JOURNAL OF CLINICAL MICROBIOLOGY, May 1988, p. 885–889
0095-1137/88/050885-05$02.00/0

Vol. 26, No. 5

Monoclonal Antibodies to *Pasteurella haemolytica* Serotype 1 Lipopolysaccharide: Demonstration of Antigenic Similarities among Several Serotypes†

JANET A. DURHAM,[1]* SUSAN M. ANTONE,[2] MADELEINE W. CUNNINGHAM,[3] AND ANTHONY W. CONFER[2]

Departments of Veterinary Parasitology, Microbiology, and Public Health[1] and Veterinary Pathology,[2] Oklahoma State University, Stillwater, Oklahoma 74078, and Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190[3]

Received 16 October 1987/Accepted 25 January 1988

Murine monoclonal antibodies (MAbs) were produced which were specific for *Pasteurella haemolytica* serotype 1 lipopolysaccharide (LPS). The MAbs also reacted with LPS present in a partially purified antigen derived from a saline extract of the organism. The epitope to which the MAbs were directed was a carbohydrate which was sensitive to oxidation with periodate, had a molecular weight between 14,000 and 25,000 as determined by immunoblotting, and was present in a crude O-antigen preparation of *P. haemolytica* LPS. The MAbs did not react with purified capsular polysaccharide from *P. haemolytica* serotype 1. In an enzyme-linked immunosorbent assay, reaction of the MAbs with LPS obtained from 14 gram-negative bacteria failed to detect any cross-reactivity with *P. haemolytica* LPS. However, the MAbs detected antigenic similarities among *P. haemolytica* serotypes 1, 5, 6, 7, 8, and 12 and, to a lesser extent, 4 and 14. These studies indicate that the LPS-O-antigens from several *P. haemolytica* serotypes have similar epitopes and may be partially responsible for shared antigenicity among serotypes.

Pasteurella haemolytica biotype A, serotype 1, is the major cause of bovine pneumonic pasteurellosis, a severe fibrinous pneumonia of feedlot cattle (11). *P. haemolytica* serotype 2 is frequently isolated from the nasal cavity of healthy cattle (18, 19), whereas other serotypes are isolated less frequently from cattle.

Lipopolysaccharide (LPS) is perhaps one of the most important constituents of the gram-negative cell wall and is well known for its biological activities, including modification of metabolic activity and phagocytic function (24), alteration of migration of neutrophils (26) and macrophages (31), and mitogenesis of lymphocytes (29). Similar biological activities have been associated with LPS from *P. haemolytica* serotype 1 (13, 17, 35).

The typical LPS molecule is composed of three regions consisting of lipid A, core oligosaccharides, and O-antigen polysaccharides. In contrast to the core oligosaccharide, which is structurally similar among many gram-negative bacteria, the O-antigen is more chemically diverse among bacteria and is highly antigenic (30). However, it is the O-antigen which is responsible for the serological cross-reactivity among certain gram-negative organisms; for example, antibodies to *Yersinia enterocolitica* (serotype O:9) cross-react with *Brucella abortus* (7, 8) and antibodies to *Brucella abortus* cross-react with *Escherichia coli* (O:157) (37). Cross-reactivity among serotypes of a bacterial species can also be contributed to the diversity of LPS, such as that seen with *Legionella pneumophila* (10) and *Pasteurella multocida* (34).

The antigenic diversity of *P. haemolytica* has led to the identification of at least 15 serotypes based on capsular antigens as determined by indirect hemagglutination (3), agglutination (20), nucleic acid homology (2), and polyacrylamide electrophoresis (38). This manuscript reports the characterization of monoclonal antibodies (MAbs) to *P. haemolytica* serotype 1 LPS and demonstrates antigenic cross-reactivity of LPS among several *P. haemolytica* serotypes.

MATERIALS AND METHODS

Bacterium. *P. haemolytica* biotype A, serotype 1, was originally obtained from a feedlot calf and maintained as described previously (32). Serotypes 5 and 9 were isolated from tracheal swabs of feedlot cattle showing symptoms of respiratory disease (George Burrows, College of Veterinary Medicine, Oklahoma State University, Stillwater). Serotypes 2, 3, 4, 6, 7, 8, 10, 11, 12, and 13 and an untypable strain were obtained from Glynn Frank (National Animal Disease Center [NADC], Ames, Iowa). Serotypes 14 and 15 were obtained from Michael Collier (University of Wisconsin, Madison).

