Monoclonal Antibody for Identification of *Bacteroides gingivalis* Lipopolysaccharide

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A monoclonal antibody, BBG-25, raised in BALB/c mice demonstrated specificity for *Bacteroides gingivalis* lipopolysaccharide. Immunoblotting indicated that this monoclonal antibody does not cross-react with lipopolysaccharide prepared from enterobacterial organisms or from other *Bacteroides* species.

Anaerobes comprise a significant proportion of the oral and dental microflora, and a number of these anaerobic bacteria have been implicated as pathogenic (14). The anaerobic, black-pigmented, gram-negative oral bacterium *Bacteroides gingivalis*, in particular, has been associated with clinical signs of adult chronic periodontitis (17, 19, 21-23, 25, 26).

For a pathogen to be successful in causing disease, it must enter the host, multiply in host tissue, avoid or not stimulate host defenses, and damage the host (20). Several bacterial surface components and end products of growth and metabolism have been implicated as virulence factors which may be involved in host tissue destruction in periodontal disease (5, 18). One such component that has received much attention as a potential virulence factor is lipopolysaccharide (LPS).

LPS is a major component of the outer membrane of gram-negative bacteria. It is essential for maintaining the integrity and functional conformation of the membrane and, consequently, of the intact cell. A wide range of physiological reactions expressed during many gram-negative bacterial infections have been attributed to this compound and, in particular, to the lipid A component (2, 3, 11, 15).

Bacteroides LPS are atypical. The biological potency of *B. gingivalis* LPS is low compared with that of LPS from gram-negative enteric organisms (8, 12), except for its potency in stimulating bone resorption of fetal rat bones in organ culture (4, 6) and its inhibition of bone collagen synthesis in fetal rat calvaria (10). Furthermore, LPS from all *Bacteroides* species analyzed to date lack 2-keto-3-deoxyoctulosonic acid, L-glycero-D-mannoheptulose, and 3-hydroxytetradecanoic acid (8, 12), components of the core and lipid A regions of LPS (11, 16) that are generally used as markers for the presence of LPS. The absence of these characteristic components has made the detection of *B. gingivalis* LPS in bacterial preparations extremely difficult.

We recently reported the production of monoclonal antibodies (MAbs) to *B. gingivalis* 381 (1). Briefly, after immunization of BALB/c mice with Formalin-treated cells, the spleens were aseptically removed and a single-cell suspension of spleen cells was fused with Sp2/0-Ag14 myeloma cells. After incubation, hybridoma supernatant was tested for reactivity to *B. gingivalis* 381 by enzyme-linked immunosorbent assay and for specificity by immunofluorescence microscopy against a selected group of gram-negative and gram-positive bacteria. Positive cultures were cloned by

limiting dilution, and wells that contained clones positive and specific for *B. gingivalis* by enzyme-linked immunosorbent assay were expanded. These MAbs, which cross-reacted with all *B. gingivalis* strains tested but did not cross-react with other oral bacterial species such as fusobacteria (1), demonstrated specificity for different antigenic preparations from the parent organism such as membrane (MAb BBG-12), fimbriae (MAbs BBG-24, -26, -30, and -31), and LPS (MAb BBG-25) (P. Chen, D. P. Dickinson, S. J. Millar, R. E. Schifferle, and M. J. Levine, J. Dent. Res., vol. 65, abstr. 876). The study described in this paper was undertaken to determine the specificity of this anti-LPS MAb towards LPS from other *B. gingivalis* strains.

