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Immunological detection of *Bacteroides fragilis* in clinical samples

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Summary. A monospecific polyclonal antiserum, prepared against *Bacteroides fragilis* common polysaccharide antigen purified by polyacrylamide gel immunoblot detected *B. fragilis*, *B. thetaiotaomicron*, *B. ovatus* and *Prevotella melaninogenica* in pus samples from various anatomical sites by immunofluorescence microscopy of the pus. With standard clinical laboratory culture methods, 36% of 147 samples were positive for one or more of the above bacteria. Of these, *B. fragilis* accounted for 33%. By immunofluorescent labelling of pus with the common antigen antiserum the detection of these bacteria in the samples increased to 50%. All nine of the blood cultures in which *B. fragilis* was detected by culture contained bacteria positive for the common antigen. Immunofluorescent labelling of pus samples with a selection of monoclonal antibodies specific for surface polysaccharides which are known to be antigenically variable in culture *in vitro* and in an animal model of infection showed that these polysaccharides are also variable in natural infection. The results indicate that the common polysaccharide antigen, in contrast to the variable surface polysaccharides, is a suitable target for the immunodetection of *B. fragilis* in clinical samples from a range of anatomical sites.

Introduction

Bacteroides fragilis is the gram-negative obligately anaerobic bacterium most commonly isolated from clinical specimens. It has been isolated, either alone or as a component of mixed infection, from a range of sites in the body, including the peritoneal cavity, genito-urinary tract, blood, lungs and perianal area.¹ The pathogenic contribution of *B. fragilis* in these mixed infections is apparent when the anaerobes are not taken into account in the treatment of the infection and this fails to resolve.² *B. fragilis* is a commensal of the gut and can account for up to 13% of the faecal flora whereas other members of the "fragilis group" of *Bacteroides* spp. (*Bacteroides sensu stricto*), such as *B. vulgatus*, predominate. However, in the adherent colonic mucosal flora, *B. fragilis* is the predominant *Bacteroides* spp. with an estimated incidence of c. 42%.³

As a result of difficulties in maintaining the viability of *B. fragilis* during the transportation of clinical

specimens and the time taken to culture the bacteria, direct immunological detection of *B. fragilis* in clinical material by immunofluorescence microscopy has been studied by several workers. These studies have involved the use of a commercially available kit (Fluoretec, Pfizer Diagnostics) based on pooled rabbit polyclonal antisera specific for type strains of *Bacteroides* spp. (*B. fragilis*, *B. vulgatus*, *B. distasonis*, *B. ovatus* and *B. thetaiotaomicron*). The detection of *Bacteroides* spp. by immunofluorescence when compared with culture were reported as 81%,⁴ 87%⁵ and 97%.⁶ The usefulness of this kit as a monitor of faecal contamination of water has also been examined;⁷ however, it was not considered to be sufficiently sensitive for the routine monitoring of faecal contamination of disinfected drinking water. The potential diagnostic use of a mouse monoclonal antibody (MAb) specific for the core region of the lipopolysaccharide (LPS) has also been investigated.⁸ Although this MAb gave good specific labelling of *B. fragilis* and reacted with 96% of the clinical isolates examined, it labelled only c. 10% of the bacteria within a given strain. We have shown previously that

labelling with MAbs specific for polysaccharide epitopes demonstrates within-strain antigenic variation in *B. fragilis*⁹ and it appears that these variable epitopes are immunodominant. Polyclonal antisera raised to whole bacterial cells are also strain specific. This could explain the lack of agreement between the detection of *B. fragilis* by culture and immunofluorescence microscopy in these earlier studies. Therefore, there is a need to identify a non-variable antigen common to *B. fragilis* that could form the basis of an immunodiagnostic test.

In the present study, the suitability of the common polysaccharide antigen described by Poxton and Brown¹⁰ was investigated. This polysaccharide antigen migrates behind the rough form of the LPS and before the smooth form of the LPS on polyacrylamide gel electrophoresis and was reported to be common to seven strains of *B. fragilis* examined by immunoblotting with mono-specific polyclonal antiserum.

This study examined, by immunofluorescence microscopy, the reactivity of a monospecific polyclonal antiserum raised against this polysaccharide antigen with pus samples (and the corresponding pure culture isolates) from a range of different body sites and blood culture samples. The corresponding reactivity of a number of MAbs specific for variable polysaccharide antigens of *B. fragilis* was also examined.