Immunizing antigen. Antigenic material was extracted from logarithmic-phase *P. haemolytica* by using phosphate-buffered saline (PBS) as described previously (21). The extract was subjected to chromatofocusing, and the initial antigenic peak was pooled and concentrated (28). This peak, termed carbohydrate-protein subunit (CPS), is a high-molecular-weight (>200,000) complex carbohydrate-protein compound (16). The 2-keto-3-deoxyoctonate-protein ratio is variable among lots, ranging from <0.1 to 5.3 μg/ml. The protein-carbohydrate ratio ranges from 0.6 to 2.1.

LPS. LPS was obtained from *P. haemolytica* serotype 1 by the phenol-water extraction method of Westphal and Jann (40). The resulting LPS was determined to be antigenic by an enzyme-linked immunosorbent assay (ELISA) that detects *P. haemolytica* antigens (28). Lyophilized LPS was reconstituted in distilled water to a concentration of 5 mg (dry weight) per ml. The LPS contained 0.45 mg of 2-keto-3-deoxyoctonate per ml, 6.5×10^4 U of endotoxin per ml (13), and undetectable protein content (<0.1 μg of protein per ml)

* Corresponding author.

† Journal article 5214 from the Agricultural Experiment Station, Oklahoma State University, Stillwater.

(5). Polyacrylamide gel electrophoresis (PAGE) and silver staining results of the LPS were consistent with patterns for other bacterial LPS (S. Muir, personal communication). To separate lipid A and the O-antigen, the LPS was acid hydrolyzed by the procedure of Grollman and Osborn (23).

Capsular polysaccharide. Serotype-specific capsular polysaccharide was isolated from *P. haemolytica* serotype 1 as described (1). The protein content was <1% by weight (5). By nuclear magnetic resonance spectroscopy, the polysaccharide structure was found to be identical to that previously described for *P. haemolytica* serotype 1 (A. Mort, unpublished data).

MAb production. MAbs were produced as described by Cunningham and Russell (14) and Goding (22). Six-week-old male BALB/c mice were immunized intraperitoneally four times at 2-week intervals with 0.2 ml of an emulsion containing equal volumes of CPS (100 μg [dry weight] per mouse) and Freund complete adjuvant, given with the first injection, or Freund incomplete adjuvant, given with later injections. Four days before fusion, the mice were injected intraperitoneally with 200 μg of CPS in sterile PBS. This was followed by a daily injection of 400 μg of CPS for the next 3 days. Mice that were not sacrificed for the fusion were bled for positive control serum. Negative control serum was obtained from age- and sex-matched BALB/c control mice.

After removal, spleens were minced and rinsed in Iscove modified Dulbecco medium (IMDM) (Gibco Laboratories, Grand Island, N.Y.) containing antibiotics as described previously (14). Spleen cells were mixed at a 1:1 ratio with murine myeloma cells (x63-Ag8.6.5.3) in 35% sterile polyethylene glycol (pH 8.5). Following fusion, the cells were centrifuged and resuspended in IMDM with antibiotics and 20% fetal bovine serum (HyClone, Logan, Utah) as described by Cunningham and Russell (14). Cell cultures were fed with IMDM containing 20% fetal bovine serum, 5 mM hypoxanthine, 0.02 mM aminopterin, and 0.8 mM thymidine (Sigma Chemical Co., St. Louis, Mo.). Hybridomas were cloned three times by limiting dilution at <1 cell per well.

