CROSS-REACTIONS OF PNEUMOCOCCAL TYPES

QUANTITATIVE STUDIES WITH THE CAPSULAR POLYSACCHARIDES*

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(Received for publication, June 25, 1964)

Classification of the pneumococci has been rendered difficult by the frequent discovery of atypical strains, many of which were eventually given individual type numbers. Avery (1) recorded such variants of Type II, and of these his IIA and IIB were later designated Types V and VI (2). Chemical relationships between the three serological types were clarified in a recent series of quantitative immunochemical studies (3, 4 *a*–4 *c*, 5). Similarly, an atypical Type III strain (6) soon became Type VIII (7) while the reason for the relationship gradually emerged as a result of chemical (8 *a*, 8 *b*), immunological (9), and quantitative immunochemical investigations (10 *a*–10 *c*).

We have studied the chemistry of the capsular polysaccharides of Types VII and XVIII (11, 12) and quantitative data are herewith given on the qualitatively long-known cross-reaction (2, 14 *a*) between these types. Types XVIII and VIII also cross-react (12), as do II and XIX (14 *b*), VI and XIV (4 *b*), and X and XIV, among others. Although the relationship of Types II and XX was discovered many years ago (13 *a*, 13 *b*, 14 *a*, 14 *b*), quantitative data on this instance of cross-reactivity are now given for the first time. In this connection, the X-XX cross-reaction, also long-known (13 *a*, 14 *a*, 14 *b*), was drawn into the study. The chemical basis of each cross-reaction is also discussed insofar as permitted by the present knowledge of the structures of the capsular polysaccharides, the principal determinants of pneumococcal type specificity. Perhaps the inadequacy of this present knowledge will stimulate further work in the field.

Materials and Methods

Specific Polysaccharides.[1]—S II, S VII, S VIII, S XIV, part of the S XVIII, and SXIX were furnished by E. R. Squibb and Sons, New York, kindness of Mr. T. D. Gerlough, and

* Supported by a grant, G-19968, from the National Science Foundation.

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[1] Designated by S, followed by the type number.

except for S XIX, were further purified (15 *a*, 15 *b*). S X and S XX (16, 17) were kindly supplied by Dr. Rachel Brown.

Antisera.—The antipneumococcal (anti-Pn) horse sera were supplied by the Division of Laboratories and Research, Department of Health, State of New York, courtesy of Miss Jessie L. Hendry, and by the Bureau of Laboratories, New York City Department of Health, kindness of Miss Annabel W. Walter. The anti-Pn II rabbit globulin was a gift from E. R. Squibb and Sons, through Mr. T. D. Gerlough.

Quantitative Estimations of Precipitated Antibody Nitrogen.—Microanalyses were carried out as in earlier papers, with use of a cold box for drainage of the tubes after centrifugation and inversion (10 *c*). Homologous reactions were set up at 0°C and left at 0°C for 48 hours, while cross-reactions were set up similarly and allowed to stand in a cold bath at 0°C for 2 weeks or longer, with frequent twirling of the tubes, except that 6 to 10 days usually sufficed for extensive, rapidly flocculating reactions.

RESULTS AND DISCUSSION

The Pneumococcal II-XX Cross-Reaction.—(*Cf.* references 13 *a*, 13 *b*, 14 *a*, 14 *b*, and Table I) Although not more than 12 per cent of precipitable type-specific antibody is involved in any instance, the reaction is reciprocal and occurs in all of the Types II and XX horse and rabbit antisera thus far tested except one weak "diagnostic" rabbit serum.

The type-specific antigenic determinant S II is composed of 1,3-linked L-rhamnose, 1,4,6-linked branch points of D-glucose, and D-glucuronic acid both as non-reducing end-groups and as residues linked 1,4- (3, 15 *a*). S XX has long been considered to contain D-glucose because of the strong reactivity of anti-Pn XX with polyglucoses (18, 19), and the presence of glucose has recently been confirmed chromatographically along with galactose and glucosamine (17). Even if the glucose in S XX were linked 1,6- as in the dextrans which strongly precipitate anti-Pn XX (13 *b*, 19), or 1,4,6- as in S II, the configurations surrounding the glucose residues in the two capsular polysaccharides must be very different, consisting, as they do, of different sugars not necessarily similarly linked. It is therefore not surprising that the cross-reaction of II and XX involves only a small portion of the precipitable antibody in the antisera. When this is removed, other cross-reactive polyglucoses such as glycogen or dextran precipitate only slightly less antibody than from the intact sera. Similarly, prior absorption of anti-Pn II or XX with glycogen leaves a high proportion of the antibody precipitable with S XX or S II, respectively.

