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Production and Characterization of Monoclonal Antibodies Specific for Lipooligosaccharide of *Serpulina hyodysenteriae*

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***Serpulina* (*Treponema*) *hyodysenteriae* is the causative agent of swine dysentery, a contagious mucohemorrhagic disease of the colon. Diagnosis of swine dysentery is extremely difficult because of the presence of cross-reactive antibodies to the proteins of *S. hyodysenteriae* and *Serpulina innocens*, a nonpathogenic inhabitant of the porcine large intestine. Therefore, monoclonal antibodies (MAbs) against the serotype-specific lipooligosaccharide (LOS) antigens of *S. hyodysenteriae* were produced to rapidly differentiate *S. hyodysenteriae* from *S. innocens*. Whole-cell preparations of *S. hyodysenteriae* serotypes 1 through 7 were used as antigens. MAbs were characterized by an indirect enzyme-linked immunosorbent assay with whole-cell or LOS antigen and by Western blot (immunoblot) analysis with whole-cell lysates as antigen. A total of 12 LOS-specific MAbs which could identify and differentiate the seven original serotypes of *S. hyodysenteriae* were produced. The MAb serospecificities are as follows: MAb 9G8, serotype 1; MAb 31D9, serotype 2; MAb 7D3, serotypes 2 and 7; MAb 24B7, serotype 3; MAb 13C2, serotype 4; MAb 18E9, serotype 4; MAb 2B7, serotype 6; MAb 1D2, serotypes 2, 5, and 7; MAb 9C5, serotypes 2, 5, and 7; MAb 11C9, serotype 7; MAb 11E10, serotype 7; and MAb 6G11, serotype 7.**

Serpulina (*Treponema*) *hyodysenteriae* is an anaerobic, beta-hemolytic spirochete found in the porcine large intestine (21). The spirochete is the etiological agent of swine dysentery (5, 7, 8), a contagious disease characterized by a mucohemorrhagic diarrhea (5, 8). *Serpulina innocens*, a nonpathogenic inhabitant of the porcine large intestine, is very similar to *S. hyodysenteriae* in both morphology and growth characteristics and shares many of the same surface antigens (3, 11, 15, 20). Numerous serological tests with sera from pigs that have recovered from *S. hyodysenteriae* infection have demonstrated the presence of cross-reactive antibodies between *S. hyodysenteriae* and *S. innocens* (10, 13, 14). The cross-reactions are known to be due to similar protein antigens, specifically, axial filament (endoflagella) proteins (19). This cross-reactivity has made the differentiation of *S. hyodysenteriae* and *S. innocens* difficult (9). One group of antigens that is not shared between the two spirochetes is the lipooligosaccharide (LOS) complex. This complex can be extracted from the cell wall of the spirochete with hot (68°C) phenol (90%)–water and is recognized by homologous serotype-specific sera from convalescent-phase swine (1, 2, 25). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining of *S. hyodysenteriae* LOS have revealed two characteristic bands that ranged between 18 and 24 kDa, with the separation differences being unique to each serotype. Seven serotypes of *S. hyodysenteriae* were originally identified on the basis of LOS reactions to hyperimmune rabbit sera, and an additional two serotypes have recently been reported from Canada (23, 24).

The production of monoclonal antibodies (MAbs) which would differentiate *S. hyodysenteriae* from *S. innocens* would be useful as a diagnostic tool and would provide a more efficient method of classifying *S. hyodysenteriae* isolates into their respective serotypes. Therefore, the objectives of the research described here were to produce and characterize MAbs specific for the seven original serotypes of *S. hyodysenteriae.*