Materials and methods

Bacterial strains

The strains used in this study were *B. fragilis* NCTC 9343 (National Collection of Type Cultures, Colindale Avenue, London), NCTC 10584, ATCC 23745 (American Type Culture Collection, Rockville, MD, USA); *B. vulgatus* NCTC 10583; *B. thetaiotaomicron* NCTC 10582; *B. ovatus* ATCC 8483; *B. distasonis* ATCC 8503; and clinical isolates of *B. fragilis* and other *Bacteroides* spp. obtained from Craigavon Area Hospital, Belfast City Hospital and Royal Victoria Hospital NI (designated LS, JC and including one metronidazole resistant strain, BCH1Mz^r), the Free University, Amsterdam, NL (designated BE) and University of Edinburgh, Scotland (designated GNAB). The *Escherichia coli* and *Staphylococcus aureus* strains used were recent clinical isolates from the bacteriology laboratory at Craigavon Area Hospital, Northern Ireland.

Specimens

The clinical samples were all obtained in Northern Ireland. The majority came from Craigavon Area Hospital, Craigavon; however, a few were also obtained from South Tyrone Hospital, Tyrone, Daisy Hill Hospital, Newry and the Royal Victoria Hospital, Belfast.

Ninety-eight pus samples (Study 1) which had been sent to the laboratories for routine diagnostic testing

were examined in detail and information concerning treatment was recorded from the patient's case history where available. These samples were subject to routine laboratory culture techniques (as detailed below) and examined by immunofluorescence microscopy for their reactivity with rabbit polyclonal antiserum to *B. fragilis* common polysaccharide antigen (CAg antiserum) and seven mouse MAbs specific for *B. fragilis* as detailed below. The pure culture isolates of *B. fragilis*, other *Bacteroides* spp. and *Prevotella melaninogenica* obtained from these samples were also examined by immunofluorescence microscopy for their reactivity with the CAg antiserum and the seven MAbs. A further 49 pus samples (Study 2) were examined for the presence or absence of *B. fragilis* by routine diagnostic methods and their reactivity with the CAg antiserum by immunofluorescence microscopy.

Ten blood culture bottles of which nine were culture-positive for *Bacteroides* spp. were examined for their reactivity with the CAg antiserum and three of these were also examined with the MAbs.

The pus samples were obtained by surgical drainage or aspiration and were placed in sterile bottles in volumes of 1–20 ml. The pus samples were either plated out directly (as detailed below) or inoculated into Brain Heart Infusion Broth (Unipath) for 24 h. Blood culture bottles (Roche Diagnostic System) were inoculated with 10 ml of the patient's blood and incubated at 37°C aerobically and anaerobically for a total of 7 days. The cultures were examined by eye for bacterial growth three times a day for the first 48 h and once a day for the next 5 days. If growth was apparent in the anaerobic blood culture bottle, a sample was seeded to anaerobic blood agar and the identification procedures performed as detailed below.

Identification by culture

Samples were routinely plated on the following agar media: horse blood agar (BA; Unipath); anaerobic horse blood agar (ABA; Gibco); colistin-nalidixic acid agar (CNA; Unipath); anaerobic blood agar plus gentamicin 50 µg/ml (ABA + GM). After inoculation, antibiotic disks containing penicillin (1 unit) and gentamicin (10 µg) were applied to the BA and CNA plates and the plates were incubated at 37°C in an aerobic atmosphere with CO₂ 10%. Disks containing penicillin (1 unit) and metronidazole (5 µg) were applied to the ABA + GM plate and gentamicin (10 µg), penicillin (1 unit) and metronidazole (5 µg) to the ABA plate. Cultures were incubated at 37°C in an atmosphere of H₂ 10%, N₂ 80% and CO₂ 10% in an anaerobic cabinet (Forma Scientific).

The aerobic plates were examined for colonies after 24 and 48 h and the isolates were gram-stained. Coliform isolates were identified with the API20E system (bioMérieux), *S. aureus* with Staphaurex (Murex Diagnostic), and *Streptococcus* spp. and *Enterococcus* spp. with the Lancefield serotyping kit Streptex (Murex Diagnostic). Streptococci which

could not be classified with the Streptex kit were identified with the API20 STREP (bioMérieux). Small pin head colonies with a characteristic sweet smell that were identified as Group A, C or F, or were otherwise unidentifiable, were classified as *Str. milleri*.

The anaerobic plates were examined after at least 48 h and metronidazole-sensitive organisms were gram-stained. Single colonies were re-streaked on ABA and after 24 h the following tests were performed with the pure cultures: API20A (bioMérieux), ATB 32A (bioMérieux) and the Mastring ID 8 (Mast Laboratories).

