IMMUNOCHEMICAL STUDIES ON BLOOD GROUPS

XLVII. The I Antigen Complex – Precursors in the A, B, H, Le^a, and Le^b Blood Group System – Hemagglutination-Inhibition Studies*

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The majority of human cold autoagglutinins are directed against a red cell antigen termed I(1). About 1 in 10,000 adults are deficient in this antigen, but have larger amounts of a related antigen termed i (1, 2 and footnote 1). I antigen differs from other blood group antigens in that its expression on the red cell is generally low at birth and increases during the first 18 months (3). No individual has yet been found to be devoid of all traces of the antigen (4). Cord red cells are deficient in I but rich in i antigen. Family studies indicate that the production of I antigen is genetically determined (4) and it has been postulated that a very common gene, *Z*, is necessary for the conversion of i into I; the very rare adult lacking I might then be homozygous for a recessive gene, *z*, which would not facilitate the change of i into I.[2] Studies with enzymes which destroyed I antigen on erythrocytes led to the inference that β-linked *N*-acetyl-D-glucosamine and β-linked D-galactose residues were involved in I specificity (5).

The lack of a readily available source of water-soluble I antigen has greatly hampered further elucidation of its structure. Recently, however, human saliva and milk have been shown to contain a substance capable of specifically inhibiting hemagglutination of I red cells by some anti-I sera (6); such an inhibitable antiserum gave a precipitin line, only in the cold, by agar gel-diffusion when it was tested with whole milk or with defatted and deproteinized milk (7). Strong inhibition by milk, however, was found only in an occasional serum (6, 7).

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[1] Wallace, J. 1960. Cited in referene 4. 425.

[2] Jenkins, W., and W. L. Marsh 1961. Cited in reference 4. 427.

This study establishes the similarity in analytical data of a partially purified glycoprotein from milk and a glycoprotein OG, which lacked A, B, H, Le^a, and Le^b activity, isolated from ovarian cyst fluid of a Nigerian (8); both of these showed I activity. From the oligosaccharides isolated after alkaline borodeuteride degradation of purified OG substance, and in terms of the over-all composite structure proposed for the oligosaccharide moiety of the blood group A, B, H, Le^a, and Le^b antigens (9–11), OG was inferred to be a precursor in this system (12). The high I activity of OG as determined by hemagglutination-inhibition studies with one anti-I serum established the I gene as closely involved in the development of the A, B, H, Le^a, and Le^b determinants. I activity has also been produced by periodate oxidation and Smith degradation of human ovarian cyst blood group A and B substances and of hog A + H gastric mucin. A considerable proportion of cow stomach blood group substances previously studied in this laboratory (13) have now been found to be potent I materials, as have certain fractions (P1) (14) obtained by mild acid hydrolysis of A and B substances.

Anti-I sera differ from one another strikingly in their ability to react with milk, with OG, and with various cow substances, as assayed by hemagglutination inhibition, indicating that they possess a spectrum of specificities.

Materials and Methods

Isolation of I Antigen from Milk.—A 350 ml sample of milk from a mother of blood group A was centrifuged at 15,000 rpm for 30 min and the fat layer which formed was removed. The defatted milk was heated in a boiling water bath for 15 min to inactivate enzymes and was centrifuged. The supernatant was dialyzed repeatedly against distilled water. The dialyzable fraction was concentrated *in vacuo* and lyophylized, 29.2 g. This fraction was devoid of I activity by hemagglutination inhibition when tested at 50 mg/ml. The nondialyzable fraction was precipitated with 2.5 volumes of 95% ethanol after the addition of sodium acetate. The precipitate was dissolved in water and the ethanol precipitation was repeated twice, yielding 462 mg. One half of this was taken up in water and deproteinized by the Sevag procedure (15, cf. 16), dialyzed against distilled water, concentrated *in vacuo*, and lyophylized, yielding 6 mg (milk fraction C).

