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Characterization of the Cross-Reaction Between Type 19F(19) and 19A(57) Pneumococcal Capsular Polysaccharides: Compositional Analysis and Immunological Relation Determined with Rabbit Typing Antisera

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The immunological relation, physicochemical characteristics, and chemical composition of types 19F(19) and 19A(57) within the cross-reactive group 19 pneumococcal capsular polysaccharides were studied. By using rabbit hyperimmune diagnostic antisera in agglutination, immunodiffusion, quantitative precipitation, and absorption assays, extensive cross-antigenicity and cross-immunogenicity were demonstrable between the disease-associated types 19F(19) and 19A(57). Types 19B(58) and 19C(59), rarely associated with human disease, were extensively cross-reactive with each other but reacted weakly with types 19F(19) and 19A(57). Both types 19F(19) and 19A(57) polysaccharides contained trace amounts of protein and nucleic acid and had comparable molecular sizes as determined by gel filtration. Compositional analysis showed type 19F(19) to contain rhamnose, glucose, *N*-acetylmannosamine, and a phosphate ester. Type 19A(57) contained these four moieties, and in addition, contained fucose, galactose, and *N*-acetylglucosamine. Plans for using this information to evaluate current and proposed formulation of multivalent pneumococcal polysaccharide vaccines are discussed.

To date, there are 83 serologically defined pneumococcal capsular polysaccharide types, many of which are serologically related (19, 26, 27; E. Lund and J. Henrichsen, Methods Microbiol., in press). Two nomenclatures evolved, based upon the use of absorbed rabbit typing antisera by means of the quellung reaction, to classify related pneumococcal types (4, 19, 26; Lund and Henrichsen, in press). The Danish system sought to combine closely related types into groups; types within a group were assigned the same number followed by different letters (19, 31). In the Danish system, each type was also assigned an antigenic formula based upon cross-absorption tests with "factor" sera. These factor sera, prepared by absorption with cross-reacting organisms, were used to differentiate cross-reacting types within groups. In the U.S. system, each type was chronologically assigned a number in order of their recognition, i.e., the latest detected types carry the highest numbers (13, 14).

Recently, a U.S. government license was awarded for the manufacture and sale of a pneumococcal vaccine composed of 14 capsular polysaccharides (39; W. Vann, M. Robinson, J. Powell, C.-J. Lee, R. Austrian, and J. B. Robbins, manuscript in preparation). The choice of capsular types for this vaccine was based upon surveys of types among pneumococcal disease isolates (1, 6, 7, 15, 18, 32, 38; Lund and Henrichsen, in press). Most of these surveys utilized typing antisera from the Statens Seruminstitut (SSI) that do not distinguish among the cross-reactive types within each group. Accordingly, the distribution of these disease isolates within cross-reactive groups has not been extensively studied.

Unabsorbed antisera prepared with any type of the 19 group will yield a quellung reaction with all the Danish group 19 types: 19F, 19A, 19B, and 19C (equivalent to U.S. types 19, 57, 58, and 59, respectively) (13, 14, 19, 31). [Pneumococcal capsular types will be referred to by the two nomenclatures by first depicting the Danish and then the U.S. system, e.g., 19F(19).] Two unpublished studies indicate that types 19F(19) and 19A(57) account for almost all of the group 19 pneumococcal illnesses in the U.S. and Denmark (R. Austrian and J. Henrichsen, unpublished data). Because of its predominance among disease isolates in the U.S., type 19F(19)

was initially chosen for vaccine production (Vann et al., manuscript in preparation). In Denmark, however, from 1970 to 1977, types 19F(19) and 19A(57) accounted for approximately the same isolates from cases of bacteremia and meningitis (Lund and Henrichsen, in press; Austrian and Henrichsen, unpublished data).

The cross-reactions of the group 19 pneumococcal capsular polysaccharides pose several problems of special interest for vaccine development. (i) What is the structural basis for the serological relation between group 19 types 19F(19) and 19A(57)? (ii) What is the quantitative immunochemical relation between these related types? (iii) Are types 19F(19) and 19A(57) serum antibodies induced in humans by injection of purified polysaccharides serologically related in a similar fashion as those of rabbit antisera prepared by multiple injection of whole Formalin-treated bacterial cells? In addition, the report of multiple antibiotic resistance, including penicillin, among type 19A(57) disease and carrier isolates have rendered these questions even more pertinent and their answers of immediate importance (2).

In this report, the immunochemical relation between pneumococcal capsular polysaccharides types 19F(19) and 19A(57) was studied with rabbit diagnostic antisera from two sources. We report also the physical-chemical characteristics and monosaccharide components of types 19F(19) and 19A(57) pneumococcal capsular polysaccharides.

MATERIALS AND METHODS

Polysaccharides. Pneumococcal capsular polysaccharides, type 19F(19) lot no. 8556-73 and 19A(57) lot no. 8814-127, were prepared from pneumococcal strains obtained from Robert Austrian [type 19F(19)] and from the reference collection prepared by Bernice Eddy at the Bureau of Biologics [19A(57)]. The capsular types of the strains used for production were confirmed by quellung reactions with factor antisera from the SSI by Robert Austrian, University of Pennsylvania. The organisms were grown in a 175-liter fermentor inoculated by a 10-liter growth from the original seed culture. During the 8-h fermentation, the temperature was 37°C, the pH was monitored to 7.0 to 7.4, and glucose was added in excess. The polysaccharides were extracted by fractional precipitation with organic solvents and treatment with trypsin and deoxyribonuclease (44). Polysaccharides were stored at 3 to 8°C in a vacuum desiccator.

