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Filamentous Capsulated Streptococci from the Human Respiratory Tract

I. Antigenic Attributes of Provisional Capsular Type 83 and Its Relationship to Streptococci of So-Called Group M

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Two immunologically reactive polysaccharides have been isolated from the cell walls and from culture filtrates of a filamentous alpha-hemolytic streptococcus provisionally designated capsular type 83. Both polysaccharides were purified by diethylaminoethyl-cellulose chromatography. Analysis indicates that the capsular polysaccharide consists of galactose and phosphorus, whereas the cell wall polysaccharide contains galactosamine, glucosamine, glucose, and phosphorus. On the basis of immunochemical experiments, it is suggested that the capsular polysaccharide is composed of galactose-phosphate units with terminal galactose residues at the nonreducing end. It has also been found that the capsular antigen of streptococcus type 83 is shared by a number of streptococcal strains classified in Lancefield's group M. The cell wall polysaccharide of streptococcus type 83 cross-reacts with antibody to the C_s, or cell wall-like capsular, polysaccharide of *Diplococcus pneumoniae*, and this cross-reactivity may be a reflection that the streptococcal antigen possesses certain structural features which are similar to those of pneumococcal C and C_s polysaccharides.

A number of attempts have been made to classify the large and heterogeneous group of alpha- and nonhemolytic streptococci of the human respiratory tract on the basis of a variety of their attributes (9, 12, 37). Recognition of the fact that many of these organisms possess both capsular and cell wall carbohydrate antigens (16, 17, 26-28, 40) has prompted an extended study to determine whether or not these cellular polymers could be used to develop an immunological scheme for their classification (2) in a manner analogous to that employed for the identification of certain other streptococci, such as those of Lancefield's groups B, F, and G (19, 30).

Alpha- and nonhemolytic streptococci of the human respiratory tract may be divided arbitrarily into two groups of morphological variants: those which grow as single cells, pairs, and short chains giving rise to smooth colonies with regular surfaces and edges, and those which grow in long chains or "filaments" of hundreds of cells, forming colonies with rough surfaces and irregular edges. Among 325 strains of this latter group, 248 have been identified as belonging to one or another of 30 capsular serotypes. Three of these streptococcal capsular antigens are related immunologically to the polysaccharides of pneumococcal capsular types 59, 72, or 75, respectively. The remaining 27 streptococcal capsular antigens do not cross-react with a polyvalent antiserum, "omni-serum" (21), to any of the known pneumococcal capsular polysaccharides. To avoid ambiguity with any of the 82 recognized pneumococcal capsular serotypes, the capsular antigens of these streptococcal strains have been given provisional numerical type designations beginning with the Arabic numeral 83 until their classification can be more fully established.

In addition, it has been found that hydrochloric acid extracts (18) of 40% of strains of capsulated filamentous streptococci examined react in capillary precipitin tests (39) with a potent antiserum to the C_s, or cell wall-like capsular, polysaccharide of pneumococcus (6), whereas intact cells fail to do so in the capsular precipitin, or Quellung, reaction. From these observations, it has been inferred that the precipitate formed results from the interaction of a streptococcal cell wall antigen with antibody to pneumococcal C_s polysaccharide. The

fact that this reaction occurs with extracts of a significant proportion of streptococcal strains of diverse capsular types suggests that their cell wall antigens may be closely similar or identical and may form the basis for their classification as a distinct group analogous to certain of the Lancefield groups of beta-hemolytic streptococci.

In the studies reported below, some attributes of filamentous streptococci producing the capsular antigen provisionally designated type 83 are described, including biochemical investigations of the capsular and cell wall carbohydrates of the prototypic strain, the latter of which cross-reacts with the C_8 polysaccharide of pneumococcus. The relationship of the capsular antigen of this organism to an antigen of streptococcal strains of Lancefield's so-called group M of both animal and human origins will be discussed.

MATERIALS AND METHODS

Bacterial strains. The prototypic strain of the filamentous alpha-hemolytic streptococcus provisionally designated capsular type 83 was recovered from the sputum of a patient in Philadelphia with an acute respiratory infection. The organism probably bore no causal relationship to the patient's illness. It will be referred to hereafter as streptococcus type 83. Strain C.C. 3 of streptococcal type 83 was isolated from the blood of a patient in Chicago.

Pneumococcal strains R36NC. $R6_{30}$, and A66R2 have been described previously (4).

Three strains of streptococci of presumed canine origin and classified in Lancefield's group M were obtained from R. C. Lancefield, Rockefeller University, New York, N.Y. They were: D168A "x", D168A/0/10, and D168C. Ten strains of human origin and two of presumed canine origin were received from R. M. Cole, National Institute of Allergy and Infectious Diseases, Bethesda, Md. They included strains 52x11, 55x634, and 60x269 from human blood cultures, 55x47 from a human abscess, 54x35, 54x73, 54x83, and 54x136 from human throat cultures, and strains D168A (ATCC 9934) and D168B (ATCC 9935).

