Fucosyltransferase activities in human lymphocytes and granulocytes

Blood group *H*-gene-specified α-2-L-fucosyltransferase is a discriminatory marker of peripheral blood lymphocytes

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Human lymphocytes and granulocytes were examined for blood group *H*-gene-specified α-2-L-fucosyltransferase, α-3-L-fucosyltransferase and blood group *Le*-gene-specified α-4-L-fucosyltransferase. No α-4-L-fucosyltransferase was detectable in either cell type. α-3-L-Fucosyltransferase was readily demonstrable in both lymphocytes and granulocytes and the levels of activity per 10^6 cells were approximately the same. In contrast, α-2-L-fucosyltransferase was detectable in lymphocytes but not in similar numbers of granulocytes. The absence of α-2-L-fucosyltransferase was confirmed in granulocytes isolated from the blood of eighty unselected donors and was independent of ABO blood group or ABH secretor status.

Human blood *Lymphocyte* *Granulocyte* *α-L-fucosyltransferase* *Blood group* H *gene* *Lymphocyte marker*

1. INTRODUCTION

The human α-2-L-fucosyltransferase that catalyses the transfer of L-fucose from GDP-L-fucose to Type 1 (Galβ(1 → 3)GlcNAc) or Type 2 (Galβ(1 → 4)GlcNAc) chains to form blood group H-active structures (Fucα(1 → 2)Galβ(1 → 3 or 4)GlcNAc) has variable expression in different tissues [1]. In blood the enzyme has been found in plasma [2], red cells, lymphocytes and platelets [3]. An α-3-L-fucosyltransferase that acts on Type 2 chains to form the structure Galβ(1 → 4)[Fucα(1 → 3)]GlcNAc occurs in the same components of blood [2,3] but the *Le* gene associated α-4-L-fucosyltransferase, which catalyses the transfer of L-fucose to the 4-*O*-position of *N*-acetylglucosamine in Type 1 chains [1], has not been found in any of these sources. Granulocytes have not previously been examined for fucosyltransferase activities. This paper records the observation that these cells express α-3-L-fucosyltransferase as strongly as do lymphocytes but fail to exhibit detectable levels of *H* gene-specified α-2-L-fucosyltransferase or *Le* gene-specified α-4-L-fucosyltransferase.

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2. MATERIALS AND METHODS

GDP-L-[^{14}C]fucose (292 Ci/mol) was purchased from Amersham (UK), Ficoll-Paque and Percoll from Pharmacia (UK) and Dextran 150 from Fisons (UK). Phenyl β-D-galactoside was obtained from Koch-Light (UK). *N*-Acetyllactosamine glycosidically linked to a spacer arm, Galβ(1 → 4)GlcNAc-*O*-$(CH_2)_8CO_2CH_3$(LNAc-R), was generously supplied by Professor R.U. Lemieux, University of Edmonton, Canada, and synthetic lacto-*N*-biose I

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2.1. *Cell separations*

Separation of red cells, lymphocytes and granulocytes from freshly drawn, heparinised, blood was achieved either by a two-stage procedure on Ficoll-Paque and Dextran 150 [4] or by a single-step method on a discontinuous gradient of Percoll [5]. The separated cells were thoroughly washed with 0.15 M NaCl and finally resuspended in this solution. Aliquots were removed for (a) cell counts in an improved Neubauer chamber and (b) identification of the cell types in smears stained with Leishmann's stain.

2.2. *Fucosyltransferase assays*

GDP-L-[^{14}C]fucose was used as the donor substrate and phenyl β-D-galactoside, LNAc-R, and lacto-*N*-biose I were the acceptor substrates for α-2-L-fucosyltransferase [6], α-3-L-fucosyltransferase [7] and α-4-L-fucosyltransferase [7], respectively. The incubation mixtures are given in the tables. At the end of the incubation period the reaction mixtures were subjected to paper chromatography as in [6,7] and the radioactive products were counted in a scintillation counter. The positional linkages of the transferred [^{14}C]fucose were established by means of linkage specific α-2-L-fucosidase and α-3/4-L-fucosidase isolated from *Trichomonas foetus* [8].

RESULTS

3.1. *Comparison of α-2-, α-3- and α-4-L-fucosyltransferase activities in lymphocytes and granulocytes isolated from the blood of an ABH secretor and a non-secretor individual*

Sixty ml of blood from a group A_1 secretor individual and a group O non-secretor individual [9] were each fractionated into red cells, lymphocytes and granulocytes by the Ficoll-Paque/Dextran procedure [4] and the separated white cells were tested for fucosyltransferase activity. With lacto-*N*-biose I as acceptor no product with the chromatographic mobility of Galβ(1 → 3)[Fucα(1 → 4)]GlcNAc was detectable in the reaction products formed with either of the cell preparations; indicating that α-4-L-fucosyltransferase is absent or present in amounts too low to be detectable. The lymphocyte

Table 1

Distribution of α-2- and α-3-L-fucosyltransferase activities in lymphocytes and granulocytes separated from the blood of two unrelated individuals

ABO blood group	Secretor status	Cells	No. of cells tested	Incorporation of [^{14}C]fucose by			
				2-Fucosyltransferase		3-Fucosyltransferase	
				2-linked product (cpm)	cpm/10^6 cells	3-linked product (cpm)	cpm/10^6 cells
0	Non-secretor	Lymphocytes	5.9×10^6	21 800	3700	108 000	18 300
		Granulocytes	5.5×10^6	0	0	123 000	20 400
A_1	Secretor	Lymphocytes	4.5×10^6	15 400	3400	84 000	18 700
		Granulocytes	4.8×10^6	0	0	55 700	11 600

