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Production and Characterization of Monoclonal Antibodies Directed against *Bordetella pertussis* Lipopolysaccharide

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Hybrid cell lines producing monoclonal antibodies against *Bordetella pertussis* lipopolysaccharide (LPS) were established. The specificity of the antibodies was ascertained by enzyme-linked immunosorbent assay (ELISA) and ELISA-inhibition experiments with LPS and delipidated polysaccharide fragments (PS-1 and PS-2) prepared from *B. pertussis* LPS. Monoclonal antibody 9-1-H5 reacted with *B. pertussis* LPS only, whereas monoclonal antibodies 6-4-H6 and 9-2-A8 reacted with PS-1 and PS-2 as well as *B. pertussis* LPS. The antibodies did not react with LPS prepared from *B. parapertussis* and *B. bronchiseptica* in an LPS-specific ELISA. A monoclonal antibody-based sandwich ELISA was developed for detection of *B. pertussis* LPS. This assay had a detection limit of *B. pertussis* LPS in concentrations ranging from 0.16 to 0.32 μg/ml. The assay was also shown to be specific for the detection of whole *B. pertussis* bacteria. No cross-reactions were observed with strains of *Branhamella catarrhalis*, *Neisseria meningitidis*, *Streptococcus miteor*, *Haemophilus influenzae*, or *Legionella pneumophila*. The monoclonal antibodies might be useful for the detection of soluble antigens and whole bacteria in clinical samples and for studies of the immunochemical structure of *B. pertussis* LPS.

The disease whooping cough is caused by the bacterium *Bordetella pertussis*. In the early phase of the disease, the bacteria attach to and multiply on the cilia of the epithelial cells of the respiratory tract (31). The bacteria produce a number of substances such as pertussis toxin (31), filamentous hemagglutinin (12), adenylate cyclase (39), heat-labile toxin (31), endotoxin (lipopolysaccharide [LPS]) (31), etc. The pathogenesis of the disease is not fully understood, but it is believed that several of these substances contribute to the manifestations of the disease (36).

The biological properties of *B. pertussis* LPS resemble those observed with LPS from gram-negative enteric bacteria, e.g., pyrogenicity, adjuvancy, local Shwartzman reaction, and histamine hypersensitivity (3). In contrast, the chemical properties of *B. pertussis* LPS differ from those of most enterobacterial LPS studied. LPS preparations obtained by phenol-water extraction contain two different LPS molecules, LPS-1 and LPS-2 (23). Upon mild acid hydrolysis of LPS-1, a polysaccharide (PS-1) containing a nonphosphorylated 3-deoxy-2-octulosonic acid (KDO) is released from lipid A. When LPS-2 is subjected to a somewhat stronger acid hydrolysis, it releases a polysaccharide (PS-2) containing a phosphorylated KDO (24). Although much effort has been put into elucidation of the chemical structure of *B. pertussis* LPS, the complete structure remains obscure (8–11, 23, 24, 27, 28). A branched heptasaccharide representing ca. 50% of PS-1 has been described (28).

The serology of *B. pertussis* LPS is confusing. Although it has been claimed that the LPS serotype should be phase specific (21, 22), others have also reported intraphase differences (1, 2). Furthermore, Peppler reported that *B. pertussis* strains could be classified into two different serotypes designated ab (wild type) and b (variant) by using polyclonal antisera (30). The aim of this study was to produce and characterize monoclonal antibodies directed against *B. pertussis* LPS. These antibodies may be useful for the detection of soluble antigen and whole bacteria in clinical samples, for studies of the immunochemical structure of *B. pertussis* LPS, and for improving the serological characterization of *B. pertussis*.

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MATERIALS AND METHODS

Bacterial strains and cultivation. *B. pertussis* 18530 (phase 1) was obtained from T. Kuronen, Central Public Health Laboratory, Helsinki, Finland. *B. pertussis* 44122/c (phase 4) and the clinical isolates *B. pertussis* 636/83, 23/85, 29/84, 60/84, 210/84, D382/83, and 500/85 (all in phase 1) and *B. parapertussis* ATCC 15237 and the clinical isolates 39/82, 229/82, 670/85, 704/85, 328/83, 89/82, 40/85, and 2/86 were obtained from P. Askelöf, National Bacteriological Laboratory, Stockholm, Sweden. *B. bronchiseptica* B2533/83, B2786/83, 809/86, 755/86, and 754/86 were obtained from E. Olsson, National Veterinary Laboratory, Uppsala, Sweden. *B. pertussis* 44122/c and 18530 were cultivated in Stainer-Scholte medium (35) at 35°C in an aerated steel fermentor (Electrolux AB, Stockholm, Sweden) at a constant pH of 7.2. All other strains were cultivated at 35°C in 100-ml Erlenmeyer flasks in the same medium. *B. pertussis* strains were cultivated for 36 h, whereas *B. parapertussis* and *B. bronchiseptica* strains were cultivated for 24 h.