Bacterial media. All strains were grown in fresh beef heart infusion broth supplemented with Neopeptone (Difco Laboratories, Inc., Detroit, Mich.) (1).

Preparation of bacterial cell walls and carbohydrates. Cell walls were prepared by the method described by Bleiweis et al. (5) from streptococcus type 83. Antigens were extracted from cell walls with cold trichloroacetic acid according to the procedure described by Park and Hancock (31). Capsular antigens were isolated from culture filtrates by the method described by Michel and Krause (25).

Analytical methods. Analyses for hexosamines and amino acids were performed by the method of Sparkman et al. (38) as modified by Karakawa and Krause (15). Total hexosamine and rhamnose were determined by the methods of Rondle and Morgan (34) and of Dische and Shettles (11), respectively. Glucose and galactose were assayed by the method of Curtis and Krause (10), employing, respectively, glucostat and galactostat reagents (Worthington Biochemical Corp., Freehold, N.J.). Total phosphorus was determined by the method described by Chen et al. (8).

Chromatography. Capsular and cell wall carbohydrates were purified by diethylaminethyl (DEAE)-cellulose chromatography (column, 30 by 2.5 cm) by methods described previously (15). Paper chromatography was performed in a solvent of *n*-butyl alcohol-pyridine-water (6:4:3 by volume) by the multiple ascent technique of Pazur et al. (32). Purified carbohydrates were hydrolyzed in 0.1 N HCl in a boiling-water bath. Samples of 5 μliters were removed and examined for the presence of reducing sugars. Reducing sugars on the chromatograms were detected by silver nitrate and aniline oxalate reagents. The sugar components of the polysaccharides were identified by their R_f values as well as by their characteristic reactivity with specific reagents (32).

Serological methods. Rabbit antisera were prepared according to a procedure described previously (4). Rabbits immunized with pneumococcal transformants of capsular type 83 gave rise to antibody of consistently higher titer than those immunized with the filamentous capsulated streptococcal strain. Qualitative and quantitative precipitin tests were performed by the method of McCarty and Lancefield (23). Quantification of antibody protein was determined by the method described by Lowry et al. (20). Methods for double diffusion in agar and for immunoelectrophoresis have been described previously by Karakawa and Kane (14). Acid extracts of streptococci were prepared by the method of Lancefield (18), and capillary precipitin tests were performed with the technique of Swift (39). Antisera for identifying group M streptococci were obtained from Difco Laboratories, Detroit, Mich., and from Wellcome Laboratories, Beckenham, England. An additional group M antiserum prepared with strain D168A "x" was obtained from R. C. Lancefield.

Transformation of pneumococci with streptococcal deoxyribonucleic acid. Partially purified preparations of deoxyribonucleic acid (DNA) of streptococcus type 83 were obtained by lysing suspensions of washed organisms in 0.1 N phosphate buffer, pH 8, containing 3% sodium citrate with a crude preparation of the enzymes of *Streptomyces albus* (22). The lysed suspension was clarified by centrifugation, and the DNA was recovered from the supernatant fluid by precipitation with 3 vol of ethanol. Transformation of noncapsulated pneumococci was carried out by a method described previously (4).

RESULTS

Isolation of antigens from streptococcus type 83. Previous studies have shown that the cell wall polysaccharides of pneumococcus are readily degraded by the conventional methods used for the isolation of carbohydrate antigens from bacterial cells. (T. Y. Liu, personal communication). Because the prototypic strain of

streptococcus type 83 had been shown to possess an antigen cross-reacting with the acid-labile cell wall (C) and C_s polysaccharides of pneumococcus, it was apparent that a prerequisite for the isolation of the streptococcal cell wall antigen involved the selection of an appropriately mild procedure for its extraction. In an attempt to minimize degradation of the carbohydrate antigen, cell walls of the streptococcus were extracted by using 10% trichloroacetic acid at 4 C for 8 h. The trichloroacetic acid-soluble fraction was separated from the insoluble cell wall residues by centrifugation, dialyzed against distilled water, and concentrated by flash evaporation. The concentrated preparation was deproteinized by shaking with a chloroform-butyl alcohol mixture as described by Sevag (35), dialyzed, and concentrated. Illustrated in Fig. 1 are the results of an immunoelectrophoretic analysis of the crude trichloroacetic acid-soluble fraction isolated from the streptococcus. In this experiment, the trichloroacetic acid extract was placed in the center well and subjected to electrophoresis for 45 min at 250 V. After electrophoresis, antiserum to whole cells of streptococcus type 83 was placed in the upper trough, and anti-C_s pneumococcal serum was placed in the lower trough. The crude trichloroacetic acid extract contained at least two distinct antigens: an acidic polymer which formed a precipitin band with the homologous streptococcal antiserum, and a heterogeneous antigen which formed a broad diffuse band only with the anti-C_s pneumococcal serum (Fig. 1).

