Activation of the Alternate Complement Pathway by Peptidoglycan from Streptococcal Cell Wall

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Activation of the alternate complement pathway in human serum by several bacterial components was compared. Peptidoglycan from group A streptococcal cell walls was the most active material, on a weight basis, followed by cell walls, protoplast membranes, and whole cells. The group-specific carbohydrate was inactive. Treatment of peptidoglycan with low concentrations of lysozyme or short periods of sonic treatment enhanced complement activation. High concentrations of lysozyme or extended sonic treatment of peptidoglycan destroyed or greatly reduced the capacity to activate complement. Lysozyme treatment of group A streptococcal cell walls or lipopolysaccharide had no measurable effect. Activation of the alternate complement pathway by group D streptococcal cell walls was destroyed by lysozyme. Activity of peptidoglycan was not inhibited by N-acetyl glucosamine, N-acetyl muramic acid, or D-alanine-D-alanine. Conversion of C3 and factor B by peptidoglycan was shown to occur by immunoelectrophoresis and crossed immunoelectrophoresis.

The alternate complement pathway (ACP) can be activated by several microbial components. Lipopolysaccharide (LPS) from gram-negative bacterial cell walls is a well-known example (11). Our understanding of the chemical structures from gram-positive bacteria responsible for activating the ACP is less precise. Tauber et al. (23) have demonstrated activation of the ACP in human serum by cytoplasmic membranes and cell walls from group A streptococci and several other gram-positive bacteria. Pneumococcal cell walls have also been reported to activate the ACP in guinea pig serum (24). Dhingra et al. (6) showed that pneumococcal cell walls could inhibit phagocytosis and that this activity may be associated with the anti-complementary activity of mucopentapeptide (peptidoglycan). Bokisch (4) has shown that peptidoglycan extensively subjected to sonic treatment can activate complement in human serum. However, he failed to observe significant activation of the ACP.

Purified group A streptococcal cell walls consist of a peptidoglycan-polysaccharide complex that has been well characterized (14). The group-specific carbohydrate moiety consists of a backbone of rhamnose with rhamnose side chains terminating with N-acetyl-glucosamine residues. The peptidoglycan moiety is made up of repeating units of the disaccharide N-acetyl-glucosamine-muramic acid, with the carboxyl group of muramic acid being substituted by either the tetrapeptide L-ala-D-glu-L-lys-D-ala or the pentapeptide L-ala-D-glu-L-lys-D-ala-D-ala (20). In many instances there is a D-alala-D-ala dipeptide bridge between the free amino group of lysine and the C-terminal D-alanine of an adjacent peptide.

In this report we examine the capacity of streptococcal cell wall components and membranes to activate the ACP. The results provide definitive evidence that the peptidoglycan moiety of bacterial cell walls is a very effective activator of the ACP in human serum.

MATERIALS AND METHODS

Organisms. Group A streptococcus, strain D-58; Group D streptococcus, strain F24; Chromobacter violaceum; and Bacillus cereus were grown in Trypticase soy broth (Baltimore Biological Laboratories, Baltimore, Md.). Cells were collected from an 18-h culture and washed three times with saline. An ultraviolet-killed, dried culture of Micrococcus lysodeikticus was obtained from Difco Laboratories, Detroit Mich.

Bacterial cell walls and components. Cells from 18-h broth cultures were collected by continuous-flow centrifugation and washed three times with cold 0.85% NaCl. The packed cells were suspended to approximately a 15% suspension (wt/vol) in pH 7.0, 0.067 M phosphate buffer plus 0.08 M NaCl (PBS). An equal volume of no. 12 Ballotini glass beads and one drop of tri-butyl phosphate were added, and the cells were broken by treatment in a Braun MSK cell ho-
mogenizer (Bronwill Scientific Inc., Rochester, N.Y.) for 3 min with flowing CO₂ to keep the temperature close to freezing. The beads were allowed to settle, the supernatant was removed, and the beads were washed several times. The combined supernatant and washings were diluted with PBS to about five times the volume of the unbroken suspension to reduce viscosity. After centrifugation at 10,000 x g for 30 min, the top layer of sediment was carefully washed from a lower layer of unbroken cells with PBS. The suspension of cell walls was washed four times in a similar manner, with complete dispersion obtained in each resuspension by treatment in a Raytheon 9KC sonic oscillator (Raytheon Manufacturing Co., Waltham, Mass.) for 30 s. They were then washed three times with deionized water and lyophilized. This is the crude cell wall preparation.

