Activities of fusocylantransferases in sera of leukaemic patients: platelet origin of serum α-6-L-fucosyltransferase

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Alterations of serum glycosyltransferase levels have been described in a number of neoplastic diseases including leukaemias and lymphomas (for references see Weiser et al., 1982). Conflicting results were reported for plasma or serum α-2-3-fucosyltransferase (EC 2.4.1.69) activity in acute myelogenous leukaemia (AML) patients. In some papers, elevation of the enzyme activity in these patients, and its decline to normal values in clinical remission, were described (Chou et al., 1977; Khilananit et al., 1977; Kessel et al., 1980). Another group reported completely opposite results, i.e. a decline of the serum enzyme activity in untreated patients and an increase of activity of the enzyme in remission (Kuhns et al., 1980). Activities of two other α-1-fucosyltransferases present in human serum, i.e. of α-3- and α-2-6-fucosyltransferase (EC 2.4.1.152 and EC 2.4.1.68 respectively), were also reported. Thus plasma α-3-L-fucosyltransferase was found to be elevated in patients with chronic granulocytic leukaemia (CGL) (Kessel et al., 1980) and those with non-Hodgkin's lymphoma (Khilananit et al., 1978). In the latter disease plasma enzyme activity was claimed to be correlated with estimated tumour burden (Khilananit et al., 1978). The activity of α-6-L-fucosyltransferase was reported elevated in plasma of all individuals

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receiving chemotherapy regardless of the disease status (Khilanani et al., 1978).

It should be pointed out, however, that the exact cellular source and mechanism of entry of fucosyltransferases (and other glycosyltransferases) into blood plasma are largely unknown. Evidence is available that α-2-fucosyltransferase is entirely of bone marrow origin (Mulet et al., 1977). On the other hand, haemopoietic tissue contributes only a part of serum glycosyltransferases specified by blood group A and B genes (Wrobel et al., 1974; Kościelak et al., 1976).

Products of different α-fucosyltransferases are variably expressed in blood cells. Thus the FucZβ2Gal structure (H antigen) is present in glycoconjugates of erythrocytes (for references see Kościelak, 1983) but not in those of granulocytes and lymphocytes (Fukuda et al., 1985; Zdebiska et al., 1985). The structure Galβ1-4[FucO1-3]GlcNAc was found in granulocytes (Zdebiska et al., 1985) along with α-3-fucosyltransferase (Greenwell et al., 1983) activity. This structure is not present in a meaningful quantity in erythrocytes. Moreover, expression of fucose-containing glycoconjugates changes during the course of erythropoiesis (for references see Fukuda, 1985). Since leukaemias are haemopoietic in origin, determination of serum α-fucosyltransferases may in theory be helpful for prognosis and even diagnosis of these diseases, providing it is known where the enzymes come from.

Here we report results of assays of α-2, α-3- and α-6-fucosyltransferases in sera of patients with AML and some other neoplastic diseases of haemopoietic tissue, i.e. of all three α-L-fucosyltransferases that are present in human sera. We also describe the source of serum α-6-fucosyltransferase which is derived from blood platelets.

Materials and methods

Patients. The study was made on sera of 32 patients with various neoplastic diseases of haemopoietic tissue. The patients were diagnosed on the basis of accepted clinical and laboratory criteria. Control serum samples were obtained from normal blood donors. All samples were kept frozen at −20°C and were thawed only once just before transferase assays.

Radioactive nucleotide sugars and enzyme acceptors. Labelled GDP-[14C]fucose (200 mCi/ml) was purchased from the Radiochemical Centre, Amersham, U.K. Pheny-β-naphthylamine, α-fucocianidin and β-galactosidase (Sigma) were purchased from the Sigma Chemical Company. α-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer (‘IV NeuAcα1-3,4 Cer) was isolated from human erythrocyte stroma by the method outlined previously (Kościelak et al., 1973). The glycolipid was purified to homogeneity by h.p.l.c. with a Knaure HPLC apparatus (pumps, model 64, programmer, model 50) on a column (0.8 cm × 150 cm) packed with unmodified silica Lichrosorb SI 60, 10 μm diameter (Merck), in chloroform. The column was eluted with chloroform/methanol/water (10:80:10, by vol.) and chloroform/methanol/water (90:10:0.5, by vol.), programmed so that the concentration of the first solvent mixture increased from 20 to 60% final concentration.