Pure culture in brain heart infusion broth with glycerol 10%, pus samples and blood cultures were stored in liquid nitrogen for future examination.

Preparation of antiserum

Monospecific polyclonal antiserum was raised to *B. fragilis* NCTC 9343 as described previously. Briefly, the monospecific polyclonal antiserum described by Poxton and Brown¹⁰ was used to identify the common polysaccharide antigen on guide strips of nitrocellulose after SDS-PAGE and immunoblotting. Small pieces of nitrocellulose containing the common antigen were cut from the unlabelled part of the nitrocellulose and dissolved in dimethylsulphoxide. This was mixed with an equal volume of Freund's complete adjuvant (Difco) and 0.1-ml amounts were inoculated subcutaneously at four sites on the back of a New Zealand White rabbit. Subsequently, the antigen in Freund's incomplete adjuvant was inoculated four times at c. 2-week intervals. A further two inoculations of antigen in PBS were made at approximately monthly intervals and the rabbit was bled after each booster dose. Antisera were tested by both immunofluorescence microscopy and immunoblotting.

Immunofluorescence microscopy

Samples (10 µl) from blood culture bottles or pus were applied to one well of a multi-well slide (Flow Laboratories). Four doubling dilutions in phosphate-buffered saline (PBS) were made along the length of the slide. For pure bacterial cultures, a suspension of c. 10⁷ bacteria/ml in PBS (30 µl) was applied to the slide. A duplicate series of dilutions of each sample was made on the same slide. The slides were air-dried and then fixed in methanol 100% for 10 min at -20°C. Slides with pus or blood culture samples were blocked with undiluted human serum for 10 min. For single labelling, the slides were incubated with either undiluted murine MAb supernates or polyclonal rabbit antiserum diluted 1 in 100 in PBS for 45 min, washed in PBS and then incubated for 45 min with sheep anti-rabbit fluorescein conjugate (Sigma) diluted 1 in 100 in PBS. After a final wash, the slides were examined with a Leitz fluorescence microscope. For dual labelling, the slides were incubated with MAb supernates, washed, incubated with polyclonal rabbit antiserum, washed and incubated with sheep anti-rabbit

fluorescein and goat anti-mouse rhodamine diluted 1 in 100 before a final wash.¹¹ To estimate the sensitivity of the labelling, Evan's Blue (Gurr; 0.05% w/v) was included during incubation with the conjugate. The slides were mounted with glycerol PBS containing an anti-photobleaching agent (Citifluor; Agar Scientific Ltd, Essex). The proportion of bacteria labelled with the antisera was determined by eye and confirmed by photographing the same field of view with filters suitable for viewing the green fluorescence of the fluorescein followed by filters suitable for viewing the red fluorescence of the Evan's blue staining with a fluorescence microscope. The percentage of bacteria labelled with the antisera was estimated by counting the total number of bacteria in each of the photographs. A minimum of 200 bacteria were counted for each estimation. Similarly, where samples were doubly labelled with a MAb and polyclonal antiserum, fields of view were photographed with filters suitable for viewing either the fluorescein (green) or rhodamine (red) dyes. Double-colour photographic exposure of the same field with the two different filter types sequentially were also examined.

Clinical samples were scored positive by immunofluorescence if at least three positive bacterial cells were observed in a sample after extensive examination of the micro-well slide. Each slide was examined for c. 10 min. The intensity of fluorescence was also noted.

Results

Sensitivity of labelling with the polyclonal antiserum to CAg

A number of different strains of *B. fragilis* and related bacteria were examined for their reactivity with rabbit polyclonal anti-serum specific for CAg by immunofluorescence microscopy. The total number of bacteria was estimated microscopically after being stained with Evan's blue. All the strains of *B. fragilis* examined were positive for CAg and between 80 and 100% of the bacteria were labelled with the polyclonal antiserum (table I). *B. ovatus* and *B. thetaiotaomicron*

Table I. Sensitivity of labelling of *B. fragilis* strains with the antiserum to the CAg

Strain no.	Bacteria positive (%)	Strain no.	Bacteria positive (%)
NCTC 9343		LS54*	99
EDL population	103	LS66*	98
SC population	95	LS67*	97
LC population	92	BE1	80
NCTC 10584	99	BE3	89
ATCC 23745	96	JC6	98
GNAB 4	86	JC15	100
GNAB 82	96	JC17	98
GNAB 92	98	JC19	100
BCH1 Mz ^r	100		

EDL, electron dense layer; SC, small capsule; LC, large capsule.
*Clinical isolates from the current study.