The partially purified cell walls, consisting primarily of the mucopeptide-mucopolysaccharide complex, were prepared as follows. Cell walls were suspended in PBS (10 mg/ml) and treated with 0.025% ribonuclease and deoxyribonuclease at 37°C for 4 h. They were washed once with PBS and treated with 0.25% trypsin at 37°C for 4 h. After washing three times with PBS and three times with deionized water, they were lyophilized. The rhamnose content is 30 to 35%, and the nitrogen content is about 6%. Group D cell walls were prepared in a similar manner except that they were heated to 62°C for 46 min to prevent autolysis. The mucopeptide moiety was obtained by formamide extraction of partially purified cell walls as described by Krause and McCarty (15). Cell walls were suspended in freshly distilled formamide (5 mg/ml) and heated with constant stirring for 1 h at 170°C. After cooling to 4°C for 2 h, the insoluble mucopeptide was sedimented at 35,000 x g for 30 min. This sediment was extracted five times in hot formamide, washed three times with deionized water, and dialyzed against water at 4°C for 24 h. After lyophilization, the product is a buff to tan powder, with a rhamnose content of 1 to 5% and a nitrogen content of 13%. The preparation used in this study had a rhamnose content of 4.2%. Peptidoglycans from C. violaceum and B. cereus were prepared by a phenol extraction procedure as described previously (1). Cyttoplasmic membranes were prepared from protoplasts from group A streptococci as described (22). LPS prepared by the Westphal phenolic extraction method from Escherichia coli O26:B6 (lot no. 619166), E. coli O55:B5 (lot no. 585002), and Salmonella typhimurium (lot no. 597584) were obtained from Difco Laboratories, Detroit, Mich. Zymosan (immunological reagent, lot no. 2455) was obtained from Nutritional Biochemicals, Cleveland, Ohio.

Lysozyme treatment of peptidoglycan, cell walls, and LPS. Three-times-crystallized egg white lysozyme was obtained from Sigma Chemical Co., St. Louis, Mo. Peptidoglycan was suspended in 0.01 M PBS (pH 7.4) at a concentration of 2 mg/ml. To several tubes, an equal volume of egg white lysozyme was added to give a final concentration ranging from 0.01 to 100 μg of lysozyme per mg of peptidoglycan. Cell walls and LPS were treated similarly, using lysozyme at a final concentration of 100 μg/mg. All tubes were incubated at 37°C for 6 h. In one case it was desirable to remove lysozyme after digestion of the peptidoglycan. Peptidoglycan was suspended at a concentration of 20 mg/ml in 0.1 M ammonium acetate buffer, pH 7.0. An equal amount of lysozyme was added and incubated at 37°C for 6 h. The solubilized peptidoglycan was dialyzed against water, and the dialyzable fraction was treated with bentonite to remove any residual lysozyme. The lysozyme-solubilized peptidoglycan was then lyophilized, resuspended, and relyophilized to remove all remaining volatile salt.

Sonic treatment. Sonic treatment was performed in a Raytheon 9KC sonic oscillator. The sample (1 to 5 ml) in a plastic tube was placed in the cup of the sonic oscillator, which contained 5 ml of water. This is a rather mild treatment when compared with using a sonic probe. Unless otherwise noted, all substances were sonically treated for 1 min at maximum voltage to facilitate even dispersion. Peptidoglycan from group A streptococci was sonically treated for 10 min to effect even suspension. Preparations of group A streptococcal peptidoglycan and cell walls, as well as zymosan, were also sonically treated for 1 to 180 min to get different particle sizes.

