

## Monoclonal Antibodies Specific for *Escherichia coli* J5 Lipopolysaccharide: Cross-Reaction with Other Gram-Negative Bacterial Species

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Received 20 April 1984/Accepted 21 June 1984

Four monoclonal antibodies against *Escherichia coli* J5 were studied. Each of these monoclonal antibodies reacted with purified lipopolysaccharides from *E. coli* J5, the deep rough mutant *Salmonella minnesota* Re595, *Agrobacterium tumefaciens*, and *Pseudomonas aeruginosa* PAO1 as well as with the purified lipid A of *P. aeruginosa*. Enzyme-linked immunosorbent assays using the outer membranes from a variety of gram-negative bacteria demonstrated that these lipid A-specific monoclonal antibodies interacted with between 84 and 97% of the gram-negative bacterial species tested. One of the monoclonal antibodies, 5E4, was shown to interact with 34 of the 35 outer membrane or lipopolysaccharide antigens tested. Immunoenzymatic staining of Western electrophoretic blots of separated *P. aeruginosa* outer membrane components was used to demonstrate that antibody 5E4 interacted with a similar fast-migrating band, corresponding to rough lipopolysaccharide, from all 17 serotype strains and all 14 clinical isolates of *P. aeruginosa*. Similarly, iodinated goat anti-mouse immunoglobulin was used to detect the binding of monoclonal antibody 8A1 to a fast-migrating band on Western electrophoretic blots of purified lipopolysaccharides from *Klebsiella pneumoniae* and both smooth and rough strains of *E. coli*, *Salmonella typhimurium*, and *S. minnesota*. These results suggest considerable conservation of single antigenic sites in the lipid A of gram-negative bacteria.

The surface (outer) monolayer of the outer membrane of gram-negative bacterial cells contains, as its major lipidic component, lipopolysaccharide (LPS). LPS molecules from different bacterial species have the same general chemical composition, comprising three major parts covalently bound to one another (17, 20). The lipid A part, consisting of five or six fatty acids attached to diglucosamine phosphate, inserts into the outer monolayer of the outer membrane (20, 23, 27). Covalently attached to this is the rough core region of LPS, usually consisting of 11 to 14 saccharides including unique octoses and heptoses and a variety of hexoses substituted with phosphate and ethanolamine. The rough core is often, but not always, capped with a repeating tri- to pentasaccharide structure of variable length which bears the name O antigen and can constitute the major antigenic structure of gram-negative cells. Although substantial variation in the O antigen composition within a single bacterial species (e.g., *Escherichia coli* has over 100 different possible O antigens) and somewhat less variation in rough core composition (7, 9) has been observed, the lipid A composition may be even less variant (20). For example, many bacteria have a lipid A consisting of diglucosamine phosphate substituted with 3 to 4 hydroxy fatty acids (which are relatively rare in nature [17, 20]), two saturated fatty acids and phosphate. The lipid A is usually covalently attached to the octose 2-keto-3-deoxyoctonate (KDO) which forms the proximal part of the rough core. The chemical similarity of the lipid A's of different bacterial species is underlined by their similar actions on host tissues (1). Hence, lipid A's bear the general name endotoxins. In addition, most or all lipid A's activate a clotting enzyme in the standard lipid A assay, the *Limulus* amoebocyte assay (1, 24). However, the chemical similarity of the lipid A's of a wide range of different bacteria has not as yet been formally proven. Interestingly, active vaccination

with LPS or whole cells of the rough *E. coli* strain J-5 and passive vaccination with antisera to strain J-5 LPS protects animals (2, 19) and possibly also humans (2) against bacteremia from a variety of gram-negative bacterial species. In this paper, we present data which favor the hypothesis that this is due in part to antigenic similarities between the lipid A regions of the LPSs of *E. coli* and other species of bacteria. To demonstrate this, Bogard et al. (W. C. Bogard, Jr., D. L. Dunn, and P. C. Kung, submitted for publication) have generated monoclonal antibodies against *E. coli* J5 whole cells which are reactive with lipid A, and we used these here to demonstrate substantial conservation of single antigenic sites in the LPS of 14 species of bacteria.

