Serotype-Specific Protection Against *Treponema hyodysenteriae* Infection in Ligated Colonic Loops of Pigs Recovered from Swine Dysentery

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Resistance to *Treponema hyodysenteriae* (serotypes 1, 2, 3, and 4) infection was evaluated in ligated colonic loops in pigs recovered from swine dysentery. Lesions were present in most loops from recovered swine inoculated with heterologous serotypes; however, lesions were not present in loops of recovered swine inoculated with homologous serotypes.

Inoculation of susceptible pigs with *Treponema hyodysenteriae* results in production of swine dysentery (SD), a syndrome which is characterized by mucohemorrhagic catarrhal enteritis (4, 13). Lesions occur only in the large intestine and persist there for several days; they eventually subside in animals which survive (5). After recovery is complete, pigs have been shown to be immune to oral rechallenge with feces from infected pigs or with pure cultures of *T. hyodysenteriae*; however, the challenge reexposure of pigs in these studies has been made with the same strain (7, 11). Recently, various strains of *T. hyodysenteriae* have been grouped into four distinct serotypes based on reactions obtained between cellular extracts of *T. hyodysenteriae* and hyperimmune serum in a gel precipitin test (2). Information on the cross-protective properties of the various serotypes in recovered swine is not available at present, but is needed as part of the process of developing an efficacious method for biological control of the disease.

Secondary specific-pathogen-free pigs (*n* = 16), 8 to 12 weeks old, were obtained from the Indian Creek Pig Co., Maxwell, Iowa, from a herd which had no previous history of SD. Fecal samples from the pigs were free of *T. hyodysenteriae* as detected by selective culture methods before the initial exposure and reexposure of the pigs to the various serotypes (12).

Pathogenic *T. hyodysenteriae* isolates B234 (serotype 1), B204 (serotype 2), B169 (serotype 3), and A-1 (serotype 4) (2) were cultured in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (6, 10). Viable cell numbers in the inoculum were determined as previously described (10).

Two pigs each were inoculated with isolates B234 (1 × 10⁹ colony-forming units [CFU]), B204 (9 × 10⁹ CFU), B169 (9 × 10⁹ CFU), and A-1 (9 × 10⁹ CFU) on two consecutive days. Clinical signs of SD appeared in 3 to 7 days; pigs were then allowed to recover for 35 to 50 days. Cecal canulas were then placed in both recovered (*n* = 8) and control (*n* = 8) pigs (1) to allow the colons to be flushed of fecal debris before colonic loops were surgically installed (14). Five colonic loops (5 to 7 cm in length) with control interloops were installed in recovered and control pigs 1 week after installation of the cecal canulas. Each of the four serotypes (10 ml, 10⁷ organisms per ml) or sterile Trypticase soy broth was injected into separate loops, and the pigs were killed and necropsied at 60 h postinoculation. The loops were opened longitudinally and examined for lesions and for the presence of *T. hyodysenteriae*. Segments of each loop were fixed in 10% buffered Formalin, embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin, and examined for microscopic lesions (3, 8). Tissues from designated loops were frozen in a 10% methyl cellulose suspension, sectioned at 6 μm in a cryostat, and fixed in cold acetone (4°C) for 30 min.

Antiserum was produced in rabbits against whole-cell *T. hyodysenteriae* antigens (serotypes 1, 2, 3, and 4) as previously described by Baum and Joens (2). The sections described above were treated with a 1:400 dilution of antiserum of designated serotypes (2) for 30 min; they were then washed and stained with fluorescein-labeled anti-rabbit immunoglobulin G (IgG) serum (1:400 dilution; Miles Laboratories, Elkhart, Ind.) for 30 min. After being washed, the tissues
were counterstained with Evans blue (0.5%), mounted, and examined by fluorescent microscopy (8).