The second half of the ethanol-precipitated material was extracted with 90% phenol and precipitated by the addition of ethanol to a 20% concentration (16, 17). After a second phenol extraction and precipitation with 20% ethanol, it was dissolved, dialyzed against distilled water, and lyophylized, yielding 47 mg. (milk 20% 2 ×).

Other Blood Group Substances.—The following materials were used:

Human substances devoid of A, B, H, Le^a, and Le^b activity (precursor substances); OG fraction 1 (10% 2×), OG.2 (20% from 10% 2×), OG.3 (20% 2×), and OG.4 (10% from first 20%) (8). Fl (18) donated by Prof. W. T. J. Morgan; Cyst 3(from Dr. Harold Baer) (19); Carlson fractions (20).

Human Le^a substances (9): N-1 phenol insoluble fraction, N-1-1 (10% 2×), N-1-3(20 % 2×), N-1 (10% of first 20 % and phenol insoluble of first 10%); Le^a Morgan NANA removed (cf 9), Le^a MT (donated by Dr. D. M. Marcus) (8, 21).

Human B substances: Beach phenol insoluble, Beach phenol insoluble P1[3](14), Beach α-galactosidase–treated (22), PM phenol insoluble and PM phenol-insoluble P1 fraction (23).

Human A substances: MSS (10% 2×) and MSM (10% 2×) (24); MSS A enzyme and HNO_2-treated (25); McDon (26). Cyst 7 (Baer) (27), Cyst 14 (A_2) (28). A sample of meconium from a group A baby (Lyons).

Human H substances: JS phenol insoluble (24) and its sequential periodate oxidation products, 1st through 5th stages (10). Tighe (29). Human H substance donated by Prof. W. T. J. Morgan.

Hog A substances: Hog 18, Hog 18P1, Hog 15P1 (30).

Hog H substances: Hog mucin Baker B-2H (31, 32). Hog mucin (A + H) prepared by isopropanol fractionation. Hog 6 (33), Hog 6P1, Hog 13 phenol insoluble, Hog 13P1 fraction, Hog 29, Hog 29P1 (30), Hog 54 (34).

Cow materials (13)*:* P1 fractions of cows 21, 40, and 45 (13, 23).

Horse material (35, 36): Horse 4 P1 fraction (23).

Bacterial polysaccharides: Pneumococcus polysaccharide S XIV (Gil) (37) and β-galactosidase–treated S XIV (34, 38). Pneumococcus Forssman antigen (39). A sample of anthrax polysaccharide (40, 41) Native fetuin and sialic acid-free fetuin isolated from pooled fetal calf serum were donated by Dr. R. G. Spiro (42, 43).

Periodate Oxidation and Smith Degradation of Human Cyst A and B and Hog A + H substances.—Human blood group A substance, 476 mg MSS (10% 2×), (24) and B substance, 487 mg Beach phenol insoluble (44), were each dissolved in 30 ml of water, an equal volume of 0.12 M sodium periodate was added, and the solution was left at 4°C in the dark (10). Hog mucin A + H substance (16), 7.63 g, was dissolved in 250 ml of water and an equal volume of 0.24 M sodium periodate was added. When uptake was essentially complete, the A substance had consumed 3.45 moles of periodate per 1000 g, the B substance 3.53 moles per 1000 g and the hog A + H substance 2.78 moles per 1000 g. After the addition of excess ethylene glycol, the solutions were dialyzed and the nondialyzable portion reduced with sodium borohydride (1 mg/ml of blood group substance) for 16 hr at room temperature. The solution was then neutralized with HCl, made up to 0.5 N with concentrated HCl, and left at room temperature for 24 hr. After exhaustive dialysis, the nondialyzable portion was lyophylized; yields were: MSS, 277 mg (56%); Beach, 262 mg (54%); hog A + H substance, 2.6 g (34%). These are designated MSS 10% 2× 1st IO_4, Beach phenol insoluble 1st IO_4, and hog mucin A + H 1st IO_4.