MATERIALS AND METHODS

Bacteria and culture conditions. The strains of *Serpulina* spp. used for the production and characterization of MAbs are described in Table 1. Each strain of all the serotypes was obtained from as many sources as possible to eliminate strain-specific MAbs (18). In addition, isolates of low and high passage numbers were used to make sure that all of the LOS epitopes were part of the antigen pool. Both high- and low-passage-number strains B204 and B256 as well as lyophilized strains B78, B234, B169, ACK300/8, B256, and B1555a were provided by Michael J. Wannemuehler, Iowa State University, Ames. High-, medium-, and low-passage-number strains B204, B234, FM 88-90, FMV 89-3323, B256, B359, B1555a, and 4/71 were provided by Gerald E. Duhamel, University of Nebraska, Lincoln. High- and low-passage-number strain B140; low-passage-number strains B78, G-Strain, B204, DJ215, B169, A1, B8044, B6933, ACK300/8, and B1555a; and purified LOSs from strains B204, B6933, B169, G-Strain, A1, B1555a, B8044, ACK300/8, B140, and B256 were from the laboratory of Lynn A. Joens, University of Arizona, Tucson. Strains B204, B78, and B256 were provided by Neil S. Jensen and Thad B. Stanton, National Animal Disease Center, Ames, Iowa. Strains B78, B234, B204, and B256 were also purchased from the American Type Culture Collection (ATCC; Rockville, Md.). A field strain isolated (low passage number) at the Department of Veterinary Diagnostic Investigation, Kansas State University, was used to produce MAbs specific for serotype 2.

Cultures were grown on Trypticase soy agar with 5% whole sheep blood (blood agar plate [BAP]) in anaerobe jars at 37°C. BJ agar (BAP with 5% pig feces extract and five antimicrobial agents: spiramycin [25 mg/ml], rifampin [12.5 mg/ml], vancomycin [6.25 mg/ml], colistin [6.25 mg/ml], and spectinomycin [200 mg/ml]) (22) was used for the isolation of *S. hyodysenteriae* from one clinical specimen.

Preparation of antigens. The antigen used for hyperimmunization of mice consisted of plate (BAP)-harvested cells (whole-cell antigen) resuspended in physiological saline. Essentially, the cells were harvested by washing the surfaces of BAPs with saline. Suspensions of spirochetes were placed in sterile tubes and were incubated for 3 days aerobically to kill the spirochetes. Suspensions were tested for contamination and spirochete death by incubation on BAPs at 37°C for 3 days under both aerobic and anaerobic conditions. Suspensions were then pooled, and a protein determination was performed on a small aliquot by the Pierce bicinchoninic acid (Pierce, Rockford, Ill.) method. The cells were adjusted with sterile saline to 0.5 mg/ml on the basis of the protein content and were stored frozen at −20°C.

Lysates of *S. hyodysenteriae* and *S. innocens* were produced as described by Joens et al. (12). Essentially, the cells were cultured for 24 h in Trypticase soy

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TABLE 1. *S. hyodysenteriae* strains used to evaluate MAb specificity[a]

Strain	Serotype	Origin
B78[b]	1	Iowa
B234[c]	1	Missouri
G	1	Mexico
Den191[d]	1	Denmark
B140	2	Minnesota
B204[e]	2	Iowa
DJ215	2	Japan
KSU 90-17491	2	Kansas
B169	3	Canada
A1	4	England
B8044	5	Missouri
B6933	6	Illinois
ACK 300/8	7	The Netherlands
FM 88-90	8	Canada
FMV 89-3323	9	Canada

[a] The MAbs were also tested against *S. innocens* B256 (U.S. type strain, ATCC 29796) and 4/71 (type strain in the United Kingdom) and weakly beta-hemolytic *Serpulina* sp. strains B359 and B1555.
[b] Type strain, ATCC 27164.
[c] ATCC 31287.
[d] Tested only by Western blot analysis with MAb 9G8.
[e] ATCC 31212.

broth supplemented with 5% fetal bovine serum under an atmosphere of H_2-CO_2 (50:50) at 37°C. The cells were harvested by centrifugation, resuspended in sterile phosphate-buffered saline (PBS), and lysed by sonication (low setting for 10 min in an ice bath). The lysate was adjusted to 0.5 mg of protein per ml and was stored at −70°C until it was used for the enzyme-linked immunosorbent assay (ELISA) and Western blots (immunoblots).

LOS was extracted from *S. hyodysenteriae* (2) and was purified as described by Halter and Joens (6). Cells were harvested and extracted with hot (68°C) phenol-water. The LOS was pelleted from the aqueous fraction by ultracentrifugation (30,000 × *g*) for 2 h. The pellets were resuspended in a minimum volume of sterile distilled H_2O, adjusted to 100 μg of carbohydrate per ml, and stored at 4°C until they were used for the ELISA.

