Human Leukemic Myeloblasts and Myeloblastoid Cells Contain the Enzyme Cytidine 5'-Monophosphate-N-acetylneuraminic Acid:Galβ1-3GalNAcα(2-3)-sialyltransferase

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ABSTRACT

We have examined the role of CMP-NeuAc:Galβ1-3GalNAc-R α(2-3)-sialyltransferase in fresh leukemia cells and leukemia-derived cell lines. Enzyme activity in normal granulocytes using Galβ1-3GalNAcα-α-nitrophenol as a substrate was 1.5 ± 0.7 nmol/mg/h whereas activity in morphologically mature granulocytes from 6 patients with chronic myelogenous leukemia (CML) was 4.2 ± 1.6 nmol/mg/h (P < 0.05). Myeloblasts from 5 patients with CML in blast crisis showed enzyme activity levels of 2.5 nmol/mg/h. From 2 patients with CML, both blasts and granulocytes were obtained, with higher enzyme activity in the patients' blasts (7.1 nmol/mg/h) than in their granulocytes (4.9 nmol/mg/h) in both cases, suggesting that the increase in enzyme activity is related to the differentiation or proliferation status of the CML cells. However, similarly high enzyme levels were also seen in myeloblasts from acute myeloblastic leukemia patients (5.6 ± 1.4 nmol/mg/h) and in some acute myeloblastic leukemia-derived cell lines (KG1a and HL60), suggesting that increased levels of this enzyme are not directly correlated with the presence of the Ph1 chromosome. This α(2-3)-sialyltransferase activity can also be detected in normal peripheral blood lymphocytes and exhibits increased activity in chronic lymphocytic leukemia cells and acute lymphoblastic leukemia. These data suggest that the level of enzyme activity may vary with growth rate and maturation status in myeloid and lymphoid hematopoietic cells. Finally, we have identified a glycoprotein in acute myeloblastic leukemia cells that serves as a substrate for the α(2-3)-sialyltransferase. The desialylated form of the glycoprotein was resialylated in vitro by the purified placental form of this α(2-3)-sialyltransferase and exhibits a molecular weight of about 150,000.

INTRODUCTION

CML is characterized by early release of myeloid cells from bone marrow into the peripheral blood and a marked increase in the circulation time of the leukemic granulocytes (1). We and others have shown that CML cell membranes are more highly sialylated than normal granulocyte membranes (2-4). Consistent with these data is the observation that the binding of the galactose-specific lectin of Ricinus communis (RCA1) to CML granulocytes is significantly increased after neuraminidase treatment (5). In vitro studies of granulocyte function in CML compared to normals have demonstrated decreased adhesiveness (6), decreased chemotaxis (7), and reduced membrane binding of the chemotactic peptide N-formylmethionyl-leucylphenylalanine (8). These altered functions are partially reversible by removal of membrane sialic acid with neuraminidase, suggesting a role for aberrant sialylation in the abnormal cell behavior (8). Sialic acids are common constituents of both the O- and N-linked glycan chains of glycoproteins (9), as well as of many glycolipids (10). They are found in a variety of linkage patterns to galactose, GalNAc, GlcNAc, or other NeuAc moieties. Nevertheless, there seem to be many more sialyltransferases than there are sialic acid linkages, thus supporting the contention (11) that the activity of these enzymes in various tissues is probably largely regulated by the strict substrate specificity of each sialyltransferase. For example, the α(2-3)-sialyltransferases (EC 2.4.99.4) which have been purified from porcine submaxillary gland (12, 13) and human placenta (14), specifically sialylate the galactosyl residue of Galβ1-3GalNAc-R via an α-2 linkage but cannot synthesize the NeuAcα2-3Galβ1-4GlcNAc-R product. These sialyl transferases will also use the gangliosides GM3 and GD1b as substrates (13, 14) as well as asialo-GM1, since these glycolipids contain the required unsubstituted Galβ1-3GalNAc-R sequence (reviewed in Ref. 15).

