Characterization and comparative bactericidal activity of monoclonal antibodies to *Bordetella pertussis* lipo-oligosaccharide A

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Spleen cells from mice immunized with a *Bordetella pertussis* N-lauroyl sarcosine membrane extract (SME) were used to generate hybridoma cells lines producing monoclonal antibodies (mAbs). Seven mAbs were shown to be specific to *B. pertussis* lipo-oligosaccharide (LOS) by immunoblotting of SME or purified LOS following SDS-PAGE. All mAbs reacted with the *B. pertussis* Tohama I strain of the LOS AB phenotype, and did not react with the atypical variant strain 134 of the LOS B phenotype. The immune reactivity of the mAbs was retained after treatment of SME with proteinase K and was lost after sodium periodate treatment. No cross-reactivity was observed with the mAbs when tested against *B. parapertussis* and other Gram-negative bacteria. However, all mAbs reacted with *B. bronchiseptica*. Binding assays with live *B. pertussis* cells demonstrated that mAbs strongly reacted with cell surface exposed antigenic determinants. High bacterial cell lytic capability was observed for five of these mAbs. Concentrations between 0.22 and 2.2 µg mAb ml⁻¹ (0.1 and 1 µg per 450 µl assay) purified by protein A were required to kill at least 50% of the bacteria. Competition immunoassays with biotinylated antibodies showed that the bacteriolytic and non-bacteriolytic mAbs were directed to different epitopes of the *B. pertussis* LOS A.

Introduction

Effective vaccination with whole cell pertussis vaccine has had a marked effect in reducing the incidence, and preventing the recrudescence, of infection by *Bordetella pertussis*. Possible side effects of these vaccines have affected immunization programmes in several countries (Romanus *et al.*, 1987; Sato *et al.*, 1984). These findings have given impetus to research aimed at identifying factors responsible for the pathogenicity and immunogenicity of this respiratory tract pathogen. Cell surface exposed components of the outer membrane may interact directly with the host, contributing to virulence, and in addition are targets for antibodies which might be protective against *B. pertussis* infection. Proteins and lipo-oligosaccharides (LOS) are the major constituents of the outer membrane and both have received considerable attention as possible determinants of virulence and immunity.

The LOS of *B. pertussis*, like the LOS of *Neisseria gonorrhoeae* (Schneider *et al.*, 1984), *N. meningitidis* (Tsai

et al., 1983) and *Haemophilus influenzae* type b (Flesher & Insel, 1978; Inzana, 1983), appears to have no O-antigen repeat units, but consists of a lipid A moiety covalently linked to the structure equivalent of the core oligosaccharide of the enteric lipopolysaccharide molecule (Hitchcock *et al.*, 1986). The biological activities of *B. pertussis* LOS resemble those of LOS of other Gram-negative bacteria (Nakase *et al.*, 1970; Watanabe *et al.*, 1990). However, as shown by Ayme *et al.* (1980), the lipid A and lipid X fractions obtained after acidic hydrolysis do not have the same activities as the ones described for other organisms. Earlier studies using passive immune lysis, immunodiffusion and bactericidal analysis with rabbit antisera defined two distinct serotypes of *B. pertussis* LOS (Ackers & Dolby, 1972; Aprile & Wardlaw, 1973). Subsequent chemical analysis of *B. pertussis* LOS preparations has confirmed these results and identified the presence of two different oligosaccharides bound to lipid A. The two major carbohydrate-containing compounds, LPS-I and LPS-II, were present in a ratio of 2:3 (Le Dur *et al.*, 1978, 1980). Peppler (1984) and Li *et al.* (1988), using SDS-PAGE followed by immunoblotting with polyclonal and monoclonal antibodies, also detected the presence of two different LPS

Abbreviations: i.p., intraperitoneally; LOS, lipo-oligosaccharide(s); mAb, monoclonal antibody; SME, N-lauroyl sarcosine membrane extract.

components: a slow migrating dominant A band and a fast migrating minor B band. It is possible that the LPS-I and LPS-II might correspond to the A and B bands as suggested by Chaby & Caroff (1988); however, more immunological studies are required to clarify the relationship between these different LOS preparations.

In this paper we have utilized monoclonal antibodies (mAbs) to characterize *B. pertussis* LOS, and identified distinct antigenic epitopes on the LOS A molecule. Our results show that one of these epitopes is involved in bactericidal activity.

