Leukocytes express a major sialoglycoprotein, leukosialin, of which the apparent molecular weight (mol wt) can be variable according to the differences in O-glycans attached to this molecule. In the present study, we analyzed the structures of O-glycans attached to leukosialin present in various T-lymphocytic leukemia cells. T-lymphoid cells from patients with acute T-lymphocytic leukemia express a large amount of the branched hexasaccharides, NeuNAcα2→3Galβ1→3(NeuNAcα2→6)GalNAc, which are also expressed in activated normal T lymphocytes, but that are almost absent in resting normal T lymphocytes. T-lymphoid cells from patients with chronic T-lymphocytic leukemia, on the other hand, mainly express the tetrasaccharides NeuNAcα2→3Galβ1→3(NeuNAcα2→6)GalNAc on leukosialin, but they also express a small significant amount of the hexasaccharides. The same hexasaccharides can be detected in thymocytes. The increased amount of the hexasaccharides in acute leukemia is associated with increased activity of β1→6GalNAc-transferase, a key enzyme in forming the hexasaccharides. Immunoblot analysis of cell lysates showed that monoclonal antibody (MoAb) T-305 reacts preferentially with leukosialin of high mol wt containing the hexasaccharides. These findings indicate that T-lymphocytic leukemia cells reexpress the oligosaccharides present in immature cells.

MATERIALS AND METHODS

Cells. The human erythroleukemia K562 and promyelocytic leukemia HL-60 and T-lymphoblastoid HSB-2 were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and 2 mmol/L glutamine. Lymphocytes from the peripheral blood (PB) of normal individuals and patients were prepared as described previously by centrifugation in Histopaque-1077 (Sigma, St Louis, MO). Human thymocytes were obtained when a thymus was removed surgically for cardiac surgery. In all, 16 cases of acute T-lymphocytic leukemia (T-ALL) and six cases of chronic T-lymphocytic leukemia (T-CLL) were subjected to analysis.

Isolation of leukosialin and labeling of carbohydrates. Sialic acid residues on the cell surface were labeled by sodium periodate oxidation followed by NaBH₄ reduction, and galactose and N-acetylgalactosamine residues on the cell surface were labeled by the galactose oxidase/NaBH₄ method after treatment with Vibrio cholerae neuraminidase. The former method labels the intact sialic acid-containing oligosaccharides whereas the latter method shows the neutral, backbone oligosaccharides remaining after removal of sialic acid. Carbohydrates of leukemic cell lines were metabolically labeled with [3H]glucosamine as described previously. Cells were incubated with glucose-free RPMI 1640 medium with 10% dialyzed FCS and 2 mmol/L glucose, complemented with 5% complete RPMI 1640 medium containing 10% FCS and 2 mmol/L glucose. [3H]Glucosamine (30 Ci/mmol, Du Pont-New England Nuclear, Boston, MA) was added at 10 μCi/mL, and the cells were labeled for 18 hours at 37°C.

Immunoprecipitation of leukosialin. After each labeling, the cells were harvested and washed twice with phosphate-buffered saline (PBS)/EDTA, lysed with PBS containing 1% NP-40 in 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 μg/mL leupeptin, and aprotinin. The cell lysates were clarified by centrifugation. An aliquot of each supernatant was used for immunoprecipitation using MoAb T-305. The immunoprecipitated proteins were resolved on 15% SDS-polyacrylamide gel electrophoresis followed by fluorography.

From the La Jolla Cancer Research Foundation Cancer Research Center, and the Scripps Clinic and Research Foundation, La Jolla, CA.

Submitted July 30, 1990; accepted November 20, 1990.

Supported by Grant No. CA 33895 from the National Cancer Institute, Bethesda, MD.

Address reprint requests to Minoru Fukuda, PhD, La Jolla Cancer Research Foundation, 1001 N Torrey Pines Rd, La Jolla, CA 92037.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1991 by The American Society of Hematology.