Table II. Detection of *Bacteroides* spp. and related genera in pus samples by culture and immunolabelling with antiserum to CAg (Study I)

Source of specimen	Number of samples received	Number culture-positive for		CAg positive, <i>Bacteroides</i> and <i>Prevotella</i> culture-negative
		<i>B. fragilis</i> *	Other <i>Bacteroides</i> and <i>Prevotella</i> spp.	
Perianal abscess	15	11	0	2
Abdominal abscess	10	4	1	2
Pilonidal abscess	9	2	5	1
Bartholin's abscess	6	1	0	0
Ischiorectal abscess	4	2	1	1
Vaginal abscess	1	1	0	0
Diverticular abscess	1	1	0	0
Groin abscess	3	1	0	2
Groin neoplasm abscess	1	1	0	0
Colostomy	1	1	0	0
Abscess at pacemaker	1	0	0	0
Haematoma	1	0	0	0
Skin wounds	31	0	0	2
Pleural fluid	2	0	0	1
Gall bladder abscess	2	0	0	1
Liver abscess	3	0	0	0
Brain abscess	2	0	0	0
Pancreatic abscess	1	0	0	0
Peritoneal fluid	1	0	0	0
Parotid abscess	1	0	0	0
Subphrenic abscess	2	0	0	0
Total	98	25	7	12

*All these samples were positive when directly labelled with the CAg antiserum.

Table IIIA. Bacteria isolated from *B. fragilis* culture-positive pus samples

Organisms isolated	Abscess site
<i>B. fragilis</i>	Perianal (2), colostomy (1)
<i>B. fragilis</i> , <i>E. coli</i>	Perianal (2), ischiorectal (1)
<i>B. fragilis</i> , <i>Str. milleri</i>	Abdominal (1), perianal (2), groin (1), pilonidal (1)
<i>B. fragilis</i> , <i>Str. milleri</i> , <i>E. coli</i>	Perianal (3), abdominal (1), vaginal (1), ischiorectal (1)
<i>B. fragilis</i> , <i>Str. agalactiae</i>	Perianal (1), pilonidal (1)
<i>B. fragilis</i> , <i>Str. viridans</i>	Abdominal (1)
<i>B. fragilis</i> , <i>E. coli</i> , <i>Str. milleri</i> , <i>Str. agalactiae</i>	Bartholin's (1)
<i>B. fragilis</i> , <i>E. coli</i> , <i>Str. agalactiae</i>	Diverticular (1), groin neoplasm (1)
<i>B. fragilis</i> , Group C streptococci	Perianal (1)
<i>B. fragilis</i> , <i>P. melaninogenica</i>	Abdominal (1)

Table IIIB. Bacteria isolated from *Bacteroides* spp. and *Prevotella* spp. culture-positive pus samples

Organisms isolated	Abscess site
<i>P. melaninogenica</i>	Abdominal wound (1)
<i>P. melaninogenica</i> , <i>Peptostreptococcus</i> sp.	Pilonidal (1)
<i>P. melaninogenica</i> , <i>E. coli</i> , <i>Str. milleri</i>	Pilonidal (2)
<i>B. thetaiotaomicron</i> , <i>E. coli</i> , <i>Str. milleri</i> , <i>Proteus mirabilis</i>	Ischiorectal (1)
<i>Bacteroides</i> sp., <i>Str. milleri</i>	Pilonidal (1)
<i>Bacteroides</i> sp., <i>Str. milleri</i> , <i>Peptostreptococcus</i> sp.	Pilonidal (1)

Table IV. Bacteria isolated on repeat culture from CAg-positive but *Bacteroides* and *Prevotella* culture-negative pus samples

Organisms initially isolated	Abscess site
None	Buttock (skin wound)*†, labial (skin wound), abdominal†
<i>E. coli</i>	Gall bladder, pilonidal†, groin, perianal, abdominal*†
<i>Str. milleri</i>	Pleural fluid, ischiorectal*†
<i>E. coli</i> , <i>Str. milleri</i>	Perianal*
<i>S. aureus</i> , <i>Str. pyogenes</i>	Groin†

**B. fragilis* isolated after re-culture of the sample.

†Positive with one or more *B. fragilis*-specific MAb (see table IX).

were labelled with lower intensity but similar sensitivity to *B. fragilis*, and *B. distasonis* gave negative results.

