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NOTES

A Monoclonal Antibody Reactive with a Common Epitope of *Moraxella* (*Branhamella*) *catarrhalis* Lipopolysaccharides

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A hybrid cell line producing a monoclonal antibody (MAb) against *Moraxella* (*Branhamella*) *catarrhalis* lipopolysaccharide (LPS) was established. The specificity of the MAb 1B12 to purified rough LPSs from six strains of *M. catarrhalis* was ascertained by enzyme-linked immunosorbent assay (ELISA), competitive-inhibition ELISA, and immunoblotting. MAb 1B12 bound to live bacterial cells and culture supernatants from a total of 34 strains of *M. catarrhalis*, including 12 strains with different LPS serotypes. No cross-reactions with smooth and rough LPSs from selected enterobacterial and nonenterobacterial strains, with other respiratory pathogens, or with *Neisseria* species were observed. These data suggest that MAb 1B12 recognizes a common epitope of *M. catarrhalis* LPS which differs from serotype determinants.

During the last decade, *Moraxella* (*Branhamella*) *catarrhalis* has been increasingly recognized as a pathogenic organism which causes various types of infections (1, 5, 27, 31). *M. catarrhalis* is now generally regarded as a major pathogen of bronchopulmonary infection and acute and chronic otitis media, comparable to *Haemophilus influenzae* and *Streptococcus pneumoniae* (16, 18, 22, 25).

Lipopolysaccharide (LPS) is a critical structural, antigenic, and pathogenic component of the outer membrane of gram-negative bacteria. Although the pathogenesis of *M. catarrhalis* is not fully understood, a recent study indicated that the LPS of *M. catarrhalis* contributes to its pathogenic potential (10). We have previously reported that *M. catarrhalis* strains possess a rough LPS exhibiting two to four bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (29). This rough LPS, the so-called lipooligosaccharide, is observed in nonenteric pathogens such as *H. influenzae* and *Neisseria gonorrhoeae* (9, 26, 30). The oligosaccharide structure is relatively conserved compared with the structure observed among the O side chain of LPS from different gram-negative bacteria (24). It has also been shown that the three LPS serotypes are distinguished according to LPS antigenicity (30). The biochemical structures of two of these LPS serotypes (A and C) have been characterized recently (6, 7, 19, 20). In this paper, we report the production of a monoclonal antibody (MAb) directed to *M. catarrhalis* LPS and demonstrate the specificity of this MAb for a common epitope of *M. catarrhalis* LPS which is present on all three LPS serotypes.

A total of 22 strains of *M. catarrhalis* were isolated from patients with lower respiratory tract infections at the clinic of the Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University. Twelve additional strains of *M. catarrhalis*, for which the LPS serotypes had been determined previously (30), were also examined. Clinical isolates of *M. catarrhalis* (87-115, 87-122, 87-94, 87-101, 88-23, 88-186, and 89-31) were grown overnight on brain heart infusion broth (Difco, Detroit, Mich.) at 37°C and harvested by centrifugation. Cells were washed twice and immediately lyophilized. LPSs were obtained by using the modified lysozyme-phenol extraction technique (13). Rough LPSs were also prepared from *H. influenzae* 84-44 and *Pseudomonas aeruginosa* 5276, which were isolated from patients with lower respiratory tract infections (28). Rough and smooth LPSs, purified from *Salmonella minnesota*, *Escherichia coli* O111:B4, *Klebsiella pneumoniae*, and *P. aeruginosa* immunotype 1 by the methods of Galanos et al. (11) and Westphal and Jann (32), respectively, were obtained from List Biological Laboratories, Campbell, Calif. MAb 1B12, a murine antibody of the immunoglobulin G2a subclass, was prepared by fusing spleen cells from a BALB/c mouse (Shizuoka Agricultural Cooperation Association for Laboratory Animals, Shizuoka, Japan) immunized with formalin-treated *M. catarrhalis* 87-115 with the SP/O-Ag14 mouse myeloma fusion partner (23) and screening the resulting hybridomas by enzyme-linked immunosorbent assay (ELISA) employing microtiter wells coated with LPS from *M. catarrhalis* 87-115. MAb binding to LPS was measured in polystyrene microtiter plates coated with 25 μg of purified LPS per ml suspended in coating buffer and left to stand overnight as previously described (24). MAb 1B12 was cloned and adapted as an ascites tumor in pristane-primed BALB/c mice. The ascites fluid MAb concentration (2.9 mg/ml) was determined with a radial immunodiffusion kit (Tago, Burlingame, Calif.).

