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Fucosyltransferase Expression in Human Platelets and Leucocytes

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The activities of α-2-L-fucosyltransferase and α-3-L-fucosyltransferase were measured in human platelets and leucocytes from normal donors. α-2-L-Fucosyltransferase was found in platelets but not in leucocytes. In contrast α-3-L-fucosyltransferase was not detected in platelets but was present in leucocytes where it was demonstrated in the neutrophil, monocyte and lymphocyte fractions.

Haemopoietic tissue contains several fucosyltransferases which catalyse the transfer of L-fucose from GDP-L-fucose to oligosaccharide structures on glycoproteins and glycolipids. Two enzymers are involved in the synthesis of known antigenic determinants. α-2-L-Fucosyltransferase (E.C.2.4.1.69) transfers L-fucose to terminal non-reducing β-D-galactosyl residues on glycoproteins and glycolipids forming H structures (Fucα1-2Galβ-R) which are the precursors of the A and B antigenic determinants. A second enzyme, α-3-L-fucosyltransferase (E.C. 2.4.1.152), transfers L-fucose to the O-3 position of *N*-acetylglucosamine in Type 2 chain *N*-acetyllactosamine (Galβ1-4GlcNAc) structures. The resultant 3-fucosyllactosamine structure is the determinant recognised by anti-SSEA 1 [1], My-1 [2] and similar (CD15) monoclonal antibodies [3]. A third enzyme, α-6-L-fucosyltransferase (E.C. 2.4.1.68), has recently been reported to be present in platelets [4].

In normal individuals, H antigens (both intrinsic and passively acquired) are expressed on platelets [5] but are not present on granulocytes [6, 7] and can only be demonstrated on lymphocytes from secretors in whom they are passively adsorbed from plasma [8]. In contrast, antibodies with specificity for 3-fucosyllactosamine do not react with platelets or lymphocytes [9] but react strongly with neutrophils and with monocytes treated with neuraminidase to remove masking sialic acid residues [9, 10].

It is therefore of interest to determine whether, in normal cells, the activity of specific fucosyltransferases relates to the expression of the corresponding antigens. If there is a direct relationship, it is to be expected that platelets express α-2-, but not α-3-L-fucosyltransferase, that neutrophils and monocytes express α-3- but not α-2-L-fucosyltrans-

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ferase and that lymphocytes express neither enzyme. Previous studies have described α-2-L-fucosyltransferase activity in platelets [11] and α-3-L-fucosyltransferase activity in neutrophils [12], but reports of α-3-L-fucosyltransferase in platelets [11] and both α-2- and α-3-fucosyltransferase activity associated with lymphocytes [11, 12] are surprising in view of the failure to detect the corresponding antigens on these cells. We have therefore re-examined the α-2-, and α-3-L-fucosyltransferase activities in normal human platelets and white cells and report the results of our studies in platelets, neutrophils, monocytes and lymphocytes, in which the purity of cell preparations was carefully assessed.

Materials and Methods

Materials

Ficoll-Paque and Percoll were purchased from Pharmacia (UK), Dextran 150 from Fisons (UK), and RPMI 1640 from Flow Laboratories (UK).

GDP-L-[^{14}C]fucose (254 Ci/mol) was obtained from NEN DuPont (UK) Ltd. and phenyl β-D-galactoside from Koch-Light (UK). *N*-Acetyllactosamine was kindly supplied by Dr A.S.R. Donald, Clinical Research Centre, Harrow, U.K.

Donor Samples

Samples (15-60 ml) of venous blood were obtained from 42 healthy laboratory volunteers or blood donors. Serum was separated after the blood had been allowed to clot at room temperature for 2 h. Cells were purified from blood anticoagulated with Na_2EDTA, or with trisodium citrate if defibrination was required. All the donors were ABO grouped using standard techniques.

Cell Fractionation

Neutrophils were isolated from EDTA-anticoagulated blood from 20 donors following Dextran sedimentation of red cells for 40 min at room temperature and centrifugation at 400 × *g* for 30 min on a Ficoll-Paque gradient [13]. Contaminating red cells were removed by hypotonic lysis for 30 sec, and the cells washed twice in 0.15 M NaCl containing 10 mM Tris buffer pH 7.2 (TBS).

