ANTIGENIC RELATIONSHIPS BETWEEN GROUPS B AND G STREPTOCOCCI*, ‡

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(Received for publication, July 7, 1964)

Previous reports have directed attention to the chemical basis for the antigenic specificity of the group carbohydrates for Groups A, A-variant, C, C-variant, and G hemolytic streptococci (1-5). In this paper attention will be directed to the carbohydrate antigen of Group B streptococci.

During the course of routine serological identification of streptococci by the capillary precipitin technique cross-reactions have been noted between Groups B and G. Previous investigations on the Group G carbohydrate have identified L-rhamnose as the major component of the determinant of antigenic specificity (5). The data reported here demonstrate chemical similarities between the Groups B and G carbohydrates and suggest that the antigenic determinant of Group B specificity is closely related to that of Group G.

Materials and Methods

Streptococcal Strains.—Group B cell walls were prepared from strains D136C and O90R obtained from Dr. Rebecca C. Lancefield, The Rockefeller Institute.

Preparation of Cell Walls and Group-Specific Carbohydrate.—Cell walls were prepared by previously described methods (6), and the group carbohydrate was extracted by the hot formamide procedure (7).

Analytical Methods.—Analyses for rhamnose, glucose, glucosamine, muramic acid, and amino acids were performed as described previously (3, 6). Galactose was measured on hydrolyzed material with the "galactostat" reagent.[1]

Precipitin Analysis.—Quantitative precipitin analyses and the preparation of rabbit antisera were performed by previously described methods (8).

EXPERIMENTAL

Composition of Group B Cell Walls.—In addition to the group-specific carbohydrate, the trypsinized cell walls of Group B normally contain a type-specific

* This investigation was supported in part by Research Grant No. HE 08027 National Heart Institute, and Training Grant No. 5 T1 GM 807 Research Training Grants Branch, National Institute of General Medical Sciences; and a Grant-in-Aid from the St. Louis Heart Association.

‡ Presented in part April 13, 1964 at the annual meeting of the American Association of Immunologists in Chicago.

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[1] Worthington Biochemical Corp., Freehold, New Jersey.

"S" polysaccharide antigen. Because the material extracted from cell walls by the formamide procedure contains both antigens, pure preparations of the group carbohydrate are obtained by additional chemical separatory procedures, or by the extraction of the group antigen from Group B mutants which are devoid of the S type antigen. Both of these alternatives were employed in the present studies. The chemical purification of the group carbohydrate from an

TABLE I

Composition of Groups B and G Cell Walls, the Soluble Carbohydrates, and the Insoluble Residues following Hot Formamide Extraction

	Cell walls			Formamide treatment					
				Extracted carbohydrate			Formamide residue		
	B strain 090R	B strain D136C*	G strain B549	B strain 090R	B strain D136C	G strain B549	B strain 090R	B strain D136C	G strain B549
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Rhamnose	21.5	18.4	19.6	50.2	50.5	40.7	0.9	0.9	5.5
Hexosamine	11.3	11.0	8.2	—	—	—	—	—	—
Glucosamine	—	—	—	12.3	11.4	0.0	10.3	11.4	10.4
Galactosamine	—	—	—	0.0	0.0	20.6	0.0	0.0	0.0
Galactose	2.8	6.0	8.9	8.9	11.0	23.7	—	—	—
Muramic Acid	6.6	6.5	3.3	‡	‡	‡	9.2	8.3	6.6
Alanine	17.4	16.7	16.9	‡	‡	‡	24.6	25.5	24.9
Glutamic Acid	8.4	8.3	7.3	‡	‡	‡	12.8	12.1	11.1
Lysine	9.0	8.5	8.0	‡	‡	‡	12.1	11.3	11.5
Glycine	0.4	0.5	0.9	‡	‡	‡	0.5	0.8	0.9

The analytical data for Group G are from Curtis, S. N., and Krause, R. M., *J. Exp. Med.*, 1964, **119**, 997.

* Cell walls of this strain contain 1.7 per cent glucose which is probably a constituent of the Type III antigen.

‡ Less than 1 per cent.

extract which is an antigenic mixture is dependent upon the fact that the S antigen is insoluble in 1.5 volumes of alcohol (9). Group carbohydrate from a Type III strain, D136C, was prepared in this fashion. In addition, Group B carbohydrate, devoid of type antigen, was also obtained from strain 090R, a rough colony variant, which lacks the type antigen. This strain was isolated by Dr. Rebecca C. Lancefield by growing the wild type in the presence of homologous type-specific antiserum.

