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Distribution of a Monoclonal Antibody-Recognized Protective Protein Immunogen on the Outer Membranes of *Pasteurella multocida* Rabbit Isolates

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The distribution of a monoclonal antibody (MAb)-recognized protective protein immunogen on the outer membrane of 153 *Pasteurella multocida* rabbit isolates was determined by dot blot (DB) analysis. MAb 1608 reacted with 36 (24%) of the 153 clinical isolates. The DB-positive clinical isolates expressed capsular antigens A, D, and nontypable and somatic antigens 2, 3, 10, 12, 15, and nontypable. Western blot (immunoblot) analysis with adsorbed and eluted MAb 1608 confirmed that the antigenic determinant identified was located on the cell surface. With MAb 1608 as a probe for antibody-accessible radioimmunoassay, 31 of 36 DB-positive *P. multocida* rabbit isolates were shown to have surface-exposed and antibody-accessible antigenic determinants, while 44 of 44 DB-negative isolates were negative by antibody-accessible radioimmunoassay. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed DB-negative *P. multocida* isolates both with (6 of 13, 46%) and without (7 of 13, 54%) the 37.5-kilodalton protein. This study establishes that the protective antigenic determinant of the 37.5-kilodalton outer membrane protein is present in 24% of rabbit clinical isolates tested and is detectable in *P. multocida* strains distributed among the major somatic types (3, 10, 12, and 15) and the capsular types (A and D) commonly isolated from rabbits in North America.

Pasteurellosis due to *Pasteurella multocida* infection is a serious and widespread problem in laboratory rabbits (4, 6, 15). The disease not only may cause high morbidity and sometimes high mortality (8), but also may interfere with the interpretation of research data.

Recently, potassium thiocyanate extract vaccines of *P. multocida* have been found to protect rabbits against homologous challenge (13, 17). Protective hyperimmune sera from rabbits immunized with potassium thiocyanate extract are directed predominantly against an antigenic determinant on a 37.5-kilodalton (kDa) outer membrane protein of *P. multocida* (12). A monoclonal antibody (MAb) directed against a 37.5-kDa outer membrane antigenic determinant has been obtained from mice immunized with outer membrane vesicles of *P. multocida* strain UT-1. This MAb, designated MAb 1608, has an immunoglobulin G2a isotype and a protein-binding site and protects mice and rabbits (Y.-S. Lu, S. Afendis, and S. P. Pakes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, B250, p. 66; Y.-S. Lu, S. J. Afendis, and S. P. Pakes, submitted for publication) against homologous challenge. Passively immunized mice also were protected against heterologous challenges by two *P. multocida* strains that are positive for the antigenic determinant identified by MAb 1608. No protection occurred when inoculated mice were exposed to a heterologous *P. multocida* isolate that lacks the MAb 1608-recognized immunogen.

If the MAb 1608-recognized immunogen is to prove useful in developing a vaccine, it should be expressed by a significant percentage of *P. multocida* rabbit isolates encountered in clinical disease. Furthermore, it should not be restricted to a single capsular or somatic serotype. The study reported herein was designed to determine the prevalence of this antigenic determinant among rabbit clinical isolates and the capsular and somatic serotypes of the isolates expressing the MAb 1608-recognized antigenic determinant.

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MATERIALS AND METHODS

Bacterial isolates and culture media. The *P. multocida* 3:A (UT-1) strain, an isolate from a rabbit with suppurative rhinitis, was used as an immunizing agent to produce MAb 1608.

The 153 isolates of *P. multocida* used in the experiment were recovered from rabbits with clinical disease, including rhinitis, pneumonia, peritonitis, lymphadenitis, conjunctivitis, tympanitis, pyometritis, and cutaneous abscesses. *P. multocida* organisms lyophilized in horse serum containing 7.5% glucose or maintained on stock culture agar were grown on blood agar plates and then in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C. All organisms used for experiments were harvested from cultures in the log phase of growth unless otherwise stated.