Complement assay. A kinetic method has been devised by Boackle and Pruitt (2) and Boackle et al. (3) to measure activation of the complement pathway by a variety of substances. This method uses sensitized sheep erythrocytes (EA) which undergo complement-mediated lysis. Continuous spectrophotometric readings of absorbance are taken after addition of EA to serum which has previously been incubated with test material. The time required for 50% of the cells to lyse is dependent upon the amount of complement remaining after incubation of the serum with the test material. Relative complement concentration was measured from standard curves prepared by making dilutions of serum.

Two recent discoveries have made it possible to modify the kinetic method to measure activation of the ACP. This modification utilizes the fact that classical pathway function requires both Mg⁺⁺ and Ca²⁺, whereas activation of the ACP requires only Mg⁺⁺ (9). By using ethylene glycol bis-amino tetracetic acid (EGTA) buffer (Sigma Chemical Co.), which is a strong chelator of Ca²⁺ but only weakly binds Mg⁺⁺, it is possible to selectively block classical pathway function while leaving the ACP intact. More recently it has been demonstrated by Platta-Mills and Ishizaka (17) that normal rabbit red cells are capable of activating the ACP in EGTA-buffered normal human serum. This report has been confirmed by Schreiber et al. (21), and evidence suggests that rabbit red cells react with initiating factor of human serum.

The kinetic method has been modified to measure ACP activation by using EGTA with added Mg⁺⁺ instead of buffer containing both Ca²⁺ and Mg⁺⁺ and by using rabbit red cells instead of EA. This method has recently been described (13; R. B. Polhill, Jr., K. M. Pruitt, and R. B. Johnston, Jr., Fed. Proc. 34:4323, 1975).

Veronal-buffered saline with 0.1% gelatin and 0.01 M ethylenediaminetetraacetate (VBS-EDTA buffer), pH 7.5, was prepared by standard methods (18). VBS containing 0.002 M Mg⁺⁺ and 0.008 M EGTA, pH 7.5 (VBS-EGTA-Mg buffer), was prepared
as described by Platts-Mills and Ishizaka (17). EGTA is a strong chelator of Ca<sup>2+</sup> but only weakly binds Mg<sup>2+</sup>, allowing activation of the ACP but blocking Cl binding.

Whole blood was collected from New Zealand white rabbits directly into Alsever solution. Before use, the cells were washed three times in VBS-EDTA buffer, incubated at 37°C for 60 min in VBS-EDTA buffer, washed three times in VBS-EGTA-Mg buffer, and suspended in the same buffer. The red blood cell concentration was adjusted so that when 0.2 ml of rabbit red blood cells was added to 1.4 ml of VBS-EGTA-Mg buffer, the optical density was 0.7 at 700 nm.

Serum was collected from normal individuals by venipuncture. The blood was allowed to clot at room temperature for 1 h and at 0°C for 2 h. The clotted blood was then centrifuged at 2,000 rpm for 20 min in an IEC PR-2 refrigerated centrifuge. The serum was removed and diluted 1:3 with VBS-EGTA-Mg buffer and incubated for 10 min at 37°C. Serum was used fresh for all experiments.

Standard curves were prepared as follows. To eight tubes were added 0.1 ml of 0.01 M PBS and 0.3 ml of VBS-EGTA-Mg buffer. To these tubes were added, in duplicate, either a 1:3, 1:6, 1:9, or 1:12 dilution of human serum in VBS-EGTA-Mg buffer. The tubes were mixed and incubated at 37°C for 60 min. The standards were kept at 0°C until assayed. The tubes were then warmed for 3 min at 37°C, and 0.2 ml of rabbit red cells was added. Continuous spectrophotometric measurements were made at 4-s intervals at 700 nm on a Beckman model 25 spectrophotometer with the cell chamber at 37°C. The time to 50% lysis (T<sub>1/2</sub>) was determined, and 1/T<sub>1/2</sub> was plotted against relative complement concentration (1:3 dilution = 33.3%, 1:6 dilution = 16.6%, etc.).

