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A Bactericidal Monoclonal Antibody Specific for the Lipooligosaccharide of *Bordetella pertussis* Reduces Colonization of the Respiratory Tract of Mice after Aerosol Infection with *B. pertussis*

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A mouse immunoglobulin G3 monoclonal antibody specific for the core oligosaccharide moiety of the lipooligosaccharide (LOS) of *Bordetella pertussis* has been shown to have complement-dependent bactericidal activity. This monoclonal antibody exhibits bactericidal activity against strains of *B. pertussis* that express the LOS A phenotype. In addition this monoclonal antibody was effective in reducing colonization by *B. pertussis* in both the lungs and tracheas of mice after aerosol infection.

Bordetella pertussis expresses two forms of a rough-type lipooligosaccharide (LOS), which have been referred to as LOS A and LOS B (7, 9). The two forms of LOS can be resolved as two distinct molecular weight species in silver-stained sodium dodecyl sulfate-polyacrylamide gels of protease-treated *B. pertussis* cell lysates (9). The A form appears as a slow-migrating band, and the B form appears as a fast-migrating band. The difference between the two forms of LOS has been shown to be due to three additional *N*-acetyl amino sugars in the core oligosaccharide moiety of LOS A that are not present in LOS B (3, 6). The structure and biological activity of *B. pertussis* LOS are similar to those of LOSs of other gram-negative bacteria that contain lipid A and a core oligosaccharide moiety containing 2-keto-3-deoxyoctonate, exhibiting lethal toxicity in mice, pyrogenicity in rabbits, and mitogenicity in cell cultures (8, 13).

A previous study (1) has evaluated the immunogenicity and protective activity of *B. pertussis* LOS. It showed that *B. pertussis* LOS bound to carrier proteins elicits bactericidal antibodies which are not protective to mice challenged with a lethal intracranial inoculum of *B. pertussis* 18323. Others have reported (4) that polyclonal antibody elicited by *B. pertussis* lipopolysaccharide (LPS) is protective in an intratracheal rat infection model.

Monoclonal antibodies (MAb) specific for *B. pertussis* LOS which have bactericidal activity toward *B. pertussis* in in vitro complement-dependent assays have been described (2). Until now, no in vivo protection activity of MAb to *B. pertussis* has been reported. In this report we show that an immunoglobulin G3 mouse MAb, designated G10F8C3, not only has complement-dependent bactericidal activity against *B. pertussis* but is also able to reduce colonization of the respiratory tract of adult BALB/c mice after aerosol infection.

G10F8C3 reacts only with the A band of the *B. pertussis* LOS on immunoblots and loses this reactivity after treatment of whole cells or blotted antigen with periodate (7). The observation that LOS A contains three additional amino sugars not present in LOS B suggests that these three additional distal saccharides in the core oligosaccharide moiety could be part of the antigenic determinant for G10F8C3.

Bactericidal activity of G10F8C3 was determined after purification from mouse hybridoma tissue culture supernatants by protein A-sepharose (Sigma Chemical Co., St. Louis, Mo.) affinity chromatography. Bactericidal assays were performed with sterile microtiter plates (Nunc, Roskilde, Denmark) or 1.5-ml microcentrifuge tubes. Assay mixtures (80 μl total) contained a suspension of 1,000 to 3,000 viable *B. pertussis* cells in phosphate-buffered saline (PBS), pH 7.4, with 1% Casamino Acids, 0.5 mM MgCl, 0.15 mM CaCl, and antibody. Assay mixtures were allowed to react for 30 min at 37°C. After incubation, 20 μl of either active or heat-inactivated (1 h at 60°C) complement from precolostral calf serum was added to the reaction mixtures. After further incubation at 37°C for 45 min, a portion of the reaction mix (10 μl) was plated onto Bordet-Gengou (BG) agar plates and the plates were incubated at 37°C. Bactericidal activity was calculated as the percentage of *B. pertussis* CFU recovered in assay mixtures incubated for 45 min at 37°C compared with the number of colonies recovered prior to the addition of complement. Data obtained were analyzed for statistical significance by the Student *t* test.