Sera Containing Cold Agglutinins.—24 sera with cold agglutinins were examined by hemagglutination inhibition. Kof., Too., and Sch. (45), and Ort., Nay, and Phi. were gifts from Dr. H. G. Kunkel; A.D., M.A., and E.W. (46), A.T. (47), Hi (Case C, reference 48), S.C., and J.R. were from Dr. J. V. Dacie; M.G. (49), J.M., and Per. were from Dr. W. Rosse; Schu. and Mat. from Dr. R. E. Rosenfield; Step (50) and Ho from Dr. M. Crookston; Ma. (Math. in 51), Da. (51, 52), and Obe from Doctors. R. S. Evans, M. Harboe, and R. C. Williams respectively; Tho. (Mr. T. in reference 53).

From cold agglutinin titers with Group OI, Oi_{adult}, and Oi_{cord} cells, 21 of the sera were typed as anti-I and 3 as anti-i (Ho., Mat., and Tho.). 19 were also examined for associated A, B, H, Le^a, Le^b, P, M, and N specificity by titration against the following red cell types: OIM; OIN; Oi_{cord}; Oi_{adult}; OhI (Bombay); OI Le (a-b-); OIp; OI Lu (a-b-); A_1I; A_1i_{adult};

[3] P1 fractions are the nondialyzable portions remaining after mild acid hydrolysis, pH 1.5–1.8, at 100° C for 2 hr; this results in the removal of most of the fucosyl end groups and of some blood group A or B active oligosaccharide side chains and probably in some depolymerization.

and BI. The sera were also titrated against rabbit and Cynomologus irus monkey[4] red cells. Six of the sera were used in absorption and elution studies designed to separate possible IA-, IB-, or IH-specific components. All of the tests gave the results expected of anti-I or anti-i; no evidence of associated specificities were found.

Analytical Methods.—Nitrogen, hexosamine, *N*-acetylhexosamine, methylpentose (fucose), and hexose (galactose) were determined by the colorimetric methods previously described (16, 54). Galactosamine was determined by the method of Ludowieg and Benmaman (55).

Hemagglutination Assay.—Cold agglutinin titers were assayed at 4°C by the microtiter technique as previouly described (56).

Hemagglutination-Inhibition Studies.—For hemagglutination-inhibition studies sera were diluted in isotonic saline to give 16 hemagglutinating units. Serial twofold dilutions of inhibitor were made in isotonic saline using the microtiter technique. To each dilution of inhibitor, an equal volume of cold agglutinin was added, followed by one volume of a 1% suspension of group OI erythrocytes. In tests with anti-i sera Oi_{cord} red cells were used. Agglutination was assessed microscopically after 2 hr in ice water (56).

TABLE I

Analytical Composition of Milk and OG Glycoprotein Fractions

Substance	N	Methylpentose (fucose)	Hexose (galactose)	Hexosamine*	*N*-acetylhexosamine*‡	Galactosamine*
	%	%	%	%	%	%
Milk Fraction C	6.4	5.4	17.6	17.2	16.3	2.6
Milk 20% 2×	9.1	7.8	13.9	21.0	16.4	2.5
OG 20% 2×	6.8	2.4	32.5	29.0	25.6	10.3

* After hydrolysis at 100°C for 2 hr in 2 N HCl.

‡ In the colorimetric method for *N*-acetylhexosamine, *N*-acetyl-D-galactosamine gives ⅓ the color of *N*-acetyl-D-glucosamine, while in the hexosamine analysis, both give equal color yields per unit weight.