In preliminary experiments, each substance was tested at concentrations ranging from 1 to 100 μg. On this basis, concentrations were selected which gave kinetic plots from which the T<sub>1/2</sub> could be calculated accurately. Activity was assayed by adding 0.1 ml of each test material, in 0.1 ml of PBS, to separate tubes. Then 0.3 ml of VBS-EGTA-Mg buffer was added to all tubes, mixed, and incubated for 10 min at 37°C to ensure that there was no available Ca<sup>2+</sup>. One milliliter of a 1:3 dilution of human serum in VBS-EGTA-Mg buffer was then added, mixed, and incubated at 37°C for 60 min with mixing at 15-min intervals. After incubation, samples were stored at 0°C until assayed (usually within 2 to 6 h). The tubes were warmed to 37°C, 0.2 ml of rabbit red blood cells was added, and spectrophotometric readings were taken as with the standards. The time to T<sub>1/2</sub> was determined, and the amount of complement present was calculated by least-squares regression analysis from the standards. The percent complement removed was calculated as follows.

\[
\% \text{ Complement removed} = \frac{\text{Concentration control} - \text{concentration after incubation with test material}}{\text{Concentration of control}} \times 100
\]

**Inhibition studies.** N-acetyl glucosamine and N-acetyl muramic acid were obtained from Sigma Chemical Co. D-Alanyl-d-alanine was obtained from Vega-Fox, Tuscon, Ariz. Inhibition studies were performed as follows: 100 μg of N-acetyl-glucosamine, N-acetyl muramic acid, D-alanyl-d-alanine, or lysozyme-solubilized peptidoglycan in 0.1 ml of VBS-EGTA-Mg buffer was added to 0.1 ml of PBS containing 1 μg of peptidoglycan. VBS-EGTA-Mg buffer was added to make a final volume of 0.4 ml, and the mixture was incubated for 10 min at 37°C. One milliliter of human serum was then added, mixed, and incubated at 37°C for 60 min. The percent complement removed was measured as described under Complement assay. Controls consisted of peptidoglycan and inhibitors alone.

**Electrophoresis.** Goat anti-human C3 and goat anti-human factor B (C3PA) were obtained from Atlantic Antibody, Westbrook, Me. Cobra venom factor was obtained from Cordis Labs, Miami, Fla. EA were prepared as described by Rapp and Borsos (18). Preceding electrophoresis, human serum was mixed with peptidoglycan or test material in the following manner. To 0.1 ml of test material was added 0.3 ml of VBS-EGTA-Mg buffer. One milliliter of a 1:3 dilution of human serum was added and mixed. The tubes were then incubated at 37°C for 60 min. The tubes were centrifuged, and the supernatant was used for electrophoresis. Conversion of C5→C6 was examined by crossed immuno-electrophoresis according to the method of Laurrel (16). Conversion of Factor B→B<sub>2</sub> was examined by immuno-electrophoresis as described by Scheidegger (19). Results were compared with serum incubated without test material (negative control) and with serum incubated with cobra venom factor (positive control).

**RESULTS**

**Activation of the ACP by streptococcal cell components.** Activation of the ACP by several cell preparations and isolated components of group A streptococci is shown in Table 1. Zymosan, *M. lysodeikticus* whole cells, peptidoglycans from *C. violaceum* and *B. subtilis*, and LPS from three sources are included for comparative purposes. Results are expressed as the mean and standard deviation of the percent complement removed from three normal human sera. Of the group A streptococcal components tested, peptidoglycan is by far the most active, followed by cell wall, protoplast membrane, and whole streptococcal cells, in that order. The group-specific carbohydrate showed no activity. Under the conditions we used, the peptidoglycan is much more active than LPS, zymosan, or *M. lysodeikticus* cells. It is possible, however, that under other conditions, such as increased sonic treatment, the activity of these preparations may increase. As a control, EA were incubated with human serum diluted 1:3 in VBS-EGTA-Mg buffer. No lysis of EA was observed, confirming that the classical pathway was blocked under these buffer conditions. As another control, peptidoglycan was incubated with rabbit red cells.
in VBS-EGTA-Mg buffer. Peptidoglycan by itself did not cause lysis of rabbit red cells.

