ABH GLYCOSYLTRANSFERASE LEVELS IN SERA AND RED CELL MEMBRANES FROM Hz AND Hm VARIANT BLOODS

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1. Introduction

The A and B blood group antigens of human fluids and tissues are carbohydrate structures attached to glycoproteins or glycolipids [1,2]. These substances are built up by A and B gene specified glycosyltransferases acting on appropriate H precursors which all exhibit the terminal disaccharide structure: α-L-Fuc(1→2) Gal-R [3].

A and B enzymes catalyse the addition of α-*N*-acetyl-D-galactosamine and α-D-galactose respectively on the carbon 3 position of the non-reducing β-D-galactose of H substrates [3].

The production of H substance is under the control of a genetic system, unrelated to *ABO* locus, termed *Hh*. The latter is characterized by an highly frequent allele *H*, producing the α-2-L-fucosyltransferase [4,5] and by a very rare allele *h*, considered to be an amorph [6,7]. Indeed the H enzyme has not been found either in sera and in RBC membranes from 'Bombay' (O_h^A, O_h^B) thought to be homozygous for the recessive *h* gene, nor in 'Parabombay' (A_h, B_h) individuals which may arise from poorly active gene at the *Hh* locus [5,8,12]. However, the A and/or B enzymes are normally expressed among such individuals, but are unable to fulfil their catalytic function due to the lack of H reactive structures. It is generally admitted that the complete expression of H substance is controlled by two other genetic systems.

(i) The secretor system *Sese*, which appears as a regulator of H enzyme synthesis at the site of H glycoprotein production. Indeed, the H enzyme is not found in milk or submaxillary glands from non-secretors *sese* [4,5]. However, the H enzyme is found in sera and bone marrow from both secretors *Se* and non-secretor *sese* which suggest that the enzyme is produced in numerous cells and later on poured into the blood stream [5,10,13]. The direct product of the *Se* gene is unknown.

(ii) The *Zz* system has been postulated in order to explain some rare H variants characterized by a selective deficiency of H antigen on red cells but normal expression of H substance in saliva [14,16]. It is assumed that the *Zz* system regulates H enzyme synthesis at the site of H glycolipid production, at least in erythroblasts. By analogy with Am phenotypes, such variants have been named Hm phenotypes.

The most common form of Hm phenotypes is inherited as a recessive character in individuals with a combination of two *z* genes [16]. Three families in which the Hm phenotypes were inherited as a dominant trait have been described, suggesting the occurence of a dominant allele *Zm* at the *Zz* locus [15–18].

Accordingly, following our studies on H variants [12] it was of interest to determine if the α-2-L-fucosyltransferase is normally active in sera and RBC membrane from Hm bloods, both of the recessive and dominant inheritances.

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2. Materials and methods

The H variants from 6 unrelated families were collected. Red cells and sera were stored at –20°C until used. Hm samples from Trn. and Jur. families were collected in Czechoslovakia and kindly sent by Dr Hrubiško (Bratislavia). Among French samples, the Let. family sample originates from Dr Kling (Metz), Dup. family and Bon. sample from Dr Jouvenceaux (Lyon) and Thi. from our unit.

UDP–D-[^{14}C]galactose (274 mCi/mmol) was obtained from Nen Chemicals (Frankfurt) and *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine from Koch-Light (London).

Reagents and methods used to estimate A, B and H glycosyltransferases in sera and RBC membranes have been described [12,19,20]. The level of UDPGal: Gl*N*Ac-β4-D-galactosyltransferase was estimated in RBC membranes by adding the following components in total vol. 175 μl: 100–200 μg erythrocyte membrane protein; sodium cacodylate buffer pH 7.0, 4 μmol; $MnCl_2$, 2 μmol; *p*-nitrophenyl-*N*-acetyl-D-glucosamine, 0.25 μmol; UDP-D-[^{14}C]galactose, 0.65 nmol; 10 μl Triton X-100 5% (v/v). The mixture was incubated for 18 h at 37°C and the reaction product isolated by single-step chromatography in ethylacetate–pyridine–water (10 : 4 : 3). The eluted radioactive *p*-nitrophenyl-*N*-acetyl-D-galactosamine released 100% of D-[^{14}C]galactose following degradation (48 h at 37°C) by a Jack bean galactosidase specific of galactose β-(1→4) linked [21].

