Expression of GD₃ Ganglioside in Childhood T-Cell Lymphoblastic Malignancies¹

William D. Merritt,² James T. Casper, Stephen J. Lauer, and Gregory H. Reaman

ABSTRACT

Lymphoblasts from seven children with T-cell lymphoblastic malignancies and three children with non-T, non-B acute lymphoblastic leukemia (ALL) were analyzed for ganglioside content. Nonmalignant T-cells from thymus served as controls. Both ganglioside and glycoprotein sialic acid were increased approximately 3-3.5-fold in T-cell disease compared to thymic tissue when expressed on a per cell basis, but not on a per milligram protein basis. Thin-layer chromatography of the isolated ganglioside fraction from T-cell lymphoblasts revealed two major resorcinol-positive bands. One ganglioside comigrated with II₃-a-/V-acetylneuraminosyl-gangliotetraosylceramide (GM₂), the major ganglioside in normal lymphoid tissue, and the other ganglioside comigrated with authentic II₃-a-/V-acetylneuraminosyldiacylglycerol (GD₃). In three different solvent systems, Neuraminidase treatment of the latter ganglioside yielded (GD₁) and lactosylceramide, hydrolysis products of GD₃. Scanning densitometry revealed that whereas thymus cells contained 78 ± 0.3% GD₃, non-T, non-B lymphoblasts contained from 22 to 86% GD₃, consistent with a corresponding decrease in GM₂. The shift to increased GD₃ was observed in the blasts from all seven T-cell patients, but not in the blasts from the non-T, non-B patients studied. Only trace quantities of GD₁ were detected from two continuous T-ALL cell lines, HSB2 and RPMI 8402. The results demonstrate a consistently significant increase in ganglioside GD₃ in uncultured, patient-derived T-cell ALL lymphoblasts when compared to non-T, non-B patients studied. Therefore, GD₃ may represent a tumor-associated antigen for the T-cell subclass of childhood lymphoblastic malignancy.

INTRODUCTION

Transformation of cells by oncogenic viruses or by chemicals consistently results in one or more changes in the composition of neural glycolipids and/or sialylated glycolipids (gangliosides). Altered expression of glycolipids appears to be related to a block in ganglioside biosynthesis to an inappropriate pathway (for reviews see Refs. 1–4). For instance, during hepatocarcinogenesis of the rat, synthesis of the disialoganglioside, GD₃, is blocked, resulting in lowered expression of complex gangliosides, particularly trisialoganglioside (5, 6). On the other hand, GD₃ is inappropriately expressed in human melanoma cells (7, 8). In studies of gangliosides of human leukemias, GD₃ is expressed to varying extents in the blasts of patients with chronic lymphocytic leukemia (9), acute lymphoblastic leukemia (ALL), and acute myeloblastic leukemia (10, 11). We have studied the ganglioside composition in malignant blasts of six children with acute T-cell lymphoblastic leukemia (T-Cell ALL), one with T-cell lymphoblastic lymphoma, and three with non-T, non-B ALL. We have found that GD₃ was a major ganglioside in each of the T-cell malignant lymphoblastic samples studied, whereas this ganglioside was barely detectable or absent in the non-T, non-B leukemias and normal thymus that we evaluated.

MATERIALS AND METHODS

Isolation and Characterization of Lymphocytes from Human Lymphoid Tissue and Lymphoblasts from Patients with Acute Lymphoid Malignancies. Fresh tonsils and adenoid tissue or lymphomas were obtained immediately after surgical removal; any highly inflamed tissue was discarded. Thymus tissue was freshly obtained after surgical removal from children undergoing cardiac surgery. Tissues were washed in McCoy's medium (tonsils and adenoids) or RPMI 1640 (thymus) with penicillin (100 µg/ml) and streptomycin (100 units/ml), and trimmed free of hematomas and visible connective/vascular tissue. Single cell preparations from each tissue were prepared as described (12). The cells were examined for viability by trypan blue exclusion, and the final cell pellet was stored at −20°C for glycolipid extraction.

Lymphoblasts were separated from whole blood, bone marrow, or pleural fluid from leukemic patients by either plasmagel sedimentation in Hanks' balanced salt solution (13), or by Ficoll-Hypaque density gradient centrifugation, as previously described (14). Cells were washed two times in PBS, checked for viability by trypan blue exclusion, and the percentage of lymphoblasts was determined by Wright's staining. Cells were further characterized with respect to various T-cell and leukemia-associated (CALLA) cell surface antigens using sheep erythrocyte rosetting assays (14, 15) or immunofluorescent techniques with heterologous or monoclonal antibodies as previously described (12, 15–17). Lymphoblasts in all cases were positive for terminal deoxynucleotidyl transferase (18) and negative for surface immunoglobulin, myeloperoxidase, chloroacetate esterase, and α-naphthylbutyrate esterase.