RESULTS

Analytical Properties of the Milk Substances—The two I-active preparations from milk showed somewhat lower values for galactose, hexosamine, N-acetylhexosamine, and galactosamine than did the OG precursor material (Table I). The milk fraction C prepared by the chloroform method had considerably less N than that obtained by phenol-ethanol fractionation. Although the methylpentose value was higher than that for the OG fractions, it was nevertheless considerably lower than is usually found for A, B, H, and Le^b substances from ovarian cyst fluid and from saliva, and was about that value reported for Le^a substances (see 17). The low values for the other constituents would suggest that the milk material was about two-thirds as pure as the other blood group substances. The *N*-acetylgalactosamine value is considerably lower than is usually found with blood group substances from human ovarian cyst fluid and

[4] Kindly supplied by the Laboratory for Experimental Medicine and Surgery in Primates, New York.

could indicate a fundamental structural difference between the milk and ovarian cyst substances.

I Activity of Various Blood Group Substances with Anti-I Ma—Table II summarizes the I-activity assays by hemagglutination inhibition, relative to milk fraction C as a standard, using anti-I serum Ma which had previously been found to be inhibitable by milk and to form a precipitin line with it in gel diffusion (7). Since the titration end points varied from one assay to another, results are given in three columns, in each instance relative to the end point for milk fraction C determined at the same time. The 20% 2× fraction of milk was somewhat less active than fraction C. It is evident that the four fractions of OG inhibited very well and were as potent as the milk fraction C. Of the other substances which could be considered as possible precursors of the A, B, H, Le^a and Le^b substances, only Fl (18) showed substantial activity, cyst 3 (19) and the Carlson material (20) being much less potent. Thus, two precursor-type substances OG and Fl showed substantial I activity when assayed with anti-I Ma.

I activity was found in a large number of other blood group substances (Table II). Of the various fractions obtained from an Le^a substance (N-1) (9), the 20% 2× fraction and the fraction reprecipitable at 10% ethanol from the first 20% ethanol precipitate showed considerable activity, while the phenol-insoluble and the 10% 2× fraction showed much less activity. Two other samples of Le^a substance, MT and the Morgan preparation, were also much less active.

Ovarian cyst A, B, and H substances, hog mucin A + H substances, and horse A, B, and inactive substances showed negligible I activity.

Of considerable interest was the finding that some cow blood group substances showed substantial I activity, in some instances (cows 14, 16, 21, 25, 26, 46) being as good as the milk and OG preparations. Substances from other cows, however, were almost totally inactive (Table II) and some cow substances showed intermediate activity.

Also of great significance was the finding that A and B substances which were essentially devoid of I activity could be converted to products with substantial I activity. Thus the P1 fractions of human cyst B substance, Beach phenol insoluble, and of human saliva B substance, P.M. phenol insoluble, obtained by mild acid hydrolysis, developed considerable potency in inhibiting hemagglutination of I erythrocytes by anti-I Ma. Indeed, in some assays the Beach phenol insoluble P1 was almost as active as the milk and OG materials. Similarly, a one-step periodate oxidation and Smith degradation of human A, human B, and hog mucin A + H substances resulted in the appearance of substantial I activity. Some I activity was also found in pneumococcal Type XIV polysaccharide after enzymatic removal of terminal galactose with β-galactosidase.

Table III summarizes the results of a screening study on the capacity of the

TABLE II

Inhibition by Various Blood Group Substances Relative to Milk of Agglutination OI Erythrocytes by Anti-I (Ma) at 4°C

Substance	Minimum concentration (μg/ml) giving complete inhibition relative to end point with milk fraction C		
Milk Fraction C	3.3*	6.7*	13.4*
Milk 20% 2×	40		
Human precursor substances			
OG.1 (10% 2×)‡	5.4		
OG.2 (20% from 10%)	4.3		
OG.3 (20% 2×)	2.6		9.3
	4.7		4.7
	4.3		
OG.4 (10% from 20%)	2.8		
Cyst 3		110	
Cyst 3 phenol sol			20.6
Cyst 3 10% ppt			94
Fl Morgan		17	
Carlson original	>6000		
Carlson purified	>1900	>1700	
Carlson hydrolysed	194		
Human Le^a substances			
N-1-1 10% 2×		265	465
N-1 10% of 1st 20%			43
N-1-3 20% 2×		13.5	54
N-1 phenol insol from 1st 10%			350
N-1 phenol insol			683
MT		62.5	
Morgan NANA removed	315		
Human B substances			
Beach phenol insol	635		635
	860		
Beach phenol insol P1	3.5		
	8.0		
Beach phenol insol 1st IO_4	124		74.6
Beach phenol insol α-galactosidase treated	905		
PM phenol insol			1000
PM phenol insol P1	22		200