Lectin studies utilizing peanut agglutinin, which binds most avidly to Galβ1-3GalNAc moieties, have suggested that the aberrant sialylation in CML cell membranes occurs on O-linked glycans (6). We have shown previously that an enzyme which specifically catalyzes the synthesis of NeuAcα2-3Galβ1-3GalNAc-R is present in human granulocytes and has increased activity in CML granulocytes, possibly accounting for the aberrant sialylation and playing a pathophysiological role in CML (16).

CML granulocytes may represent a population of cells less mature than normal granulocytes, in which case the increased α(2-3)-sialyltransferase would reflect relative immaturity of the leukemic cells. Myeloid cells exhibiting a less differentiated phenotype are readily available from both CML patients (in myeloid blast crisis) as well as from patients with acute myeloblastic leukemia. Relatively undifferentiated cells are also available in the form of leukemia-derived cell lines, some of which, e.g., K562 (17), EM2, and EM3 (18) were derived from Ph1-positive leukemic blasts. Thus we have studied the levels of sialyltransferase activity in fresh leukemia samples as well as leukemia-derived cell lines of both myeloid and lymphoid lineages. Finally, we have attempted to identify some of the glycoproteins which serve as the natural substrates for this enzyme in intact cells.

MATERIALS AND METHODS

Cells and Cell Lines. More than 95% morphologically mature granulocytes from both CML and normal samples were obtained from...
EDTA-anticoagulated peripheral venous blood after dextran sedimentation, ammonium chloride lysis, and layering of leukocytes onto a double gradient of Hypaque and dextran, as described previously (6, 19). Leukemic blast cells (as defined by hematological and phenotypic criteria) were obtained from patients with acute leukemia and CML in myeloid blast crisis having greater than 70% blasts in the peripheral blood. Mononuclear cell fractions were prepared from heparinized peripheral venous blood by Ficoll-Hypaque density gradient centrifugation as reported previously (19). Hematopoietic cell lines were obtained from the American Tissue Culture Collection (Rockville, MD) or from colleagues in Toronto. Cell lines were maintained in RPMI 1640 with 10% heat-inactivated fetal calf serum in a 5% CO₂ atmosphere at 37°C. All cells and cell lines were washed 3 times in saline and stored at -70°C as pellets of 2 x 10⁶ cells.

Sialyltransferase Assays. Enzyme assays were set up as previously described (8, 19). Briefly, frozen pellets of 2 x 10⁶ cells were resuspended in 2 ml of 0.2 M NaCl, washed in saline, treated with 10 units of DNase (Sigma, St. Louis, MO), washed again in saline, and solubilized in 1% Triton X-100 at 4°C for 20 min. Debris was removed by centrifugation at 1000 x g for 10 min. The supernatant, a solubilized total cell membrane preparation, was used for the assays. Aliquots (20 µl) of this supernatant (typically containing 40–60 µg of protein) were incubated with 10 µl of 0.05 M Tris-HCl (pH 7.2), 10 µl of 0.5 mM CMP-[4-'²⁴C]sialic acid (0.06 µCi, 25 mCi/mmol; New England Nuclear), and 10 µl of the substrate Galβ1–3GalNAcα-ONP (2 mM final concentration). The Km for CMP-[4-'²⁴C]sialic acid was previously shown to be 0.18 mM, and that for Galβ1–3GalNAcα-ONP was 0.3 mM (16). Reaction mixtures were incubated at 37°C for 1 h, and the reaction was terminated by addition of 15% (w/v) trichloroacetic acid and 5% (w/v) phosphotungstic acid and the resulting precipitate incorporated into organic counting scintillation fluid. Glacial acetic acid (7 µl) was added to the contents of each scintillation vial. After extensive washing away of unbound material, the bound glycoproteins were slowly eluted with cell lysis buffer supplemented with 4% D-galactose. The eluted fraction (10 ml) was dialyzed and concentrated against 3- x 1-liter volumes of 0.01 M Tris-saline, pH 7.4. The glycoprotein solution was brought to 70% with ethanol and the proteins were precipitated overnight at -70°C. The precipitated glycoprotein fraction was pelleted at 10,000 x g for 10 min. The pellet was dissolved in 0.1 M NaCl, 7.4 M glycine, and 1 mM EDTA at pH 11. The solution was dialyzed against 20 mM Tris-HCl, pH 8.0, containing 0.5 M NaCl. The dialyzed solution was then applied to a large column of Dowex AG 2-X8, Cl⁻ form, 200-400 mesh (Bio-Rad Laboratories). Enzyme activity was assayed in 2 ml of 0.1 M NaCl, 0.5 M morpholinoethanesulfonic acid (pH 6.0), containing 40 mM MnCl₂, 10 mM 12 mM UDP-[14C]galactose (0.05 µCi, 337.0 mCi/mmol; New England Nuclear) and 0.05 M Tris-HCl, pH 7.4, containing 0.5% nonidet P-40, and 1 mM Mg²⁺ and Mn²⁺. Solubilized glycoprotein solution (50 µl) was incubated with 10 µCi CMP-[4-¹⁴C]sialic acid diluted as above and 0.05 milliunit of purified placental 2–3-sialyltransferase for 1 h at 37°C. The reaction was terminated by addition of 25 µl (x5) SDS-PAGE gel sample buffer. The reaction tubes were boiled and the products were resolved by SDS-PAGE and fluorography (22). Similar experiments were performed using CML granulocytes.