0006-4971/91/7707-0015$3.00/0


©1991 by The American Society of Hematology.
tin, 1 μg/mL aprotinin, and 5 mmol/L of sodium tetrathionate as protease inhibitors. The supernatant, after brief centrifugation, was used as a total cell lysate. Leukosialin was immunoprecipitated by rabbit antileukosialin serum (provided by Dr. Sven Carlsson) followed by addition of *Staphylococcus aureus* (Pansorb, Calbiochem, La Jolla, CA) as described previously. Aliquots of the immunoprecipitates were analyzed by SDS-polyacrylamide gel eleetrophoresis (PAGE, 8% acrylamide gels) and visualized by fluorography after treatment with Enlightning (Du Pont-NEN).

**Analysis of O-glycans attached to leukosialin.** The immunoprecipitates were digested with Pronase (nuclease-free, Calbiochem), and the large glycopeptides were isolated by gel filtration on Sephadex G-50 as described previously. The column was 1.0 × 110 cm, and 1.1 mL was collected for each fraction at a flow rate of 6 mL/h. The O-glycans were released from the large glycopeptides by alkaline borohydride treatment and isolated by gel filtration on Sephadex G-50 as described previously. The released oligosaccharides were then applied to a column (1.0 × 108 cm) of Bio-Gel P-4 (4–400 mesh). The flow rate was 3 mL/h, and each fraction contained 1.0 mL. Both Sephadex G-50 and Bio-Gel P-4 were equilibrated with 0.1 mol/L NH₄HCO₃. The oligosaccharides separated by Bio-Gel P-4 were then analyzed by high-performance liquid chromatography (HPLC) on a column (0.4 × 25 cm) of amino-bonded silica (Lichrosorb-NH₂, Merck, Cherry Hill, NJ), using a Varian 5000 HPLC apparatus. The mobile phase was 3% acetic acid in a mixture of acetonitrile/H₂O, pH 5.5 with triethylamine, and the flow rate was 1.0 mL/min. For sialylated oligosaccharide analysis, the composition of mobile phase was isocratic at 80% acetonitrile for 5 minutes, followed by a gradient to 50% acetonitrile in 75 minutes. For separation of neutral oligosaccharides, the composition of the mobile phase was isocratic at 50 minutes at 90% acetonitrile, followed by a gradient to 60% acetonitrile in 75 minutes. Standard oligosaccharides were obtained from leukosialin present in HL-60 and K562 cells as described previously.

**Immunologic detection of leukosialin and leukosialin with complex O-glycans.** Cells were lysed in the same lysis buffer used for radioactively labeled cells and the lysates were centrifuged briefly. The supernatants were boiled in sample buffer and subjected to SDS-PAGE using 8% acrylamide gels. Proteins in the gel were then analyzed by high-performance liquid chromatography (HPLC) for 5 minutes, followed by a gradient to 50% acetonitrile in 75 minutes or 1 hour, the reaction was stopped with 0.4 mL 20 mmol/L sodium tetraborate, 10 mmol/L EDTA (pH 9.1) and the mixture was passed through a column (0.5 × 4 cm) of Dowex 1 × 8 (CI⁻) equilibrated in water. The column was washed with 2.5 mL water, and the total eluate was used for counting.

**UDP-GlcNAc: Galβ1→3GalNAc β1→6N-acetylgalcosaminyltranserase.** The assay mixture contained, in a total volume of 25 μL: 50 mmol/L cacodylate buffer, pH 7.4, 20 mmol/L MnCl₂, 0.1% Triton X-100, 0.1% BSA, 0.1 mmol/L UDP-[¹⁴C]galactose (100,000 cpm/mmol), 10 mmol/L GlcNAc, and 50 or 100 μg protein. After incubation at 37°C for 30 minutes or 1 hour, the reaction was stopped with 0.4 mL 20 mmol/L sodium tetraborate, 10 mmol/L EDTA (pH 9.1) and the mixture was passed through a column (0.5 × 4 cm) of Dowex 1 × 8 (CI⁻) equilibrated in water. The column was washed with 2.5 mL water, and the total eluate was used for counting.