Detection of *Bacteroides* spp. in pus samples

The source of the 98 pus samples examined in Study 1, the culture results for *Bacteroides* spp. and related genera and the reactivity with the CAg antiserum by immunofluorescence microscopy are shown in table II. The bacteria isolated from samples positive for *B. fragilis*, *Bacteroides* spp. and the related genus *Prevotella* by initial culture are shown in tables IIIA and IIIB respectively. Pus samples that were culture-negative for *Bacteroides* and *Prevotella* spp., but

positive by immunofluorescence with the CAg antiserum are detailed in table IV. These 12 specimens which were negative for *Bacteroides* and *Prevotella*

Table V. Detection of *B. fragilis* in pus samples by both culture and immunolabelling with antiserum to the CAg. (Study 2)

Source of specimen	Number of samples received	Number culture-positive for <i>B. fragilis</i> *	CAg-positive, <i>Bacteroides</i> culture-negative
Perianal abscess	9	9	0
Pilonidal abscess	8	4	2
Unspecified pus	7	3	1
Abdominal abscess	4	2	1
Scrotum abscess	4	1	2
Pelvic abscess	2	0	2
Mastoid maxillary sinus	3	0	2
Suprapubic abscess	1	1	0
Pleural cavity	1	1	0
Hernia wound abscess	1	0	1
Pouch of Douglas	1	0	0
Neck abscess	1	0	0
Quinsy	1	0	0
Wound abscess	1	0	0
Abdominal drain fluid	1	0	0
Tibial abscess	1	0	0
Breast abscess	1	0	0
Back abscess	1	0	0
Gall bladder abscess	1	0	0
Total	49	21	11

*All except one of these samples were also positive when labelled with the CAg antiserum.

spp. on initial routine testing in the laboratory, but immunofluorescence-positive with the CAg antiserum, were retrieved from storage in liquid nitrogen and re-cultured. Of these, *B. fragilis* was isolated from four (table IV).

Of a further 49 pus samples examined in Study 2, 21 were positive by culture for *B. fragilis*. Eleven of the culture-negative samples were positive by immunofluorescence with CAg antiserum (table V). Immunofluorescence labelling of a typical pus sample is illustrated in fig. 1. With the exception of one sample in Study 2, if a sample was positive for *B. fragilis* on culture it was also positive by immunofluorescence with the CAg antiserum. Of the samples positive for *Bacteroides* spp. or *P. melaninogenica*, or both, only one (no. 3), which was positive for an unidentified *Bacteroides* sp., was negative by immunofluorescence.

In total, 36 % of the pus samples were positive for *B. fragilis* and related bacteria by culture and 50 % were positive by immunofluorescence microscopy with the CAg antiserum.

Investigation of the patients' case histories

The antibiotic treatment recorded in the case histories of patients whose specimens were either CAg antiserum- or culture-positive for *B. fragilis* is given in table VI. Although metronidazole was prescribed before drainage of the abscesses, samples nos. 11 and 15 were still positive for *B. fragilis* on culture. One sample (no. 68) was culture-negative and the antibiotic treatment was changed to co-fluampicil; however, the current study indicated that this sample was CAg-positive. Examination of the case histories also revealed that four patients had recurrent infections at the same anatomical site (table VII).

Fig. 1. Micrograph of pus from a groin neoplasm labelled with rabbit antiserum to CAg and anti-rabbit immunoglobulin fluorescein conjugate ($\times 100$).

Table VI. Antibiotics* administered to patients with either *B. fragilis* and related bacteria culture- and CAg-positive (A) or only CAg-positive (B) pus samples. (Study 1)

Sample no.	Antibiotic treatment	
	before drainage	after drainage
A		
4	Co-fluampicil	NI
11	Cefuroxime and metronidazole	Cefuroxime and metronidazole
14	Co-fluampicil	NI
15	Gentamicin and metronidazole	Gentamicin and metronidazole
18	NI	Ampicillin and metronidazole
23	Co-fluampicil	NI
56	Flucloxacillin	NI
66	Metronidazole and cefuroxime	Metronidazole and cefuroxime
73	NI	Erythromycin
75	Amoxycillin	NI
91	Flucloxacillin	NI
96	Co-fluampicil	NI
B		
28	NI	Gentamicin
68	Co-amoxiclav and metronidazole	Co-fluampicil
70	Co-fluampicil	NI
71	Co-fluampicil	NI

NI, not indicated.

*Information obtained from patients' case histories, where available.