In the following experiments, attempts were made to separate the two distinct antigens present in the trichloroacetic acid extract of streptococcus type 83. The crude trichloroacetic acid fraction was subjected to gel filtration on a Bio-gel P-60 column (90 by 2.5 cm) as described previously (13). Two major fractions were eluted from the column: a fraction reacting with antiserum to streptococcus type 83, and a fraction reacting with anti-C_s pneumococcal serum. Depicted in Fig. 2 are the paper chromatography analyses of the acid hydrolysates of both antigens. Note that the fraction reacting with antiserum to streptococcus type 83, designated the crude capsular fraction, consisted essentially of galactose, glucosamine, and galactosamine. In contrast, the fraction reacting with anti-C_s pneumococcal serum consisted predominantly of glucose, galactose, and hexosamines. Noteworthy is the fact that the cell wall antigen of streptococcus type 83 contains some of the same constituents as pneumococcal C and C_s polysaccharides (Fig. 2), i.e., glucose and galactosamine (29). Subsequent immunological analysis indicated that the presence of galactose in the preparation of the cell wall antigen of streptococcus 83 was due, in part, to contamination of the cell wall preparation with capsular antigen. Furthermore, the preparation of capsular antigen also contained elements of the streptococcal mucopeptide, namely, glucosa-

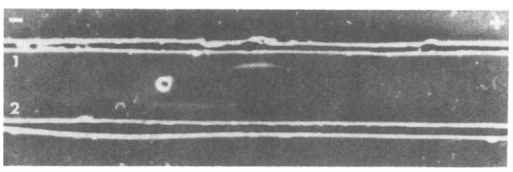

FIG. 1. *Immunoelectrophoresis of a crude trichloroacetic acid extract (1 mg/ml) of the prototypic strain of filamentous streptococcus type 83. Well: crude antigens of streptococcus type 83. Top trough: antiserum to streptococcus type 83. Bottom trough: anti-pneumococcal C_s serum.*

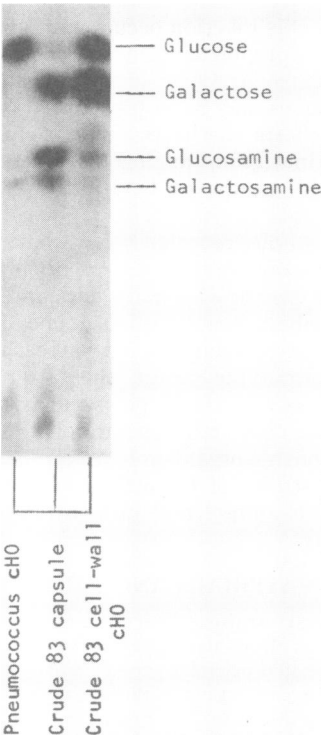

FIG. 2. *Analysis of the acid hydrolysates of the two immunologically active fractions (type 83 capsular polysaccharide [0.2 mg/ml]; type 83 cell wall material [1 mg/ml]) isolated from the trichloroacetic acid extract of streptococcus type 83 by Bio-gel P-60 filtration. Hydrolysis was performed with 0.1 N HCl at 100 C for 30 min. Whatman no. 1 paper was used in ascending chromatography in an n-butyl alcohol-pyridine-water solvent system (6:4:3).*

mine. Additional attempts to isolate purified capsular and cell wall antigens from whole cells or culture filtrates of streptococcus type 83 are described below.

Isolation of a capsular and of a cell wall antigen from culture filtrates of streptococcus type 83. In the following experiments, capsular and cell wall antigens were isolated from culture filtrates of streptococcus type 83 by the modified method of Michel and Krause as described by McDonald and Karakawa (24). Twenty liters of culture filtrate were concentrated with polyethylene glycol (Union Carbide, 20 M), deproteinized, dialyzed, and lyophilized as described previously. The lyophilized product was subjected to DEAE-cellulose chromatography on a column (30 by 2.5 cm) and eluted with varying molarities of $(NH_4)_2CO_3$ buffer, pH 8.6. Two fractions, designated fractions I and II, were eluted from the column (Fig. 3). Analysis of the two fractions by capillary precipitation clearly indicated that fraction I represented the capsular antigen of streptococcus type 83, whereas fraction II represented the cell wall antigen. Table 1 shows the results of chemical analyses of fractions I and II. It should be noted that fraction I (the capsular antigen) consisted essentially of galactose and phosphorus, whereas fraction II (the cell wall antigen) consisted of glucose, galactose, hexosamine, and phosphorus. Significantly, fraction I was devoid of all the major constituents of the streptococcal mucopeptide (Table 1). Although fraction II contained small amounts of the major component of fraction I as well as other components of the mucopeptide, the results presented here indicate the feasibility of separating the capsular and cell wall antigens of streptococcus type 83 by DEAE-cellulose chromatography.