To determine whether the MAb had any functional biological activity in vivo, we employed the adult mouse respiratory infection model (10). Adult female BALB/c mice were injected intraperitoneally with 75 or 150 μg of either G10F8C3 or a nonrelevant mouse immunoglobulin G1 MAb, N5-2, which is specific for a viral membrane protein. Antibody for injection was prepared in sterile PBS. Twenty-four hours after passive transfer, all mice were aerosol infected with *B. pertussis* Tohama, as described by Kimura et al. (5). On days 0, 1, 5, 9, and 14 postinfection, groups of five mice were sacrificed and lungs and tracheas were removed and homogenized. Homogenates were diluted in sterile PBS and plated onto Bordet-Gengou agar plates to determine the numbers of viable *B. pertussis*. Datum points on graphs represent mean log 10 (±standard deviation) *B. pertussis* CFU recovered from five mice per time point.

In our bactericidal assay system (Table 1) the indicated concentrations of MAb were mixed with either active com-

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TABLE 1. Concentration-dependent, complement-mediated bactericidal assay of MAb G10F8C3 versus strain Tohama I

Antibody	Concn (μg/ml)	% Reduction in bacterial CFU with:	
		Complement[a]	Heat-inactivated complement[a]
G10F8C3	10	99.5[b] ± 0.4	−18.1[c] ± 29.6
	1.0	77.7[b] ± 3.7	−9.7 ± 0.7
	0.1	46.1[b] ± 4.8	−7.3 ± 14.9
N5-2	10	6.4 ± 17.0	−1.1 ± 13.1
None[d]		26.7 ± 4.8	−9.5 ± 13.8

[a] Numbers represent average percent bactericidal activity of G10F8C3, N5-2, or no antibody against *B. pertussis* Tohama I ± standard deviation of triplicate reaction mixtures.
[b] $P < 0.01$ compared with value for N5-2 or no antibody.
[c] Negative number denotes percent increase in number of colonies recovered from assay mixtures 45 min after addition of inactive complement.
[d] Bactericidal activity of active or inactive complement under assay conditions with no antibody.

plement or heat-inactivated complement. In reaction mixtures containing active complement and G10F8C3, a concentration-dependent bactericidal activity was observed. Control reaction mixtures containing either no antibody or the nonrelevant antibody exhibited significantly lower bactericidal activity than did the lowest concentration of G10F8C3 ($P < 0.01$). Increases in bacterial counts in test groups containing inactive complement indicate that serum elements in the complement source had no inhibitory effect on *B. pertussis* in this assay. Several *B. pertussis* strains were tested in our complement-mediated bactericidal assay to determine whether G10F8C3 was also bactericidal toward other *B. pertussis* strains shown to have LOS A and LOS B phenotypes (Table 2). The data presented here are consistent with the observations of Archambault et al. (2) in demonstrating bactericidal activity of immunoglobulin G3 MAb specific for *B. pertussis* LOS. In this report, we show that G10F8C3 was bactericidal to all assayed strains that express the LOS A phenotype. G10F8C3 was not found to be bactericidal to the atypical strain BP134, which does not express LOS A (Table 2). The bactericidal activity of

TABLE 2. Bactericidal activity of MAb G10F8C3 against *B. pertussis*

Strain	LPS phenotype[a]	% Bactericidal activity[b]
Tohama I	AB	91.0 ± 3.4
BP*824* Vir⁻	AB	100 ± 0
BP114	AB	76.8 ± 6.6
BP165	AB	76.6 ± 2.6
BP321	AB	100 ± 0
BP325	AB	96.6 ± 1.0
BP347	AB	100 ± 0
BP353	AB	91.7 ± 5.5
BP354	AB	89.5 ± 6.9
BP359	AB	86.4 ± 3.4
18-323	AB	99.0 ± 1.4
BP134	B	−7.6[c] ± 2.6

[a] LOS A and B phenotypes determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in this study and by Li et al. (7).
[b] Numbers represent percent bactericidal activity of G10F8C3 at a concentration of 10 μg/ml against *B. pertussis* strains ± standard deviation of duplicate reaction mixtures.
[c] Negative number denotes percent increase in number of colonies recovered from assay mixtures containing active complement after 45 min of incubation at 37°C.

