THE ENZYMIC PRODUCTS OF THE HUMAN *A* AND *B* BLOOD GROUP GENES IN THE SERUM OF "BOMBAY" O_h DONORS

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Received 28 July 1972

1. Introduction

The rare "Bombay" O_h blood group phenotype is characterised by the absence of A, B or H substances from the erythrocytes or secretions and the presence of anti-A, B and H agglutinins in the plasma [1]. Individuals belonging to this group were predicted to be of the genotype *hh* [2, 3] and thus to lack the H-active structures that are formed by the enzymic product of the *H*-gene, the common allele at this genetic locus. H-active structures are the acceptor substrate for the *A*-gene specified *α-N*-acetylgalactosaminyl-transferase and the *B*-gene specified *α-D*-galactosyl-transferase [4–7]; the lack of A- and B-specific structures in the "Bombay" O_h individuals can thus be explained by the absence of the *H*-gene product and it is unnecessary to assume a defect in, or failure of expression of, the enzymic products of the *A* and *B* genes. In normal blood group A and B individuals the transferases associated with the *A* and *B* genes have recently been demonstrated in serum or plasma [8–11]. Therefore, to test the hypothesis that these genes are expressed normally at the enzymic level in "Bombay" O_h individuals, serum or plasma from nine donors of this phenotype were examined for *α-N*-acetylgalactosaminyl- and *α-D*-galactosyl-transferases. Whenever possible, the findings on the sera were correlated with family data which enabled the probable ABO genotype of the O_h donors to be deduced. The results demonstrated that the enzymes are readily detectable in the sera from the "Bombay" O_h donors and that the assay of these serum transferases provides a method for establishing whether *A* and *B* genes are carried by donors of this phenotype.

2. Materials and methods

Unlabelled uridine diphosphate D-galactose (UDP-galactose) was purchased from Sigma London Chemical Company Ltd. and UDP-[^{14}C] galactose (240 mCi/mmole) from the Radiochemical Centre, Amersham, England. Unlabelled uridine diphosphate *N*-acetyl-D-galactosamine (UDP-*N*-acetylgalactosamine) was prepared by the method of Carlson et al. [12] and UDP-*N*-acetyl-[^{14}C] galactosamine (43 mCi/mmole) was purchased from the New England Nuclear Corporation, Frankfurt, Germany. 2′-Fucosyllactose (*O*-α-L-fucosyl-(1→2)-*O*-β-D-galactosyl (1→4)-D-glucose) was supplied by Dr. A. Gauhe.

Paper chromatography was carried out in ethyl acetate–pyridine–water (2:1:2 by vol, upper phase; solvent *a*) on Whatman No 40 paper. Radioactive peaks were detected with a 7201 Packard Radiochromatogram Scanner and counted in a Nuclear Chicago Scintillation counter, Series 720.

Serum samples from the "Bombay" O_h donors S.A., GOV. and SOO. (see table 1) were provided by Miss Phyllis Moores of the Natal Blood Transfusion Service, South Africa, who also supplied the following details: Both parents of S.A. were group O and therefore her genotype is *OO*. GOV. and SOO. are brothers whose parents were group O and A_1B. The brothers both have group B wives; GOV. has one group B child and SOO. has one group O child and five group B children. Serum from the donor V.D'A. was supplied by Dr. R. Sacchi, Centro Transfusionale Bologna, Italy. The family pedigree of this donor [13] demonstrates that V. D'A's genotype must be A_2O or *OO*. Serum samples from three donors, P.D., M.S. and SUB. were provided by Dr. H.M. Bhatia, Blood Group Reference Centre,

Table 1
The α-*N*-acetyl-D-galactosaminyl- and α-D-galactosyltransferase activities in sera from nine "Bombay" O_h donors

Serum donor	ABO group Phenotype	Genotype*	Radioactivity incorporated into 2′-fucosyllactose as: α[^{14}C]Gal cpm	% of total	α[^{14}C]GalNAc cpm	% of total	Predicted genotype
S.A.	O_h	*OO*	0	0	0	0	
G.F.	O_h	Not known	0	0	0	0	*OO*
V.D'A.	O_h	*OO* or A_2O	0	0	13,184	6.5	A_2O
P.D.	O_h	Not known	20,848	10	21,530	11	*AB*
M.S.	O_h	Not known	19,124	9.5	0	0	*BO*
SUB	O_h	*AO, BO, AB* or *OO*	30,658	15	32,214	16	*AB*
SCH	O_h	*OO*	0	0	0	0	
GOV	O_h	A_1O or *BO*	0	0	17,229	8.6	A_1O
SOO	O_h	A_1O or *BO*	16,247	8.1	0	0	*BO*
F.J.	A_1B	A_1B	18,510	7.7	15,954	7.3	
W.M.	O	*OO*	0	0	0	0	

