Enzymic Control of the Expression of the X Determinant (CD15) in Human Myeloid Cells During Maturation: The Regulatory Role of 6'-Sialyltransferase

By Patricia O. Skacel, Andrew J. Edwards, Coral T. Harrison, and Winifred M. Watkins

To establish the basis for the reduced expression of the X determinant on leukemic blasts and the changes in antigenic expression that occur during myeloid maturation, the presence on myeloid cells of X and related structures was examined in conjunction with studies on the activities of the glycosyltransferases involved in their biosynthesis. Expression of X and sialyl-X was weak on blasts in comparison with neutrophils despite the presence of the requisite precursor structures. Much higher levels of 3'-fucosyltransferase activity were found in blasts than in neutrophils when nonsialylated substrates were used, but, whereas the enzyme in neutrophils reacted equally well with 3'-sialylated and nonsialylated acceptors, the enzyme in blasts showed a marked preference for nonsialylated substrates. 6'-Sialyltransferase activity was strong in blasts but was not detectable in neutrophils, whereas a much lower level of 3'-sialyltransferase activity was present in both blasts and neutrophils. Dimethyl sulfoxide-induced maturation of HL60 cells was associated with (1) a decrease in both 6'-sialyltransferase and 3'-fucosyltransferase activities, (2) a change in the substrate specificity of 3'-fucosyltransferase towards that found in mature cells, and (3) increased cell surface expression of sialyl-X. These results suggest that the reduced expression of X in myeloblasts is related to the presence of the strong 6'-sialyltransferase, which uses the precursor substrate at the expense of the 3'-fucosyltransferase and prevents the synthesis of X and sialyl-X. The developmental regulation of the levels of 3'- and 6'-sialyltransferases, and the level and specificity of the 3'-fucosyltransferases, therefore controls the expression of X and its degree of sialylation.

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From the Divisions of Immunochemical Genetics and Transplantation Biology, MRC Clinical Research Centre, Middlesex, UK.

Supported by the Medical Research Council and a grant from the Leukaemia Research Fund, UK.

Address reprint requests to Patricia Skacel, MB, Department of Haematology, Northwick Park Hospital, Watford Rd, Harrow, Middlesex HA1 3UJ, UK.

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MATERIALS AND METHODS

Cell Isolation

Neutrophils from seven normal volunteers were isolated from EDTA anticoagulated blood by density gradient centrifugation on

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Ficoll-Paque at 40g for 30 minutes.29 Erythrocytes contaminating the residual pellet were removed by hypotonic lysis in water restored to 0.15 mol/L saline after 30 seconds.

Leukemic blast cells from seven patients with AML were isolated from marrow taken into heparinised (20 U/mL) RPMI. Cells were harvested from the mononuclear layer after centrifugation on Ficoll-Paque at 800g for 20 minutes. Leukemia was classified into French-American-British (FAB) subtypes on the basis of morphology and cytochemistry of marrow smears.30 All cells were washed twice in 0.15 mol/L NaCl containing 10 mmol/L Tris-HCl pH 7.2 before final suspension in 0.15 mol/L NaCl (for glycosyltransferase assays) or phosphate-buffered saline (PBS) containing 2% fetal calf serum (FCS) and 0.1% azide (for marker studies). Cells were counted in a hemocytometer. Purity and morphology of cell suspensions were assessed from cytocentrifuge preparations stained with May-Grünwald-Giemsa.

**Enzyme Treatment**

Neuraminidase was used to remove terminal sialic acid residues from cells before marker studies. Cells suspended in PBS/FCS (10 x 10^6 cells in 300 μL) were incubated with 30 μL *Vibrio cholerae* neuraminidase 1.0 U/μL (Koch-Light Ltd, Haverhill, UK) for 30 minutes at 37°C. The cells were washed twice before final suspension in PBS/FCS/azide.

**Cell Culture**

HL60 cells were obtained from the European Collection of Animal Cell Cultures (PHLS Centre for Applied Microbiology and Research, Porton Down, UK) and grown in Falcon tissue culture flasks in RPMI (Flow Laboratories, High Wycombe, UK) supplemented with 10% heat-inactivated (56°C for 30 minutes) FCS and 2 mmol/L glutamine without antibiotics at 37°C in a humidified atmosphere of 5% CO₂. Cells were passaged twice weekly to maintain exponential growth.