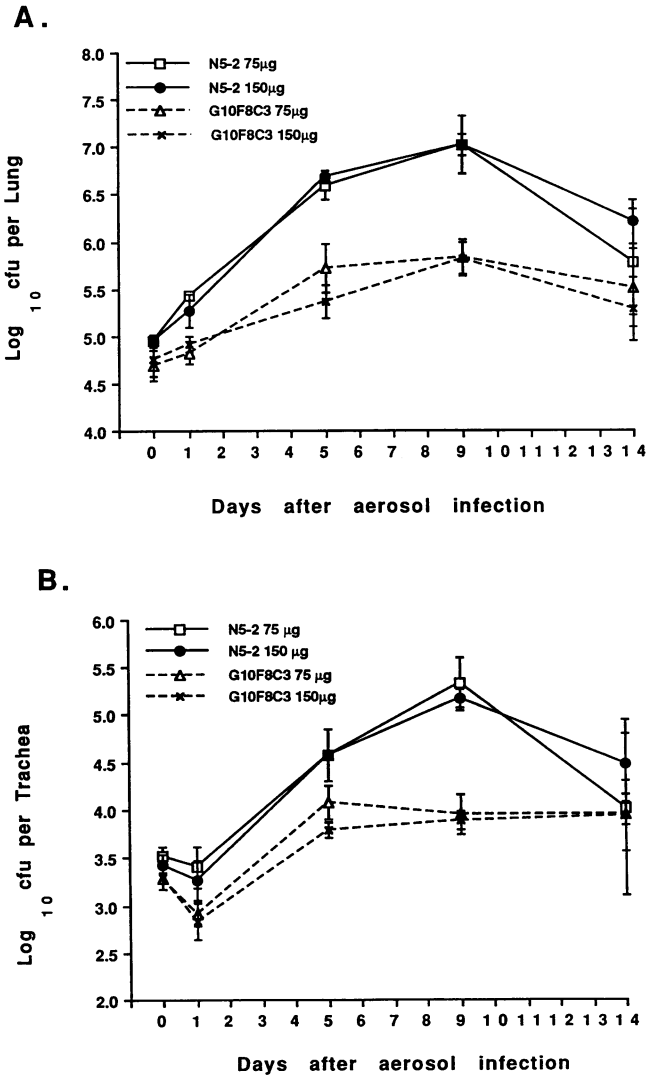

FIG. 1. *B. pertussis* colonization of the lungs (A) and tracheas (B) of BALB/c mice passively immunized with 75 or 150 μg of either control MAb N5-2 or anti-LOS MAb G10F8C3. The plots show the geometric means ± standard deviations (bars) for five mice per time point.

G10F8C3 was complement dependent for all strains assayed. Assay mixtures containing active complement without G10F8C3 or heat-inactivated complement exhibited no significant bactericidal activity (Table 1). These observations imply that epitopes specific for LOS A are targets for complement-mediated bactericidal activity.

BP824, a genetically altered *B. pertussis* strain deleted for the *bvg* (Vir) genes, which also expresses the LOS A and LOS B phenotypes, was also tested in this assay. The results (Table 2) show that the absence of virulence factors in BP824 has no effect upon the bactericidal activity of G10F8C3.

In addition to in vitro bactericidal activity, we have also determined that G10F8C3 exhibits functional activity in vivo in reducing the respiratory colonization by *B. pertussis* in aerosol-infected mice. Groups of mice were passively immunized with either MAb G10F8C3 or N5-2. Twenty-four hours after passive transfer, mice were challenged by aerosol infection with *B. pertussis* Tohama. Significant reductions in

colonization were observed in groups that received G10F8C3, compared with that in control groups that received N5-2. This reduction occurred in both the lungs and tracheas (Fig. 1; $P < 0.02$ for days 1, 5, and 9). Fourteen days after aerosol infection, colonization was also reduced in control groups by natural immunity.

