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13. ABSTRACT

The report describes work on the polysaccharide and teichoic acid cell wall antigens of the streptococci. It is shown that the chemical modification of such antigens provides them with the ability to sensitize red blood cells, and such cells can be used to measure antibody levels in blood sera. Methods for the extraction of polysaccharide and teichoic acid antigens from the streptococci have been developed, and the chemical composition and serological specificity of these antigens has been defined. The role of the streptococcal cell wall in the uptake of DNA and subsequent genetic transformation has been investigated.



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| genetic transformation | | | | | | |
| Streptococcal disease | | | | | | |
| antibody measurement | | | | | | |

# Final Progress Report

## Structure and Biological Activity of the Streptococci

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## 1. Aims of the program.

The research program of this laboratory is devoted to (a) studies on the chemical activity, genetic properties and organization of the streptococcal cell and (b) the relation of these properties to the pathogenicity of the microorganism. The possible role of the streptococcus as a source of sensitizing antigen, or other product of metabolism, in upper respiratory disease, carditis, or dental disease emphasizes that such studies are necessary for an understanding of the mechanism of the disease process. The ability of the microorganism to survive, multiply and produce disease in vivo is due in large part to the immunological-chemical-genetic activity of the streptococcal cell. This research program is directed toward those objectives.

## 2. Research progress.

### a. Measurement of serum antibodies to streptococcal disease in humans.

Previous studies in this laboratory described the early work on the use of stearoyl derivatives of streptococcal polysaccharide antigens for the sensitization of red blood cells and the measurement of serum antibodies. Our work has continued and 2 publications have resulted (J. Bact. 100:641, 1969; Int. Arch. Allergy Applied Immun., 40:820. 1971). The adsorption of the $H_3$-ester to the red cell has been demonstrated and properties of the system have been studied. It has also been demonstrated that, with the addition of complement, antibody titers can be measured based on red cell lysis. It is likely that the procedure could be applied to other bacterial antigens, i.e. teichoic acids. A group A streptococcal preparation was esterified and used to sensitize red cells. The agglutination titer in A antiserum was low, however additional work is required to define conditions for the esterification of polymers such as teichoic acids.

This procedure has been employed by other workers to measure group A antibodies in sera from patients with streptococcal glomerulonephritis (Ortiz et al., Arch. Int. Med. 126:640-646, 1970; Potter et al., J. Inf. Dis. in press (June) 1972). Methods presently available (ASO, AH, etc.) measure antibodies to extracellular streptococcal products, however, our procedure measures antibodies to an intracellular streptococcal pound, a component of the cell wall.

It appears this method could be used to measure antibodies against any bacterial polysaccharide--containing material (including the teichoic acids). In addition to a diagnostic aid, it would be of value to determine the effectiveness of vaccination.

b. Chemical structure and serological specificity of streptococcal polysaccharide cell wall antigens.

The group E polysaccharide has been studied in some detail (Inf. Immun. 3:653-658, 1971). These streptococcal strains produce bacteremia in man and lymphadenitis in swine. The antigen has been isolated and purified by methods previously developed in this laboratory (J. Bact. 90:667, 1965). Partial hydrolysis of the antigen released 5 oligosaccharides which were isolated and purified by column chromatography. These were identified as di-, tri- and tetra-saccharides. They were analyzed by chemical analysis, borohydride reduction, inhibition of immunological activity, and susceptibility to enzyme action. The repeating unit in the polysaccharide has been identified as O-B-D-glucosyl-(1-2)-O-$\alpha$-L-rhamnosyl-(1-4)-L-rhamnose. The terminal glucose possess the major responsibility for the specificity of the antigen. The group F antigen also possess B-D-glucose in terminal position however it is adjacent to N-acetylglucosamine (Michel et al., J. Gen. Microb. 37:381. 1964). Thus it appears that the specificity of these antigens depends on finite differences in their structure.

A new polysaccharide antigen of the group A streptococci has been isolated and identified (Inf. Immun. 3:385-389, 1971). It is composed of glucosamine, glucose and rhamnose, with the amino sugar possessing the principal serological specificity.

c. The structure and specificity of streptococcal teichoic acids.

A group A streptococcal teichoic acid was isolated and purified, and shown to be a glycerol-phosphate-alanine-glucosamine polymer. Serological specificity was due to N-acetylglucosamine and D-alanine methyl ester, with the former possessing principal responsibility. The glucosamine and alanine appear to be labile to the method of extraction. A glucose-glucosamine polymer was also isolated from the same organism. Its specificity was due to glucosamine. Group A antiserum, even after adsorption with heterologous cells, contained antibodies to both of these polymers. These polymers are thus specific for group A cells. (J. Bact. 102:747-752, 1970).

The results of this and an earlier study indicate that group A antiserum, produced in rabbits against whole cells and subsequently adsorbed with whole cells of a strain from another serological group, contains at least three antibodies: (i) anti-group A polysaccharide, (ii) anti-glucose: glucosamine polymer (polysaccharide G) and (iii) anti-glycerol:phosphorus: alanine:glucosamine polymer. The role of each antibody in the identification of a streptococcus would depend primarily on the amount of each antibody per unit volume of serum and the method used to extract antigens from the cell. The usual practice of 0.1 N HCl at 100 C to extract the antigens might reduce the specificity of the teichoic acid polymer; however the glucose:glucosamine polymer might be resistant as is the group A

rhamnose:glucosamine antigen. The extent of distribution of the glucose: glucosamine polymer among serological types other than 3 and 14 is not known at present.

