Serological Activity of Staphylococcal Polysaccharide

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ABSTRACT

The polysaccharide from cell walls of coagulase-positive staphylococci coated both latex particles and tanned red cells for agglutination by human sera and by specific staphylococcal antisera. Treatment with trypsin or autoclaving destroyed the capacity of polysaccharide to coat particles but did not affect precipitation of antibody. Periodic acid destroyed both properties. The teichoic acid portion of the staphylococcal polysaccharide displayed precipitin activity similar to polysaccharide, but it did not coat either latex particles or tanned red cells. Teichoic acid did, however, inhibit specific agglutination of polysaccharide-coated particles or cells.

The polysaccharides of cell walls of coagulase-positive staphylococci contain polymers of teichoic acids and of mucoproteptides. Teichoic acids of coagulase-positive staphylococci are either α- or β-N-acetylmuramyl ribitol teichoic acids; cell walls of some strains of coagulase-positive staphylococci contain predominantly β-N-acetylmuramyl ribitol teichoic acid, and cell walls of other strains contain predominantly α-N-acetylmuramyl ribitol linkages (6, 8, 11).

The mucoproteptides in cell walls consist of polymers of N-acetylmuramic and N-acetylmuramic acid or of N-acetylmuramic and N,O-diacytymuramic acid with four amino acids, alanine, glycine, glutamic acid, and lysine (3, 6, 8, 11). The complex of teichoic acids and mucoprotein has been designated polysaccharide (4).

In the present study, staphylococcal antisera were found to agglutinate latex particles and tanned red cells, both coated with staphylococcal polysaccharide, but not with teichoic acid. Nonetheless, teichoic acid was capable of inhibiting the agglutination of polysaccharide-coated red cells or latex particles.

MATERIALS AND METHODS

Preparation and detection of antigens. The method used for preparation of staphylococci by ultrasonic means has been described previously (7, 14). Teichoic acids were prepared by the methods described by Morse (8).

The various antigens in ultrasonic supernatant fluids were separated by continuous-flow electrophoresis on a refrigerated Spinco continuous-flow electrophoresis apparatus in barbital buffer of pH 8.6 and 0.05 ionic strength. Supernatant fluid was applied at the rate of 1 ml/hr. The voltage was maintained constant at 650 v with the current varying from 40 to 50 ma.

Staphylococcal antigens were also separated on a 2.5 × 38 cm diethylaminoethyl (DEAE) cellulose column at 4 C with a gradient from 0.05 to 0.3 m phosphate. Fractions were collected in 50-drop samples.

Both the fractions from electrophoresis and from column chromatography were dialyzed against distilled water, lyophilized, and reconstituted in saline at a concentration of 2 mg/ml. The antigens in ultrasonic supernatant fluids of staphylococci and in various fractions were identified in Ouchterlony plates by the methods described previously (7, 14).

Hemagglutination and latex agglutination. Latex particles were coated by incubating 1:10 dilution of latex particles (Dow Chemical Co., Midland, Mich.) in pH 8.6 borate buffer with 20 μg/ml of electrophoretically isolated polysaccharide for 60 min at 37 C with continuous mixing by a magnetic stirrer. The coated particles were then washed three times in borate buffer and resuspended in borate buffer plus 1% normal rabbit serum.

Sheep red cells were tanned by the methods described by Morse (8). Agglutination of coated latex particles and of coated tanned red cells by antisera was tested using the Takatsy loop microtitrator from Intercontinental Scientific Corp. New York, N.Y.

Preparation of antisera. Antisera against Formalin-killed staphylococci were prepared in rabbits by the method described by Oeding (12). Sera from patients with and without staphylococcal infections were frozen at −60 C until used. Pooled concentrated human γ-globulin at a concentration of 165 mg/ml was obtained from Merck, Sharp & Dohme, West Point, Pa.