Injection of mice. BALB/c mice were purchased from Charles River and were hyperimmunized with whole cells (0.25 mg of protein per ml) of either *S. hyodysenteriae* or *S. innocens* mixed with Ribi adjuvant (0.5 mg of MPL [monophosphoryl lipid A], 0.5 mg of S-TDM [synthetic trehalose dicorynomycolate], 40 μl of Squalene, and 0.2% Tween 80 in water; Ribi Immunochem, Hamilton, Mont.). Following the Ribi protocol, female BALB/c mice received 0.2 ml of immunogen intraperitoneally on days 0, 21, and 35. An additional intravenous injection was administered 3 days prior to the fusion of the splenocytes. The mice were then sacrificed by CO_2 asphyxiation for hybridoma production or were rested for 4 to 6 months before repeating the intravenous injection procedure and sacrificing the mice by CO_2 asphyxiation.

Production of hybridomas. Microtiter plates were prepared for the fusion by adding 3×10^3 mouse peritoneal macrophages in Dulbecco's modified Eagle medium supplemented with hypoxanthine-aminopterin-thymidine medium (Sigma, St. Louis, Mo.) to each well on the day before the fusion. The plates were incubated in 8.5% CO_2 at 37°C. Hybridomas were produced by fusing unfractionated splenocytes with 2×10^8 Ag8 myeloma cells (X63Ag8.653; ATCC) or half of the splenocytes with 1×10^7 Ag8 cells with polyethylene glycol (molecular weight, 1,300 to 1,600; Sigma) (4). The fusion mixture was distributed equally into 2,400 wells for a whole spleen or 1,200 wells for a half spleen in 96-well plates.

Preparation of ELISA plates for hybridoma screening. The titer of the coating antigen (0.5 mg of total protein per ml) was determined by a standard block titration with MAbs specific for genus-specific flagellar polypeptides (data not shown). By this method, whole-cell suspensions of the various spirochete strains could be standardized on the basis of the amount of axial filament protein. The optimum coating concentration of the spirochetes was determined to be 0.5 μg per well (total protein) for most suspensions. Plates were coated for 4 h at 37°C, washed (PBS plus 0.1% Tween 80 and 0.5% horse serum), and stored with the coating antigen at −20°C.

ELISA for screening of hybridomas. The ELISA method for the screening of hybridomas has previously been described in detail (27, 28). Briefly, 0.1 ml of spent hybridoma medium was added to wells coated with spirochetes. Following incubation for 10 min at 37°C, the wells were washed seven times (PBS plus 0.1% Tween 80 and 0.5% horse serum), and the spirochete antigens were reacted with 0.1 ml of anti-mouse immunoglobulin G (IgG) (heavy and light chains) horseradish peroxidase conjugate (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) diluted 1:2,500 in washing buffer. The reaction mixture was incubated for 10 min at 37°C, washed as described above, and developed by adding substrate (H_2O_2 plus ABTS [2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid)] in citrate buffer; pH 4; solutions B and A, respectively; Kirkegaard & Perry Laboratories). A_{414} readings were taken with an automatic plate reader (MR 600; Dynatech Instruments, Inc., Torrance, Calif.) following incubation times of 10, 20, 40, and 60 min at room temperature. An optical density reading of 0.05 or higher was considered a positive reaction.

Evaluation of MAb specificity. MAbs positive for the reference strains were also screened for their cross-reactivities to other serotypes and species by the ELISA. Plates were coated with lysates from the seven reference strains of *S. hyodysenteriae* (B234, B204, B169, A1, B8044, B6933, and ACK300/8), the *S. innocens* type strain B256, and lysates from the weakly beta-hemolytic strains B359 and B1555a as well as a homogenate of a BJ agar plate. The ELISA was performed as described above.

MAbs were also reacted with purified LOS (17) in the ELISA (as described above) to examine the specificity of the MAb for the LOS antigen. Wells were coated with LOS from strains of each of the seven serotypes at a concentration of 250 ng per well for 4 h at 37°C. Positive reactions were predetermined (data not shown) and included those reactions with optical densities of 0.05 and higher. MAbs showing specificity for a member(s) of a given serotype were then evaluated by Western blot analysis on whole-cell lysates.