IGG glycopeptide was isolated from pronase digests of human IgG (16 g), product of the Institute of Haematology, Warsaw, Poland, by fractionation on Sephadex G-25, Sephadex G-75 and Dowex 50W×2 (200–400 mesh) columns exactly as described by Narasimhan et al. (1979). The main carbohydrate peak (containing approx. 85 mg of hexose and approx. 10 mg of protein) eluted from Dowex 50W×2 was desalted on a Bio-Gel P-10 column (1.5 cm × 35 cm) and then subjected to acid hydrolysis with 0.1 M HCl at 80°C for 1 h to remove sialic acid, fucose and bisecting N-acetylglucosamine residues (Longmore & Schachter, 1982). The material was again desalted on a Bio-Gel P-4 column (1.5 cm × 35 cm) and then exhaustively digested with jack bean β-galactosidase (Sigma) by the method of Li & Li (1970). Briefly, the partially hydrolysed glycopeptide (80 mg) was treated with 0.4 ml of 0.2 M-citrate buffer (pH 4.0) and the enzyme (2 ml, 4 units). The mixture was incubated for 8 h at 37°C. Subsequently, additional enzyme (1 ml) was added and the incubation was continued for 40 h under toluene. The mixture was then filtered successively through Bio-Gel P-10 (2 cm × 30 cm) and Bio-Gel P-10 (2 cm × 35 cm) columns and the appropriate hexose-containing peaks were collected. The final eluate was evaporated under reduced pressure.

On the basis of methylation analysis employing g.l.c.–m.s. technique (for methods see Zdebiska et al., 1985) it was concluded that the main component of the preparation was biantennary GnMN glycopeptide (Longmore & Schachter, 1982) which is derived from blood platelets. α-2-Fucosyltransferase which is derived from blood platelets.
Results

Serum α-2-L-fucosyltransferase was assayed in 15 normal blood donors, eight patients with AML, two patients with erythroleukaemia (EL), five patients with CGL and six patients with chronic lymphocytic leukaemia (CLL). Sera of blood donors yielded α-2-L-fucosyltransferase values which ranged from 56.4 to 128.0 pmol of [14C]fucose incorporated into acceptor substrates and a mean value of 91.3 (s.d. 23.2) pmol. The enzyme activity was higher in four A and two B blood group individuals (mean 111.4 pmol, range 94.8-128.0 pmol) than in five blood group O individuals (mean 66.0 pmol, range 56.4-76.3 pmol). Sera of patients with AML, of whom five were of blood group A, one of B, one of O and one of AB, had α-2-L-fucosyltransferase values ranging from 12.8 to 74.2 pmol of [14C]fucose incorporated into acceptor substrates and a mean value of 41.3 (s.d. 11.4). The enzyme activity was lower in four patients (three of blood group A and one of B) who were not yet treated with cytostatic drugs (mean 33.4, s.d. 9.4, range 23.0-46.0). The highest enzyme value of 74.2 pmol was recorded in patient K.F. (blood group A), who was in clinical remission. Identical, low enzyme values of 30.8 pmol were observed in two blood group B patients with EL. One of these patients had not yet been given chemotherapy.

Results obtained on patients with CGL and CLL were under treatment with cytostatic drugs. The effect of the drugs on α-2-L-fucosyltransferase was studied with NeuAcα2L-Cer, which is known to be a specific substrate for the enzyme (Johnson & Watkins, 1985; Hanson & Zopf, 1985). The reaction was linear during at least 24h. Endogenous incorporation was negligible.

α-6-L-Fucosyltransferase was assayed in sera of our patients. The attempt was unsuccessful with α-6-L-fucosyltransferase activities with grossly abnormal granulocyte, lymphocyte and platelet counts as exhibited by some of our patients. The attempt was unsuccessful with α-3-L-fucosyltransferase (data not shown).

α-6-L-Fucosyltransferase was assayed in sera of individual patients

Table 1. Serum α-3- and α-6-L-fucosyltransferases in patients with AML and normal blood donors

