations with the other principal surface antigens, O and H. *Klebsiella pneumoniae* strains have been grouped into 72 capsular types (17), and *Streptococcus pneumoniae* strains have been grouped into 83 types (8). However, in all these cases, only a few types are normally associated with disease.

The capsule of *B. fragilis* is the major virulence factor that has been defined to date and is responsible for abscess formation. However, it may be that not all *B. fragilis* capsules are able to induce abscesses. The ability to induce abscesses is not linked to a particular chemical composition of the capsule but, rather, to a distinctive structural motif comprising at least a positive charge (a free amino group) and a negative charge in the repeating unit of the polysaccharide (21, 22). A chemically and antigenically different capsule does not necessarily indicate a difference in virulence, provided that the opposite-charge motif is preserved. Further definition of these relationships will require chemical characterization of capsular polysaccharides from additional *B. fragilis* strains.

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