Serological Reactions of the Genus *Peptostreptococcus*

MITCHELL B. GRAHAM* AND WILLIAM A. FALKLER, JR.

Department of Microbiology, University of Maryland Dental School, Baltimore, Maryland 21201

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White male New Zealand rabbits were immunized with soluble antigen preparations (SP) of the following gram-positive anaerobic cocci: *Peptostreptococcus anaerobius* ATCC 27337 and VPI 5737; *P. micros* VPI 2618; *Streptococcus morbillorum* ATCC 27527; *P. parvulus* VPI 5229; and *P. productus* ATCC 27340. SP were reacted with homologous and heterologous rabbit antisera in immunodiffusion, immunoelectrophoresis, indirect fluorescent-antibody, and tanned-cell passive hemagglutination tests. Even though each antiserum reacted strongly with its homologous SP, no interspecies reactivity was observed except between *P. productus* antisera and *P. parvulus* SP by the passive hemagglutination test. Antisera prepared to both strains of *P. anaerobius* reacted with the other strain in all serological tests.

The gram-positive anaerobic cocci, which include members of the genus *Peptostreptococcus*, are not easily identified to the species level. Graves et al. (1) and Wideman et al. (8) reported that inhibition of growth of *P. anaerobius* by sodium polyanethol-sulfonate could be used to identify this particular member of the genus. Porschen and Spaulding (6) prepared rabbit antisera to clinical isolates of the genera *Peptococcus* and *Peptostreptococcus* in an attempt to find a genus-specific or species-specific antigen. They observed no shared antigens except with antisera to *Peptococcus magnus*, which reacted with several strains of this species in fluorescent-antibody tests. Wells and Field (7) used gas-liquid chromatography to analyze the long-chain fatty acids produced by members of the two genera. They reported that this procedure could not be used for identification of species and concluded that additional biochemical and serological tests, guanine-cytosine ratios, or deoxyribonucleic acid homology studies might prove helpful.

In this communication, we report on results obtained from the serological testing of antigenic preparations from typed species of the genus *Peptostreptococcus* with hyperimmune rabbit antisera prepared to homologous and heterologous species.

MATERIALS AND METHODS

Bacterial strains and media. *P. anaerobius* ATCC 27337 and VPI 5737, *P. micros* VPI 2618, *Streptococcus morbillorum* ATCC 27527 (formerly called *P. morbillorum*), *P. parvulus* VPI 5229, and *P. productus* ATCC 27340 were used. All strains were grown in Schaedler broth (BBL) except *P. parvulus*, which was grown in Schaedler broth supplemented with 0.2%

fetal calf serum (Grand Island Biological Co.). Each strain was incubated in a BBL anaerobe jar with GasPak at 37°C. All strains were incubated 18 to 24 h except *P. parvulus*, which was incubated 42 to 48 h. The cells were harvested by centrifugation at 10,000 × *g* for 20 min and washed three times in 0.15 M NaCl (saline).

Soluble antigen preparation (SP). Before sonic treatment, 1.78 g (wet weight) of each strain was resuspended in 5.0 ml of saline, to which was added 1.25 ml of 5-μm glass beads. Suspensions of each organism were sonically treated in a dry ice-alcohol bath, using a Branson Sonifier with probe tip at 6 A for 16 bursts of 30 s each. After sonic treatment, the glass beads were removed by centrifugation at 70 × *g* for 10 min. The sonically treated preparations were centrifuged at 10,000 × *g* for 10 min, and the resulting supernatant fluids were designated SP. SP were used to prepare rabbit antisera and as an antigen preparation in immunodiffusion (ID) and immunoelectrophoresis (IE). For use in the tanned-cell passive hemagglutination test, SP were saturated with (NH₄)₂SO₄. The sediments were resuspended and again saturated. The resulting precipitates were resuspended to original volume, dialyzed in saline, and designated ASP. The protein concentrations of SP and ASP were determined by the method of Lowry et al. (4).