Salmonella typhimurium LPS (List Laboratories, Irvine, Calif.), *Escherichia coli* LPS (Sigma Chemical Co., St. Louis, Mo.), and *Salmonella minnesota* LPS, including all the mutant forms (generous gifts from Otto Luderitz), were used. LPS purified from *B. gingivalis* 381 was prepared as previously described (10). LPS was extracted from *B. gingivalis* SUNYAB A7A1-28, A7A2-10, and 9-14K-1; *Bacteroides intermedius* ATCC 25261 and 25611, NCTC 9336, and SUNYAB 20-3; *Bacteroides asaccharolyticus* ATCC 25260 and 27067; *Bacteroides loescheii* ATCC 15930 and Virginia Polytechnic Institute strain DIC-20, *Bacteroides endodontalis* ATCC 35406; and *Bacteroides corporis* ATCC 33547 by a modification of the phenol-water procedure described by Westphal and Jann (24). *B. gingivalis* 381 was included as a positive control. Briefly, all strains were grown on blood agar plates anaerobically in an atmosphere containing 85% nitrogen, 5% carbon dioxide, and 10% hydrogen. Cells were scraped from the blood agar plates and suspended in 0.15 M sodium chloride. The cells were washed three times in saline, suspended in distilled water, and heated to 65 to 68°C. An equal volume of 90% phenol preheated to 65 to 68°C was added, and the extraction was allowed to proceed for 15 min. After being cooled to 10°C, the mixture was separated into aqueous and phenol phases by centrifugation at 12,000 × g for 30 min. The phenol phase was reextracted with water as described above, and the aqueous phases were combined. The excess phenol in the aqueous phase was removed by dialysis, and the aqueous phase was lyophilized. This resulting fraction was the LPS preparation used for testing cross-reactivity of the LPS preparations from the different *Bacteroides* species.

The reactivity of the anti-*B. gingivalis* LPS MAb to LPS-containing fractions from different *Bacteroides* species, in addition to purified LPS preparations from members of the family *Enterobacteriaceae* and *B. gingivalis* 381, was deter-

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TABLE 1. Immunodot determination of the reactivity of the anti-LPS MAb BBG-25 with enteric LPS preparations^a

LPS prepn	Concn (μ g/well)	Reactivity of BBG-25 at dilution:	
		1:100	1:500
<i>S. minnesota</i>	5	—	—
R.60	5	—	—
R.345	5	—	—
R.5	5	—	—
R.595	5	—	—
<i>S. typhimurium</i>	5	—	—
<i>E. coli</i>	5	—	—
<i>B. gingivalis</i>			
Peak 2 ^b	1	++	+
Peak 3 ^b	1	++	+

^a +, Positive; ++, strong; —, negative. Reactivity of normal ascites (1:500) and goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (1:3,000) was negative.

^b Purified LPS preparations from *B. gingivalis* 381 from Sephadex G-100 chromatography (10).

mined by immunoblotting. Immunoblotting on nitrocellulose was performed on a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Richmond, Calif.). The bacteria and the different LPS preparations were placed in duplicate wells of the Bio-Dot apparatus and allowed to gravity flow through the nitrocellulose for 30 min to 1 h. Blocking buffer (250 μ l per well; 20 mM Tris hydrochloride [pH 7.5] with 1% bovine serum albumin and 500 mM NaCl) was then applied to the samples by the same method. Each well was then washed with 400 μ l of washing buffer (20 mM Tris hydrochloride, 500 mM NaCl, 0.05% Tween 20 [pH 7.5]) and dried under vacuum. The anti-LPS MAb, BBG-25, at appropriate dilutions (see Tables 1 and 2), was then applied to each well and allowed to gravity flow for 1 h. The samples were washed under vacuum as previously described. Goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (100 μ l/well; 1:3,000 dilution: Bio-Rad) was added and allowed to gravity flow for 30 min to 1 h, followed by three washes of washing buffer under vacuum. The nitrocellulose membrane was removed and placed in the color development solution (1 part of 4-chloro-1-naphthol in methanol [3 mg/ml] and 5 parts of 0.018% hydrogen peroxide in 20 mM Tris hydrochloride–500 mM NaCl [pH 7.5]) for 3 to 5 min. The nitrocellulose membrane was rinsed three times in washing buffer, rinsed thoroughly in distilled water, and dried for storage.