### MATERIALS AND METHODS

**Bacterial strains, growth media, and antigen preparation.** All bacterial strains used were from our culture collection and have been described previously (4, 5, 9-11, 16). Outer membranes were isolated by the one-step sucrose density gradient procedure described by Hancock and Carey (8) after growth on either 1% (wt/vol) proteose peptone no. 2 (most strains), 0.8% (wt/vol) tryptone-0.5% (wt/vol) yeast extract-0.5% (wt/vol) NaCl (*Salmonella* and *E. coli* strains), or *Yersinia* broth (*Yersinia* strain [4]). LPSs were isolated by the technique of Darveau and Hancock (5); lipid A of *Pseudomonas aeruginosa* was isolated from the LPS of *P. aeruginosa* H103 as previously described (13). The LPSs from *Agrobacterium tumefaciens* PLT6467, PLT5-1005, and PLT4 were a kind gift from M. Thomashow (Washington State University, Pullman, Wash.).

**Isolation of monoclonal antibodies.** Hybridomas secreting the described monoclonal antibodies were isolated essentially by the methods of Kohler and Milstein (12) at Centocor, Philadelphia, Pa. Whole heat-killed *E. coli* J5 cells were used as an injecting antigen to prime mice before removal of their spleen cells and fusion. Different injection protocols were

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utilized before the isolation of individual hybridomas (Bogard et al. submitted for publication). Monoclonal antibodies were purified from ascites by protein A-Sepharose chromatography (14).

**Antigen analysis techniques.** Monoclonal antibodies were screened by enzyme-linked immunosorbent assay (ELISA) as described previously (10, 14), except that the antigen-coated plates were incubated with the monoclonal antibody overnight at 23°C. In all cases, 20 µg of outer membrane per ml and 5 µg of LPS per ml had been determined in control experiments as the optimal concentrations of coating antigen at which antigen was not limiting. These concentrations were used for all ELISAs. The color developed after the addition of substrate was read after 45 min with an ELISA reader (Titertek Multiscan; Flow Laboratories, McLean, Va.) set at 405 nm. Usually, results were averaged over two or three individual experiments involving duplicate determinations. The following negative controls were usually performed to ensure that the results were due to interaction of the monoclonal antibody with the antigen: (i) omission of antigen, (ii) omission of monoclonal antibody, (iii) omission of second antibody, (iv) inclusion of *Bacillus subtilis* (gram-positive) cell envelope as the first antigen, or (v) use of the *P. aeruginosa* LPS O antigen type 5-specific monoclonal antibody MA1-8 (10) in place of the monoclonal antibodies described here, together with *E. coli* CGSC 6041 outer membranes as the antigen. In each case, ELISA readings of less than 0.1 were obtained. The maximum background reading on each microtiter plate was subtracted from all results on that plate. As a positive control, *P. aeruginosa* H103 outer membranes were used as the antigen with monoclonal antibody MA1-8 as the first antibody (10).

Some of these data were confirmed by ELISA in which the antigen was adsorbed onto nitrocellulose filter paper (STHA 09610, Millipore Corp., Bedford, Mass.) by using a Millititer filtration system (Millipore Corp.). Otherwise, the method of ELISA used closely followed our previously described technique (14). Radioimmunoassays for initial screening of monoclonal antibodies were performed by the technique of Dechtol (6).

**Western electrophoretic blotting procedure and immunological analysis of proteins.** Separation of outer membrane antigens was performed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis with a modification of the method of Tsai and Frasch (26) in which the solubilization buffer contained 40 mM EDTA, pH 6.8, and the running gel contained 14% acrylamide and 0.25% bisacrylamide. The separated outer membrane components were transferred to nitrocellulose by the Western blot method described by Towbin et al. (25). Subsequent immunostaining of outer membrane components closely followed the method of O'Connor and Ashman (18), except that phosphate-buffered saline (137 mM NaCl, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, 20.4 mM Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O, 3.1 mM NaN<sub>3</sub>, 2.68 mM KCl [pH 7.4]) was used as the buffer in all steps and incubation of the monoclonal antibody with the electroblotted outer membrane components was for 18 h at 23°C. Alternatively, iodinated goat anti-mouse immunoglobulin F(ab')<sub>2</sub> was used to reveal the position of monoclonal antibody binding, as previously described (15).