**UDP-Gal: GlcNAc β1→4galactosyltranserase.** The assay mixture contained, in a total volume of 25 μL: 50 mmol/L cacodylate buffer, pH 7.4, 20 mmol/L MnCl₂, 0.1% Triton X-100, 0.1% BSA, 0.1 mmol/L UDP-[¹⁴C]galactose (100,000 cpm/mmol), 10 mmol/L GlcNAc, and 50 or 100 μg protein. After incubation at 37°C for 30 minutes or 1 hour, the reaction was stopped with 0.4 mL 20 mmol/L sodium tetraborate, 10 mmol/L EDTA (pH 9.1) and the mixture was passed through a column (0.5 × 4 cm) of Dowex 1 × 8 (CI⁻) equilibrated in water. The column was washed with 2.5 mL water, and the total eluate was used for counting.

**CMP-NeuNAc: Galβ1→3GalNAc β1→6N-acetylgalcosaminyltranserase.** The assay mixture contained, in a total volume of 25 μL: 50 mmol/L cacodylate buffer, pH 7.4, 20 mmol/L MnCl₂, 0.1% Triton X-100, 0.1% BSA, 0.1 mmol/L CMP-[¹⁴C]NeuNAc (20,000 cpm/mmol), 100 μg asialo-bovine submaxillar mucin (BSM), and 50 or 100 μg protein. After incubation at 37°C for 30 minutes or 1 hour, the reaction was stopped with 0.4 mL ice-cold water. The products were analyzed as described above for β1→6N-acetylgalcosaminyltranserase.

**CMP-NeuNAc: Galβ1→4α-sialyltranserase.** The assay mixture contained, in a total volume of 25 μL: 50 mmol/L cacodylate buffer, pH 6.5, 1% Triton X-100, 0.1% BSA, 0.5 mmol/L CMP-[¹⁴C]NeuNAc (20,000 cpm/mmol), 100 μg asialo-bovine submaxillar mucin (BSM), and 50 or 100 μg protein. After incubation at 37°C for 30 minutes or 1 hour, the reaction was stopped with 0.4 mL ice-cold water. The products were analyzed as described above for β1→6N-acetylgalcosaminyltranserase.
from various leukemic cells after SDS-PAGE. The results indicate that leukosialin from T-ALL has a higher apparent mol wt than leukosialin from T-CLL (Fig 1A). The apparent mol wt of leukosialin from T-CLL (lanes 7 and 8) was slightly higher than that of leukosialin from a normal individual (lane 10).

As shown previously, leukosialin from peripheral T lymphocytes of normal individuals contains almost exclusively the tetrascarharides, \( \text{NeuNAc} \alpha_2 \rightarrow 3 \text{Gal} \beta_1 \rightarrow 3(\text{NeuNAc} \alpha_2 \rightarrow 6) \text{GalNAc} \), whereas leukosialin from activated T lymphocytes contains mainly the more complex hexascarharides \( \text{NeuNAc} \alpha_2 \rightarrow 3 \text{Gal} \beta_1 \rightarrow 3(\text{NeuNAc} \alpha_2 \rightarrow 3 \text{Gal} \beta_1 \rightarrow 4\text{GlcNAc} \beta_1 \rightarrow 6) \text{GalNAc} \), which were also shown to be present in leukosialin from the HSB-2 leukemic cell line.\(^\text{12}\) Leukosialin from K562 cells contains almost exclusively the same tetrascarharide expressed on resting T lymphocytes.\(^\text{12}\) K562 cells and HSB-2 cells were therefore used as markers for low-mol-wt and high-mol-wt forms of leukosialin, respectively. The difference in leukosialin mol wt can be observed even after sialic acid residues are removed. After this treatment, leukosialin from T-ALL cells showed apparent mol wt similar to leukosialin of HSB-2 cells (Fig 1B, lane 5). In contrast, leukosialin from T-CLL is heterogenous in mol wt, migrating at the positions between K562 leukosialin and T-ALL leukosialin (Fig 1B, lane 4). The same results were obtained in all 16 cases of T-ALL and in all six cases of T-CLL. These results suggest that leukosialin from T-CLL contains more complex saccharides than normal T lymphocytes, and T-ALL probably contains more complex saccharides than T-CLL.