Bacterial strains. Staphylococcal strains of known serological types were furnished by Jay Cohen, Communicable Disease Center, Atlanta, Ga.; their antigens have been described previously (2).

Treatment of antigens. Electrophoretically isolated
polysaccharide was treated with trypsin, autoclaving, or periodic acid, and was tested for precipitins and for ability to coat latex particles or tanned red cells before and after treatment.

We incubated 2 mg/ml of isolated polysaccharide overnight at 37 C with 200 µg/ml of trypsin before testing. The same concentration of polysaccharide was autoclaved at 120 C for 1 hr. In tests with periodic acid, 0.02 M periodic acid in phosphate buffer (pH 7) was incubated in the dark at 25 C for 5 hr with equal parts of 2 mg/ml of polysaccharide. The reaction was stopped by adding 0.2 volume of 10% dextrose in water.

RESULTS

Several known antigens could be detected in the supernatant fluids of washed coagulase-positive staphylococci after ultrasonic vibration by use of Ouchterlony gel diffusion plates. These included the group protein antigen (Jensen's protein antigen A), the group polysaccharides, and the individual type-specific antigens which have been previously designated by the letters a to m.

The various antigens were separated by continuous-flow electrophoresis and by DEAE column chromatography. Rabbit antisera agglutinated latex particles and tanned sheep cells which were coated with fractions containing only polysaccharide precipitins.

Latex particles coated with electrophoretically isolated Cowan I polysaccharide were agglutinated by sera from rabbits immunized with Wood 46, Cowan I, Cowan II, or F-21 staphylococci, but not by sera from the same rabbits before immunization. Sera from immunized rabbits agglutinated coated latex particles at dilutions ranging from 1:256 to 1:1,024. In addition, 10 human sera agglutinated coated latex particles at dilutions ranging from 1:32 to 1:1,024.

Polysaccharides isolated from Cowan I staphylococci by either electrophoresis or by column chromatography formed lines of identity in Ouchterlony plates with chemically prepared teichoic acid from Wood 46 staphylococci (Fig. 1). Both polysaccharide and teichoic acid inhibited the agglutination by antisera of latex particles coated with polysaccharide (Table 1). However, staphylococcal antisera did not agglutinate latex particles incubated with teichoic acid with precipitin activity ranging from 0.1 to 100 times that of polysaccharide.

Treatment of polysaccharide or teichoic acid with trypsin or autoclaving did not alter the precipitin activity of either compound (Table 2). However, latex particles incubated with treated polysaccharide were not agglutinated by antisera. Periodic acid destroyed precipitin activity of both polysaccharide and teichoic acid. In addition, latex particles which were incubated with polysaccharide treated with periodic acid were not agglutinated by antisera.

Untreated sheep cells incubated with several concentrations of Cowan I polysaccharide were not agglutinated by rabbit antisera or by human sera. However, tanned sheep cells coated with

![Fig. 1. Formation of lines of identity by precipitin bands between staphylococcal antisera (central well) and electrophoretically isolated Cowan I polysaccharide (upper outer well) or Wood 46 teichoic acid (lower outer well).](image)

**TABLE 1. Inhibition by polysaccharide or teichoic acid of agglutination of latex particles coated with Cowan I polysaccharide**

<table>
<thead>
<tr>
<th>Conc of inhibitor µg/ml</th>
<th>Reduction in agglutination titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polysaccharide</td>
</tr>
<tr>
<td>0</td>
<td>1,024</td>
</tr>
<tr>
<td>0.2</td>
<td>1,024</td>
</tr>
<tr>
<td>0.4</td>
<td>512</td>
</tr>
<tr>
<td>0.8</td>
<td>256</td>
</tr>
<tr>
<td>1.6</td>
<td>256</td>
</tr>
<tr>
<td>3.2</td>
<td>64</td>
</tr>
<tr>
<td>6.4</td>
<td>64</td>
</tr>
</tbody>
</table>