Preparation of outer membrane vesicles of *P. multocida*. Outer membrane vesicles were prepared by a modification of the method of McDade and Johnson (16).

MAb production. MAb 1608 was produced from mice immunized with *P. multocida* UT-1 outer membrane vesicles, as described previously (18).

DB analysis. Dot blot (DB) analysis was used to determine the presence of the specific antigenic determinant of *P. multocida* in 153 clinical *P. multocida* rabbit isolates. *P. multocida* isolates were grown to confluence on dextrose starch agar (Difco Laboratories). Organisms (ca. 10^8 cells) were harvested and washed twice in 0.01 M phosphate-buffered saline (PBS) (pH 7.2). Cell pellets were suspended in 1 ml of sterile distilled water, vortexed vigorously for 5 min, and stored at −20°C until used. For DB analyses, 10-μl portions of cell suspension were added to separate wells of a Bio Dot Apparatus (Bio-Rad Laboratories, Richmond,

Calif.) containing a piece of nitrocellulose paper (NCP) prewetted in PBS. Samples were dried onto the paper by vacuum for 1 h at room temperature. The NCP containing the sample was then processed in the same manner as that described for Western blot (WB; immunoblot) analysis. Negative controls included sample without antigen and cell suspensions of *Mycoplasma pulmonis* or *Pseudomones aeruginosa*. Homologous *P. multocida* served as the positive control.

MAb 1608 adsorption and elution. A 500-μl portion of MAb 1608 was mixed with 500 μl of various isolates of washed *P. multocida* live cells (ca. 10^8 CFU) at 4°C for 1 h and centrifuged at 12,000 × *g* for 5 min. The supernatant was added to 500 μl of fresh intact *P. multocida* cells, and the procedure was repeated three times. The final supernatant was saved and used as adsorbed MAb. All *P. multocida* cell suspensions were kept at 4°C to ensure that cells remained intact.

The eluted MAb was obtained by mixing MAb with intact *P. multocida* cells and collecting the sediment after centrifugation at 12,000 × *g* for 5 min. The sediment containing intact bacteria and adsorbed antibodies was mixed with a fresh sample (500 μl) of MAb, and the same procedure was repeated three times. The final pellet containing bacteria-antibody complexes was washed with PBS and suspended in 0.2 M glycine hydrochloride (pH 2.8). The supernatant containing eluted MAb was removed and adjusted to pH 7.2 with 1 mM Tris hydrochloride (pH 8.0). The eluted MAb containing 0.1% bovine serum albumin was stored at −20°C until used.

WB immunoanalysis. Outer membrane vesicles of *P. multocida* UT-1 prepared by lithium chloride extraction were used as the antigen. The membrane antigens were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to NCP by the methanol-Tris-glycine method (19). After transfer, the NCP strips were incubated with PBS-T (0.01 M PBS, 0.05% Tween 20, pH 7.2) containing 2% bovine serum albumin for 1 h at room temperature to block the nonspecific binding sites. The NCP strips were then incubated with hybridoma culture supernatant (1:2 dilution in PBS-T) overnight at 4°C and washed three times with PBS-T at the end of incubation. The NCP was reacted next with a 1:1,000 dilution (in PBS-T) of horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (heavy and light chain specific; Organon Teknika, Malvern, Pa.) for 8 h at 4°C, washed three times (15 min each) with PBS-T, and incubated with a 1:1,000 dilution of horseradish peroxidase conjugates of rabbit anti-goat immunoglobulin G (Organon Teknika) overnight at 4°C. The strips were washed three times in PBS-T and immersed in 50 mM Tris buffer (pH 7.4) containing 4-chloro-1-naphthol (0.5 mg/ml) and hydrogen peroxide (0.01%) for color development. The color reaction was terminated by flooding the NCP with distilled water.