The Pneumococcal X-XX Reaction.—(*Cf.* Table I) During the preceding study an additional Type XX antiserum became available from a horse that had been immunized with Types X and XX pneumococci because of the strong cross-reactivity which had been noted between these types (13 *a*, 14 *a*, 14 *b*). This antiserum also precipitated S II. To determine whether this were due to the anti-Pn X or to the anti-Pn XX, separate portions of the C-absorbed antiserum were precipitated at the maxima with S X and S XX. Both supernatants failed to precipitate with S II and both showed far less residual precipitable

antibody with the reciprocal polysaccharide of the X-XX pair than would have been expected from the separately determined anti-S X and anti-S XX content of the original C-absorbed serum (Table I).

While the only anti-Pn X on hand gave negligible precipitation with S II, the monospecific anti-Pn XX H616 yielded 44 μg of antibody N in this reaction, and its removal, together with an additional 52 μg of antibody N by dextran, failed to reduce appreciably the cross-precipitation with S X (Table I, footnote§). S X is said to contain galactose, galactosamine, glucosamine, and ribitol phosphate; S XX was found to contain galactose, glucose, and glucosamine (17). Since S II lacks galactose and glucosamine, the sugars common to S X and S XX, the limited chemical data available are in accord with the quantitative serological values in indicating that the II-XX and X-XX cross-reactions rest on different chemical bases. However, the disappearance of precipitation with S II after absorption of the X-XX antiserum with S X would then require explanation and might be due to the presence of X-XX cross-reactive determinants on the antibody molecules which are also reactive in the II-XX cross-precipitation.

The Pneumococcal II-XIX Reaction.—Agglutination of these types was originally stated to be non-reciprocal (14 *b*). Cross-precipitation was reciprocal but slight in the two antisera studied (Table I). From the little known of the chemistry of S XIX (17, 20) the reaction would appear to be due to similarly linked residues of D-glucose or L-rhamnose in the capsular polysaccharides of the two types, but an unimpressively small proportion of antibody is involved.

Types VI and XIV.—This reciprocal cross-reaction was discussed in reference 4 *b* and was attributed to an immunological similarity between non-reducing end-groups of D-galactose, such as are present in S XIV, and the 1,2-linked D-galactose occurring in S VI.

Types VII and XIV.—Cross-precipitation was definite in all three anti-Pn VII sera and in the only potent anti-Pn XIV serum available (Table II). In this instance, also, terminal non-reducing residues of D-galactose are probably involved (11), although the participation of other sugars common to the two capsular polysaccharides is not excluded.

The VII-XVIII Cross-Reaction.—This was one of the earliest found among the pneumococcal types (2, 14 *a*). Quantitative data on this reciprocal reaction are given in Table II. Cross-reactivity was very weak in the direction S XVIII anti-Pn VII. S XVIII is known to contain D-glucose residues linked both 1,6- and 1,4-, and it is possible that the D-glucose in S VII (11) is similarly bound. L-Rhamnose may also be involved in the relationship, since the residues of this sugar are resistant to oxidation by periodate in both S VII and S XVIII.