3. Results and discussion

By definition, Hm individuals are characterized by a selective decrease of H antigen on their RBC and normal amount of H substance in saliva [14,16]. For reasons which will appear later, we have subdivised these H variants in two categories as refered to 'Hz' and 'Hm'. These phenotypes are inherited as recessive or dominant traits [22], respectively.

3.1. *First type of H variants (Hz phenotypes)*

Nine blood samples with H negative RBC and normal H secretory saliva were studied in 4 unrelated families (Dup., Trn., Thi., Bon.).

The recessive mode of inheritance of the Hz character in Dup. family is shown in fig.1a. As previously demonstrated with 'Bombay' samples [5,8,12] the H enzyme is absent or catalytically inactive in sera and RBC membranes from all these Hz individuals (table 1). However, the A and/or B enzyme levels fall in the normal range, which confirm the true genotypes deduced from family studies. Very clearly, in spite of common features with 'Bombay' bloods, the Hz cannot be explained by the inheritance of a double dose of *h* gene because their saliva contains a normal amount of H substance. The lack of α-2-L-fucosyltransferase activity in Hz sera, suggests that the serum enzyme does not originate from secretory organs still able to produce H substance. Moreover, this assumption is consistent with the similar level of H enzyme which have been found in secretor and non-secretor sera [10]. It was noticed

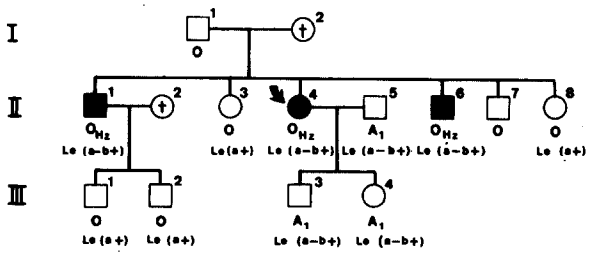

Fig.1a

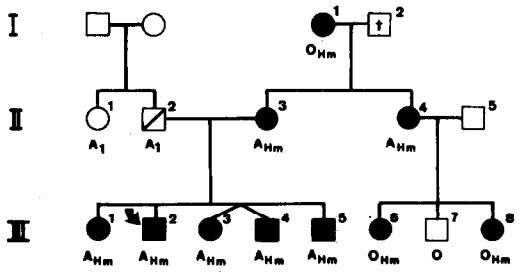

Fig.1b

Fig.1. Genealogy of typical Hz and Hm families. Fig.1a. Pedigree of Dup. family. Fig.1b. Pedigree of Let. family. Symbols: (●, ■) Hz or Hm variants; (▣) atypical H variant.

Table 1
ABH glycosyltransferases levels in sera and RBC ghosts from Hz variants

Sample	Genotype	In serum		In RBC ghosts			
		A_1 (pH 6.0)	A_2 (pH 7.0)	B	H	A	B	H
Dup. n	O^{o}_{Hz}				0			0
m	O^{o}_{Hz}				0			0
e	O^{o}_{Hz}				0			0
Trn. h	O^{o}_{Hz}				0			
a	O^{B}_{Hz}			12 900	0		35 900	0
m	O^{B}_{Hz}			13 400	0			
jc	O^{B}_{Hz}			23 000	0			
jb	O^{B}_{Hz}			13 600	0			
Thi. a	O^{o}_{Hz}				0			0
Bon.	O^{A1}_{Hz}	28 900	20 800		0	51 300[a]		0
Controls	O				7040 (14)			4310 (4)
	A_1	28 000 (16)			9380 (16)	21 260 (6)		5170 (3)
	A_2		16 420 (17)		8490 (17)	2655 (5)		6020 (3)
	A_1B	25 370 (14)		11 480 (14)	9100 (17)	52 930 (2)	31 720 (2)	4350 (2)
	A_2B		12 750 (16)	8250 (16)	6445 (16)			
	A_1A_2	29 200 (9)	24 550 (9)		11 390 (10)			8400 (1)
	B_I			5820 (10)	6310 (10)		51 530 (6)	8000 (7)
	B_{II}			12 300 (10)	4880 (10)			

[a] This activity falls in the normal range of controls. Indeed, only mean values have been reported for control samples in which large individual variations of A and B enzymes in RBC have been noticed

Serum enzyme activities are expressed as cpm of radioactive sugar donor incorporated by serum in standard assay conditions [12,20]. In RBC ghosts the enzyme levels are calculated in cpm/mg protein. Zero values mean no detectable enzyme activity

that Hz bloods II-1, II-4 and II-6 from the Dup. family (fig.1a) were grouped Le(a-b+) on their erythrocytes but gave negative reactions with anti-H reagents. This observation is a further demonstration that Lewis substances are not synthesized by RBC but rather passively acquired from plasma [23,24]. The Lewis antigens taken up by Hz RBC are glycosphingolipids of unknown origin where obviously the expression of H is not suppressed. Then, if Hz phenotypes occur by action of the hypothetical *Zz* system, these individuals have at least one copy of *Se* and *H* genes and a double dose of *z* gene.