AA-RIA. The antibody-accessible radioimmunoassay (AA-RIA) (11) was used to determine whether the 37.5-kDa antigenic determinant present in clinical *P. multocida* rabbit isolates was exposed on the cell surface and antibody accessible. *P. multocida* clinical isolates were grown overnight on blood agar and suspended in cold 0.01 M PBS (pH 7.2) to a concentration of 10^8 CFU/ml. MAb 1608 was purified from hybridoma culture supernatant by passing it over a protein A-Sepharose 4B column (7). Purified MAb 1608 was radioiodinated by the chloramine-T method (10) to an activity of 8×10^7 cpm/μg of protein. A total of 10^7 cells in 100 μl of PBS were mixed with 10^6 cpm of radioiodinated MAb 1608 and 500 μl of PBS containing 10% (vol/vol) heat-inactivated fetal calf serum–1% NaN_3 and incubated for 2 h at 4°C with gentle rocking. After incubation, 25 μl of a carrier organism (2.5×10^8 cfu of *P. multocida* 82-247 lacking the 37.5-Kd determinant) was added to the suspension. This mixture was washed three times in PBS-fetal calf serum and suspended in 500 μl of solubilization buffer (12). Radioactivity was measured by a Gamma Trac 1191 automatic gamma counter (TmAnalytic, Elk Grove Village, Ill.). Results are expressed as counts per minute of labeled antibody bound to *P. multocida* cells.

Capsular and somatic antigen determination. All of the 36 DB-positive *P. multocida* isolates and 44 of the 117 DB-negative *P. multocida* isolates recovered from rabbits with rhinitis, cutaneous abscesses, conjunctivitis, lymphadenitis, pneumonia, pyometritis, and tympanitis were selected randomly and analyzed for *P. multocida* capsular and somatic antigens, as described previously (2, 3, 14).

RESULTS

Prevalence of the MAb 1608-recognized antigenic determinant among *P. multocida* rabbit clinical isolates. Of the 153 *P. multocida* rabbit clinical isolates examined by DB analysis, 36 (24%) developed dark spots, indicating the presence of the MAb 1608-recognized antigenic determinant. The three negative controls did not develop dark spots, and the positive control developed a dark spot as expected.

To confirm the reliability of *P. multocida* cell suspensions in distilled water as antigen sources for DB analysis, we compared the results of sonicated *P. multocida* and *P. multocida* cell suspensions of the same 10 *P. multocida* isolates (5 each of DB-positive and DB-negative isolates). The results were identical regardless of whether sonicated cells or cell suspensions of *P. multocida* were used. Furthermore, we compared SDS-PAGE protein profiles of five different supernatants obtained from *P. multocida* cell suspensions in distilled water and their corresponding *P. multocida* outer membrane vesicles extracted by lithium chloride. The results from the two groups were essentially identical.

Capsular and somatic antigens of DB-positive and representative DB-negative *P. multocida* clinical isolates. The distribution of capsular and somatic antigens among the *P. multocida* clinical isolates tested is presented in Table 1. Some DB-negative and DB-positive isolates expressed more than one somatic antigen. Of the 36 *P. multocida* DB-positive isolates tested, 14 (39%) were capsular type A, 4 (11%) were type D, and 18 (50%) were nontypable. One (3%) expressed somatic antigen 2; 17 (47%), 3; 21 (58%), 10; 4 (11%), 12; and 1 (3%), 15. Seven (19%) isolates were nontypable.

Of the 44 *P. multocida* DB-negative isolates, 16 (36%) were capsular type A, 5 (11%) were type D, and 23 (52%) were nontypable. Two (5%) expressed somatic antigen 1; 2 (5%), 2; 10 (23%), 3; 1 (2%), 7; 14 (32%), 10; 5 (12%), 12; and 1 (2%), 14. Some 23 (52%) were nontypable.

Use of AA-RIA to determine presence of antigenic determinants among *P. multocida* rabbit clinical isolates. AA-RIA was used to screen 80 *P. multocida* clinical isolates for the presence and accessibility of the MAb 1608-recognized antigenic determinant on the cell surface (Table 1). Of the 36 DB-positive isolates tested, 31 were AA-RIA positive (mean cpm, 32,398; range, 5,601 to 194,853), whereas 44 of the 44 DB-negative isolates were AA-RIA negative (mean cpm, 675; range, 70 to 1,804).