SDS-PAGE and Western blotting. The method used for SDS-PAGE and Western blot analysis has previously been described in detail (16, 18). Hybridoma supernatants were diluted 1:2 in 5% milk (Lucerne powdered milk diluted in distilled H_2O) and were incubated with the blots overnight at 25°C. Following three washes (PBS and 0.1% Tween 20) (17), biotinylated anti-mouse IgG (heavy and light chains; Kirkegaard & Perry Laboratories) diluted 1:400 was reacted with the blots for 45 min at 25°C. After an additional three washes, horseradish peroxidase-labeled avidin (Kirkegaard & Perry Laboratories) diluted 1:400 in washing buffer was added to the blots for 45 min at 25°C. The blots were developed by adding substrate consisting of H_2O_2 (solution B) and 4-chloro-1-naphthol (solution A) (50:50) (Kirkegaard & Perry Laboratories).

RESULTS

ELISA with lysates of whole spirochetes. The reactions of the hybridoma supernatants against lysates of *Serpulina* spp. are given in Table 2. MAbs 9G8, 7D3, 24B7, 13C2, 18E9, and 2B7 reacted with *S. hyodysenteriae* serotypes 1, 2, 3, 4, 4, and 6, respectively, and demonstrated specificity for a single serotype. MAbs 1D2 and 9C5 showed multiple specificities for serotypes 2, 5, and 7. None of the eight MAbs tested reacted with *S. innocens* B256 or with weakly beta-hemolytic spirochete strain B359 or B1555a by the ELISA.

ELISA with purified lipopolysaccharide. The reactions of the hybridoma supernatants against hot phenol-water-extracted LOS (250 ng) are given in Table 3. The results are consistent with those of the ELISA in which whole-cell lysates were used as coating antigen and were also consistent with the results of an indirect fluorescent-antibody test (IFAT) with intact acetone-fixed spirochetes (data not shown), with the following exceptions. MAb 7D3 reacted with the LOS of serotype 7 but not with whole-cell lysates of serotype 7. This cross-reactivity was also demonstrated by IFAT, in which weak fluorescence of some spirochetes was observed and which appeared to be the result of a wide variation in the level of expression of the cross-reactive epitope. MAbs 1D2 and 9C5 both produced a strong positive reaction to serotype 5 LOS in the ELISA with whole-cell lysates, reacted to serotype 5 LOS on Western blots (Fig. 1C) (the Western blot for MAb 9C5 is not shown), and produced strong fluorescence on intact spirochetes in the IFAT. The low (MAb 1D2) and negative (MAb 9C5) ELISA reactions with serotype 5 LOS could not be explained, unless the cross-reactive epitope was damaged by the hot phenol-water in the purification process. The low serotype-specific reactivity of MAb 2B7 in ELISAs with both whole-cell lysates and LOS of serotype 6 could not be explained because the MAb reacted well by Western blotting (Fig. 1F) and produced strong fluorescence in the IFAT. Additionally, MAbs 11C9, 11E10, and 6G11, which showed serotype specificity for serotype 7, and MAb 31D9, which was serotype specific for

TABLE 2. Results of testing MAbs in an indirect ELISA with lysates of *S. hyodysenteriae* or *S. innocens* as antigen[a]

Strain	Serotype	Hybridoma no.							
		9G8	7D3	24B7	13C2	18E9	2B7	1D2	9C5
B234	1								
G	1	0.210	0.007	0.005	0.002	0.004	0.009	−0.001	−0.003
B204	2	0.000	0.250	0.003	0.001	0.000	0.002	0.161	0.138
90-17491	2	0.001	0.171	0.001	−0.010	0.003	0.001	−0.005	−0.002
B169	3	−0.005	0.005	0.288	−0.009	−0.002	0.004	−0.006	0.011
A1	4	0.000	0.002	−0.001	0.063	0.165	0.002	0.000	0.005
B8044	5	−0.003	0.002	0.001	−0.005	−0.006	−0.006	0.143	0.192
B6933	6	0.018	0.000	0.004	−0.003	−0.003	0.072	−0.002	0.003
ACK300/8	7	0.003	0.013	0.005	−0.011	−0.006	−0.007	0.658	0.478
B256[b]		0.012	0.002	0.002	−0.015	−0.007	−0.013	0.000	−0.008
B359[b]		0.000	0.010	0.000	0.000	0.000	−0.013	0.003	0.002
1555a[b]		−0.002	0.004	0.002	−0.007	0.000	−0.005	0.007	−0.008

