Electrofocuseding Pattern of Fucosyltransferase Activity in Human Leukemic Cells

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ABSTRACT

Fucosyltransferase (FT) activity of normal lymphocytes, normal granulocytes, and various types of human leukemic cells and electrofocusing pattern of FT activity in human leukemic cells and normal lymphocytes were examined using asialofetuin as an acceptor. Levels of FT activity in normal lymphocytes were higher than those of normal granulocytes in which FT activity was almost undetectable. The FT activity was higher in blast cells of acute myeloblastic leukemia and chronic myelogenous leukemia in blast crisis than in blast cells of acute lymphoblastic leukemia and the chronic phase of chronic myelogenous leukemia. The level of FT activity was lower in cells of chronic lymphocytic leukemia than that of normal lymphocytes, but it was higher than that of normal granulocytes. Three main isoelectric forms of FT in leukemic blast cells were identified by isoelectrofocusing, and they each had a characteristic focusing point: around pH 4.5 (peak 1); pH 4.9 (peak 2); and pH 5.2 (peak 3). In blast cells of myeloid leukemia, the activity of peak 3 was markedly higher than those of peaks 1 and 2. In blast cells of lymphoid leukemia, the activity of peak 3 was also the highest, but the activity of peak 2 was higher than that in myeloid blast cells. In normal lymphocytes, the major isoelectric form of FT was focused at around pH 4.9 and peak 3 was undetectable. These results indicated apparent differences not only in FT activity but also in isoelectric forms of FT between myeloid leukemic cells and lymphoid leukemic cells.

INTRODUCTION

Glycoconjugates participate in intercellular adhesion, cellular recognition, and cell differentiation (1, 2). Changes in surface glycoproteins and glycolipids have been investigated in relation to malignant transformation of mammalian cells (3–5). The precise sequential actions of certain glycosyltransferases are necessary for the formation of any one of these glycoconjugates (6). The role of surface glycosyltransferase activity in malignant transformation of cells has been postulated (5, 7, 8). Among the glycosyltransferases, the FTs are a group of enzymes which catalyze the transfer of fucose from a GDP-fucose donor onto appropriate glycoprotein, glycolipid, or low molecular weight acceptors (6, 9). Several different FTs have been identified (9, 10). The H-gene-specified enzyme, α-2-L-FT and the Le-gene-specified enzyme α-3/α-4-L-FT catalyze the transfer of fucose to the C-2 position of the terminal nonreducing β-d-galactosyl residue and to the C-3/C-4 position of the subterminal N-acetyl-d-glucosaminyl residue, respectively. The α-3-L-FT, which is different from both the H- and Le-dependent FTs, transfers fucose to the C-3 position of the subterminal N-acetyl-d-glucosamine or d-glucose residue. α-6-L-FT has been shown to transfer fucose to the asparagine-linked N-acetyl-d-glucosamine residue.

Serum FT activity has been reported to be elevated in many types of cancer patients and decreased after successful treatment (11–14). Recently, Kessel et al. (15) investigated the electrofo-

Received 9/7/84; revised 1/16/87; accepted 2/24/87.
1 This work was partially supported by a Grant-in-Aid from the Japanese Government.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: FT, fucosyltransferase; AML, acute myeloblastic leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; N-EM, N-ethylmaleimide; DTT, dithiothreitol; FT-IF, electrofocusing pattern of fucosyltransferase activity; TdT, terminal deoxynucleotidyltransferase.

MATERIALS AND METHODS

Patients and Cell Lines. We studied the blast cells from 4 cases of AML, 7 cases of CML (4 in the chronic phase and 3 in the blastic phase), 8 cases of ALL, 2 cases of CLL, and 2 cases of T-cell lymphoma. Normal lymphocytes and granulocytes were obtained from healthy volunteers who had given us their informed consent. The diagnosis and classification of leukemia were made using cytochemistry (17), cell surface marker analysis, TdT assay, and chromosome analysis.

We also studied five human leukemic cell lines: two myeloid cell lines (HL-60, K562); one B-cell line (BALL); and two T-cell lines (CCRF-CEM, MOLT-3).

Cell Separation. Peripheral blood or bone marrow blood was obtained from patients with various types of leukemia. Peripheral blood was also obtained from healthy volunteers. The involved lymph nodes from patients with malignant lymphoma were teased in culture medium and then passed through a fine steel mesh to make a single-cell suspension. Mononuclear cells were isolated from peripheral blood, bone marrow aspirate, and lymph node cell suspension by centrifugation through Ficoll-Hypaque gradients. Normal granulocytes were separated using dextran-sodium metrizoate at 4°C, and erythrocytes were removed after hypotonic shock.