Granulocytic differentiation was induced with DMSO. Cells were seeded at an initial concentration of 2.5 x 10^6/ml into duplicate flasks containing fresh medium with or without 1.3% DMSO. After periods of growth between 30 minutes and 9 days, cells were harvested from the culture medium by centrifugation at 800g for 5 minutes and examined for morphologic evidence of differentiation.31 Viability was greater than 95% in all experiments, as judged by Trypan Blue dye exclusion. In addition, cytochrome c reduction was used to assess functional differentiation of induced cells.31

KG1 cells were kindly supplied by Dr M.Y. Gordon (Institute of Cancer Research, London, UK). They do not differentiate in response to DMSO32 and were cultured in identical conditions to HL60 cells as controls, to exclude a direct effect of DMSO on glycosyltransferase activity.

**Indirect Immunofluorescence**

**MoAbs.** LEuM1 (CD15) was purchased from Becton Dickinson Immunocytometry Systems (Mountain View, CA), CSLEX1 was kindly provided by Dr P. Terasaki (UCLA Tissue Typing Laboratory, Los Angeles, CA) as affinity-purified antibody to a concentration of 2.8 ng/mL. Antibodies 1B2 (hybridoma supernatant) and FH3 (ascitic fluid) were a gift of Prof S. Hakomori (Biomembrane Institute, Seattle, WA). Antibodies were used at maximal concentrations to just prevent neutrophil agglutination and thus allow direct comparison of staining intensity between blasts and neutrophils. Working dilutions were LEuM1 1:100, CSLEX1 1:100, 1B2 1:5, and FH3 1:100.

Antibody binding was assessed by indirect immunofluorescence on fresh and enzyme-pretreated cells. Cells (0.5 x 10^6 in 50 μL PBS/FCS/azide) were incubated with MoAb in the dilution indicated above. An irrelevant antibody was used as a negative control.

Fluorescein-conjugated F(ab')₂ rabbit antimouse Ig (Dako Ltd, High Wycombe, UK) was used as second antibody at a dilution of 1:25. Both incubation stages were performed for 30 minutes at 4°C. Stained cells were analyzed by cytofluorimetry in a FACStar PLUS (Becton Dickinson Immunocytometry Systems).

**Glycosyltransferase Assays**

**Materials.** GDP-L-[³⁵C]fucose (254 Ci/mmol), CMP-[⁴C]sialic acid (262 Ci/mmol), and UDP-[³⁵C]lactose (270 Ci/mmol) were purchased from Amersham (Aylesbury, UK). NAL, lactose, 3', sialyl-NAL, 6'-sialyl-NAL, 3'-sialyllactose, 6'-sialyllactose, and cold GDP-L-fucose were kind gifts of Dr A.S.R. Donald (Division of Immunoochemical Genetics, CRC). Fucin and alisofetuin were obtained from Sigma (Poole, UK) and phenyl D-galactoside from Koch-Light Ltd.

Assays were performed on 20 μL aliquots of cell suspension in 0.15 mol/L NaCl containing 0.8 x 10⁶ neutrophils or 0.4 x 10⁶ leukemic cells. Immediately before assay, cells were lysed by preincubation in 0.25% Triton-X100 (BDH Ltd, Poole, UK) for 60 minutes at 4°C.

**2-Fucosyltransferase.** 2-Fucosyltransferase was assayed as described previously with phenyl D-galactoside as acceptor molecule.33

**α-3-Fucosyltransferase assay with low molecular weight acceptors.** Incubation mixtures contained: cell suspension 20 μL, 1% Triton-X100 5 μL, NAL 0.75 μmol, GDP-fucose 7.5 nmol, GDP-L-[³⁵C]fucose 0.3 nmol, ATP 0.25 μmol, MnCl₂ 1.0 μmol, Na cacodylate pH 7.0 5.0 μmol. The total volume was 65 μL. After incubation at 37°C for 2 hours the radioactive product, 3-fucosyl-NAL, was separated from nucleotide sugar donor and free sugar by descending paper chromatography on Whatman DE 81 paper (Whatman Ltd, Haidstone, UK) in propan-1-ol/ethyl acetate/pyridine/H₂O (5:1:1:3, by vol) for 12 to 16 hours and identified by its mobility relative to lactose (R₅o 1.0).