ELISA. An ELISA was used to screen culture supernatants for antibody reactive with CPS by the method of Voller et al. (Dynatech Laboratories, Inc., Alexandria, Va.). The optimal antigen and reagent concentrations were determined by a checkerboard titration method (S. M. Antone, M.S. thesis, Oklahoma State University, Stillwater, 1986). Briefly, microdilution wells were coated with 0.5 μg of CPS and washed after each step of incubation with PBS containing 0.05% Tween 20, pH 7.2 (PBS-Tween). Positive and negative control mouse sera diluted 1:500 in PBS-Tween containing 1% bovine serum albumin and culture supernatants were reacted with CPS-coated wells. Alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Sigma Chemical Co.) was then reacted with the wells, and the reaction was developed by using *p*-nitrophenyl phosphate (1 mg/ml) in diethanolamine hydrochloride (pH 9.6) and 1 mM $MgCl_2$. After a 30-min incubation, the reaction was stopped with 3 N NaOH, and the A_{405} was determined (model EL307; Bio-Tek Instruments, Inc., Burlington, Vt.). The antibody-producing hybridomas were considered positive when the well tested attained an A_{405} value of at least 0.5. MAb reactivity to carbohydrate- and non-carbohydrate-influenced epitopes in the CPS was performed in the ELISA by the periodate oxidation technique described by Woodard et al. (41).

SDS-PAGE and immunoblotting. Discontinuous sodium dodecyl sulfate (SDS)-PAGE consisting of a 2% acrylamide stacking gel and a 10% acrylamide resolving gel (27) was used to separate the different antigen preparations. Electrophoretic transfer of proteins to a nitrocellulose membrane was performed in a Mini Transphor TE22 (Hoefer Scientific Instruments, San Francisco, Calif.) by the procedure of Towbin et al. (39).

The antigens were detected immunoenzymatically following consecutive incubations with MAb supernatant, biotinylated protein A (1:300) (Amersham Corp., Arlington Heights, Ill.), and streptavidin-horseradish peroxidase complex (1:400) (Amersham). Antigenic bands were observed following incubation in a solution of 0.5 mg of 4-chloro-1-naphthol per ml in PBS containing 0.03% H_2O_2. The reaction was stopped by the addition of distilled water, and the blots were allowed to air dry.

Isotyping and quantitation of immunoglobulins. The isotype of the MAbs was determined by an ELISA with a mouse monoclonal subisotyping kit containing rabbit anti-mouse immunoglobulin G1 (IgG1), IgG2a, IgG2b, IgG3, IgM, and IgA (HyClone Laboratories). The immunoglobulin concentration of the culture supernatants was determined in an ELISA (36); the concentrations were extrapolated from a regression line established by using A_{405} values and concentrations of a purified mouse IgG3 standard (Chemicon International, Inc., El Segundo, Calif.).

Bacterial species and *P. haemolytica* serotype specificity. The ELISA was used to determine whether the hybridoma supernatants recognized LPS from other gram-negative bacteria. LPSs from *Escherichia coli* (serotypes O26:B6, O55:B5, O111:B4, O127:B8, O128:B12, J5, and EH100), *Salmonella typhimurium*, *Serratia marcescens* (Sigma Chemical Co.), *Proteus vulgaris*, *Klebsiella pneumoniae* (RIBI Corp., Hamilton, Mont.), *Brucella abortus* 19 and 2308 (M. Phillips, NADC), and *Pasteurella multocida* type 3 (R. Rimler, NADC) were used to coat microtiter plates at 10 μg/ml. Culture supernatants and control sera were incubated for 1 h, and then alkaline phosphatase-conjugated goat anti-mouse immunoglobulin was added at a 1:200 dilution for 1 h. The remaining procedure was as described above.

Formalinized *P. haemolytica* serotypes 1 through 15 and an untypable strain were used to coat microtiter plates. The reaction of the MAbs to the various serotypes was detected by the ELISA as described above. Formalinized whole-cell preparations of *P. haemolytica* serotypes 1, 2, 5, 6, 9, and an untypable strain were separated by electrophoresis, transferred to nitrocellulose, and reacted with the antibody supernatants as described above.

RESULTS

Three hybridomas which produced antibodies to CPS, as determined by high A_{405} values, were cloned by limiting dilution and designated E1F10, H6B5, and H12E11. The immunoglobulin class secreted by all three hybridomas was mouse IgG3, with 5 to 10 μg of antibody per ml being secreted. The MAbs reacted with various antigen preparations of *P. haemolytica* serotype 1 by ELISA (Table 1). The MAbs failed to react to CPS after treatment with sodium-*m*-periodate, indicating that these antibodies reacted with a carbohydrate epitope in the CPS. The MAbs did not react with either the periodate- or nontreated serotype 1-specific capsular polysaccharide. Each MAb reacted with *P. haemolytica* serotype 1 LPS, and reactivity was lost after periodate treatment. After acid hydrolysis of LPS, the MAbs reacted more intensely with the core polysaccharide-O-antigen moiety than with the lipid A (Table 1).

