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Changes of β-1,4-*N*-acetylglucosaminyltransferase III (GnT-III) in patients with leukaemia

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Changes in the activity and transcription of UDP-*N*-acetyglucosamine: β-D-mannoside β-1,4-*N*-acetylglucosaminyltransferase III (GnT-III: EC 2.4.1.144) were investigated in haematological malignancies. GnT-III activity was elevated in patients with chronic myelogeneous leukaemia in blast crisis (CML-BC) and patients with multiple myeloma (MM); whereas most of the normal healthy subjects and patients with other haematological malignancies, including CML in its chronic phase, showed negligible activity. The GnT-III transcript of leukaemic cells from various haematological diseases showed a single band with a similar size. The ratio of GnT-III activity per normalized transcript in CML-BC was considerably higher than in the other conditions, which provided the possibility that in CML-BC the transcript or the enzyme protein might be more stable, or that a post-translational modification of the enzyme might enhance its activity. Furthermore, a lectin blot analysis of patient specimens and a lectin fluorescence study of CML cell lines revealed that E_4-PHA binding to surface glycoproteins correlated with GnT-III activity, indicating that more bisecting GlcNAc was added to these glycoproteins, catalysed by elevated GnT-III in CML-BC.

Keywords: *N*-acetylglucosaminyltransferase III, chronic myelogeneous leukaemia, E_4-PHA

Abbreviations: GnT-III, UDP-*N*-acetyglucosamine: β-D-mannoside β-1,4-*N*-acetylglucosaminyl transferase III; CML, chronic myelogeneous leukaemia; AML, acute myelogeneous leukaemia; ALL, acute lymphoblastic leukaemia; CLL, chronic lymphocytic leukaemia; MM, multiple myeloma; DSA, *Datura stramonium*; E_4-PHA, E_4-phytohaemagglutinin; Con A, Concanavalin A; high-performance liquid chromatography; GlcNAc, *N*-acetylglucosamine; PBS, phosphate-buffered saline; BSA, borine serum albumin; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate.

Introduction

UDP-*N*-acetylglucosamine: β-D-mannoside β-1,4-*N*-acetylglucosaminyl transferase III (GnT-III) is involved in the formation of a bisecting *N*-acetylglucosamine (GlcNAc) [1], as shown in Fig. 1. High activities of this enzyme have been observed in hepatic nodules of rat liver during hepatocarcinogenesis [2], in ascites hepatoma cells, AH-66 [3] and in LEC rat liver during hepatocarcinogenesis [4]. In our laboratory, an assay method for GnT-III activity has been developed, using a pyridylaminated bi-antennary sugar chain as a substrate [5], and subsequently we have purified rat GnT-III, and cloned cDNA for rat GnT-III [6] and human GnT-III [7]. Regarding haematological malignancies, GnT-III activity was changed during the differentiation of HL-60 cells [8] and was modified by interleukin 6 in myeloma cell lines [9].

CML is a haematological malignancy in which myeloid cells at various stages of maturation are markedly increased in peripheral blood in the chronic phase, and is characterized by the Philadelphia (Ph^1) chromosome found in more than 90% of CML patients [10]. Abnormalities in the carbohydrate structures on CML cells have been reported by several investigators. It has been reported that granulocytes of CML in the chronic phase were highly sialylated in association with the elevated activity of CMP-NeuAc: Gal β1-3 GalNAc α-R α2-3-sialyltransferase [11] (EC.2.4.99.4) and UDP-GlcNAc: Gal β1-3 GalNAc α-R β1-6 GlcNAc transferase [12] (EC 2.4.1.102; core 2 β6-GlcNAc transferase). These enzymes are mainly involved in the formation of O-glycans and little information has been provided about the enzymes responsible for N-glycan synthesis in CML and other leukaemia cells.

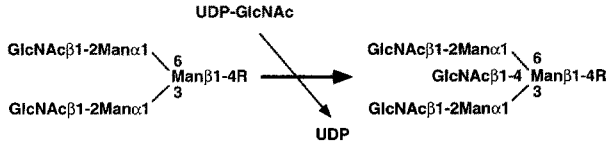

Figure 1. Reaction catalysed by GnT-III. R and Man indicates GlcNAcβ1-4 GlcNAc-2-aminopyridine and mannose, respectively.