In some experiments, α-3-fucosyltransferase activity with non-sialylated and sialylated acceptors was compared. These assays contained 0.75 μmol NAL or a sialylated derivative, 3'- or 6'-sialyl-NAL, and 0.3 nmol GDP-L-[³⁵C]fucose. Cell suspension, Triton-X100, ATP, MnCl₂, and Na cacodylate were added as described above. Assays with NAL were incubated for 30 minutes only to ensure linear incorporation of [³⁵C]fucose into acceptor (up to 15% use of GDP-[³⁵C]fucose) and allow direct comparison with the activity measured with sialyl-NAL. A longer incubation period of 4 hours was necessary using the sialylated acceptors to ensure sufficient incorporation of [³⁵C]fucose for accurate measurement of product. Incorporation was linear during this period. Radioactive products were separated as described above for assays with NAL and by high-voltage paper electrophoresis (4,000 V, 90 minutes) on Whatman 3 MM paper in 40 mmol/L pyridine acetate buffer pH 5.4 for assays with sialyl-NAL. The product was identified by its mobility relative to picric acid (R₅o 0.6). By either separation procedure the recovery of total counts was quantitative.

**α-3-Fucosyltransferase assay with glycoprotein acceptors.** Assays were performed with 400 μg of fetuin or asialofetuin in 20 μL 0.25 mol/L cacodylate buffer pH 7.0. In addition, reaction mixtures contained the following: cell suspension 20 μL, 1% Triton-X100 5 μL, GDP-L-[³⁵C]fucose 0.3 nmol, ATP 0.25 μmol, and MnCl₂ 1.0 μmol (total volume 65 μL). Control assays to assess endogenous acceptor were performed without glycoprotein. After incubation at 37°C for 8 to 16 hours the reaction mixture was subjected to descending paper chromatography on Whatman No. 40 paper in propan-1-ol/ethyl acetate/pyridine/H₂O (5:1:1:3, by vol).
Radiolabeled glycoprotein remained at the origin and was separated from nucleotide sugar donor and free sugar.

**Sialyltransferase activity.** Assays were performed with NAL or asialofetuin as acceptor molecules and contained the following: cell suspension 20 μL, 1% Triton-X100 5 μL, NAL 0.75 μmol or asialofetuin 400 μg, CMP-[14C]sialic acid 0.4 nmol, MnCl₂ 0.25 μmol, Na cacodylate, pH 6.4, 5.0 μL (total volume 60 μL). Reaction mixtures were incubated for 4 hours at 37°C. With NAL, the radioactive products were separated from nucleotide donor and free sugar by high voltage paper electrophoresis (4,000 V, 90 minutes) on 3 MM paper in 40 mmol/L pyridine acetate buffer pH 5.4. The 3'- and 6'-sialyl-NAL reaction products obtained using NAL as acceptor have identical mobilities in the above system (Rₚ₀ 0.7) and, therefore, to distinguish 3'- and 6'-sialyltransferase activities measured with this acceptor further, assays were performed in triplicate. The reaction products were pooled and the two isomers were further separated as follows. After electrophoresis the total product was identified by radiochromatogram scanning, eluted overnight with 100 mmol/L pyridine acetate buffer pH 5.4, evaporated, resuspended in H₂O, and subjected to descending paper chromatography on Whatman No. 40 paper in ethyl acetate/pyridine/H₂O (10:4:3 by volume). 3'- and 6'-sialyl-NAL were identified by their mobilities relative to 3'-sialyllactose (1.4 and 1.0, respectively). In sialyltransferase assays with asialofetuin, the separation of products by chromatography was identical to that used for the 3-fucosyltransferase assay.

