NOTES

Agglutinating Monoclonal Antibodies That Specifically Recognize Lipooligosaccharide A of *Bordetella pertussis*

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Monoclonal antibodies that specifically agglutinate strains of *Bordetella pertussis* having serotype 1 agglutinogen were uniquely reactive with the electrophoretically slow-migrating A form of lipooligosaccharide. These monoclonal antibodies should be useful for the structural analysis of *B. pertussis* lipooligosaccharide and for the establishment of a better-defined serogroup for *Bordetella* species.

Two chemically and immunologically distinct lipopolysaccharides (LPSs) are produced by *Bordetella pertussis* (7, 10). They exhibit endotoxic activities similar to those of the LPSs of other gram-negative bacteria (6) and are structurally similar in that they contain lipid A and have an oligosaccharide core containing 2-keto-3-deoxyoctonate (9). They do not, however, contain the long-chain polysaccharide (O antigen) characteristic of the LPS of enteric organisms (5) and have been designated lipooligosaccharides (LOSs) as an alternative to the nomenclature of R-type LPSs (11, 15). By sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the LOSs of *B. pertussis* appear as a slow-migrating A band and a fast-migrating B band (10), similar to those of *Neisseria meningitidis* (13). Here we describe two monoclonal antibodies that recognize LOS A of *B. pertussis* and agglutinate only those strains of *B. pertussis* which express the A form of the LOS.

Hybridomas were obtained by standard procedures (4) after immunizing BALB/c mice with partially purified preparations of fimbriae from *B. pertussis* 114 or 325 (2, 16). After fusion with the plasmacytoma cell line SP 2/0, the hybridoma cell culture supernatants were screened for antibodies which agglutinated *B. pertussis* strains having specific agglutinogen serotypes. In addition to obtaining monoclonal antibodies specific for agglutinogen 2 or 6 (Li et al., unpublished data), two hybridomas, G10F8C3 and E8D8B1, produced monoclonal antibodies which agglutinated *B. pertussis* strains of various serotypes (Eldering agglutinogens 1 to 6) (Table 1). Both monoclonal antibodies were of the immunoglobulin G3 subclass and were partially purified from ascites by repeated precipitation at 4°C after dialysis against 10 mM Tris buffer (pH 8) (1). The ability of the *B. pertussis* strains to express LOS A correlated with their ability to be agglutinated by the monoclonal antibodies (Table 1). Four strains of *Bordetella bronchiseptica* and three strains of *Bordetella parapertussis* were not agglutinated, nor were other gram-negative bacteria, such as *Escherichia coli, N. meningitidis, Neisseria gonorrhoeae, Salmonella typhimurium, and Haemophilus influenzae*. The nature of the agglutinogen was defined by the following series of experiments, and though most of the data shown were obtained with monoclonal antibody G10F8C3, identical immunoblot results were obtained with monoclonal antibody E8D8B1. Cell lysates of *B. pertussis* were separated by SDS-PAGE (15% polyacrylamide), transferred to nitrocellulose, and analyzed by the Western blot (immunoblot) technique (12). Monoclonal antibody G10F8C3 detected a single diffuse band migrating near the gel front on those lanes containing lysates of agglutination-positive *B. pertussis* strains (Fig. 1a). Initial studies suggested that the antigen was heat and protease resistant. To determine if the antigen was an LOS, *B. pertussis* cells were incubated with pronase (final concentration, 1 mg/ml) at 37°C for 1 h and separated by SDS-PAGE, and the gel was silver stained for LOS by the method of Tsai and Frasch (14) (Fig. 1b). Two silver-stained bands (bands A and B) were detected in *B. pertussis* strains that were agglutinated by the monoclonal antibodies, and the slower-migrating LOS A band comigrated with the immunoreactive band (Fig. 1a). *B. pertussis* 134, 10901, and 11615, which were not agglutinated by the monoclonal antibodies, did not express immunoreactive or silver-stained LOS A (Fig. 1). To verify the antigenic specificities of the monoclonal antibodies, LOSs purified from strains Tohama I and 134 (gifts from M. S. Peppler, University of Alberta, Edmonton, Alberta, Canada) were analyzed by SDS-PAGE and Western blotting. Silver staining confirmed that the purified LOS of Tohama I was of the AB type, while strain 134 contained only LOS B (Fig. 2a). On Western blots, the monoclonal antibody reacted only with the A band of Tohama I LOS (Fig. 2b). The monoclonal antibodies apparently recognized an oligosaccharide component of LOS, since reactivity was lost after the treatment of whole cells or blotted antigen with 100 mM periodate for 30 min at room temperature (data not shown).