* Deduced from family studies (see Materials and methods).
Abbreviations: Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine.
Reaction mixture: 0.6–1.0 nmole UDP-[^{14}C]galactose (200,000–240,000 cpm) or 3.3 nmole UDP-*N*-acetyl-[^{14}C]galactosamine 200,000 cpm); 2.0 μmole $MnCl_2$ (except for mixture with plasma from donor SCH. which contained 4.0 μmole $MnCl_2$); 1.0 μmole ATP; 0.5 μmole 2′-fucosyllactose; 7.0 μmole sodium cacodylate buffer, pH 6.5; 20 μl serum or plasma. Total volume of the reaction mixture 200 μl. Incubated at 37° for 18 hr.

Bombay, India. No family data was available on donor P.D.. Donor M.S. had a group B parent, one group B sib and two group O children [14]. Donor SUB's parents were group A and B and data on his sibs indicate that the parents' genotypes must be *AO* and *BO* [14]. Citrated plasma from donor SCH. was sent by Dr. Ch. Salmon, Centre Departemental de Transfusion Sanguine, Paris, who gave the donor's genotype as *OO*. No family data was available on donor G.F. whose serum was provided by Dr. B.P.L. Moore of the Canadian Red Cross Blood Transfusion Service, Toronto, Canada. Whole blood, serum or plasma was received from all these donors within two to three days after withdrawal of the blood and the samples were transported under refrigeration. Separated serum or plasma that was not used on the day of arrival was stored at −18° and then used immediately after the sample had thawed out.

The transferases in the sera were assayed by two methods. The first was the transfer of *N*-acetyl-[^{14}C] galactosamine or [^{14}C] D-galactose from their respective uridine derivatives to the low molecular weight acceptor, 2′-fucosyllactose. The reaction conditions are given in table 1. At the end of the incubation period the neutral sugars were separated from the charged compounds as described previously [15] and chromatographed in solvent *a*. The anomeric linkage of the transferred *N*-acetylgalactosamine was determined by treatment with an α-*N*-acetylgalactosaminidase from *Lumbricus terrestris* [16] that was free from β-activity. The linkage of the transferred galactose was determined by treatment with purified α and β-galactosidases from *Trichomonas foetus* [17, 18].

The second method for the detection of the transferases was conversion of normal group O cells to A- and/or B-active cells by the transfer of sugars from the unlabelled nucleotide derivatives [19,11]. The sera from the "Bombay" O_h donors were absorbed with packed washed, group O, A, and B red cells to remove the anti-H, -A and -B agglutinins. The reaction

Table 2
Conversion of human group O cells to group A and B reactive cells by the transferases in sera from "Bombay" O_h donors.

Serum donor	ABO group		Haemagglutination end-point after treatment of O cells with serum and:				Reactivity of converted cells
			UDP-Gal		UDP-GalNAc		
	Phenotype	Genotype	Anti-A	Anti-B	Anti-A	Anti-B	
S.A.	O_h	*OO*	<1:2	<1:2	<1:2	<1:2	
G.F.	O_h	Not known	<1:2	<1:2	<1:2	<1:2	
V.D'A.	O_h	*OO* or A_2O	<1:2	<1:2	1:4	<1:2	Weak A
P.D.	O_h	Not known	<1:2	1:256	1:128	<1:2	AB
M.S.	O_h	Not known	<1:2	1:128	<1:2	<1:2	B
SUB	O_h	*AO,BO,AB* or *OO*	<1:2	1:256	1:256	<1:2	AB
SCH.	O_h	*OO*	<1:2	<1:2	<1:2	<1:2	
GOV.	O_h	A_1O or *BO*	<1:2	<1:2	1:256	<1:2	A
SOO	O_h	A_1O or *BO*	<1:2	1:128	<1:2	<1:2	B
E.S.	B	*BB* or *BO*	<1:2	1:256	<1:2	<1:2	B
W.W.	A_1	Not known	<1:2	<1:2	1:128	<1:2	A
W.M.	O	*OO*	<1:2	<1:2	<1:2	<1:2	

Reaction mixture: 100 μg UDP-galactose or UDP-*N*-acetylgalactosamine; 1 μmole $MnCl_2$; 0.5 μmole ATP; 5 μl packed group O erythrocytes; absorbed serum 50 μl. Total volume of the reaction mixture 70 μl. Incubated for 6 hrs. at 37°.

mixture used for the cell conversion experiments is given in table 2. At the end of the incubation period the red cells were collected by centrifuging at 2,000 rpm and washed twice with several volumes of 0.9% NaCl. A 1% suspension of the washed cells was tested for haemagglutination as described previously [20]. Human anti-A and anti-B sera were used as the test reagents and the end-point of agglutination was taken as the last dilution of the serum at which clumps of two or three cells were visible under the low power of the microscope.