## RESULTS

**Preliminary analysis of the monoclonal antibodies.** Four hybridomas were isolated after separate cell fusions in which one of the two fusion partners was spleen cells isolated from mice injected with heat-killed *E. coli* J5 cells. The purified monoclonal antibodies secreted by these hybridomas were

screened by radioimmunoassay with whole cells and LPSs from the rough mutant *E. coli* J5 (2), the deep rough mutant *Salmonella minnesota* Re595 (27), and other strains and were shown to interact with each of these antigens. Three of these monoclonal antibodies, 5E4, 8A1, and 1D4, were shown by interaction with subclass-specific antisera to be of the immunoglobulin G1 subclass; antibody 6B2 was of the immunoglobulin G2a subclass. Only two of these monoclonal antibodies, 5E4 and 8A1, interacted strongly with commercial, purified lipid A from *S. minnesota* Re595 (catalog no. 437632; Calbiochem-Behring Corp., La Jolla, Calif.), demonstrating a positive interaction with as little as 100 pg of lipid A as the coating antigen. Antibodies 6B2 and 1D4 showed no detectable response with 10 µg of that lipid A.

**Titration of monoclonal antibodies.** When the amount of antigen coating the plates was maintained at 20 µg/ml = 0.4 µg per well for outer membranes or 5 µg/ml = 0.1 µg per well for LPS and when the amount of monoclonal antibody was varied, an increased ELISA optical density reading at 405 nm was obtained as antibody amounts were increased. The data could be reasonably fitted to a linear reciprocal plot of ELISA reading after 45 min (reflecting the amount of antibody-antigen complex) against the reciprocal of the monoclonal antibody concentration (Fig. 1). The results for antibody 5E4 suggested a maximal optical density reading at 405 nm in ELISA of 0.6 to 1.1 for the four samples and  $K_d$  values (antigen-antibody binding constants) from a Steward-Petty plot (22) of 91, 98, 112, and 148 nM for *A. tumefaciens* PLT-51005 LPS, *Azotobacter vinelandii* OP outer membranes, *E. coli* PCO479 outer membranes, and *P. aeruginosa* PAO1 outer membranes, respectively, as antigens.

**Cross-reaction among gram-negative bacteria.** Outer membranes and LPSs from a variety of species and genera of gram-negative bacteria were screened by ELISA for interaction with the four monoclonal antibodies (Table 1). Antibod-

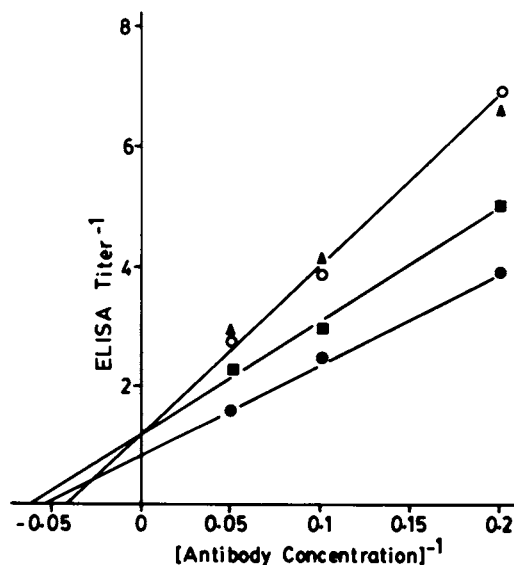


FIG. 1. Steward-Petty (22) plot of ELISA antibody titrations of monoclonal antibody 5E4 against different antigens. The substrate concentration is expressed in micrograms per milliliter, and the ELISA titer is expressed as absorbance at 405 nm recorded 45 min after the addition of paranitrophenol phosphate. Each point is an average of two to four separate determinations. Antigens were *P. aeruginosa* PAO1 outer membranes (●), *A. tumefaciens* PLT5-1005 LPS (▲), *E. coli* K-12 strain CGSC 6044 outer membranes (■), and *A. vinelandii* OP outer membranes (○). All lines were drawn by linear regression with correlation coefficients of 0.99 or greater.