Culture of Human T-Cell Leukemia Lines. The cell lines RPMI 8402 (T₁₈,₁) and CCRF-HSB2 (T₂₀) (19, 20) were obtained from the laboratory of Dr. J. Minowada, Roswell Park Memorial Institute, Buffalo, NY. Karyotyping of the cultured cell lines was performed in the cytogenetics laboratory of Milwaukee Children’s Hospital, and the presence of Epstein-Barr virus was analyzed by immunofluorescence staining for us by Dr. Warner Henle, Joseph Stokes Jr. Research Institute, Philadelphia, PA. The cells were cultured in RPMI 1640 media (GIBCO, Grand Island, NY) in the presence of penicillin (100 µg/ml), streptomycin (100 units/ml), 4 mm 4-(2-hydroxyethyl)-1-piperazineneethanesulfonic acid, and 10% heat-inactivated fetal bovine serum (GIBCO). Cells were cultured in a humidified 5% CO₂, 37°C incubator, and were split two times per week. For analysis of gangliosides, cells were cultured in 350-ml volumes in closed-roller bottles.

Isolation and Purification of Gangliosides. Cell pellets were homogenized in a small volume of dH₂O and homogenized with a polytron homogenizer. Aliquots were removed for assay of protein and sialic acid. Lipids were extracted by overnight stirring of the homogenate in chloroform:methanol in the ratio, 1:1:0:1 (v/v/v) of chloroform:methanol:homogenate. The precipitated material was collected by filtration through a sintered glass filter and then reextracted twice with chloroform:methanol:water, 1:1:0:1 (v/v/v). The combined lipid extracts were evaporated by rotary evaporation at 45°C, and gangliosides and neutral glycolipids were separated by the procedure of Ledeen et al. (21). Specifically, the residue was dissolved in chloroform:
methanol:water, 60:30:8 (v/v/v), and placed on a DEAE-Sephadex A-25 column (5 g of resin/2.5 × 10^8 cells). Nonacidic lipids, including neutral glycolipids, and acidic lipids, including gangliosides, were eluted separately. After removal of solvent, lipids in the latter fraction were saponified with 0.1 M methanolic NaOH to destroy phospholipids (22). The mixture was dialyzed in the presence of EDTA against distilled H_2O for 48 h, and then lyophilized. The lyophilized material was dissolved in chloroform:methanol, 4:1 (v/v), to a 2-g column of silicic acid (Biosil; Bio-Rad Industries). Sulfatides and other contaminants were eluted with chloroform:methanol, 80:20 (v/v), and gangliosides were eluted with chloroform:methanol, 1:1 (v/v) (21). The ganglioside fraction was dried under nitrogen, dissolved in chloroform:methanol, 1:1 (v/v) and stored at –20°C for further analysis.

Individual gangliosides were purified from T-cell leukemia extracts by preparatory TLC. Chromatography was on 250 μ silica gel H 20- x 20-cm plates (Analtech, Newark, DE) with the solvent system chloroform:methanol:0.22% CaCl_2, 60:40:9 (v/v/v). Silica gel H was used instead of silica gel G to improve the recovery of the gangliosides away from the silica gel. Individual gangliosides were located with iodine vapor, scraped, and then eluted by overnight shaking in chloroform:methanol:water, 1:1:0.1 (v/v/v).

Analytical Procedures. Gangliosides were separated by TLC on precoated silica gel 60 TLC or HPTLC plates in either n-propanol: 0.22% CaCl_2, 8:2 (v/v) (Solvent A), chloroform:methanol:0.22% CaCl_2, 60:40:9 (v/v/v) (Solvent B), or chloroform:methanol:28% NH_4OH:water, 60:40:3.5:5 (v/v/v/v) (Solvent C). Ganglioside standards were spotted in lanes alongside experimental lanes. Standards included GM_1, isolated from human liver (23); SGP, isolated from human red blood cells (24); human brain GD_3 and Tay-Sachs brain GM_2, gifts from Dr. Robert Yu, Yale University; and GM_1 and GD_2 (Supelco, Bellefonte, PA). Ganglioside bands were visualized by resorcinol staining (25). The relative proportions of individual gangliosides were assessed by scanning densitometry using a Schimadzu model CS-930 TLC scanning densitometer, with drifting baseline and peak integration capability.