MAb 1B12 bound to the LPSs purified from all seven strains of *M. catarrhalis* (Table 1). However, MAb 1B12 did not bind to smooth LPSs from wild-type *S. minnesota*, *E. coli* O111:B4, *K. pneumoniae*, or *P. aeruginosa* immunotype 1, to rough LPSs from *H. influenzae* and *P. aeruginosa*, or to rough LPSs or lipid A from *S. minnesota*. MAb 1B12 exhibited similar reactivities, as detected by ELISA, with LPSs from six different *M. catarrhalis* strains (Fig. 1A). The maximum reactions were ob-

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TABLE 1. Binding activity of MAb 1B12 in ELISA[a]

LPS and bacterial strains	Level of binding activity
Rough LPS	
M. catarrhalis	
87-115	++
87-122	++
88-186	++
88-23	++
87-94	++
89-31	+
87-101	++
H. influenzae 84-44	−
P. aeruginosa 5276	−
S. minnesota	
R60 (Ra)	−
R345 (Rb)	−
R5 (Rc)	−
R595 (Re)	−
lipid A	−
Smooth LPS	
S. minnesota wild type	−
E. coli O111:B4	−
K. pneumoniae	−
P. aeruginosa immunotype 1	−

[a] MAb-containing ascites fluid was assayed at a concentration of 2.9 μg/ml. The optical densities at 405 nm are scored as follows: 0 to 0.49, −; 0.5 to 0.99, +; >1.00, ++.

served at MAb concentrations of 2.9 to 29.0 μg/ml. The specificity of *M. catarrhalis* LPS-reactive MAb 1B12 was further confirmed by a competitive-inhibition ELISA. Tenfold dilutions of the LPS suspension were preincubated with MAb 1B12 at a concentration of 2 μg/ml, which yielded an optical density of 1.0 when assayed alone in ELISA. The mixture was then assayed as described above. The LPS of each *M. catarrhalis* strain inhibited MAb reactivity with homologous LPS in a dose-dependent manner (Fig. 1B). Purified LPS samples were sonicated, boiled, and electrophoresed in a Laemmli SDS-PAGE system with a 4% stacking gel and 14% separating gel. Gels were fixed by overnight incubation in a solution containing 40% ethanol and 5% acetic acid and were silver stained. LPS was transferred from identical unstained companion gels onto nitrocellulose membranes, which were then incubated

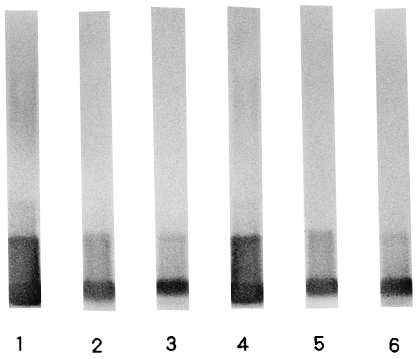

FIG. 2. Immunoblotting of purified LPSs from six *M. catarrhalis* strains demonstrating binding of MAb 1B12. Lane 1, strain 87-115; lane 2, strain 87-122; lane 3, strain 88-186; lane 4, strain 88-23; lane 5, strain 87-101; lane 6, strain 87-94.

with 1B12 MAb and developed as previously described (24). All LPS samples from the six *M. catarrhalis* strains exhibited similar bands in the low-molecular-weight region (data not shown). MAb 1B12 reacted strongly with all LPSs from the six *M. catarrhalis* strains, and the same two bands were observed on immunoblots (Fig. 2).

Live bacterial cells or culture supernatants of *M. catarrhalis* and other strains were examined by an indirect sandwich ELISA. MAb 1B12 was diluted in carbonate coating buffer at a concentration of 1.5 μg/ml and was incubated overnight at 4°C in polystyrene microtiter plates. Each bacterial strain was cultured overnight at 37°C in 100 ml of brain heart infusion broth. Brain heart infusion broth was supplemented with 5% Fildes enrichment (Bact, Detroit, Mich.) for the culture of *H. influenzae* and with 5% rabbit blood for the culture of *S. pneumoniae*. Free LPS of *M. catarrhalis* released into the culture supernatant has been shown to be essentially identical to cellular LPS (12). The wells were washed with phosphate-buffered saline (PBS)-Tween, and 100 μl of appropriately diluted bacterial suspension or bacterial culture supernatants was incubated per well at 25°C for 30 min. The final two steps, separated by PBS-Tween washes, were as follows: addition of 100 μl of rabbit serum (dilution, 1/1,000), hyperimmune to formalin-killed *M. catarrhalis* 87-115, and addition of 100 μl of alkaline phosphatase-conjugated goat antibodies to immunoglobulin (dilution, 1/500; Tago, Burlingame, Calif.) and addition of