Strains of *B. parapertussis* and *B. bronchiseptica* which were not agglutinated by the monoclonal antibodies nor by the U.S. Reference Agglutinogen Factors 1 through 6 Antisera were also analyzed by SDS-PAGE (Fig. 3a) and immunoblotting with G10F8C3 (Fig. 3b). After SDS-PAGE and silver staining, some of the strains of *B. parapertussis* and *B. bronchiseptica* appeared to have high-molecular-weight
As described (8) with monoclonal antibodies to B. pertussis LOS A would cross-react with the LOSs of other bacterial genera. Strains of N. gonorrhoeae, N. meningitidis, H. influenzae, S. typhimurium, and E. coli were pronase-digested and separated by SDS-PAGE. The LPS profiles of the different gram-negative bacteria were heterogeneous, as indicated by silver staining (Fig. 4a). None of the rough or smooth types of LPS molecules reacted with the monoclonal antibody to B. pertussis LOS A after Western blot analysis of a duplicate gel (Fig. 4b).

Agglutinogen 1 was common to all B. pertussis strains agglutinated by the monoclonal antibodies to LOS A (Table 1). The U.S. Reference Agglutinin Factor 1 Antiserum (Eldering agglutinin 1 polyclonal antiserum) reacted with LOSs of B. bronchiseptica, B. parapertussis, and E. coli. N. meningitidis, N. gonorrhoeae, S. typhimurium, and H. influenzae did not agglutinate with B. pertussis LOSs.

### Table 1. Agglutination of bacterial strains by monoclonal antibodies reactive with B. pertussis LOS A

<table>
<thead>
<tr>
<th>Organism and strain</th>
<th>Agglutination serotype</th>
<th>LOS phenotype</th>
<th>Agglutination titer</th>
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<tbody>
<tr>
<td>B. pertussis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>460</td>
<td>1.2.3.4.6.</td>
<td>A B</td>
<td>256</td>
</tr>
<tr>
<td>Tohama I</td>
<td>1.2.3.4.</td>
<td>A B</td>
<td>2048</td>
</tr>
<tr>
<td>Tohama 325</td>
<td>1.2.3.4.</td>
<td>A B</td>
<td>64</td>
</tr>
<tr>
<td>150</td>
<td>1.2.3.4.</td>
<td>A B</td>
<td>128</td>
</tr>
<tr>
<td>BP 338</td>
<td>1.2.3.4.</td>
<td>A B</td>
<td>8</td>
</tr>
<tr>
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<td>1.3.6</td>
<td>A B</td>
<td>128</td>
</tr>
<tr>
<td>432</td>
<td>1.3.6</td>
<td>A B</td>
<td>256</td>
</tr>
<tr>
<td>BP 353</td>
<td>1.3.6</td>
<td>A B</td>
<td>128</td>
</tr>
<tr>
<td>BP 354</td>
<td>1.3.6</td>
<td>A B</td>
<td>512</td>
</tr>
<tr>
<td>Tohama III</td>
<td>1.3.6</td>
<td>A B</td>
<td>64</td>
</tr>
<tr>
<td>BP 326</td>
<td>1.3.6</td>
<td>A B</td>
<td>256</td>
</tr>
<tr>
<td>134</td>
<td>1.2.3.4.6.</td>
<td>B</td>
<td>0.2% Formalin</td>
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<tr>
<td>10901</td>
<td>Nontypeable</td>
<td>B</td>
<td>1.024</td>
</tr>
<tr>
<td>11615</td>
<td>Nontypeable</td>
<td>B</td>
<td>1.024</td>
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<td>B. bronchiseptica</td>
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<td></td>
</tr>
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<td>058</td>
<td>A B</td>
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<tr>
<td>106</td>
<td>A B</td>
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</tr>
<tr>
<td>207</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>299</td>
<td>A B</td>
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<td></td>
</tr>
<tr>
<td>B. parapertussus</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>480</td>
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</tr>
<tr>
<td>482</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>497</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
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<td></td>
</tr>
<tr>
<td>N. meningitidis</td>
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<td>N. gonorrhoeae</td>
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<tr>
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<td>H. influenzae</td>
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</table>