TABLE 1. Cross-reaction of anti-lipid A monoclonal antibodies with the outer membranes, cell walls, LPS, or lipid A of various bacteria

Species	Strain	Sample	ELISA optical density reading after 45 min <sup>a</sup>			
			5E4	8A1	1D4	6B2
<i>Escherichia coli</i>	CGSC 6041	OM <sup>b</sup>	0.2 (+)	0.2 (+)	0.1 (+)	0.2 (+)
	CGSC 6044	OM	0.3 (+)	0.5	(+)	
	PCO479	OM	0.3 (+)	0.5	(+)	
<i>Salmonella typhimurium</i>	SGSC 205	OM	0.3	0.3 (-)	0.5	0 (+)
	SGSC 206	OM	0.1	0	0	0.2
	SGSC 227	OM	0.3	0.1 (+)	0.3	0.2 (+)
<i>Yersinia pestis</i>	EV76	LPS	0.1	0	0.1	0
<i>Edwardsiella tarda</i>	E79054	OM	0.1 (+)	0.2	0.4	0.1
<i>Pseudomonas aeruginosa</i>	PAO1	OM	0.2 (+)	0.4 (+)	0 (+)	0.2 (+)
	AK1160	OM	0.1	0.3	0	0
	K799	OM	0.3 (+)	0.5		
	Z61	OM	0.2	0.3	0	0
	H223	OM	0.3	0.3		
	CFP1M	OM	0.3 (+)	0.5		
	CFP1NM	OM	0.4 (+)	0.6		
	CFC1M	OM	0.3 (+)	0.4		
	CFC1NM	OM	0.2 (+)	0.4		
	CF4349	OM	0.3 (+)	0.5		
	CF221	OM	0.2 (+)	0.5		
	CF1278	OM	0.3 (+)	0.4		
	PAO1	Lipid A	0.4	0.6	0.1	0.3
	PAO1715	LPS	0.3	0.3		
	PAO1716	LPS	0.3	0.3		
	PAO1670	LPS	0.3	0.1	0.1	0.5
<i>Pseudomonas fluorescens</i>	ATCC 13525	OM	0	0	0.2	0
<i>Pseudomonas putida</i>	ATCC 4359	OM	0.2	0.4		
<i>Pseudomonas anguilliseptica</i>	ET7601	OM	0.4	0	0.5	0.1
	ET2	OM	0.3	0.2	0	0.2
<i>Pseudomonas cepacia</i>	ATCC 25416	OM	0	0.1 (-)	0.2	0.2 (+)
<i>Pseudomonas</i> sp.	CF283	OM	0.6 (+)	0.6		
<i>Azotobacter vinelandii</i>	OP	OM	0.4	0.5	0	0.1
<i>Aeromonas salmonicida</i>	NCMB 2020	OM	0.2 (+)	0.1	0.2	0.1
<i>Aeromonas hydrophila</i>	ET2	OM	0.4	0.4		
<i>Vibrio cholerae</i>	PS7910	OM	0.2 (+)	0.5	0.1	0.1
<i>Vibrio anguillarum</i>	ET208	OM	0.3 (+)	0.4	0	0.1
<i>Agrobacterium tumefaciens</i>	PLT 6467	LPS	0.7 (+)	0.8 (+)	0.1	0.1
	PLT 5-1005	LPS	0.2	0.3 (+)	0.1	0
	PLT 4	LPS	0.4	1.0 (+)		
<i>Bacillus subtilis</i>	BGSC1	Cell walls	0	0	0	0
<i>Streptococcus faecalis</i>		Whole cells	0	0	0	0
<i>Staphylococcus aureus</i>		Whole cells	0	0	0	0