TABLE 1. Distribution of capsular and somatic antigens and accessibility of MAb 1608-recognized antigenic epitope among *P. multocida* clinical isolates from rabbits

Strain	Isolation site[a]	Antigenic type		AA-RIA	
		Capsular	Somatic	cpm	Interpretation[b]
DB-positive isolates					
87-1353	RH	A	NT[c]	12,026	+
87-1280	RH	A	NT	1,324	−
87-910	CO	A	10	9,151	+
87-645	AB	A	NT	4,402	+
87-586	AB	NT	NT	3,821	−
87-582	RH	NT	NT	6,066	+
86-362	RH	NT	3	25,388	+
86-329	AB	A	10	14,979	+
85-765	RH	A	3, 10	77,853	+
85-763	RH	A	3, 10	13,511	+
85-423	RH	NT	3, 10	26,707	+
85-409	RH	A	10, 12	13,236	+
84-567	CO	NT	3	9,983	+
84-439	RH	NT	3, 10	21,572	+
84-167	RH	A	10	12,349	+
84-166	RH	NT	3	7,307	+
84-151	CO	NT	3	5,939	+
84-150	RH	A	10	49,981	+
84-142	TY	D	3, 10	7,343	+
83-572	PN	NT	3, 10, 12	27,804	+
83-171	CO	NT	10	6,116	+
82-390	RH	A	12	2,702	−
82-282	PN	D	NT	5,601	+
82-245	AB	NT	3, 10	23,906	+
82-216	PN	NT	3, 10	33,588	+
81-556	PN	NT	10, 12	25,441	+
81-545	PN	NT	NT	134,742	+
81-544	PN	NT	3	11,522	+
81-509	RH	A	10	21,259	+
81-474	PE	NT	3	33,898	+
81-472	PN	NT	3, 10	124,423	+
81-224	TY	NT	3, 10	194,853	+
81-88	PN	D	10	12,687	+
80-468	PN	A	10	537	−
L-182	RH	D	2, 10, 15	1,188	−
UT-1	RH	A	3	30,720	+
DB-negative isolates					
87-887	CO	A	NT	977	−
87-885	RH	A	NT	1,192	−
87-817	PY	NT	10	1,068	−
87-727	RH	A	NT	1,285	−
87-646	TY	A	3, 10	967	−
87-345	AB	A	NT	1,372	−
87-250	AB	A	10	1,410	−
87-249	LA	A	12	1,101	−
87-247	TY	A	NT	1,023	−
86-328	PY	D	2, 10, 14, 15	349	−
86-325	CO	D	10, 15	978	−
86-313	PY	D	3, 10	715	−
86-304	RH	D	10	682	−
85-566	CO	A	NT	700	−
85-392	PN	D	3, 10	741	−
84-252	RH	NT	1	935	−
84-3	PN	A	3, 10	1,445	−
83-363	RH	NT	NT	451	−
83-127	TY	NT	NT	1,508	−
82-247	CO	NT	1	880	−
82-241	CO	A	NT	468	−
81-291	TY	NT	3, 7	554	−
81-247	CO	A	NT	172	−
81-231	TY	NT	3, 10	1,804	−
81-228	RH	A	12	1,054	−
81-158	PN	NT	NT	128	−
81-97	CO	NT	NT	83	
81-62	AB	NT	NT	137	−
81-39	AB	NT	3, 10	234	−
80-595	PN	NT	NT	86	−
80-493	PN	NT	3, 10	196	−
80-492	PN	NT	2, 12	891	−
80-312	PN	A	NT	751	−
80-192	TY	NT	NT	319	−
80-22	PN	NT	3, 10	198	−
79-250	PN	NT	3, 10, 12	266	−
79-19	RH	NT	NT	996	−
L-227	RH	NT	NT	70	−
L-216	RH	NT	NT	278	−
L-209	RH	A	NT	127	−
L-203	RH	NT	NT	254	−
L-154	RH	NT	NT	84	−
L-123	RH	A	12	135	−
L-33	RH	NT	NT	644	−

[a] AB, Cutaneous abscess; CO, conjunctivitis; LA, lymphadenitis; PE, peritonitis; PN, pneumonia; PY, pyometritis; RH, rhinitis; TY, tympanitis.