By SDS-PAGE and immunoblotting, all three MAbs reacted similarly with antigens in CPS and LPS, demonstrating

TABLE 1. Reactivity[a] of MAbs with various *P. haemolytica* serotype 1 antigen preparations

Antigen	Reactivity (A_{405})				
	MAb			Control sera[b]	
	E1F10	H6B5	H12E11	Positive	Negative
CPS	1.881	1.912	1.951	>1.999	0.053
$NaIO_4$ treated	0.135	0.229	0.164	1.857	0.126
LPS	1.006	0.999	0.932	0.750	0.028
$NaIO_4$ treated	0.020	0.004	0.030	0.517	0.004
Capsular polysaccharide	0.008	0.002	0.008	0.431	0.013
$NaIO_4$ treated	0.040	0.016	0.027	0.398	0.004
Lipid A	0.121	0.093	0.091	0.374	0.008
O-antigen	0.224	0.220	0.232	0.665	0.006

[a] Reactivity was measured by ELISA at an antigen concentration of 10 μg/ml. The value of duplicate samples was expressed as A_{405} minus the A_{405} of the PBS control.

[b] Positive control sera were from CPS-hyperimmunized BALB/c mice. Negative control sera were from age- and sex-matched nonimmunized BALB/c mice.

multiple, equally spaced bands in a molecular size range of 14 to 25 kilodaltons (kDa) (Fig. 1). Periodate treatment of the Western blots (immunoblots) of the CPS and LPS negated this reaction.

The MAbs did not react with LPS from any other gram-negative bacteria tested, including *P. multocida* type 3 (Table 2). However, each MAb had intense cross-reactivity among *P. haemolytica* serotypes 1, 5, 6, 7, 8, and 12 and less reactivity with serotypes 4 and 14 (Table 3). There was no detectable binding with the other seven serotypes or the untypable strain. Likewise, cross-reactivity was observed with the MAbs among serotypes 1, 5, and 6 in an immunoblot (Fig. 2).

DISCUSSION

The three MAbs characterized in the present study reacted with CPS and LPS. Upon oxidation with sodium-*m*-periodate, the reactivity seen against CPS and LPS was lost. Periodate oxidation cleaves the carbohydrate-vicinal hydroxyl groups of the antigen without altering the structure of the polypeptide chains (4). Therefore, loss of reactivity to LPS after oxidation confirms the anticarbohydrate reactivity of the MAbs and indicates that specificity is for the polysaccharide component of LPS. Failure of the MAbs to react to the serotype-specific capsular polysaccharide, which is not sensitive to periodate oxidation (1), and a more intense reaction with the core-O-chain moiety of LPS than the lipid A fraction support the concept that these MAbs are directed against the polysaccharide moieties of LPS. Because of the lack of reaction with LPS from gram-negative organisms other than *P. haemolytica* in the present study, the MAbs were specific for *P. haemolytica* LPS. Loss of reactivity of the MAbs with CPS after periodate oxidation and the similar LPS patterns for LPS and CPS observed with SDS-PAGE immunoblotting indicate the presence of LPS in the CPS.

Previous studies with *P. haemolytica* serotype 4 (33) indicated that acid hydrolysis cleaved the core oligosaccharide (1.9 kDa) and O-chains, which were then separable by gel filtration. In the present study, the core-O-chain-rich supernatant was dialyzed extensively in tubing with a 6- to 8-kDa size exclusion prior to use in the ELISA. This should cause loss of free core molecules. Also, gel filtration of our dialyzed core-O-chain supernatant produced only one major peak greater than 6 kDa (J. A. Durham and B. A. Lessley,

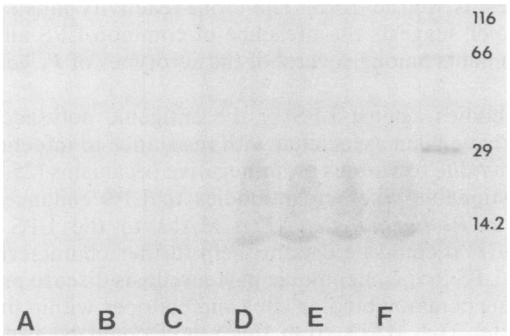

FIG. 1. Immunoblot analysis of *P. haemolytica* serotype 1 CPS antigens with MAbs. Lanes A to C, CPS was treated with sodium-*m*-periodate prior to antibody incubation. Lanes D to F, CPS was not treated with sodium-*m*-periodate. Lanes A and D, E1F10; lanes B and E, H6B5; lanes C and F, H12E11. Molecular weight markers (10^3) are shown.