Rabbit typing antisera. Antisera were prepared by repeated intravenous injection of Formalin-treated strains of type 19F(19) or 19A(57) organisms (31; Lund and Henrichsen, in press). The sera were stored at 3 to 8°C with 0.01% thiomersal. Type 19 antiserum (lot no. 19-17), type 57 antiserum (R19A-03), a mixture of both antisera (R19-13, 31, 44) were obtained from the New York State Public Health Laboratories, (NYSPHL) Albany, N.Y. Types 19F(19) and 19A(57) antisera were also produced by the SSI.

Immunodiffusion. Polysaccharides and antisera were placed in 1% agarose (Marine Colloids, Rockport, Maine), dissolved in 0.01 M sodium phosphate-buffered saline (pH 7.4), and allowed to react for 24 h at 4°C. The gels were then photographed with the use of indirect light.

Capsular reaction assays. Twofold dilutions of antisera were examined under the microscope for their capacity to induce quellung and agglutination reactions with a loopful of the antisera dilutions and suspensions of Formalin-treated cells (4; Lund and Henrichsen, in press). The end point was defined as the highest antisera dilution yielding a definite agglutination, and the titer is expressed as the reciprocal dilution.

Precipitation analysis. Equal volumes (0.2 ml) of antisera and polysaccharide solutions, prepared in 0.15 M NaCl–0.01 M NaN_3, were mixed in 12-ml acid-washed conical centrifuge tubes, capped, and incubated at 37°C for 1 h. The tubes were incubated at 4°C for 3 days with frequent agitations and then centrifuged at 3,000 rpm at 4°C for 30 min. The supernatants were decanted, and the precipitates were resuspended and washed three times with 3 ml of phosphate-buffered saline. The washed precipitates were suspended in 5.0 ml of 0.8% sodium lauryl sulfate, and their absorbance was measured at 280 nm in a Gilford spectrophotometer. Their precipitating antibody content was estimated by assuming an extinction coefficient of 14.0 for rabbit immunoglobulin. The supernatants were assayed for their antigen and antibody content by countercurrent immunoelectrophoresis (11). Antisera were absorbed by incubation with 1.0 mg of polysaccharide at 37°C for 1 h and overnight at 3 to 8°C and centrifuging the suspension at 15,000 rpm at 4°C for 2 h. An additional absorption was done with 0.1 mg of polysaccharide per ml of serum if the supernatants, assayed by countercurrent immunoelectrophoresis, showed precipitating activity.

Chemicals. Rhamnose, fucose, mannose, glucose, galactose, D-glucuronic acid, galacturonic acid, mannosamine, glucosamine, galactosamine, glucose-1-phosphate, glucose-6-phosphate, and *N*-acetyl neuraminic acid were from Sigma Chemical Co., St. Louis, Mo. *N*-acetylmannosamine, *N*-acetylglucosamine, and *N*-acetylgalactosamine were from Calbiochem, La Jolla, Calif. The gas chromatography (GC) accessories were from Supelco, Bellefonte, Pa. The derivatizing agent Trisil and the GC column conditioner Silyl-8 were obtained from Pierce Chemicals, Rockford, Ill. Glucostat and Galactostat reagents were bought from Worthington Biochemicals, Freehold, N. J.

Analytical methods. Elemental analysis was done by the National Institutes of Health microanalytical laboratory. Moisture was determined by the thermogravimetric method (46). Nucleic acid was measured by absorption of the solution at 260 nm, assuming E1% at 1 cm to be 20.0. Protein was assayed by the method of Lowry et al. (24). Methylpentose and uronic and sialic acids were determined by colorimetric techniques (8, 40, 45). Polysaccharides were hydrolyzed with 2 N methanesulfonic acid at 115°C for 5 h and analyzed for hexosamines in a Beckman 121M amino

acid analyzer (3, 20, 23). The polysaccharides were also hydrolyzed with 4 N HCl for 1 h and analyzed for glucose and galactose by an enzymatic method (22, 43). Molecular size was characterized by measuring Kd, distribution coefficient, by gel filtration on 4B (lot no. 3325) and CL2B (lot no. 3772) Sepharose columns (1.9 by 100 cm) equilibrated with 0.2 M ammonium acetate and 0.1% sodium azide (46).

GC. A Perkin-Elmer model 990, fitted with a dual column and a dual-flame ionization detector, and a Hewlett-Packard 3380A integrator were used for GC. The reference and the sample columns were pretreated with Silyl-8 and conditioned at 300°C. The following conditions were used: column, ⅛ inch (OD) by 6 feet (ca. 0.32 [OD] by 182.88 cm) packed with 3% OV-17 on Chromasorb W; carrier gas, nitrogen; flow rate, 25 ml/min; injector temperature, 320°C; detector temperature, 300°C; column temperature, 130°C, held for 4 min at 6 or 8°C/min, 270°C. The polysaccharide (0.5 to 1.0 mg) was treated with 2 N aqueous HCl or 2 N methanolic HCl in a 2-ml Reacti-Vial (Supelco) with a Teflon-coated screw-cap and heated at 70 or 100°C. Flushing sample vials with nitrogen was found to be unnecessary. The hydrolysis was stopped at intervals, and the solution was lyophilized. The residue was treated with Trisil (0.5 ml) and heated at 70°C for 30 min with occasional stirring. Portions (5 μl) were injected into the column, and the peaks were identified by comparing their retention times with the trimethylsilyl derivatives of the authentic mono- and disaccharides prepared under similar conditions.