Preparation of LPS. LPS was extracted from *B. pertussis* 44122/c and 18530, *B. parapertussis* ATCC 15237, and *B. bronchiseptica* B 2533/83 by the hot phenol-water method (37) and purified by high-speed centrifugation as described previously (18). LPS from *B. pertussis* 23/85, 29/84, 60/84, 210/84, D382/83, 636/83, and 500/85, *B. parapertussis* 2/86, and *B. bronchiseptica* B2786/83 was extracted by the rapid-isolation micromethod (20). Briefly, bacteria from 10-ml cultures were suspended in 300 μl of distilled water and mixed with an equal volume of 90% phenol at 65 to 70°C for 15 min. The aqueous phase was adjusted to 0.5 M in NaCl, and LPS was precipitated with 95% ethanol. After >6 h at −20°C, LPS was reprecipitated. The final precipitate was

suspended in 50 μl of distilled water and stored at −20°C until used.

Preparation of PS-1 and PS-2. *B. pertussis* 18530 LPS was hydrolyzed into polysaccharide fragments (PS-1 and PS-2), essentially as described by Le Dur et al. (23). Briefly, 280 mg of LPS was suspended in 120 ml of aqueous trifluoroacetic acid (pH 3.0) and stirred for 120 h at 50°C. The reaction mixture was cooled and centrifuged at 16,000 × *g* for 30 min. The supernatant, containing PS-1, was fractionated by gel filtration on a Bio-Gel P-10 column (2.6 by 95 cm; Bio Rad Laboratories, Richmond, Calif.) connected to a refractometer (R 403; Waters Associates, Inc., Milford, Mass.) with distilled water as eluant. The precipitate was dissolved in 150 ml of 0.25 M hydrochloric acid and incubated for 30 min at 100°C. The reaction mixture was cooled and centrifuged at 160,000 × *g* for 2 h. The supernatant, containing PS-2, was fractionated on a Bio-Gel P-10 column as described above.

Protein content. The protein content of LPS preparations was measured by the method of Lowry et al. (26). Protein concentrations of purified monoclonal antibodies were calculated from A_{280} values with the absorbance constants ($A^{0.1\%}$) for immunoglobulin G (IgG) (1.4) and IgM (1.2), respectively (19).

Immunization. Female BALB/c mice, 6 to 10 weeks of age, were each immunized intraperitoneally with 0.1 mg of *B. pertussis* 18530 LPS once a week for 6 to 7 weeks.

Fusion and cloning. Hybridomas were established as described previously (18). Briefly, B lymphocytes from immunized mice were fused with mouse myeloma cells from the myeloma cell line SP2/0-Ag14 (34) by using polyethylene glycol 4000 (E. Merck AG, Darmstadt, Federal Republic of Germany) as the fusion agent. Primary selection of hybrids was performed by growing the cells in 96-well microtiter plates (no. 3042; Becton Dickinson Labware, Oxnard, Calif.) on HAT medium (25) in a tissue culture incubator at 37°C with 80% humidity and 5% CO_2. Hybrid cells of interest were recloned by limiting dilution.

Production of antibodies. Monoclonal antibodies were produced by one of two methods. (i) The hybridoma cells were grown in 250-ml tissue culture flasks (no. 3075; Costar Data Packaging, Cambridge, Mass.) in RPMI 1640 medium (GIBCO Laboratories, Glasgow, Scotland) supplemented with 10% fetal calf serum, L-glutamine (1 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). (ii) The antibodies were produced in pristane (Sigma Chemical Co., St. Louis, Mo.)-primed mice (32) by injecting 5 × 10^6 cells intraperitoneally into the mice. The antibodies were stored at −20°C until they were used.