[b] Results are considered positive if the counts per minute are at least eightfold greater than the mean counts of the 44 negative isolates (mean cpm, 675).

[c] NT, Nontypable.

The results of AA-RIA were compared with those of WB analyses, using unadsorbed MAb, adsorbed MAb, and eluted MAb (Table 2). Seven of the 12 positive isolates containing cell surface-exposed and antibody-accessible antigenic determinants as revealed by WB analysis were positive by AA-RIA. The six DB- and WB-negative *P. multocida* isolates were negative for cell surface-exposed antigenic determinants by AA-RIA. To evaluate the antibody accessibility of the antigenic determinant of the five DB-positive AA-RIA-negative *P. multocida* isolates, we used outer membrane vesicles of *P. multocida* UT-1 as antigen and unadsorbed, adsorbed, and eluted MAb 1608 in WB analysis. Each of the isolates was used to prepare adsorbed and eluted MAbs. Antibody activity was detected in unadsorbed and adsorbed MAb 1608 but not in the eluted MAb 1608 (Table 2).

WB and SDS-PAGE analyses of selected DB-positive and DB-negative *P. multocida* isolates. The protein detected by MAb 1608 was compared in 12 DB-positive *P. multocida* isolates by WB analysis, using outer membrane vesicles of individual *P. multocida* isolates as antigen. The proteins reactive with MAb 1608 in these strains migrated identically when resolved by SDS-PAGE in 10% polyacrylamide gel (data not shown). Thirteen other *P. multocida* strains that failed to react with MAb 1608 in DB analysis also did not react with MAb 1608 in WB analysis. The 13 DB- and WB-negative *P. multocida* isolates were further evaluated for the presence of a 37.5-kDa outer membrane protein by SDS-PAGE. Six of the 13 (46%) DB-negative isolates possessed a 37.5-kDa protein, whereas the other 7 isolates (54%) lacked a 37.5-kDa protein.

DISCUSSION

Previous studies suggest that effective *P. multocida* vaccines may be produced only against isolates of the same

TABLE 2. Reactivity of MAb 1608 to 18 *P. multocida* rabbit isolates by DB analysis, WB analysis, and AA-RIA

Isolate	DB reactivity[a]	WB reactivity[b]			AA-RIA reactivity (cpm)[c]	Presence of 37.5-kDa protein
		Unadsorbed MAb	Adsorbed MAb	Eluted MAb		
86-329	+	+	−	+	+ (14,979)	+
85-763	+	+	−	+	+ (13,511)	+
85-423	+	+	−	+	+ (26,707)	+
84-439	+	+	−	+	+ (21,572)	+
84-166	+	+	−	+	+ (7,307)	+
83-171	+	+	−	+	+ (6,116)	+
UT-1	+	+	−	+	+ (30,720)	+
87-1280	+	+	+	−	− (1,324)	+
87-586	+	+	+	−	− (3,821)	+
82-390	+	+	+	−	− (2,702)	+
80-468	+	+	+	−	− (537)	+
L-182	+	+	+	−	− (1,188)	+
86-313	−	+	+	−	− (715)	+
84-252	−	+	+	−	− (935)	−
82-247	−	+	+	−	− (880)	−
81-291	−	+	+	−	− (554)	+
81-228	−	+	+	−	− (1,054)	−
80-492	−	+	+	−	− (891)	−