Substance	Minimum concentration (μg/ml) giving complete inhibition relative to end point with milk fraction C		
Milk Fraction C	3.3*	6.7*	13.4*
Human A substances			
McDon		>100	
McDon P1		50	
MSM 10% 2×		1090	
MSS 10% 2×	>1620		>1470
MSS A enz, HNO_2 treated		>710	
MSS 10% 2× 1st IO_4	735		735
Cyst 7		250	
Cyst 14(A_2)		1400	
		>1600	
Lyons meconium		1190	
Human H substances			
JS phenol insol	>1070		
JS phenol insol 1st IO_4	545		
JS phenol insol 2nd IO_4	295		
JS phenol insol 3rd IO_4	902		
JS phenol insol 4th IO_4	1260		
JS phenol insol 5th IO_4	1240		
Tighe		>9000	
Human H substance (Morgan)		100	
Hog A substances			
Hog mucin (A + H) 10% 2×			>1430
Hog mucin (A + H) 10% 2× 1st IO_4		25	
		50	
Hog mucin fraction 4		100	
Hog 15 P1	500		
Hog 18	1820		
Hog 18 P1		909	
Hog H substances			
Hog mucin Baker (B-2H)		13.5	
Hog 6	>1820		
Hog 13	>910		
Hog 6P1, 13 P1	1820		
Hog 29	>910	500	
Hog 54	>950		
Hog 29 P1	1110		

* End points for milk in individual experiments are compared with end points for other polysaccharides in assays run at the same time.

‡ 10% 2× refers to the fraction obtained by two precipitations at 10% ethanol from phenol. Other fractions are denoted in an analogous manner.

TABLE II—*Continued*

Substance	Minimum concentration (μg/ml) giving complete inhibition relative to end point with milk fraction C		
Milk Fraction C	3.3*	6.7*	13.4*
Cow blood group substances			
10% Fractions			
Cows 14, 21, 25			2
Cow 26			2
			6.2
Cow 46			7.8
Cow 16			15.6
Cow 22, 13			31.2
Cow 43			33
Cow 23		34	62.4
Cow 20			62.4
Cows 15, 17, 48			250
Cows 12, 37			500
Cows 32, 28, 18			1000
33			2000
34			>2000
Phenol insoluble			
Cow 21			15.6
			50
Cow 22			125
Cows 23, 15			1000
Cows 17, 12, 18			2000
P1 Materials			
Cow 21	14.2		
Cow 45	7.1		
Cow 40	22.8		

Substance	Minimum concentration (μg/ml) giving complete inhibition relative to end point with milk fraction C		
Milk Fraction C	3.3*	6.7*	13.4*
Horse blood group substances			
Horse 3		125	
		900	
25% Precipitates			
Horse 2		250	
Horse 6		390	
Horse 1		625	
Horse 4	1820		
Horse 4 P1	910		
Phenol insoluble			
Horse 6		1000	
Horse 3		900	
Bacterial Polysaccharides			
Pneumococcus XIV (Gil)		129	
Pneumococcus β-galactosidase treated		32	
Pneumococcus Forssman polysaccharide		>1010	
Anthrax polysaccharide	>100 >910		
Fetuin			
Native		>910	
Sialic acid-free		>610	