The reaction mixtures for α-2-L-fucosyltransferase contained in a total volume of 160 μl; GDP-[^{14}C]fucose, 840 pmol (280 000 cpm); phenyl β-D-galactoside, 2 μmol; ATP, 0.5 μmol; Tris–HCl buffer (pH 7.2), 5 μmol; NaN_3, 0.8 μmol; Triton X-100, 500 μg; 100 μl of lymphocyte or granulocyte cell suspension (about 5×10^6 cells). The reaction mixtures for α-3-L-fucosyltransferase were similar except that 2 μmol $MnCl_2$ were added, LNAc-R (100 μg) was used as acceptor substrate in place of phenyl β-D-galactoside, Tris–HCl buffer (pH 8.0) replaced the pH 7.2 buffer and 100 μg bis(*p*-nitrophenyl) phosphate was added to inhibit esterase action on the LNAc-R substrate. The mixtures were incubated for 64 h at 37°C and the products of α-2- and α-3-L-fucosyltransfer were separated and characterised by means of their chromatographic mobilities and susceptibility to linkage-specific α-L-fucosidases as in [6,7]

Table 2

Range of α-2- and α-3-L-fucosyltransferase activities in the lymphocytes and granulocytes prepared from 80 unselected blood samples

Enzyme source	ABO group	No. of samples	Incorporation of [^{14}C]fucose (cpm/10^6 cells) by			
			2-Fucosyltransferase		3-Fucosyltransferase	
			Range	Mean	Range	Mean
Lymphocytes	O	34	395–3138	1600	1135–16925	8800
	B	8	494–1919	1300	3472–22246	10000
	A	28	363–4805	2000	591–15847	6800
	AB	10	371–8067	2500	4322–13515	8500
Granulocytes	O	34	–	0	1087–19787	7300
	B	8	–	0	1595–11623	6500
	A	28	–	0	1100–20663	7100
	AB	10	–	0	1132–16173	4400

The reaction mixtures were the same as in table 1 except that about 1×10^6 cells and 210 pmol (70000 cpm) GDP-[^{14}C]fucose were used for each incubation mixture and the total volume was 120 μl. The mixtures were incubated for 16 h at 37°C

and granulocyte preparations from both donors showed strong α-3-L-fucosyltransferase activity but whereas α-2-L-fucosyltransferase was readily detectable in the lymphocyte preparations none was found in the granulocytes isolated from the blood of either donor (table 1).

In case the failure to detect α-2-L-fucosyltransferase in granulocytes arose from enzyme inhibition caused by the materials used for the cell separations a second set of experiments was carried out on blood from the same two donors fractionated on a discontinuous Percoll gradient [5]. The results were essentially similar in that α-3-L-fucosyltransferase was detectable in both lymphocyte and granulocyte preparations, whereas α-2-L-fucosyltransferase activity was found only in the lymphocyte fractions.

3.2. *Lymphocytes and granulocytes from eighty unselected blood samples tested for expression of α-2- and α-3-L-fucosyltransferase activity*

The absence of the α-2-L-fucosyltransferase from the granulocytes isolated from both a blood group O non-secretor and a group A secretor individual indicated that this failure of expression was not related to ABO blood group or secretor status. In order to determine whether the finding was a general one white cells isolated from blood samples from 80 unselected donors presenting at the North London Blood Transfusion Centre were examined. The blood (5 ml) was fractionated by the Ficoll-Paque/Dextran procedure [4]. α-2-L-Fucosyltransferase activity was found in all the lymphocyte preparations but not in any of the granulocyte fractions whereas both cell types from all the donors had α-3-L-fucosyltransferase activity (table 2).

4. DISCUSSION

The absence of α-4-L-fucosyltransferase activity in granulocytes accords with earlier observations [2,3] suggesting that the *Le* gene is not expressed in haemopoietic tissue. The absence of α-2-L-fucosyltransferase, however, distinguishes the granulocyte preparations from the lymphocytes. The lack of this transferase in 80 unselected individuals, of whom only about 25% would be ABH non-secretors [9], indicated that in granulocytes the expression of the *H* gene-specified enzyme is not under the control of the secretor gene *Se*. Granulocytes have a greater abundance than lymphocytes of peroxidase and alkaline phosphatase [10] and these enzymes are valuable markers for distinguishing granulocytic cells. The α-2-L-fucosyltransferase may similarly

prove useful as a marker of peripheral blood lymphocytes. Further work is required to determine whether the transferase is present in lymphocytic precursor cells and in all lymphocyte subclasses.

Recent evidence [11,12] has shown that certain hybridoma-derived monoclonal antibodies that distinguish human granulocytes from other cells of peripheral blood are specific for the carbohydrate structure Galβ(1 → 4)[Fucα(1 → 3)]GlcNAc. The appearance on the granulocyte cell surface of this antigenic determinant correlates with the presence of the α-3-L-fucosyltransferase in these cells and with the absence of the α-2-L-fucosyltransferase since these two enzymes functioning in concert would be expected to give the difucosyl structure Fuc(1 → 2)Galβ(1 → 4)[Fucα(1 → 3)]GlcNAc in which the antigenic specificity of the monofucosyl determinant would be masked by the presence of the terminal non-reducing α-2-linked fucosyl residue.

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