B-GENE SPECIFIED 3-α-D-GALACTOSYLTRANSFERASE ACTIVITY IN HUMAN B BLOOD GROUP VARIANTS

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1. Introduction

The human blood group A-specified 3-α-*N*-acetyl-D-galactosaminyl-, B-specified 3-α-D-galactosyl- and H-specified 2-α-L-fucosyltransferases occur in soluble form in sera [1–6] and as membrane-bound enzymes in erythrocytes [1,7–10] from individuals with common ABO phenotypes. They are associated with two different genetic systems: the first two are related to the ABO locus and the third to the Hh locus. These activities have been also studied in unusual blood groups such as 'Bombay' phenotypes [7,8,11,12], A variants [9,13], B variants: B weak [14]; B_m [10,14,15]; and cisAB [16–18].

In this work, B variants from B_3, B_x and B_{el} subgroups [19] were investigated for A, B and H blood group glycosyltransferase activities using low molecular weight acceptors or (and) O red cell conversion. Studies on *N*-acetylglucosamine:β-D-galactosyltransferase activity in the sera were included as controls of state of preservation. Moreover, when activities were not detected, attempts were made to concentrate potential weak B-transferase.

2. Materials and methods

Our study is based on nine B variant samples belonging to seven families (B_3: Car, Pan, Sal; B_x: Opa, Voi; B_{el}: Aba, Iss), the immunological and thermodynamic properties of these variants were reported in [20–22]. Sera were stored at −80°C for several months and at −20°C for a shorter time, red cells were kept frozen at −196°C until used. Erythrocyte membranes were prepared from fresh or stored red cells according to [23]. The protein content of the red cell membrane preparations was estimated by a modified Lowry's method [24].

UDP-*N*-acetyl-[I-^{14}C]galactosamine (47.2 mCi/mM) and UDP-[^{14}C]galactose (283 mCi/mM) were purchased from the New England Nuclear Corp. They were used in 50% and 2% ethanolic solution, respectively. GDP-[^{14}C]fucose (118 mCi/mM) was purchased from the Radiochemical Centre (Amersham) and used in 2% ethanolic solution. Unlabelled UDP-*N*-acetylgalactosamine was prepared according to [25,26]; UDP-galactose was purchased from Sigma Chemical Co. 2′-Fucosyllactose (2′FL: *O*-α-L-fucopyranosyl-(1–2)-*O*-β-D-galactopyranosyl-(1–4)-D-glucopyranose) was extracted from human milk. Lacto-*N*-biose I (LNB I: *O*-β-D-galactopyranosyl-(1–3)-*N*-acetyl-D-glucosamine) came from Sefochem and *N*-acetylglucosamine from Sigma Chemical Co.

Transferase assays in human sera: standard and extended conditions for A and B transferase assays have been described [5,13,18] as well as conversion of O red cells [18] and *N*-acetylglucosamine:β-D-galactosyltransferase assays [9]. Incubations for H-transferase activity contained in 55 μl, the following: 25 μl serum, 0.9 μmol LNB I, 0.9 nmol GDP-[^{14}C]fucose (1.9×10^5 cpm), 0.6 μmol $MgCl_2$, 2.5 μmol Tris–HCl buffer (pH 7.2), 1 μmol ATP and 1 μmol NaN_3; incubated for 72 h at 37°C.

Transferase assays in erythrocyte membranes: for all these experiments, 250 μg erythrocyte membrane protein were used as enzymic source. A-transferase activity was estimated according to [9]. B-transferase assays were carried out in 150 μl and contained: erythrocyte membranes, 0.4 μmol 2′FL, 0.6 nmol UDP-[^{14}C]Gal (3×10^5 cpm), 1.25 μmol $MnCl_2$, 6.0 μmol Hepes buffer (pH 6.5) and 0.3% (v/v) Triton X-100.