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The presence of bisecting GlcNAc inhibits the binding of the lectin DSA (*Datura stramonium*) to tri- or tetra- Galβ-1-4GlcNAc antennary in N-glycans [13]. Another lectin, E_4-PHA (E_4-*phytohemmagglutinin*) exibits specific binding to bisecting GlcNAc in N-glycans [14]. These lectins can, therefore, be used in concert to investigate the presence of bisecting GlcNAc. The present study was undertaken to investigate the expression of GnT-III in various haematological malignancies. GnT-III activity and mRNA expression were found to be elevated in CML-BC. The effect of this change in GnT-III activity on the attachment of bisecting GlcNAc to N-glycans of glycoproteins on the cell surface was also investigated.

Materials and methods

Cells and cell lines

Peripheral blood or bone marrow samples were obtained before treatment from nineteen patients with CML-BC (ten in myeloid crisis, five in lymphoid crisis and four in megakaryocytic crisis), twelve patients with CML in the chronic phase, sixteen with acute myelogeneous leukaemia (AML), twelve with acute lymphoblastic leukaemia (ALL), eleven with chronic lymphocytic leukaemia (CLL), fourteen with multiple myeloma (MM) and ten healthy individuals. AML groups consisted of three M1, five M2, two M3, four M4, one M5 and one M6, and ALL included four L1 and eight L2, according to French-American-British criteria [15]. Myeloid cells of all patients with CML were shown to carry Ph^1 chromosome. Samples were obtained after informed consent. The diagnosis of disease was established by morphology, cytochemical staining, and phenotype analysis.

Mononuclear cell fractions were isolated from heparinized peripheral blood and bone marrow aspirate by Ficoll-Hypaque density gradient centrifugation. All samples were then washed three times in phosphate-buffered saline (PBS) and stored in liquid nitrogen as pellets of 1×10^7 cells. Samples selected for this study were shown to contain more than 90% malignant cells, confirmed by May-Giemsa staining.

The KU812 cell line and its subclone, KU812F, were established from cells of a CML patient [16], and were supplied by the Japanese Cancer Research Resources Bank (Tokyo, Japan). These lines were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Nalgene, Victoria, Australia) and antibiotics.

Reagents

Aprotinin, phenylmethylsulfonyl fluoride, and leupeptin were purchased from Sigma. Biotinylated DSA and biotinylated E_4-PHA, and fluorescein isothiocyanate (FITC)-conjugated DSA and E_4-PHA were obtained from Seikagaku Kogyo (Tokyo, Japan).

Preparation of crude extract and determination of GnT-III

Enzyme assays for GnT-III were set up as previously described [17]. In brief, frozen pellets of patient samples or cultured cells were quickly thawed out, washed three times with cold PBS, resuspended in cold PBS, and sonicated for 10 min at 4°C. The crude enzyme preparations were incubated with a pyridylaminated bi-antennary sugar chain as a substrate [18] at 37°C for 2 h. In a preliminary study, we confirmed that the enzyme activities were linear during the 2 h incubation. The reaction buffer for the GnT-III assay consisted of 125 mM 2-(*N*-morpholino) ethane sulfonate (MES), pH 6.25, containing 0.77 mM substrate, 20 mM UDP-GlcNAc, 10 mM $MnCl_2$, 200 mM GlcNAc, and 0.5% Triton-X. The samples were boiled for 1 min to stop the reaction, centrifuged at 15 000 rpm for 5 min to remove the debris, and applied to an HPLC system. The HPLC system consisted of a TSK-gel ODS 80TM column (4.6 × 150 mm) eluted with 0.02 M ammonium acetate buffer, pH 4.0, containing 0.3% *n*-butanol at a flow rate of 1.2 ml min^{-1}. Under these elution conditions, the bisecting GlcNAc product was completely separated from the substrate. The elution profiles of the products of GnT-IV and GnT-V overlapped with the substrate [17]. The amount of GnT-III product formed was determined from the fluorescence intensity, by comparison with a pyridylaminated bi-antennary sugar chain standard (339 pmol). The specific activity of the enzyme was obtained by averaging of triplicates prepared from each patient or cell line, and expressed as nmol of GlcNAc transferred h^{-1} mg $protein^{-1}$. The protein concentration was determined with a BCA kit (Pierce).