various blood group substances to inhibit hemagglutination of I erythrocytes by various anti-I sera. It is evident that the sera differ substantially in their specificity from anti-I Ma (see also Table II). With anti-I Ort, only milk, OG 20% from 10% and 20% 2×, F_4, Beach P1, the first IO_4 steps of Beach, and hog mucin A + H showed I activity relatively similar to anti-I Ma. However, cows 46, 26, and 21 were relatively inactive with Ort while they were very active with Ma. Beach phenol insoluble and the N-1 fractions were active with Ort but relatively inactive with Ma. All of the other anti-I sera behaved quite differently in that very large amounts of all substances tested were required. Indeed with one group, Nay, Phi, A.T., Schu., Obe, M.G., and J.M., none of the materials were active in the highest concentrations tested. The largest amounts used were limited with certain materials by the small amounts available.

The other anti-I sera showed various inhibition patterns, although in each

TABLE III

Inhibition by various Blood Group Substances of Hemagglutination of OI Erythrocytes by Anti-I and Anti-i Sera

Sera	Inhibitors: Minimum concentration giving complete inhibition (μg/ml)																	
	Milk		OG	Fl	Cows					Beach (human B)		MSS (human A)		N − 1 (human Le^a)		Hog mucin A + H	
	Fraction C	20% 2×	20% from 10%*		26	28	32	46	21	Phenol insoluble	P1	10% 2×	1st IO_4	Phenol insoluble	10% of 1st 20%	Original material	1st IO_4
Anti-I																	
Ma.	3–13	40	4	17	2–6.5	1000	1000	8	2	635 860	3.5–8	>1620	735	683	43	>1440	50
Ort.	0.1–1	1.2	0.5	0.4	125	250	250	500	125	9.4‡	7.0	735	11.8	15	1.0	180	25
		3.0	0.4 (0.2,1)				500	500		19.7	20	>405	16.6			397	110
Nay, Phi., A.T. Schu., Obe., M.G., J.M.	>100	>600 or >1200	>1270	>1050	>2000 or nt§	>2000 or nt	>2000	>2000	>2000 or nt	>1260	>910	>1470	>1510	>1290	>1390	>1440	>1600 >800
Step., Too., E.W. Hi, S.C.	>100	>600 or >1200	635–1270 (640)	>1050	>2000 or nt	>2000 or nt	>2000	>2000 or nt	>2000 or nt	>1260	>910	>1470	>1510	>1290	>1390	>1440	>1600 >800
Sch., Da.	>100	>300	318–1270	1050	500–1000	>2000	2000 or >2000	1000	nt	>1260	>910	>1470	>1510	>1370	>1390	>1440	>800
Per., J.R., A.D., M.A.	>100	>1200 or >600	158–365 (80)	1050	nt	>2000 or nt	>2000 (JR 250)	>2000 or nt	1000–2000 (Per. nt)	>1260	>910	>1470	>1510	>1290	>1390	>1440	>1600 >800
Kof.	>100	>1200	40	131	nt	>2000	1000	>2000	1000	>1260	>910	>1470	>1510	>1370	>1390	>1440	>1600
Anti-i																	
Tho., Mat.	>100	>1200 (Mat. nt)	159–320 (20,37,160)	1050	nt	nt	>2000	nt	>2000	>1260	>910	>1470	>1510	>1290	>1390	>1440	>1600
Ho.	>100	nt	>1270 >1270 (>1280)	>1050	nt	nt	>2000	nt	>2000	>1260	>910	>1470	>1510	>1290	>1390	>1440	>1600

* Values for OG 20% 2× in parentheses.

‡ Beach phenol insoluble Ist IO_4 fraction gave an end point of <0.6 μg.