RESULTS

In the first series of experiments, levels of α(2–3)-sialyltransferase activity were measured in granulocytes from 6 normal volunteers, granulocytes from 6 patients with CML in chronic phase, and myeloblasts from 5 patients with CML in myeloid blast crisis (Table 1). Using the synthetic substrate Galβ1–3GalNAcα-ONP, which is a specific acceptor for this sialyltransferase, enzyme activities were 1.5 ± 0.7 (SD) nmol/mg/h in normal granulocytes, 4.2 ± 1.6 nmol/mg/h in CML granulocytes, and 6.7 ± 2.2 nmol/mg/h in CML myeloblasts, suggesting a hierarchy in which leukemic blast cells have higher enzyme levels than leukemic granulocytes, which in turn have higher enzyme levels than normal granulocytes. In 2 patients with CML blast crisis, individual myeloblast and granulocyte preparations were obtained. Higher sialyltransferase activity was observed in the patients’ blasts than their granulocytes in both cases (Table 1).

To study the distribution of this enzyme in other cell types of the hematopoietic system, a number of lymphoid and myeloid-derived cell lines were collected and assayed for α(2–3)-sialyltransferase activity. In these preliminary experiments, a
range of enzyme activity levels was detected ranging from 4.5 nmol/mg/h for KG1a cells to only 0.3 nmol/mg/h for the B-lymphoma-derived Daudi cell line. Significantly, however, there were no differences in α(2-3)-sialyltransferase activity between those cell lines which contained the Ph1 chromosome (K562, EM2, and EM3) and those which did not (KG1, KG1a, HL60, U937, Nalm-6, HOON, Daudi, GH-1, HSB-2, and Jurkat) (data not shown).

In the next series of experiments, a large number of chronic and acute leukemia samples of both lymphoid and myeloid types were collected and assayed for α(2-3)-sialyltransferase activity. As shown in Table 2A, myeloblasts from 17 untreated AML patients contained relatively high sialyltransferase levels (5.6 ± 3.7 nmol/mg/h), similar to the levels detected in myeloblasts of 8 CML blast crisis samples (6.2 ± 2.2 nmol/mg/h).