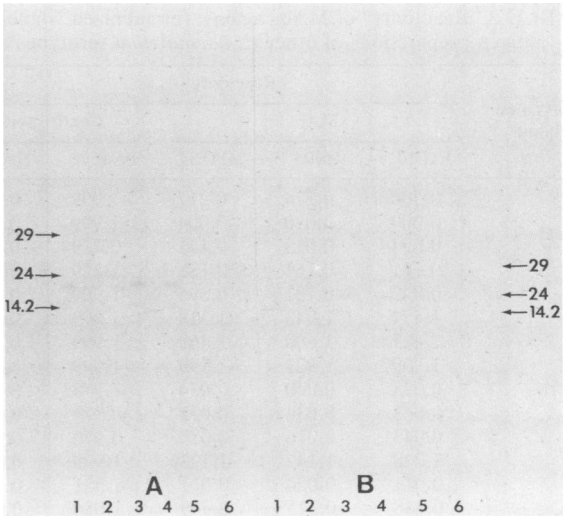

FIG. 2. Immunoblot analysis of formalinized whole-cell preparations of *P. haemolytica* serotypes 1, 2, 5, 6, 9, and the untypable strain with two MAbs, E1F10 (A) and H6B5 (B). Lane 1, Serotype 1; lane 2, serotype 2; lane 3, serotype 5; lane 4, serotype 6; lane 5, serotype 9; lane 6, untypable strain. Approximate molecular weight markers (10^3) are shown. The immunoblots are from two separate gels, giving rise to the different molecular weight marker positions.

TABLE 2. Reactivity[a] of MAbs produced against *P. haemolytica* serotype 1 with LPSs of various gram-negative bacteria

LPS source	Reactivity (A_{405})				
	MAb			Control sera[b]	
	E1F10	H6B5	H12E11	Positive	Negative
Expt 1					
Pasteurella haemolytica	1.006	0.999	0.932	0.750	0.028
Pasteurella multocida type 3	0.022	0.036	0.016	0.190	0.008
Serratia marcescens	0.008	0.025	0.004	0.126	0.002
Salmonella typhimurium	0.009	0.006	0.026	0.067	0.000
Klebsiella pneumoniae	0.006	0.023	0.002	0.122	0.000
Proteus vulgaris	0.008	0.015	0.019	0.149	0.004
Brucella abortus 19	0.000	0.019	0.005	0.066	0.022
Brucella abortus 2308	0.000	0.000	0.000	0.071	0.001
Expt 2					
Pasteurella haemolytica	0.503	0.433	0.441	0.897	0.013
Escherichia coli O26:B6	0.015	0.004	0.010	0.277	0.009
Escherichia coli O55:B5	0.013	0.012	0.014	0.260	0.000
Escherichia coli O111:B4	0.006	0.011	0.016	0.116	0.021
Escherichia coli O127:B8	0.007	0.005	0.004	0.177	0.012
Escherichia coli O128:B12	0.001	0.000	0.003	0.193	0.002
Escherichia coli J5	0.010	0.008	0.013	0.130	0.004
Escherichia coli EH100	0.003	0.000	0.002	0.162	0.006

[a] As in Table 1, footnote *a*, but triplicate samples.
[b] See Table 1, footnote *b*.

unpublished data), further supporting the observation that the MAbs were directed against the O-antigen of *P. haemolytica*.