Ganglioside acid was measured by the method of Hammond and Papermaster (26) at 549 μm, after digestion of gangliosides in 0.1 M H_2SO_4 for 1 h at 80°C. Glycoprotein sialic acid was measured from the chloroform-methanol-insoluble residues collected on sintered glass filters after lipid extraction of cells (procedure modified from Ref. 27). Samples (10 mg) were digested for 2.5 h at 80°C to optimize sialic acid hydrolysis, and digests were placed on a column of BioRad AG 1-× 8 acetate ion exchange resin. Sialic acid was eluted with 1.5 ml 1 N formate, and 0.5-ml samples were assayed in triplicate as above. Various concentrations of N-acetylneuraminic acid (Sigma) were processed in parallel throughout the procedure. Proteins were measured by a linear formate, and 0.5-ml samples were assayed in triplicate as above. Various concentrations of N-acetylneuraminic acid (Sigma) were processed in parallel throughout the procedure. Proteins were measured by a linear transform modification of the Folin-Lowry procedure (28).

Neuraminidase Digestion of GD_3. Ganglioside. The isolated GD_3 (8 nmol sialic acid/treatment) was resuspended in 100 μl of 0.05 M sodium acetate buffer, pH 5.7, with either 150, 50, or 15 munits neuraminidase (Clostridium perfringens, Type X; Sigma), (29). Incubations were for 10 min to 24 h at 37°C, and were terminated by addition of 2 ml of chloroform:methanol, 2:1 (v/v). The precipitated protein was removed by centrifugation, and the soluble components were partitioned by addition of 0.4 ml water, vortexing, and recentrifugation (270 × g, 15 min). The lower phase was dried under N_2, and the lipids were resuspended in a small volume of chloroform:methanol, 2:1 (v/v) and separated by HPTLC, using the solvent system, chloroform:methanol:water, 50:20:3.3 (v/v/v). Bands were visualized by spraying with orcinol reagent.

RESULTS

Lymphoblastic Malignancies. Surface marker characterization of the malignant cells from seven of the ten patients demonstrated that the cells were of T-cell origin. This was demonstrated by sheep erythrocyte rosetting and/or reactivity with either an antithymic antibody or with a monoclonal antibody against the sheep erythrocyte receptor (OKT11) (Table 1). The remaining ALL patients studied were non-T, non-B based on absence of T-cell markers and surface membrane immunoglobulin. These cells were examined for expression of CALLA, and were either CALLA* (2 cases) or CALLA+ (1 case). All samples demonstrated terminal deoxynucleotid transferase activity.

Quantitation of Ganglioside and Glycoprotein Sialic Acid. Sialic acid was measured colorimetrically in the purified ganglioside fractions and lipid-free residue from pooled thymic tissue (12 samples), and the lymphoblasts from six separate T-cell ALL patients. The results showed that both ganglioside and glycoprotein sialic acid were increased approximately 3-3.5-fold in leukemia cells compared to thymic cells. Measurements of the ganglioside fractions showed that T-cell leukemias contained 11.80 ± 4.3 nmol sialic acid/10^8 cells, and the thymocytes contained 4.3 ± 2.0 nmol/10^8 cells. Similarly, the leukemia samples contained 133.0 ± 12 nmol glycoprotein sialic acid/10^6 cells whereas thymocytes contained 38.0 ± 4 nmol/10^6 cells. On the other hand, T-cell ALL cells and thymocytes contained equal amounts of ganglioside and glycoprotein sialic acid when expressed on a per mg protein basis (0.5 and 3.9 nmol/mg protein respectively).

Ganglioside TLC of Malignant T-Lymphoblasts. The purified ganglioside fraction of malignant lymphoblasts in each of seven T-cell patients with malignancies were analyzed by TLC. Fig. 1 shows the ganglioside patterns of three leukemia samples (lanes 5-7) compared to tonsil and adenoid lymphocytes (lane 3) and thymocytes (lane 4). The patterns show that the ganglioside profile from T-malignant blasts is more similar to that of thymus gangliosides than that of tonsil and adenoid lymphocyte gangliosides. However, the leukemia cells have a lowered amount of the major doublet ganglioside found in thymus (Fig. 1, single arrow), which chromatographed with standard GM_3, and a proportionally larger amount of a second but very minor doublet ganglioside (Fig. 1, double arrow). The latter ganglioside was also observed in tonsil and adenoid lympho-