Rabbit antisera. At least two white male New Zealand rabbits (3 to 4 kg) were injected twice subcutaneously in the back (with a 1-week interval between injections) with 1 mg of protein of SP of each strain in Freund complete adjuvant (Difco). These injections were followed by two intraperitoneal injections of 1 mg of protein of SP at weekly intervals. After 7 days, the rabbits were bled, and the prepared sera were stored at -20°C until used. In addition, several rabbits were given booster injections of 1 mg of protein of SP intraperitoneally.

ID and IE. Double diffusion in two dimensions was performed on glass slides using 1.0% agarose (Calbiochem) in 0.05 M borate-buffered saline, pH 9.0. Glass slides overlaid with 3.0 ml of the 1.0% buffered agar

FIG. 1. Immunodiffusion of rabbit anti-*P. anaerobius* VPI 5737 sera and homologous and heterologous SP. Center well: Rabbit anti-*P. anaerobius* VPI 5737 sera. Peripheral wells (SP > 5.0 mg of protein/ml): (1) *P. anaerobius* ATCC 27337; (2) *P. anaerobius* VPI 5737; (3) *P. micros* VPI 2618; (4) *S. morbillorum* ATCC 27527; (5) *P. productus* ATCC 27340; (6) *P. parvulus* VPI 5229.

gel were used for IE. The slides containing SP were electrophoresed in Veronal buffer, pH 8.6 (5).

Indirect fluorescent antibody (IFA). The following modification of Proschén and Spaulding's method (6) was used. A drop from a 0.1% suspension (determined from the packed cell volume after centrifugation at $800 \times g$ for 20 min) of each strain was allowed to air dry on a glass slide and fixed for 1 min with cold alcohol. Rabbit antisera and fluorescein-labeled goat anti-rabbit 7S globulin sera were diluted 1:10 and 1:8, respectively. As controls, bacterial cells were treated with normal rabbit sera and counterstained with fluorescein-labeled goat anti-rabbit 7S globulin sera.

Tanned-cell passive hemagglutination. A microtiter modification of the method in Kabat and Mayer (3) was utilized. Fifty microliters of heat-treated (56°C for 30 min) and sheep erythrocyte (SRBC)-absorbed rabbit antisera were serially diluted, using 0.2% gelatin in saline as diluent. Twenty-five microliters of tanned sensitized SRBC (1.25%) was then added to each well. Antisera and cell buttoning controls were included. After the reagents were added to each well, the plates were agitated for 1 min and placed in a 37°C incubator for 30 min, followed by incubation at room temperature for 3 h. The hemagglutination patterns were then recorded (1 to 4+), and the plates were placed in the refrigerator overnight. The following morning hemagglutination patterns were rechecked.

RESULTS

Typically two to three precipitin lines were observed by ID when each SP was reacted at varying dilutions with its homologous antisera.

The SP concentration giving optimum reactivity (maximum number of lines) with its undiluted homologous antisera was reacted with undiluted antisera from each species. No precipitin lines were observed. Antisera prepared to each of the two *P. anaerobius* strains showed at least two lines of identity when reacted with SP to either strain and no reactivity with heterologous SP (Fig. 1). In addition, when each SP (protein concentration of 6 to 10 mg/ml) was electrophoresed and reacted with its homologous antisera, 6 to 12 precipitin lines were observed. The reaction of *P. anaerobius* SP with its homologous antisera is seen in Fig. 2, well a. No reactivity was observed when each SP was reacted with antisera to the heterologous species by IE. In addition, when *P. anaerobius* SP were electrophoresed and reacted with antisera to the other strain, several precipitin lines were shared (Fig. 2, well b).

In IFA testing, each antiserum showed positive fluorescence with homologous cells when diluted to a titer of at least 1:160. As in previous serological tests, *P. anaerobius* antisera reacted with the heterologous strain (Fig. 3), but no interspecies reactivity was observed. All test controls were negative.

In an attempt to detect antibody reactive with passively SP-sensitized tanned cells, each antiserum was reacted with tanned cells sensitized with varying concentrations (5.0 to 0.01 mg of protein/ml) of the ASP preparation. It was observed for the majority of strains that sensitization with 1 mg/ml gave the highest detectable antibody titers. The results obtained from reaction of SP (1 mg/ml)-sensitized cells with homologous and heterologous antisera can be seen in Table 1. Again no interspecies reactivity was observed with the exception of the three *P. productus* antisera, which each gave a titer of 1:32 when reacted with *P. parvulus* sensitized cells. In addition, antisera prepared to both strains of *P. anaerobius* gave a titer of at least 1:512 with the heterologous SP-sensitized cells.