[a] Wells were coated with 0.5 μg of lysate on the basis of protein content. Numbers enclosed in boxes are positive values.
[b] Nonpathogenic porcine strain.

serotype 2 in the ELISA with whole-cell lysates, Western blots, and IFAT (data not shown), also showed serotype specificities in the ELISA with LOS. MAb 31D9 was the only MAb that showed serotype specificity for serotype 2 in all of the assays which were used. The high background of MAb 1D2 on *S. innocens* B256 of 0.025 was considered to be an artifact of nonspecific binding because there was no cross-reactivity on Western blots (Fig. 1C) and no fluorescence was observed on the IFAT. A summary of the serotype specificities and isotypes of the 12 LOS-specific MAbs is given in Table 4.

Western blot analysis. Figure 1 shows the reactions of representative MAb supernatants 9G8 (serotype 1), 7D3 (serotype 2), 24B7 (serotype 3), 13C2 (serotype 4), 2B7 (serotype 6), and 1D2 (serotype 7), all of which produced by Western blotting bands to their corresponding serotypes at the 16- to 19-kDa range. Again, MAbs 9G8, 24B7, 13C2, and 2B7 showed specificities for single serotypes, while MAb 7D3 showed reactivity to both serotypes 2 and 7 and MAb 1D2 showed cross-reactivity to serotypes 2, 5, and 7. The minor bands in the higher-molecular-mass range were demonstrated to be biotin by the addition of labeled avidin in the absence of biotinylated anti-mouse IgG (heavy and light chains) (data not shown).

DISCUSSION

The data presented here demonstrate the LOS specificity of the nine MAbs to serotypes 1, 2, 3, 4, 6, and 7 of *S. hyodysenteriae*. Cross-reactivity was demonstrated with the three other MAbs, MAbs 7D3, 1D2, and 9C5, to serotypes 2 and 7 or serotypes 2, 5, and 7. MAbs 9C5 and 1D2 were derived from a serotype 7 *S. hyodysenteriae* fusion, while MAb 7D3 was produced from a serotype 2 fusion. Apparently, shared LOS epitopes are present on these isolates, which is consistent with the work of Mapother and Joens (25). They demonstrated the cross-reactivities of LOSs extracted from *S. hyodysenteriae* serotypes 5 and 7 to rabbit antisera specific for serotype 2 LOS. They further demonstrated that adsorption of the rabbit sera with serotype 2 LOS removed the reaction. Epitope specificity studies would have to be performed in order to confirm that the cross-reaction is to the same epitope. No MAbs which reacted specifically to serotype 5 LOS were generated. However, positive identification of serotype 5 isolates is possible by performing elimination experiments with serotype-specific MAbs for serotype 2 (MAb 31D9) and serotype 7 (MAbs 11C9, 11E10, and 6G11) *S. hyodysenteriae.*

The difficulty in producing MAbs specific for the LOS of *S. hyodysenteriae* was believed to be due to the competitiveness of the immunodominant protein antigens of the endoflagella. It was estimated that the ratio of producing the 12 unique LOS antibody-secreting hybridomas was 1:500. In addition, a majority of the hybridomas that were produced secreted antibodies specific for sheep blood antigens present in the culture medium. This was because the spirochete preparations used for hyperimmunization and screening were neither washed nor centrifuged so that essential epitopes on the LOS would not be lost or damaged.