Types VII and XIX.—Cross-precipitation was weak (Table II) except in anti-Pn VII 937C. The antibody involved seemed largely different from that taking part in the smaller precipitation with S XIV, since the S XIX-absorbed

TABLE I

Antibody Nitrogen Precipitated in Reciprocal Cross-Reactions of Pneumococcal Types II and XX, X and XX, and II and XIX

Type-specific polysaccharide	Amount used	Antipneumococcal sera, Type								
		II H513	II H1054 C*	II R globulin C*	X H627 C*	XX H616 C*	X, XX H488C*	488 C absorbed with S X	488C absorbed with S XX	XIX H631 C*
	μg	μg	μg	μg	μg	μg	μg	μg	μg	μg
Homologous	For max. pptn.	3600	1051	<4330	864	355	<733 with S X 913 with S XX	390 with S XX	230 with S X	2250
S II	10				3‡					
	20									6
	50						31		0	8
	100					40§		0‖		
	150						32			
	200					44§				
S XX	50	48¶	17**	76‡‡	139					
	100	56¶	15**	73‡‡	163					
	200	51¶								
S X	50					32				
	150					35				
S XIX	30	15§§								
	60	20§§								

0°C; data calculated to 1.0 ml antiserum. H, horse; R, rabbit.

Sera II 513, II 1054C, and XX 616C were precipitated with oyster glycogen A_{1b} (see following footnotes). The supernatants of the first two yielded 42 and 9 μg N, respectively, with S XX; the third supernatant gave 29 μg N with S II. Serum XX 616C gave 62 and 71 μg N with dextran N236; the supernatant gave 18 μg N with S II.

* Absorbed with pneumococcal C-substance. Serum 513 contained only traces of anti-C.

‡ 25 μg S II precipitated 1 μg N.

§ Supernatants plus oyster glycogen A_{1b} (21) at the 0.6 mg level gave 33 μg N as against 41 μg with intact serum; supernatants plus dextran N236 at the 0.1 mg level, 52 μg N as against 62 μg with intact serum; the II, dextran-absorbed supernatants gave 29 μg N with S X at the 50 μg level.

‖ An attempt to coprecipitate any II–anti-XX complexes with S II plus S XX gave only 390 μg N.

¶ Supernatants plus glycogen A_{1b} at the 3 mg level gave 218 μg N; at the 6 mg level, 201 μg N; values for the intact serum, 198 and 215 μg N.

** Supernatants plus glycogen A_{1b} at the 0.5 mg level gave 12 μg N as against 17 μg with intact serum.

‡‡ Supernatants plus glycogen A_{1b} at the 1 mg level at 0°C overnight gave 38 μg N; intact serum, under somewhat different conditions, gave 45 μg N.

§§ Supernatants plus S XX at the 50 μg level gave 35 μg N.

TABLE II

Antibody Nitrogen Precipitated from Horse Sera at 0°C in the Reciprocal Cross-Reactions of Pneumococcal Polysaccharides of Types VII and XIV, VII and XVIII, and VII and XIX

Polysaccharide	Amount used	Antipneumococcal horse sera, Type						
		VII 895C*	VII 937C*	VII 1074C*	XIV 635C*	XVIII 495C*	XVIII 632C*	XIX 631C*
	μg	μg	μg	μg	μg	μg	μg	μg
Homologous	At max. pptn.	579	893	500	1010	2200	308	2250
S VII	20							2
	30				29‡			
	50					48	21§	1
	60				34‡			
	150					72§	18	
	300					69		
S XIV	25			20				
	33	7	27					
	50			24				
	100	4	35					
	150			15				
	200		35					
S XVIII	25	7		4				
	40	7	0					
	50			3				
	75							
	110		0					
90 per cent alkali-degraded S XVIII	25	3		3				
	50		0					
	75	2		3				
	150							
S XIX	50	1	70‖	7				
	150	1	70‖	10				

Calculated to 1.0 ml of antiserum.

* Absorbed with pneumococcal C-polysaccharide.

‡ Supernatants plus oat glucan gave 20 μg N; intact serum gave 17 μg.

§ Data from reference 12, 75 μg S VII used for serum 632C.

‖ Supernatants plus S XIV, karaya, dextran N236 gave 22, 95, 46 μg N, respectively. Values for intact antiserum 937C, 35, 116, 46 μg N, respectively.