Immunoglobulin class and subclass. Immunoglobulin class, subclass, and light chain were determined by the immunodiffusion method of Ouchterlony with 1% agarose (Pharmacia, Uppsala, Sweden) in 10 mM phosphate-buffered saline (pH 7.2) and specific rabbit antiserum to mouse IgM, IgG (7S), IgG1, IgG2a, IgG2b, and IgG3 kappa and lambda chains (Bionetics Laboratory Products, Charleston, S.C.).

ELISA. Antibody production was measured by enzyme-linked immunosorbent assay (ELISA) (13), performed with 96-well microtiter trays (Dynatech M 129 A; Flow Laboratories, Irwine, Scotland) as described previously (18). Briefly, each well was coated with 125 μl of *B. pertussis* 18530 LPS (20 μg/ml) in 10 mM phosphate-buffered saline (pH 7.2) and 0.02% NaN_3 and left overnight at 22°C. Remaining binding sites were blocked with 1% bovine serum albumin in 10 mM phosphate-buffered saline, and 100 μl of cell culture supernatants or ascitic fluid was added to each well. The immune reaction was performed by adding 100 μl of horseradish perodixase-conjugated rabbit anti-mouse immunoglobulin (DAKO, Copenhagen, Denmark). As substrate, 100 μl of 3 mM 1,2-phenylenediaminedihydrochloride (Fluka, Buchs, Switzerland) in a substrate buffer consisting of 25.7 ml of 0.2 M Na_2HPO_4, 24.3 ml of 0.1 M citric acid, and 50 ml of deionized water (pH 5.0) was used. Immediately before use, 40 μl of H_2O_2 was added to the substrate solution. The optical density at 492 nm was measured by using a Titertek Multiscan spectrophotometer (Flow Laboratories). An optical density of >0.2 above background was considered to be a positive result. As a negative control, wells were incubated with RPMI 1640 medium or ascitic fluid from mice injected with cells of the myeloma SP2/0-Ag14.

ELISA inhibition. Inhibition of the monoclonal antibodies (culture supernatant) with LPS and polysaccharide fragments was performed with glass tubes as described previously (18). Briefly, monoclonal antibodies were incubated with the inhibitors, serially diluted in 10 mM phosphate-buffered saline (pH 7.2), for 30 min at 22°C. The remaining antibodies, not neutralized by the inhibitors, were measured by ELISA as described above. The 50% inhibitory value was recorded as the concentration of LPS or polysaccharide fragments needed to obtain a 50% decrease in the optical density as compared with that in control tubes with no inhibitors added.

Purification and biotinylation of antibodies. The monoclonal antibodies 6-4-H6 and 9-2-A8 were purified from ascites fluid on a protein A-Sepharose CL-4B column (Pharmacia, Uppsala, Sweden). Monoclonal antibody 9-1-H5 was purified by ammonium sulfate precipitation (30 to 45% fraction). Purified antibodies were dialyzed overnight at 4°C against 0.2 M borate buffer (pH 8.5) and diluted to a concentration of 1 mg/ml. A 120-μl amount of *N*-hydroxysuccinimidobiotin (Sigma), dissolved in dimethyl sulfoxide to 1 mg/ml, was added to 1 ml of antibody solution. The mixture was incubated for 2 h at 22°C and then dialyzed overnight at 4°C against 10 mM phosphate buffer (pH 7.2)–0.5 M NaCl–1% Tween 20 (38).

Sandwich ELISA. The sandwich ELISA was performed with 96-well microtiter trays (Titertec PVC EIA 173; Flow Laboratories). Wells were coated overnight at 22°C with purified monoclonal antibodies (2 μg/ml) in 50 mM carbonate buffer (pH 9.6). The remaining binding sites were blocked by incubation for 15 min at 22°C with 1% bovine serum albumin in 10 mM phosphate-buffered saline–Tween 20. The wells were rinsed with phosphate-buffered saline–Tween 20. Samples (100 μl each) of LPS serially diluted in phosphate-buffered saline or samples of whole bacteria were added in duplicate to each well. The trays were first incubated for 1 h at 37°C and then overnight at 4°C. After the wells were rinsed with phosphate-buffered saline–Tween 20, biotinylated monoclonal antibodies, diluted in 10 mM phosphate buffer–0.5 M NaCl–1% Tween 20 (pH 7.2), were added to each well and incubated with peroxidase-avidin (DAKO, Copenhagen, Denmark) diluted 1/500 in phosphate buffer-NaCl-Tween 20. Dilutions of biotinylated antibodies were 1/5,000 (6-4-H6), 1/500 (9-1-H5), and 1/2,500 (9-2-A8), respectively. The substrate reaction was performed as described above. An optical density of 0.2 above background was considered a positive result.