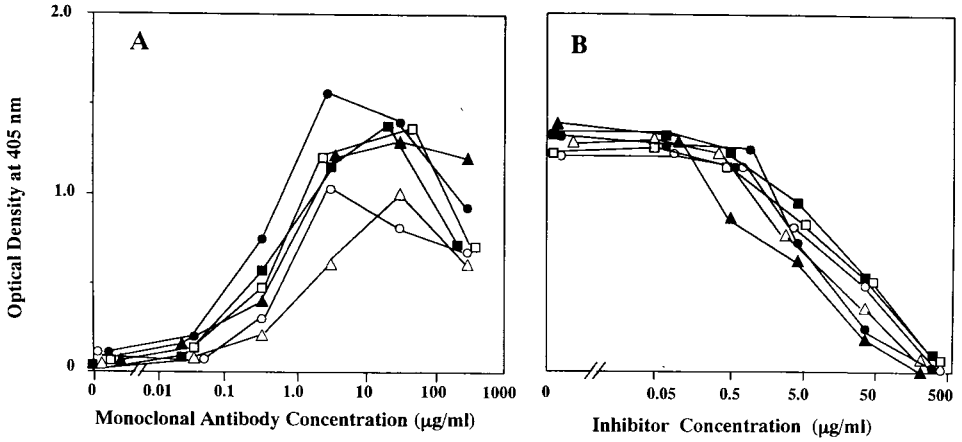

FIG. 1. (A) Binding of MAb 1B12 to purified LPSs from six respiratory pathogenic *M. catarrhalis* strains in antigen capture ELISA. (B) Dose-dependent inhibition of binding of MAb 1B12 to LPSs from six *M. catarrhalis* strains by the homologous LPS in a competitive-inhibition ELISA.

TABLE 2. Binding activity of MAb 1B12 to whole bacteria and culture supernatant in an indirect sandwich ELISA[a]

M. catarrhalis strain (serotype)[b]	Level of binding activity with:					
	Bacterial inoculum (CFU/ml)			Culture supernatant (dilution)		
	10^8	10^7	10^6	1:1	1:10	1:100
87-115 (ND)	++	−	−	++	+	−
87-122 (ND)	++	+	−	++	+	−
88-33 (ND)	++	+	−	++	+	−
88-51 (ND)	++	−	−	+	−	−
88-88 (ND)	++	+	−	++	+	−
88-151 (ND)	+	−	−	+	+	−
88-159 (ND)	++	−	−	++	++	+
88-176 (ND)	++	+	−	++	++	+
90-25 (ND)	+	−	−	+	−	−
90-28 (ND)	+	−	−	+	+	−
90-29 (ND)	++	++	−	++	+	−
90-71 (ND)	++	+	−	++	+	−
90-147 (ND)	++	−	−	++	+	−
90-151 (ND)	++	+	−	+	−	−
91-41 (ND)	++	+	−	++	+	−
91-43 (ND)	+	−	−	+	−	−
91-68 (ND)	++	+	−	++	+	−
91-71 (ND)	+	−	−	+	−	−
91-76 (ND)	+	−	−	+	−	−
91-239 (ND)	++	+	−	+	−	−
91-254 (ND)	+	−	−	+	−	−
91-256 (ND)	+	−	−	+	−	−
A-20 (A)	+	−	−	+	−	−
B-23 (A)	+	−	−	+	−	−
D-1 (A)	++	+	−	++	+	−
M-5 (A)	+	+	−	+	−	−
A-21 (B)	+	−	−	+	−	−
A-30 (B)	+	−	−	+	−	−
D-13 (B)	+	−	−	+	−	−
F-17 (B)	+	−	−	+	−	−
V-19 (C)	++	+	−	+	+	−
O-29 (C)	+	−	−	++	−	−
E-15 (C)	+	−	−	+	−	−
T-15 (NT)	+	+	−	++	+	−

[a] Data are optical densities at 405 nm and were scored as follows: 0 to 0.29, −; 0.3 to 0.99, +; >1.00, ++.
[b] ND, not determined; NT, nontypeable.