<sup>a</sup> All ELISAs employed 20 µg of outer membranes or 5 µg of LPS as antigens and 20 µg of monoclonal antibodies. Results are recorded as optical density at 405 nm (after liberation of phosphate from paranitrophenylphosphate) after 45 min. Data are usually the averages of two or three sets of duplicate determinations with standard deviations ranging from 2 to 30% but usually less than 10%. The + and - in brackets refer to positive or negative interactions, respectively, in Western blot experiments similar to those shown in Fig. 2 and 3.

<sup>b</sup> OM, Outer membranes.

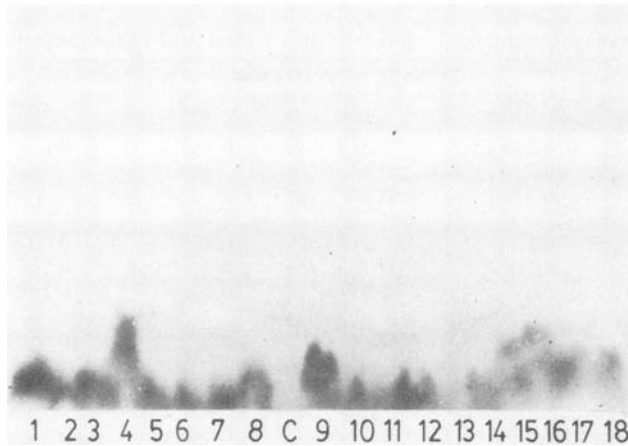


FIG. 2. Western electrophoretic blot of separated outer membranes of strains of the 17 *P. aeruginosa* serotypes after reaction with monoclonal antibody 5E4. The blot was made by electrophoretic transfer of the separated outer membranes from SDS-polyacrylamide gels to nitrocellulose paper. These electrophoretic blots were then interacted with the monoclonal antibody followed by a goat anti-mouse immunoglobulin alkaline phosphatase-conjugated antibody and subsequent addition of substrate (Naphthol As Mx phosphoric acid and fast red Tr salt). The outer membranes by lanes were as follows: 1, serotype 17; 2, serotype 16; 3, serotype 15; 4, serotype 14; 5, serotype 13; 6, serotype 12; 7, serotype 11; 8, serotype 10; 9, serotype 9; 10, serotype 8; 11, serotype 7; 12, serotype 6; 13, serotype 5; 14, serotype 4; 15, serotype 3; 16, serotype 2; 17, serotype 1; 18, *P. aeruginosa* PAO1. C, Control lane containing *P. aeruginosa* outer membrane protein F purified free of LPS. The actual American Type Culture Collection numbers of the serotype strains are given in reference 14.

ies 1D4 and 6B2 showed considerable cross-reaction, demonstrating interaction with 65 to 70% of the antigens tested. However, nearly half of these interactions were quite weak, giving ELISA readings (absorbance at 405 nm) of around 0.1. In contrast to the results with *S. minnesota* lipid A, both 1D4 and 6B2 interacted with *P. aeruginosa* lipid A, although 1D4 interacted quite weakly. This could be due to differences in the methods of isolating lipid A, to the fact that *S. minnesota* lipid A was isolated from a deep rough mutant of *S. minnesota* whereas *P. aeruginosa* lipid A was isolated from a wild-type isolate, or to contamination of *P. aeruginosa* lipid A by other rough core components. We were unable to confirm this last possibility.

Antibodies 5E4 and 8A1 showed extensive cross-reactions with the outer membranes of many gram-negative bacteria; they interacted with all except 1 and 3 antigens, respectively, of the 35 antigens tested. In addition to their strong reactions with *S. minnesota* lipid A noted above, both antibodies reacted strongly with *P. aeruginosa* lipid A. None of the antibodies above interacted with the gram-positive cells (or cell walls) tested, i.e., *B. subtilis*, *Staphylococcus aureus*, and *Streptococcus faecalis*.