The behavior of the *z* gene does not appear as a general switch of H glycolipid production, but rather as a more specific regulator of H glycolipid substance of RBC. It is suggested that if most of the serum enzyme originates from red cells, the genetic block caused by *zz* genes in Hz individuals fully explains the absence of α-2-L-fucosyltransferase, both in their sera and RBC membranes. However, the identification

of A_{Hz} variants [14] also provides evidence that the *H* gene of these individuals is not completely silent. Finally, as mentioned above, the terminology Hz seems more appropriate for such H variants.

3.2. *Second type of H variants (Hm phenotypes)*

The Dup. family:

In the Jur. family investigated at the present time, the trait is inherited accross 3 generations but, on the contrary, of the recessive Hz variants studied above, the H antigen of Jur.'s RBC is only partly reduced when tested with anti-H reagents from *Ulex europaeus* and *Laburnum alpinum* [17,18]. When the sera from 2 A_{Hm} and 4 A_{Hm} B individuals were examined, a strong α-2-L-fucosyltransferase activity was found (table 2). Moreover, the sera from A_{Hm} B people had a normal B transferase level and in all cases A transferase from A_{Hm} and A_{Hm} B had an optimum activity at about pH 7.0–7.5, which is characteristic of A_2 enzyme [25]. The RBC membranes of Jur. samples have not been tested, because only minute amounts of erythrocytes were available. However, from the results presented below it is very likely that A, B and H transferases are present in these red cells.

It is impossible from our data to speculate if the slightly reduced content of H antigen in Jur.'s RBC is due to a 'dominant' modifier or more simply to some allele at the *Hh* locus. According to [18] the H defect caused by a regulatory mechanism, is only effective in '*cis* position' with respect of A gene. Indeed, the A antigen from A_{Hm} and A_{Hm} B is also slightly reduced while a normal B antigen strength has been demonstrated in the latter. Alternatively, we suggest that a competition between A and B transferase for the precursor, may also explain these serological data.

The Let. family:

A second family (Let.) where the character is inherited in 3 generations is shown in fig.1b. Erythrocytes from the father II-2 are also H defective, but he was found to be a non-secretor. Nevertheless, no history of consanguinity could be demonstrated between II-2 and II-3. All children from this mating have H deficient RBC, some being secretors (III-4, III-5) and others non-secretors (III-1, III-2, III-3). Among the 3 children from II-4, 2 are of the Hm type (III-6 and III-8) and 1 is a normal group 0 (III-7).

The H red cell antigenic strength from members of Let. family is shown in table 2. The defect of H was more pronounced among A than among 0 individuals, and it was noticed that most H variants of group A had a decreased expression of A_1 antigen. Such individuals were hardly discernable from A_2 people.

Investigations of A and H transferases in sera and RBC from members of the Let. family (table 2) can be summarized as follow:

(i) The H serum enzyme activities fall in normal range as compared to common ABO blood samples.

(ii) Despite a significant drop of their H antigen, the H transferase of RBC membrane from H variants was as active as control RBC.

Such a finding was rather surprising and raised the question of a possible deficiency in type 2 chain glycolipidic substrate from which the H substance is built by enzymatic transfer of L-fucose. In order to verify this assumption, the UDPGal: GlNAc-β-4-D-galactosyltransferase activities of RBC membrane from the H variants (II-2, -3, -4; III-1, -2, -3, -5, -6, -8) and 3 normal individuals were quantified as described in section 2. No difference has been noticed between the variants and controls. Indeed the amount of D-[^{14}C]galactose transfered onto the substrate was respectively equal to 44 000 ± 4000 cpm/mg protein (control RBC) and 47 000 ± 8000 cpm/mg protein (H variants).

