IMMUNOCHEMICAL STUDIES ON BLOOD GROUPS*

VI. The Cross-Reaction between Type XIV Antipneumococcal Horse Serum and Purified Blood Group A, B, and O Substances from Hog and Human Sources

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The agglutination of human erythrocytes of blood groups A, B, and O by Type XIV antipneumococcal horse sera has been clearly demonstrated (1, 2). Purified blood group A substance from commercial pepsin precipitates with Type XIV horse antiserum (3) and in one instance, removed about one-half of the antibody from an antiserum. In addition, both the specific polysaccharide of the Type XIV pneumococcus (SXIV) and, after partial hydrolysis, the blood group A substance react with horse antianthrax sera (4). The chemical basis for these cross-reactions has been clarified by the finding that the blood group A substance, SXIV, and the C polysaccharide of the anthrax bacillus contain in common N-acetyl glucosamine and galactose (3, 4). With the demonstration that purified blood group A and O substances from individual hog stomach linings were identical in a considerable number of chemical and physical properties (5) and that both contained *N*-acetyl *d*-glucosamine, *d*-galactose, and *l*-fucose (6) it was considered that a quantitative study of the precipitin reaction of these substances with Type XIV antipneumococcal horse serum might provide information about their structural similarities and differences. The surprising result was that preparations of hog blood group A substance differed widely in their capacity to precipitate anti-SXIV although they were all of equal purity and had the same capacity to precipitate homologous anti-A formed in man (5). Similar variations in precipitability for anti-SXIV were found among various preparations of hog blood group O substance, and of blood group A and O substances from human saliva and stomach (7).

EXPERIMENTAL

The blood group A and O substances from individual hog stomach linings and the blood group A substances from human saliva, stomach, and amniotic fluid were the preparations

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that had been used in previous studies (5–7). In addition, samples of blood group substances were purified from human B and O saliva and another preparation of O substance was obtained from a human stomach by the method described for the isolation of the blood group A substance (7).

Two Type XIV antipneumococcal horse sera were used; A66, supplied by Dr. A. J. Weil of Lederle Laboratories contained both Type XIV and Type XIX antibody, and H635 (1939 bleeding) provided by the New York State Department of Health Laboratories. The two sera contained 1.08 and 0.87 mg. anti-SXIV N per ml. and both sera agglutinated human erythrocytes of blood groups A, B, and O.

The cross-reaction between the various blood group substances was studied by adding increasing quantities of blood group substance dissolved in saline to a series of 10 ml. conical centrifuge tubes each containing a measured volume of antiserum at 0°C.; the total volume was kept constant. The contents of the tubes were mixed and the tubes kept in the refrigerator for a week (8) during which period their contents were mixed twice daily. The precipitates were then centrifuged off in a refrigerated centrifuge, washed twice in the cold with chilled saline, quantitatively transferred to 10 ml. micro-Kjeldahl flasks with water and a few drops of M/2 NaOH, and analyzed for nitrogen by the Markham micro-Kjeldahl method (9). Values represent the average of duplicate analyses. Supernatants from each pair of analyses were combined and divided in half. To one portion 25 or 50 μg. blood group substance was added to test for residual cross-reacting antibody and to the other portion 0.1 ml. of antiserum was added to test for excess blood group substance. Supernatant tests were set up at 0°C., left in the refrigerator for 1 week, centrifuged in the cold, and the degree of precipitation in each tube was noted.

RESULTS

From Table I it is evident that individual preparations of hog blood group A substance vary greatly in their capacity to precipitate with Type XIV antipneumococcal horse sera. For instance, 500 μg. of the blood group A substance from hog 10 precipitated only 19 μg. N from 0.5 ml. serum H635 while equal amounts of similar products from hogs 16, 3, and 8 precipitated 39, 72, and 72 μg. N respectively. The same range of variation in precipitating power was also observed with hog blood group O substances. Tests on supernatants were carried out in all instances and invariably showed a broad zone of precipitation indicating the presence of both antigen and antibody. Several representative sets of supernatant tests are shown in Table I. From the data with hog 16 and hog 29, it is evident that 1 week in the refrigerator was adequate for maximum precipitation. With material from hog 29, no difference in capacity to precipitate Type XIV antibody was found after the solution had remained in the refrigerator for 6 weeks.