Mononuclear cells were isolated from citrated blood from 13 donors. This was defibrinated, layered onto Ficoll-Paque and spun at 1000 × *g* for 20 min. The cells harvested from the interface were washed once with RPMI and twice more with TBS. These preparations contained lymphocytes and 10-40% monocytes as judged by cytocentrifuge slides stained with May Grunwald Giemsa and were free from contaminating neutrophils or platelets.

Lymphocytes and monocytes were further purified from the mononuclear cell suspensions from six donors on a discontinuous Percoll gradient [14]. The resultant lymphocyte preparation was free from other cells and the monocyte-rich suspension contained 70-90% monocytes. All cell suspensions were counted in a haemocytometer.

Platelet rich plasma was separated from EDTA-anticoagulated blood from 30 separate donors by centrifugation at 400 × *g* for 10 min. Platelets were isolated after a second

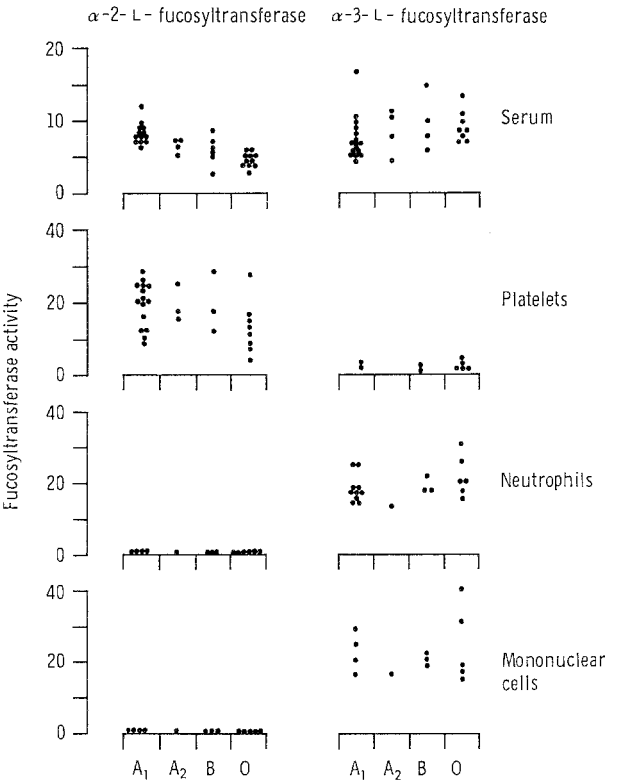

Figure 1. Activity of α-2-L-fucosyltransferase and α-3-L-fucosyltransferase in the serum, platelets, neutrophils and mononuclear cells from normal donors. Activity is expressed as pmol [^{14}C]fucose transferred/h per 100 μl serum in the serum assays or per mg protein in assays of cell suspensions.

centrifugation at 1000 × *g* for 7 min and washed twice in TBS containing 0.5% EDTA. All platelet suspensions, when prepared this way and examined closely, were found to be contaminated with mononuclear cells. To ensure maximum removal of such cells, further low speed centrifugations at 400 × *g* were performed once or twice more as necessary, before washing in TBS to remove EDTA. This final suspension was counted in a Coulter S plus IV electronic cell counter to enable measurement of the white cell contamination in addition to the platelet count.

All serum and cell samples were assayed immediately or stored at -70°C.

Fucosyltransferase Assays

α-2-L-Fucosyltransferase assays contained the following: serum or cell suspension, 20 μl; neutral ATP, 0.5 μmol; GDP-[^{14}C]fucose, 0.16 nmol; Tris-HCl pH 7.2, 5.0 μmol; phenyl β-D-galactoside, 2.0 μmol; 10% Triton X-100, 5 μl (cell assays only). Total volume, 100 μl.

α-3-L-Fucosyltransferase assays were as above except that *N*-acetyllactosamine (1.0 μmol) was used as acceptor instead of phenyl β-D-galactoside and $MnCl_2$ (2.0 μmol) was added to the reaction mixtures.

Table 1. α-3-L-Fucosyltransferase activity in purified lymphocyte and monocyte preparations from six donors.