Mass spectrometry. GC-mass spectrometry was performed with an LKB model 9000 GC-mass spectrometer with 3% OV-17 on Chromasorb Q columns with helium as carrier gas (30 ml/min). Column conditions were as in conventional gas-liquid chromatography; the source temperature was maintained at 280°C. The energy of the bombarding electrons and the ionizing current were 70 eV and 60 μA, respectively. Mass spectra were taken with a scan speed of 2 to 3 s (electronic mass, 20 to 600 or 20 to 900) (9, 17).

Liquid chromatography. A Japan Electronic and Optical Laboratories (JOEL) amino acid analyzer 9AH model was modified to carry out the neutral sugar analysis. The column (6.3 mm by 22 cm) was packed with Hamilton HP-AN90 (10-mm particle size) in lithium form and eluted with 90% ethanol in water. A 1-ml amount of polysaccharide solution (1 mg/ml) was treated with 4 N HCl and heated under nitrogen at 100°C for 1 h and then cooled and lyophilized. The residue was dissolved in 90% ethanol in water (10 ml) and a portion (0.8 ml) was loaded on the cationic column. The peaks were identified by comparison with the retention times of the authentic standards.

RESULTS

Physical-chemical. The results of physicochemical analyses of types 19F(19) and 19A(57) pneumococcal polysaccharides are listed in Table 1. Both preparations contained negligible amounts of protein and nucleic acid and had comparable molecular sizing characteristics. This latter property is related to the amount of antibody precipitated by capsular polysaccharides from typing antisera (Vann et al., manuscript in preparation). Both polysaccharides contained phosphorus [3.78% for type 19F(19) and 2.56% for type 19A(57)]. Type 19F(19) polysaccharide contained *N*-acetylmannosamine, glucose, and methylpentose. Type 19A(57) contained *N*-acetylglucosamine and galactose in addition to the three monosaccharide components detected in 19F(19). No sialic or uronic acid was detected in either polysaccharide.

Immunodiffusion. Figure 1 shows the immunodiffusion reactions of the two polysaccharides with SSI and NYSPHL typing antisera. The 19F(19) polysaccharide precipitated with types 19F(19) and 19A(57) antisera from each source. A precipitin spur extending from 19F(19) over the 19A(57) was observed when these two polysaccharides were reacted with type 19F(19) antisera from SSI and NYSPHL. Similarly, type 19A(57) polysaccharide precipitated with a spur overriding 19F(19) polysaccharide when both were reacted with NYSPHL or SSI 19A(57) antisera. More antigenic sites were revealed, therefore, by homologous reactions of the two group 19 polysaccharides and their antisera. The type 19F(19) and 19A(57) antisera (NYSPHL lot no. R19-13, 31, 44), prepared by simultaneous injection of 19F(19) and 19A(57) organisms, demonstrate a spur from type 19F(19) over 19A(57) polysaccharides. Not shown is the failure of types 19B(58) and 19C(59) antisera to precipitate with either types 19F(19) or 19A(57) polysaccharides.

Agglutination titers. The titers of the four SSI unabsorbed group 19 antisera, against the

TABLE 1. *Physical-chemical characteristics of pneumococcal capsular polysaccharides types 19F(19) and 19A(57)*[a]

Polysaccharide type	Elemental analysis (%)				Protein (%, wt/wt)	Nucleic acid (%, wt/wt)	*N*-acetylglucosamine (%)	*N*-acetylmannosamine (%)	Glucose (%)	Galactose (%)	Methyl pentose (total) (%)	Molecular size (Kd)	
	Carbon	Hydrogen	Nitrogen	Phosphorus								CL2B Sepharose	4B Sepharose
19F(19)	39.7	6.39	3.00	3.75	2.0	1.4	ND[b]	9.5	14.5	ND	29.2	0.42	0.12
19A(57)	40.6	6.45	3.37	2.56	1.8	0.4	6.9	8.9	10.3	12.5	19.1	0.77	0.30

[a] All values were corrected for moisture content as determined by thermogravimetric analysis.
[b] ND, None detected.

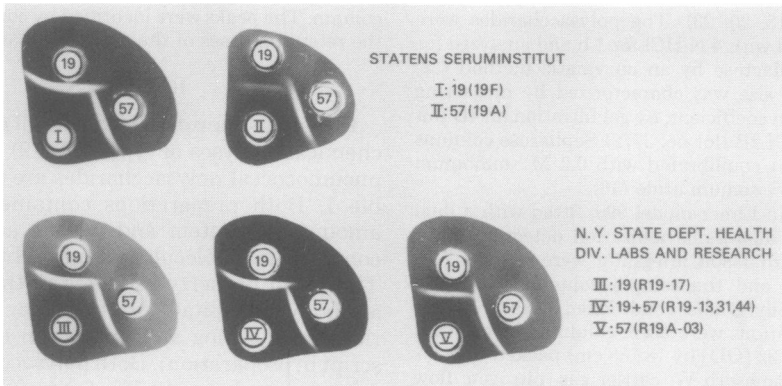

FIG. 1. *Immunodiffusion analysis of types 19F(19) and 19A(57) pneumococcal capsular polysaccharides reacted with typing antisera from NYSPHL and SSI. The concentration of the polysaccharides was 0.5 mg/ml.*

homologous and cross-reacting strains, are shown in Table 2. The general pattern, rather than the absolute values, is of interest because titers of individual animals may vary during the course of immunization and differ among animals and are related to the strain used for immunization. All strains induce the highest antibody titer to the homologous type, but type 19F(19) induces a comparable response to 19A(F). All four types induce a comparable homologous response.