RESULTS

Cloning. Three hybridomas producing antibodies directed against *B. pertussis* LPS were established from two different fusion experiments. Hybridoma 6-4-H6 originated from a

TABLE 1. Characteristics of monoclonal antibodies and their titers in ELISA

Monoclonal antibody	Class	Titer[a]	
		In vitro	Ascites
6-4-H6	IgG3	320	64,000
9-1-H5	IgM	640	32,000
9-2-A8	IgG3	640	32,000

[a] Endpoint titers of cell culture supernatants or ascites fluid in ELISA. Endpoints were defined as the highest dilution in a twofold serial dilution still giving an optical density at 492 nm of >0.2 above background. Wells were coated with LPS from *B. pertussis* 18530.

mouse immunized for 6 weeks with *B. pertussis* LPS, whereas hybridomas 9-1-H5 and 9-2-A8 were derived from a mouse immunized with LPS for 7 weeks.

Class and subclass. The results of double-diffusion experiments by the Ouchterlony technique are shown in Table 1. All three hybridomas produced antibodies with kappa light chains.

Antibody production. The hybridomas were cultivated in tissue culture flasks for a minimum of 3 months, producing antibodies in titers ranging from 320 to 640 as determined by ELISA (Table 1). The monoclonal antibodies were also produced in vivo by growing the cells as ascites in mice. The titers of such preparations ranged between 32,000 and 64,000 (Table 1).

Preparation of PS-1 and PS-2. The polysaccharide fragments PS-1 and PS-2 were prepared from *B. pertussis* 18530 LPS. The protein content of the LPS preparation was 1.6%, and the nucleic acid content was estimated to be ca. 2% as measured by A_{260}. The amounts of PS-1 and PS-2 obtained were 15.5 and 12.8 mg, respectively.

ELISA inhibition. ELISA inhibition of monoclonal antibodies (culture supernatant) was performed with LPS and delipidated polysaccharide fragments (PS-1 and PS-2) prepared from *B. pertussis* 18530 as inhibitors. LPS, PS-1, and PS-2 inhibited completely the monoclonal antibodies produced by hybridoma 6-4-H6. The amounts of inhibitor needed to obtain 50% inhibition of the antibodies were 70 μg/ml (LPS), 260 μg/ml (PS-1), and 200 μg/ml (PS-2), respectively (Fig. 1A). Monoclonal antibody 9-1-H5 was inhibited by LPS but not by PS-1 or PS-2, in the concentrations tested. LPS at 45 μg/ml was required to obtain 50% inhibition of this antibody (Fig. 1B). LPS, as well as PS-1 and PS-2, inhibited the antibodies produced by hybridoma 9-2-A8 with 50% inhibitory values of 28 μg/ml (LPS), 60 μg/ml (PS-1), and 50 μg/ml (PS-2) (Fig. 1C).

TABLE 2. Titers of monoclonal antibodies obtained against different LPS preparations in ELISA

Strain	Titer of monoclonal antibody[a]:		
	6-4-H6	9-1-H5	9-2-A8
B. pertussis			
44122/c	128,000	32,000	32,000
23/85	128,000	32,000	32,000
29/84	128,000	32,000	16,000
210/84	64,000	8,000	2,000
D382/83	16,000	32,000	8,000
500/85	1,000	2,000	1,000
636/83	64,000	2,000	32,000
60/84	4,000	2,000	2,000
B. parapertussis			
ATCC 15237	<1,000	<1,000	<1,000
2/86	<1,000	<1,000	<1,000
B. bronchiseptica			
B2533/83	<1,000	<1,000	<1,000
B2786/83	<1,000	<1,000	<1,000

[a] Endpoint titers of ascites fluid. Titers were defined as described in Table 1.