Presented in Table I are the chemical compositions of Group B cell walls, the formamide-extracted and purified carbohydrate, and the mucopeptide residue. Inclusion in the table of previous data for the analogous fractions of Group G affords a comparison of the Group B components to those of Group G. It is to be noted that rhamnose is a major constituent of the cell walls for both

groups and that galactose and hexosamine are detected in appreciable quantities. Typically, muramic acid, alanine, glutamic acid, lysine, and glycine are found in the cell walls of both groups, and except for the lower content of muramic acid in the Group G cell walls, the mole ratios are comparable. Although not recorded in the table, a fifth amino acid, comprising 1 to 2 per cent of the Group B cell walls, and tentatively identified as serine on the basis of paper chromatography, was consistently detected. Reexamination of Group G cell walls reveals a similar amino acid in comparable concentration.

The formamide residues of Group B cell walls consist of approximately 10 per cent glucosamine, 9 per cent muramic acid, 25 per cent alanine, 12 per cent of both glutamic acid and lysine, 1 per cent serine, and less than 1 per cent

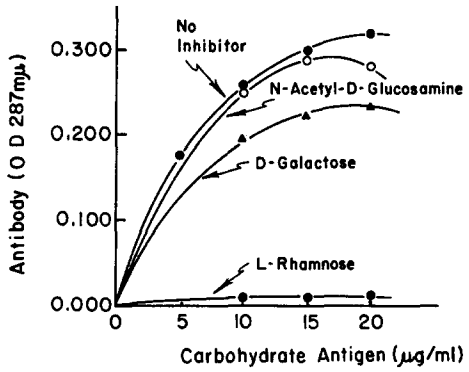

FIG. 1. Inhibition of the precipitin reaction between Group B carbohydrate and Group B antiserum. The final concentration of the inhibitors was 55 μmoles per ml.

glycine, a composition similar to the mucopeptide for Group G as well as the other groups of streptococci.

The compositions of the group-specific carbohydrates of both Groups B and G, in common with those of Groups A, A-variant, and C streptococci, are characterized by a large percentage of rhamnose. The Group B polysaccharide has one-half as much galactose as does the Group G carbohydrate. A distinctive difference between Groups B and G carbohydrates is that glucosamine is identified in the former and galactosamine in the latter. Group B carbohydrate extracted by hot formamide contains only traces of mucopeptide constituents, a finding consistent with the results of similar extractions of the other streptococcal groups so far examined.

Precipitin Inhibition with the Constituent Sugars.—Quantitative precipitin inhibition studies were undertaken to discover which of the constituent sugars was the significant component of the antigenic determinant. The results are depicted in Fig. 1. It is to be noted that the addition of 1 per cent, or 55 micromoles per ml, of L-rhamnose to the Group B antigen-antibody system markedly

inhibits the precipitin reaction, whereas the other constituent sugars of the carbohydrate have no significant inhibitory effect. In this connection it should be emphasized that L-rhamnose also inhibits the Group G precipitin reaction (5), a finding which suggests, in view of data reported here, that this sugar is a major feature of the antigenic determinants for both Groups B and G carbohydrates.

In previously reported experiments, the effectiveness of various sugars as inhibitors of the Group G precipitin reaction was compared to that of L-rhamnose. The results of similar inhibition experiments with Group B carbohydrate

TABLE II

Inhibition of the Groups B and G Precipitin Reactions with Various Sugars

Inhibitor, 55 μmoles/ml	Inhibition	
	Group B reaction	Group G* reaction
	per cent	*per cent*
L-Rhamnose	97.7	95.5
L-Mannose	78.4	57.8
L-Arabinose	34.3	21.2
L-Glucose	27.5	14.5
D-Galactose	26.3	14.5
D-Fucose	25.4	23.7
D-Glucose	10.0	10.0
L-Fucose	9.9	6.4
D-Xylose	9.5	13.7
D-Arabinose	9.5	11.7
D-Mannose	8.5	8.3
N-Acetylglucosamine	7.6	0.0

* From Curtis, S. N., and Krause, R. M., *J. Exp. Med.*, 1964, **119**, 997.

performed at antigen-antibody equivalence are shown in Table II, and the previous Group G data are included for comparison. L-Mannose is the most effective inhibitor other than L-rhamnose in both the Group B and G reactions, whereas D-mannose is ineffective. The D-isomer of fucose (a methyl pentose) inhibited more effectively than the L-isomer; on the other hand, L-arabinose was more effective than D-arabinose. Upon close inspection of these findings, it is clear that a sugar is an effective inhibitor if it possesses the configuration at carbons 2 and 4 similar to that of L-rhamnose, while those sugars which are unlike L-rhamnose in this respect are less effective inhibitors. These data suggest that the steric arrangement about carbons 2 and 4 is a major feature of the antigenic specificity of the rhamnose molecule.