Assay of Fucosyltransferase Activity. Cells were suspended at a density of 1 × 10^6 cells/ml in 0.32 M sucrose solution, pH 7.0, containing 1 mM EDTA, 1.4 mM 2-mercaptoethanol (Wako, Osaka, Japan), and 0.1% v/v Triton X-100 (Sigma Chemical Co., St. Louis, MO). The cell suspension was sonicated by four 15-s bursts with cooling and centrifuged at 7000 × g for 20 min. The supernatant was used for enzyme assay.

Asialofetuin was used as an acceptor for fucosyltransferase. Terminal N-acetyleneuraminic acid was removed from fetuin (Sigma) by hydrolyzing it with 0.1 N H_2SO_4 at 80°C for 1 h and processing it according to the methods described by Spiro (18). The enzyme assay was carried out by the modified methods of Chou et al. (19) and Hoflack et al. (20). Twenty µl of crude enzyme solution were incubated with 100 µM Tris-HCl buffer solution, pH 7.4, containing 0.3% (v/v) Triton X-100, 10 mM MgCl_2, 2 mM ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 0.5 mM UMP (Sigma), 500 µg asialofetuin, and 3.74 µM GDP-[14C]fucose (New England Nuclear, Boston, MA) in a total volume of 100 µl at 37°C for 20 h. [14C]Fucose incorporated into asialofetuin was precipitated by trichloroacetic acid (21), and the precipitate was solubilized with protocol and counted with a liquid scintillation spectrophotometer. FT activity was expressed as the net radioactivity by subtracting the endogenous activity determined by omitting asialofetuin from the assay system.

Effect of thiol reagents on fucosyltransferase activity was examined by the addition of thiol-blocking reagent, N-EM (10 µM), and a thiol reagent, dithiothreitol (DTT) (10 µM), to the assay solution.

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About 30 to 60 μl of the crude enzyme solution were electrofocused on an ampholine PAG plate (pH 4–5.5; LKB, Bromma, Sweden) at 500 V for 12 h with the gel bed chilled to 4°C. The gel was then divided into 30 fractions, their pH was measured, and the individual gel pieces were eluted with 500 μl of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.0). The eluates were concentrated to about 40 μl at 4°C. Assay of fucosyltransferase activity was carried out as above on an ampholine PAG plate (pH 4–6.5; LKB, Bromma, Sweden) at 20 KHz for a total of 45 s with cooling. Sonicates were centrifuged at 105,000 × g for 1 h at 4°C. The supernatant was used.

Surface Markers. T-cells were identified by their ability to spontaneously bind sheep erythrocytes (22) and by reactivity with monoclonal anti-T-antibodies (OKT3 and OKT6; Ortho Pharmaceutical Corporation, Raritan, NJ) (23, 24). Cell surface immunoglobulin was detected using fluorescein isothiocyanate-labeled goat anti-human immunoglobulin antiseraum [Fab(α); fragment; Cappel, Cochranville, PA].

Assay of TdT. TdT was assayed in extracts of cells by the method of Coleman et al. (25) after a slight modification. Cells were suspended in 0.25 M potassium phosphate, pH 7.5, containing 1 mM 2-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride and disrupted by sonication at 20 KHz for a total of 45 s with cooling. Sonicates were centrifuged at 105,000 × g for 1 h at 4°C. The supernatant was used for the assay of TdT. The reaction mixture for the TdT assay contained 0.3 M potassium cacodylate (pH 7.0), 1 mM [3H]dGTP (100 cpm/pmol), 1 mM MnCl2, 0.125 unit oligo(dA)20-32, 1 mM 2-mercaptoethanol, 0.1 mg/ml bovine serum albumin, and enzyme extract in a total volume of 100 μl. The reaction mixture was incubated at 35°C for 30 min, and the aliquot was collected on a Whatman GF/C filter disc. The acid-insoluble radioactivity of the disc was counted in a toluene scintillation spectrophotometer. The level of TdT activity in normal bone marrow cells was less than 2 nmol/108 cells and levels higher than this value were considered TdT positive.