Table VII. Bacteria isolated from successive samples obtained from the same patient* based on information in patients' case histories

Sample no.	Abscess site	Date	Culture result
23	Ischiorectal	14.02.89	* <i>B. fragilis</i> , <i>Str. milleri</i>
		12.08.90	<i>B. fragilis</i> , <i>Str. milleri</i>
70	Pilonidal	29.11.89	* <i>Str. milleri</i>
		28.05.90	<i>Str. milleri</i> , <i>B. fragilis</i>
80	Ischiorectal/perianal	14.07.89	No growth
		04.04.90	<i>Str. milleri</i> , <i>B. fragilis</i> , <i>P. melaninogenica</i>
		30.04.90	* <i>Str. milleri</i> , <i>B. fragilis</i>
		24.08.90	No growth
		12.03.91	<i>Str. milleri</i> , <i>B. fragilis</i> , <i>P. melaninogenica</i>
95	Ischiorectal/perianal	29.09.90	* <i>Str. milleri</i> , <i>B. fragilis</i> , <i>E. coli</i>
		23.12.87	<i>Str. milleri</i> , <i>B. fragilis</i> , <i>E. coli</i>

*Denotes pus sample examined in Study 1.

Detection of *B. fragilis* from blood culture bottles

Nine blood culture bottles from which *B. fragilis* was isolated, and one from which *B. distasonis* was isolated, were examined for their reactivity with the CAg antiserum. All nine cultures positive for *B. fragilis* were also positive by immunofluorescence with the CAg antiserum; however, the *B. distasonis*-positive blood culture bottle was CAg-negative. Fig. 2 illustrates typical labelling of a blood culture sample.

Reactivity of *B. fragilis* clinical samples and pure cultures with murine MAbs

Twenty-five pus samples (from Study 1) and three blood culture bottles, which were all CAg positive, were examined by immunofluorescence microscopy

for their reactivity with six murine MAbs specific for the surface polysaccharides of *B. fragilis* associated with the large capsule and electron-dense layer populations. The corresponding pure culture isolated was also examined in each case. MAb QUBf5 is specific for the polysaccharide which forms on PAGE and immunoblotting a pattern similar to that of the O-antigen of other bacterial species. The other MAbs are specific for higher molecular mass polysaccharide associated with the large capsule and electron-dense layer populations of *B. fragilis*.⁹

Four of the pus samples did not react with any of the MAbs when examined directly and the rest reacted with a variable selection of the MAbs. All of the pure culture isolates of *B. fragilis* obtained from these samples were positive for at least one of the MAbs

Fig. 2. Micrograph of blood culture labelled with rabbit antiserum to CAg and anti-rabbit immunoglobulin fluorescein conjugate ($\times 100$).

(table VIII). There was no apparent relationship between the site of origin of the sample and the MAb labelling. Double labelling with both the CAg antiserum and the MAbs confirmed that the MAbs label a varying proportion of the *B. fragilis* cells present within a pure culture isolate. This was also the case when pus and blood culture samples were examined directly, without prior culture. Fig. 3 illustrates double labelling of a blood culture. MAb QUBf11, which reacted with all of the pure cultures and 24 of the 28 samples, labelled only 7–15% of the bacterial population. Labelling also indicated that outer membrane vesicles were present in the pus samples.

Examination of the samples that were culture negative for *B. fragilis* and related bacteria, but were positive with the CAg antiserum, indicated that seven of 12 of these samples also reacted with at least one of the MAbs (table IX). Samples from which *Bacteroides* spp. (other than *B. fragilis*) or *P. melaninogenica*, or both, were cultured and which were positive with the CAg antiserum (table IIIB), were negative with all the MAbs. This suggests that the polysaccharides recognised by these MAbs are specific to *B. fragilis*.

Sixteen of the pure culture isolates from Study 1 were examined for their reactivity with a MAb (Bf4) specific for a high molecular mass polysaccharide associated with the small capsule population of *B. fragilis*.¹² An estimated 1%, or less, of the bacteria within these populations were labelled with the MAb. Similarly, the proportion of the bacteria positive with the CAg antiserum, which were also positive with MAb Bf4 when pus samples were examined directly, was $\leq 1\%$.

