Aberrant Sialylation of Granulocyte Membranes in Chronic Myelogenous Leukemia

By M. A. Baker, R. N. Taub, C. H. Whelton, and A. Hindenburg

Peripheral blood granulocytes from patients with chronic myelogenous leukemia (CML) were studied for accessibility of membrane sialic acid and galactose residues to sodium borohydride-3H radiolabeling after oxidation with sodium metaperiodate (Pl/B3H4) or with galactose oxidase (GO/B3H4). Granulocytes from untreated patients with chronic myelogenous leukemia showed increased radiolabeling with Pl/B3H4 and decreased labeling with GO/B3H4 when compared to normal granulocytes. Granulocytes from leukemic patients receiving chemotherapy showed normal labeling patterns. Gel electrophoresis of membrane extracts showed that the changes in Pl/B3H4 and GO/B3H4 reactivity of CML cells were distributed over all membrane protein bands. Our data suggest that CML granulocyte membrane proteins are aberrantly sialylated, with decreased accessibility of galactose residues, and that these changes may be reversed by clinical drug treatment.

**CHRONIC MYELOGENOUS LEUKEMIA**

(CML) is characterized by hyperleukocytosis, due in part to premature release from bone marrow of cells at all stages of myeloid differentiation and to prolonged intravascular circulation of leukemic neutrophils.1-3

We have previously shown that CML granulocytes are deficient in binding sites for peanut lectin (specific for gal-B-1,3-galNAc determinants), but agglutinate excessively with the lectin limulin (specific for sialic acid determinants), and that these changes are associated with diminished granulocyte adherence to column-packed nylon wool.4 This suggests that, as in other blood cell systems,5-7 changes in membrane glycoconjugates may play a role in the pathophysiologic behavior of CML granulocytes. We have therefore studied the granulocytes from untreated and treated CML patients after oxidation-reduction tritium labeling to more directly determine alterations in surface membrane sialic acid and galactose residues.

**MATERIALS AND METHODS**

Peripheral blood was obtained by venipuncture from CML patients and normal volunteers and anticoagulated with EDTA. Following removal of red cells by dextran sedimentation and ammonium chloride lysis, washed leukocytes were layered onto a double gradient of Hypaque and dextran to obtain a neutrophil-rich fraction.8 Cells at all stages of myeloid differentiation and to prolonged intravascular circulation of leukemic neutrophils.1-3

Peripheral blood granulocytes from patients with chronic myelogenous leukemia (CML) were studied for accessibility of membrane sialic acid and galactose residues to sodium borohydride-3H radiolabeling after oxidation with sodium metaperiodate (Pl/B3H4) or with galactose oxidase (GO/B3H4). Granulocytes from untreated patients with chronic myelogenous leukemia showed increased radiolabeling with Pl/B3H4 and decreased labeling with GO/B3H4 when compared to normal granulocytes. Granulocytes from leukemic patients receiving chemotherapy showed normal labeling patterns. Gel electrophoresis of membrane extracts showed that the changes in Pl/B3H4 and GO/B3H4 reactivity of CML cells were distributed over all membrane protein bands. Our data suggest that CML granulocyte membrane proteins are aberrantly sialylated, with decreased accessibility of galactose residues, and that these changes may be reversed by clinical drug treatment.

Surface compounds were extracted and solubilized by incubation of the cells in 1% Triton X-100 and 0.25 M phenylmethylsulfonylfluoride at 4°C for 5 min. The supernatants were recovered following centrifugation at 5,000 g for 5 min at 4°C and were counted in a scintillation counter.

SDS polyacrylamide gel electrophoresis was performed on 3H-sodium borohydride-labeled cell surface extracts, with labeled samples obtained from equivalent cell numbers. Supernatants were mixed with 4% SDS, 10% 2-mercaptoethanol, and 2% glycerol, boiled for 90 sec, applied to a slab gel, and electrophoresed by the method of Laemmli.9 Fixed gels were stained with Coomassie blue or fluorographed by the method of Bonner and Laskey10 as follows: gels were dried after treatment with Enhance solution (NEN Canada Ltd.) and covered with Kodak X-Omat AR film for 7 days at -80°C. Films were developed by automatic processing and examined both visually and by Joyce-Loebl Chromoscan densitometry.

**RESULTS**

Studies were performed on granulocytes obtained from seven CML patients in the chronic phase who had never received chemotheraphy and from ten CML patients who had been clinically treated with chemotherapy. Granulocytes from several normal subjects and three nonleukemic leukocytic patients were used as controls. All CML patients were positive for the Philadelphia chromosome. Total white blood cell counts of untreated CML patients were uniformly high, with a mean of 94.0 x 10⁹/liter and a range of 54–200 x 10⁹/liter. The treated CML patients had a mean total white count of 18.5 x 10⁹/liter, with a range of 9.6–45.4 x 10⁹/liter. The patients had been treated with intermittent busulfan (8 patients) or hydroxyurea (2 patients), and at the time of testing, all patients had been off chemotherapy for at least 2 wk. The 7 normal volunteers were healthy laboratory staff.
with a mean white count of $5.7 \times 10^9$/liter (range 4.8–7.0 $\times 10^9$/liter). The 3 nonleukemic samples were obtained from patients with bacterial infection, all of whom were receiving antibiotics but not corticosteroids; their white counts were 17.3 $\times 10^9$/liter, 25.8 $\times 10^9$/liter and 35.4 $\times 10^9$/liter.

