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Identification of a Novel Group of *Serpulina hyodysenteriae* Isolates by Using a Lipopolysaccharide-Specific Monoclonal Antibody

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A monoclonal antibody to *Serpulina hyodysenteriae* 8930 was produced and was used to probe pronase-treated cell lysates of *S. hyodysenteriae* isolates in immunblots. The results showed that the monoclonal antibody was specific for only five closely related *S. hyodysenteriae* isolates: 8930, 5380, 70A, RMIT 88, and RMIT 97.

Serpulina hyodysenteriae is the etiological agent of swine dysentery (6, 13). The isolates of the organism were initially divided into four serotypes (1) on the basis of an agarose double gel immunodiffusion test (ADGP) with lipopolysaccharide (LPS) extracted from each isolate and hyperimmune rabbit sera raised to selected isolates. Subsequently, more serotypes have been identified by this immunodiffusion test (9).

Unfortunately, some new serotypes cross-react with the rabbit antisera raised against reference strains of serotypes 1 and 2. This cross-reactivity can be eliminated by cross-adsorbing the rabbit sera raised against any new serotypes with the reference strains of serotypes 1 and 2. However, the need for cross-adsorption indicates that the rabbit antiserum typing system has some anomalies and so needs to be reviewed or a more discriminating typing system needs to be devised.

Hampson et al. (4, 5) suggested that the rabbit antiserum typing system be revamped, proposing that *S. hyodysenteriae* isolates be classified into five serogroups, with each serogroup being defined by the reactivities of their LPSs with rabbit antisera raised to reference strains for each serogroup.

Recently, Hampson (3) has extended the number of serogroups to eight, with some serogroups represented by one isolate. However, this system does not address the problems of the rabbit antiserum typing system. Consequently, there is a need for an alternative *S. hyodysenteriae* typing scheme. The aim of the present work was to produce monoclonal antibodies to *S. hyodysenteriae* 8930, a reference strain for a group of isolates (designated *S. hyodysenteriae* 5380-like) that were similar but difficult to type by the existing rabbit antiserum typing system.

Monoclonal antibodies to *S. hyodysenteriae* 8930 were produced by the method of Goding (2) by using polyethylene glycol 1500 (Boehringer Mannheim) as the fusing agent. Hybridomas were selected by using Dulbecco's modification of Eagles medium containing hypoxanthine, aminopterin, and thymidine (8). Hybridoma supernatants were screened for antibodies to *S. hyodysenteriae* 8930 by an enzyme-linked immunosorbent assay (ELISA) with sonicated *S. hyodysenteriae* 8930 as the antigen (5 μg per well). Hybridomas that produced antibodies to *S. hyodysenteriae* 8930 were cloned by limiting dilution and were used to produce monoclonal antibody ascites in male BALB/c mice (2).

The *S. hyodysenteriae* isolates examined in immunoblots with monoclonal antibodies to *S. hyodysenteriae* 8930 were obtained from veterinary laboratories in Australia and overseas or were isolated from pigs with clinical swine dysentery. Isolation and maintenance of these isolates were performed by the methods of Songer et al. (12). The other enteric bacteria used in the present study were grown on nutrient agar plates or blood agar plates incubated at 37°C (11). Table 1 lists the *S. hyodysenteriae* isolates used in the present study. In addition, the following enteric bacteria were used in the present study: *Bacillus subtilis*, *Campylobacter coli*, *Campylobacter fetus* subspecies *fetus*, *Campylobacter fetus* subspecies *veneralis*, *Campylobacter hyointestinalis*, *Campylobacter jejuni*, *Campylobacter mucosalis*, *Campylobacter pyloridis*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas vulgaris*, *Serpulina innocens*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella flexneri*, *Shigella sonnei*, *Yersina enterocolitica*, and *Serratia marcescens*.

Bacterial cell lysates, which were used as the antigen in immunoblots, were prepared by harvesting and washing (three times) the organisms in 10 mM Tris-HCl (pH 7.4) and boiling them for 5 min in 100 mM Tris-HCl–15% glycerol–2% sodium dodecyl sulfate (SDS)–2 mM phenylmethylsulfonyl fluoride (pH 6.8). The supernatants (cell lysates) were retrieved by centrifugation at 13,500 rpm in an Eppendorf centrifuge. Estimation of the protein content of cell lysates was determined by a modification of the Lowry method (10). Pronase-treated cell lysates were prepared by incubating 200 μg of the cell lysate with 20 μg of pronase (Boehringer Mannheim) for 16 h at 37°C.

SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting (Western blotting) of cell lysates and pronase-treated cell lysates were performed by the methods of Laemmli (7) and Towbin et al. (14), respectively.

Screening of hybridoma supernatants by ELISA detected 21 that contained antibodies to *S. hyodysenteriae* 8930. Ten supernatants that were strongly positive by ELISA were used in separate immunoblots with *S. hyodysenteriae* 8930 cell lysate as the antigen.