The results shown here are from one experiment in which each substance was tested on three different human sera. In the past year we have tested three different preparations of peptidoglycan and several preparations of cell walls with similar results.

**Effect of solubilization by lysozyme or sonic treatment.** Additional evidence that peptidoglycan is the active component of the streptococcal cell wall was provided by measuring the effect of lysozyme on the ACP activity of isolated peptidoglycan. Lysozyme is a muramidase which cleaves the β(1,4) bond between N-acetyl-muramic acid and N-acetyl-glucosamine, resulting in eventual solubilization of the peptidoglycan and, if used in high enough concentrations, degradation to disaccharide-peptide units. Group A and group D streptococcal cell walls as well as LPS were also tested. Lysozyme concentrations ranged from 0.01 to 100 µg per mg of peptidoglycan.

Low concentrations of lysozyme enhanced ACP activation by peptidoglycan, whereas higher concentrations of lysozyme progressively destroyed activity (Fig. 1). Although the enhancement is not statistically significant, increased activity has been consistently observed with several preparations of peptidoglycan and low concentrations of lysozyme. This indicates that complement activation is dependent upon the particle size of peptidoglycan. In our buffer system, the ACP was not activated by peptidoglycan suspensions which had been extensively degraded by lysozyme treatment. After treatment with lysozyme, there was no significant change in the capacity of group A cell walls to activate the ACP. In contrast, the ACP activity of group D cell walls was almost completely destroyed. It has previously been shown that group A streptococcal cell walls are very resistant to the lytic effects of lysozyme (12), whereas group D cell walls are readily lysed. As expected, endotoxin activity was not affected by lysozyme. The results also rule out the possibility that the cell walls and peptidoglycan are contaminated with endotoxin. Lysozyme alone does not activate the ACP (Fig. 1).

To explore further the effects of particle size on activation of the ACP, peptidoglycan was fragmented mechanically rather than enzymatically. Peptidoglycan, group A streptococcal cell wall, and zymosan were sonically treated for from 1 to 180 min and tested for activation of the ACP. Analogous to the lysozyme treatment, there was enhancement of ACP activation with peptidoglycan preparations treated with up to 30 min of sonic treatment (Fig. 2). Thereafter, ACP activation decreased, reaching a very low level after 180 min of sonic treatment. In contrast, group A streptococcal cell wall and zymosan, which are much more resistant to degradation by sonic treatment, showed only slight changes in their complement-activating ability.

**Inhibition studies.** We attempted to identify more precisely the structure of peptidoglycan which was responsible for activating the ACP. For this purpose, experiments were designed to detect blocking of ACP activation by purified components of peptidoglycan or peptidoglycan fragments solubilized by lysozyme. N-acetyl glucosamine, N-acetyl muramic acid, d-alanine, or lysozyme-solubilized peptidoglycan was mixed with peptidoglycan to detect inhibition of ACP activation. None of the compounds tested could inhibit complement activation by peptidoglycan, nor could they activate the ACP alone (Table 2).

**Immunoelectrophoresis.** Evidence that the anti-complementary effect of peptidoglycan is the result of activation of the ACP rather than the classical pathway has depended thus far upon the activity proceeding in Ca²⁺-free conditions. It is possible that the peptidoglycan inhibits complement activity by binding to any component of the complement system. More direct evidence that peptidoglycan activates the ACP is provided by the demonstration of molecular alteration of C3 and factor B in Ca²⁺-free buffer (VBS-EGTA-Mg buffer). Crossed immunoelectrophoresis and immunoelectrophoresis were performed to demonstrate this conversion.