[a] *P. multocida* cells suspended in water were used as antigens in DB analysis.
[b] Outer membrane vesicles of *P. multocida* UT-1 were reacted with unadsorbed or eluted MAb 1608. Adsorbed and eluted MAb 1608 were prepared by incubating MAb 1608 with various isolates of intact *P. multocida* cells and eluting from *P. multocida* cell-antibody complexes, respectively.
[c] Results are considered positive if the counts per minute are at least eightfold greater than the mean counts of the 44 negative isolates (mean cpm, 675).

somatic (9) or capsular (1, 5) type. The results reported here, however, demonstrate that the protective immunogen recognized by MAb 1608 is expressed by clinical strains representing most of the major somatic types (types 3, 10, 12, and 15) and the two capsular serotypes (A and D) commonly found in North American rabbits. Previous passive immunization studies tend to support the notion that it is the presence of this protein antigenic determinant that affords immunity. Mice were protected from heterologous challenge by two *P. multocida* strains bearing the MAb 1608-recognized antigenic determinant and expressing somatic antigens 3 and 10 and capsular antigens A or nontypable, but they were not protected against challenge by a strain not bearing the determinant and expressing somatic antigens 3 and 10 and nontypable capsular antigens (Lu et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987; Lu et al., submitted). Thus, it confirms that the protein antigenic determinant is one of the targets for protective antibodies.

Of the 153 rabbit *P. multocida* isolates evaluated in this study, 36 (24%) expressed the MAb 1608-recognized antigenic determinant. This result indicates that additional protective antigenic determinants on the 37.5-kDa or other outer membrane proteins of *P. multocida* must be identified and combined to prepare an ideal *P. multocida* vaccine for rabbits, one that provides protection against the broad spectrum of clinical isolates.

The antigen against which vaccine-induced antibodies are directed should be readily accessible on intact cells, thus allowing protective antibodies to immediately identify invading *P. multocida* organisms and act to defend the host. Our previous studies with adsorbed and eluted antibodies in WB analyses demonstrated a cell surface location for the MAb 1608-recognized antigenic determinant on the three strains of *P. multocida* examined (Lu et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987; Lu et al., submitted). The technique proved too cumbersome and time-consuming for screening large numbers of isolates. We therefore adopted the AA-RIA procedure and confirmed its reliability in comparison tests with the WB technique. As expected, all 44 DB-negative isolates were also negative by AA-RIA; however, 5 of the 36 DB-positive isolates were AA-RIA negative. This discrepancy may be explained by inaccessibility of the antigenic determinant in intact *P. multocida* cells. This explanation is supported by the observation that MAb 1608 antibody adsorbed with each of the DB-positive AA-RIA-negative isolates showed no loss of reactivity against homologous antigen (Table 2). In addition, the eluted MAb 1608 for each of the isolates failed to react with homologous antigen. Lysing the *P. multocida* cells for DB analysis may result in mechanical distortion or some other alteration that exposes the antigen that was inacessible to antibody in intact *P. multocida* cells.

Selected DB-negative *P. multocida* isolates were further evaluated for the presence of the 37.5-kDa protein by SDS-PAGE. As predicted, 7 of the 13 DB-negative isolates lacked the protein, but, unexpectedly, the other 6 isolates had the protein. The lack of reactivity may be due to the absence or inaccessibility of the antigen due to steric hindrance by other cell surface macromolecules such as proteins or lipopolysaccharides.

In conclusion, it is encouraging that the *P. multocida* isolates that expressed the protective antigenic determinant also expressed the somatic and capsular antigens commonly associated with disease. Efforts at vaccine development should focus on identification of cell surface, antibody-accessible, protein antigenic determinants that are shared by many somatic and capsular serotypes of *P. multocida* clinical isolates.

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LITERATURE CITED

1. **Bains, R. V. S.** 1955. A preliminary examination of the antigens of *Pasteurella multocida* type 1. Br. Vet. J. **111**:492–498.