Generally, the polysaccharide O-side chains of the LPS molecule are responsible for serotype specificity among many gram-negative organisms (9). However, in the present study, the MAbs detected cross-reacting antigens among several serotypes of *P. haemolytica*, supporting the concept that *P. haemolytica* serotyping is based on capsular polysaccharide or some other surface antigen and not LPS. Also, strong serologic cross-reactivity among *P. haemolytica* serotypes 1, 5, 6, 7, 8, and 12 and, to a lesser degree, serotypes 4 and 14 indicates shared epitopes among their O-antigens. In a comparative study among five serotypes (1, 2, 5, 6, and 9) and one untypable strain of *P. haemolytica*, biological and antigenic similarities among serotypes 1, 5, and 6 were observed (M. J. Gentry, A. W. Confer, and S. G. Holland, Vet. Microbiol., in press). In the present study, when reacted against formalinized whole-cell preparations of serotypes 1, 2, 5, 6, 9, and an untypable strain in an immunoblot, each MAb recognized similar antigenic areas among serotypes 1, 5, and 6. The reactivity to the low-molecular-weight bands indicates reaction to a fast-resolving antigen located within the LPS. Burrells et al. (6) showed strong cross-reactivity among serotypes 1, 2, 5, 6, 7, 8, 9, and 12 by using a sodium salicylate extract of *P. haemolytica*. Salicylate extracts contain both protein and polysaccharide antigens (15). Therefore, some of the serological cross-reactivity seen by Burrells et al. (6) could be related to shared protein epitopes as well as LPS. The cross-reactivity illustrated in this paper suggests the presence of common LPS antigenic determinants among several of the serotypes of *P. haemolytica*.

Antibodies against LPS or the antigenic polysaccharide chains have been associated with resistance to infection and mortality due to various gram-negative organisms (25, 29). It is questionable whether antibodies to LPS enhance resistance to *P. haemolytica* (12). MAbs to the LPS of *P. haemolytica* could be used to help further characterize the role of LPS in the pneumonic pasteurellosis disease process, including common binding sites and epitopes within the LPS molecule. The MAbs from this study could be used in a screening kit, such as latex bead agglutination, to rapidly determine the presence of *P. haemolytica* in pneumonic lesions or to determine whether *P. haemolytica* grown from the nasal passages of cattle is low-virulence serotype 2 or a more likely virulent serotype such as serotype 1, 5, or 6.

TABLE 3. Reactivity[a] of MAbs against formalinized whole-cell antigen preparations of other *P. haemolytica* serotypes[b]

Serotype or subunit	Reactivity (A_{405})				
	MAb			Control sera[c]	
	E1F10	H6B5	H12E11	Positive	Negative
1	0.506	0.274	0.217	>1.999	0.297
2	0.000	0.010	0.004	>1.999	0.092
3	0.024	0.013	0.151	0.729	0.129
4	0.181	0.161	0.188	1.229	0.112
5	0.842	0.732	0.895	>1.999	0.202
6	1.474	1.043	1.318	>1.999	0.246
7	1.481	1.600	1.469	>1.999	0.199
8	>1.999	1.922	>1.999	>1.999	0.201
9	0.023	0.040	0.034	1.978	0.133
10	0.043	0.034	0.032	0.974	0.107
11	0.043	0.036	0.019	0.970	0.071
12	1.228	1.142	1.123	>1.999	0.249
13	0.011	0.024	0.011	1.581	0.110
14	0.348	0.315	0.349	1.086	0.002
15	0.010	0.013	0.006	0.731	0.012
Untypable	0.027	0.006	0.047	1.636	0.085
CPS	>1.999	>1.999	>1.999	>1.999	0.354

[a] See Table 1, footnote *a*.
[b] When the plates were coated, concentrations were equivalent based on A_{650} values of approximately 1.8.
[c] See Table 1, footnote *b*.

ACKNOWLEDGMENTS

This research was supported in part by special grant 86-CRSR-2-2880 from the U.S. Department of Agriculture.

We thank Sherl Holesko for typing the manuscript.