RESULTS

Fucosyltransferase Activity of Leukemic Cells. Fig. 1 shows the FT activity of normal lymphocytes, normal granulocytes, and various types of human leukemic cells. Normal lymphocytes showed a higher level of FT activity than normal granulocytes the FT activity of which was almost undetectable. The FT activity of blast cells from AML showed a higher level of FT activity than blast cells from ALL. Blast cells from the chronic phase of CML showed FT activity as high as those from AML, and cells from the chronic phase showed almost the same level of FT activity as normal lymphocytes. Leukemic cells from CLL showed a lower level of FT activity than normal lymphocytes.

The FT activity of human leukemia cell lines is summarized in Table 1. HL-60 cells had the highest level of activity. K562 and MOLT 3 cells had comparable levels of enzyme activity. T-cell-derived CCRF-CEM cells and B-cell ALL-derived BALL cells had lower levels of activity than these 3 cell line cells.

Electrofocusing Pattern of Fucosyltransferase. In the blast cells obtained from cases with AML, the FT-EF revealed three main peaks of activity. Peak 1 was focused around pH 4.5, peak 2 around pH 4.9, and peak 3 around pH 5.2 (Fig. 2). In the blast cells obtained from two cases of AML, peak 3 showed the highest activity, but the activity of the other two peaks remained low (Fig. 2). Fig. 3 shows a representative profile of FT-EF in blast cells of ALL null cell type. In ALL, FT-EF showed three peaks of activity. As in the case of AML, peak 3 was the highest in the blast cells obtained from null cell-type ALL. In contrast to AML, however, peaks 1 and 2 were much higher in ALL (Fig. 3). In the cases of T-cell lymphoma, FT-EF also showed three peaks of activity. Peak 3 had the highest activity, and peak 1 had the lowest (Fig. 3). In the case of TdT-positive blast crisis of CML, peak 2 and peak 3 were high and peak 1 was almost undetectable. In the case of TdT-negative blast crisis, however, only peak 3 was detectable (Fig. 4).

In HL-60 cell line cells, peak 3 was the highest and peaks 1 and 2 were almost undetectable. An extra peak, which was focused around pH 5.5, was identified. In K562, peak 3 was also the highest and peaks 1 and 2 were almost undetectable (Fig. 5). In CCRF-CEM cell line, peak 3 activity was the highest, and peak 2 showed approximately one-fifth the activity of peak 3. Peak 1 was undetectable. Similar results were obtained with MOLT 3 cells and BALL cells (Fig. 6).

Table 1 Fucosyltransferase activity in human leukemia cell line cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Fucosyltransferase activity [14C]fucose incorporated (dpm)/2 × 106 cells</th>
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<tr>
<td>HL-60</td>
<td>12,518 ± 2,228</td>
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<tr>
<td>K562</td>
<td>4,432 ± 621</td>
</tr>
<tr>
<td>MOLT-3</td>
<td>4,414 ± 247</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>2,972 ± 324</td>
</tr>
<tr>
<td>BALL</td>
<td>1,758 ± 66</td>
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*Mean ± SD of 3 separate assays.

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<td>100</td>
<td>25</td>
<td>10</td>
<td>5</td>
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<td>BML1</td>
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Fig. 1. FT activity of normal lymphocytes (Lym.), normal granulocytes (Grn.), and various types of human leukemia cells. FT activity was measured using asialofetuin as described in "Materials and Methods." Each point was based on the data obtained by a single assay done on each patient's cells and each normal subject's cells. C.P., chronic phase. B.C., blastic crisis.

Fig. 2. Electrofocusing patterns of fucosyltransferase activity in blast cells from two patients with AML. Thirty μl of crude enzyme solution equivalent to 3 × 106 cells (5367 dpm) in AML1 and 3 × 106 cells (5920 dpm) in AML2 were electrofocused as described in "Materials and Methods." Data represent one-half the amount of [14C]fucose incorporated into asialofetuin acceptor by enzyme fractions which focused at the specified pH values. There are three main components of FT activity, which focused at around pH 4.5 (peak 1), pH 4.9 (peak 2), and pH 5.2 (peak 3).
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FT-EF of normal lymphocytes showed a pattern different from those of ALL and AML. Peak 2 had the highest activity and peak 1 was almost undetectable (Fig. 7). The FT-EF of normal granulocytes could not be obtained because FT activity was almost undetectable in granulocytes as shown in Fig. 1.

Effect of Thiol Reagents on Fucosyltransferase Activity. The specific activity of FT of the blast cells obtained from the majority of the cases of acute leukemia showed a tendency to be weakly inhibited by the addition of a thiol-blocking reagent N-EM. A tendency of slight inhibition of the specific activity of FT of the blast cells obtained from acute leukemia was also observed by the addition of DTT in a majority of the cases.