The same type of variation in capacity to precipitate with Type XIV antiserum was observed with blood group A substance from human saliva and stomach (Table II) as well as with materials prepared by the same methods from O saliva and stomach. Data are included on the relative viscosity and on the capacity of the various samples to precipitate anti-hog A formed in man (7). For instance, the substances from human stomachs 2 and 3 both showed

the same capacity to precipitate anti-A but that from stomach 2 was about twice as potent in precipitating antibody from Type XIV antipneumococcal serum. Similarly, the products from B. K. precipitated considerably more antibody in the cross-reaction than did A. $B._4$ or W. $H._1$ 10 per cent precipitates

TABLE I

Cross-Reaction of Purified Blood Group A and O Substances from Individual Hog Stomach Linings with Type XIV Antipneumococcal Horse Sera

Amount blood group substance added	Total nitrogen precipitated																
	Blood group A substances										Blood group O substances						
	Hog 3	Hog 8	Supernatant + Hog 8	Supernatant + Anti-body	Hog 10	Supernatant + Hog 10	Supernatant + Anti-body	Hog 15	Hog 16* (a)	Hog 16* (b)	Hog 13	Hog 19	Hog 25	Hog 27	Hog 29‡ (a)	Hog 29‡ (b)	Hog 29‡ (c)
μg.	μg.	μg.			μg.			μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.
0.5 ml. H635																	
50	15	24	++±	+	13	++	++	8	12	9	16	12	7	24	23	21	18
100	22	32	++	+	8	++	++	13	18	15	27	20	8	33	28	29	25
150	31	42	++	+	13	++	++	17	24	19	39	24	14	46	35	36	31
250	46	54	++	+	16	++	++	23	30	25	43	23	18	64	44	46	39
500	72	72	+±	+±	19	++	++	35	39	38	64		23	80	63	66	60
0.5 ml. A66																	
50		19	++++	+	16	++	+						17	31			
100		25	+++±	++±	20	++	+±						24	48			
150		28	+++	+++	20	+	+±						31	54			
200		33	+++	+++	21	+±	++						32	77			
300		36	+++	+++									37	73			
400		42			17								43	96			
500					18	+±	++										
η rel.§ (5)...	1.65	1.39			1.71			1.64		1.58	1.58	1.27	1.56	1.40		1.65	

* Set (*b*) was identical with set (*a*) but tubes were allowed to remain in the refrigerator for 2 weeks instead of 1 week.

‡ Sets (*b*) and (*c*) run 6 weeks after set (*a*), set (*c*) was washed and analyzed after 2 weeks in the refrigerator. Fresh solution used for set (*a*).

§ Relative viscosity of 0.2 per cent solution in 0.9 per cent saline.

(7) although they showed only about one-half the potency in precipitating anti-A.

The preparations from amniotic fluid and from human stomach 1 were unusually potent in precipitating with Type XIV antiserum although they showed only 17 per cent of the capacity of other samples in precipitating anti-A. Blood group B substance from human saliva also precipitated with Type XIV antiserum, essentially similar data being obtained with the phenol-insoluble and 10 per cent precipitate.

TABLE II

Cross-Reaction of Purified Blood Group Substances from Human Sources with Type XIV Antipneumococcal Horse Sera

Amount substance added	Total nitrogen precipitated																				
	Human saliva															Amniotic fluid	Human stomach				
	A substance											B substance		Substance from O saliva		A substance	A substance				O substance
	A. B_4	G. C.		W. H_1		W. H_2		B. K.		M. S.	W. G.	S. E.		F. P.	Bd		1	2	3	4	5
	10% ppt.	Phenol-insol.	10% ppt.	Phenol-insol.	10% ppt.	Phenol-insol. undigested	10% ppt.	Phenol-insol.	10% ppt.	Phenol-insol.	Phenol-insol.	Phenol-insol.	10% ppt.	10% ppt.	10% ppt.	Phenol.-insoluble					
$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$
0.5 ml. H635																					
50	19	18		18	15	10	16	20	16	31	22	20	12	30	11	44	22	18	11	17	30
100	30	26		30	23	20	29	32	29	47	38	31		47	16	70	37	33	16	22	45
150	34	36		39	29	28	39	46	41	61	46	36		62	25	86	45	38	18	27	56
250	46	50		50	43	40	60	63	60	74	61	42	46	86	29	108	52	46	23	39	72
500	61	60		81	55	58	102	86	81	88		56	65	105	42	125	65	62	29	50	
0.5 ml. A66																					
50		17	18		16							14	9			27					
100		25	29		25							17	14			43					
150		36	35		34							20	16			51					
250												26	24			63					
500					40							37	33			88					
η rel (7)...	1.25	1.23	1.25	1.35	1.23	1.13	1.13	1.27	1.13							1.20		1.11		1.13	
Relative capacity to precipitate anti-A (7).....	100	90	100	78	100	25	80; 100	48	46	86	40					17	17	100	100	63	