Methods

Bacterial strains and growth conditions. *B. pertussis* strains 9340, 9797 (18323), 12742, 12743 and 8767, and *B. bronchiseptica* strain 19395 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). *B. pertussis* Tohama I strain and *B. pertussis* mutant strain 134 were a gift from M. S. Peppler, University of Alberta, Edmonton, Canada. Ten clinical isolates of *B. pertussis*, four of *B. paraptussis* and one of *B. bronchiseptica* were obtained from the Ontario Public Health Laboratory, Ottawa, Ontario, Canada, and from R. Higgins, Faculty of Veterinary Medicine, St-Hyacinthe, Quebec, Canada. Other Gram-negative bacteria were obtained from our own collection. All *Bordetella* strains were cultivated at 36 °C on charcoal agar plates. Specificity of *B. pertussis* strains was tested by slide agglutination using commercially available *B. pertussis* specific antisera (Difco).

Outer membrane protein and LOS preparations. N-Lauroyl sarcosine membrane extract (SME) from *B. pertussis* 9340 was prepared as previously described by Barenkamp *et al.* (1981). Protein content was determined by the Lowry method. LOS was prepared from *B. pertussis* 9340 by the phenol/water extraction procedure described by Inzana (1983).

Immunization of mice. Balb/c mice, 6 to 10 weeks of age, were immunized intraperitoneally (i.p.) with 50 µg of *B. pertussis* SME in incomplete Freund's adjuvant once a week for 4 to 5 weeks. A final inoculation of 30 µg of SME alone was given i.p. 4 d before the fusion procedure. Hyperimmune sera were obtained from immunized mice by cardiac puncture before spleen removal.

Production and purification of mAbs. Hybridomas were generated as described previously (Brodeur *et al.*, 1985). Hybrid clone supernatants were tested for antibody production by ELISA, as previously described (Hamel *et al.*, 1987), using as coating antigen 100 µl of *B. pertussis* SME (10 µg protein ml⁻¹) in 0.05 M-NaHCO₃ buffer, pH 9.6, in each well of a 96-well microtitre plate. *B. pertussis* antibody-positive cells were subcloned by limiting dilution. Selected subclones were grown for antibody production in serum-free cell culture medium, or as ascites as previously described (Brodeur & Tsang, 1986). mAbs were purified as previously described (Hamel & Brodeur, 1990). The immunoglobulin class, subclass, and light-chain specificity were determined by indirect ELISA using commercial reagents (Fisher Biotech).

SDS-PAGE and immunoassay procedures. SME or LOS preparations were resolved by electrophoresis using the discontinuous buffer system of Laemmli (1970), and Western blot analysis performed as previously described by Hamel *et al.* (1987). The LOS structure of each *Bordetella* strain was determined after SDS-PAGE followed by a silver stain (Tsai & Frasch, 1982). *B. pertussis* Tohama I and 134 strains expressing the

AB and B LOS phenotype, respectively, were used as reference strains. A dot-enzyme immunoassay, performed as described previously (Lussier *et al.*, 1989), was used for specificity determination of mAbs against a panel of *B. pertussis*, *B. paraptussis* and *B. bronchiseptica* ATCC strains, clinical isolates and other Gram-negative bacteria. *B. pertussis* cells were adjusted to 1 × 10⁸ c.f.u. ml⁻¹. SME was used at a concentration of 20 µg protein ml⁻¹.

Proteolytic treatment and sodium periodate oxidation of *B. pertussis* SME or LOS. Digestion of *B. pertussis* SME or LOS with proteinase K was performed as previously described (Hamel *et al.*, 1987). The immune reactivity of mAbs with the enzyme-treated SME or LOS were thereafter tested by the dot-enzyme immunoassay as described above. mAb BM-1 directed against an outer membrane protein of *B. pertussis* SME was used as a control. Sodium periodate oxidation (Woodward *et al.*, 1985) of *B. pertussis* SME was conducted in order to determine whether the mAbs were directed against carbohydrate antigenic determinants of *B. pertussis* LOS, as previously described by Martin *et al.* (1990).

Antibody accessibility radioimmunoassay. Adsorption of mAbs at the surface of live bacteria was performed as previously described (Martin *et al.*, 1988). Bacteria grown for 2 d on charcoal agar plates were suspended in hybridoma culture supernatant to a concentration of 2 × 10⁹ c.f.u. ml⁻¹.