Effect of N-EM on FT-EF was examined using human leukemic cell lines. In CCRF-CEM, peak 3 was not affected by the presence of N-EM, whereas the activity of peak 2 of FT seemed to be suppressed. In MOLT 3, a similar effect of N-EM on the FT activity was observed with a tendency to weak suppression of peak 2.

DISCUSSION

Using asialofetuin as an acceptor, we have demonstrated the difference in FT activity between normal lymphocytes and normal granulocytes. Normal lymphocytes had higher levels of FT activity than normal granulocytes in which FT activity was almost undetectable. The present assay methods for FT with the use of asialofetuin will detect both α-2- and α-3-transferase activity, the former transferring fucose to terminal galactose and the latter transferring fucose to subterminal N-acetylgalcosamine residue. This assay presumably does not measure FT activity which requires a terminal N-acetylgalcosamine residue.

Fig. 3. Electrofocusing patterns of fucosyltransferase activity in blast cells of human lymphoid leukemia. A, lymphoblasts obtained from a patient with null cell-type ALL. Enzyme solution equivalent to 1 x 10⁷ cells (13,247 dpm) was subjected to electrofocusing. B and C, lymphoblasts from patients with T-cell lymphoma. Enzyme solutions equivalent to 4 x 10⁶ cells (8,786 dpm) and 4 x 10⁶ cells (7,294 dpm) were subjected to electrofocusing. Data represent one-half the amount of [14C]fucose into an asialofetuin acceptor by enzyme fractions which focused at specified pH values.

Fig. 4. Electrofocusing patterns of fucosyltransferase activity in blast cells of CML blast crisis. A, blast cells obtained from a patient with TdT-positive blast crisis, showing high peak 2 and peak 3 activities. Enzyme solution equivalent to 1 x 10⁷ cells (8,150 dpm) was subjected to electrofocusing. B, blast cells obtained from a patient with TdT-negative blast crisis, showing only peak 3 activity. Data represent one-half the amount of [14C]fucose incorporated by enzyme fractions which focused at the specified pH values.

Fig. 6. Electrofocusing patterns of fucosyltransferase activity in human lymphoid leukemia cell lines. A, CCRF-CEM. Enzyme solution equivalent to 3 x 10⁶ cells (4,290 dpm) was used for electrofocusing. B, Molt 3. Enzyme solution equivalent to 3 x 10⁶ cells (6,621 dpm) was used for electrofocusing. C, BALL. Enzyme solution equivalent to 6 x 10⁶ cells (5,460 dpm) was used for electrofocusing. Data represent one-half the amount of the [14C]fucose incorporated into an asialofetuin acceptor by enzyme fractions, which focused at the specified pH values.

Fig. 7. Electrofocusing pattern of fucosyltransferase activity in normal human lymphocytes. Enzyme solution equivalent to 8 x 10⁶ cells (2,100 dpm) was used for electrofocusing. Data represent one-half the amount of the radioactive fucose incorporated by enzyme fractions which focused at the specified pH values.
According to the recent study of Greenwell et al. (26), human granulocytes are devoid of H-gene-specified α-2-L-fucosyltransferase, and the absence of this enzyme distinguished the granulocytes from the lymphocytes. They considered that this enzyme might be useful as a marker of peripheral blood lymphocytes. We also found a difference in FT activity between normal lymphocytes and normal granulocytes although we used a different acceptor. The present assay system could be measuring the H-gene-specified α-2-L-fucosyltransferase, but it could also be detecting another fucosyltransferase which uses asialofetuin as an acceptor.

In this study, we demonstrated elevated levels of FT activity in blast cells of human leukemia, especially of AML and the blastic phase of CML. Interestingly, FT activity was lower in more mature forms of leukemia cells from the chronic phase of CML, which were sedimented at the interphase by Ficoll-Hypaque centrifugation, and both mature granulocytes of the chronic phase of CML and normal granulocytes showed the lowest level of FT activity which was almost undetectable. The FT activity of blast cells from ALL was equivalent to that of more mature cells of chronic phase CML and higher than that of leukemic cells from CLL.

The FT activity of human leukemia cell lines showed a similar tendency, lymphoid cell lines having a lower activity than myeloid cell lines of which HL-60 had markedly high enzyme activity.