Figure 2 shows the fluorogram of leukosialin from other lymphoblastoid diseases. The results indicate that leukosialins from patients with T lymphoma, hairy cell leukemia, and AIDS exhibit higher mol wt than leukosialin from normal individuals. Consistent with the previous report, B-lymphocytic leukemia expresses a small amount of leukosialin, although its mol wt is close to that of normal T lymphocytes (compare lanes 1 and 3). It is also noteworthy that an HIV-positive patient with no symptoms expresses a low-mol-wt form of leukosialin (lane 5), whereas an AIDS patient expresses a high-mol-wt form of leukosialin (lane 6). Further studies on more cases of these diseases are necessary to confirm these results, however.

**Structures of O-glycans attached to leukosialin on leukemic cells.** To elucidate the cause of differences observed in molecular sizes of leukosialin, O-glycans attached to leukosialin were analyzed in two typical types of leukemia, T-ALL and T-CLL. Cell-surface carbohydrates were labeled by the periodate/\( \text{NaB}[^\text{3}H] \) procedure, and leukosialin was immunoprecipitated. The samples shown in lanes 4, 5, and 7 in Fig 1A were digested by pronase, and the digests were separately applied to a column of Sephadex G-50. Figure 3A and C shows the digested glycopeptides eluted
near the void volume of the column. These high-mol-wt glycopeptides were then treated with alkaline borohydride, and the released O-glycans were isolated by Sephadex G-50 gel filtration (Fig 3B and D).

The isolated O-glycans were subjected to Bio-Gel P-4 gel filtration. Figure 4A shows that major O-glycans from T-CLL leukosialin elute at positions corresponding to the disialylated tetrasaccharide, NeuNAcα2 → 3Galβ1 → 3(NeuNAcα2 → 6)GalNAcOH, and to monosialylated trisaccharide. The latter could be resolved into two isomers, NeuNAcα2 → 3Galβ1 → 3GalNAcOH and Galβ1 → 3(NeuNAcα2 → 6)GalNAcOH, by chromatography on Li-Chrosorb-NH, (Fig 5A). The disialylated hexasaccharide NeuNAcα2 → 3Galβ1 → 3(NeuNAcα2 → 3Galβ1 → 4GlcNAcβ1 → 6)GalNAcOH and the monosialylated pentasaccharide NeuNAcα2 → 3Galβ1 → 3(Galβ1 → 4GlcNAcβ1 → 6)GalNAcOH were detected as minor oligosaccharides. In contrast, the major oligosaccharide from T-ALL leukosialin was the disialylated hexasaccharide. The disialylated tetrasaccharide, together with small amounts of the monosialylated pentasaccharide and the monosialylated trisaccharide, was also present (Figs 4B and 5B). These results, summarized in Table 1, indicate that leukosialin with high mol wt contains more of the hexasaccharides than leukosialin with low mol wt.

To confirm these results, cells were labeled by the galactose oxidase/NaB\[^{3}H\] procedure after sialidase treatment, and leukosialin was immunoprecipitated with anti-
leukosialin antibodies. This protein (lanes 4 and 5 of Fig 1B) was digested with pronase, glycopeptides of high mol wt were isolated by Sephadex G-50 gel filtration, and O-glycans were released from the glycopeptides by alkaline borohydride treatment. The isolated released O-glycans were then subjected to Bio-Gel P-4 gel filtration and HPLC. Figure 6 shows that Galβ1 → 3(Galβ1 → 4GlcNACβ1 → 6)GalNAcOH and Galβ1 → 3GalNAcOH were obtained in both chromatographic conditions, but T-ALL leukosialin contains more of the asialo-tetrasaccharide than the asialo-disaccharide, whereas the reverse is true for T-CLL leukosialin. The molar ratios of the tetrasaccharide to the disaccharide were 21:79 for T-CLL leukosialin and 52:48 for T-ALL leukosialin. These ratios are consistent with the ratio obtained on sialylated saccharides (Table 1), indicating that the results obtained by these two methods reflect the actual amounts of oligosaccharides.