TABLE 3. Results of testing MAbs in an indirect ELISA with LOS from *S. hyodysenteriae* as antigen[a]

Strain	Serotype	Hybridoma no.											
		9G8	7D3	31D9	24B7	18E9	13C2	2B7	1D2	9C5	11C9	11E10	6G11
G	1	0.151	0.033	0.018	0.032	0.019	0.020	0.022	0.020	0.004	0.014	0.009	0.010
B204	2	0.017	0.416	0.068	0.021	0.016	0.019	0.034	0.078	0.018	0.010	0.008	0.010
B140	2	−0.007	0.638	0.255	0.016	0.022	−0.009	−0.022	0.128	0.230	0.000	−0.003	−0.002
B169	3	−0.007	0.023	−0.004	0.154	0.022	0.017	0.010	0.016	−0.005	0.007	0.003	0.009
A1	4	−0.000	0.024	−0.005	0.021	0.577	0.217	0.017	0.019	0.001	0.008	0.004	0.004
8044	5	−0.006	0.005	−0.006	0.018	0.017	−0.002	0.015	0.030	0.013	0.000	−0.004	−0.007
6933	6	0.000	−0.001	0.016	0.016	0.014	0.010	0.059	0.013	−0.011	0.001	0.000	−0.001
ACK300/8	7	0.013	0.133	0.028	0.007	0.010	−0.008	0.008	0.794	0.714	0.435	0.371	0.466
B256[b]		0.019	0.021	0.000	0.001	0.017	−0.017	0.016	0.025	−0.009	0.013	0.009	0.011
1555[b]		−0.002	0.004	0.000	0.005	0.018	−0.014	0.016	0.010	−0.011	0.011	0.012	0.007

[a] Wells were coated with 250 ng of LOS on the basis of the carbohydrate content. Numbers enclosed in boxes are considered positive values.
[b] Nonpathogenic porcine spirochetes.

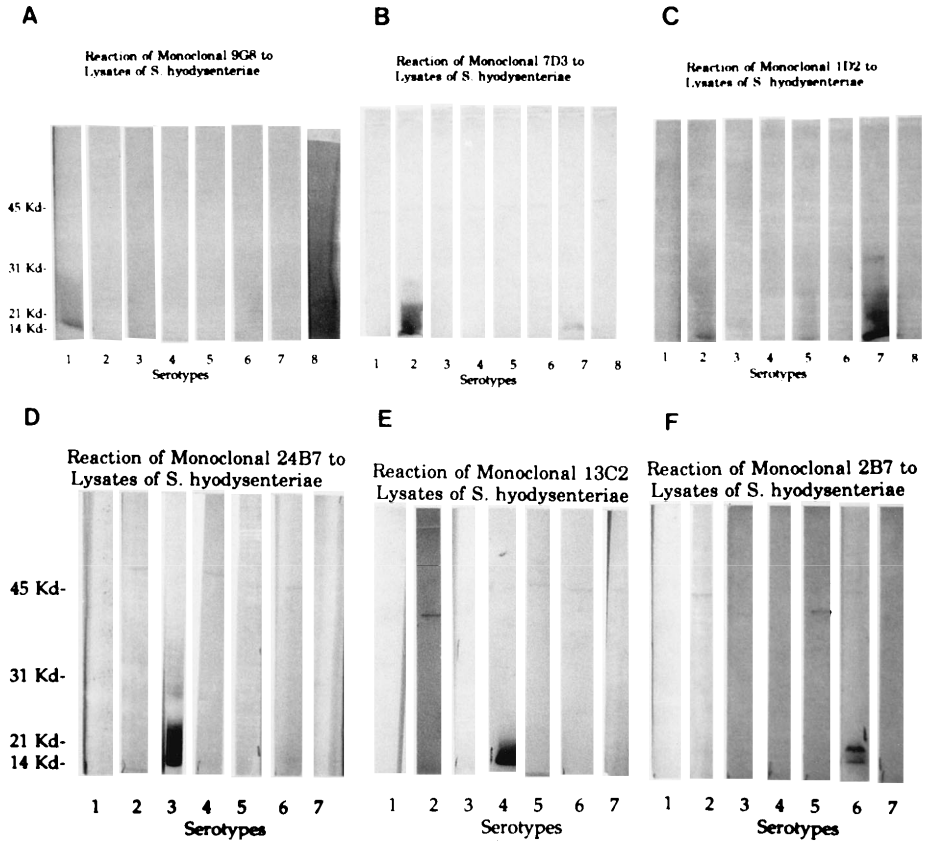

FIG. 1. Western blots of *S. hyodysenteriae* LOS reacted to MAb 9G8 (A), MAb 7D3 (B), MAb 1D2 (C), MAb 24B7 (D), MAb 13C2 (E), and MAb 2B7 (F). The serotype-specific reaction was of each MAb to a diffuse antigen of between 14 and 18 kDa.