Previous studies from our laboratory on the streptococcal group O cell wall polysaccharide antigen showed that several non-group protein antigens were removed from the cell by extraction with dilute HCl at 100 C (Mukasa and Slade, Inf. Immun., in press). The protein antigens of the streptococci which have been studied immunologically and chemically have been designated as type antigens. They occur most frequently in group A and are designated M, R and T. No other protein antigens of the streptococci have been identified and described. It was thus of considerable interest to investigate these antigens of the group O streptococci and to compare them with the other streptococcal protein antigens. The O streptococci are beta hemolytic and occur in the human throat and respiratory tract. They cause subacute endocarditis in man.

d. Genetic transformation of streptococci.

The transformation with extracellular DNA of streptococci to antibiotic resistance has been described in published work from this laboratory ( J. Bact. 83:443, 1962; 88:595, 1964; 91:2216, 1966). These studies were concerned with the characteristics and properties of the transformation process in these bacteria and were described in some detail in an earlier progress report.

During the past 3 years we have studied transformation in group H streptococci which had been isolated from root canals of human teeth. The synthesis of the group and type polysaccharide antigens was employed as a marker (J. Bact. 96:1225, 1968). The transfer of each of these markers was only successful in those strains which did not already possess a group or type antigen. The addition of a capsule-type polysaccharide antigen has been found to occur in pneumococci by transformation ( Austrian et al., J. Exptl. Med. 110:571, 1959). Thus in the streptococci it would a appear that the polysaccharide antigen (which is not a capsule in the same sense as in the pneumococcus) occupies an essential spatial position in the wall, and the synthesis of a 2nd such molecule is repressed by control of one or more of the enzymes involved.

We also noted in these studies that the synthesis of the type II antigen always resulted in beta hemolysis. The transformation of this marker to a streptococcal strain not possessing a type antigen resulted in synthesis of the antigen and beta hemolysis. It is likely that these are closely linked on the cell genome. A reaction or reactions essential for type II polysaccharide synthesis is considered to be responsible for hemolysis of the red cell.

Our recent studies (Deddish and Slade, J. Bact 105:779-786, 1971) have demonstrated the binding of DNA by the streptococcal cell wall. The role of the cell wall in transformation has never been described. Information from other laboratories has been limited to the uptake of DNA by viable bacterial cells. We have shown that both homologous and heterologous (B. subtilis, calf thymus) DNA is bound by competent and noncompetent

streptococcal cell walls, however the competent cell walls bind 1.5 - 2.1 times as much DNA as the non-competent walls. Our results also showed that the "binding sites" were removed by trypsin. Trypsin was also able to remove about 40% of the bound DNA and 60% of this DNA was still capable of causing transformation. Antibodies against the competent cell wall prevented the DNA binding and transformation.

It is our belief that 2 "binding sites" exist on the streptococcal cell wall. One is not concerned in transformation whereas the other is of such a nature that binding eventually results in integration and expression of the marker.

3. Significance of the research.

These studies on the streptococcal antigens covering the past 10 years has provided us with information on (1) methods of extraction of the antigens from the cell without loss of immunological specificity, (2) chemical composition and structure of the antigens, and (3) relation of antigenic specificity to structure. Such information is essential for the development of a vaccine. It has also been determined that these antigens can be chemically modified, and as such can be used to measure antibody levels in human sera. Previous methods have not been sufficiently sensitive to achieve such measurements. Thus this method can be used to monitor antibody levels during natural or artificial immunization

4. Research publications during the report period.


a. Deddish, P. and H. E. Slade. 1969 Detection of Polysaccharide, Teichoic Acid, and Protein Antigens in Bacterial Colonies on an Agar Surface. J. Bact. 97:1352-1356.

b. Pavlovskis, O. and H.D. Slade. 1969. Adsorption of $^{3}H$-Fatty Acid Esters of Streptococcal Groups A and E Cell Wall Polysaccharide Antigens by Red Blood Cells and Their Effect on Hemagglutination. J. Bact. 100:641-646.

c. Matsuno, T. and H. D. Slade. 1970. Composition and Properties of a Group A Streptococcal Teichoic Acid. J. Bact. 102:747-752.

d. Deddish, P. and H. D. Slade. 1971. Binding of Deoxyribonucleic Acid by Cell Walls of Transformable and Nontransformable Streptococci. J. Bact. 105:779-786.

e. Pavlovskis, O. and H. D. Slade. 1971. Factors Affecting the Adsorption of Esterified Streptococcal Group A and E Cell Wall Polysaccharide Antigens to Red Blood Cells and Their Effect on Antibody Measurement. Int. Arch. Allergy 40:820-833.

f. Matsuno, T. and H. D. Slade. 1971. Group A streptococcal Polysaccharide Antigens. Inf. Immun. 3:385-389.

g. Soprey, P. and H. D. Slade. 1971. Chemical Structure and Immunological Specificity of the Streptococcal Group E Cell Wall Polysaccharide Antigen. Infec. Immun. 3:653-656.