MAb specific for the core oligosaccharide moieties of other bacterial LPSs have been isolated that exhibit cross-reactivity between strains and are protective against infection (12). The present study is the first to show in vivo activity of a MAb that is cross-reactive with several *B. pertussis* strains. This MAb is bactericidal against both virulent and nonvirulent *B. pertussis* strains which express the LOS A phenotype. Although the in vivo mode of action of MAb G10F8C3 is uncertain, it was observed that this MAb is effective in reducing lung colonization in mice after a respiratory infection with *B. pertussis*. In support of these data, Shahin et al. (11) have recently demonstrated protective activity of anti-*B. pertussis* LOS MAb that reduce leukocytosis and death in an infant mouse model of pertussis. The high degree of conservation of the oligosaccharide moiety recognized by G10F8C3 in several *B. pertussis* strains (with the exception of the atypical BP134 strain) demonstrates the potential of employing this antigenic determinant as a component in new pertussis vaccines to elicit a protective response against *B. pertussis* infections.

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REFERENCES

1. **Ackers, J. P., and J. M. Dolby.** 1972. The antigen of *Bordetella pertussis* that induces bactericidal antibody and its relationship to protection of mice. J. Gen. Microbiol. **70:**371–382.
2. **Archambault, D., P. Rondeau, D. Martin, and B. R. Broudeur.** 1991. Characterization and comparative bactericidal activity of monoclonal antibodies to *Bordetella pertussis* lipo-oligosaccharide. J. Gen. Microbiol. **137:**905–911.
3. **Caroff, M., R. Chaby, D. Karibian, J. Perry, C. Deprun, and L. Szabo.** 1990. Variation in the carbohydrate regions of *Bordetella pertussis* lipopolysaccharide: electrophoretic, serological, and structural features. J. Bacteriol. **172:**1121–1128.
4. **Gotto, J., T. Eckhardt, J. Scott, and D. Woods.** 1992. Protective effect of *Bordetella pertussis* lipopolysaccharide in the rat intratracheal challenge model of pertussis, abstr. E-15, p. 146. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992. American Society for Microbiology, Washington, D.C.
5. **Kimura, A., K. T. Mountzouros, D. A. Relmam, S. Falkow, and J. L. Cowell.** 1990. *Bordetella pertussis* filamentous hemagglutinin: evaluation as a protective antigen and colonization factor in a mouse respiratory infection model. Infect. Immun. **58:**7–16.
6. **LeDur, A., R. Chaby, and L. Szabo.** 1980. Isolation of two protein-free and chemically different lipopolysaccharides from *Bordetella pertussis* phenol-extracted endotoxin. J. Bacteriol. **143:**78–88.
7. **Li, Z. M., J. L. Cowell, M. J. Brennan, D. L. Burns, and C. R. Manclark.** 1988. Agglutinating monoclonal antibodies that specifically recognize lipooligosaccharide A of *Bordetella pertussis*. Infect. Immun. **56:**699–702.
8. **Moreau, M., R. Chaby, and L. Szabo.** 1984. Structure of the reducing heptasaccharide of polysaccharide 1 isolated from the *Bordetella pertussis* endotoxin. J. Bacteriology **159:**611–617.
9. **Peppler, M. S.** 1984. Two physically and serologically distinct polysaccharide profiles in strains of *Bordetella pertussis* and their phenotype variants. Infect. Immun. **43:**224–232.
10. **Sato, H., K. Izumiya, H. Sato, J. L. Cowell, and C. R. Manclark.** 1980. Aerosol infection of mice with *Bordetella pertussis*. Infect. Immun. **29:**261–266.
11. **Shahin, R. D., J. Hamel, B. Brodeur, and M. F. Leef.** 1992. Analysis of protective and nonprotective monoclonal antibodies specific for *Bordetella pertussis* lipooligosaccharide, abstr. D-153, p. 121. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992. American Society for Microbiology, Washington, D.C.
12. **Terashima, M., I. Uezumi, T. Tomio, M. Kato, K. Irie, T. Okuda, S. Yokota, and H. Noguchi.** 1991. A protective human monoclonal antibody directed to the outer core region of *Pseudomonas aeruginosa* lipopolysaccharide. Infect. Immun. **59:** 1–6.
13. **Wantanabe, M., H. Takimato, Y. Kumazawa, and K. Amano.** 1990. Biological properties of lipopolysaccharides from *Bordetella* species. J. Gen. Microbiol. **136:**489–493.