Reactivity of monoclonal antibodies with different LPS preparations. The specificity of the monoclonal antibodies was further studied by ELISA with LPS preparations from different strains of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* as coating antigen. Antibodies (ascites) produced by hybridoma 6-4-H6 reacted with *B. pertussis* LPS with titers ranging from 1,000 to 128,000. Antibodies produced by hybridoma 9-1-H5 reacted with *B. pertussis* LPS with titers ranging from 2,000 to 32,000, whereas hybridoma 9-2-A8 produced antibodies with titers ranging from 1,000 to 32,000. No reactivity was observed with any of the monoclonal antibodies to LPS from strains of *B. parapertussis* or *B. bronchiseptica* (Table 2). Furthermore, the antibodies were negative in ELISA when tested against LPS prepared from strains of *Haemophilus influenzae* type b (10 different strains), *Neisseria meningitidis* group Y, *Pseudomonas aeruginosa* O6, *Yersinia enterocolitica* O9, *Escherichia coli* O55:B5, and *Vibrio cholerae* Ogawa and Inaba.

Sandwich ELISA. A sandwich ELISA for the detection of

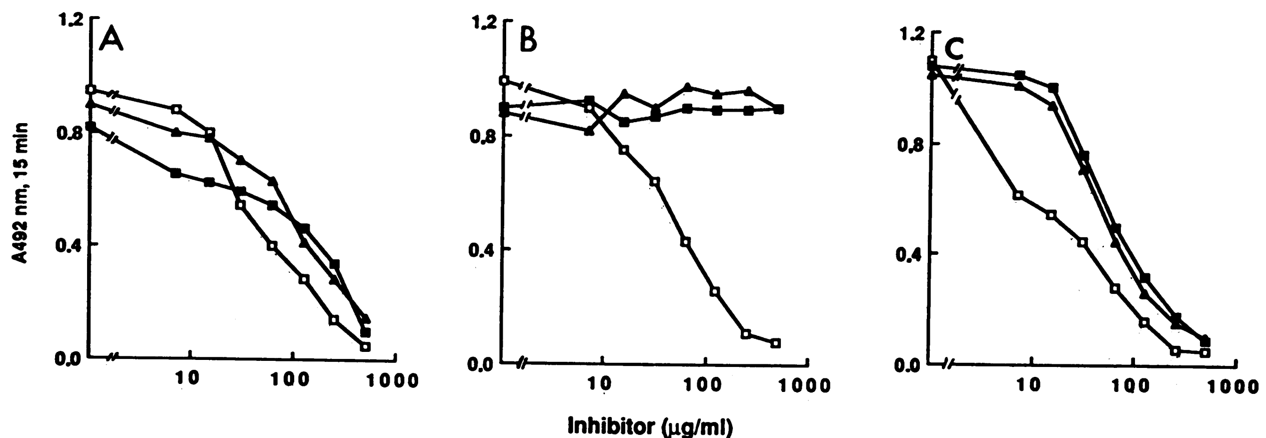

FIG. 1. ELISA inhibition of monoclonal antibodies 6-4-H6 (A), 9-1-H5 (B), and 9-2-A8 (C) with LPS (□), PS-1 (■), and PS-2 (▲) from *B. pertussis* 18530. The microtiter tray was coated with LPS from *B. pertussis* 18530.

TABLE 3. Amount of *B. pertussis* LPS detected in the sandwich ELISA

Strain	Antigen (μg/ml) detected[a] with					
	Biotinylated 9-1-H5 and following capture MAb[b]:			Biotinylated 9-2-A8 and following capture MAb:		
	6-4-H6	9-1-H5	9-2-A8	6-4-H6	9-1-H5	9-2-A8
B. pertussis						
44122/c	0.63	1.25	0.63	0.32	1.25	0.63
18530	0.16	1.25	0.16	0.16	0.63	0.16
B. parapertussis						
ATCC 15237	>20	>20	>20	>20	>20	>20
2/86	>20	>20	>20	>20	>20	>20
B. bronchiseptica						
B 2533/83	>20	>20	>20	>20	>20	>20
B 2786/83	>20	>20	>20	>20	>20	>20

[a] The minimum amount of antigen needed was defined as the highest dilution in a twofold serial dilution still giving an optical density at 492 nm of >0.2 above background.
[b] MAb, Monoclonal antibody.