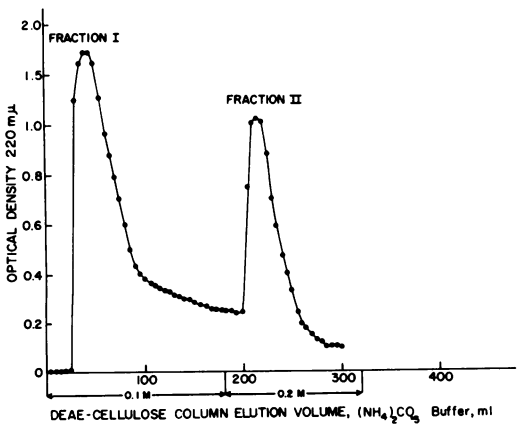

FIG. 3. *Distribution of carbohydrate antigens in eluates from DEAE-cellulose chromatography of a 50-mg sample of a trichloroacetic acid extract of streptococcus type 83.*

TABLE 1. *Chemical composition of the capsular and cell wall antigens and mucopeptide of streptococcus type 83*

Component	Capsule (fraction I) (μmol/mg)	Cell wall CHO (fraction II) (μmol/mg)	Mucopeptide (μmol/mg)
Glucose	0.14	0.77	ND[a]
Galactose	3.80	0.51	ND
PO_4	1.50	0.10	ND
Glucosamine	<0.10	0.56	0.99
Galactosamine	<0.10	0.13	0.10
Muramic acid	<0.10	<0.10	1.19
Alanine	<0.10	0.19	1.71
Glutamic acid	<0.10	0.27	0.87
Glycine	<0.10	0.52	0.24
Lysine	<0.10	<0.10	0.80
Aspartic acid	<0.10	<0.10	0.28
Threonine	<0.10	0.06	0.13
Serine	<0.10	0.14	0.20

[a] ND, not done.

Isolation of the streptococcal type 83 capsular antigen from pneumococcal transformants. Crude preparations of DNA were prepared from a streptomycin-resistant mutant of streptococcus type 83 according to the method described. With these preparations, it was possible to transform three noncapsulated pneumococcal strains, and both streptomycin-sensitive pneumococcal transformants bearing the type 83 streptococcal capsule and streptomycin-resistant noncapsulated transformants were recovered. Rabbits immunized with the capsulated pneumococcal transformants gave rise to antibodies which reacted both with the transformed pneumococci and with the donor strain of streptococcus type 83. To demonstrate the nonidentity of the streptococcus and the capsulated pneumococcal transformants, cell wall antigens of streptococcus type 83, the pneumococcal transformant R36NCT83, and the noncapsulated pneumococcus R36NC were prepared. Depicted in Fig. 4 are the elution patterns of acid hydrolysates of cell walls of the three strains. Shown in the bottom frame are the results of chemical analysis of the streptococcal cell wall hydrolysate. Note that the concentration of glucosamine in the streptococcal cell wall is relatively high compared with that of galactosamine. In contrast, the composition of the cell wall of the pneumococcal capsular transformant, depicted in the middle frame, differs markedly from that of the streptococcus in that it contains approximately equimolar