RNA preparation and Northern blot analysis

For Northern blot analysis, mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation and stored in liquid nitrogen. Total cellular RNA was prepared by acid guanidine thiocyanate extraction and preparative ultracentrifugation [19]. Extracted RNAs (20 μg) were size-fractionated by electrophoresis through 1.0% formaldehyde-agarose gel and transferred onto Zeta-probe membrane (Bio Rad, Richmond, CA, USA) by capillary action. For a probe, the human GnT-III cDNA *Sac* I fragment (1.5 kb) [7] was radiolabelled with [α-^{32}P]dCTP using a Multiprime DNA labelling system (Amersham, UK). Prehybridization was performed in 50% formamide, 6 × SSC (1 × SSC, 15 mM sodium citrate and 150 mM NaCl, pH 7.0), 5 × Denhardt's solution, and 0.5% SDS at 42°C for 4 h. The filter was hybridized overnight at 42°C in the prehybridization solution containing 10% polyethyleneglycol 8000 and ^{32}P-labelled probe, and the blots were washed twice at 55°C with 2 × SSC and 0.1% SDS for 30 min. The membranes were subjected to autoradiography at –80°C for 1–3 days. Probe stripping was performed by washing the membranes twice at 95°C with 0.1 × SSC and 0.5% SDS for 20 min, according to the manufacturer's instruction. The stripped membrane was rehybridized to a ^{32}P-labelled β-actin probe (Nippon Gene, Tokyo, Japan). After development, the intensity of signal band was measured with a densitometer (Shimazu, CS-9000, Japan).

Lectin blot analysis

Cell pellets stored in liquid nitrogen were quickly thawed out, washed three times with cold PBS, and lysed in a cold lysis

buffer for 20 min at 4°C. The buffer contained 140 mM NaCl, 20 mM Tris-HCl pH 7.8, 10% glycerol, 1% Nonidet P-40, 0.1% sodium azide, 1 mM phenyl-methylsulfonyl fluoride, 0.15 U ml^{-1} aprotinin, 10 mM EDTA, and 10 μg ml^{-1} leupeptin. Insoluble material was removed by centrifugation at 15 000 rpm for 15 min at 4°C. Total protein content of the lysate was determined with the BCA kit. Thereafter, cell lysates (3 μg) were boiled for 5 min in a sample buffer containing 0.125 M Tris-HCl (pH 6.8), 2% SDS, and 10% glycerol and subjected to 8% SDS-polyacrylamide gel electrophoresis. The separated proteins were electrophoretically transferred onto a nitrocellulose membrane. Non-specific binding sites on the filter were blocked by incubating the membrane in PBS-T (PBS containing 0.05% Tween-20) supplemented with 3% BSA (bovine serum albumin). After washing with PBS-T three times, filters were then probed with biotinylated E_4-PHA (10 μg ml^{-1}) or biotinylated DSA (10 μg ml^{-1} for 1 h at room temperature. The blots were washed with PBS-T three times and incubated with avidin-horseradish peroxidase complexes (Vector Laboratories Inc., CA, USA) for 30 min. After washing, the membranes were developed using enhanced chemiluminescence (Amersham, UK), according to the manufacturer's protocol.

Fluorescence microscopy

KU812 and KU812F cells were harvested from the culture, washed twice with PBS, and resuspended at a density of 1×10^4 cells per ml in PBS. Thereafter, the cells were stained with FITC-conjugated E_4-PHA (10 μg ml^{-1}) or FITC-conjugated DSA (10 μg ml^{-1}) at 4°C for 30 min. After washing twice, an aliquot of the cell suspension was placed on a slide and mounted in 10% glycerol in PBS. The images of fluorescence were viewed with an epifluorescence microscope (IIRS, Zeiss, Germany) and photographed with a 20 s exposure using a 400 × objective.

Results

Enzyme activity of GnT-III in cell samples from patients with haematological diseases and in cell lines

Figure 2 shows the levels of GnT-III activity in various leukaemia cells. GnT-III activity was above 1.00 nmol h^{-1} mg $protein^{-1}$ in fifteen out of nineteen samples of CML-BC, whereas all twelve samples of CML in chronic phase were below 0.25 nmol h^{-1} mg $protein^{-1}$. The GnT-III activities of two CML-BC samples were more than 10 nmol h^{-1} mg $protein^{-1}$ (12.39 and 17.53 nmol h^{-1} mg $protein^{-1}$ in myeloid crisis and basophilic crisis, respectively), and another two CML samples were 8.33 nmol h^{-1} mg $protein^{-1}$ in myeloid crisis and 6.35 in lymphoid crisis.