**Table 1. Activation of ACP by various microbial preparations**

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration (µg/ml of 1:3 serum)</th>
<th>% Complement removed/µg of test material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A streptococci, whole cells</td>
<td>20</td>
<td>0.68 ± 0.47</td>
</tr>
<tr>
<td>Group A, whole cells (trypsinized)</td>
<td>20</td>
<td>1.03 ± 0.55</td>
</tr>
<tr>
<td>Group A, cell wall</td>
<td>5</td>
<td>5.37 ± 0.34</td>
</tr>
<tr>
<td>Group A, peptidoglycan</td>
<td>1</td>
<td>28.72 ± 8.69</td>
</tr>
<tr>
<td>Group A, carbohydrate</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Group A, protoplast membrane</td>
<td>10</td>
<td>2.95 ± 0.40</td>
</tr>
<tr>
<td><em>M. lysodeikiticus</em>, whole cells</td>
<td>20</td>
<td>3.55 ± 0.76</td>
</tr>
<tr>
<td>Zymosan</td>
<td>20</td>
<td>0.90 ± 0.25</td>
</tr>
<tr>
<td><em>E. coli O26:B6</em>, LPS</td>
<td>10</td>
<td>3.14 ± 2.28</td>
</tr>
<tr>
<td><em>E. coli O55:B5</em>, LPS</td>
<td>50</td>
<td>0.40 ± 0.09</td>
</tr>
<tr>
<td><em>S. typhimurium</em>, LPS</td>
<td>5</td>
<td>7.60 ± 1.34</td>
</tr>
<tr>
<td><em>B. cereus</em>, peptidoglycan</td>
<td>10</td>
<td>3.19 ± 1.00</td>
</tr>
<tr>
<td><em>C. violaceum</em>, peptidoglycan</td>
<td>10</td>
<td>1.77 ± 1.6</td>
</tr>
</tbody>
</table>

* Several concentrations were tested; these are the concentrations which provided most precise measurement of complement activation.
Fig. 1. Activation of complement by bacterial products after treatment with lysozyme. C, Complement.

Table 2. Attempts to inhibit activation of the ACP by peptidoglycan

<table>
<thead>
<tr>
<th>Material*</th>
<th>% Complement removed/µg of test material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A streptococcal peptidoglycan</td>
<td>28.79 ± 9.04</td>
</tr>
<tr>
<td>Peptidoglycan + D-alanyl-D-alanine</td>
<td>28.68 ± 8.50</td>
</tr>
<tr>
<td>Peptidoglycan + muramic acid</td>
<td>28.85 ± 8.53</td>
</tr>
<tr>
<td>Peptidoglycan + N-acetyl glucosamine</td>
<td>28.39 ± 8.60</td>
</tr>
<tr>
<td>Peptidoglycan + lysozyme-solubilized peptidoglycan</td>
<td>28.72 ± 8.69</td>
</tr>
<tr>
<td>D-Alanyl-D-alanine</td>
<td>0</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>0</td>
</tr>
<tr>
<td>N-acetyl glucosamine</td>
<td>0</td>
</tr>
<tr>
<td>Lysozyme-solubilized peptidoglycan</td>
<td>0</td>
</tr>
</tbody>
</table>

* One microgram of peptidoglycan plus 100 µg of inhibitor or 100 µg of inhibitor only.

Fig. 2. Activation of complement by group A streptococcal cell walls, peptidoglycan from group A cell walls, and yeast zymosan after sonic treatment for various periods of time. C, Complement.

of C3→C3i and factor B→B. Figures 3 and 4 show that these alterations are produced by peptidoglycan. Cobra venom factor was used as a positive control. No conversion of C3 or factor B was obtained with EA under identical conditions.
COMPLEMENT ACTIVATION BY PEPTIDOGLYCAN

FIG. 3. Conversion of C3 to C3i. Crossed immunoelectrophoresis of human serum diluted 1:3 with VBS-EGTA-Mg buffer and incubated with (A) PBS, (B) 10 µg of peptidoglycan, or (C) cobra venom factor. Electrophoresis was performed in agarose with 0.05 ionic-strength Veronal buffer, pH 8.6, containing 1 mM calcium lactate. Electrophoresis in the second direction was run in the same agarose buffer containing goat antiserum against human C3.