Relationship between Groups B and G Carbohydrate.—The unanticipated finding that L-rhamnose is a primary feature of the antigenic determinant for

both Groups B and G carbohydrates indicates the immunologic basis for the cross-reactivity between these antigens, and provides an explanation for the cross-reactions between Groups B and G streptococci which are frequently noted during the course of routine serological identification. These antigenic relationships are graphically depicted by quantitative precipitin analyses in which both Groups B and G carbohydrates are tested with homologous and heterologous antisera. As illustrated in Fig. 2, the Group B carbohydrate cross-reacts significantly with Group G antiserum, and conversely, the Group G

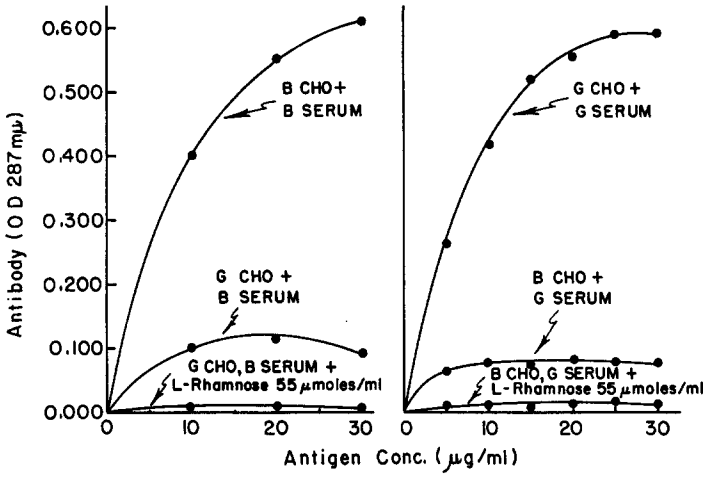

FIG. 2. Groups B and G quantitative precipitin reactions with homologous and heterologous antisera, and the inhibition of the cross-reactivity with L-rhamnose. B CHO, Group B carbohydrate; G CHO, Group G carbohydrate.

carbohydrate gives an appreciable cross-reaction with Group B antiserum. It is to be noted that both cross-reactions are inhibited by L-rhamnose.

Despite the similarity between Groups B and G antigens, it is feasible with cross-absorptions, to prepare group-specific antisera. The immunological relationships of these cross-absorptions are tabulated in Table III. Absorption was performed by the addition of purified heterologous carbohydrate to the serum in sufficient quantity to precipitate all cross-reactive antibodies. The reactivity of the anti-B and the anti-G sera prior to absorption with heterologous carbohydrate is similar to the results depicted in Fig. 2. After absorption of the sera with heterologous carbohydrate the cross-reactivity is eliminated. The reactivity with the homologous antigen persists, although the precipitate is diminished from that obtained with unabsorbed sera. Thus, in the reaction between 40 μg of Group B carbohydrate and unabsorbed B serum the optical density value of the dissolved antibody precipitate was 0.530, whereas

the value with absorbed sera was 0.440. Clearly, the antisera following heterologous absorption are less reactive with homologous antigen, suggesting that heterologous absorption removed a fraction of the homologous antibodies.

The elimination of the cross-reactivity of the sera following absorption with homologous carbohydrate is indicated by the quantitative precipitin tests depicted in Fig. 3. Absorption was performed by the addition to the sera of the homologous, purified carbohydrate in sufficient quantity to precipitate the antibody at equivalence. Data are included to show the homologous and het-

TABLE III

Elimination of the Cross-Reactivity of the Groups B and G Antisera following Absorption with Heterologous Carbohydrate

Serum	Antigen	Antigen conc.	OD 287 mμ	Serum	Antigen	Antigen conc.	OD 287 mμ
		μg				μg	
Group B	B	20	0.485	Group G	G	25	0.464
		30	0.520			30	0.465
		40	0.530			35	0.520
					B	10	0.055
	G	15	0.067				
		20	0.070			15	0.064
		25	0.070			20	0.064
Group B absorbed with G CHO	B	20	0.415	Group G absorbed with B CHO	G	25	0.390
		30	0.440			30	0.363
		40	0.440			35	0.365
	G	20	0.000		B	15	0.000

The analytical values represent the assay of antibody, measured spectrophotometrically at 287 mμ, after dissolution of the washed antigen-antibody precipitates.