3. Results

Nine serum samples from "Bombay" O_h donors were each tested for α-*N*-acetylgalactosaminyl and α-galactosyl-transferase activity with 2′-fucosyllactose as acceptor (table 1). The sera from a normal A_1B donor and a group O donor were included as controls. The A_1B serum and four of the "Bombay" O_h sera (V.D'A., P.D., SUB. and GOV.) transferred *N*-acetyl-[^{14}C]galactosamine to the trisaccharide to give a compound ($R_{lactose}$, 0.5 in solvent *a*) which corresponded in its chromatographic properties to the tetrasaccharide previously synthesized with 2′-fucosyllactose as acceptor when group A tissues were used as the enzyme source [5]. The labelled *N*-acetylgalactosamine was released by the α-*N*-acetylgalactosaminidase preparation; thus confirming that the sugar was transferred in α-linkage.

When UDP-[^{14}C]galactose was used as the sugar donor, the A_1B serum, and four of the "Bombay" O_h sera (P.D., M.S., SUB. and SOO.) had α-galactosyltransferase activity. The product ($R_{lactose}$, 0.4 in solvent *a*) co-chromatographed with the tetrasaccharide identified as *O*-α-D-galactosyl-(1→3)-[*O*-α-L-fucosyl-(1→2)-] *O*-β-D-galactosyl-(1→4)-D-glucose [21]. The radioactive galactose was cleaved from the tetrasaccharide by α-galactosidase and was not susceptible to the corresponding β-enzyme. Two of the "Bombay" O_h donors, known to be of the genotype *OO* (S.A. and SCH.), had neither α-*N*-acetylgalactosaminyl nor α-galactosyltransferase activity; serum from one O_h

Table 3
The β-galactosyltransferase activity in sera from "Bombay" O_h donors

Serum Donor	ABO phenotype	β[^{14}C]Gal incorporated into *N*-acetylglucosamine	
		cpm	%
S.A.	O_h	155,422	76
G.F.	O_h	130,350	65
V.D'A.	O_h	147,436	74
P.D.	O_h	169,662	85
M.S.	O_h	148,782	71
SUB.	O_h	142,748	71
SCH.	O_h	211,068	100
GOV.	O_h	105,000	45
SOO	O_h	114,300	49
E.S.	B	156,346	78

Reaction mixture: The same as in table 1 with UDP-[^{14}C]galactose as the sugar donor and with *N*-acetylglucosamine (0.5 μmole) as the sugar acceptor in place of 2'-fucosyllactose.

donor of unknown genotype was also devoid of these enzyme activities. The amount of radioactive sugar incorporated into 2'-fucosyllactose under the conditions given in table 1 was not the maximum obtainable; increasing the time of incubation or the amount of serum in the reaction mixture resulted in greater incorporation. However, no α-*N*-acetylgalactosaminyl or α-galactosyltransferase activity was detectable in the samples S.A., G.F. and SCH. even after prolonged incubation with 50 μl of serum or plasma. In the "Bombay" O_h sera that had α-*N*-acetylgalactosaminyl and/or α-D-galactosyl transferases, the activities were retained largely unimpaired after storage of the sera for several months at −18°.

Absorption of the "Bombay" O_h sera with A, B and O erythrocytes to remove the agglutinins did not decrease the enzyme activities as measured by the transfer of labelled sugars to 2'-fucosyllactose. The absorbed sera were therefore tested for their capacity to convert group O cells into A or B active cells in the presence of unlabelled UDP-*N*-acetylgalactosamine or UDP-galactose [19, 11]. Sera from the normal group A_1B and O donors were included as controls. Four of the "Bombay" O_h sera (V.D'A., P.D., SUB. and GOV.) converted the O cells into A active cells when UDP-*N*-acetylgalactosamine was included in the incubation mixture, although in one instance, (serum V.D'A.) the treated cells were only weakly agglutinated by anti-A serum (table 2). More prolonged incubation of the group O cells with this particular serum sample did not increase the haemagglutination titre of cells with anti-A serum. With the other three O_h sera the degree of conversion to A was comparable with that given by the serum from a group A_1 donor. Similarly, the four O_h sera that transferred D-galactose to 2'-fucosyllactose (P.D., M.S., SUB. and SOO.) converted O cells to B-reactive cells when UDP-galactose was a component of the reaction mixture (table 2). The group O cells did not develop agglutinability with either anti-A or anti-B sera when incubated with the nucleotide sugars and the absorbed sera or plasma from the O_h donors S.A., G.F. or SCH.