Much lower enzyme levels were seen in leukaemia cells from AML, ALL, and CLL patients. In AML and ALL cells, the GnT-III activity was below 0.5 nmol h^{-1} mg $protein^{-1}$ in all samples except one AML and one ALL sample. The samples

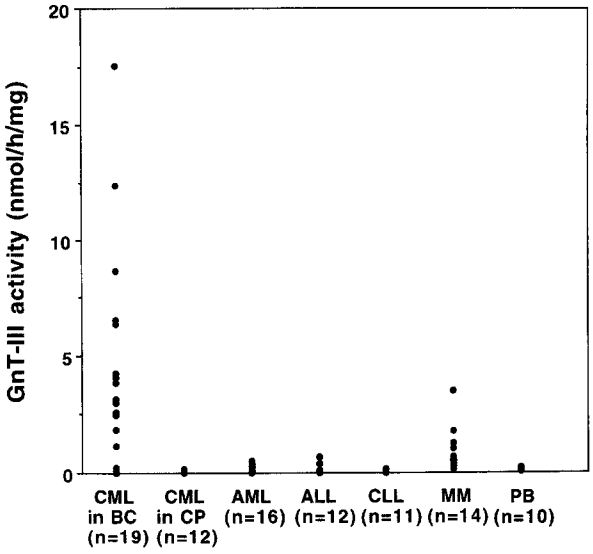

Figure 2. GnT-III activity in haematological diseases and normal subjects. Crude extracts were prepared from cells and the GnT-III activity was assayed as described in 'Materials and methods'. BC and CP denotes blast crisis and chronic phase, respectively. PB indicates mononuclear cells isolated from healthy individuals. The values of GnT-III activity were the average of triplicates for each sample. The figure in parenthesis indicates the number of patients investigated in each group.

from CLL patients showed negligible GnT-III activity (below 0.02 nmol h^{-1} mg $protein^{-1}$), except one sample from a CLL patient who had a monoclonal gommopathy (M-proteinaemia) of unknown origin. In myeloma samples, the enzyme activity ranged from 0.19 to 3.47 nmol h^{-1} mg $protein^{-1}$. The GnT-III activity of mononuclear cells isolated from peripheral blood of seven healthy individuals was less than 0.02 nmol h^{-1} mg $protein^{-1}$ and the activity of the other three was below 0.25 nmol h^{-1} mg $protein^{-1}$.

Based on a Mann-Whitney statistical analysis, the GnT-III activity of leukaemia cells prepared from CML-BC was significantly higher than that of cells from CML patients in chronic phase, the AML, ALL CLL groups, healthy individuals ($p < 0.01$), or the MM group ($p < 0.05$). The GnT-III activity of the cells from CML in the chronic phase was not significantly different from that of cells from patients with other haematological malignancies, except for the CML-BC group.

We also examined the enzyme activity of two CML cell lines, KU812 and KU812F. Interestingly, in the CML cell lines, KU812 had a GnT-III activity of 1.12 nmol h^{-1} mg $protein^{-1}$, while its subclone KU812F showed negligible activity (below 0.02 nmol h^{-1} mg $protein^{-1}$). The GnT-V activity was also examined simultaneously in these patient cell samples and cell lines. Cell samples from CML-BC, CML in chronic phase, AML, ALL, CLL, MM, and PB had a GnT-V activity (mean pmol h^{-1} mg $protein^{-1}$ ± SD) of 563 ± 83, 609 ±