Bactericidal assay. The bactericidal activity of ascitic fluid or designated protein A-purified mAbs was tested *in vitro* as previously described (Brodeur *et al.*, 1985). All ascites were heat-inactivated at 56 °C for 15 min. Ascitic fluid was diluted in PBS containing 0.15 mM-CaCl₂, 0.5 mM-MgCl₂ and 0.1% bovine serum albumin (buffer-BSA). Bacteria (50 µl), adjusted to 2 × 10⁴ c.f.u. ml⁻¹, were dispensed into a tube containing 90 µl of each antibody dilution and 280 µl of the buffer-BSA, before adding 30 µl of guinea-pig serum as a source of complement. Duplicate antigen/antibody mixtures were incubated either in the absence of complement or with heat-inactivated (56 °C, 30 min) guinea-pig serum to serve as additional controls. After incubation for 45 min at 37 °C, 50 µl of each mixture was plated onto charcoal agar plates. The plates were incubated for 3 to 5 d at 36 °C, after which time bacterial colonies were counted. Both negative and positive controls were included in each experiment. In each bactericidal assay, approximately 200 c.f.u. were counted on control negative plates. The bactericidal titre for each antibody was measured as the reciprocal of the highest dilution giving less than 50% cell survival. The bactericidal activity of the mAbs was evaluated against all *B. pertussis* and *B. bronchiseptica* ATCC strains and clinical isolates. As a control for background, mouse antibody ascitic fluid containing mAb P2-10 specific to *H. influenzae* type b outer membrane protein (Martin *et al.*, 1990) was treated in an identical manner.

Competition ELISA using biotinylated antibodies. Protein-A-purified *B. pertussis* LOS-specific mAbs BL-1, BL-2 or BL-5 were dialysed for 4 h at 4 °C against 0.1 M-NaHCO₃ pH 8.4, and mixed with biotinyl-ε-aminocaproic acid N-hydroxysuccinimide ester (Calbiochem) at a molar ratio of four biotin molecules to one immunoglobulin for 4 h at room temperature in the dark with constant agitation. The mixture was then dialysed overnight at 4 °C against PBS, pH 7.2.

To perform the competition assay, SME-coated ELISA plates were first incubated with hybridoma supernatant or protein-A-purified antibody for 1 h at 37 °C, washed three times with PBS-Tween, and exposed for 15 min at room temperature to biotinylated antibodies diluted such that an A₄₅₀ of 0.7 to 0.8 would be obtained in the absence of competing antibody. Thereafter, plates were washed with PBS-Tween, and then incubated with streptavidin-horseradish peroxidase conjugate diluted in 3% BSA in PBS for 45 min at room temperature. As substrate, 100 µl of 0.05 M-o-phenylenediamine dihydrochloride (Sigma) in 0.1 M-citric acid, pH 4.5, and 0.0006% H₂O₂ was added in

each well. A_{450} was read 15 min after the substrate was added to each well.

The following formula was used to estimate the percentage of inhibition of binding: $\{1 - [(Absorbance\ with\ inhibitor - background) - (Absorbance\ without\ inhibitor - background)]\} \times 100$, where background represents absorbance in wells without mAb or containing an irrelevant mAb.

Results

Characterization of mAbs

Seven hybridoma secreting mAbs (BL-1 to BL-7) specific to *B. pertussis* were generated. Initially, cell culture supernatants were screened by ELISA using SME as coating antigen. All mAbs were IgGs except for BL-3, which is an IgM, and they all possessed a κ light chain. mAbs were tested by dot-enzyme immunoassay for reactivity against a panel of *Bordetella* strains, and other Gram-negative bacteria (Table 1). The seven mAbs

reacted with all *B. pertussis* strains obtained from ATCC and with the 10 clinical isolates tested so far. None of the mAbs reacted with *B. parapertussis* or other Gram-negative bacteria. However, all mAbs cross-reacted with the four *B. bronchiseptica* strains expressing the LOS AB phenotype.

SDS-PAGE of purified *B. pertussis* LOS and Western blot analysis revealed that these mAbs were directed against LOS epitopes (Fig. 1). Similar results were obtained when SME or whole bacteria digested with proteinase K were used instead of purified LOS (data not shown). Sodium periodate and proteinase K treatments were used in a dot-enzyme immunoassay to confirm the carbohydrate nature of the LOS epitopes recognized by mAbs. No reduction of mAb reactivity against LOS was noted after enzymic treatment of SME protein. On the other hand, treatment with sodium periodate abolished mAb reactivity against SME, whilst reactivity of mAb BM-1, which is directed against a protein of *B. pertussis*, was not affected by this treatment.