**β-4-Galactosyltransferase assays.** β-4-Galactosyltransferase assays contained the following: cell suspension 20 μL, 1.0% Triton-X100 5 μL, N-acetylglucosamine 0.25 μmol, MnCl₂ 1.0 μmol, UDP-[14C]galactose 0.14 nmol, Na cacodylate buffer pH 7.0, 5 μmol. Incubations were performed at 37°C for 10 minutes. The radioactive product was separated by descending paper chromatography on DE81 paper in ethyl acetate/pyridine/H₂O (5:5:1:3, by volume). 3'- and 6'-sialyl-NAL were identified by their mobilities relative to 3'-sialyllactose (1.4 and 1.0, respectively). In β-4-galactosyltransferase assays, glucose activity was estimated on duplicate samples by a modification of the Bradford method. Glycosyltransferase incubation mixtures contained between 20 and 50 μg protein. Incorporation of radioactive sugars was linear with respect to protein concentration and incubation time.

**RESULTS**

**Cell Marker Studies**

The FAB subtypes of leukemic samples 1 through 4 were M4, M1, M2, and M1, respectively. Cytospin preparations of the mononuclear cell fractions isolated from marrow contained over 90% monomorphic blast cells in all but case 3, in which approximately 40% of the cells were more mature (promyelocytes and myelocytes).

The MoAbs used in this study recognize the X determinant and related oligosaccharide structures. Their specificities are shown in Table 1.

**LeuM1.** LeuM1 recognizes the X (CD15) determinant that is strongly expressed on mature neutrophils. The results in normal neutrophils and in three of the leukemic samples are illustrated in Fig 1a. The majority of cells from all four leukemic samples were negative with LeuM1 at the antibody dilutions used in this study. In case 3, the positive fraction, when separated on the basis of light scattering, corresponded to the granulated population of promyelocytes and myelocytes identified on the cytospots. Similar results were obtained using the antibody FH3.

**CSLEX1.** In a proportion of cases of AML, the expression of X is enhanced by prior treatment of the cells with neuraminidase, which suggests that antigenic masking by sialic acid may occur. We investigated this possibility using CSLEX1, an antibody that recognizes sialyl-X, the sialylated derivative of the X determinant. Neutrophils were strongly reactive with CSLEX1 (Fig 1b) and therefore express sialyl-X as well as X. The majority of cells from cases 1, 2, and 4, by contrast, were only weakly reactive with the antibody. Again, in case 3 stronger reactivity appeared to be associated with cell maturity. Therefore, sialylation of

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**Table 1. Structures Recognized by Antibodies Specific for the X Determinant and Related Carbohydrate Antigens**

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Antigen Recognized</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LeuM1</td>
<td>X determinant (CD15)</td>
<td>Galβ1-4GlcNAc</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>FH3</td>
<td>X determinant</td>
<td>Galβ1-4GlcNAc</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CSLEX1</td>
<td>Sialyl-X</td>
<td>NeuAc2-3Galβ1-4GlcNAc</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IB2</td>
<td>N-acetylactosamine</td>
<td>Galβ1-4GlcNAc</td>
<td>39</td>
</tr>
</tbody>
</table>

---

**Fig 1.** Cytofluorimetric analysis of neutrophils and mononuclear preparations of leukemic marrows using MoAbs directed to carbohydrate sequences. LeuM1 (a), CSLEX1 (b), and IB2 before (c) and after (d) neuraminidase treatment were used. Negative control trace using an irrelevant antibody is also shown (—).
the X determinant did not account for the reduced expression in myeloblasts compared with neutrophils. As an alternative means of assessing sialylation of X, the reactivity of cells with LeuM1 was compared before and after neuraminidase treatment. The results obtained were consistent with those obtained with CSLEX1 but this method was less sensitive.

**IB2**. To establish whether, despite high levels of the biosynthetic enzyme 3-fucosyltransferase in blasts, poor expression of X and sialyl-X might be explained by lack of a suitable precursor structure, we examined reactivity of the cells with IB2, an antibody that recognizes the terminal disaccharide NAL that is the precursor of the X determinant. Expression of sialyl-NAL, the precursor of sialyl-X, was assessed by comparing reactivity with IB2 before and after neuraminidase treatment of the cells. Untreated neutrophils were positive as shown in Fig IC, but reactivity was considerably enhanced by neuraminidase pretreatment (Fig Id). On mature cells, therefore, that express both X and sialyl-X, the terminal NAL disaccharide structures exist in both sialylated and nonsialylated forms. In contrast, all four blast cell populations were negative initially but become strongly positive after neuraminidase treatment. This result suggests that, on leukemic cells, the NAL structures are present, but only as sialylated derivatives, sialyl-NAL.