LITERATURE CITED

1. **Adlam, C., J. M. Knights, A. Mugridge, J. C. Lindon, P. R. W. Baker, J. E. Beesley, B. Spacey, G. R. Craig, and L. K. Nagy.** 1984. Purification, characterization, and immunological properties of the serotype-specific capsular polysaccharide of *Pasteurella haemolytica* (serotype A1) organisms. J. Gen. Microbiol. **130**:2415–2426.
2. **Biberstein, E. L., and C. K. Francis.** 1968. Nucleic acid homologies between the A and T types of *Pasteurella haemolytica*. J. Med. Microbiol. **1**:105–108.
3. **Biberstein, E. L., M. Gills, and H. Knight.** 1960. Serological types of *Pasteurella hemolytica*. Cornell Vet. **50**:283–300.
4. **Bobbitt, J. M.** 1956. Periodate oxidation of carbohydrates. Adv. Carbohydrate Chem. Biochem. **11**:1–41.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
6. **Burrells, C., H. B. Evans, and A. M. Dawson.** 1983. Antigenic relationships between the serotypes of *Pasteurella haemolytica* demonstrable by enzyme-linked immunosorbent assay (ELISA). Vet. Microbiol. **8**:187–198.
7. **Caroff, M., D. R. Bundle, and M. B. Perry.** 1984. Structure of the O-chain of the phenol-phase soluble cellular lipopolysaccharide of *Yersinia enterocolitica* serotype O:9. Eur. J. Biochem. **139**:195–200.
8. **Caroff, M., D. R. Bundle, M. B. Perry, J. W. Cherwonogradsky, and J. R. Duncan.** 1984. Antigenic S-type lipopolysaccharide of *Brucella abortus* 1119-3. Infect. Immun. **46**:384–388.
9. **Chester, I. R., P. M. Meadow, and T. L. Pitt.** 1973. The relationship between the O-antigen lipopolysaccharides and serological specificity in strains of *Pseudomonas aeruginosa* of different serotypes. J. Gen. Microbiol. **78**:305–315.
10. **Ciesielski, C. A., M. J. Blaser, and W.-L. L. Wang.** 1986. Serogroup specificity of *Legionella pneumophila* is related to lipopolysaccharide characteristics. Infect. Immun. **51**:397–404.
11. **Collier, J. R.** 1968. Pasteurellae in bovine respiratory disease. J. Am. Vet. Med. Assoc. **152**:824–828.
12. **Confer, A. W., R. J. Panciera, and D. A. Mosier.** 1986. Serum antibodies to *Pasteurella haemolytica* lipopolysaccharide: relationship to experimental bovine pneumonic pasteurellosis. Am. J. Vet. Res. **47**:1134–1138.
13. **Confer, A. W., and K. R. Simons.** 1986. Effects of *Pasteurella haemolytica* lipopolysaccharide on selected functions of bovine leukocytes. Am. J. Vet. Res. **47**:154–157.
14. **Cunningham, M. W., and S. M. Russell.** 1983. Study of heart reactive antibody in antisera and hybridoma culture fluids against group A streptococci. Infect. Immun. **42**:531–538.
15. **Donachie, W., N. J. L. Gilmour, and I. R. Poxton.** 1982. Cell surface antigens of *Pasteurella haemolytica*: characterization of the protective antigen in a mouse model. Soc. Gen. Microbiol. Q. **9**:M13.
16. **Durham, J. A., A. W. Confer, D. A. Mosier, and B. A. Lessley.** 1986. Comparison of the antigens associated with saline solution, potassium thiocyanate, and sodium salicylate extracts of *Pasteurella haemolytica* serotype 1. Am. J. Vet. Res. **47**:1946–1951.
17. **Emau, P., S. N. Giri, and M. L. Bruss.** 1986. Comparative effects of smooth and rough *Pasteurella haemolytica* lipopolysaccharides on arachidonic acid, eicosanoids, serotonin, and histamines in calves. Circulatory Shock **20**:239–253.
18. **Fox, M. L., R. G. Thomson, and S. E. Magwood.** 1971. *Pasteurella haemolytica* of cattle: serotype, production of beta-galactosidase and anti-bacterial sensitivity. Can. J. Comp. Med. **35**: 313–317.
19. **Frank, G. H., and P. C. Smith.** 1983. Prevalence of *Pasteurella haemolytica* in transported calves. Am. J. Vet. Res. **44**:981–985.
20. **Frank, G. H., and G. E. Wessman.** 1978. Rapid plate agglutination procedure for serotyping *Pasteurella haemolytica*. J. Clin. Microbiol. **7**:142–145.