Discussion

The results confirm that the heterogeneous labelling pattern of MAbs specific for high molecular mass polysaccharides of *B. fragilis*, previously observed in culture collection strains and in bacteria grown in a mouse model of peritoneal infection,¹³ also occurred in the recent clinical isolates of *B. fragilis* obtained in the study. Variable labelling was also apparent when pus samples were examined directly, without prior culture. Polysaccharides associated with the large capsule, small capsule and electron-dense layer populations^{9,12} were all detected in the pus samples with the MAbs. This indicates that antigenic variation in the polysaccharides of *B. fragilis* is apparent during the course of natural infection and raises the possibility that antigenic variation of surface polysaccharides is related to the virulence of *B. fragilis*. Interestingly, there was no obvious relationship between the labelling pattern of the MAbs and a particular site of infection. It is possible that variable antigens are the immunodominant antigens in *B. fragilis* as inoculation with whole cells produces antisera that do not label all the bacterial cells in other strains^{14,15} and are thus too insensitive for use as a diagnostic test for the presence of *B. fragilis*. Furthermore, all the polysaccharide-specific MAbs that were raised with whole bacteria as an inoculum were specific for the antigenically variable polysaccharides which are not expressed by all the bacteria within a given population of *B. fragilis*.^{16–18} It is possible that these immunodominant variable polysaccharides mask the common polysaccharide. The unsuitability of the variable polysaccharides as targets

Table VIII. Reactivity of clinical samples (S) and the corresponding pure culture isolate (C) of *B. fragilis* with MAbs specific for *B. fragilis* NCTC 9343 surface polysaccharides

Site and sample no.	Reaction with MAb QUBf											
	5		7		8		9		10		11	
	C	S	C	S	C	S	C	S	C	S	C	S
Perianal/ischiorectal abscess												
14	+	+	+	+	-	-	+	-	+	+	+	+
16	-	-	-	-	-	-	+	-	+	-	+	-
23	-	-	-	-	-	-	-	-	-	-	+	+
26	-	-	-	-	-	-	-	-	+	-	+	-
52	-	-	-	-	+	+	-	-	-	-	+	+
54	+	+	+	+	+	+	-	-	+	-	+	+
61	-	-	-	-	+	+	-	-	+	+	+	+
67	+	+	+	+	+	+	-	-	-	-	+	+
75	-	-	+	+	+	+	-	-	-	-	+	+
80	+	+	+	+	+	+	+	+	+	+	+	+
87	-	-	-	-	+	+	-	-	+	+	+	+
92	+	+	-	-	+	+	-	-	+	+	+	+
95	+	+	-	-	+	+	-	-	+	+	+	+
Abdominal abscess												
11	-	-	-	-	-	-	+	-	+	-	+	+
13	-	-	-	-	-	-	-	-	-	-	+	-
66	+	+	+	-	-	-	+	+	+	-	+	+
89	-	-	+	+	+	+	-	-	-	-	+	+
Pilonidal abscess												
56	+	+	-	-	-	-	-	-	+	+	+	+
91	-	-	-	-	+	+	-	-	+	+	+	+
Vaginal/Bartholin's abscess												
18	+	+	+	+	+	+	-	-	-	-	+	+
76	-	-	-	-	-	-	-	-	+	+	+	+
Diverticular abscess												
84	+	+	-	-	-	-	+	+	+	+	+	+
Groin abscess												
73	-	-	-	-	+	+	-	-	+	+	+	+
Colostomy												
15	-	-	-	-	-	-	-	-	-	-	+	-
Groin neoplasm												
27	+	+	-	-	+	+	+	+	+	+	+	+
Blood culture												
39	+	+	+	+	+	+	-	-	-	-	+	+
55	+	-	-	-	+	+	-	-	+	+	+	+
98	-	-	-	-	+	+	-	-	-	-	+	+

C, pure culture; S, pus sample.

for the immunological detection of *B. fragilis* in clinical samples is emphasised. The sensitivity of labelling is not sufficient to allow detection of *B. fragilis* in all instances. It is unlikely that even a mixture of these MAbs could be used effectively as some samples and isolates reacted with only one MAb.

In contrast, a monospecific polyclonal antiserum specific for the CAg of *B. fragilis*¹⁰ labelled between 80 and 100% of the bacteria within 16 strains of *B. fragilis* obtained from culture collections and isolates from Northern Ireland, Edinburgh and Amsterdam, and also populations of strain NCTC 9343 enriched for the large capsule, small capsule or electron-dense layer. The antiserum recognised *B. fragilis*, *B. thetaiotaomicron*, *B. ovatus* and *P. melaninogenica* but not *B. vulgatus* or *B. distasonis*. Why this polysaccharide is common to some species of *Bacteroides* and *Prevotella* but not other species of *Bacteroides*

is unclear. Interestingly, there is evidence for the horizontal transfer of genes between *Bacteroides* and *Prevotella* spp.¹⁹ By labelling bacteria directly in pus samples, the detection of these species was increased above that obtained by culture only. The culture-negative immunofluorescence microscopy-positive samples are unlikely to represent false positives, as (i) four of these yielded viable *B. fragilis* when the isolation procedures were performed a second time from the stored pus samples, with particular care to minimise the exposure of the material to air and not as part of the normal day-to-day activity of the diagnostic laboratory, and (ii) a number of culture-negative samples were also positive by immunofluorescence microscopy with MAbs specific for *B. fragilis* polysaccharides (table IX) which, because of the specificity of the MAbs, indicates that *B. fragilis* cells were present in these specimens but were non-viable. Therefore, this