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TABLE 1. *S. hyodysenteriae* isolates used in the study

Serotype	Strain (source)[a]
1	B234 (USA), B78 (USA), Wa2528, L7273, 1415, 1165-86, 1097-86
2	B204 (USA) 933, 2612, (NSW) 4943, 3636-87, 924, 3460, 2513, 1039-86, 33249B, 33243B, 508-86, 33243A, 907, 31212 (USA, ATCC) 32486C, 33241B, 376, 320-19A
3	B169 (Canada)
4	A1, (UK) Q5392, Q5363
5	B8044 (USA)
6	B6933 (USA)
5380-like	5380, 8930, 70A, Q9374, Q4662, Q7308, 947, RMIT88, RMIT97, T11W2, 1155

[a] Unless stated otherwise, isolates were from Victoria, Australia. Isolate designations beginning with the letter Q, e.g., Q5392, were from Queensland, Australia; USA, United States; NSW, New South Wales, Australia; UK, United Kingdom. ATCC, B204 isolate from the American Type Culture Collection.

The present investigation indicated that each hybridoma produces antibodies that recognized *S. hyodysenteriae* 8930 LPS. This result was confirmed in immunoblots with pronase-treated *S. hyodysenteriae* 8930 cell lysates as the antigen (data not shown). One hybridoma, 3E7, was cloned, monoclonal antibody ascites were generated, and its specificity was examined in immunoblots by using pronase-treated cell lysates of *S. hyodysenteriae* representing all known serotypes or serogroups. Similar examinations were performed with selected enteric bacteria. The immunoblots involving the *S. hyodysenteriae* isolates (Table 1) revealed that the antibody was specific for *S. hyodysenteriae* 8930, 5380, 70A, RMIT 88, and RMIT 97 (Fig. 1). When the monoclonal antibody ascites were tested against a selection of enteric bacteria (see above), it did not recognize any of these bacteria (Fig. 2). The monoclonal antibody recognizes only the LPSs of a select group of *S. hyodysenteriae* isolates.

The results presented here indicate that a monoclonal antibody directed against the LPS of *S. hyodysenteriae* 8930 is able to specifically identify select strains of *S. hyodysenteriae*, namely, *S. hyodysenteriae* 8930, 5380, 70A, RMIT 88, and RMIT 97. Furthermore, the monoclonal antibody did not cross-react with any previously classified *S. hyodysenteriae* serotypes or with a selection of other enteric pathogens. The monoclonal antibody raised to *S. hyodysenteriae* 8930 can be used to identify and specifically classify this new group of *S. hyodysenteriae* isolates.

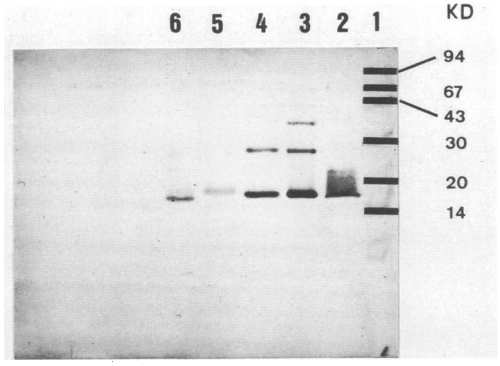

FIG. 1. Immunoblot with pronase-treated, SDS-PAGE-separated cell lysates as antigen and probing with the anti-*S. hyodysenteriae* 8930 LPS monoclonal antibody. Lanes: 1, molecular mass markers (14, 20, 30, 43, 67, and 94 kDa); 2 to 6, *S. hyodysenteriae* isolates 8930, 5380, 70A, RMIT 88, and RMIT 97, respectively.

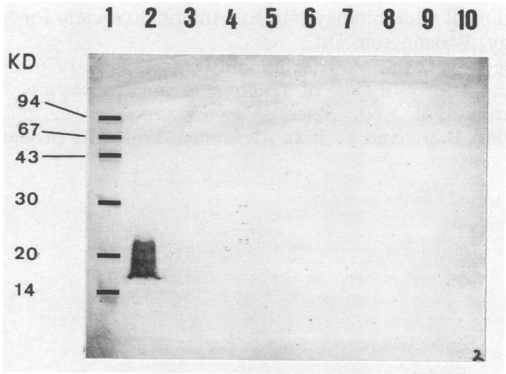

FIG. 2. Immunoblot with pronase-treated, SDS-PAGE-separated cell lysates and probing with the anti-*S. hyodysenteriae* 8930 LPS monoclonal antibody. Lanes: 1, molecular mass markers (14, 20, 30, 43, 67, and 94 kDa); 2, *S. hyodysenteriae* 8930; 3, *S. innocens*; 4, *Enterobacter aerogenes*; 5, *Serratia marcescens*; 6, *Morganella morganii*; 7, *Proteus vulgaris*; 8, *Shigella flexneri*; 9, *Shigella sonnei*; 10, *Escherichia coli*.

The means of identifying isolates with a monoclonal antibody raised to a reference strain for the *S. hyodysenteriae* 5380-like isolates described here indicates that the production of monoclonal antibodies to the reference strains of previously identified serotypes or serogroups could provide a new, more efficient method of classifying *S. hyodysenteriae* isolates.

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