21. **Gentry, M. J., R. E. Corstvet, and R. J. Panciera.** 1982. Extraction of capsular material from *Pasteurella haemolytica*. Am. J. Vet. Res. **43**:2070–2073.
22. **Goding, J. W.** 1983. Monoclonal antibodies: principles and practice. Academic Press, Inc., New York.
23. **Grollman, A. P., and M. J. Osborn.** 1964. *O*-Phosphorylethanolamine: a component of lipopolysaccharide in certain gram-negative bacteria. Biochemistry **3**:1571–1574.
24. **Henricks, P. A. J., M. E. vanderTol, R. M. W. M. Thyssen, B. S. van Asbeck, and J. Verhoef.** 1983. *Escherichia coli* lipopolysaccharides diminish and enhance cell function of human polymorphonuclear leukocytes. Infect. Immun. **41**:294–301.
25. **Kirkland, T. N., and E. J. Ziegler.** 1984. An immunoprotective monoclonal antibody to lipopolysaccharide. J. Immunol. **132**: 2590–2592.
26. **Klesius, P. H., W. H. Chambers, and R. D. Schultz.** 1984. Effect of bacterial lipopolysaccharide on bovine polymorphonuclear neutrophil migration in vitro. Vet. Immunol. Immunopathol. **7**: 239–244.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
28. **Lessley, B. A., A. W. Confer, D. A. Mosier, M. J. Gentry, J. A. Durham, and J. A. Rummage.** 1985. Saline-extracted antigens of *Pasteurella haemolytica*: separation by chromatofocusing, preliminary characterization, and evaluation of immunogenicity. Vet. Immunol. Immunopathol. **10**:279–296.
29. **Morrison, D. C., and J. L. Ryan.** 1979. Bacterial endotoxins and host responses. Adv. Immunol. **28**:293–415.
30. **Morrison, D. C., and R. J. Ulevitch.** 1978. The effects of bacterial endotoxins on host mediation systems. Am. J. Pathol. 93:527–617.
31. **Nagao, S., A. Tanaka, K. Onozaki, and T. Hashimoto.** 1982. Differences between macrophage migration inhibitions by lymphokines and muramyl dipeptide (MDP) or lipopolysaccharide (LPS): migration enhancement by lymphokines. Cell. Immunol. **71**:1–11.
32. **Newman, P. R., R. E. Corstvet, and R. J. Panciera.** 1982. Distribution of *Pasteurella haemolytica* and *Pasteurella multocida* in the bovine lung following vaccination and challenge exposure as an indicator of lung resistance. Am. J. Vet. Res. **43**:417–422.
33. **Perry, M. B., and L. A. Babiuk.** 1984. Structure of the polysaccharide chain of *Pasteurella haemolytica* (serotype 4) lipopolysaccharide. Can. J. Biochem. Cell Biol. **62**:108–114.
34. **Rimler, R. B., and W. E. Brown.** 1982. Comparison of the serological response of chickens to *Pasteurella multocida* and its lipopolysaccharide. Avian Dis. **26**:842–846.
35. **Rimsay, R. L., J. E. Coyle-Dennis, L. H. Lauerman, and P. G. Squire.** 1981. Purification and biological characterization of endotoxin fractions from *Pasteurella haemolytica*. Am. J. Vet. Res. **42**:2134–2138.
36. **Schoenfeld, Y., J. Rauch, H. Massicotti, K. Syamal, J. Andre-Schwartz, B. D. Stollar, and R. S. Schwartz.** 1983. Polyspecificity of monoclonal lupus auto-antibodies produced by human-human hybridomas. N. Engl. J. Med. **308**:414–420.
37. **Stuart, F. A., and M. J. Corbel.** 1982. Identification of a serological cross-reaction between *Brucella abortus* and *Escherichia coli* O:157. Vet. Rec. **110**:202–203.
38. **Thompson, D. A., and D. L. Mould.** 1975. Protein electrophoresis pattern of *Pasteurella haemolytica*. Res. Vet. Sci. **18**:342–343.
39. **Towbin, H., T. Staehlin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
40. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides: extraction with phenol-water and further applications for the procedure. Methods Carbohydr. Chem. **5**:83–91.
41. **Woodward, M. P., W. W. Young, Jr., and R. A. Bloodgood.** 1985. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. J. Immunol. Methods **78**:143–153.