B. pertussis LPS was established. LPS from *B. pertussis* 44122/c and 18530, *B. parapertussis* ATCC 15237 and 2/86, and *B. bronchiseptica* B 2533/83 and B 2786/83 were used as antigens. LPS, in concentrations ranging from 20 ng/ml to 20 μg/ml, was added to microtiter trays previously coated with LPS-specific monoclonal antibodies.

Monoclonal antibodies 6-4-H6 and 9-2-A8 were equally effective as capture antibodies and slightly better than monoclonal antibody 9-1-H5 when used with biotinylated antibody 9-1-H5. The sensitivity of the assay increased when biotinylated antibody 9-2-A8 was used as the second antibody. The highest sensitivity was obtained with antibody 6-4-H6 as the capture antibody with biotinylated antibody 9-2-A8. This combination detected *B. pertussis* LPS at 0.16 and 0.32 μg/ml in two different preparations, respectively. LPS from *B. parapertussis* and *B. bronchiseptica* was not detected at the concentrations tested (Table 3). High background values was obtained with biotinylated antibody 6-4-H6 as second antibody.

The influence of a shorter incubation period with antigen was studied in an experiment with biotinylated monoclonal antibody 9-1-H5 as the second antibody. Incubation for 1 h at 37°C was compared with incubation for 1 h at 37°C followed by overnight incubation at 4°C. The amount of antigen required for a positive ELISA reaction increased with the shorter incubation period.

Triethylamine is known to increase the solubility of LPS (16). However, no increase in sensitivity was observed when LPS dissolved in 36 mM triethylamine was used compared with LPS dissolved in phosphate-buffered saline. Furthermore, the sensitivity of the sandwich ELISA was not increased by the use of a either pool of monoclonal antibodies (defined polyclonal serum) as the capture antibody or a pool of biotinylated antibodies as the second antibody.

The sandwich ELISA was also evaluated for the detection of whole bacteria, cultivated overnight in Stainer-Scholte medium (35). Monoclonal antibodies 6-4-H6, 9-1-H5, and 9-2-A8 were used as capture antibodies, with biotinylated antibody 9-2-A8 as the second antibody. The incubation period with antigen was 1 h at 37°C. All *B. pertussis* strains tested were positive in the sandwich ELISA, whereas all *B. parapertussis* and *B. bronchiseptica* strains were negative when antibodies 6-4-H6 and 9-2-A8 were used as capture antibodies (Fig. 2A and C). When antibody 9-1-H5 was used as the capture antibody, all *B. pertussis* strains were positive, whereas all but one of the *B. parapertussis* and all the *B. bronchiseptica* strains were negative (Fig. 2B). Strains of *Branhamella catarrhalis*, *N. meningitidis* groups B and Y, *Streptococcus miteor*, *H. influenzae* types a and b, and *Legionella pneumophila* were all negative in the sandwich ELISA. The number of bacteria tested was 8×10^6 to 2.5×10^9 CFU/ml.

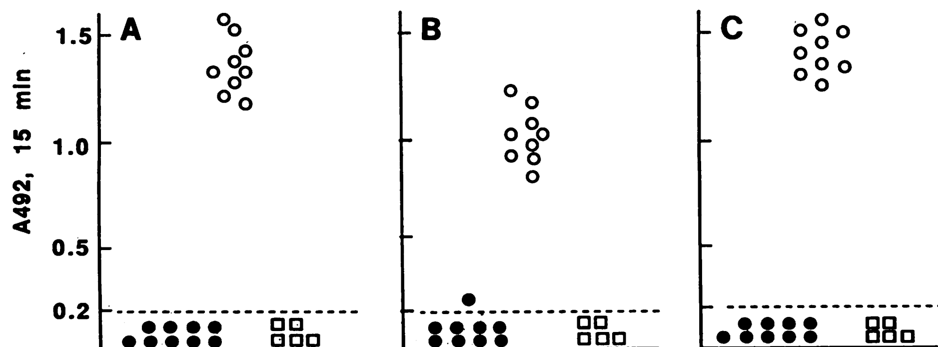

FIG. 2. Sandwich ELISA absorbance values of *B. pertussis* (○), *B. parapertussis* (●), and *B. bronchiseptica* (□) cultures. The assays were performed in microtiter wells coated with monoclonal antibodies 6-4-H6 (A), 9-1-H5 (B), and 9-2-A8 (C).