* Bordetella strains were grown on Bordet Gengou blood agar medium, and other gram-negative strains were cultured by routine procedures. Cells were harvested, washed, and treated with 0.2% Formalin.

- Agglutination was performed as previously described (8) with Eldering agglutinogen polyclonal antiserum (3).
- LOS profile was determined by silver staining, and designations were based on the nomenclature of Peppler (10). The A and B designations for strains of B. bronchiseptica and B. parapertussis which may have a number of silver-stained bands denote the presence of silver-stained bands corresponding to the A and B forms of B. pertussis LOS.
- Agglutination assays were performed with monoclonal antibody preparations (640 µg of protein per ml) partially purified as described in the text. A titer is reported as the inverse of the maximum dilution of antibody which agglutinated the bacteria.
- Not agglutinated by monoclonal antibody.
- Agglutinated by typing sera.

forms of LPSs. B. parapertussis 482 and 497 formed a typical ladder pattern, more like that of the LPSs of enteric bacteria that contain O antigen (Fig. 3a; 5). The B. parapertussis strains did not have silver-stained bands corresponding to LOS A of B. pertussis, but a band comigrating with LOS B was present. The LOSs of these B. parapertussis strains did not react after immunoblotting with monoclonal antibody G10F8C3 (Fig. 3b). Three of the four strains of B. bronchiseptica had broad silver-stained areas corresponding to LOSs A and B of B. pertussis, and the LOS A bands of B. bronchiseptica reacted with the monoclonal antibody to B. pertussis LOS A (Fig. 3b). Indirect immunofluorescence assays indicated that the monoclonal antibodies bound to the surface of LOS A bands of B. bronchiseptica (data not shown). These results suggest that LOS A can be a surface antigen on B. bronchiseptica but that it may not be available in sufficient density to serve as an agglutinogen.

**FIG. 1.** Agglutination of bacterial strains by monoclonal antibodies reactive with B. pertussis LOS A. (a) Immunoblot with 20 µg of monoclonal antibody G10F8C3 per ml; (b) duplicate gel stained with silver for LOS by the method of Tsai and Frasch (14). The B. pertussis strains used were as follows: 460 (lane 1), Tohama I (lane 2), 150 (lane 3), BP 338 (lane 4), 114 (lane 5), 432 (lane 6), BP 353 (lane 7), BP 354 (lane 8), Tohama III (lane 9), BP 326 (lane 10), Tohama 325 (lane 11), 134 (lane 12), 10901 (lane 13), and 11615 (lane 14). The positions of LOS bands A and B are shown.

Since other gram-negative bacteria also express an LOS phenotype (5, 13, 15), it was of interest to determine whether the monoclonal antibody to B. pertussis LOS A would cross-react with the LOSs of other bacterial genera. Strains of N. gonorrhoeae, N. meningitidis, H. influenzae, S. typhimurium, and E. coli were pronase-digested and separated by SDS-PAGE. The LPS profiles of the different gram-negative bacteria were heterogeneous, as indicated by silver staining (Fig. 4a). None of the rough or smooth types of LPS molecules reacted with the monoclonal antibody to B. pertussis LOS A after Western blot analysis of a duplicate gel (Fig. 4b).