**Glycosyltransferase Activities**

2-Fucosyltransferase. 2-Fucosyltransferase activity could not be demonstrated in neutrophils or in the blast cell preparations, as reported previously.

3-Fucosyltransferase. 3-Fucosyltransferase, which catalyzes the final step in the synthesis of the X determinant, transfers fucose to NAL structures (see Fig 2) at the ends of type 2 chains on glycoproteins and glycolipids. In confirmation of our previous observations, activity of this enzyme was readily demonstrable in neutrophils but was present in much higher levels in leukemic blasts (Table 2).

**Fig 2. Pathways of fucosylation and sialylation of NAL (Galβ1-4GlcNAc-R) end groups. Transfer of fucose, by 3-fucosyltransferase, to the subterminal GlcNAc residue results in the synthesis of X, whereas synthesis of sialyl-X proceeds via initial sialylation of the terminal Gal residue, by 3'-sialyltransferase, followed by fucosylation.**

Transfer of sialic acid in d-6 linkage to the terminal Gal residue, as shown by the left-hand pathway, blocks the synthesis of both X and sialyl-X.

### Table 2. Glycosyltransferase Activities in Neutrophils and Leukemic Myeloblasts

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>2-Fucosyltransferase</th>
<th>3-Fucosyltransferase</th>
<th>Sialyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 7)</td>
<td>0</td>
<td>737 ± 109</td>
<td>347 ± 100</td>
</tr>
<tr>
<td></td>
<td>(330-863)</td>
<td>(192-447)</td>
<td></td>
</tr>
<tr>
<td>AML blasts</td>
<td>0</td>
<td>8,466 ± 6,014</td>
<td>2,883 ± 223</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>(4,066-17,250)</td>
<td>(2,614-3,133)</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as cpm [14C] sugar transferred/10^6 cells/h with NAL as acceptor molecule. Mean value ± standard deviation is quoted with ranges given in parentheses.

**Sialyltransferase**. Sialyltransferase activity was present in neutrophils but was much higher in the leukemic cells (Table 2), a finding consistent with strong expression of sialyl-NAL on blasts. Incorporation of [3H] sialic acid measured with asialofetuin was virtually identical to that with NAL (6.7 ± 1.3 compared with 6.4 ± 0.5 pmol sialic acid transferred/10^6 cells/h for blasts, mean ± SD).

**Separation of 3'- and 6'-sialyltransferase products.** To explore further the lack of expression of sialyl-X on myeloblasts despite apparently strong expression of sialyl-NAL and high levels of 3-fucosyltransferase and sialyltransferase, we distinguished 3'- and 6'-sialyltransferase activities by separation of the reaction products (3'- and 6'-sialyl-NAL) of the sialyltransferase activity with NAL as acceptor. The results in Table 3 show that the activity in neutrophils was exclusively due to 3'-sialyltransferase, whereas in the leukemic cells over 70% of the total activity was due to 6'-sialyltransferase.

**DMSO-Induced HL-60 Cells**

To determine the extent to which the differences between leukemic myeloblasts and normal mature cells might reflect the changes occurring during normal myeloid maturation, we studied HL-60 cells induced to differentiate with DMSO. Morphologic evidence of maturation was present in 80% to 90% of treated cells and by 6 or 7 days most had progressed to myelocyte and metamyelocyte stages. Phorbol myristate acetate (PMA)-stimulated superoxide production (measured by cytochrome c reduction) increased from 0 to 18 nmol/10^7 cells/h by day 5. The morphologic changes were accompanied by alterations in both antigen expression and enzyme activity.