In cells of the lymphoid lineage, unstimulated peripheral blood lymphocytes contained relatively low levels of the α(2-3)-sialyltransferase activity (1.2 ± 0.3 nmol/mg/h), similar to those found in normal granulocytes (1.3 ± 0.4 nmol/mg/h). Interestingly, lymphocytes from 4 untreated chronic lymphocytic leukemia patients contained increased levels of this transferase activity (mean, 3.76 nmol/mg/h). Lymphoblasts from 4 acute lymphoblastic leukemia patients contained even higher levels of α(2-3)-sialyltransferase activity (mean, 4.7 nmol/mg/h), once again indicating a hierarchy in which leukemic lymphoblasts have the highest levels, followed by leukemic lymphocytes, and finally normal peripheral blood lymphocytes.

Samples taken from CML patients before or after chemotherapy were assayed for sialyltransferase activity. Higher levels of enzyme activity were found in the samples preceding a period of chemotherapy (Table 2A) than after treatment (Table 2B). Enzyme activities in granulocytes from treated patients were almost the same as those in normal control granulocytes. In a smaller series of CLL patients, a similar phenomenon was observed (Table 2B).

β3-Galactosyltransferase activity was determined in normal and leukemic granulocytes and leukemic myeloblasts using the substrate asialo-ovine submaxillary mucin (Table 3). Normal granulocytes and myeloblasts from CML patients in blast crisis exhibited very similar β3-galactosyltransferase activities (14.2 ± 3.0 nmol/mg/h). CML granulocytes showed lower activities (10.3 ± 4 nmol/mg/h) and AML blasts from 4 patients were higher on the average than normal granulocytes and CML blasts. Moreover, wide variations were observed among AML patients (20.5 ± 9.2 nmol/mg/h). These differences were not statistically significant.

Natural Substrates of the α2-3-Sialyltransferase. The peanut agglutinin bound/eluted fraction of a neuraminidase-treated

<table>
<thead>
<tr>
<th>Table 2</th>
<th>α(2-3)-Sialyltransferase activity* in leukemia cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patient samples</td>
</tr>
<tr>
<td>A. Untreated patients</td>
<td>CML granulocytes</td>
</tr>
<tr>
<td></td>
<td>CML myeloblasts</td>
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<tr>
<td></td>
<td>AML myeloblasts</td>
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<td>CLL lymphocytes</td>
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<td></td>
<td>ALL lymphoblasts</td>
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<tr>
<td>B. Treated patients</td>
<td>CML granulocytes</td>
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<td></td>
<td>CLL lymphocytes</td>
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<tr>
<td>C. Normal controls</td>
<td>Granulocytes</td>
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<td></td>
<td>Peripheral blood lymphocytes</td>
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</tbody>
</table>

* Mean sialyltransferase activity expressed as nmol/mg/h using the substrates Galβ1-3GalNAc-ONP or Galβ1-3GalNAc-PNP.

### Table 3 β3-galactosyltransferase activity in leukemic cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Sample no.*</th>
<th>β3-Galactosyl transferase activity (nmol/mg/h) using asialo-OSM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal granulocytes</td>
<td>6</td>
<td>14.2 ± 3.0</td>
</tr>
<tr>
<td>CML granulocytes</td>
<td>6</td>
<td>10.3 ± 4.0</td>
</tr>
<tr>
<td>CML blasts</td>
<td>5</td>
<td>14.2 ± 6.0</td>
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<tr>
<td>AML blasts</td>
<td>5</td>
<td>20.5 ± 9.2</td>
</tr>
</tbody>
</table>

* Normal granulocytes were tested from 12 normal donors pooled in pairs to obtain sufficient cells for assay.