**Immunoblotting of leukosialin with specific antibodies.**

Results from chromatographic analysis suggested to us that leukosialin with different mol wt could be visualized by immunologic detection on Western blots. Cell lysates were separated by SDS-PAGE, and the proteins were electrophoretically blotted to nitrocellulose filters. These filters were then incubated with either rabbit antileukosialin antiserum or mouse T-305 MoAb. As shown in Fig 7, the rabbit antileukosialin antiserum detects all leukosialins regardless of size (lanes 1 through 6 in A), whereas the T-305 MoAb detects only leukosialin with high mol wt, in particular leukosialin from T-ALL and HSB-2 cells (lanes 7 through 12 in A). The apparent mol wt of leukosialin recognized by T-305 is larger than that of the nonreactive form (eg, lane 4 as compared with lane 10 in Fig 7A), suggesting that T-305 recognizes the hexasaccharides attached to leukosialin. In contrast to T-ALL cells, T-CLL cells barely express any leukosialin that reacts with T-305 (lane 11 in Fig 7A). When peripheral lymphocytes of a normal individual were analyzed, the T-305 MoAb detected

---

**Table 1. Structures and Relative Amounts of O-Linked Oligosaccharides Found on Leukosialin From Normal and Leukemic T Lymphocytes**

<table>
<thead>
<tr>
<th>Leukosialin Oligosaccharides</th>
<th>T-CLL (%)</th>
<th>T-ALL (%)</th>
<th>Thymocytes (%)</th>
<th>Normal T Lymphocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NeuNAcα2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Galβ1 → 3GalNAcOH*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NeuNAcα2 → 3Galβ1 → 3GalNAcOH</td>
<td>35.0</td>
<td>27.6</td>
<td>26.0</td>
<td>30.6</td>
</tr>
<tr>
<td>NeuNAcα2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 NeuNAcα2 → 3Galβ1 → 3GalNAcOH</td>
<td>45.7</td>
<td>21.5</td>
<td>32.5</td>
<td>65.6</td>
</tr>
<tr>
<td>NeuNAcα2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 NeuNAcα2 → 3Galβ1 → 4GlcNACβ1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NeuNAcα2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Galβ1 → 3GlcNACβ1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NeuNAcα2 → 3Galβ1 → 4GlcNACβ1</td>
<td>8.4</td>
<td>19.0</td>
<td>14.6</td>
<td>1.6</td>
</tr>
<tr>
<td>NeuNAcα2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 NeuNAcα2 → 3Galβ1 → 3GalNAcOH</td>
<td>10.9</td>
<td>32.0</td>
<td>26.8</td>
<td>2.0</td>
</tr>
<tr>
<td>NeuNAcα2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 NeuNAcα2 → 3Galβ1 → 3GalNAcOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The numbers are expressed as percentage of the total O-linked oligosaccharides in a molar ratio.