The major difficulty in the diagnosis of swine dysentery is that polyclonal antisera produced against *S. hyodysenteriae* cross-react strongly with the nonpathogenic spirochete *S. innocens* and other nonpathogenic intestinal spirochetes in swine. In addition, the poor quality of the specimens received by the diagnostic laboratory inhibits isolation of the spirochete in culture (26). The MAbs described in this report are specific for the organism and could be used in a capture assay or a fluorescent-antibody assay to rapidly distinguish *S. hyodysenteriae* isolates from other contaminating intestinal spirochetes.

No commercial diagnostic reagents are available for the identification of *S. hyodysenteriae* spirochetes in specimens from swine. Collectively, the MAbs described here provide a method of rapidly screening specimens for *S. hyodysenteriae* serotypes 1 through 7. In addition, spirochetes identified with this pool of MAbs can subsequently be serotyped with each MAb separately. This will provide researchers with an epidemiological tool that can be used to map the serotype involved in an outbreak of swine dysentery.

TABLE 4. MAb isotypes and serotype specificities for the LOS of *S. hyodysenteriae*

Immunogen	Apparent serotype specificity	Hybridoma	Isotype
B234 (ATCC)	1	9G8	IgM
KSU 90-17491	2, 7	7D3	IgG
KSU 90-17491	2	31D9	IgM
B169	3	24B7	IgG
A1	4	13C2	IgG
A1	4	18E9	IgG
B6933	6	2B7	IgG
ACK300/8	7, 5, 2	1D2	IgM
ACK300/8	7, 5, 2	9C5	IgM
ACK300/8	7	11C9	IgG
ACK300/8	7	11E10	IgG
ACK300/8	7	6G11	IgG

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REFERENCES

1. **Alderton, M. R., S. C. Smith, and P. J. Coloe.** 1993. Identification of a novel group of *Serpulina hyodysenteriae* isolates by using a lipopolysaccharide-specific monoclonal antibody. J. Clin. Microbiol. **31:**1326–1328.
2. **Baum, D. H., and L. A. Joens.** 1979. Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect. Immun. **25:**792–796.
3. **Combs, B. G., and D. J. Hampson.** 1991. Use of a whole chromosomal probe for identification of *Treponema hyodysenteriae*. Res. Vet. Sci. **50:**286–289.
4. **Fazekas De St. Groth, S., and D. Scheidegger.** 1980. Production of monoclonal antibodies: strategy and tactics. J. Immunol. Methods **35:**1–21.
5. **Glock, R. D., and D. L. Harris.** 1972. Swine dysentery. II. Characterization of lesions in pigs inoculated with *Treponema hyodysenteriae* in pure and mixed cultures. Vet. Med. Small Anim. Clin. **67:**65–68.
6. **Halter, M. R., and L. A. Joens.** 1988. Lipooligosaccharides from *Treponema hyodysenteriae* and *Treponema innocens*. Infect. Immun. **56:**3152–3156.
7. **Harris, D. L., R. D. Glock, C. R. Christensen, and J. M. Kinyon.** 1972. Swine