Table 1
The α-*N*-acetyl-D-galactosaminyl-, α-D-galactosyl- and 2-α-L-fucosyltransferase activities in sera and RBC membranes from normal B and B variant donors

	Glycosyltransferase activities in sera								In RBC membranes		
	A[a]/2'FL	B/2'FL	H/LNB I	β/GlcNAc	A/O RBC[b]		B/O RBC[b]		A/2'FL	B/2'FL	H/LNB I
					Score	Titre	Score	Titre			
B II	0	26.3 ± 3.0 (21)	23 ± 9 (10)	54	0	/	83	1024	0	164 (71.9[c])	14
B I	0	11.2 ± 2.7 (107)	30 ± 9 (10)	46	0	/	71	256	0	43 (15.5[c])	11
B_3 CAR C.	0	8.7	13	44	0	/	53	64	n.t.	0[c]	16[c]
B_3 PAN R.	n.t.	10.0	27	50	0	/	80	1024	n.t.	0[c]	15[c]
A_1B_3 SAL M.	287	10.6	22	56	n.t.		n.t.		70[c]	0[c]	7[c]
B_3 SAL S.	n.t.	14.8	19	56	0	/	68	256	0[c]	0[c]	6[c]
B_X OPA R.	n.t.	0	43	50	0	/	0	/	n.t.	0[c]	12[c]
A_1B_X VOI Y.	190	0	56	33	33	16	0	/	233	0	10
A_2B_{el} ABA A.	375	0	42	50	0	/	0	/	73	0	9
B_{el} ABA L.	0	0	50	56	0	/	0	/	n.t.	0	13
B_{el} ISS M.	n.t.	0	23	104	n.t.		0	/	n.t.	0	n.t.
A_1	315	0	104	66 ± 2 (3)	73	256	0	/	121 ± 44 (4)	0	9
A_2	142	0	56	81	4	4	0	/	n.t.	0	n.t.
O	0	0	18	79 ± 7 (6)	0	/	0	/	0	0	8

[a] Assays carried out at pH 6.0 and 7.0, value taken at pH giving the best activity

[b] Reactivity of converted O cells to group A or B reactive cells tested with specific anti-A or anti-B sera are expressed as scores and titres of haemagglutination. Immune anti-A (B) or anti-B (A) had a titre of 4096 on A_1 or B cells, respectively

[c] RBC membranes prepared from frozen cells

Experiments were carried out as in β referred to *N*-acetylglucosamine:β-D-galactosyltransferase. A, B, H and β-transferase activities are expressed as pmol product formed on low molecular weight acceptors. Values in parenthesis indicate the number of samples tested

Reaction products were isolated and estimated as in [7,9,18] except for the β-galactosyltransferase product which was isolated after electrophoresis by descending chromatography in butanol/ethanol/water solvent (10/1/2, by vol.) for 72 h (R_{Gal} = 0.52).

Attempts to concentrate B-transferase activity: proteins were precipited at 60% of an ammonium sulfate saturated solution, desalted by filtration on a Sephadex G-50 column (medium) then concentrated, in batch, using Sephadex G-25 (coarse). All procedures were carried out at 4°C. Tests were performed in the extended conditions above.

3. Results and discussion

Human blood group *A*, *B* and *H*-gene specified glucosyltransferases were investigated in sera and erythrocyte membranes from nine B variants belonging to seven families using low molecular weight acceptors: 2'fucosyllactose, for A and B, lacto-*N*-biose I for H. Moreover, conversion assays of O red cells into A and B reactive cells were used. Sera and red cell membranes from common groups B (B_I and B_{II} [5]), A_1, A_2 and O were included as controls.