DISCUSSION

The serological classification of *B. pertussis* LPS is unclear. Aprile and Wardlaw (2) reported on six antigenic determinants designated A to F, using polyclonal antisera obtained by immunizing rabbits with heat-killed bacteria. Determinants A, C, E, and F were found in LPS of some phase I strains, whereas determinant D was found in one phase I strain and in one phase IV strain. All strains in their study shared determinant B, whereas determinant F was shared with LPS from *Brucella melitensis*. Peppler (30) reported that *B. pertussis* strains could be grouped into one of two distinct profiles by Western immunoblots of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of *B. pertussis* LPS. Wild-type strains were of the ab type, whereas variant strains were of the b type. As far as we are aware, no one has studied how the a and b bands found by Peppler relate to LPS-1 and LPS-2 (24).

The presence of an O side chain consisting of repeating units has not been shown for *B. pertussis* LPS. However, the blurred bands obtained after sodium dodecyl sulfate-polyacrylamide gel electrophoresis of *B. pertussis* LPS (30) resemble those obtained after sodium dodecyl sulfate-polyacrylamide gel electrophoresis of LPS from *Y. enterocolitica* O9, *Brucella abortus* (5), and *H. influenzae* (20), all being members of the *Brucellaceae* family. Both *Y. enterocolitica* O9 LPS (6) and *Brucella abortus* LPS (7) have O chains with a monosaccharide repeating unit. Similar blurred bands were obtained with *V. cholerae* O1 LPS (17), which also has a monosaccharide repeating unit. However, it might be shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of *V. cholerae* O1 LPS that these blurred bands consist of a major band containing several finely resolved bands (17). This pattern is characteristic of O chains with a monosaccharide repeating unit and is not as distinct as the stepladderlike banding pattern obtained with LPS containing oligomeric repeating units, e.g., *Salmonella typhimurium* (5, 17). It is thus quite possible that the O side chain of *B. pertussis* LPS consists of a monosaccharide repeating unit.

Monoclonal antibodies against *B. pertussis* LPS have been reported previously; however, these antibodies cross-reacted with LPS from *B. parapertussis* and *B. bronchiseptica* in ELISA (15). The monoclonal antibodies presented in this paper are specific for *B. pertussis* LPS and cause no cross-reactions with other *Bordetella* species tested. The observation that both polysaccharide fragments of *B. pertussis* LPS inhibited monoclonal antibodies 6-4-H6 and 9-2-A8 indicates that the antigen-binding epitope(s) is present in both fragments. Whether PS-2 is composed of PS-1 and an additional oligosaccharide or whether the polysaccharide fragments are essentially different carbohydrate structures with only minor regions in common must be studied further. Monoclonal antibody 9-1-H5 reacted with *B. pertussis* LPS but not with the polysaccharide fragments. This indicates that the epitope on LPS responsible for binding this antibody either is acid labile and is thus degraded during acid hydrolysis of LPS or is situated outside the PS-1 and/or PS-2 region. These monoclonal antibodies will obviously be of value in further studies of the chemical structure and immunological properties of LPS from *B. pertussis*.

Proper identification of the bacterium in suspected cases usually requires that clinical samples collected by nasopharyngeal swabs or washings be cultivated for 3 to 7 days (29). *B. pertussis* is a highly fastidious bacterium, and the successful isolation of this organism is dependent on several factors, such as a correct collection of the sample from the respiratory tract followed by transport of specimens in freshly made transport medium (33) or direct plating of the sample on freshly prepared Bordet-Gengou agar plates. A sensitive and specific method for the rapid detection of a current *B. pertussis* infection is highly desirable. Monoclonal antibodies specific for *B. pertussis* antigens may be used for direct detection of antigen in clinical samples. Such assays should be able to detect soluble antigens released from the bacteria as well as to detect living or dead bacteria. The sandwich ELISA presented in this study detected soluble *B. pertussis* LPS as well as whole bacteria. Whether the sensitivity of this assay will compete with that of other assays based on the detection of *B. pertussis* filamentous hemagglutinin and pertussis toxin in clinical samples is presently being studied.

Direct examination of smears by the fluorescent-antibody technique is sometimes successful for the detection of *B. pertussis*, although as much as 50% false-negative results may occur with polyclonal antisera (4, 14). Also, false-positive reactions are obtained owing to poor specificity of such sera (4). Use of highly specific monoclonal antibodies lacking batch-to-batch variations may circumvent these problems.

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