Precipitation analysis. Table 3 shows the maximum amount of antibody precipitated by types 19F(19) and 19A(57) polysaccharides from rabbit typing antisera. Both 19F(19) and 19A(57) polysaccharides precipitated with all antisera. Both type 19F(19) antisera yielded the most precipitate when reacted with the homologous type 19F(19) polysaccharide. The type 19F(19) polysaccharide precipitated 8.16 mg of antibody per ml from the NYSPHL serum and 4.58 mg of antibody per ml from the SSI serum. The cross-reactivity of these type 19F(19) antisera with the type 19A(57) polysaccharide was 51% with that of the homologous NYSPHL serum and 74% with the SSI serum. The NYSPHL type 19S(57) antisera had higher precipitating antibody levels than the SSI reagent when reacted with 19A(57) polysaccharide. The cross-reactivity of the two type 19A(57) antisera, when reacted with type 19F(19) polysaccharide, was 76% for NYSPHL and 41% for SSI. Absorption of the typing antisera with the polysaccharides confirmed that the homologous reagents reacted with the most antigenic sites. Type 19F(19) polysaccharide removed all of the homologous and heterologous precipitation from the type 19F(19) antisera. In contrast, absorption of both type 19A(57) antisera with type 19F(19) polysaccharide did not remove all type 19A(57) reactivity. Similar results were obtained with absorption experiments with type 19A(57) polysaccharide.

TABLE 2. *Capsular reaction titers of group 19 pneumococcal typing antisera*[a]

Capsular type	SS typing antiserum titer			
	19F(19)	19A(57)	19B(58)	19C(59)
19F(19)	128	16	8	8
19A(57)	128	64	16	16
19B(58)	32	8	64	32
19C(59)	32	16	32	128

[a] Unabsorbed rabbit antisera, prepared by multiple intravenous injections of Formalin-treated organisms of one pneumococcal capsular type, were studied for their capsular reaction with group 19 strains. The activity is expressed as the reciprocal of the highest dilution yielding microscopic agglutination (see text).

Mass spectrometry. The hydrolysis and methanolysis products of type 19F(19) polysaccharide were converted into their trimethylsilyl derivatives for GC-mass spectrometry studies. The mass spectra of methanolysis product indicated a deoxyhexose, 1-*O*-methyl hexopyranoside, hexosamine, *N*-acetylhexosamine, hexose phosphate, and a disaccharide.

GC. The retention times of the peaks observed in a representative GC of the methanolysis-Trisil reaction mixture (Fig. 2), identified as rhamnose, 1-*O*-methylglucopyranoside, *N*-acetylmannosamine, glucose-6-phosphate, and trehalose. Methanolysis was also done varying the hydrolysis time, temperature, and acid strength. Analysis of these reaction mixtures produced similar results, indicating that type 19F(19) polysaccharide is composed of three monosaccharides, rhamnose, glucose, and *N*-acetylmannosamine. The GC-mass spectroscopy analysis of the hy-

TABLE 3. *Precipitin analysis of types 19F(19) and 19A(57) rabbit pneumococcal typing antisera*[a]

Antisera	Absorbent	Pneumococcal vaccine	Antibody protein precipitated (mg/ml)	
			19F(19)	19A(57)
NYSPHL 19F(19)	None	19F(19)	8.16	4.16
NYSPHL 19A(57)	None	19A(57)	1.39	1.84
NYSPHL A-F	None	19F(19), 19A(57)	2.30	1.63
SSI 19F(19)	None	19F(19)	4.58	3.40
SSI 19A(57)	None	19A(57)	0.24	0.59
NYSPHL 19F(19)	19F(19)	19F(19)	0.00	0.00
	19A(57)		1.86	0.00
NYSPHL 19A(57)	19A(57)	19A(57)	0.00	0.00
	19F(19)		0.00	0.95

[a] Equal volumes of antisera (0.2 ml) and antigen were mixed and incubated at 37°C for 1 h. The antibody content was determined by measuring the absorbance at 280 nm of the washed precipitates as described in the text. For the absorption experiments, 5.0 ml of serum was mixed with a weighed amount of the dry mixed polysaccharides and incubated at 37°C, and then at 4°C as described in the text. The completeness of absorption was assayed by countercurrent immunoelectrophoresis.