The 3-α-D-galactosyltransferase activity was clearly detected only in B_3 and normal B sera using 2'FL as well as O red cells as substrates (table 1). Other sera from B_x and B_{el} donors were devoid of B-transferase activity even when they were tested in extended conditions. As no decrease for 2-α-L-fucosyltransferase and 4-β-D-galactosyltransferase was observed the state of preservation seemed not to be involved in these results. As described in section 2, attempts were made to concentrate potential B-transferase activity. The coefficient of concentration obtained was ~2–4. Except for B_3 and normal B sera, all the B experiments were negative (table 2). When purified B-tetra-[^{14}C]saccharide (1.25 × 10^5 cpm) was incubated in extended conditions, without the substrates, with the different samples studied here, no release of galactose was observed. Moreover, mixtures of normal B and B variant sera (1/1, v/v), in standard conditions, transferred the same quantity of [^{14}C]galactose from UDP-[^{14}C]galactose to 2'FL as the mixture used as control: normal B and O sera (1/1, v/v). These experiments allowed the exclusion of glycolytic activity towards hypothetically formed B-product and an inhibition effect towards potential B-transferase in the B variant samples studied.

The erythrocyte membrane preparations from all the B variants do not contain detectable 3-α-D-galactosyltransferase except for B_I and B_{II} samples even when 3-α-*N*-acetylgalactosaminyl- for AB variants and 2-α-L-fucosyltransferases were clearly demonstrated. If transferase activities of RBC membranes reflect those of hematopoetic tissue, this could suggest that the *B*-gene was not normally expressed in hematopoetic tissue, due perhaps to the action of a modifying gene in this cellular line, as described for B_m [10] and A_m [13] phenotypes. Although the contribution of erythroblasts to the transferase activity in the

Table 2
The 3-α-D-galactosyltransferase activity in sera from B and B_3 donors in different experimental conditions, expressed as pmol product formed

	3-α-D-Galactosyltransferase activity on 2'FL			On O RBC			
	Standard conditions	Extended conditions	Assay conc.	Standard conditions		Assay conc.	
				Score	Titre	Score	Titre
B II	26.3 ± 3.0 (21)	138	236	83	1024	95	1024
B I	11.2 ± 2.7 (107)	53	150	71	256	96	1024
B_3 CAR.	8.7	n.t.	96	53	64	83	512
B_3 PAN.	10.0	32	102	80	1024	91	1024
B_X, B_{el} samples	0	0	0	0	–	0	–

The conditions are in section 2. Results are expressed as pmol B-product formed on 2'FL or as scores and titres of haemagglutination using anti-B(A) immune serum (titre 4096 on B cells)

serum was estimated from chimera studies at ~20% [3,13], *B*-gene specified glycosyltransferase in B_3 sera is as active as in normal B, taking into account the individual variations. In any case, if this mechanism fits perfectly with the B_m and A_m phenotypes, it does not reflect the high capacity of B_3 erythrocytes to be agglutinated. More likely, the absence of B-transferase activity in B_3 RBC membrane assays could result from an altered enzyme, more labile than that of normal B, which could have some activity in intracellular medium.

As for the other B-variants, B_x and B_{el}, undetectable B-transferase activity in sera as well as in RBC membranes could arise from qualitatively or quantitatively modified enzyme. An unexcreted transferase or some regulatory or modifying functions could be involved too. Meanwhile, in all AB variants studied here and in [15], A-transferase activity remained unchanged. On the other hand, from immunological homology studies on ABO glycosyltransferases, it was proved in [27] that the genes governing blood group expression should be truly allelic. These data are not consistent with regulatory control in ABO in blood group expression; only one case has been described [28] where perhaps a modifying gene acted on A and B blood group expression of two related individuals (A/O and B/O). Unfortunately glycosyltransferase activities were not studied. Lastly, as shown in [29] for one case of cisAB inheritance, an unequal crossing over producing a chromosome with alleles for O and B enzymes could also be a possible explanation for some B variants.

Thermodynamic and immunologic studies [20–22] and these enzymic results seem to demonstrate that B variants are homogeneous in the same kindred but are heterogeneous from one family to another. It is likely that the different B variants do not arise from a single mechanism but are the expression of different and convergent phenomena.

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