*This oligosaccharide was minimally present.*

---

**Fig 5.** HPLC analysis of intact O-glycans on amino-bonded silica (Lichrosorb-NH₃) column. Oligosaccharides were separated by HPLC on a Lichrosorb-NH₃ column as described in the Materials and Methods section. (A) O-Glycans from T-CLL; (B) O-glycans from T-ALL. The elution positions of standard oligosaccharides, as shown in Fig 4, are indicated by arrows, except for 4',Galβ1 → 3( NeuNAcα2 → 6)GalNAcOH. Radioactivity (—); acetonitrile concentration (—).
Fig 6. Analysis of O-glycans after removal of sialic acid. (A and B) Bio-Gel P-4 gel filtration of neutral oligosaccharides obtained from leukosialin labeled by galactose oxidase/NaB$^3$H$_4$, procedure. Leukosialin was labeled after neuraminidase treatment of T-CLL cells (A) or T-ALL cells (B). Fractions were pooled as indicated by bars and subjected to HPLC analysis. (C and D) HPLC analysis of neutral O-glycans shown in A or B. Radioactivity (---); acetonitrile concentration (---). Arrows marked 2 and 4 indicate the elution positions of Gal$\beta$1→3GalNAcOH and Gal$\beta$1→3[Gal$\beta$1→4GlcNAc$\beta$1→6]GalNAcOH, respectively. The chromatographic conditions are described in the Materials and Methods section.

A high mol wt of leukosialin that was a minor component of the total leukosialin content (lane 2 as compared with lane 8 in Fig 7A). These results are consistent with the results obtained by structural analyses of oligosaccharides. Leukosialin becomes a high-mol-wt form when it contains more complex hexasaccharides, which in turn react with the T-305 MoAb. The rabbit antileukosialin antiserum, on the other hand, reacts with leukosialin regardless of glycosylation status because it reacts with the peptide moiety of leukosialin, as shown previously.

Comparison of leukosialin from leukemic cells, thymocytes, and normal T lymphocytes. To investigate whether the complex hexasaccharides present in leukemic cells can be regarded as reexpression of the saccharides on immature cells, we examined leukosialin from thymocytes. Figure 1 (lane 9) shows that leukosialin from thymocytes is heterogeneous in mol wt, with a major band slightly larger than leukosialin from normal T lymphocytes. O-Glycans from thymocyte leukosialin were similarly prepared and analyzed by Bio-Gel P-4 gel filtration (Fig 4D). The results indicate that thymocyte leukosialin contains a significant amount of the disialylated hexasaccharide (peak 1), in contrast to leukosialin in normal peripheral lymphocytes containing almost exclusively the disialylated tetrasaccharide (peak 2, Fig 4C). These results indicate clearly that immature T lymphocytes, isolated as thymocytes and leukemic cells, share the property of expressing the complex hexasaccharides that are barely detectable in the peripheral lymphocytes of normal individuals.

Comparison of glycosyltransferase activities between normal and leukemic lymphocytes. To understand better the mechanisms underlying the differences in O-glycans attached to leukosialin, we measured the activities of four glycosyltransferases. Among them, $\beta_1 \rightarrow 6N$-acetylgalactosaminyItransferase is a key enzyme required to form the hexasaccharide

Fig 7. Detection of leukosialin by antileukosialin antibodies and T-305 MoAb. Cell lysates were subjected to SDS-PAGE, and transferred to nitrocellulose filters, and the blots were reacted with specific antibodies. (A) Immunoblots using rabbit antileukosialin peptide antiserum (lanes 1 through 6) or T-305 MoAb (lanes 7 through 12) are shown. Cell lysates in lanes 7 through 12 are duplicates of those in lanes 1 through 6. Lanes 1 and 7, K562 cells; lanes 2 and 8, normal peripheral blood lymphocytes; lanes 3 and 9, T-ALL; lanes 4 and 10, T-ALL; lanes 5 and 11, T-CLL; lanes 6 and 12, HSB-2 cells. (B) Immunoblots using rabbit antileukosialin peptide antiserum. Lane 1, normal PBL; lane 2, T-ALL; lane 3, T-CLL; lane 4, K562 cells; lane 5, HSB-2 cells.
N-acetylglucosamine residue is added by pl 3Galp1 NeuNAca2. T lymphocytes as well as K562 cells express a negligible branched hexasaccharide NeuNAca2 amount of pl acetylglucosaminyltransferase. As shown in Table 2, normal T lymphocytes and K562 cells express negligible amounts of the 4GlcNAcpl- 6(NeuNAc2 GalNAc, and why T-ALL cells express less of the hexasaccharide but higher than normal T lymphocytes. In contrast to significant changes in B1 → 6N-acetylglucosaminyltransferase, the other three enzymatic activities measured remained constant compared to normal T lymphocytes. These results indicate that formation of the branched hexasaccharide is directly proportional to the activity of B1 → 6N-acetylglucosaminyltransferase.