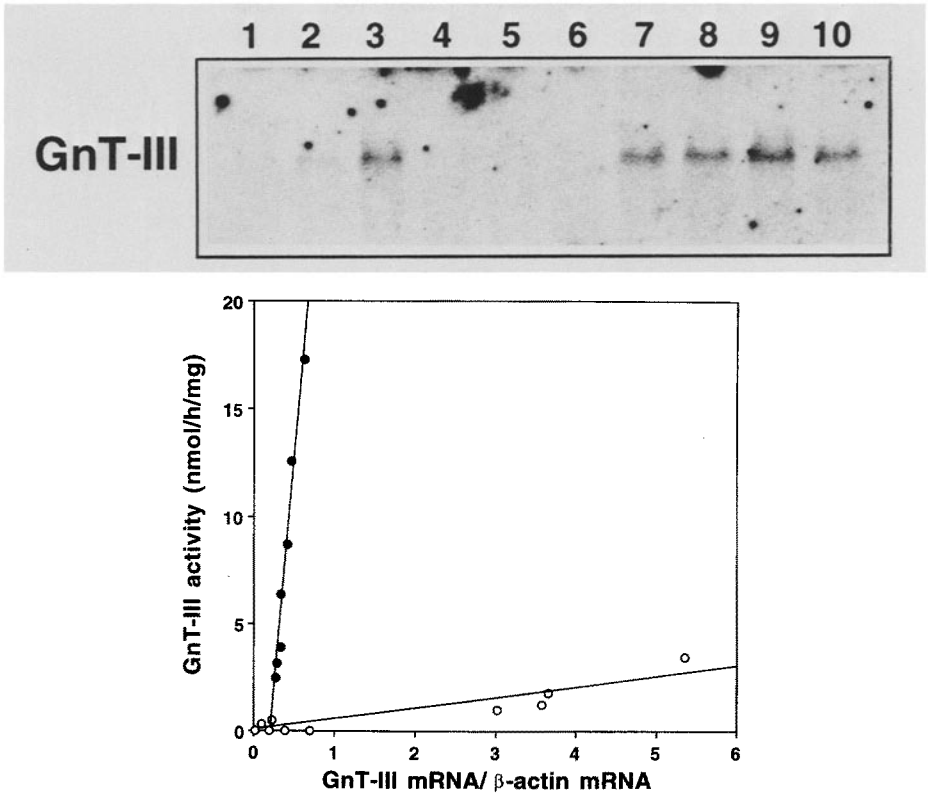

Figure 3. (A) Northern blot of GnT-III mRNA from cells of patients with haematological diseases. Total cellular RNA (20 μg) was electrophoresed, transferred to Zeta-probe membrane, and hybridized with ^{32}P-labelled cDNA probe for GnT-III as described in Materials and Methods. The samples were as follows:CML-BC (lanes 8, 9 and 10), CML in chronic phase (lane 5), AML (lane 2), ALL (lane 4) CLL (lane 1), MM (lane 3 and 7) and normal subjects (lane 6). All transcripts gave a single band of the same size. (B) Correlation between GnT-III activity and GnT-III transcript. The intensity of GnT-III transcript on the blot was determined by densitometry. B actin was used as an internal control. A regression analysis was carried out by plotting the GnT-III activity (Y axis) against a ratio of the intensity of the GnT-III transcript band to that of the actin transcript band (X axis). Samples from CML-BC, and from haematological malignancy other than CML-BC, are indicated as closed circle and open circle respectively.

103, 708 ± 87, 480 ± 58, 622 ± 77, 591 ± 68, and 749 ± 49, respectively. GnT-V activity (pmol h^{-1} mg $protein^{-1}$ ± SD) of KU812 and KU812F was 483 ± 44 and 569 ± 63, respectively. Cell lines with negligible GnT-III activity (below 0.02 nmol h^{-1} mg $protein^{-1}$) were also shown to have GnT-V activity, indicating that the viability of these cell lines was not reduced when the enzyme activity was measured.

Expression of the GnT-III transcript in haematological diseases and association with GnT-III activity

Expression of the GnT-III transcript in leukaemic cells of the haematological malignancies and the leukaemic cell lines was examined by Northern blot analysis (Fig. 3A). In all cells examined, the GnT-III transcript appeared to be a single band of about 4.7 kb, the same size as previously described [7, 20]. In order to analyse statistically whether the GnT-III activity was correlated with the amount of GnT-III transcript, a regression graph was plotted. The intensity of the GnT-III mRNA blot was normalized by using a internal β-actin sample. All clinical specimens used in the regression analysis were hybridized simultaneously on the same membrane in Northern blot analysis. Two regression lines were obtained. With samples from patients with CML-BC (closed circle), we obtained the following equation; $Y = -9.171 + 0.430 \times X$ ($r = 0.960$, $p = 0.02$). On the other hand, samples from other haematological patients (open circle) gave the equation; $Y = 0.081 + 0.0049 \times X$, ($r = 0.790$, $p = 0.05$). These results suggest that the regulation of GnT-III activity in CML-BC was different from the other haematological diseases.