**Western electroblotting analysis.** To determine the specific component of outer membranes which was interacting with the monoclonal antibodies and to confirm some of the ELISA results in Table 1, *P. aeruginosa* PAO1 outer membranes separated by SDS-polyacrylamide gel electrophoresis and transferred from the electrophoretogram to nitrocellulose paper by the Western technique (23) were interacted with monoclonal antibodies. Each of the monoclonal antibodies interacted with a single major band (Fig. 2) which had migrated in the SDS-polyacrylamide gel with a relative

mobility of around 0.8 to 0.9 compared with the bromphenol blue dye front. This band was identified as rough LPS (containing rough core and lipid A) since it comigrated with authentic rough LPS from the rough mutant strain H146 and interacted with an LPS rough core-specific (9) monoclonal antibody MA3-5 but not with an LPS O antigen-specific (10) monoclonal antibody MA1-8.

Monoclonal antibody 1D4 showed no interaction by ELISA with *P. aeruginosa* H103 outer membranes (Table 1) but strong interaction with this antigen by Western electroblotting analysis (data not shown). Similar differential interactions were observed for other antigens with this antibody, and we consider that this probably reflects the mode of antigen presentation to the antibody. Consistent with this concept, antibody 8A1 gave strong reactions with many antigens in ELISA but reacted poorly with Western blots.

Extensive cross-reactions of the antibodies 5E4 and 8A1 with the outer membranes of different *P. aeruginosa* strains was observed by ELISA analysis (Table 1). This was confirmed, in part, by Western electroblotting analysis. Antibody 5E4 interacted with a similar fast-migrating band from all 17 serotypes (Fig. 2) and 14 clinical isolates (Fig. 3) of *P. aeruginosa*. Occasionally, the interaction of antibody 5E4 with a series of closely spaced bands of lower relative mobility was also observed. Although this observation was too inconsistent to analyze properly (perhaps owing to the low affinity of the monoclonal antibodies for higher-molecular-weight LPS), these bands may represent smooth, O antigen-containing LPS which has been shown to constitute around 5 to 10% of the LPS molecules in some *P. aeruginosa* strains (9).

Similar reactions were observed when monoclonal antibody 8A1 was interacted with commercial, purified LPS (List Biological Laboratories, Ltd.) which had been separated by SDS-polyacrylamide gel electrophoresis and then transferred to nitrocellulose by the Western blotting method.

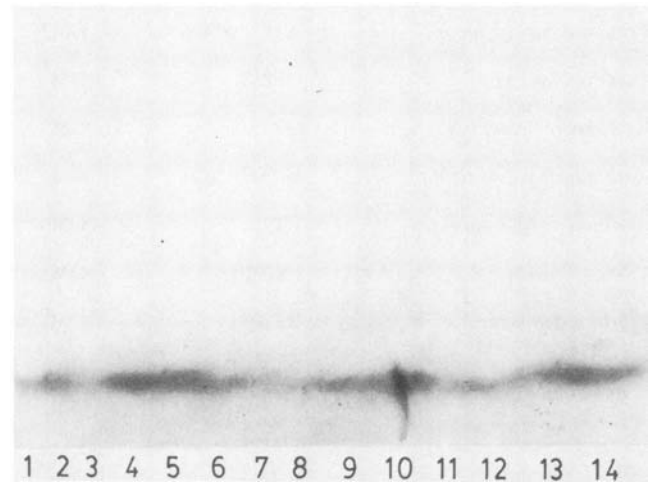


FIG. 3. Reaction of monoclonal antibody 5E4 with a Western electrophoretic blot of the separated outer membranes of clinical isolates of *P. aeruginosa* (9). The procedure outlined in the legend to Fig. 2 was followed. The outer membranes by lanes were as follows: 1, strain CF3660; 2, strain CF9490; 3, strain CF221; 4, strain CF4349; 5, strain CF284; 6, strain CF4522; 7, strain CF832; 8, strain CF3790; 9, strain CF1278; 10, strain L; 11, strain CF1452; 12, strain CF6094; 13, strain 2314; 14, strain CFP1M. This blot was made from an 11% acrylamide SDS-polyacrylamide gel (unlike that in Fig. 2, which was from a 14% acrylamide gel). Therefore, rough LPS migrated with the dye front and reacted as a tight band (cf. Fig. 2).