**DISCUSSION**

The present studies show that leukemic cells of T-cell origin express O-glycans that are more complex than those expressed on resting T lymphocytes. By gaining this more complex hexasaccharide, the apparent mol wt weight of leukosialin becomes larger in leukemic cells. At the same time, leukosialin with the hexasaccharides becomes reactive with MoAb T-305 because this MoAb recognizes the hexasaccharides attached to leukosialin. This study demonstrates a clear correlation between the attachment of the hexasaccharides and the increase of mol wt of leukosialin, based on the characterization of O-glycans attached to leukosialin. In addition, all glycoproteins visualized by the MoAb T-305 are larger than those detected in resting T lymphocytes by antileukosialin antibodies. These results indicate that leukosialin can be judged to contain the hexasaccharides when it has a high mol wt and reacts with the T-305 antibody.

The expression of the hexasaccharides appears to be correlated to the types of leukemia involved. Among T-lymphocytic leukemias, ALL expresses more high mol wt forms of leukosialin than CLL. How leukemic cells begin to express the hexasaccharides that are barely detected in normal T lymphocytes of PB is not yet known. We previously showed that normal activated T lymphocytes express the branched hexasaccharides almost exclusively. Obviously, however, these leukemic cells are nonfunctional and far different from the activated T lymphocytes that have full functional capability. A clue to understanding the appearance of the T-305-reactive hexasaccharide derives from analysis of thymocyte leukosialin, which shows a heterogeneous mol wt array; some of the molecules apparently contain the T-305-reactive hexasaccharide. Because thymocytes are precursors for T lymphocytes, the appearance of this hexasaccharide in leukemic cells can be interpreted as reappearance of a differentiation antigen that was suppressed during maturation, and this hexasaccharide can be regarded as a typical example of an onco-differentiation antigen. Thymocytes and peripheral activated T lymphocytes differ in expression of proteins other than the T-305 determinant, however. In the thymus, most large cortical thymocytes are positive for T-305 reactivity and these cells also express CD1, whereas peripheral lymphocytes are negative for CD1. Nevertheless, the conversion of quiescent cells such as resting T lymphocytes to cell division is apparently associated with the appearance of the T-305 hexasaccharides. Further studies will be required to determine if synthesis of the hexasaccharides is necessary for T cells to initiate cell division or if expression of the T-305-reactive moiety is a result of a change in differentiation state.

The present study shows that the appearance of the branched hexasaccharide is caused by the appearance of Galβ1 → 3GalNAcβ1 → 6N-acetylglucosaminyltransferase. As shown previously, the same enzyme was critical in the conversion of the tetrasaccharides to the hexasaccharides during T-cell activation. Apparently the presence of this enzyme is essential to form Galβ1 → 3(GlcNAcb1 → 6)GalNAc, which in turn is changed to Galβ1 → 3(Galβ1 → 4GlcNAcβ1 → 6)GalNAc by β-galactosyltransferase and then to the final product, NeuNac2 → 3Galβ1 → 3(NeuNac2 → 3Galβ1 → 4GlcNAcβ1 → 6)GalNAc, by the action of α2 → 6sialyltransf$	ext{a}$. It is noteworthy that the activity of α2 → 6sialyltransf$	ext{a}$