2. **Carter, G. R., and S. W. Rundell.** 1975. Identification of type A strains of *P. multocida* using staphylococcal hyaluronidase. Vet. Rec. **96:**343.
3. **Carter, G. R., and P. Subronto.** 1973. Identification of type D strains of *Pasteurella multocida* with acriflavine. Am. J. Vet. Res. **34:**293–294.
4. **Chengappa, M. M., R. C. Meyers, and G. R. Carter.** 1982. Capsular and somatic types of *Pasteurella multocida* from rabbits. Can. J. Comp. Med. **46:**437–439.
5. **Dhanda, M. R.** 1958. Immunization of cattle against hemorrhagic septicemia with purified antigens. Indian Vet. J. **36:**6–8.
6. **DiGiacomo, R. F., L. E. Garlinghouse, Jr., and G. L. Van Hoosier, Jr.** 1983. Natural history of infection with *Pasteurella multocida* in rabbits. J. Am. Vet. Med. Assoc. **183:**1172–1175.
7. **Ey, P. I., S. J. Prowse, and C. R. Jenkin.** 1978. Isolation of pure IgG1, IgG2a, IgG2b immunoglobulins from mouse serum using protein A-sepharose. Immunochemistry **15:**429–436.
8. **Flatt, R. E.** 1974. Bacterial diseases, p. 194–205. *In* S. H. Weisbroth, R. E. Flatt, and A. L. Kraus (ed.), The biology of the laboratory rabbit. Academic Press, Inc., New York.
9. **Heddleston, K. L., and P. A. Rebers.** 1972. Fowl cholera: cross-immunity induced in turkeys with formalin-killed in vivo-propagated *Pasteurella multocida*. Avian Dis. **16:**578–586.
10. **Hunter, W. N., and F. C. Greenwood.** 1972. Preparation of ^{131}I-labeled human growth hormone of high specific activity. Nature (London) **194:**495–496.
11. **Kimura, A., P. A. Gulig, G. H. McCracken, Jr., T. A. Loftus, and E. J. Hansen.** 1985. A minor high-molecular weight outer membrane protein of *Haemophilus influenzae* type b is a protective antigen. Infect. Immun. **47:**253–259.
12. **Lu, Y. S., S. P. Pakes, and L. Massey.** 1987. Hyperimmune serum from rabbits immunized with potassium thiocyanate extract of *Pasteurella multocida* protects against homologous challenge. J. Clin. Microbiol. **25:**2173–2180.
13. **Lu, Y.-S., S. P. Pakes, L. Massey, and C. Stefanu.** 1987. A potassium thiocyanate extract vaccine prepared from *Pasteurella multocida* 3:A protects rabbits against homologous challenge. Infect. Immun. **55:**2967–2976.
14. **Lu, Y.-S., S. P. Pakes, and C. Stefanu.** 1983. Capsular and somatic serotypes of *Pasteurella multocida* isolates recovered from healthy and diseased rabbits in Texas. J. Clin. Microbiol. **18:**292–295.
15. **Lu, Y. S., D. H. Ringler, and J. S. Park.** 1978. Characterization of *Pasteurella multocida* isolates from the nares of healthy rabbits and rabbits with pneumonia. Lab. Anim. Sci. **28:**692–697.
16. **McDade, R. L., Jr., and K. H. Johnson.** 1980. Characterization of serologically dominant outer membrane proteins of *Neisseria gonorrhoeae*. J. Bacteriol. **141:**1183–1191.
17. **Ringler, D. H., G. K. Peter, C. E. Chrisp, and D. Keren.** 1985. Protection of rabbits against experimental pasteurellosis by vaccination with a potassium thiocyanate extract of *Pasteurella multocida*. Infect. Immun. **49:**498–504.
18. **Robertson, S. M., C. F. Frish, P. A. Gulig, J. R. Kettman, K. H. Johnson, and E. J. Hansen.** 1982. Monoclonal antibodies directed against a surface-exposed outer membrane of *Haemophilus influenzae* type b. Infect. Immun. **36:**80–88.
19. **Towbin, H., T. Staehelin, 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.