Lectin blot analysis with E_4-PHA and DSA

Elevated GnT-III activity is likely to result in an increase in bisecting GlcNAc on N-glycans. In order to examine whether this change in glycosylation is reflected by GnT-III activity, CML, AML and ALL cell samples with various GnT-III activities were analysed. Whole cell lysates prepared from clinical specimens with haematological malignancies were separated by SDS-PAGE, blotted on to nitrocellulose and probed with the lectins E_4-PHA and DSA. Concanavalin A (Con A), like DSA, can be useful as a negative probe for bisecting GlcNAc,

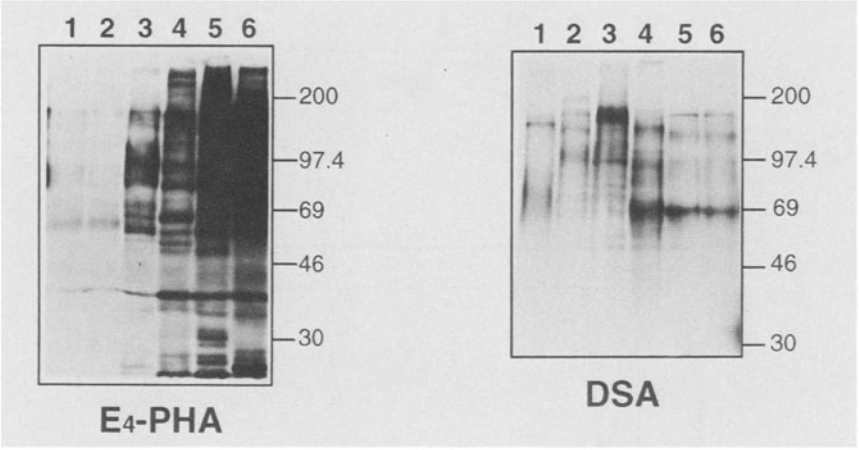

Figure 4. Western blot analysis with biotinylated E_4-PHA and DSA. Whole lysates from cells samples were separated on 8% SDS-PAGE, transferred onto nitrocellulose membranes, blocked with 3% BSA in PBS-T. Then the membranes were probed with biotinylated-E_4-PHA (*left panel*) or biotinylated-DSA (*right panel*), and followed by avidin-peroxidase. The blots were visualized by chemiluminescence. The molecular masses of marker proteins are indicated in kDa at the right side of the panel. The following samples are present in lanes 1–6: healthy individual, CLL, AML, MM, two patients with CML-BC respectively. For further details see text.

because binding of Con A to the core mannose of a bi-antennary N-linked sugar chain [21] is inhibited by a bisecting GlcNAc. In our Western blot analysis, however, use of Con A resulted in high nonspecific chemiluminescence in spite of BSA blocking of the filter. Therefore we chose E_4-PHA [14] as a positive probe and DSA [13] as a negative probe for the identification of bisecting GlcNA on cell-surface molecules.

The GnT-III activity (nmol h^{-1} mg $protein^{-1}$) of the cell samples used for lectin blot in Fig. 4 were as follows: lane 1 (healthy individual), 0.023; lane 2 (CLL), 0.024; lane 3 (AML), 0.528; lane 4 (MM), 2.58; lane 5 (CML-BC) 8.33; lane 6 (CML-BC), 12.39. In Fig. 4, lysates of cell samples from the CML patients in blast crisis showed higher affinity with E_4-PHA, compared with similar extracts from the healthy individual, and the CLL, AML, and MM patients (*left panel*). The binding of E_4-PHA appeared to be correlated with GnT-III activity, implying elevated GnT-III activity catalysed the addition of bisecting GlcNAc to the glycoproteins on CML cells in blast crisis. The affinity of DSA was, however, slightly decreased in the CML cell extracts (lanes 5 and 6) compared to the AML and MM extracts (lanes 3 and 4). This may be due to the inhibition of the binding of DSA in the presence of high amounts of bisecting GlcNAc (*right panel*).

Fluorescence microscopy with FITC-E_4-PHA and FITC-DSA

Surface glycoproteins affected by GnT-III activity was further evaluated using FITC-conjugated lectins and the KU812 cells with high GnT-III activity and its subclone KU812F with negligible GnT-III activity. In Fig. 5, elevated fluorescence with E_4-PHA was observed on KU812 cells compared to the KU812F, while fluorescence of KU812 stained with FITC-DSA was more faint than that of KU812F. These observations indicated that surface glycoproteins on the KU812 cells were affected by the elevated GnT-III activity and glycoproteins with bisecting GlcNAc were increased.