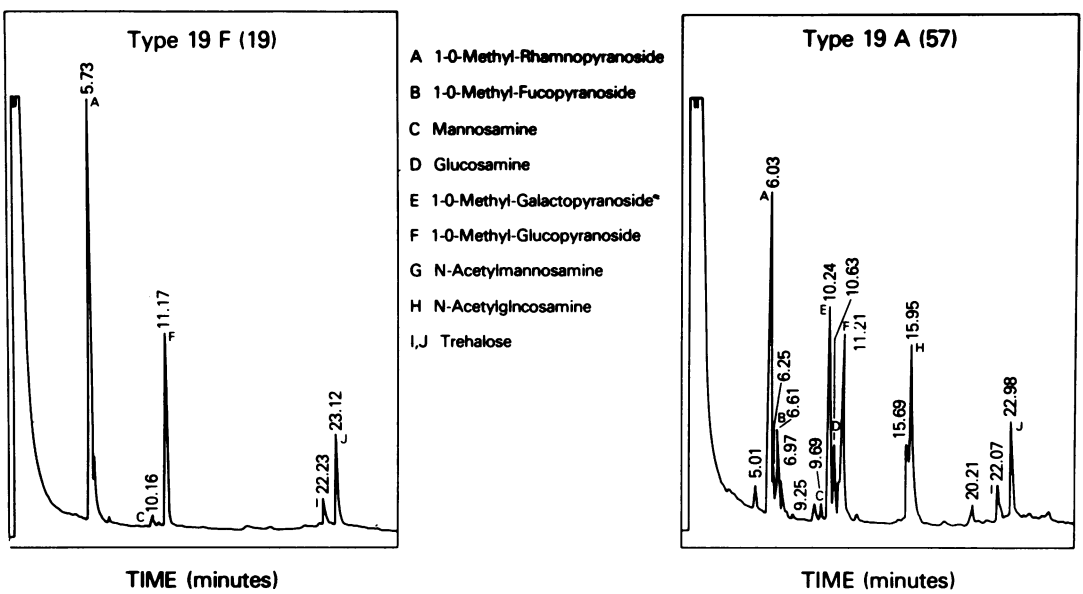

FIG. 2. *GC of trimethylsilyl derivatives of type 19F(19) and 19A(57) polysaccharides. Samples (1.0 mg) were hydrolyzed with 2.0 ml of 2 N methanolic HCl at 70°C for 3 h and lyophilized. The residue was treated with trimethylsilyl reagent at 85°C for 30 min, and the reaction product passed through the column.*

drolysis products from 1 to 3 N aqueous HCl revealed the presence of the same sugars. Similarly, the hydrolysate obtained with 2 N methane sulfonic acid was found to contain the same sugars, but the disaccharide was present in larger amounts in comparison with the monosaccharides.

Liquid chromatography. Analysis of the 2 N aqueous HCl hydrolysate (Fig. 3) revealed the presence of rhamnose and glucose but no disaccharide. Trehalose could not be detected by this method because it is not a reducing saccharide.

The type 19 polysaccharide hydrolysis product from 2 N methane sulfonic acid was also analyzed for hexosamine, as illustrated in Fig. 4, with the amino acid analyzer. The maximum amount of hexosamine was detected when the hydrolysis was stopped at 5 to 6 h. No glucosamine was detected in the product. This peak could be either mannosamine or galactosamine. Because the retention time of the hexosamine peak observed in the GC was in agreement with that of mannosamine, it would be reasonable to conclude that mannosamine is the hexosamine.

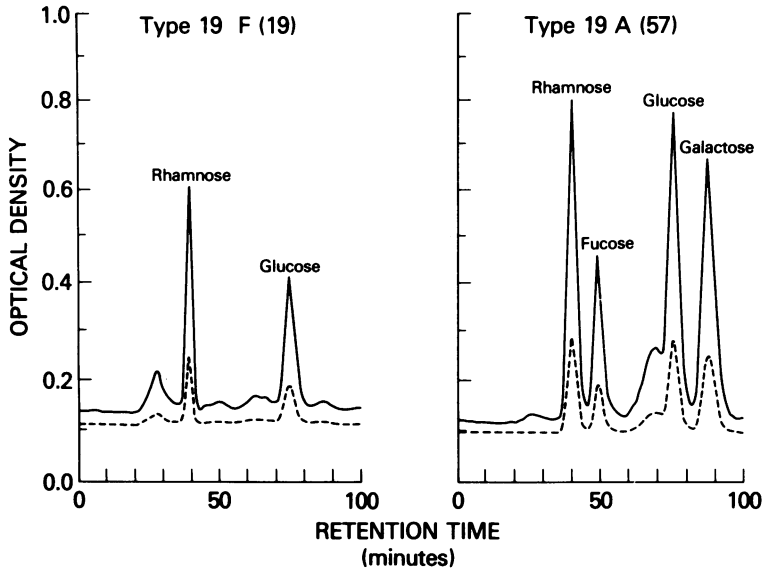

FIG. 3. *Neutral monosaccharide analysis of types 19F(19) and 19A(57) polysaccharides by liquid chromatography. Samples (1.0 mg) were hydrolyzed with 2 N HCl under nitrogen at 100°C for 1 h and lyophilized. The residues were dissolved in 90% ethanol and passed through the JEOL amino acid analyzer as described in the text.*

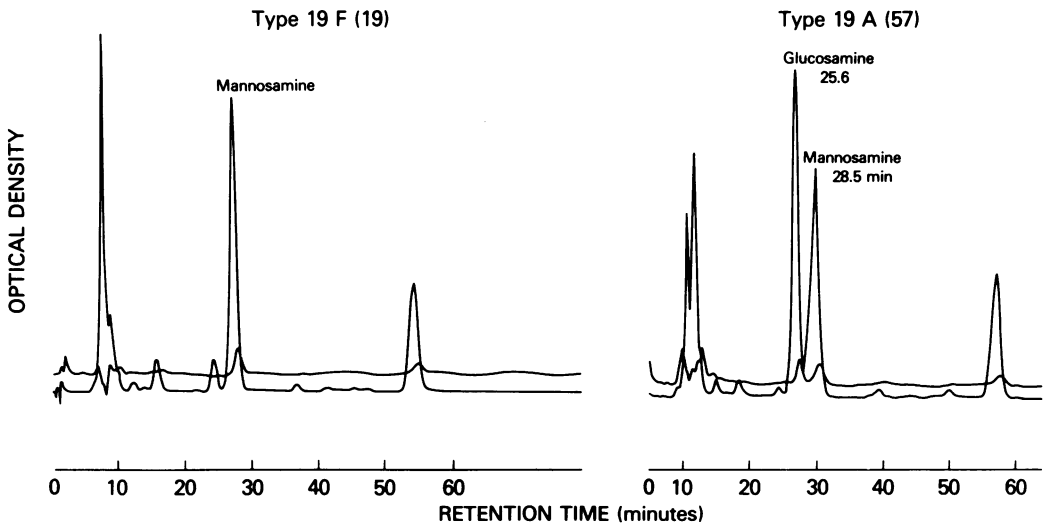

FIG. 4. *Hexosamine analysis of types 19F(19) and 19A(57) polysaccharides. Samples (1.0 mg) were hydrolyzed with 2 N HCl under nitrogen at 100°C for 1 h and lyophilized. The residue was dissolved in pH 2.2 sodium citrate buffer and applied to a Beckman model 120C amino acid analyzer.*