DISCUSSION

The data presented confirm previous observations (3) that purified hog blood group A substances precipitate with Type XIV antipneumococcal horse serum and in addition establish that hog blood group O substances and human blood group substances from individuals of groups A, B, and O also cross-react with Type XIV antipneumococcal antibody. Since these purified preparations appear to contain some of the essential constituents of the Type

XIV specific polysaccharide, *N*-acetyl-*d*-glucosamine and galactose, it seems reasonable to attribute the cross-reactivity to this similarity in chemical composition and thereby to provide a reasonable explanation for the capacity of Type XIV antiserum to agglutinate human erythrocytes of all four blood groups. Preparations of the various blood group substances also inhibit the agglutination of human A, B, or O erythrocytes by the Type XIV antiserum.

The broad zones over which supernatants from the reaction of the blood group substances with Type XIV horse antiserum invariably showed the presence of both antigen and antibody are also best interpreted on the basis of a cross-reaction similar to that between Types III and VIII antipenumococcal horse antibodies and their heterologous specific polysaccharides (10, 11).

Individual preparations of purified hog blood group A substances, all of which were shown by quantitative immunochemical assays to be of equal potency in their reactivity with anti-hog A formed in man (5), vary widely in their cross-reactivity with Type XIV antipneumococcal antibody (Table I). Similar variations apparently unrelated to the capacity of the substance to precipitate anti-A were also found for the human A substances from saliva and stomach (7). Whether the differences in the cross-reactivity of the various O substances from hog stomachs and of materials of similar composition from human group O saliva are also unrelated to their potency as group O substances cannot yet be decided since quantitative precipitin assays for anti-O are not available and assays by inhibition of hemagglutination are not sufficiently precise to provide a definitive answer. The differences in cross-reactivity among the individual A or O preparations could not be correlated with the relative viscosity of their solutions, and do not appear to be due to technical difficulties such as failure to attain maximum precipitation since identical results were obtained when precipitin analyses were (hogs 16 and 29, Table I) carried out after the tubes had been in the refrigerator for 2 weeks instead of 1 week; in addition no detectable change in precipitating capacity of the substance from hog 29 occurred after the solution had remained 6 weeks in the refrigerator.

It is not possible at present to offer an adequate explanation of these findings. However, it has previously been established by quantitative precipitin assays that solutions of hog blood group A substance are extraordinarily stable with regard to their blood group A activity, no detectable reduction in potency occurring after exposure for 2 hours at 100°C. in solutions varying in pH from 2.97 to 7.58 (12). That such individual preparations may vary in their cross-reactivity with Type XIV antipneumococcal serum and not in their blood group A activity suggests that the two activities may be associated with different portions of the complex molecule and that it may be possible under suitable conditions to alter one without affecting the other. It is apparent that any

adequate explanation of the chemical basis for the unique biological activities of the blood group substances must account for the differences herein noted.

SUMMARY

Purified blood group A, B, and O substances from hog and human sources precipitate with Type XIV antipneumococcal horse serum and provide an explanation for the observation that Type XIV antibody agglutinates human erythrocytes of all four major blood groups.

Individual preparations of A substance or O substance from either species vary in their capacity to precipitate Type XIV antibody although the hog A substances did not differ in potency toward anti-A. Similarly, no correlation between A activity and reactivity with Type XIV antibody could be found among the human A substances.

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