100 μl of *p*-nitrophenyl phosphate substrate (1 mg/ml in 10% diethanolamine; Sigma 104) for 30 min at 25°C. A_{405} was read. MAb 1B12 bound to all live bacterial cells at a density of 10^8 CFU/ml and to the undiluted bacterial culture supernatants of all 34 strains of *M. catarrhalis* (Table 2). Included among these 34 strains were strains from 11 distinct LPS serotypes and 1 nonserotypeable strain. Fourteen strains showed positive reactions at a bacterial density of 10^7 CFU/ml, but no reactions were seen at a density of 10^6 CFU/ml. MAb 1B12 also reacted with all of the undiluted culture supernatants from these 34 strains and with the 1:10 to 1:100 diluted culture supernatants from 16 strains. We also evaluated the reactivity of an LPS from *M. catarrhalis* 88-186 in the same assay. Positive reactions were observed for LPS concentrations between 5 ng/ml and 50 μg/ml (data not shown). In contrast, MAb 1B12 did not react with live bacterial cells at a concentration of 10^8 CFU/ml or with undiluted culture supernatants from seven clinical isolates of *Staphylococcus aureus*, *S. pneumoniae*, and *P. aeruginosa* (data not shown). MAb 1B12 also did not react with an LPS of *H. influenzae* or with live cells and undiluted culture supernatants from seven clinical isolates of *H. influenzae*, although Campagnari et al. reported the cross-reactions of one MAb specific for the LPS of *H. influenzae* with three *M. catarrhalis* strains (3). Similarly, MAb 1B12 did not react with live cells or culture supernatants from different *Neisseria* species (*Neisseria lactamica*, *N. sicca*, *N. meningitidis*, *N. gonorrhoeae*, and *N. perflava*), which were obtained from the American Type Culture Collection. Jonsson et al. (15) have reported cross-reactions of immunized rabbit serum against *M. catarrhalis* with beta-hemolytic streptococcus groups A and G. However, we observed no cross-reactivities of MAb 1B12 with either of two clinical isolates of beta-hemolytic streptococcus groups A and G (data not shown).

Published reports have shown that *M. catarrhalis* possesses a rough LPS with a molecular mass of 3.0 to 5.5 kDa (29, 30). It has been previously shown that three different LPS serotypes can be distinguished within *M. catarrhalis* (30). For a total of 302 strains collected from different parts of the world, 93.4% of the strains were LPS serotypeable. A recent study reported cross-reactivity among nine *M. catarrhalis* strains of different serotypes, as detected by using rabbit antisera in an immunofluorescence assay (14). As previously suggested (21), the lack of correlation of antibody titers with LPS serotype shown in this study is explainable by poor expression of the antigenic determinants of LPS on the bacterial surfaces. However, the antigenically conserved structure of the lipooligosaccharides of *M. catarrhalis* remains to be determined.

In this study, we first produced a murine MAb 1B12 that was reactive with *M. catarrhalis* LPSs and then demonstrated the specificity and cross-reactivity of this MAb among *M. catarrhalis* strains. The cross-reactions among eleven *M. catarrhalis* strains with different LPS serotypes and one nontypeable strain were also demonstrated. Therefore, the present data suggest that MAb 1B12 specifically recognizes an epitope on rough LPS which is common to *M. catarrhalis* strains. A recent study employing purified LPSs from two *M. catarrhalis* strains and anti-LPS antibodies from immunized rabbits similarly indicated that an LPS of one *M. catarrhalis* strain possessed both individual and common antigenic determinants (10). These lines of evidence indicate that at least one common epitope, which differs from serotype determinants, exists on the rough LPS of *M. catarrhalis*. The present study suggests a possible application of this MAb for the detection of soluble antigen or whole bacteria of *M. catarrhalis* in clinical specimens. It might be able to detect soluble LPS antigens in clinical samples because 5 ng of soluble LPS from *M. catarrhalis* 88-186 was sufficient for positive reactions. Further studies will be required to evaluate the possible diagnostic uses of this MAb.

Previous studies have documented an immune response to *M. catarrhalis* in patients with bronchopulmonary infections and otitis media (2, 17). Convalescent-phase immunoglobulin G antibody from patients with pulmonary infection demonstrated bactericidal activity against *M. catarrhalis* strains (4). It has also been documented that antibody to LPS of *M. catarrhalis* mediates complement-dependent bacteriolysis against homologous strains (29). Thus, in vitro and in vivo antibacterial properties of this MAb appear to be noteworthy.

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