Quantitative determination of rhamnose and glucose by colorimetric assay and mannosamine by the amino acid analyzer provided an additional method for calculation of their molar ratios. In this fashion the pneumococcal polysaccharide type 19F(19) was found to contain rhamnose, glucose, and *N*-acetylmannosamine. On the basis of these data, we estimate their ratio to be 4:2:1, respectively. Similar investigations with type 19A(57) polysaccharide indicated its composition to be approximately rhamnose (3 mol), fucose (2 mol), glucose (2 mol), galactose (2 mol), *N*-acetylmannosamine (1 mol), and *N*-acetylglucosamine (1 mol) (Table 1). The liquid chromatogram (Fig. 3) showed the rhamnose and fucose to be present in 2:1 ratio. The individual percentages of these deoxyhexoses were calculated from the value of the total methyl-

pentose and this ratio. In this case, it was not possible to identify the hexose phosphate due to the complexity of the GC.

DISCUSSION

We have started to characterize the immunological, physicochemical, and structural properties of group 19 pneumococcal capsular polysaccharides associated with serious human disease. Both types 19F(19) and 19A(57) have three monosaccharides, rhamnose, glucose, *N*-acetylmannosamine, in common. Their cross-reactivity has been characterized by cross-precipitation and by reciprocal absorption with rabbit typing antisera. Both types precipitated maximally with homologous and to a lesser extent by heterologous antisera. Types 19B(58) and 19C(59) showed comparatively less cross-reactivity with the two disease-producing group 19 types in agglutination and precipitation assays. Both types 19F(19) and 19A(57) completely absorbed the specific antibodies induced by the homologous type used for immunization. Both polysaccharides absorbed from 48.4 to 77.3% of the cross-reacting antibodies from the heterologous antisera. Types 19B(58) and 19C(59) cross-react with each other and to a much lesser extent with 19F(19) and 19A(57) by capsular reaction assays. Previous studies have shown that absorption of both 19B(58) and 19C(59) antisera with type 19A(57) organisms removes all cross-reactions of these antisera with both type 19F(19) and type 19A(57) organisms. Furthermore, absorption of 19C(59) antisera with type 19B(58) organisms removes all reactivity with 19F(19), 19A(57), and 19B(58), leaving only a weak reaction with the homologous type, 19C(59). Similiar results were obtained with absorption experiments with 19B(58) antisera and 19C(59) organisms. These results indicate that types 19B(58) and 19C(59) are serologically more related to each other than to the disease-associated types. This pattern of similiarities and differences among the types of group 19 is consistent with the "antigenic formulas" proposed by Mørch (26, 31; Lund and Henrichsen, in press). From the earlier data and that described in this report, it is evident that pneumococcal types 19F(19) and 19A(57) are cross-antigenic, cross-immunogenic, and serologically similar.

Because of the complexity of types 19F(19) and 19A(57) polysaccharides and uncontrollable anomerization of sugars after their hydrolysis, their composition was studied by more than one method. The mass spectra of the products enabled us to classify the sugars present in these polysaccharides, i.e., pentose, deoxyhexose, hexose, hexosamine, or disaccharide, etc. The GCs were utilized to identify the specific sugars. After such an identification, the mass spectra of the products were compared with the spectra of the authentic sugar samples. The liquid chromatographic techniques were used to reassess the identity of the monosaccharide units. Quantitative evaluation of the ratio of these monosaccharides utilized colorimetric techniques and amino acid analyzer method. Both 19F(19) and 19A(57) contain rhamnose, glucose and *N*-acetylmannosamine. In addition to three shared monosaccharides, type 19A(57) contains fucose, galactose, and *N*-acetylglucosamine. The demonstration of both hexose phosphate and trehalose in the hydrolysis products of each type suggests there are some shared identical linkages.

After alkaline hydrolysis and akaline phosphatase digestion, Baddiley et al. were able to demonstrate a phosphodiester bond and isolation of the repeating subunit of pneumococcal types 29 and 10A(34) (41, 42). This method seems useful for study of phosphorylated capsular polysaccharides, and studies with the group 19 polysaccharide are underway. Miyazaki et al. reported pneumococcal "type 19" polysaccharide contained rhamnose, glucose, *N*-acetylmannosamine, and phosphate in the molar ratio of 2:1:1:1 (21, 30). However, the type of the group 19 strain was not mentioned.

It is not yet clear whether glucose-6-phosphate and trehalose were formed by the cleavage of the glycosidic linkages or by the recombination of the glucose, formed during hydrolysis, with the other components under the experimental conditions. We plan further investigation with nuclear magnetic resonance analysis of the intact polysaccharide and of its fragments prepared by more mild hydrolysis conditions.