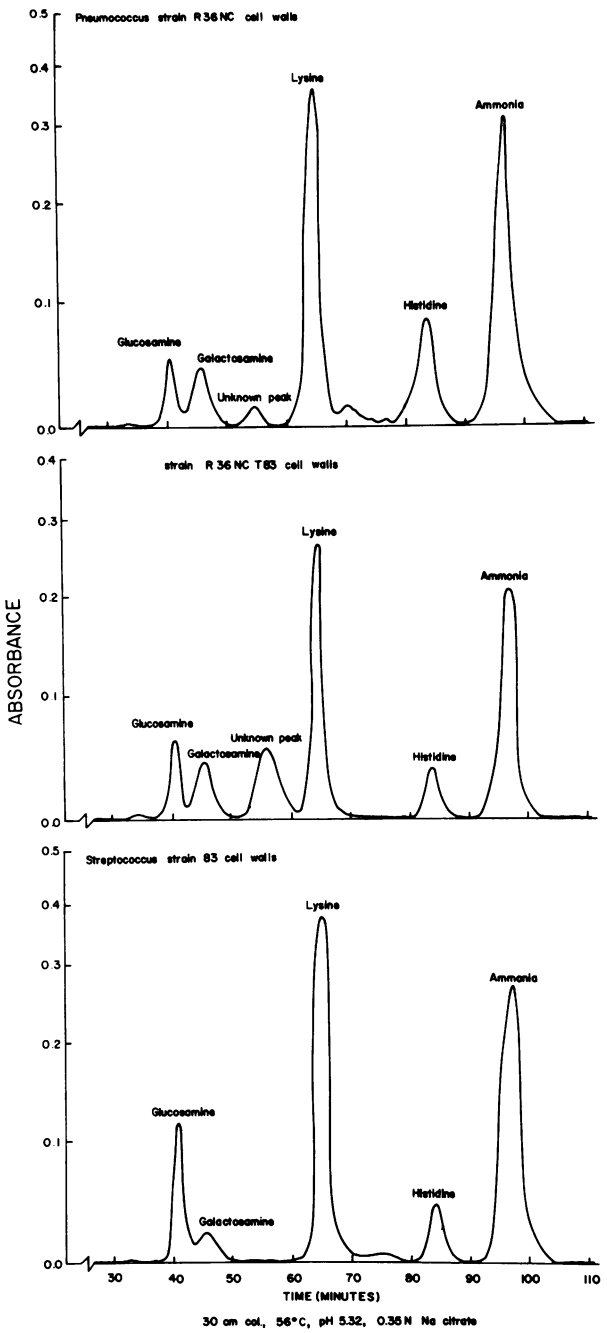

FIG. 4. *Acid hydrolysate analyses. Bottom frame: analysis of an acid hydrolysate of type 83 streptococcal cell walls. Middle frame: analysis of an acid hydrolysate of cell walls of a type 83 pneumococcal transformant. Top frame: analysis of an acid hydrolysate of cell walls of a noncapsulated pneumococcus, strain R36NC. Sample sizes, 0.2 mg.*

amounts of glucosamine and galactosamine. In addition, an unidentified amino compound was eluted in the vicinity of galactosamine. This compound was absent from the streptococcal cell wall hydrolysate. For comparison, the composition of the cell wall of the noncapsulated pneumococcus R36NC is depicted in the top frame. It is apparent that the molar ratios of

glucosamine to galactosamine in the cell walls of the noncapsulated and transformed capsulated pneumococcal strains are similar. In addition, the unidentified amino compound observed in the capsulated transformant is present also in the cell wall of the noncapsulated pneumococcal strain. At present, the identity of this amino compound is not known. These results clearly indicate that the type 83 capsular antigen isolated from cultures of the pneumococcal transformant was indeed associated with the cell wall carbohydrate of that organism and did not result from contamination of the pneumococcal culture with streptococcal cells from which the preparation of DNA was derived.

The chemical analyses of the capsular carbohydrates isolated are shown in Table 2. In all instances, galactose and phosphorus were the major constituents of the capsular carbohydrates derived from these strains. Illustrated in Fig. 5 are the results of quantitative precipitin reactions between the capsular carbohydrates isolated from the pneumococcal transformants and antiserum to streptococcus type 83. The carbohydrates isolated from the pneumococcal transformants $R6_{30}T83$, R36NCT83, and A66R2T83 all gave significant reactions with this antiserum. Additional evidence showing clearly that the capsular material isolated from the pneumococcal transformants is immunochemically identical to the capsular polysaccharide of streptococcus type 83 is depicted in Fig. 6. In each instance, the precipitin lines formed between the antiserum and the capsular antigens of the pneumococcal transformants and of streptococcus type 83 merged to form lines of identity.

Immunochemical analysis of the type 83 streptococcal capsular antigen. To identify the immunodominant determinant of the purified type 83 capsular carbohydrate, the antigen was hydrolyzed with 0.05 N HCL at 100 C for varying time intervals, and the products of hydrolysis were examined by paper chromatog-

TABLE 2. *Chemical composition of the purified capsular polysaccharides of streptococcus type 83 and pneumococcal (pnc) transformants R36NCT83, $R6_{30}T83$, and A66R2T83*

Component	Composition (μmol/mg) of strains			
	Streptococcus type 83	Pnc R36NCT83	Pnc$R6_{30}$T83	Pnc A66R2T83
Galactose	3.80	3.60	2.20	1.70
Glucose	0.14	<0.10	<0.10	<0.10
Phosphorus	1.50	0.90	1.00	0.60
Hexosamine	<0.01	<0.01	<0.01	<0.01

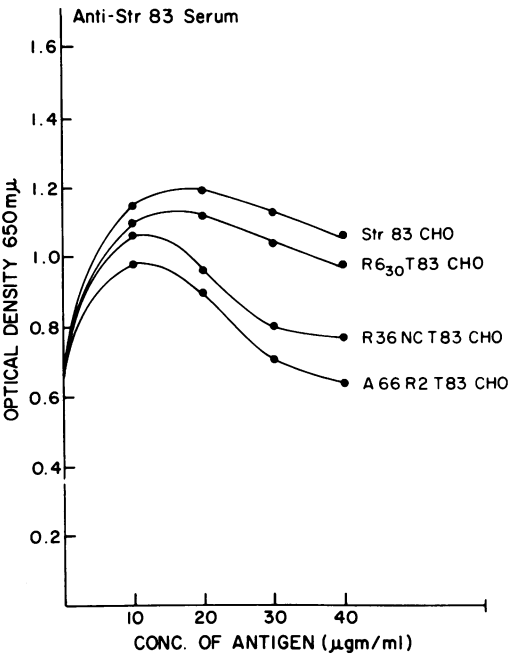

FIG. 5. *Quantitative precipitin reactions of purified capsular antigens of streptococcus type 83 and of type 83 capsular transformants of pneumococcal strains $R6_{30}$, R36NC, and A66R2 with 0.1 ml of antiserum to streptococcus type 83.*