Granulocytes from the seven patients with clinically untreated CML in the chronic phase showed diminished labeling with galactose oxidase-sodium borotritide ($^3$H) compared to neutrophils from the ten normal or leukocytotic controls (Table 1). Neutrophils from the ten CML patients receiving chemotherapy showed GO/B$^3$H$_4$ and PI/B$^3$H$_4$ labeling patterns similar to normals. Pretreatment of CML cells in vitro with *Vibrio cholerae* neuraminidase (VCN) increased GO/B$^3$H$_4$ labeling twofold and reduced PI/B$^3$H$_4$ labeling by approximately one-third, to values similar to those obtained after VCN treatment of normal cells.

SDS gel electrophoresis of labeled membrane extracts from untreated CML granulocytes showed generalized decreased labeling of all bands with GO/B$^3$H$_4$ and generalized increased labeling of all bands with PI/B$^3$H$_4$. Densitometry reading was used to give measurable estimates of relative band densities and showed the most prominent changes in the band with estimated molecular weight of 120,000 daltons. Gels and densitometry readings from one patient are illustrated in Figs. 1 and 2.

**DISCUSSION**

Peripheral blood granulocytes from patients with CML show increased membrane reactivity with sodium metaperiodate/sodium borohydride ($^3$H) and decreased reactivity of membranes with galactose oxidase when compared to normal granulocytes. SDS gel chromatography shows that these reactions occur on many membrane proteins, including the major 120,000-dalton glycoprotein described by Gahmberg and Anderson. These differences are no longer present after treatment of cells with neuraminidase and are not seen in granulocytes from patients receiving chemotherapy with busulfan. These data suggest that, in CML, there is decreased availability of membrane galactose secondary to increased or aberrant terminal sialic acid. These changes may reflect the synthesis of a unique glycoprotein by CML cells, as has been suggested by Van Beek, a relative increase in normal sialylated glycipeptides, or the synthesis of glycoproteins containing more sialyl groups per polypeptide chain. The latter possibility is most likely, as both normal and CML cells show comparable availability of membrane galactose after treatment with neuraminidase.

In our previous study, VCN treatment of normal or CML cells exposed equivalent numbers of binding sites for $^{125}$I-labeled peanut lectin, suggesting that O-linked synthesis of gal-B-1,3-gal-NAc determinants was unimpaired in CML cells. Also, we found normal agglutination of CML cells with the lectin, concanavalin A, indicating that N-linked synthesis of membrane man-

**Table 1.** Surface-Labeled Activities of Sialosyl or Galactosyl Residues of Granulocytes

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>No Neuraminidase</th>
<th>Neuraminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Galactose Oxidase</td>
<td>Periodate</td>
</tr>
<tr>
<td>Normal granulocytes (7 patients)</td>
<td>86 ± 8</td>
<td>113 ± 14</td>
</tr>
<tr>
<td>CML granulocytes (7 patients)</td>
<td>49 ± 6†</td>
<td>137 ± 6‡</td>
</tr>
<tr>
<td>CML granulocytes (10 patients, treated with busulfan)</td>
<td>65 ± 9</td>
<td>110 ± 6</td>
</tr>
</tbody>
</table>

* All tests were done on $2 \times 10^7$ granulocytes, with triplicate samples from each patient, reported as the mean and standard deviation of the counts per minute $\times 10^3$.

† $p = 0.01$ compared to normals.

‡ $p = 0.05$ compared to normals.
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nose was most likely normal as well. This again suggests that sialic acid synthesis per se may be abnormal in these cells.

The experiments of Gesner and coworkers demonstrated that cell surface sialic acid is important in maintaining the integrity of red cells or lymphocytes in the circulation.5,12

Alterations in membrane glycoconjugates, particularly sialic acid, occur during the maturation of thymocytes,13 intestinal crypt cells,14 and fetal hematopoietic cells.15 Membrane sialylation affects neutrophil chemotaxis16 and adherence to nylon wool.4

Masking of cell surface receptors for adherence and chemotaxis by sialic acid would contribute to the hyperleukocytosis and the profusion of immature forms characteristically found in the blood smear in chronic myelogenous leukemia. Accrual of sialic acid with masking of other surface carbohydrate residues and reduction of intercellular adhesion may be involved in the release of maturing granulocytes from bone marrow stromal elements, just as has been found in thymocyte maturation.13 Excessive sialylation of immature myeloid forms could thus cause their precocious release into the blood.

Similar sialoglycopeptide alterations may be related to the pathophysiology of other malignant states.7 Lloyd17 has argued that surface hypersialylation may be an important determinant of malignant cell behavior because of its tendency to reduce intercellular adhesion and to mask cell receptors for environmental regulatory stimuli. Yogeeswaran and Salk18 and Fogel et al.19 have shown that the ability of murine tumor cells to metastasize spontaneously from subcutaneous sites is positively correlated with the degree of sialylation of galactosyl and N-acetylgalactosaminyl residues in cell surface glycoconjugates. Sasaki et al. have shown that sialyltransferase activity is increased in blast cells from acute nonlymphoblastic leukemia compared to the level in normal mature granulocytes.20

Our studies of cells from busulfan-treated patients indicate that the oligosaccharide abnormalities of CML may be reversible by chemotherapy. Response to treatment may reflect the suppression of a hypersialylated subclone of CML cells, allowing the more normal precursors to predominate. It is also possible that busulfan or other antiproliferative agents used in the therapy of CML exert direct effects on membrane oligosaccharide biosynthesis.21,22

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