Fig. 1. Western blot of purified *B. pertussis* 9340 LOS demonstrating specificity of mAbs.

Table 1. Specificity of mAbs by dot-enzyme immunoassay

Bacterial strains	LOS phenotype	mAbs							
		BL-1	BL-2	BL-3	BL-4	BL-5	BL-6	BL-7	BM-1*
<i>B. pertussis</i>									
ATCC strains (5)	AB	+	+	+	+	+	+	+	+
Clinical strains (10)	AB	+	+	+	+	+	+	+	+
Tohama I strain	AB	+	+	+	+	+	+	+	+
Mutant strain 134	B	—	—	—	—	—	—	—	+
<i>B. bronchiseptica</i>									
Clinical isolates (4)	AB	+	+	+	+	+	+	+	—
ATCC Strain (1)	B	—	—	—	—	—	—	—	—
<i>Bordetella parapertussis</i> (4)	—	—	—	—	—	—	—	—	—
Gram-negative bacteria† (16)	—	—	—	—	—	—	—	—	—

* mAb directed against an outer membrane protein of *B. pertussis*.

† The bacterial strains (ATCC number) are: *Alcaligenes faecalis* (8750), *Citrobacter freundii*, *Edwardsiella tarda* (15947), *Enterobacter aerogenes* (13048), *Enterobacter cloacae* (23355), *Haemophilus influenzae* type b (Eagan strain), *Klebsiella pneumoniae* (13883), *Neisseria catarrhalis* (8176), *Neisseria perflava* (14799), *Neisseria subflava* (19243), *Proteus rettgeri* (25932), *Proteus vulgaris* (13315), *Salmonella typhimurium* (14028), *Serratia marcescens* (8100), *Shigella flexneri* (12022), *Shigella sonnei* (9290).

Table 2. Surface accessibility of mAbs

mAbs	¹²⁵ I-labelled anti-mouse immunoglobulin bound (c.p.m.)*	
	<i>B. pertussis</i>	<i>H. influenzae</i> type b
BL-1	19666	1022
BL-2	12913	873
BL-3	6966	2062
BL-4	11887	755
BL-5	11481	961
BL-6	25348	970
BL-7	25645	1073
BM-1†	4390	832
P2-10‡	325	5120
P2-4‡	1336	35995

* Mean c.p.m. of duplicate experiments.

† mAb directed against *B. pertussis* outer membrane protein.

‡ mAb specific to *H. influenzae* type b outer membrane protein.

Reactivity of mAbs with *B. pertussis* expressing different LOS

To verify their epitope specificity, all mAbs were tested against *B. pertussis* Tohama I strain of the LOS AB phenotype, mutant strain 134 of the LOS B phenotype, and strains of *B. bronchiseptica* of the AB and B LOS phenotypes (Table 1). In dot-enzyme immunoassays, the seven mAbs reacted only with strains representing the LOS phenotype AB. These results clearly indicated that all the mAbs reacted only with strains of *Bordetella* expressing LOS A.

Surface exposure of LOS A epitopes

To determine if mAbs were directed against cell surface exposed epitopes, hybridoma culture supernatants were incubated with live *B. pertussis*. All mAbs were able to bind to the surface of *B. pertussis* as measured by radioimmunoassay (Table 2). mAb BL-3, an IgM, showed the lowest reactivity with *B. pertussis* surface antigens. None of the mAbs reacted significantly with *H. influenzae* type b. mAbs P2-4 and P2-10, specific to *H. influenzae* type b outer membrane protein, did not bind on the surface of *B. pertussis*, but reacted strongly with *H. influenzae* type b.

Comparative ELISA and bactericidal activity of mAbs

Assays were performed to evaluate and compare the ability of mAbs to bind antigen and to exert bactericidal activity against *B. pertussis* and *B. bronchiseptica*. All samples from bactericidal assays were plated in duplicate and each experiment was performed several times. The ELISA titre against *B. pertussis* SME antigens and the bactericidal titre of ascites fluid containing mAbs are contrasted in Table 3. There was no correlation between ELISA and bactericidal titre. mAbs BL-6 and BL-7 had the highest ELISA titres against *B. pertussis* antigens but did not demonstrate any bactericidal activity. The ELISA titres of the five bactericidal antibodies ranged from 4000 to 128 000. mAbs BL-1 to BL-5 showed similar bactericidal activity against *B. pertussis*; however, though they adsorbed to the cell surface of *B. bronchiseptica*.