Extensive cross-reactions have been found among many bacterial capsular polysaccharides including pneumococci. For example, *Escherichia coli* K capsular polysaccharide antigens 30, 42, and 85 induce antibodies reactive with pneumococcal types 1, 4, 8, 12, and 19 (35). A nonhemolytic streptococcus, strain 4998/41 (*Streptococcus salivarius*), nongroupable with available antisera, yielded a quellung reaction with pneumococcal typing antisera 19A, 19B, 19C, and 40 (25, 33, 34). *Haemophilus influenzae* type b capsular polysaccharide cross-reacts with pneumococcal polysaccharide types 6, 15, 29 and 35 (29). These cross-reacting polysaccharides may induce "natural" serum antibodies against encapsulated organisms, such as pneumococci, meningococci, and *H. influenzae* type b (J. B. Robbins, R. Schneerson, C. E. Frasch, D. Craven, I. Ørskov, F. Ørskov, and T. Y. Liu, Acta Pathol. Microbiol. Scand., in press). In our experiments with capsular reactions and immune

precipitation between the organisms as well as purified polysaccharides and rabbit hyperimmune antisera, prepared by multiple intravenous injections of Formalin-treated bacteria, an extensive cross-reaction between types 19F(19) and 19A(57) was observed. Values of about 50 to 75% of cross-reactivity by precipitin and inhibition of precipitin formation were observed. Furthermore, injection of each pneumococcal type induced the formation of serum antibodies to both types 19F(19) and 19A(57) polysaccharides. These results suggest that either polysaccharide should induce antibodies reactive with both types 19F(19) and 19A(57) polysaccharides. Although this has not always been the case at the SSI, the higher serum antibodies induced by type 19F(19) organisms at the SSI and NYSPHL sera used in this study suggest that this polysaccharide might be a superior immunogen. The greater complexity of type 19A(57) polysaccharide as compared with the 19F(19) suggests that 19A(57) may be a better polysaccharide immunogen for vaccine usage. Another possibility is that maximum antibody synthesis for these two group 19 types may be achieved by both 19F(19) and 19A(57) represented in the vaccine. Other reasons for studying activities of group 19 pneumococcal polysaccharides are related to the observed age-related immunogenicity and stability of 19F(19) component of vaccine. Both types 19F(19) and 19A(57) are frequent causes of disease in infants and young children including those with deficient splenic function, especially sickle cell anemia (1, 5, 32, 36–38). Type 19F(19) is a poor immunogen in individuals less than 2 years old (5, 10, 12, 16). Furthermore, type 19F(19) undergoes hydrolysis, reduction in molecular size, and thereby immunogenicity more rapidly than other pneumococcal polysaccharide vaccine components (46; Vann et al., in press). Thus, further studies of group 19 pneumococcal and structurally related polysaccharides are planned to choose the most effective vaccine formulation.

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LITERATURE CITED

1. **Ammann, A. J., J. Addiego, D. W. Wara, B. Lubin, W. B. Smith, and W. C. Mentzer.** 1977. Polyvalent pneumococcal-polysaccharide immunization of patients with sickle-cell anemia and patients with splenectomy. N. Engl. J. Med. **297**:897-900.
2. **Appelbaum, P. C., A. Bhamjee, J. N. Scragg, A. F. Hallet, A. A. Bowen, and R. Cooper.** 1977. *Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. Lancet **ii**:995-997.
3. **Ashwell, G.** 1975. Hexosamine determinations. Methods Enzymol. **3**:95-97.
4. **Austrian, R.** 1976. The quellung reaction, a neglected microbiological technique. Mt. Sinai J. Med. N.Y. **43**: 699-709.
5. **Austrian, R.** 1976. Vaccine of pneumococcal caspular polysaccharides for the prevention of pneumococcal pneumonia, p. 79-91. *In* R. F. Beers, and E. G. Bassett, (ed.), The role of immunological factors in infectious, allergic and autoimmune processes, no. 8. Eighth Miles International Symposium. Raven Press, New York.
6. **Austrian, R., R. M. Douglas, G. Schiffman, A. M. Coetzee, H. J. Koornhof, S. Hayden-Smith, and R. D. Reid.** 1976. Prevention of pneumococcal pneumonia by vaccination. Trans. Assoc. Am. Physicians **89**: 184-194.
7. **Austrian, R., V. M. Howie, and J. H. Ploussard.** 1977. The bacteriology of otitis media. Johns Hopkins Med. J. **141**:104-111.
8. **Bitter, T., and H. M. Muir.** 1962. A modified uronic acid carbazole reaction. Anal. Biochem. **4**:330-334.
9. **Bjorndal, H., C. G. Hellerguist, B. Lindberg, and S. Svensson.** 1970. Gas liquid chromatography and mass spectrometry in methylation analysis of polysaccharides. Angew. Chem. Int. Ed. Eng. **9**:610-619.
10. **Borgono, J. M., A. A. McLean, P. P. Vella, A. F. Woodhour, I. Canepa, W. L. Davidson, and M. D. Hilleman.** 1978. Vaccination and revaccination with polyvalent pneumococcal polysaccharides in adults and infants. Proc. Soc. Exp. Biol. Med. **157**:148-154.
11. **Coonrod, J. D., and M. W. Rytel.** 1970. Detection of type-specific pneumococcal antigens by CIE. J. Lab. Clin. Med. **76**:107-113.
12. **Davies, J. A.** 1937. The response of infants to inoculation with type 1 pneumococcus carbohydrate. J. Immunol. **33**:1-7.
13. **Eddy, B. E.** 1944. A simplified procedure for detecting cross reactions in diagnostic antipneumococcic serum. Public Health Rep. **59**:1041-1045.
14. **Eddy, B. E.** 1944. A study of cross reactions among the pneumococcic types and their application to the identification of types. Public Health Rep. **59**:451-468.
15. **Finland, M., and M. W. Barnes.** 1977. Changes in occurrence of capsular serotypes of *Streptococcus pneumoniae* at Boston City Hospital during selected years between 1935 and 1974. Clin. Microbiol. **5**:154-166.
16. **Finland, M., and H. L. Schuman.** 1942. The type-specific agglutinin response of children with pneumococcal pneumonia. J. Immunol. **45**:215-242.
17. **Heller, S. R., D. A. Koniver, H. H. Fales, and G. W. A. Milne.** 1974. Conversational mass spectral search system—display and plotting of spectra and dissimilarity comparison. Anal. Chem. **46**:947-950.
18. **Howie, V. M., J. H. Ploussard, R. Austrian, A. J. Amman, and R. B. Johnston.** 1972. Pneumococcal serotypes and antibody responses in otitis media in children. Pediatr. Res. **6**:130-139.
19. **Kauffman, F., E. Lund, and B. E. Eddy.** 1960. Proposal for a change in the nomenclature of *Diplococcus pneumoniae* and a comparison of the Danish and American type designation. Int. Bull. Bacteriol. Nomencl. Taxon. **10**:31-40.
20. **Kortt, A. A., J. R. Wysocki, and T. Y. Liu.** 1976. Primary structure of streptococcal proteinase. J. Biol. Chem. **251**:1941-1947.
21. **Larm, O., and B. Lindberg.** 1976. The pneumococcal polysaccharides: a re-examination. Adv. Carbohydr. Chem. **33**:295-322.
22. **Lee, C. J., and J. B. Robbins.** 1978. Enzymatic measurement of glucose and galactose content of pneumococcal capsular polysaccharides. J. Biol. Stand. **6**: 444-449.
23. **Liu, T. Y., E. C. Gotschlich, E. K. Jonssen, and J. R.**