DISCUSSION

We have shown previously that CMP-NeuAc:Galβ1-3GalNAc-R α(2-3)-sialyltransferase is present in human leukocytes and has increased activity in granulocytes from patients with chronic myelogenous leukemia (16). The human placental form of this enzyme which is similar, if not identical to the leukocyte form, exhibits relatively restricted substrate specificity and will efficiently catalyze the addition of NeuAc in α(2-3) linkage to unsubstituted Galβ1-3GalNAc-R structures (14). In our previous studies (16), evidence was not found for the
presence of either the GalNAc \( \alpha \)-protein \( \alpha(2-6) \)-sialyltransferase (EC 2.4.99.3) or the (NeuAc\( \alpha \)-2\( \rightarrow \)3Gal\( \beta \)-1\( \rightarrow \)3GalNAc\( \alpha \)-R \( \alpha(2-6) \)-sialyltransferase (EC 2.4.99.7), neither of which can sialylate the Gal\( \beta \)-1\( \rightarrow \)3GalNAc\( \alpha \)-ONP (23, 24) in CML or nor-

sialyltransferase (EC 2.4.99.3) or the (NeuAc\( \alpha \)-2\( \rightarrow \)3Gal\( \beta \)-1\( \rightarrow \)3)GalNAc-R accepts a glycoprotein containing the substrate sequence as an acceptor substrate for the \( \alpha(2-3) \)-sialyltransferase (3). Compared to those of normal granulocytes, the leukosialins isolated from immature myeloblasts contained mainly short \( \alpha \)-linked oligosaccharides, including NeuAc\( \alpha \)-2\( \rightarrow \)3 Gal\( \beta \)-1\( \rightarrow \)3GalNAc and NeuAc\( \alpha \)-2\( \rightarrow \)3Gal\( \beta \)-1\( \rightarrow \)3(Neu Ac\( \alpha \)-2\( \rightarrow \)6)Gal-

NAC. The detection of those structures in immature myeloid cells correlates well with our observations that such cells contain significantly elevated levels of the \( \alpha(2-3) \)-sialyltransferase.

Since AML cells and myeloblasts from CML patients in blast crisis contained the highest levels of this \( \alpha(2-3) \)-sialyltransferase, we tried to isolate natural substrates from those cells, i.e., glycoproteins containing the substrate sequence Gal\( \beta \)-1\( \rightarrow \)3GalNAc-R. Thus, a neuraminidase-treated, PNA-agarose bound glycoprotein fraction was prepared and was shown to contain a substrate molecule for the purified placental form of this \( \alpha(2-3) \)-sialyltransferase (14). After in vitro resialylation, the product of this catalysis was resolved as a broad band both a molecular weight of approximately 150,000 in reduce SDS-PAGE. Since many of the leukosialin molecules exhibit a similar apparent size in SDS-PAGE (32), particularly after desialylation (22), it is possible that this M, 150,000 structure may be a member of the leukosialin family. No substrate structures could be isolated from CML granulocytes using similar procedures. A possible explanation for this is that the lysis of granulocytes under the conditions we used here results in the release of proteases from lysosomal vesicles which destroy the substrate molecule(s) before they can be isolated on the PNA lectin column. More likely explanations are that since leukosialins in CML cells contain longer, more complex, and/or branched \( \alpha \)-glycans than those in AML cells (3), the desialylated forms of these leukosialins would not bind to the Gal\( \beta \)-1\( \rightarrow \)3GalNAc-R-specific PNA. Furthermore, these desialylated structures would not be good substrates for the \( \alpha(2-3) \)-sialyltransferase. The biochemical nature of \( \alpha \)-linked sialylated surface glycoproteins, leukosialins, or sialophorins may be important in the physiology of leukocyte recirculation (33). The increase in expression of \( \alpha(2-3) \)-sialyltransferase in myeloid blasts and the decrease in activity with maturity to normal granulocytes may reflect a physiological role for \( \alpha \)-linked sialylation in adhesion and receptor binding. The permanence of this otherwise transient change in leukemic myeloid cells may contribute to this abnormal pathophysiology (8). It may be of interest that the \( \alpha(2-3) \)-sialyltransferase enzyme is encoded by a gene on chromosome 11 suggesting a role for this chromosome site in normal myeloid differentiation and leukemic pathophysiology (34).

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**REFERENCES**


SIALYLTRANSFERASES IN HUMAN LEUKEMIC CELLS


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