### Table 3. Measurement of 3'- and 6'-Sialyltransferase Activities in Normal Neutrophils and AML Blasts

<table>
<thead>
<tr>
<th>Patient Sample</th>
<th>Total Activity</th>
<th>6'-Sialyltransferase</th>
<th>3'-Sialyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML 1</td>
<td>19,437</td>
<td>15,738 (81)</td>
<td>3,699 (19)</td>
</tr>
<tr>
<td>2</td>
<td>23,418</td>
<td>19,220 (82)</td>
<td>4,198 (18)</td>
</tr>
<tr>
<td>3</td>
<td>12,159</td>
<td>9,156 (75)</td>
<td>3,003 (25)</td>
</tr>
<tr>
<td>4</td>
<td>27,282</td>
<td>19,926 (73)</td>
<td>7,356 (27)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2,036</td>
<td>— (0)</td>
<td>2,036 (100)</td>
</tr>
<tr>
<td>3,333</td>
<td>— (0)</td>
<td>3,333 (100)</td>
<td></td>
</tr>
<tr>
<td>3,834</td>
<td>— (0)</td>
<td>3,834 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as cpm [14C] sugar transferred/assay. Figures in parentheses indicate percentage of total activity.
Marker studies. HL60 cells express the X determinant and this was unchanged or slightly reduced in most cells after exposure to DMSO as reported by other groups. However, the expression of sialyl-X increased until by day 5 a population (20% to 30%) of the cells was strongly positive (Fig 3).

Enzyme activities. In uninduced cells elevated levels of 3-fucosyl- and sialyltransferase were present, comparable with those in leukemic blasts. After 24 hours of exposure to DMSO, a reduction in the level of both enzymes was apparent, (as illustrated in Fig 4) by which time the levels had decreased to 60% and 40%, respectively, of those measured in uninduced cells. A predominance of 6'-sialyltransferase was measured in uninduced cells similar to that found in leukemic blasts and the decrease in total sialyltransferase activity that accompanied DMSO-induced maturation was due to loss activity of this enzyme (Fig 5). 3'-Sialyltransferase remained unchanged at 24 hours and decreased only slightly by day 6.

The DMSO-related changes in enzyme activities were not due to an alteration in the rate of cell growth; this continued at an exponential rate for 48 hours after exposure to DMSO. To exclude a direct effect of DMSO on glycosyltransferase activities, we measured the activity of \( \beta \)-4-galactosyltransferase in HL60 cells that remained unchanged during the period of exposure (data not shown). 3-Fucosyl- and total sialyltransferase activities in KG1 cells exposed to DMSO for 48 hours remained at 89% and 100% of control values, respectively.

3-Fucosyltransferase Activity Measured With Sialylated and Nonsialylated Acceptors

3-Fucosyltransferase activity in blasts and HL60 cells was approximately 10-fold greater than in neutrophils when measured with asialofetuin, as was found also with NAL as acceptor. However, activity in the mature cells was similar when measured with fetuin or asialofetuin, with a slight preference for asialofetuin (see Fig 6), whereas in AML blasts and HL60 cells there was a marked difference. Activity measured with asialofetuin was approximately 5 times higher than with fetuin. Activity with nonsialylated
and sialylated low molecular weight acceptors (NAL and 3'-sialyl-NAL) showed a similar pattern (see Table 4), with the blasts again showing 5 times greater preference than the mature cells for NAL, as compared with 3'-sialyl-NAL. When HL60 cells were induced with DMSO, a marked decrease in total 3-fucosyltransferase activity measured with asialofetuin occurred (as with NAL as acceptor), but, in addition, a change in enzyme specificity was apparent in the direction of a less marked preference for asialofetuin as cell maturation progressed (Fig 6). In blasts, in contrast to the findings with sialyltransferase, a direct comparison between incorporation of [14C] fucose measured with NAL and asialofetuin showed that it was more than 100-fold greater with NAL (549 ± 391 compared with 1.1 ± 0.6 pmol fucose transferred/10^6 cells/h), which suggests that asialofetuin is a rather poor acceptor for this enzyme.

**DISCUSSION**

These results show that (1) expression of X and sialyl-X on leukemic myeloblasts is markedly reduced compared with neutrophils and (2) the activity of the biosynthetic 3-fucosyltransferase and sialyltransferases is different in these two cell types. We suggest that the differences between surface carbohydrate expression in immature and mature cells can be accounted for by their different enzyme profiles.