Discussion

Among the malignancies of the B cell lineage investigated in this study, specimens from the MM group showed elevated GnT-III activity. Immunoglobulin G commonly carries two N-linked sugar chains, some which contain bisecting GlcNAc [21]. High GnT-III activity may be occurring in myeloma cells because there is continuous production of immunoglobulin with bisecting GlcNAc. However, CLL is a haematological malignancy of mature B cells and cells from all the CLL patients, except one with monoclonal gammopathy (M-proteinemia), had negligible GnT-III activity. Previously, appreciable GnT-III activity has been reported for two IgG-secreting and two IgM-secreting B cell lines, and less activity for B cells isolated from tonsils [22]. The elevation of GnT-III activity may, therefore, be associated with a large increase in secretion of immunoglobulin, as often detected in the serum of MM patients, rather than with immunoglobulin expressed on the surface of cells, as observed for CLL and tonsillar B cells. ALL, a haematolopoietic malignancy at an immature stage in the lymphoid cell lineage, showed trace GnT-III activity, perhaps reflecting little production of immunoglobulin.

In malignancies of myeloid lineage many CML-BC cells showed GnT-III activity above 1.00 nmol h^{-1} mg $protein^{-1}$. In contrast, all of the CML cells in the chronic phase and most of AML cells showed much lower activity. The samples from four CML patients, two in myeloid, one in lymphoid, and one in basophilic crisis, showed extremely high levels of GnT-III activity, while only trace activity was seen in samples from three other CML patients, one in myeloid, one in lymphoid,