§ nt = not tested.

instance much larger amounts of inhibitor were needed than were with Ma and Ort. Thus Step, Too., EW, Hi, and S.C. showed some inhibition only with OG 20% from 10%; with anti-I Sch. and Da, inhibition was obtained with OG 20% from 10%, Fl, Cows 46 and 26 in amounts ranging from 300 to 1000 μg; with Per, J.R., A.D., and M.A., OG 20% from 10% and Fl were active in large amounts; with Kof,. these two materials were somewhat more active and certain cow substances reacted with one and not with another (Table III). It appears from the data that I specificity is not a single unique specificity but represents a spectrum of antigenic determinants.

Two of the three anti-i sera, Tho. and Mat., showed inhibition with OG 20% from 10% and with Fl in amounts ranging from 159 to 1050 μg, while the third, Ho., showed no inhibition with any of the materials tested. Thus, anti-i sera, like anti-I sera, possess a spectrum of specificities.

DISCUSSION

The findings in this study serve to establish the relation of blood group I specificity to the A, B, H, Le^a, and Le^b blood group glycoproteins. An I-active material from milk has been paritally purified and shown to possess analytical properties relatively similar to those of blood group substances from human ovarian cyst fluid. This milk substance was very potent in inhibiting hemagglutination of I erythrocytes by anti-I with only 2 (Ma and Ort) of the 21 anti-I sera tested (Table I) (see 6).

Of greater significance, however, was the finding that the precursor blood group substances OG and Fl from human ovarian cyst fluid were also highly active with these two antisera, as were several fractions of an Le^a active substance from ovarian cyst fluid; the precursor OG and Fl substances also showed some activity with a number of the other anti-I sera (Table III). In addition, certain cow blood group substances isolated earlier (13) were found to show high I activity with anti-I Ma and Ort and activity with an occasional anti-I serum. It is noteworthy that cow substances often had a lower fucose content (13) than did other blood group substances which were less active (Tables II and III).

Further support for the concept that receptors for the I specificity are internal to the A, B, H, Le^a, and Le^b determinants is shown by the increased I activity of blood group A and B substances resulting from one-step periodate oxidation and Smith degradation which converts them to precursor-like structures similar to the OG fractions (12). In addition, mild acid hydrolysis of human saliva and ovarian cyst B substances also produced a great increase in I activity.

Thus, as revealed by the two anti-I sera Ma and Ort, I specificity appears at a stage in the biosynthesis of the A, B, H, Le^a, and Le^b substances corresponding to the OG and Fl precursor substances, and the enzyme produced by the I gene must probably function to produce its product prior to those controlled by A,

B, H, and Le^a genes. I determinants may prove to be widely distributed in nature since the cow blood group substances also contain them. I determinants may well persist to a greater or lesser extent in A, B, H, Le^a, or Le^b cyst substances as a result of incomplete biosynthesis or possibly be due to degradation in the cyst cavity.

Among the material used in the survey (Table III) i antigen was detected only in OG 20% 2×, OG 20% from 10%, and Fl substances. Thus it appears that some, but not all blood group substances containing I determinants may also contain i determinants as recognized by sera Tho. and Mat. This survey did not reveal blood group substances reacting with the third anti-i serum, Ho.

The spectrum of I specificities revealed by hemagglutination inhibition with the various antisera indicates the complexity of the I system. Quantitative precipitin studies in another paper[5] confirm this conclusion and indicate that precursor, cow, and milk substances differ in their capacity to precipitate anti-I from various antisera.

SUMMARY

A partially purified blood group-like substance obtained from milk showed I activity with 2 of 21 anti-I sera. With these antisera, certain human ovarian cyst substances considered to be precursors of the A, B, H, Le^a, and Le^b substances also showed I activity comparable to the milk material. Strong I activity could be produced by one-stage periodate oxidation and Smith degradation of human ovarian cyst A and B substances, or of hog mucin A + H substance, or by mild acid hydrolysis of human saliva or ovarian cyst blood group B substance. The two sera indicate that I specificity appears at intermediate stages in the biosynthesis of the A, B, H, Le^a, and Le^b substances. Anti-I sera differ strikingly in their specificities, indicating substantial heterogeneity of the I determinants.

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