The virtual absence of reactivity of blasts with LeuM1 described here is not strictly comparable with results of other studies because we deliberately used nonsaturating concentrations of antibody and, in all but case 2, weak reactivity could be induced by increasing the antibody concentration to a level at which marked agglutination of neutrophils occurred. Lack of reactivity was not due to antigenic masking of X by sialic acid, to any major extent, because the expression of sialyl-X was also weak. The enzymic studies suggest that lack of X expression is most probably related to high levels of 6'-sialyltransferase, which uses the precursor substrate, thereby preventing the synthesis of either X or sialyl-X as shown in Fig 2. In blasts, 3-fucosyltransferase and 6'-sialyltransferase would be expected to be the major competitors for NAL substrate. How much X or 6'-sialyl-NAL is formed is therefore likely to be determined by the relative activities of the two enzymes. A strongly active 6'-sialyltransferase would use NAL end groups to form 6'-sialyl-NAL structures, at the expense of 3-fucosyltransferase. In neutrophils, 6'-sialyltransferase activity is absent, leaving 3-fucosyltransferase and 3'-sialyltransferase as the major competitors for NAL. The synthesis of X and sialyl-X will therefore depend on the relative activities of these two enzymes. Initial fucosylation of NAL by 3-fucosyltransferase would result in X but not sialyl-X, whose synthesis proceeds via sialylation, followed by fucosylation as in Fig 2. Initial sialylation of NAL by 3'-sialyltransferase would result in 3'-sialyl-NAL, which can then be converted to sialyl-X. The marker studies in neutrophils are in agreement with this pattern of activity and show surface NAL, sialyl-NAL, X and sialyl-X structures. The appearance of unsubstituted NAL end groups is likely to be a result of lower overall activities of both enzymes.

The apparent predominance of 6'-sialyltransferase activity in blasts and lack of effective competition for NAL by 3-fucosyltransferase is surprising in view of the markedly elevated level of 3-fucosyltransferase activity compared with sialyltransferase activity. There was no evidence for the presence of fucosidases on prolonged incubations of reaction mixtures, or for inhibitors. Limited availability of GDP-fucose or divalent cations might be important controlling influences; 3-fucosyltransferase in neutrophils has an absolute requirement for Mn^{2+}, whereas 3'- and 6'-sialyltransferase do not (unpublished observations). However, the higher comparative levels of 3-fucosyltransferase were evident only when the low molecular weight compound NAL was used as acceptor; with asialofetuin as acceptor molecule, 3-fucosyltransferase activity was actually considerably less than that of 6'-sialyltransferase (1.1 ± 0.6 compared with 6.7 ± 1.3 pmol sugar transferred/10^6 cells/h). It is probable, therefore, that 3-fucosyltransferase demonstrates fine substrate specificity such that the complexity of the oligosaccharide structures internal to NAL or the surface conformation of glycoprotein and glycolipid molecules influence its activity in a major way. The length and complexity of oligosaccharide moieties isolated from blasts and neutrophils are very different, and analysis of neutrophil glycoproteins suggests that glycosyltransferases acting on NAL structures at the ends of tetra-antennary N-linked chains do exhibit branch specific-

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**Table 4. Comparison of 3-Fucosyltransferase Activity in Neutrophils and AML Blasts Using Nonsialylated and Sialylated Low Molecular Weight Acceptor Molecules**

<table>
<thead>
<tr>
<th>Patient Sample</th>
<th>NAL</th>
<th>3'-Sialyl-NAL</th>
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</thead>
<tbody>
<tr>
<td>AML 5</td>
<td>8,146</td>
<td>331</td>
</tr>
<tr>
<td>6</td>
<td>7,905</td>
<td>408</td>
</tr>
<tr>
<td>7</td>
<td>5,942</td>
<td>302</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>1,085</td>
<td>245</td>
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<tr>
<td></td>
<td>936</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>851</td>
<td>181</td>
</tr>
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</table>