Donor	ABO group of donor	Incorporation of [^{14}C]fucose (pmol/h/mg protein)	
		Lymphocytes	Monocytes
1	O	36.9	40.8
2	O	31.5	29.4
3	O	33.9	49.2
4	B	33.9	27.2
5	B	17.2	41.8
6	A_1	27.0	34.6

Following incubation at 37°C for 16 h the radioactive products were separated by descending paper chromatography on Whatman No. 40 paper in ethyl acetate/pyridine/water, 10/4/3 by vol, and counted in a liquid scintillation counter [15, 16]. In assays of α-2-L-fucosyltransferase, using phenyl β-D-galactoside as acceptor, product was separated after running the chromatograms for 4-6 h. In the α-3-L-fucosyltransferase assays, however, a much longer period (40-60 h) was required to ensure adequate separation of the 3-fucosyllactosamine product from 2-fucosyllactosamine which is also synthesised when both α-2- and α-3-L-fucosyltransferase are present in the reaction mixture.

Protein was estimated by a modification of the Bradford Method [17]. The number of cells added to the reaction mixtures was adjusted so that assays contained 50-100 mg protein. However, lower amounts of protein (10-39 mg) were present in the assays on lymphocytes and monocytes because of the extremely low yields which occur when isolating relatively pure populations of mononuclear cells.

Results

The activity of α-2-L- and α-3-L-fucosyltransferase in the serum and cells from individuals of different ABO groups is shown in Fig. 1. α-2-L-Fucosyltransferase activity was readily demonstrated in platelets. The pattern of activity was similar to that found in serum, with higher levels in group A_1 compared with group O donors [15]. No activity was detected in neutrophils or mononuclear cells. Prolonged incubations (64 h) with increased amounts of donor (0.64 nmol) and acceptor (4.0 μmol) substrates carried out with mononuclear cells from two individuals failed to reveal any evidence of enzyme activity.

In contrast, α-3-L-fucosyltransferase activity was found in neutrophils and mononuclear cells in similar amounts and the levels were independent of ABO group in both cells and serum. This enzyme was barely detected in most of the platelet preparations, and the activity measured (0.5-4.3 pmol/h/mg protein) appeared to be directly related to the amount of white cell contamination. This contamination was quantified in the platelet preparations from six donors and ranged from 2-6 × 10^6/ml in suspensions containing 2-4 × 10^9/ml platelets. Extrapolation from the α-3-L-fucosyltransferase activity of

the mononuclear cell preparations from the same donors gave values which corresponded almost exactly with those found in the platelets.

Mononuclear cell suspensions obtained by standard methods using density gradient centrifugation contain lymphocytes and monocytes. To determine whether α-3-L-fucosyltransferase activity was present in both cell types, the mononuclear cells from six donors were further separated into lymphocytes and monocytes. This enzyme activity was detected in both cell types in comparable amounts as shown in Table 1.

Discussion

These studies clearly demonstrate that 1) α-2-L-fucosyltransferase is present in platelets but not in leucocytes and 2) α-3-L-fucosyltransferase is absent from platelets but is present in leucocytes where it is found in neutrophils, monocytes and lymphocytes. The results confirm previous reports in which α-2-L-fucosyltransferase was found in platelets [11] but not in neutrophils [12], and α-3-L-fucosyltransferase was demonstrated in neutrophils [12] and in mononuclear cells [11, 12].

α-2-L-Fucosyltransferase activity is therefore found in cells which synthesise H structures and is absent from those which do not. In a previous study in which α-2-L-fucosyltransferase activity was found to be associated with the mononuclear fraction of white cells [12], the fraction was isolated from anticoagulated rather than defibrinated blood. The α-2-L-fucosyltransferase activity measured in this fraction was almost certainly due to platelet contamination of the interface.

Similarly, in platelets and myeloid cells α-3-L-fucosyltransferase activity is associated with cells that have 3-fucosyllactosamine determinants expressed on the cell membrane; activity is demonstrable in neutrophils and monocytes which are recognised by antibodies such as My-1, and is absent from platelets, which are not [9, 10]. We have shown that the small amount of activity in platelets demonstrated by ourselves and others [11] is due to white cell contamination which, despite the precautions taken, is virtually impossible to eliminate completely.

In contrast, peripheral lymphocytes (most of which are T cells in normal individuals) are non-reactive with My-1 and similar antibodies [9], even following neuraminidase treatment [18], but do have demonstrable α-3-L-fucosyltransferase activity. Whether these cells have 3-fucosyllactosamine structures on their surfaces which remain cryptic, even after removal of sialic acid residues by neuraminidase, or whether lack of suitable precursor structures prevents their formation, remains to be elucidated.

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