FIG. 4. Immunoelectrophoresis. Conversion of factor B to B in human serum diluted 1:3 with VBS-EGTA-Mg buffer and incubated for 60 min at 37°C with PBS (upper well) or 5 µg of peptidoglycan (lower well). Electrophoresis was performed in agar with 0.05 ionic-strength Veronal buffer, pH 8.6, containing 0.01 M EDTA. Patterns were developed with goat antiserum against human factor B.

DISCUSSION

These studies show that the peptidoglycan structure of streptococcal cell walls is a potent activator of the alternate pathway of human complement. The group-specific carbohydrate is completely inactive, and the isolated protoplast membrane from group A streptococci displayed only one-tenth the activity of peptidoglycan. Under the conditions employed, peptidoglycan was more active on a weight basis than zymosan or three different preparations of LPS. It is possible that, with optimal dispersion, the activity of these agents might be increased, although in our experiments extended sonic treatment of zymosan did not increase activity.

Due to the numerous differences in assay method and preparation of cell components, it is difficult to compare our results directly with others. Tauber et al. (23), using a standard complement titration (CH50) method rather than the kinetic technique we used, found the cytoplasmic membrane from group A streptococci to be more active than cell walls in activating the ACP. On the other hand, Winkelstein and Tomas (24) in experiments with pneumococci
found just the opposite. Their cell membrane preparation showed less than 10% of the activity of pneumococcal cell walls. Peptidoglycan and teichoic acid accounted for about 95% of their cell wall preparation. In our hands, streptococcal cell walls were more active than cell membrane, and peptidoglycan was the most active component tested; therefore, it is doubtful that contamination of streptococcal cell walls or peptidoglycan with membrane influenced our results. In addition, treatment of peptidoglycan or group D cell walls with lysozyme, which solubilizes these structures but does not affect protoplast membrane, destroyed all the complement-activating capacity of these components. For these same reasons it is possible to exclude LPS, media components, and cell enzymes as being responsible for the activation of the ACP.

Experiments with lysozyme-treated or sonically treated peptidoglycan demonstrate that this cell wall polymer has an optimum size for maximal ACP activation. With low doses of lysozyme or short periods of sonic treatment activity increases, but if degradation proceeds further, activity is lost. Thus, treatment of peptidoglycan with 100 μg of lysozyme per mg or sonic treatment for 180 min destroyed its capacity to activate the ACP. In this respect peptidoglycan is like another polymer, dextran sulfate, which must be of a molecular weight of at least 1 × 10⁴ to be active (5).

Bokisch (4) was the first to show that peptidoglycan isolated from both streptococci and Staphylococcus epidermidis was capable of activating complement. Because activation in normal serum was 40× that in C2-deficient serum, he concluded that complement activation by peptidoglycan proceeds almost entirely by the classical pathway. It seems probable that he did not detect significant activation of the ACP because he used peptidoglycan which was extensively solubilized by sonic treatment. We have demonstrated that extensive sonic treatment destroys the capacity of peptidoglycan to activate the ACP.

Activation of complement by either the classical or alternative pathway results in the generation of a wide spectrum of biological activities with the potential to elicit acute inflammation (7), modify the immune response (8), or initiate clotting (25). It is important, therefore, to understand how bacterial products can activate the complement system directly without the requirement of antibody. Peptidoglycan is a cell wall polymer which occurs with minor chemical modification in almost all bacteria and is even more common than LPS. The potential influence of such material on protective or tissue-injuring processes mediated by complement may be significant.

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LITERATURE CITED

COMPLEMENT ACTIVATION BY PEPTIDOGLYCAN