G CHO, Group G carbohydrate; B CHO, Group B carbohydrate.

erologous reactivity of the sera prior to homologus absorption. It is to be noted that the sera following absorption with homologous antigen are no longer reactive with the heterologous carbohydrate. The elimination of the cross-reactivity following homologous absorption and the diminution in the reactivity following heterologous absorption suggest that a fraction of the antibodies reacts with both antigens.

Antigenic Relationship between Group B and the Variant Carbohydrates.—It may be recalled that in the Groups A-variant and the C-variant carbohydrates side chains of rhamnose oligosaccharides have been identified as the major determinants of antigenic specificity, whereas in the case of Groups A and C the serologic reactivity of the rhamnose side chains is masked by terminal

amino sugars. In a previous paper attention was directed to the fundamental difference between the role of rhamnose in the determinant of the variant carbohydrates on the one hand, and in the determinant of Group G carbohydrate on the other (5). This was suggested by the fact that variant and G carbohydrates do not cross-react with heterologous antisera. A similar finding was noted in the case of Group B; namely, that variant and B carbohydrates do not cross-react with heterologous antisera.

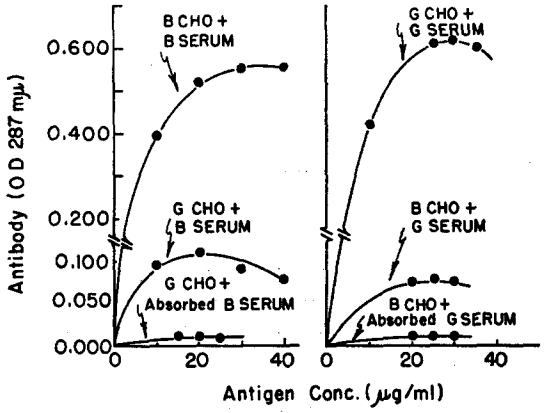

FIG. 3. Elimination of the cross-reactivity of groups B and G antisera following absorption with homologous carbohydrate.

DISCUSSION

In certain respects the chemical content of the cell walls and the carbohydrate antigen of Group B resembles that of Groups A, A-variant, C, C-variant, and G streptococci (10). The trypsinized cell walls of the latter groups of streptococci contain two major components: a mucopeptide matrix and a group-specific carbohydrate. The Group B cell walls were also found to contain these two essential components. The purified carbohydrate of all of these groups contains rhamnose and a hexosamine, and although B and G carbohydrates contain an appreciable content of galactose, the A and C carbohydrates are devoid of this sugar.

The studies reported here underscore the close chemical and antigenic relationship between the group-specific antigens of B and G hemolytic streptococci. Analytical evidence has shown that the purified carbohydrates of both groups contain rhamnose as the major constituent in addition to a hexosamine and galactose. Quantitative precipitin inhibition studies indicate that the major component of the antigenic determinant for both Groups B and G carbohydrates is a monosaccharide of L-rhamnose. The marked inhibitory effect of

L-rhamnose on the precipitin reactions suggests that this sugar is terminal to the other constituent sugars in the molecular structure of these antigens. The close antigenic relationship between the B and G carbohydrates is further substantiated by the fact that they exhibit a cross-reactivity with heterologous antisera which is inhibited by L-rhamnose. In view of these findings the antigenic specificity of these two antigens must be dependent upon either the linkage of the terminal rhamnose residue to the remainder of the molecule, or upon the particular sugar which occupies the first subterminal position. It is conceivable of course that both of these features may contribute to the specificity of the determinant.

Previous immunochemical studies have defined the close antigenic relationship between Groups A and C carbohydrates (11). The data are in agreement with the hypothesis that side chains with rhamnose-rhamnose linkages constitute a major feature of the rhamnose moieties of these carbohydrates, but that the antigenic determinants in the case of Group A are terminal *N*-acetylglucosaminide residues, whereas the determinants of Group C are terminal *N*-acetylgalactosaminide residues. On the basis of the studies reported here there is a fundamental difference from an immunochemical point of view between the A and C carbohydrates, on the one hand, and the B and G carbohydrates on the other.

SUMMARY

Trypsinized cell walls of Group B hemolytic streptococci are composed of a group-specific carbohydrate and mucopeptide. The carbohydrate extracted with hot formamide is composed of rhamnose, *N*-acetylglucosamine, and galactose. Quantitative precipitin inhibition studies have shown that L-rhamnose is the significant component of the antigenic determinant. The cross-reactivity between B and G carbohydrates is dependent upon the fact that L-rhamnose is a determinant sugar in both antigens.

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