DISCUSSION

The advent of better anaerobic techniques and the increasing awareness of their possible role in disease processes are associated with the frequent isolation of gram-positive anaerobic cocci from clinical specimens. Peptostreptococci have been isolated from many infections such as abscesses, puerperal fever, and lung gangrene (2). Their role in any disease process is poorly understood. Interest in the pathogenicity of particular members of this group of organisms has been hampered by clinical treatment not requiring identification of these organisms to the spe-

FIG. 2. Immunoelectrophoresis of *P. anaerobius* SP (ATCC and VPI) against rabbit anti-*P. anaerobius* ATCC 27337 sera. Well a: *P. anaerobius* SP ATCC 27337. Well b: *P. anaerobius* SP VPI 5737. Trough: Rabbit anti-*P. anaerobius* ATCC 27337 sera.

FIG. 3. Positive IFA testing of *P. anaerobius* ATCC 27337 whole cells reacted with rabbit anti-*P. anaerobius* VPI 5737 sera and goat anti-rabbit 7S globulin sera.

TABLE 1. Tanned-cell passive hemagglutination of ASP^a (1 mg of protein/ml)-sensitized SRBC reacted with homologous and heterologous antisera

ASP-sensitized cells	Rabbit antisera prepared to:					
	<i>P. anaerobius</i> ATCC	<i>P. anaerobius</i> VPI	<i>P. micros</i>	<i>S. morbillorum</i>	<i>P. parvulus</i>	<i>P. productus</i>
<i>P. anaerobius</i> ATCC 27337	512 ^b	1,024	— ^c	—	—	—
<i>P. anaerobius</i> VPI 5737	512	512	—	—	—	—
<i>P. micros</i> VPI 2618	—	—	512	—	—	—
<i>S. morbillorum</i> ATCC 27527	—	—	—	256	—	—
<i>P. parvulus</i> VPI 5229	—	—	—	—	128	32
<i>P. productus</i> ATCC 27340	—	—	—	—	—	256

^a (NH₄)₂SO₄ precipitate of soluble antigen preparation.^b Reciprocal of highest titer giving 2+ hemagglutination.^c —, Less than 1:2 reactivity.

cies level. Knowledge regarding the host response to these organisms must also await an understanding of the immunogenic relationships between members of this group.

It appears from this study that the specificity of the antisera to the species tested will allow a serological approach as a means of rapidly identifying these organisms. The only instance where interspecies reactivity was observed was between *P. productus* antisera and *P. parvulus* sensitized cells in the tanned-cell test, where each *P. productus* antiserum gave a titer of 1:32 when reacted with *P. parvulus* sensitized cells. However, *P. parvulus* antisera failed to react with *P. productus* SP in any of the four serological tests. Therefore the reactivity observed between *P. parvulus* and *P. productus* may represent a shared antigenic determinant(s) detected by the *P. productus* antisera. The lack of reactivity of *P. parvulus* antisera with *P. productus* SP may be due to the shared antigenic determinant(s) being in such a low concentration or of a structural configuration such that it was not immunogenic in the rabbits immunized with *P. parvulus* SP.

A previous study by Proschén and Spaulding (6) attempted to use serological techniques to identify the gram-positive anaerobic cocci. They prepared rabbit antisera to 11 different strains of peptostreptococci and peptococci, including two strains of *P. anaerobius*. When the 11 antisera were tested against other gram-positive anaerobic cocci, a high degree of strain specificity was observed. Out of a total of nine strains of *P. anaerobius* tested, one *P. anaerobius* antiserum reacted with two strains and the other antisera reacted with none. Our current study suggests

there may be species-specific antigens for *P. anaerobius*, since antisera prepared to both strains reacted strongly with the heterologous strain in all four serological tests.

The specificity of the peptostreptococcal antisera is currently being tested against known clinical isolates. If these antisera remain species specific, then their use in a fluorescent-antibody test will allow rapid identification of members of this genus and a better understanding of their participation in disease processes.

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