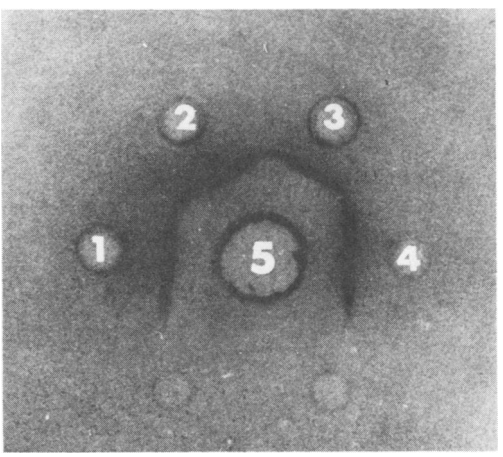

FIG. 6. *Immunodiffusion reactions in agar of the purified capsular polysaccharides of streptococcus type 83 and of several pneumococcal transformants (0.1 mg/ml). Capsular polysaccharide: well 1, pneumococcus R36NCT83; well 2, streptococcus type 83; well 3, pneumococcus $R6_{30}T83$; well 4, pneumococcus A66R2T83; well 5, antiserum to streptococcus type 83.*

raphy as described by Pazur et al. (32). Illustrated in Fig. 7 are the results of paper chromatography analyses of the acid hydrolysates of the capsular polysaccharide. Galactose was released after only 5 min of hydrolysis, whereas no disaccharides or oligosaccharides were observed in the hydrolysates; neither glycerol nor ribitol was found. The findings suggested that the galactose residues were linked to the main structure of the carbohydrate by extremely acid-labile bonds such as phosphodiester bonds. Since no oligosaccharides, such as a disaccharide or trisaccharide of galactose, were observed in the hydrolysate, it is possible that this antigen may represent a polymer of repeating galactose phosphate units. Table 3 shows the results of quantitative precipitin inhibition studies with various monosaccharide inhibitors. D-Galactose in a concentration of 0.022 μmol/ml was found to be a good inhibitor of the reaction between the type 83 streptococcal capsular polysaccharide and its homologous antiserum. Galactose-1-PO_4, however, was a better inhibitor. Approximately 67% of the precipitin reaction between the capsular polysaccharide and its homologous antiserum was inhibited by galactose-1-PO_4, whereas galactose-6-PO_4 inhibited only 20% of the same reaction. These findings suggest that terminal galactose-1-PO_4 units may represent the immunodominant determinants of the capsular antigen of streptococcus type 83.

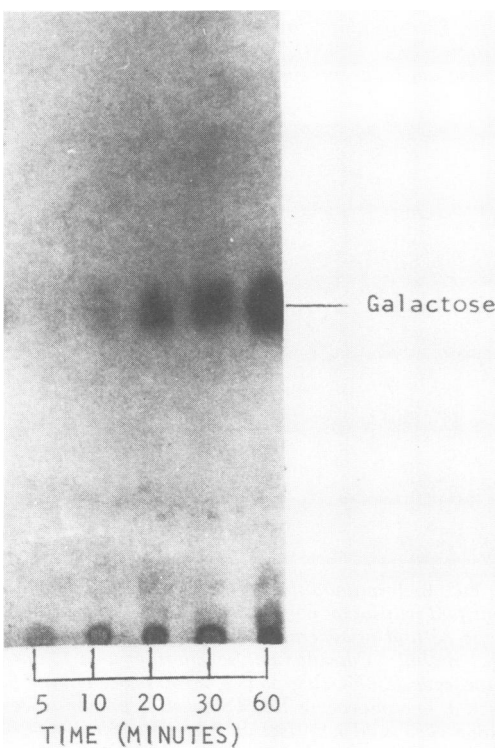

FIG. 7. *Paper chromatogram of an acid hydrolysate of purified type 83 streptococcal capsular polysaccharide. Hydrolysis was carried out with 0.05 N HCl at 100 C for 5, 10, 20, 30, and 60 min.*

TABLE 3. *Quantitative inhibition of the precipitin reaction between the capsular polysaccharide of streptococcus type 83 and its homologous antiserum by various monosaccharides*