When tested by immunoblot against purified LPS preparations from members of the family *Enterobacteriaceae*, MAb BBG-25 did not respond (Table 1). In this case, two purified LPS preparations from *B. gingivalis* 381 served as positive controls. We have previously shown (10) that these components represent high- and low-molecular-weight LPS species, Peak 2 and Peak 3, respectively, which differ in the size of the carbohydrate side chains associated with the lipid moiety. The possibility that the determinant is present on these enteric LPS molecules but is masked in some way, e.g., by steric hindrance by the polysaccharide chain, was considered. However, the finding that none of the R mutants of *S. minnesota* were reactive tends to exclude this possibility. Another possible explanation for the lack of response of enteric LPS to the MAb could be the inability of the LPS to bind to the nitrocellulose. However, by silver staining (9) we were able to demonstrate that the LPS, at levels as low as

1 μ g, was present on the nitrocellulose. These results suggest that the determinant for the MAb BBG-25 is not present in these enteric LPS molecules. A similar lack of cross-reactivity between MAbs to *B. fragilis* LPS and LPS extracted from enterobacterial organisms has previously been demonstrated by an enzyme inhibition immunoassay (7).

The LPS-containing fractions from the different *Bacteroides* species showed that MAb BBG-25 responded positively to LPS from all the *B. gingivalis* strains tested but demonstrated no cross-reactivity to LPS of any of the other *Bacteroides* species tested (Table 2). Recently, a similar result with a MAb to *B. gingivalis* LPS has been demonstrated in an enzyme-linked immunosorbent assay (13). This result indicates that there is a common antigenic determinant present in *B. gingivalis* LPS molecules that is unique to *B. gingivalis*.

The weak response of the MAb to cells of *B. gingivalis* 381, the parent organism, and to cells of the other *B. gingivalis* strains (Table 2) would suggest that the epitope for the MAb BBG-25 in intact bacteria is not readily accessible at the cell surface. Interestingly, the MAb BBG-25 demonstrates an intense response to the low-molecular-weight LPS species, Peak 3, which has shorter O-antigenic carbohydrate side chains associated with the lipid moiety (10). As the lipid component is in the outer membrane, this response suggests that the domain containing the epitope for the MAb is also closely associated with the outer membrane rather than being present as a surface antigen. This finding would be consistent with the weak response to intact bacteria.

In any study correlating a biological activity with a specific

TABLE 2. Immunodot determination of the reactivity of the anti-LPS MAb BBG-25 with *B. gingivalis* cells and LPS preparations from different *Bacteroides* species^a

Bacterium	Strain	Source ^b	Reactivity of BBG-25 (1:500) in:	
			Cell ^c	LPS ^d
<i>B. gingivalis</i>	381	S. S. Socransky ^e	+	++
	9-14K-1	SUNYAB	±	++
	A7A2-10	SUNYAB	±	++
	A7A1-28	SUNYAB	±	++
<i>B. intermedius</i>	ATCC 25261	ATCC	ND	—
	ATCC 25611	ATCC	ND	—
	NCTC 9336	NCTC	ND	—
	20-3	SUNYAB	ND	—
<i>B. asaccharolyticus</i>	ATCC 25260	ATCC	ND	—
	ATCC 27067	ATCC	ND	—
<i>B. loescheii</i>	ATCC 15930	ATCC	ND	—
	DIC-20	VPI	ND	—
<i>B. endodontalis</i>	ATCC 35406	ATCC	ND	—
<i>B. corporis</i>	ATCC 33547	ATCC	ND	—

^a +, Positive; ++, strong; ±, weak; —, negative; ND, not done. Reactivity of normal ascites (1:500) and goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (1:3,000) was negative.

^b SUNYAB, State University of New York at Buffalo, Buffalo, N.Y.; ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, England; VPI, Virginia Polytechnic Institute, Blacksburg, Va.

^c 2×10^5 cells/ml; 100 μ l per well.

^d 1.5 μ g per well.

^e Forsyth Dental Center, Boston, Mass.

bacterial component, it is important to rule out contamination by other bacterial products, such as LPS, which exhibit the same biological activity. The lack of characteristic chemical markers in *B. gingivalis* LPS has made the presence of this component in bacterial preparations very difficult to assess. Recently, LPS contamination of a surface polysaccharide extract from *B. gingivalis* was demonstrated by using this MAb (R. E. Schifferle, unpublished observation). Therefore, this anti-LPS MAb, BBG-25, which shows specificity toward *B. gingivalis* LPS, represents an immunochemical reagent that will enable us to identify *B. gingivalis* LPS in bacterial extracts.

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