In this case, the position of binding of the monoclonal antibody was revealed by the subsequent addition of iodinated goat anti-mouse immunoglobulin and autoradiography. The nine LPS samples used included both smooth and rough strains of *E. coli* (including the LPS of the strain used as the injecting antigen to raise monoclonal antibodies), *S. typhimurium*, and *S. minnesota* as well as smooth strains of *Klebsiella pneumoniae* and *P. aeruginosa* (Fig. 4A). In addition, we analyzed a commercial *S. minnesota* lipid A preparation which failed to stain by the periodate-silver method of Tsai and Frasch (26) after SDS-polyacrylamide gel electrophoresis (Fig. 4A). Each of the LPS samples and lipid A bound 8A1 to a fast-migrating band which corresponded to rough LPS (Fig. 4B). In addition, antibody 8A1 showed weaker binding to a faster-migrating band and to a band which failed to enter the stacking gel. We do not know the reason for these extra bands for the lipid A preparation, although we suspect that they represent artifacts generated during the mild acid hydrolysis required to separate lipid A from the rest of the LPS.

Monoclonal antibody 8A1 also reacted with broad, fast-migrating bands from the outer membranes of other bacterial species, including *Edwardsiella tarda*, *A. tumefaciens*, and *Vibrio anguillarum* (Table 1). In addition, the monoclonal antibody interacted strongly with a variety of narrow, slower-migrating bands. Presumably, these bands represented species of LPS which migrated more slowly for some reason such as binding to outer membrane proteins. As evidence of this, similar bands were not observed when purified LPS was used as an antigen (Fig. 4B).

Similar analyses were performed with many combinations of outer membranes and antibodies. The results are summarized in brackets in Table 1.

#### DISCUSSION

The results of our ELISA analysis strongly suggest that each of the monoclonal antibodies described here is specific for the lipid A portion of LPS. All four of the antibodies reacted with purified lipid A from *P. aeruginosa* (Table 1), and two of them, 5E4 and 8A1, also interacted strongly with commercial *S. minnesota* lipid A. Such differential interaction for antibodies 1D4 and 6B2 may be explained by induced rather than native differences in the chemical composition of these lipid A molecules since the isolation procedure for lipid A is degradative, usually involving heating to 100°C for more than 1 h in mild acid. The strong interaction of antibodies 5E4 and 8A1 with *A. tumefaciens* LPS suggests that the octose KDO is not part of the antigenic site for these monoclonal antibodies since *Agrobacterium* LPS apparently lacks KDO when analyzed colorimetrically (R. P. Darveau and R. E. W. Hancock, unpublished observations). Although the actual antigenic site recognized by the four monoclonal antibodies was not elucidated in this study, the substantial differences in patterns of interaction observed in the ELISA study (Table 1) suggest that somewhat different epitopes were recognized by each of the antibodies.

The data presented here suggest that lipid A is broadly antigenically conserved in gram-negative bacteria. Each of the monoclonal antibodies interacted with antigens from all four families (*Enterobacteriaceae*, *Pseudomonadaceae*, *Vibrionaceae*, and *Rhizobiaceae*) and from 84 to 87% of the individual species of bacteria tested. The most extensively tested antibody, 5E4, interacted with 97% of the individual antigens tested, with a single LPS band from 33 different *P. aeruginosa* strains representing all 17 serotypes (with anti-