**TABLE 2. Effect of treatment of polysaccharide or teichoic acid on agglutination of coated latex particles or formation of precipitin lines**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latex agglutination titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polysaccharide</td>
</tr>
<tr>
<td>None</td>
<td>1,024</td>
</tr>
<tr>
<td>Trypsin</td>
<td>1,024</td>
</tr>
<tr>
<td>Autoclaving</td>
<td>1,024</td>
</tr>
<tr>
<td>Periodic acid</td>
<td>1,024</td>
</tr>
</tbody>
</table>
Cowan I polysaccharide were agglutinated at dilutions ranging from 1:1,280 to 1:2,560 by sera obtained from three patients with staphylococcal diseases. In addition, antisera from rabbits immunized with F-21, Cowan I, or Wood 46 staphylococci agglutinated coated tanned red cells at maximal dilutions ranging from 1:32 to 1:5,120. Hemagglutination could be inhibited by concentrations of Wood 46 teichoic acid as low as 0.15 \( \mu \text{g/ml} \) (Table 3). Treatment of polysaccharide by autoclaving or with trypsin before incubation with tanned sheep red cells prevented hemagglutination by antisera. Tanned red cells which were incubated with concentrations of teichoic acid with precipitin activity ranging from 0.1 to 100 times that of polysaccharide were not agglutinated by antisera.

**DISCUSSION**

The \( \alpha \)- and \( \beta \)-acetylg glucosaminyl ribitol teichoic acid portions of staphylococcal polysaccharides have been shown to be major determinants of agglutination of staphylococcal cell walls by staphylococcal antiserum (6, 8). Precipitating antibodies against teichoic acid have been demonstrated in most sera from adult subjects (7, 15). In addition, immunization with isolated teichoic acid was followed by a rise in teichoic acid antibody concentrations (15).

Teichoic acids also elicit a wheal and urticarial reaction on skin testing, and, in patients with higher concentrations of teichoic acid antibodies, an Arthus phenomenon with central necrosis occurred (7, 11, 15). Mudd et al. (10) have reported that teichoic acid antibodies are required for maximal phagocytosis of coagulase-positive staphylococci.

The mucopeptide polymers of polysaccharide from coagulase-positive staphylococci have been well characterized chemically, but their immunological activities have not been investigated extensively.

**Table 3. Inhibition by Wood 46 teichoic acid of hemagglutination of tanned sheep cells coated with Cowan I polysaccharide**

<table>
<thead>
<tr>
<th>Concentration (( \mu \text{g/ml} ))</th>
<th>Hemagglutinin titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>640*</td>
</tr>
<tr>
<td>0.2</td>
<td>40</td>
</tr>
<tr>
<td>1.6</td>
<td>20</td>
</tr>
<tr>
<td>12.5</td>
<td>20</td>
</tr>
<tr>
<td>100</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Reciprocal of maximal dilution of serum from patient Hunt.

Hofstad (5) and Oeding (13) have reported that polysaccharide, but not teichoic acid, coated tanned red cells which were then agglutinated by antisera. The present study confirmed these findings but also demonstrated that, once red cells or latex particles were coated with Cowan I polysaccharide, agglutination by antisera was inhibited by Wood 46 teichoic acid and by Cowan I polysaccharide.

These findings agree with Morse's report (9) that only small amounts of teichoic acid antigen, perhaps the portion with residual mucopeptide, were removed by tanned red cells, but that hemagglutination by antisera was inhibited by very small concentrations of teichoic acid.

In addition, treatment with trypsin or autoclaving destroyed the ability of polysaccharides to coat latex particles or tanned red cells, but did not alter the precipitin activity of polysaccharide which requires the teichoic acid portion of the polysaccharide polymers (4).

These studies demonstrate that both teichoic acid and mucopeptide polymers of staphylococcal polysaccharide are necessary for the agglutination of coated latex particles and coated tanned red cells by antibodies against staphylococcal teichoic acid.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**