TABLE III

Antibody Nitrogen Precipitated from Horse Sera at 0°C in the Reciprocal Cross-Reactions of Pneumococcal Polysaccharides of Types VIII and XVIII, VIII and XIX, and X and XIV

Polysaccharide	Amount used	Antipneumococcal horse sera, Type					
		VIII 996C*	VIII 1008	X 627C*	XIV 635C*	XVIII 495C*	XIX 631C*
	μg	μg	μg	μg	μg	μg	μg
Homologous	At max. pptn.	1030	1288	864	1010	2200	2250
S VIII	25					11	
	50						124
	75					14‡	
	150						127
Reduced S VIII	50					21‡	
	150					18	
S X	50				12§		
	100				15§		
S XIV	50			60‖			
	100			79‖			
	200			54			
S XVIII	25	0					
	75	0	37¶				
	150		40¶, 47				
90 per cent alkali-degraded S XVIII	50		17¶				
	150		22¶				
S XIX	25	5					
	50		90**				
	75	5					
	150		105**				

Calculated to 1.0 ml antiserum.

* Absorbed with pneumococcal C-polysaccharide. Antiserum VIII 1008 contained only traces of anti-C.

‡ Data from reference 12. The analyses in anti-Pn XVIII and those recorded in *Biochemistry*, 1961, 1,1175, were carried out by Dr. S. Estrada-Parra. Reduced S VIII precipitated 1015 μg N from anti-Pn VIII 1008 (10 *c*).

§ Supernatants plus dextran N236 gave 13 μg N, as in the intact serum.

‖ Supernatants plus S XX at the 100 μg level gave 144 μg N (*cf.* Table I).

¶ Data from *Biochemistry*, 1962, 1,1175. Periodate-oxidized S XVIII, which still retains a portion of its D-glucose, gave 26 μg N with anti-Pn VIII 1008; periodate-oxidized alkali-degraded S XVIII, which was glucose-free, gave no precipitate.

** Supernatants plus S III at the 120 μg level gave 250 μg N; intact serum gave 331 μg N. Antiserum 1008, absorbed with oat glucan at the 50 μg level, which had previously given 121 μg N, precipitated 73 μg N with S XIX at the 50 μg level.

supernatants still gave 63 per cent as much precipitate with S XIV as did the intact serum. As knowledge of the polysaccharides of Types VII and XIX is incomplete (11, 17, 20), little more can be said.

The VIII-XVIII Cross-Reaction.—Quantitative data, taken in part from reference 12, are given in Table III. The reciprocal cross-reactivity is ascribed to the presence of 1,4–linked D-glucose residues in the repeating units of the two capsular polysaccharides (8 *b*, 12). Reduced S VIII, in which most of the 1,4–linked D-glucuronic acid is converted to D-glucose, reacts more strongly with anti–Pn XVIII than does S VIII. This is perhaps to be expected. In the reciprocal reaction, however, alkali-degraded S XVIII precipitates anti–Pn VIII less well than does the intact polysaccharide. One would expect the removal of an acetyl group and α-glycerophosphate side-chains to make the 1,4–linked glucose residues in S XVIII more accessible to anti–Pn VIII if any change were induced. Possibly the diminution of reactivity is a reflection of secondary alterations brought about by the alkali for which other chemical reactions (12) have given no indication.

The VIII-XIX Cross-Reactivity.—This instance of crossing was also noted early (13 *a*, 14 *a*, 14 *b*). Precipitation was rapid and heavy in the single anti–Pn XIX serum and one of the two anti–Pn VIII sera tested (Table III), but too little is known of the chemistry of S XIX to provide an explanation.

The X-XIV Cross-Precipitation (Table III).—This reciprocal reaction seems not to have been encountered in earlier studies. There is chromatographic evidence that S X contains galactose and glucosamine (17), and as S XIV contains both D-galactose and *N*-acetyl-D-glucosamine (22), either or both of these sugars may be involved.

SUMMARY

Data are given on the amounts of antibody nitrogen precipitated in the cross-reactions, often reciprocal, of the specific capsular polysaccharides of the pneumococcal type pairs II and XX, II and XIX, VII and XIV, VII and XVIII, VII and XIX, VIII and XVIII, VIII and XIX, X and XIV, and X and XX. Since little is known of the fine structures of the polysaccharides of Types VII, X, XIX, and XX, rigorous correlations between chemical structure and specificity can not be made, but several tentative conclusions are drawn.

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