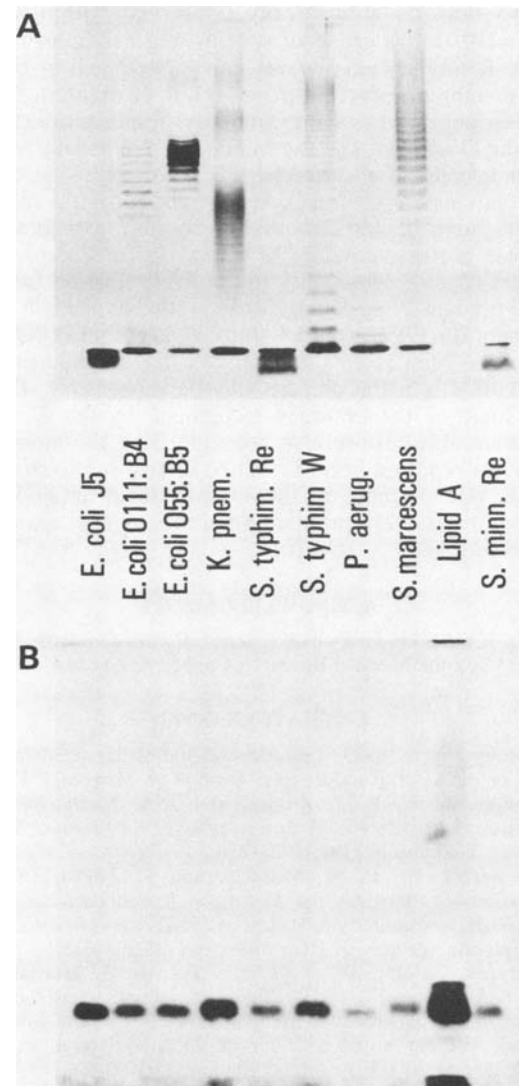


FIG. 4. Reaction of monoclonal antibody 8A1 with LPS or lipid A from different strains after separation by SDS-polyacrylamide gel electrophoresis followed by transfer to nitrocellulose by the Western electrophoretic blotting procedure. The position of the bound monoclonal antibody was revealed by incubating with radioiodinated protein A and autoradiography. (A) SDS-polyacrylamide gel electrophoretogram of the LPSs stained by the technique of Tsai and Frasch (26). (B) Western blot of LPSs stained with monoclonal antibody 8A1 and  $^{125}\text{I}$ -labeled goat anti-mouse immunoglobulin. The strain names between the panels refer to the LPSs present in the respective lanes above and below the captions.

genically different O antigens) of *P. aeruginosa*, and with 8 strains of the family *Enterobacteriaceae*. Significantly, none of the monoclonal antibodies interacted with three gram-positive strains. The simplest explanation of this extensive antigenic cross-reaction is that all lipid A moieties in gram-negative bacteria have a common evolutionary origin and have been strongly conserved over time. The reason for this strong conservation is unclear, but to date no lipid A-deficient mutant of a gram-negative bacterium has been isolated, suggesting that lipid A may be an essential component of these cells.

Such extensive conservation is unusual among outer membrane components. For example, individual outer membrane

proteins may be antigenically conserved within a single species (10, 14), a group of species (e.g., reference 14), or even a family (11). However, no cross-reaction between outer membrane proteins from strains of different families has been observed to date. Similarly, the distal portions of LPS, the O antigen, and the rough core are usually strain or species specific (e.g., references 3, 9, 10, 21).

The antigenic cross-reactions of lipid A from a variety of gram-negative bacteria provide a possible explanation for the cross-protective nature of *E. coli* J5 LPS or whole-cell vaccines (2, 19). The ability to passively transfer immunity to experimental *Pseudomonas* bacteremia by using *E. coli* J5 antiserum (2, 19) as well as antiserum to lipid A-KDO (Re LPS) of *S. minnesota* (28) suggests that antibodies to the lipid A-KDO portion of LPS may be responsible. Presumably, some of the epitopes recognized by the *E. coli* J5 polyclonal antisera are also recognized by the monoclonal antibodies reported here. It will be interesting to determine whether one or more of these monoclonal antibodies are capable of passively protecting animals against gram-negative bacteremia.

#### ACKNOWLEDGMENTS

Some of the preliminary experiments on *Pseudomonas* spp. were supported by the Medical Research Council of Canada.

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