Fig. 3. Micrographs of blood culture labelled with (i) rabbit antiserum to CAg and anti-rabbit immunoglobulin fluorescein conjugate and (ii) mouse MAb QUBf11 and anti-mouse immunoglobulin rhodamine conjugate. The same field of view was photographed with filters to show: **a**, the green fluorescence of the fluorescein; **b**, the red fluorescence of the rhodamine ($\times 100$).

study highlights the problems of detecting obligate anaerobes in clinical specimens by culture alone in a routine diagnostic laboratory and indicates that the CAg is a suitable target for immuno-detection. If the CAg antiserum alone had been used for the detection of *B. fragilis* and related bacteria, two samples that were positive by culture would have been mis-reported as negative. This represents an underestimate of 1–2 %

of the total of 147 samples examined in the study. In contrast with the current diagnostic laboratory culture methods, the incidence of *B. fragilis* and related bacteria was underestimated by *c.* 14%. Although relatively few blood culture bottles were examined, in all nine instances where *B. fragilis* was isolated, the blood culture was positive with the CAg antiserum. Thus, there is the potential for immediate confirmation

Table IX. Reactivity of pus samples negative for *B. fragilis* and related bacteria, but CAg-positive with MAbs specific for *B. fragilis* NCTC 9343 surface polysaccharides

Sample no.	Reaction with MAb QUBf					
	5	7	8	9	10	11
28	—	+	—	+	—	+
34*	—	+	+	—	—	+
35	—	+	—	—	—	+
57*	—	—	+	+	+	+
68	—	—	+	—	+	+
70*	—	—	+	—	+	+
94	+	—	+	—	+	+

—, negative; +, positive. **B. fragilis* culture positive after re-culture. Four samples were negative with all the MAbs.

of *B. fragilis* in blood culture bottles at the point when growth in the bottle is first observed. The current study examined pus samples and blood culture bottles exclusively. The usefulness of such a test with swab samples remains to be elucidated.

This study also exemplifies the classical polymicrobial infections in which *B. fragilis* are involved and which have been much studied, in particular in relation to *E. coli*.²⁰ *E. coli* was isolated in association with *B. fragilis* in c. 46% of instances; however, *Str. milleri* was present along with *B. fragilis* in 68% of cases in this study. *Str. milleri* is a facultative pathogen that has been isolated from a similar range of body sites to that of *B. fragilis*.²¹ The possible synergic interaction between *Str. milleri* and *B. fragilis* warrants further investigation.

Of a total of 45 pus samples from perianal, pilonidal or ischiorectal sites, 39 were positive for *B. fragilis* and related bacteria in this study. The high incidence of *B.*

fragilis in abscesses at these sites is interesting in that, although *B. fragilis* is present in quantity in the adherent colonic mucosal flora, it does not predominate in the faecal flora.³ It is also of interest that in both Study 1 and 2 most of these samples were from male patients (11 male and two female in Study 1; 10 male and three female in Study 2; data not shown). The reasons for this are open to speculation. As all the samples in this study comprised pus that had been drained surgically from an abscess, it is unlikely that the *B. fragilis* detected represents faecal contamination of the specimen. Four of the patients examined in this study had recurrent infections at this site (table VII). Unfortunately, the information in the patients' case histories relating to the antibiotic therapy employed was incomplete, but patient no. 70 was initially reported as culture-negative for *B. fragilis* but positive for *Str. milleri* and is recorded as being treated with co-fluampicil. This sample was positive for the CAg by immunofluorescence and *B. fragilis* was subsequently cultured from the pus when it was re-examined (table IV). This patient had a recurrence of infection at this site 6 months later, from which both *B. fragilis* and *Str. milleri* were cultured.

In conclusion, this study confirms that the immunodominant surface polysaccharides of *B. fragilis* are antigenically variable in both recent clinical isolates and in bacteria present in pus. Thus, these polysaccharides are not suitable targets for the immunodetection of *B. fragilis* in clinical samples. However, CAg is a suitable target and increased the detection of *Bacteroides* and related genera by c. 14%.

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