There are several isoelectric forms of FT in human sera and tissues (27, 28). Electrofocusing study by Kessel et al. (15) detected three forms of FT in plasma from patients with leukemia and infectious diseases using asialofetuin as an acceptor. They demonstrated that the isoelectric points of these three forms were pl 4.7, 5.1, and 5.6. They thought that the isoelectric form with a pl of 4.7 was derived from early myeloid precursor cells and that the isoelectric form with a pl of 5.6 was from the malignant clone in CML (16). However, they did not directly examine these isoelectric forms of FT prepared from various cell types.

We also demonstrated three isoelectric forms of FT in blast cells of various types of leukemia and in cells of human leukemic cell lines by electrofocusing with a pH range from 4 to 6.5. Each of the forms had a characteristic isoelectric point: around pl 4.5, 4.9, and 5.2. We arbitrarily designated them as peaks 1, 2, and 3, respectively. We found some differences in FT-EF between human myeloid leukemic blast cells and lymphoid leukemic blast cells. In both myeloid and lymphoid blast cells, the major form was peak 3. Blast cells of AML showed very low peak 1 and peak 2 activities. In contrast, blast cells of null cell-type ALL showed higher relative peak 1 and peak 2 activities than AML blast cells. Lymphoid cells obtained from patients with T-cell lymphoma had a similar pattern.

Similar results were obtained by the analysis of FT-EF of several human leukemic cell lines. Human myeloid cell lines (HL-60 and K562) showed a predominant peak 3 activity with scanty peak 1 and peak 2 activities, whereas human lymphoid cell lines (CCRF-CEM and MOLT 3) showed a higher activity of peak 2 besides a predominant peak 3. Although these isoelectric forms may have different kinds of FT activity in terms of linkages formed, the analysis of FT by isoelectrofocusing might serve as a tool for differential diagnosis of myeloid and lymphoid leukemia.

In FT-EF of blast cells obtained from patients with CML, TdT-positive blast cells had high peak 2 and peak 3 activities, whereas TdT-negative blast cells had only peak 3 FT activity. Since TdT-positive blasts are thought to be of lymphoid origin and those without TdT of myeloid origin, FT isoelectric forms might reflect the cell origin in CML blast crisis. Because no difference was found in the levels of plasma FT activity between myeloid crisis and lymphoid crisis of CML (14), FT-EF of blast cells will deserve further investigation with regard to the differentiation of these two types of blast crisis.

Although it was not definite, the isoelectric form that focused at pH 4.9 (peak 2) seemed to be abolished or decreased by the addition of N-EM. Since H-gene-specified FT is inhibited by N-EM (19), the isoelectric form with a pl of 4.9 may have a characteristic similar to the H-gene-specified FT.

In both myeloid and lymphoid leukemia cells, the major isoelectric form of the enzyme was focused at around pH 5.2 (peak 3). Interestingly, the major isoelectric form of the enzyme in normal lymphocytes was focused at around pH 4.8 and the isoelectric form with a pl of 5.2 was undetectable. Although direct evidence is lacking, the isoelectric form with a pl of 5.2 (peak 3) may be related to the immaturity of hematopoietic cells or malignant transformation of these cells. FT-EF must be examined in various maturation stages of hematopoietic cells in order to clarify this point. According to the study of plasma FT by Kessel et al. (16), the isoelectric form with a pl of 5.6 was considered to be derived from a malignant clone in CML. The differences in pl values between cellular and plasma enzymes might reflect the differences in the content of ionizable groups such as sialic acid on the enzymes but the correspondence of the two was not clarified in the present study. Little is known about the origin of each isoelectric form of serum FT. FT activity is found in milk (29), mammary gland (30), submaxillary gland (31), gastric mucosa (32), small intestinal mucosa (33), and hematopoietic cells (16, 34). In general, elevated levels of serum glycosyltransferase in many malignant diseases are thought to be due to the shedding process or secreting process from malignant cells (11, 12, 35) and are considered to be good indicators of tumor masses in the body. Serum FT from leukemic patients has been reported to be sensitive to the inhibition by N-EM (16, 36). However, FT activity of leukemic cells determined with the use of asialofetuin has been reported to be insensitive to N-EM and DTT (36). Therefore, the major isoelectric form of serum FT obtained from leukemic patients may not be directly released from leukemic blast cells. Further investigation is necessary to elucidate the relationship between serum FT activity and FT activity of leukemic blast cells.

ACKNOWLEDGMENTS

We thank Dr. Tadashi Kasahara, Department of Parasitology and Medical Zoology, Jichi Medical School, for supplying leukemic cell lines used in this study. We also thank Keiko Kikumoto and Ikuko Tsukada for their skillful technical assistance.

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