Sera obtained from recovered and control pigs before inoculation were tested in an enzyme-linked immunosorbent assay (9) against lipopolysaccharide (2) from the respective serotype. Each loop was cultured at necropsy for the presence of T. hyodysenteriae as described by Songer et al. (12). Also, numbers of T. hyodysenteriae on the mucosal surface were determined for one recovered and one control pig exposed to the four serotypes. Sections (1 cm²) were cut from the wall of the loop, suspended in 9.0 ml of phosphate-buffered saline (pH 7.2), and vigorously washed. The resulting suspensions were serially diluted in phosphate-buffered saline, and the dilutions were cultured on selective agar (12). T. hyodysenteriae isolated from loops at necropsy were serotyped as described by Baum and Joens (2).

Gross lesions were not detected in loops from pigs recovered from SD and reexposed to the homologous serotype (Table 1). In contrast, lesions were present in 17 of 24 (70.8%) loops in recovered pigs exposed to heterologous serotypes. In control pigs (previously unexposed pigs), lesions were detected in 26 of 32 (81.2%) loops exposed to the four serotypes. Lesions were similar in loops from both recovered and control pigs and consisted of hyperemia, edema, catarhal inflammation, and mild hemorrhage. Fluid to mucoid exudate in excess of 50 ml was found in 20% of the loops with lesions. Microscopic lesions included hyperemia, epithelial cell erosion, mucosal cell hyperplasia, and crypt dilation and were correlated with the presence of gross lesions in infected loops.

Antibody activity to the homologous lipopolysaccharide antigen was detected in sera from recovered pigs before loop reexposure. Cross-reactions between sera from recovered pigs and lipopolysaccharides from heterologous isolates were not detected. Sera from controls contained no antibody detectable by enzyme-linked immunosorbent assay at the time of exposure. T. hyodysenteriae organisms were detected by fluorescent antibody staining in all colonic tissues with detectable gross lesions. The organism was present at necropsy in large numbers both on the luminal surface and in the crypts in loops from recovered pigs exposed to heterologous serotypes. In contrast, T. hyodysenteriae was detected by fluorescent antibody staining on the colonic luminal surface but not in the crypts of recovered pigs reexposed to the homologous serotype.

Virulent T. hyodysenteriae was recovered at necropsy from infected loops of control pigs. In addition, T. hyodysenteriae was recovered from loops of recovering pigs reexposed to homologous and heterologous isolates. Serotypes of isolates obtained from the loops were in all cases identical to those inoculated. Mucosal counts varied among the loops, especially those from the recovered pig. Counts of approximately 10⁸ organisms per cm² were found on the mucosal surface of loops which had gross and microscopic lesions. Loops from recovered pigs reexposed to the homologous serotype had a mucosal count of approximately 10⁹ organisms per cm². Also, a loop exposed to a heterologous isolate which failed to develop detectable lesions had an average mucosal count of 6 × 10⁸ organisms per cm².

Pigs recovered from the disease induced by isolate B204 are immune when reinoculated oral-
ly with the same isolate (7). We report here similar results in ligated colonic loops in convalescent pigs inoculated with the homologous isolate. However, we also showed that loops from recovered pigs inoculated with different serotypes developed SD lesions. The serotypes of *T. hyodysenteriae* are identified by the reactions of the lipopolysaccharides of the organism in a gel-precipitin test (2). The results of this study and of previous work (9) indicate that the immune response to SD in recovered pigs is partially directed against this antigen.

The correlation between numbers of *T. hyodysenteriae* in crypts and total wall counts has been made previously (8, 14). The absence of *T. hyodysenteriae* from crypts of loops with homologous challenge and the low numbers of *T. hyodysenteriae* organisms support the hypothesis that the ability to enter into and proliferate in the crypts is an important determinant in the pathogenesis of this disease. The present results suggest that one aspect of protection involves the blocking of this event, but whether or not this event is mediated by antibody is unknown.

These results indicate that the immune response of pigs infected with *T. hyodysenteriae* is at least partially serotype specific. Therefore, more efficient protection against SD may result from a vaccine that includes a pool of antigens from the known serotypes.

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LITERATURE CITED