Table 3. Bactericidal activity of mAbs

mAbs	Ig class and subclass	<i>B. pertussis</i>		<i>B. bronchiseptica</i>	
		ELISA titre*	Bactericidal titre†	ELISA titre	Bactericidal titre
BL-1	IgG1, κ	128 000	500	64 000	0
BL-2	IgG3, κ	32 000	1 000	32 000	0
BL-3	IgM, κ	32 000	500	16 000	0
BL-4	IgG3, κ	32 000	1 000	32 000	0
BL-5	IgG3, κ	4 000	500	800	0
BL-6	IgG1, κ	128 000	0	4 000	0
BL-7	IgG1, κ	128 000	0	4 000	0
BM-1‡	IgM, κ	16 000	0	0	0
P2-10§	IgG2a, κ	0	0	0	0
C57	Polyclonal	32 000	500	4 000	0

* ELISA was performed with *B. pertussis* 9340 SME as coating antigen. ELISA titre is expressed as the reciprocal of the highest ascites fluid dilution giving an absorbance value ≥ 0.1 , 60 min after the enzyme substrate was added to each well.

† Bactericidal titre was expressed as highest ascites fluid dilution demonstrating at least 50% reduction of c.f.u. The bactericidal activity of the mAbs was evaluated against all ATCC strains and clinical isolates of *B. pertussis* and *B. bronchiseptica*.

‡ mAb directed against *B. pertussis* outer membrane protein.

§ mAb directed against *H. influenzae* type b outer membrane protein.

|| Hyperimmune mouse serum specific to *B. pertussis* SME.

Fig. 2. Bactericidal activity of anti-LOS mAbs on *B. pertussis* strain 9340. Purified mAbs BL-1 (●), BL-2 (▲), or BL-5 (○) and P2-10 (■) were used at different concentrations.

Fig. 3. Inhibition of binding of biotinylated mAbs to *B. pertussis* SME by unlabelled mAbs was tested by ELISA. Biotinylated mAbs BL-1 (solid bars), mAb BL-2 (hatched bars), mAb BL-5 (open bars).

tica (data not shown), none of the mAbs were bactericidal against this bacterium.

Comparisons between BL-1, BL-2 and BL-5 were extended using purified mAbs instead of ascites fluid (Fig. 2). Between 0.22 and 2.2 µg purified mAbs BL-1, BL-2, or BL-5 ml⁻¹ (0.1 and 1 µg per 450 µl assay) were required to kill 50% of the bacteria. The bactericidal activity was dose-dependent, with 22.2 µg mAb ml⁻¹ (10 µg per 450 µl assay) showing almost complete killing. No bactericidal activity was observed in controls using either complement alone, heat-inactivated complement and mAbs, complement with control anti-*H. influenzae* type b specific mAb, or mAbs alone without complement. These results ruled out the possibility that agglutination might be responsible for any reduction of c.f.u. in the assay.

Determination of *B. pertussis* LOS epitopes associated with bactericidal activity

The seven mAbs were classified on the basis of shared versus independent epitopes by means of competitive binding of paired mAbs to *B. pertussis* SME. For each test, an excess of one unlabelled mAb was assayed for its capacity to inhibit binding to SME of another mAb labelled with biotin. The competition studies, using three biotinylated mAbs, allowed the identification of at least two distinct LOS A antigenic determinants, one of which is involved in bactericidal activity (Fig. 3). Bacteriolytic mAbs BL-1 to BL-5 recognized the same or overlapping epitopes. On the other hand, the non-bacteriolytic mAbs BL-6 and BL-7 recognized a LOS A epitope distinct from that defined by the previous group of mAbs. Our results did not indicate whether these latter mAbs recognized the same epitope.

Discussion

The pathogenesis of *B. pertussis* infection is not fully understood, but it is believed that several components produced by the bacteria, such as pertussis toxin (Askelof *et al.*, 1990), filamentous haemagglutinin (Kimura *et al.*, 1990), adenyl cyclase (Hewlett *et al.*, 1989; Rogel *et al.*, 1989), heat labile toxin (Nakase & Endoh, 1988) and endotoxin (LOS) (Chaby & Caroff, 1988), all contribute to the manifestations of the disease. Although the LOS of *B. pertussis* has previously been characterized both biochemically and immunologically, and has been implicated in virulence and immunity, localization of immunologically active epitopes on the structure of the LOS has not yet been well defined. In this work, mAbs directed against epitopes in the oligosaccharide portion of the LOS of *B. pertussis* were used to further characterize this pathogen. By this approach we have identified antigenic determinants on the LOS A of *B. pertussis* some of which are associated with bactericidal capability.

