A Research Note

IMMUNOGENIC PROPERTIES OF SLIME FROM PROPIONIBACTERIA[1]

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Abstract

Slime, produced by members of the genus *Propionibacterium*, was isolated, and attempts were made with it to induce antibody formation in biological systems to demonstrate the possible use of anticapsular antibodies for serological typing. Slime produced by three different species of *Propionibacterium* did not produce detectable precipitating antibodies in rabbits. Killed whole cells of *Propionibacterium zeae* induced specific agglutinin formation. Antibodies produced against whole cell antigens of *P. freudenreichii* and *P. shermanii* exhibited substantial heterologous agglutination reactions.

The immunological properties of capsular materials are commonly known. For historical background, a review of work done before 1948 is available *(3)*. In recent years, the immunogenic properties of capsular materials have served as valuable tools to classify microorganisms *(3, 7)*. Possession of highly polymerized polysaccharide capsular antigens has permitted separation of pneumococci into approximately 75 types *(10)*. The classical work of Lancefield *(4)* demonstrated the value of polysaccharide antigens for serological classification of group-B streptococci.

The need for serological classification of *Propionibacterium* was mentioned by Malik et al. *(6)* in their taxonomic evaluation of this genus. Previously Werkman and Brown *(13)* had reported a serological study of 11 species of this genus and found extensive cross agglutination. They used preparations of O antigen and made no mention of capsular or slime antigens. Recently, Skogen *(9)* demonstrated capsule formation and slime production in several species of *Propionibacterium*. By analyzing slime hydrolysate using paper and thin-layer chromatography he determined that mannose and lesser amounts of glucose and galactose were present. No mention was made of the presence or absence of proteins or individual amino acids in the slime. Our investigation was undertaken to evaluate the immunogenic properties of slime from *Propionibacterium* in an attempt to exploit the possible use of anticapsular or antislime antibodies in typing members of the genus *Propionibacterium*.

Materials and Methods

Organisms used

Three strains of propionibacteria that produced copious amounts of slime *(9)* were used in this study. They were *Propionibacterium zeae* strain no. 74, *Propionibacterium shermanii* strain no. 47, and *Propionibacterium freudenreichii* strain no. 1. These strains were routinely maintained in sodium lactate broth *(12)*.

Preparation of whole cell antigens

Cultures grown in broth were killed with formalin, and cells harvested by centrifugation. The cells were resuspended in 0.05 M phosphate-buffered saline and were heated for 20 min in a water bath at 70 C. Formalin was added to a final concentration of 0.01%. The suspensions were brought to a final concentration of 1×10^9 bacteria/ml on the McFarland scale [an arbitrary optical density scale *(2)*] before injection.

Slime preparation

The method of Lindeberg *(5)* was used to isolate and purify the slime of the three slime-producing strains of propionibacteria.

Cultures were inoculated into 1 liter of glucose broth (containing 2.0% glucose, 1.0% Trypticase, 1.0% yeast extract, 0.025% K_2HPO_4, in distilled water. Incubation was at 21 C for 21 days. Cells were removed by centrifugation, and the supernatant fluid containing the loose slime was collected. Two volumes of 95% ethanol were slowly added to the clear supernatant liquid, and the fibrous mass, formed by mixing the ethanol with the supernatant fluid, was spooled onto a glass rod and removed. Slime was resuspended in distilled water and reprecipitated with ethanol. It was then collected and dried on a watch glass for 24 hr at 45 C. Dried slime was weighed and resuspended in distilled water to a final concentration of 10 mg/ml. Resuspended slime was dialyzed against cold, running, tap water for 24 hr, lyophilized, and then stored at 4 C. Lyophilized slime was used for further antigenic preparations.

Assessment of immunogenicity

Immunogenicity of propionibacterial slime was assessed by injecting New Zealand albino rabbits (2.2-2.8 kg) with either wholecell suspensions or isolated slime. When whole cells were used, increasing doses (0.5, 1.0, 2.0, and 2.0 ml) were injected intravenously on three successive days each week during a 4-week period. Animals were bled by intracardial puncture 9 days after the final injection, and sera were stored at −20 C unless used immediately.