Wysocki. 1971. Studies on the meningococcal polysaccharides. J. Biol. Chem. **246**:2849–2858.

24. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
25. **Lund, E.** 1950. Antigenic relationship between pneumococci and non-hemolytic streptococci. Acta Pathol. Microbiol. Scand. **27**:110–118.
26. **Lund, E.** 1970. On the nomenclature of the pneumococcal types. Int. J. Syst. Bacteriol. **20**:321–323.
27. **Lund, E., A. Munksgaard, and S. M. Stewart.** 1972. A new pneumococcus type. Acta Pathol. Microbiol. Scand. Sect. B **80**:497–500.
28. **Lund, E.** 1971. Distribution of Pneumococcus types at different times and different areas, p. 49–59. *In* M. Finland, W. Marget, and K. Bartman, (ed.), Bacterial infections. Changes in their causative agents—trends and possible basis. Springer-Verlag, Berlin.
29. **MacPherson, C. F., H. E. Alexander, and G. Leidy.** 1949. Quantitative determination in type-specific antisera to *Haemophilus influenzae* of the antibody that cross-reacts with encapsulated pneumococci. J. Immunol. **57**:443–446.
30. **Miyazaki, T., and T. Yadomae.** 1971. Polysaccharides of type XIX. II. The type specific polysaccharide and its chemical behavior. Carbohydr. Res. **16**:153–159.
31. **Mørch, E.** 1943. Serological studies on the pneumococci, p. 108. Oxford University Press, London.
32. **Mørch, E.** 1944. On the frequency of 70 Pneumococcus types in Denmark. Acta Pathol. Microbiol. Scand. **21**: 149–164.
33. **Mørch, E.** 1944. Serological relationship between a Streptococcus strain and some pneumococcus types. Acta Pathol. Microbiol. Scand. **21**:96–101.
34. **Mørch, E.** 1945. Capsular antigens common to a streptococcus strain and some pneumococcus types. Acta Pathol. Microbiol. Scand. **22**:159–164.
35. **Ørskov, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, Chemistry and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. **41**:667–710.
36. **Pearson, H. A.** 1977. Sickle cell anemia and severe infectious disease due to encapsulated bacteria. J. Infect. Dis. **136**(Suppl.):S25–S30.
37. **Powars, D., and W. A. Schroeder.** 1975. Rapid diagnosis of sickle cell anemia at birth by microcolumn chromatography. Pediatrics **55**:630–635.
38. **Seeler, R. A., W. Metzger, and M. A. Mufson.** 1972. *Diplococcus pneumoniae* infections in children with sickle cell anemia. Am. J. Dis. Child. **123**:8–10.
39. **Smit, P., D. Oberholzer, S. Hayden-Smith, H. J. Koornhof, and M. R. Hilleman.** 1977. Protective efficacy of pneumococcal polysaccharide vaccines. J. Am. Med. Assoc. **238**:2613–2616.
40. **Svennerholm, L.** 1958. Analysis of sialic acid. Acta Chem. Scand. **12**:547–552.
41. **Venkata Rao, R., J. Buchanan, and J. Baddiley.** 1966. The type-specific substance from Pneumococcus type 10A. Biochem. J. **100**:801–811.
42. **Venkata Rao, E., M. J. Watson, J. G. Buchanan, J. Baddiley.** 1969. The type- specific substance from Pneumococcus type 29. Biochem. J. **111**:547–576.
43. **Washka, M. E., and E. W. Rice.** 1961. Determination of glucose by an improved enzymatic procedure. Clin. Chem. **7**:542–549.
44. **Weibel, R. E., P. P. Vella, A. A. McLean, A. F. Woodhour, W. L. Davidson, and M. R. Hilleman.** 1977. Studies in human subjects of polyvalent pneumococcal vaccines. Proc. Soc. Exp. Biol. Med. **156**:144–150.
45. **Williams, C. A., and M. W. Chase.** 1968. Methylpentose determination. Methods Immunol. Immunochem. **2**: 292–294.
46. **Wong, K. H., O. Barrera, A. Sutton, J. May, D. H. Hochstein, J. D. Robbins, J. B. Robbins, P. D. Parkman, and E. B. Seligmann.** 1977. Standardization and control of meningococcal vaccines, group A and group C polysaccharides. Biol. Stand. **5**:197–215.
47. **World Health Organization.** 1976. Technical report series, no. 588. World Health Organization, Geneva.