Although α-galactosyltransferase activity was demonstrable in only certain of the "Bombay" O_h samples, incubation of the sera with UDP-[^{14}C] galactose and the monosaccharide *N*-acetylglucosamine revealed strong β-galactosyltransferase activity in all the sera (table 3). A labelled disaccharide was formed that co-chromatographed with *N*-acetyllactosamine (*O*-β-D-galactosyl-(1→4)-*N*-acetylglucosamine) and was completely hydrolysed by β-galactosidase.

4. Discussion

The transfer of *N*-acetylgalactosamine from UDP-*N*-acetylgalactosamine and of D-galactose from UDP-galactose, in α-linkage to the H-active trisaccharide, 2'-fucosyllactose, by transferases in certain "Bombay" O_h serum samples, demonstrates that enzymes with the specificity expected for the products of the *A* and *B* genes may, as predicted, be present in the sera of individuals of the "Bombay" O_h phenotype (table 1). Confirmatory evidence for the specificity of the transferases was obtained by the conversion of group O erythrocytes into A- or B-active cells by the corresponding sera in the presence of added nucleotide sugars (table 2).

The ABO genotypes of only two of the nine "Bombay" O_h donors were known with certainty (S.A. and SCH.) and these were both homozygous *OO*. In agree-

ment with this genotype no α-*N*-acetylgalactosaminyl or α-D-galactosyltransferases were detectable in the serum from these donors, although both had strong β-galactosyltransferase activity (table 3). The serum from one other donor (G.F.), for whom no family data was available, behaved similarly in that only β-galactosyltransferase activity was demonstrable; it can therefore be predicted that the genotype of G.F. is *OO*.

Family studies on the relatives of the two brothers GOV. and SOO. indicate that their genotypes must be either A_1O or *BO*. The finding of an α-*N*-acetylgalactosaminyltransferase in the serum GOV., with no trace of the *B*-gene specified enzyme, and of an α-galactosyltransferase in the serum SOO., in the absence of the *A*-gene specified enzyme, demonstrates that GOV. carries an *A* gene and is therefore of the genotype A_1O, and that SOO. carries a *B* gene and is of the genotype *BO*. The donor V.D'A., whose genotype is known to be either *OO* or A_2O [13], had an α-*N*-acetylgalactosaminyltransferase and hence carries an *A* gene. With this serum the incorporation of *N*-acetyl-[^{14}C]galactosamine into the low molecular acceptor was more striking than the results obtained in the cell conversion experiments in which only weak A activity developed. The reason for this one discrepancy is not known although it may be attributable to the fact that the gene carried by this donor is A_2 and not A_1.

The ABO blood groups of donor SUB.'s family permit one to deduce only that his genotype is either *AO*, *BO*, *AB* or *OO* [14]. The demonstration of a strong α-*N*-acetylgalactosaminyl- and α-galactosyltransferase clearly indicates, however, that he is carrying both the *A* and *B* genes. Similarly, donor P.D., for whom no family data was available, had both the *A*- and *B*-gene specified transferases, and is thus of the genotype *AB*. Donor M.S. has a normal group O child and hence must be carrying an *O* gene [14]. The blood groups of other members of the family indicate that he may be carrying a *B* gene but there is insufficient information for an unequivocal decision to be reached on this point. The presence of an α-galactosyltransferase in his serum confirms, however, that his genotype is *BO*.

In none of the nine "Bombay" O_h samples examined were the α-*N*-acetylgalactosaminyl- or α-galactosyltransferases found in the serum at variance with those to be expected from the known family data. Provided that reasonably fresh serum samples are used, tests for the *A*- and *B*-gene specified transferases should thus be a dependable method for ascertaining the true ABO group of donors of the "Bombay" O_h phenotype.

Acknowledgements

We thank Miss Phyllis Moores, Dr. H.M. Bhatia, Dr. R. Sacchi, Dr. B.P.L. Moore and Dr. Ch. Salmon for the samples of "Bombay" O_h bloods, Dr. A. Gauhe for the sample of 2'-fucosyllactose, Mr. A. Yates for the α- and β-galactosidase preparations and Miss Lynne Roberts for the α-*N*-acetylgalactosaminidase preparation. We are grateful to Mr. Michael Topping for the preparation of unlabelled UDP-*N*-acetylgalactosamine and to Miss Susan Beck for technical assistance. This work was supported by a grant from the Medical Research Council.

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