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>All patients (n = 12)</th>
<th>Untreated (n = 3)</th>
<th>Under treatment (n = 4)</th>
<th>Presented 4-8 weeks after treatment (n = 5)</th>
<th>As above but in remission (n = 3)</th>
<th>Blood donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14.3 (13.9)</td>
<td>34.0</td>
<td>5.7</td>
<td>9.4</td>
<td>9.0</td>
<td>17.0</td>
</tr>
<tr>
<td>B</td>
<td>23.9 (23.0)</td>
<td>17.1</td>
<td>15.7</td>
<td>34.6</td>
<td>37.3</td>
<td>64.6</td>
</tr>
<tr>
<td>O</td>
<td>14.9 (14.9)</td>
<td>19.0</td>
<td>4.6</td>
<td>4.8</td>
<td>4.6</td>
<td>13.2</td>
</tr>
<tr>
<td>AB</td>
<td>14.9 (14.9)</td>
<td>3.0</td>
<td>11.0</td>
<td>16.4</td>
<td>21.5</td>
<td>30.9</td>
</tr>
<tr>
<td>A-3-FT*</td>
<td>10.5-55.0</td>
<td>18.0-55.0</td>
<td>1.0-12.0</td>
<td>5.0-15.0</td>
<td>5.0-14.0</td>
<td>4.5-70.0</td>
</tr>
<tr>
<td>A-6-FT*</td>
<td>4.0-58.0</td>
<td>14.7-25.0</td>
<td>4.0-20.0</td>
<td>15.0-58.0</td>
<td>15.0-58.0</td>
<td>23.3-125.0</td>
</tr>
<tr>
<td>A-3-FT†</td>
<td>4.0-15.0</td>
<td>14.7-25.0</td>
<td>4.0-20.0</td>
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<td>15.0-58.0</td>
<td>23.3-125.0</td>
</tr>
</tbody>
</table>

* pmol of [14C]fucose incorporated into NeuAcα2L-Cer/24 h per 100 μl of serum.
† pmol of [14C]fucose incorporated into stripped IgG glycopeptide/16 h per 100 μl of serum.

Fig. 1. Platelet count versus the activity of α-6-L-fucosyltransferase in sera of individual patients

Patients initial diagnosis are above and below the curve respectively. Patient J.K. with thrombocytosis had not been properly diagnosed at the time of presentation.

Table 1: Serum α-3- and α-6-L-fucosyltransferases in patients with AML and normal blood donors

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blood samples were collected with heparin as anticoagulant and then centrifuged at 3000 rev./min for 30 min.

Discussion

Although this study was made with a limited number of patients, we can wholly confirm the results of Kuhns et al. (1980) of a diminution of α-2-α-fucosyltransferase activity in sera of patients with AML. The enzyme was also low in two patients with EL, the disease resulting most often from the proliferation of a malignant proerythroblastic clone (Wintrobe, 1981). At this stage of erythropoiesis ABO antigens are not yet well expressed (see Fukuda, 1985). Thus a low α-2-α-fucosyltransferase activity in sera of patients with EL is not unexpected.

Serum α-3-α-fucosyltransferase activity seems to be elevated in untreated patients with AML but the enzyme activity is adversely affected by chemotherapy and stays low even in patients in remission. A low serum α-3-α-fucosyltransferase activity in blood group A donors cannot be explained at present. Obviously more sera should be studied.

Most interesting results were obtained on the activity of serum α-6-α-fucosyltransferase, which is clearly confined within intact platelets. Thus a low enzyme activity in sera of patients receiving chemotherapy is readily explained since platelet counts invariably fall during such treatment. It had previously been assumed that serum α-4-α-fucosyltransferase activities should reflect tumour burden and its cellular composition (Kessel et al., 1980; Kuhns et al., 1980). The present paper shows clearly that platelets are the major source of at least one α-4-α-fucosyltransferase. This finding as well as the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets (Skacek & Watkins, 1987) may provide a clue for the conflicting reports on serum or plasma α-2-α-fucosyltransferase activity, as well as for the reported presence of α-2-α-fucosyltransferase in blood platelets.

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Structure, biosynthesis and genetics of the Sd" antigen

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The Sd" determinant is the sole antigen of the Sd blood group system (see Race & Sanger, 1975) and is inherited as a Mendelian dominant character. Sd"-active materials are present in the blood and urine of 92% of the population, in another 4% these reactivities are weak or lacking, and the remaining 4% are true Sd"- individuals with anti-Sd" in their serum. As well as this, naturally occurring human anti-Sd", certain lectins, notably Dolichos biflorus, react with the Sd" antigen but are less specific. With such reagents cells from Sd(−) individuals are agglutinated to a different degree and may be classed as Sd(−) or Sd(−+)−. A few extremely rare individuals have very strongly reacting cells and are classified as 'Cad' after the Mauritian family in which such cells were first observed (Cazal et al., 1968). The exact genetic relationship between Cad and Sd" has still to be established.

In urine the major carrier of the Sd" determinant has been shown (Morgan et al., 1979) to be the Tamm-Horsfall (T-H) glycoprotein (Tamm & Horsfall, 1950). Analytically, T-H glycoproteins from Sd(+) and Sd(−) individuals differ only in that the Sd(+) samples contain 1–2% N-acetylgalactosamine whereas the Sd(−) glycoproteins have less than 0.2%, indicating that this sugar is part of the Sd determinant (Soh et al., 1980). This finding reinforced earlier observations which showed that N-acetylgalactosamine inhibited the agglutination of Sd(+) cells with