dysentery. I. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. Vet. Med. Small Anim. Clin. **67:**61–64.
8. **Harris, D. L., and R. J. Lysons.** 1992. Swine dysentery. *In* A. Leman, B. Straw, W. Mengeling, S. D'Allaire, and D. Taylor (ed.), Diseases of swine, 7th ed. Iowa State University Press, Ames.
9. **Hudson, M. J., T. J. L. Alexander, and R. J. Lysons.** 1976. Diagnosis of swine dysentery: spirochaetes which may be confused with *Treponema hyodysenteriae*. Vet. Rec. **99:**498–500.
10. **Hunter, D., and C. N. Saunders.** 1977. Diagnosis of swine dysentery using absorbed fluorescent antiserum. Vet. Rec. **101:**303–304.
11. **Jensen, N. S., T. A. Casey, and T. B. Stanton.** 1990. Detection and identification of *Treponema hyodysenteriae* by using oligodeoxynucleotide probes complementary to 16S rRNA. J. Clin. Microbiol. **28:**2717–2721.
12. **Joens, L. A., D. L. Harris, and D. H. Baum.** 1979. Immunity to swine dysentery in recovered pigs. Am. J. Vet. Res. **40:**1352–1354.
13. **Joens, L. A., D. L. Harris, J. M. Kinyon, and M. L. Kaeberle.** 1978. Microtitration agglutination for detection of *Treponema hyodysenteriae* antibody. J. Clin. Microbiol. **8:**293–298.
14. **Joens, L. A., J. M. Kinyon, D. H. Baum, and D. L. Harris.** 1978. Immunofluorescent studies on *Treponema hyodysenteriae*, p. M.2. *In* Proceedings of the International Pig Veterinary Society. International Pig Veterinary Society, Zagreb, Yugoslavia.
15. **Joens, L. A., and R. B. Marquez.** 1986. Molecular characterization of proteins from porcine spirochetes. Infect. Immun. **54:**893–896.
16. **Joens, L. A., R. B. Marquez, and M. Halter.** 1993. Comparison of outermembrane fractions of *Serpulina* (*Treponema*) *hyodysenteriae*. Vet. Microbiol. **35:**119–132.
17. **Joens, L. A., N. A. Nord, J. M. Kinyon, and I. T. Egan.** 1982. Enzyme-linked immunosorbent assay for detection of antibody to *Treponema hyodysenteriae* antigens. J. Clin. Microbiol. **15:**249–252.
18. **Joens, L. A., and M. E. Nuessen.** 1986. Bactericidal effect of normal swine sera on *Treponema hyodysenteriae*. Infect. Immun. **25:**757–760.
19. **Kent, K. A., R. Sellwood, R. M. Lemcke, M. R. Burrows, and R. J. Lysons.** 1989. Analysis of the axial filaments of *Treponema hyodysenteriae* by SDS-PAGE and immunoblotting. J. Gen. Microbiol. **135:**1625–1632.
20. **Kinyon, J. M., and D. L. Harris.** 1979. *Treponema innocens*, a new species of intestinal bacteria and emended description of the type strain of *Treponema hyodysenteriae*. Int. J. Syst. Bacteriol. **29:**102–109.
21. **Kinyon, J. M., D. L. Harris, and R. D. Glock.** 1977. Enteropathogenicity of various isolates of *Treponema hyodysenteriae*. Infect. Immun. **15:**638–646.
22. **Kunkle, R. A., and J. M. Kinyon.** 1988. Improved selective medium for the isolation of *Treponema hyodysenteriae*. J. Clin. Microbiol. **26:**2357–2360.
23. **Li, Z., M. Belanger, and M. Jacques.** 1991. Serotyping of Canadian isolates of *Treponema hyodysenteriae* and description of two new serotypes. J. Clin. Microbiol. **29:**2794–2797.
24. **Li, Z., N. Jensen, M. Belanger, M. L'Esperance, and M. Jacques.** 1992. Molecular characterization of *Serpulina* (*Treponema*) *hyodysenteriae* isolates representing serotypes 8 and 9. J. Clin. Microbiol. **30:**2941–2947.
25. **Mapother, M. E., and L. A. Joens.** 1985. New serotypes of *Treponema hyodysenteriae*. J. Clin. Microbiol. **22:**161–164.
26. **Taylor, D. J., R. J. Lysons, J. Bew, R. Stevenson, and R. M. Lemcke.** 1985. Survival of *Treponema hyodysenteriae* in samples of dysenteric pig feces sent by post and stored at room temperature. Vet. Rec. **116:**48–49.
27. **Westerman, R. B., G. W. Fortner, K. W. Mills, R. M. Phillips, and J. M. Greenwood.** 1993. Use of monoclonal antibodies specific for the ā determinant of K88 pili for detection of enterotoxigenic *Escherichia coli* in pigs. J. Clin. Microbiol. **31:**311–314.
28. **Westerman, R. B., K. W. Mills, R. M. Phillips, G. W. Fortner, and J. M. Greenwood.** 1988. Predominance of the ac variant in K88-positive *Escherichia coli* isolates from swine. J. Clin. Microbiol. **26:**149–150.