Inhibitor	Concn (μmol/ml)	% Inhibition
D-Galactose	0.022	58.3
α-Φ-Nitro-galactose	0.013	56.2
β-Φ-Nitro-galactose	0.013	51.0
Galactose-1-PO_4	0.011	67.5
Galactose-6-PO_4	0.011	20.5

Relationship of streptococcus type 83 to streptococci of so-called Lancefield's group M. Group M in the Lancefield scheme of classification of beta-hemolytic streptococci was established by Fry (41) and included a number of strains isolated from the tonsils of dogs. In 1959, Skadhauge and Perch (36) reported that hydrochloric acid extracts of several strains of alpha-hemolytic streptococci isolated from the oral cavity of healthy humans and from the blood of patients with endocarditis were precipitated strongly by diagnostic group M antiserum prepared by Wellcome Laboratories. Similar reactions were described by Rifkind and Cole (33) with an antiserum to one of Fry's group M strains, SHC 194 (also designated D168B, ATCC 9935). In the course of a survey of filamentous streptococci from the human respiratory tract of a variety of provisional capsular types for their possible relationship to any of the recognized Lancefield groups of streptococci, it was observed that acid extracts of streptococcal strains of type 83 were also precipitated by group M antiserum prepared by Wellcome Laboratories. When the same group M antiserum was used in the Quellung reaction, the type 83 streptococcal strains gave positive reactions identical to those obtained with type 83 streptococcal antiserum. This observation indicated that the reaction between the group M antiserum and the type 83 streptococcal strains involved a capsular antigen rather than one from the streptococcal cell wall.

A number of strains classified as group M streptococci both of animal and of human origin were obtained from R. C. Lancefield and R. M. Cole. Extracts of these strains and group M

antigen from Difco Laboratories were examined in capillary precipitin tests with several antisera prepared for the identification of streptococci of group M and with antiserum to streptococcus type 83 (Table 4). Several strains both of animal and human origins reacted strongly with the Wellcome group M antiserum and type 83 streptococcal antiserum, but not with group M antiserum prepared by Difco Laboratories nor with antiserum to strain D168A "x" prepared by Dr. Lancefield. When cells of the reacting strains were examined in the Quellung reaction, all gave unequivocally positive results, indicating again that the reactive antigen was capsular in nature. Double diffusion tests in agar gels were performed with acid extracts of the capsulated strains (15), Difco group M antigen, and Wellcome group M antiserum and type 83 streptococcal antiserum. An extract of streptococcus type 83 was included also. Extracts of some but not all strains both of animal and human origins formed lines of identity with the extract of streptococcus type 83 (Fig. 8). The Difco group M antigen gave a strong line of precipitation which spurred slightly over that of streptococcus type 83.

TABLE 4. *Capillary precipitin reactions of acid extracts of streptococcus type 83, group M streptococci, and Difco group M antigen with various antisera*

Antigen	Antiserum			
	Streptococcus type 83	Wellcome group M	Difco group M	Lancefield D168A "x"
Streptococcus type 83	++[a]	++	−	−
D168A "x"	−	−	−	+
D168/0/10	−	−	−	+
D168B	++	++	−	−
D168C	++	++	+	±
52x11	−	−	−	−
54x35	−	−	−	−
54x73	−	−	−	−
54x83	−	−	−	−
54x99	−	−	+	±
54x136	−	−	+	+
C.C.3	++	++	−	−
Difco group M	++	++	−	±

[a] Intensity of precipitin reactions graded from negative (−) to two plus (++) depending upon amount of precipitate.

The foregoing observations demonstrate clearly that a number of reagents used currently for the identification of streptococci of Lancefield's group M are related to a capsular antigen identical with or closely similar to the capsular polysaccharide of streptococcus type 83. The utility of the capsular precipitin reaction in localizing this antigen on the surface of streptococcal cells is clearly borne out by these experiments. In the light of these findings, it would appear that the validity of Lancefield's streptococcal group M requires further study to