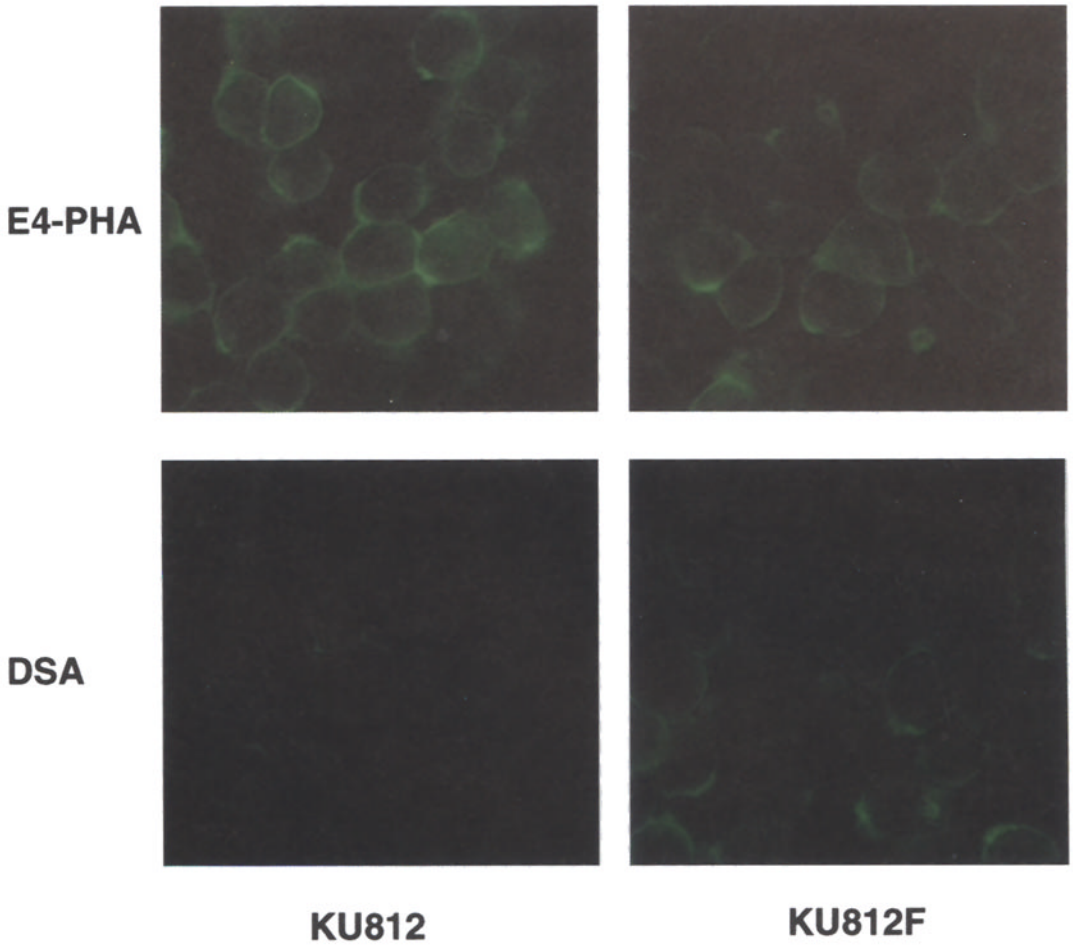

Figure 5. Fluorescence microscopy on KU812 and KU812F cells. KU812 cells (*right panels*) and its subclone KU812F (*left panels*) were stained with FITC-conjugated E_4-PHA (*upper panels*) or FITC-conjugated DSA (*lower panels*), as described in Materials and methods. Stained cells were observed with epifluorescence microscope and photographed.

and one in megakaryocytic crisis. No significant changes, such as the leukaemic cell phenotype, M-proteinaemia, or the co-existence of a non-haematological disorder, were found in the patients with elevated GnT-III activity.

Isolated mononuclear cells from the peripheral blood of healthy individual consist of lymphocytes and monocytes. The cells from the patients with haematological malignancies have a different origin from normal mononuclear cells, for example, AML and CML are the malignant equivalent of myeloid and plasma precursor cells, respectively. So mononuclear cells from the healthy donors are not the best control in this type of investigation. Currently, it is very difficult to prepare an enriched population of myeloid or plasma precursor cells from normal individuals.

The Ph^1 chromosome is a marker of CML. It is caused by a reciprocal translocation between the long arm of chromosomes 9 and 22, t (9:22) (q34; q11). The *GnT-III* gene has been localized to chromosome 22q. 13. 1. in the normal human genome using *in situ* hybridization [7]. The Ph^1 translocation between the long arm of chromosomes 9 and 22, t (9:22) (q34; q11) does not appear to directly promote the GnT-III activity, because there are two segments separating the *GnT-III* gene from the *bcr* region. Moreover, samples of CML patients with the Ph^1 chromosome in the chronic phase, showed low GnT-III activity. Some additional factors, which accompany conversion into blast crisis, must contribute to the high expression of GnT-III activity.

The mechanism by which several patterns of enzyme expression are observed is not clear. Several mechanisms are possible; alternative splicing of the mRNA for the enzyme to produce isoforms with different activities; regulation of promotor activity by growth factors; cytokines or protein kinases; changes in stability of the transcript; alterations in the turnover rate of the enzyme; and post-translational modifications of the enzyme, such as phosphorylation, glycosylation or proteolytic processing. No significant differences

in the *GnT-III* genes were found in haematological cells [20]. Moreover, the transcripts from the various cell types studied were single components of the same size, as shown in the present investigation and our previous study [20]. This indicates that the *GnT-III* gene appears to be present as a single copy without isoforms.

The different relationship between GnT-III activity and the amount of GnT-III mRNA for CML-BC cells compared with other haematological cells was unexpected. It suggested that in CML-BC, the stability of the transcript might be higher, the half-time of GnT-III might be more prolonged, or that post-translational modification might enhance the activity of GnT-III.

In a previous investigation, the CD45 on KU812 cells exibited a higher affinity for E_4-PHA and a lower affinity for DSA than did the CD45 on KU812F cells, indicating that bisecting GlcNAc was added to CD45, catalysed by the elevated GnT-III activity [20]. In this investigation, whole cell lysates from patients with CML-BC showed higher affinity to E_4-PHA and lower affinity to DSA in association with high GnT-III activity, suggesting that bisecting GlcNAc was added to N-glycans attached to many glycoproteins not only CD45 on cells lines, but also in cells from CML patients.

In conclusion, our results revealed that elevated expression of GnT-III is characteristic of CML cells in blast crisis. The mechanism for this finding is now under investigation in our laboratory. Elevated GnT-III activity is characteristic of this condition, and clinically it could be used as a disease marker for CML cells converted into blast crisis.

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