(iii) The A transferase of all sera is maximally active at pH 6.0 which is a property of A_1 enzymes [25]. In these variants, the A_1 antigens are not completely developed because only a limited amount of H precursor is available.

The cause of the H antigen decrease in RBC from Let. family is not clear since transferases are active as measured on low molecular weight acceptors. It was shown that H substance reached a normal level in saliva as estimated by usual 2-fold dilutions assays. Obviously, the method does not give a precise quantitative estimation and it cannot be excluded that H

Table 2
Characteristics of Hm variant bloods

Sample	Probable blood group variant	ABH in[a] saliva	Antigens on RBC[b]				Glycosyltransferases[c]					
			Lectin anti-H		Human anti-A	*Dolichos biflorus*	In serum				In RBC ghosts	
			1/8	1/1			A_1 (pH 6.0)	A_2 (pH 7.0)	B	H	A	H
Jur. family:												
O.Vy	$A_{Hm}B$						5380	10 450	11 800	12 300		
F.Bi	$A_{Hm}B$						5670	11 300	13 900	13 900		
B.Ju	$A_{Hm}B$		weak		reduced		4400	10 300	12 250	13 640		
B.Na	A_{Hm}						5680	11 450	0	16 260		
F.Ju	A_{Hm}	A,H	reduced				3950	9730	0	15 640		
M.Na	$A_{Hm}B$		reduced		reduced		5100	14 190	14 310	13 350		
Let. family:												
I-1	O		49	98						16 750		
II-1	A_1			11								4120
II-2	A_1	ns	0	12	93	90	30 580	19 230		14 470	14 750	11 420
II-3	A_{Hm}	A,H	0	5	94	10	22 130	14 270		10 110	19 180	4370
II-4	A_{Hm}	A,H	0	5	90	18	22 480	13 710		27 720		
III-1	A_{Hm}?	ns	0	32	93	91						
III-2	A_{Hm}?	ns	0	0	98	10	24 530	13 245		22 310	49 030	4760
III-3	A_{Hm}?	ns	0	0	93	10	24 710	14 840		28 300	30 880	6060
III-4	A_{Hm}	A,H	0	0		32						
III-5	A_{Hm}	A,H	0	5	93	16					88 200	4450
III-6	O_{Hm}		62	95						12 200		
III-7	O		92	100						9330		
III-8	O_{Hm}		50	93						14 570		

[a] ns = non-secretor of ABH substances

[b] In Jur. family, the results on RBC antigens have been compiled from [15–17]. With Let. samples the lectin anti-H used was *Ulex europaeus*

[c] Enzyme activities as defined in table 1

Controls of agglutination percentage were as follow: (i) human anti-A: A_1 (95 ± 3); A_2 (90 ± 3). (ii) *Dolichos biflorus:* A_1 (95 ± 3); A_2 (0–10). (iii) *Ulex europaeus* diluted 1/8: O (87 ± 8); A_2 (67 ± 9); A_1 (20 ± 7); undiluted O (100 ± 5), A_1 (50)

secretion is also affected to some extent. In these conditions, the most likely genetical background of such phenotypes is the occurence of a rare allele at the *Hh* locus, conveniently named Hm [26,27] in order to make the difference with the Hz phenotypes having a lack of α-2-L-fucosyltransferase.

However, the sharp contrast between the normal level of H enzyme in Hm RBC and their reduced H antigen content does not fit with such a concept and it is suggested now, that these variants could be explained by a genetic variation not related to the *Hh* locus. Then, either the defect is created in early steps of the glycosylation process of carbohydrate chains or the H transferase is deprived of substrate by a membrane enzyme having an high affinity for the 2-chain glycolipids.

These possibilities are under study but if true they may signify that the terminology Hm is a misnomer.

The apparent similarity between Am and Hm phenotypes established from serological studies [14] is not confirmed by blood group enzyme investigations. Indeed, the A transferase of Am sera is only 30–50% as active as controls [19] but is completely absent or inactive in RBC [20] which indicates that Am phenotypes arise either from a modifier or a rare allele at the *ABO* locus.

The results obtained bring some information on the origin of serum α-2-L-fucosyltransferase. Indeed, when the H antigen is missing on RBC, no H enzyme appears in plasma even if H substance is normally secreted in saliva (Hz individuals). Conversely, when the H enzyme is expressed on RBC, the H transferase is always found in plasma. We have concluded that serum α-2-L-fucosyltransferase mainly originates from hematopoietic tissue.

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