### Table 2. Glycosyltransferase Activities in Normal Resting T Lymphocytes and Leukemic Cells

<table>
<thead>
<tr>
<th>Glycosyltransferase</th>
<th>Normal T Lymphocytes</th>
<th>K-562</th>
<th>HL-60</th>
<th>HSB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galβ1 → 3GalNAcβ1 → 6GlcNAc</td>
<td>0.04 ± 0.01</td>
<td>&lt; 0.02</td>
<td>0.54 ± 0.03</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>GalNAcα2 → 6NeuNAc</td>
<td>0.47 ± 0.04</td>
<td>0.34 ± 0.03</td>
<td>0.33 ± 0.02</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Galβ1 → 3GalNAcα2 → 3NeuNAc</td>
<td>0.13 ± 0.01</td>
<td>0.20 ± 0.03</td>
<td>0.22 ± 0.09</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>GlcNAcα1 → 4Gal</td>
<td>16.2 ± 1.6</td>
<td>16.6 ± 1.1</td>
<td>11.9 ± 1.3</td>
<td>14.1 ± 2.9</td>
</tr>
<tr>
<td>β1 → 6GlcNAcα2 → 6NeuNAc</td>
<td>2.9</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glycosyltransferase activities were assayed as described in the Materials and Methods section. Enzyme activities are shown as the values of donor substrate incorporated into exogenous acceptor (nmol/h/mg protein). The mean and SE of three preparations are given. The detection limit for these assays was approximately 0.02 nmol/h/mg protein.
ferase is not changed in leukemic cells. The β1 → 6N-acetylgalactosaminyltransferase is probably present in earlier Golgi cisternae than α2 → 6sialyltransferase, as was shown for other glycosyltransferases involved in O-glycan synthesis. In this case, the β1 → 6N-acetylgalactosaminyltransferase adds N-acetylgalactosamine at C-6 of N-acetylgalactosamine before the α2 → 6sialyltransferase adds a sialic acid to the same position. The branched hexasaccharide is therefore formed by β1 → 6N-acetylgalactosaminyltransferase which α2 → 6sialyltransferase is present or not. It will be of significance to determine the subcellular localization of these glycosyltransferases to test this hypothesis.

The present study also provides a basis for diagnosis and prognosis of leukemia of T-cell origin. When a patient's leukemic cells are shown to express high-mol-wt leukosialin predominantly, that patient must be suspected of having a significant number of T-lymphoblastoid cells. In addition, when a patient with T-CLL gains the high-mol-wt form of leukosialin, it is likely that the proportion of lymphoblasts increases in PB lymphocytes. These results suggest strongly that the prognostic status of a patient can be assessed by determining the mol wt of leukosialin and the reactivity with T-305 antibody. Developing therapy by using this hexasaccharide as a target also may be possible, although it is clear that immature thymocytes also express these saccharides. We hope that further studies using polyclonal antileukosialin antibodies and MoAb T-305 will provide a tool to assess the diagnosis, prognosis, and potential therapy of hematologic disorders of T-lymphocyte origin.

O-Glycans in murine cells appear to be different from those present in human cells described in the present report. Lefrancois et al reported that activated cytotoxic T lymphocytes of the murine system express carbohydrate antigens that distinguish activated cytotoxic T lymphocytes from resting T lymphocytes or helper T lymphocytes. MoAb specific to murine cytotoxic T lymphocytes were later shown actually to recognize the Cad determinant. Because murine cytotoxic T lymphocytes express leukosialin of higher mol wt than that expressed in resting T lymphocytes, O-glycans with the Cad determinant probably are present in leukosialin from activated T lymphocytes but not in resting T lymphocytes in the murine system. Despite this apparent difference in oligosaccharide structures between human and mouse cells, it is of significance that differentiation antigens specific to activated T lymphocytes are of carbohydrate nature in both systems. It will be interesting to determine if the changes in oligosaccharide structure play some role in T-cell circulation in the blood or in the altered functions of T lymphocytes after activation or in leukemogenesis.

ACKNOWLEDGMENT

We thank Ted Shih for technical assistance, Dr Mark A. Williams for critical reading of the manuscript, and Henny Bierhuizen for secretarial assistance.

REFERENCES

18. Fox RI, Hueniken M, Fong S, Behar S, Royston I, Singhal
T-lymphocytic leukemia expresses complex, branched O-linked oligosaccharides on a major sialoglycoprotein, leukosialin

O Saitoh, F Piller, RI Fox and M Fukuda