Previous investigations involving mAbs directed against LOS have concentrated primarily on the provision of a database for use in subsequent studies of epidemiology, typing and diagnosis of *B. pertussis* infection. In an earlier study (Frank & Parker, 1984), eleven mAbs were produced to *B. pertussis* antigens. These mAbs reacted with LOS of *B. pertussis* and also with LOS from *B. paraptussis* and *B. bronchiseptica*. Unfortunately, lack of appropriate technology hindered the localization of specific epitopes for these mAbs. More recently, other investigators have produced mAbs to LOS that appear to be primarily responsible for species specificity (Gustafsson *et al.*, 1988). In contrast to

the study by Frank & Parker (1984), none of these mAbs reacted with *B. parapertussis* and *B. bronchiseptica*. It is surprising to note that none of the mAbs described by Gustafsson *et al.* (1988) reacted with *B. bronchiseptica* even though two of these mAbs recognized binding epitopes present on delipidated polysaccharide fragments of both LPS-I and LPS-II, prepared according to the procedure described by Le Dur *et al.* (1978, 1980). Without immunoblots of *B. pertussis* and *B. bronchiseptica* LOS, we cannot establish that these mAbs are specific for the LOS A and/or LOS B bands. The lack of reactivity of these mAbs with *B. bronchiseptica* might be explained by assuming that the two strains used in these experiments were of the LOS phenotype B. Li *et al.* (1988) have also confirmed that several strains of *B. bronchiseptica* have broad silver-stained areas on SDS-PAGE corresponding to LOS phenotype AB or B of *B. pertussis*. The mAb specific to *B. pertussis* LOS A reacted with the LOS A band of *B. bronchiseptica* but not with the *B. bronchiseptica* strain of the LOS phenotype B.

As far as we are aware, biological activity was never associated with any mAbs specific to *B. pertussis* LOS. The mAbs described in this paper were initially identified and selected from five different fusions on the basis of their reactivity with surface exposed epitopes in *B. pertussis* LOS. Specificity studies by dot-immunoassay demonstrated that the seven mAbs reacted with all *B. pertussis* of the LOS phenotype AB tested so far. No cross-reaction was observed with a panel of 16 Gram-negative bacteria, nor with *B. parapertussis*. All the mAbs reacted with four isolates of *B. bronchiseptica* of the LOS phenotype AB, but not with a strain of the LOS phenotype B. Western blot analysis confirmed that mAbs reacted only with the A band of *B. pertussis* and *B. bronchiseptica* LOS and not with *B. pertussis* strain 134 or *B. bronchiseptica* strains containing only LOS B. The mAbs apparently recognized an oligosaccharide component of LOS since reactivity was lost after treatment of outer membrane preparations with sodium periodate. It is also of interest to note that the pattern of reactivity in competitive binding assays allowed the identification of at least two distinct LOS A antigenic regions. Five of the mAbs recognizing the same or overlapping epitopes on the LOS A molecule were capable of inducing bactericidal activity *in vitro*. This biological activity was shown to be antibody-dose-dependent and complement-dependent.

It is somewhat surprising to find that none of the mAbs were bactericidal for *B. bronchiseptica* even though they reacted with a surface accessible antigenic determinant on the LOS A molecules (data not shown). Li *et al.* (1988) have reported that LOS A may not be available in sufficient density at the surface of *B. bronchiseptica* to serve as an agglutinin. This observation might explain

the lack of bactericidal activity of our mAbs against *B. bronchiseptica*. The availability of a LOS antigenic variant (strain 134) has given us the opportunity to evaluate the effect of changes in LOS phenotype on the survival of *B. pertussis* in the presence of specific antibody and complement. None of the mAbs were bactericidal for this *B. pertussis* variant strain. It is thus evident that *B. pertussis* and *B. bronchiseptica* share common immunogenic structures within their LOS A bands. However, the role of these epitopes in immunity is still unclear. Protective human immunity to many Gram-negative diseases has been closely correlated with the presence of serum bactericidal antibodies. The evaluation of the *B. pertussis* LOS A epitopes as a target for protective antibodies remains to be investigated.

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