Isolated slime was used as the antigenic dose at an initial rate of 0.1 mg/kg body weight of the individual rabbits. During the first week, rabbits were given two subcutaneous injections of 1 ml slime plus 1 ml Freund's complete adjuvant. During the second week, two 1-ml intramuscular injections of slime plus Freund's complete adjuvant, in a 1:1 ratio, were given at 3-day intervals. Freund's complete ad-

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juvant (7) is a suspension of killed mycobacteria in a mixture of paraffin oil and an emulsfying agent. During the third week, adjuvant was discontinued, and only the slime suspension was injected by the intraperitoneal route. Two such injections were given at 3-day intervals. One week after completion of this series of injections, rabbits were bled by intracardial puncture. Sera were stored at −20 C unless used immediately as noted.

To determine the effect of increasing doses on antibody production, isolated slime was injected intradermally at 0.85 mg/kg body weight along with adjuvant. Two such injections were given for 2 weeks. During the third week, adjuvant was discontinued, and only slime was injected by the intravenous route. Rabbits were allowed to rest 1 week and were bled by intracardial puncture. Sera were stored at −20 C.

Serological methods

Bacterial agglutination was done in 13 × 100 mm tubes. The titer was recorded by reading the antigen antibody reaction patterns after holding overnight in a refrigerator after 3-hr incubation at 50 C.

The ring test for rapid detection of precipitating antibody was carried out in tubes, using Pasteur pipettes to introduce the components. The procedure was followed as outlined by Campbell et al. (2).

Capillary precipitation tests were done by the method of Swift et al. (11), with reading after 12-hr refrigeration.

Agar-gel diffusion was prepared using the slide method of Schubert et al. (8). Antisera were tested in a satellite pattern to determine the relationship between strains. Slides were incubated 2 days at 35 C in a moist chamber.

The Neufeld *Quellung* reaction was determined by the method outlined by Campbell et al. (2).

Sheep red blood cells were sensitized for passive hemagglutination by mixing equal volumes of tannic acid-treated erythrocytes and antigen (1). Following antisera addition, tubes were checked for agglutination after 3- and 12-hr incubation at room temperature.

Complement fixation was done according to the method of Campbell et al. (2), employing previously titrated complement and specific hemolysin. To overcome the anticomplementary reaction of the immune serum, the test was done with an initial serum dilution of 1:128.

RESULTS AND DISCUSSION

Immunogenicity of isolated slime

Antisera from rabbits injected with isolated slime material did not contain detectable levels of precipitating antibodies when assayed by ring-test precipitation, indirect hemagglutination, gel diffusion, and complement fixation tests.

Immunogenicity of formalinized cells

Immunogenic properties of killed whole cells of the three species of *Propionibacterium* were studied by agglutination tests. Homologous antigen reacted with the three antisera that had been obtained. Presence of cross agglutination observed with the antisera of both *P. freudenreichii* and *P. shermanii* supports the earlier findings of Werkman and Brown (13). Likewise, the antiserum to *P. zeae* exhibited no cross-reaction. Attempts to detect anticapsular or antislime antibodies by using the *Quellung* reaction were unsuccessful.

Results of this study indicate that slime produced by three species of *Propionibacterium* did not induce detectable precipitating antibody formation in rabbits. It is possible that slime from propionibacteria, like pneumococcal polysaccharides, may be antigenic only in certain species of mammals. Obviously it is not antigenic in rabbits and might act as a hapten. This work further indicates that subsequent attempts to induce antibody formation against propionibacterial slime should be done in species of animals other than rabbits. Since chickens are better producers of precipitating antibody than are rabbits [Wolf et al. as cited by Campbell et al. (2)] their use for this purpose would seem logical.

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