**FIG. 2.** B. pertussis lysates and purified LOSs. (a) Pronase-treated cell lysates or purified LOS separated by SDS-PAGE and stained with silver as described in the legend to Fig. 1; (b) immunoblot of a duplicate gel detected with monoclonal antibody G10F8C3. Lanes: 1, Tohama I cell lysate; 2, Tohama I LOS (5 µg); 3, 134 LOS (5 µg); 4, 134 cell lysate. The positions of B. pertussis LOS bands A and B are shown.
FIG. 3. Comparison of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* by SDS-PAGE and immunoblot. (a) Pronase-treated whole-cell lysates separated by SDS-PAGE and stained with silver as described in the legend to Fig. 1; (b) Western blot of a duplicate gel with monoclonal antibody G10F8C3. Lanes: 1, *B. pertussis* Tohama I; 2, *B. parapertussis* 480; 3, *B. parapertussis* 482; 4, *B. pertussis* 497; 5, *B. bronchiseptica* 056; 6, *B. bronchiseptica* 106; 7, *B. bronchiseptica* 207; 8, *B. bronchiseptica* 209. The positions of LOS bands A and B are shown.

FIG. 4. SDS-PAGE (a) and immunoblot (b) of gram-negative organisms. Pronase-treated whole-cell lysates were separated by SDS-PAGE and silver stained, or transfers were reacted with monoclonal antibody G10F8C3 as described in the legend to Fig. 1. Lanes: 1, *B. pertussis* Tohama I; 2, *N. gonorrhoeae*; 3, *N. meningitidis*; 4, *H. influenzae*; 5, *S. typhimurium*; 6, *E. coli*. The positions of *B. pertussis* LOS bands A and B are shown.

FIG. 5. Comparison of the *Bordetella* antigens recognized by U.S. Reference Factor 1 Antiserum (a) and monoclonal antibody (b). Duplicate nitrocellulose transfers containing whole-cell lysates were analyzed by Western blotting after incubation with factor 1 polyclonal antiserum (diluted 1:300) or with monoclonal antibody G10F8C3. Lanes: 1, *B. pertussis* Tohama I; 2, *B. pertussis* 460; 3, *B. pertussis* 114; 4, *B. pertussis* BP 353; 5, *B. pertussis* Tohama III; 6, *B. pertussis* 134; 7, *B. parapertussis* 480; 8, *B. parapertussis* 482; 9, *B. bronchiseptica* 207; 10, *B. bronchiseptica* 058. The position of LOS band A is shown.

strongly with LOS A on immunoblots of *Bordetella* cell lysates (Fig. 5a), as did monoclonal antibody G10F8C3 (Fig. 5b). These results indicate that this polyclonal antiserum contained antibodies reactive with LOS A and suggest that LOS A might correspond to agglutinogen factor 1. However, LOS A and agglutinogen factor 1 are apparently not identical, since agglutinogen factor 1 antiserum agglutinated *B. pertussis* 134, which did not express LOS A (Fig. 2) and was not agglutinated by the monoclonal antibodies (Table 1). Also, LOS purified from Tohama I (10) inhibited the agglutination of this strain by the monoclonal antibodies but failed to block the agglutination of Tohama I strain by the polyclonal agglutinogen factor 1 antiserum (data not shown). Although LOS A cannot be equated with agglutinogen 1, it is clear that this LOS is an agglutinogen found on many pathogenic strains of *B. pertussis*.

In summary, the results presented here suggest that the agglutinating monoclonal antibodies G10F8C3 and E8D8B1 recognize an oligosaccharide epitope on an LOS that is unique for strains of *B. pertussis* and *B. bronchiseptica* having the LOS AB profile. Since it has been suggested that LPS can be used as a phenotypic marker for *B. pertussis* (4), the monoclonal antibodies described here could be useful in establishing a better-defined serotyping system for *B. pertussis* in which LOS A might be considered a typing antigen. The monoclonal antibodies might also be useful for
the estimation of contaminating levels of endotoxin in vaccine preparations and for further studies on the structure of *B. pertussis* LOS.

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**LITERATURE CITED**