Results are expressed as cpm [14C] sugar transferred/10^6 cells/h.
Gangliosides with terminal 2-6 sialyl type 2 chains and internal fucose residues have been shown to be much higher in malignant than in normal tissues and it is possible that 2-6 sialyl type 2 chains on leukemic myeloblasts could also be internally fucosylated. However, such fucose would not give rise to X or sialyl-X structures and would not have been detected in this study. Although the X determinant can be demonstrated on both glycoprotein and glycolipid isolated from HL60 cells, it is not known whether on blasts it occurs preferentially on one or the other. Glycolipid acceptors were not used in this study but clearly the use of different and more complex acceptors, together with detailed examination of the exact distribution of X during early stages of cell maturation, will be necessary to fully appreciate these complex interdependent mechanisms.

The expression of X during normal myeloid maturation does appear to be developmentally regulated. It first appears at or just before the promyelocyte stage but is absent in earlier precursors. Lack of expression on leukemic blasts due to high levels of 6'-sialyltransferase may, therefore, simply be a feature of immaturity rather than leukemic transformation with the occurrence of X and/or sialyl-X influenced in a major way by the interaction between 6'-sialyltransferase, 3'-sialyltransferase, and 3-fucosyltransferase which, in turn, will be determined by the precise stage of maturation arrest of the leukemic cells.

The strong expression of X on HL60 cells, which also have high levels of 3-fucosyltransferase and 6'-sialyltransferase, suggests that in these cells, both enzymes compete effectively for substrate. The changes in enzyme activity after exposure to DMSO also suggest a developmentally regulated reduction in 3-fucosyl- and 6'-sialyltransferase activities. These changes were accompanied by a population of cells with increased expression of sialyl-X, presumably because the decrease in both enzyme levels encouraged more effective competition for NAL by 3'-sialyltransferase.

The reduced activity with sialylated substrates demonstrated by the 3-fucosyltransferase in blasts is also likely to have an important regulatory influence on the expression of sialyl-X. The marker studies did not distinguish between surface 3'- and 6'-sialyl-NAL structures, but even if a significant proportion of the sialyl linkages were α-2,3 it is probable that these structures could not have been converted to sialyl-X by the 3-fucosyltransferase present at this stage of maturation. Whether the observed differences in substrate specificity of the 3-fucosyltransferase during cell maturation are due to changes in a single enzyme or whether two distinct enzymes are expressed independently is not clear. However, Johnson and Watkins reported similar differences in purified 3-fucosyltransferases from CGL cells and normal neutrophils, and both enzymes appeared to be distinct from the 3-fucosyltransferase isolated from plasma. Two 3-fucosyltransferases are also present in mutant CHO cells lines and show different specificities for sialylated substrates. Interestingly, the introduction of a 3-fucosyltransferase cDNA from HL60 cells into wild type CHO cells by transfection resulted in demonstrable 3-fucosyltransferase activity that was distinct from the two described in mutant cells but resembled the HL60 enzyme described here.

The biologic role of sialyl-X is unknown. However, it is tempting to speculate that it may be important in cellular adhesion mechanisms, particularly those that determine the release of mature neutrophils from the bone marrow into the circulation. X and sialyl-X structures are abundant on most, if not all, N-linked glycoproteins isolated from the circulation and other in vitro abnormalities of adherence. The precise nature of this aberrant sialylation is not known, but whether it involves increased expression of sialyl-X and how this relates to altered glycosyltransferase activity is worthy of further study. Elevated activity of an α2,3-sialyltransferase that acts on Galβ1-3GalNAc residues in O-linked oligosaccharides has recently been demonstrated in CGL cells, but to what extent this is responsible for increased sialylation of functional importance is not clear because in neutrophils only one glycoprotein, leukemia-lin, has been found to contain predominantly O-linked structures. The development of molecular techniques such as transfection of cloned glycosyltransferases to alter terminal glycosylation sequences will greatly improve our understanding of the biologic importance of cell surface carbohydrate structures and the factors regulating their synthetic control in malignant transformation and during normal differentiation.

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Enzymic control of the expression of the X determinant (CD15) in human myeloid cells during maturation: the regulatory role of 6-sialytransferase

PO Skacel, AJ Edwards, CT Harrison and WM Watkins