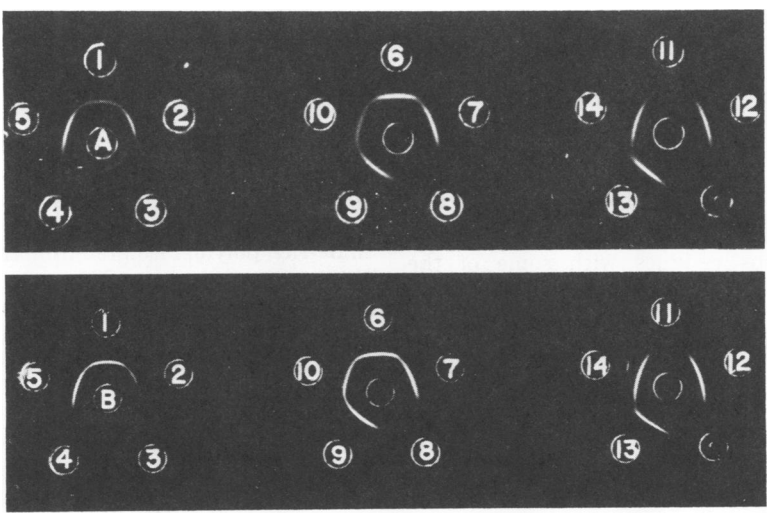

FIG. 8. *Immunodiffusion reactions in agar of acid extracts of streptococcus type 83, strains of group M streptococci, and Difco group M antigen with antisera to streptococcus type 83 and Wellcome group M antiserum. Upper frame: center wells (A), streptococcus type 83 antiserum; lower frame: center wells (B), Wellcome group M antiserum. Outer wells: 2, 5, 7, 9, 12, and 14, streptococcus type 83; well 1, strain D168B; well 3, strain C.C. 3; well 4, strain 69x169; well 10, strain D168C; well 11, strain 54x99; well 13, Difco group M antigen.*

identify a group-specific cell wall carbohydrate if it is to maintain its place with other groups in this scheme of classification.

DISCUSSION

The results described indicate that the prototypic strain of the filamentous alpha-hemolytic streptococcus provisionally designated capsular type 83 produces two distinct carbohydrate antigens; a cell wall polysaccharide and a capsular polysaccharide. Both polymers can be extracted readily from whole cells with cold trichloroacetic acid or from concentrated culture filtrates and purified by DEAE-cellulose chromatography. Chemical analyses indicate that the capsular antigen consists of galactose and phosphorus, whereas the cell wall antigen of the streptococcus consists of galactosamine, glucosamine, glucose, and phosphorus. Immunochemical evidence is consistent with the view that the cell wall antigen of the prototypic streptococcus type 83 possesses antigenic determinants which are similar to the immunodeterminants of the C_s or cell wall-like capsular polysaccharide of pneumococcus (6). Detailed analysis of the immunochemical relationship between the cell wall antigens of a filamentous streptococcus and of pneumococcus will be presented in another communication.

With regard to the capsular antigen, timed acid hydrolysis with 0.05 N HCl indicated that galactose residues probably occupy a terminal position and are linked by extremely acid-labile bonds. Quantitative precipitin inhibition studies using purified capsular material and specific inhibitors clearly showed that galactose-1-PO_4 is a potent inhibitor of the precipitin reaction between the type 83 capsular antigen and its homologous antiserum. In view of those observations, it is suggested that the streptococcal capsular antigen of provisional streptococcal type 83 may consist of a backbone of galactose and phosphorus residues with some of the galactose residues linked to the main chain by acid-labile bonds.

Demonstration that a number of strains of streptococci of both animal and human origins, designated previously as group M streptococci, have capsular antigens immunologically identical or closely similar to that of streptococcus type 83 raises questions regarding their classification. It has been shown by others (33, 36) that streptococci in this group possess a number of antigens, some resistant and some sensitive to heat and to protolytic enzymes. The present studies suggest that the thermostable, enzyme-resistant antigen demonstrated by Rifkind and Cole (33) is a capsular antigen and that antisera to it should not be used to characterize group M streptococci, if such characterization, by analogy with other streptococcal groups, is to be based upon a cell wall carbohydrate. The usefulness of the Quellung reaction in distinguishing capsular from cell wall antigens is clearly evident.

At the present time, relatively few alpha- and nonhemolytic strains of streptococci from the human respiratory tract have been studied intensively, and the classification of most strains is based largely upon their biochemical activities which are somewhat variable. Because of the relative lack of knowledge of the antigenic structure of these organisms, studies have been initiated to determine whether or not it is possible to develop a suitable scheme for their classification based, by analogy with other streptococci, on their chemically definable capsular and cell wall polysaccharides. The filamentous streptococcus described in this report is one of a number of strains of diverse capsular types producing a cell wall polysaccharide which cross-reacts with the C_s or cell wall-like capsular polysaccharide of pneumococcus. It is noteworthy that not all strains of filamentous streptococci of capsular type 83 and of other provisional capsular types have cell wall antigens which cross-react with pneumococcal C and C_s polysaccharides. This observation suggests that the cell wall antigens of these streptococci, like those of the several Lancefield groups of beta-hemolytic streptococci, exhibit a significant degree of antigenic diversity. The association of the same capsular antigen with different cell wall antigens is reminiscent of certain of the capsular antigens of group F streptococci which may be produced by streptococci devoid of the group F antigen (25).

The results presented indicate the feasibility of classifying certain alpha-hemolytic streptococci into distinct serological groups on the basis of their cell wall antigens. The hexosamine-rich polymer isolated from the cell walls of the prototypic strain of streptococcus type 83 may represent a common structural feature of other filamentous streptococci. With the availability of distinct cell wall antigens, capsular antigens, and suitable monospecific antisera, it is possible that a serological scheme can be developed for the grouping and typing of these organisms.

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