Table 1 Characteristics of lymphoblastic malignancies analyzed

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Surface phenotype*</th>
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<tbody>
<tr>
<td>1</td>
<td>T-ALL</td>
<td>M</td>
<td>4.5</td>
<td>ER*(77%),T-Ag*(70%),CALLA-</td>
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<td>2</td>
<td>T-ALL</td>
<td>F</td>
<td>11</td>
<td>ER*(57%),T-Ag*(72%),CALLA-</td>
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<tr>
<td>3</td>
<td>T-ALL</td>
<td>M</td>
<td>12</td>
<td>ER*(63%),T-Ag*(70%),CALLA-</td>
</tr>
<tr>
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<td>T-lymphoblastic</td>
<td>F</td>
<td>11</td>
<td>ER*(85%),T-Ag*(95%),CALLA-</td>
</tr>
<tr>
<td>5</td>
<td>T-ALL</td>
<td>M</td>
<td>16</td>
<td>ER*(2%),T-Ag*(40%),CALLA-</td>
</tr>
<tr>
<td>6</td>
<td>T-ALL</td>
<td>M</td>
<td>3</td>
<td>ER*(96%),OKT11*(97%),B4,CALLA+</td>
</tr>
<tr>
<td>7</td>
<td>T-ALL</td>
<td>F</td>
<td>5</td>
<td>ER*(95%),OKT11*(97%),B4,CALLA+</td>
</tr>
<tr>
<td>8</td>
<td>non-T, non-B ALL</td>
<td>F</td>
<td>9</td>
<td>ER*,OKT11*,B4,sig,CALLA+</td>
</tr>
<tr>
<td>9</td>
<td>non-T, non-B ALL</td>
<td>M</td>
<td>5</td>
<td>ER*,T-Ag,sig,CALLA+</td>
</tr>
<tr>
<td>10</td>
<td>non-T, non-B ALL</td>
<td>M</td>
<td>2.5</td>
<td>ER*,T-Ag,sig,CALLA+</td>
</tr>
</tbody>
</table>

* ER, percentage of sheep erythrocyte rosette formation at 4°C; T-Ag, percentage of expression of thymocyte antigen; CALLA, expression of common acute lymphoblastic leukemia antigen; OKT11 and B4, expression of T11 and B4 antigens, respectively; sig, expression of surface membrane immunoglobulin. All samples were positive for terminal deoxynucleotid transferase.
GD₂ IN MALIGNANT T-LYMPHOBLASTS

TLC and Neuraminidase Treatment of Purified Ganglioside from Malignant T-Lymphoblasts. Based on the migration of the two major gangliosides of the mixed ganglioside fractions in the GM₁ and GD₂ regions, purified upper and lower doublet gangliosides were tested for comigration with GM₁ and GD₂ standards in three different solvent systems. As seen in Fig. 3, the two major T-lymphoblast gangliosides chromatographed with identical Rₜ values to GM₁ and GD₂, respectively, in each solvent system. The results also indicate that the doublet gangliosides are indeed single ganglioside species, with differences in the ceramide portion accounting for slight differences in the migration of each band of the doublets.

Standard GD₂ and the lower doublet ganglioside of T-lymphoblasts were treated with *C. perfringens* neuraminidase to compare the ganglioside and neutral glycosphingolipid neuraminidase products of these gangliosides (Fig. 4). Incubation of brain GD₂ standard with 150 munits neuraminidase for short times (10–40 min) resulted in loss of GD₂ and appearance of both GM₁ and lactosyl ceramide. Longer periods of time (2 and 24 h) were required to hydrolyze GM₁ completely to lactosyl ceramide. Neuraminidase treatment of the ganglioside from T-lymphoblasts also resulted in glycolipids which migrated with GM₁ and lactosyl ceramide, although this ganglioside was more sensitive to neuraminidase than brain GD₂. 150 munits treatment for 10 min resulted in complete hydrolysis of the ganglioside to lactosyl ceramide, 15 munits for 10 min resulted in appearance of GM₁ with no lactosyl ceramide, and 50 munits resulted in both GM₁ and lactosyl ceramide (not shown). These results are consistent with the preliminary identification of the lower doublet ganglioside of T-lymphoblasts, based on the three-solvent TLC, as GD₂.

Comparison of Gangliosides of Malignant T-Lymphoblasts to Gangliosides of Non-T, Non-B Lymphoblasts. Gangliosides were extracted from cells obtained from three non-T, non-B ALL patients (two CALLA⁻ and one CALLA⁺) to determine if high levels of GD₂ were common to childhood lymphoblastic leukemias and lymphomas in general. The results of the TLC chromatogram of the purified gangliosides showed that the lower doublet ganglioside observed in malignant T-lymphoblasts (GD₂) was not found in these non-T, non-B leukemic blasts. Rather, a ganglioside which cochromatographed with the major ganglioside of human red blood cells, SPG, was the major ganglioside other than GM₁ in these cells (Fig. 5).

Quantitative Scanning Densitometry of Ganglioside Profiles of Childhood Lymphoblastic Malignancies. Results of scanning densitometry of the ganglioside profiles of thymocytes, blasts from six T-ALL patients and one T-cell lymphoma patient, and blasts from three non-T, non-B ALL patients are shown in Fig. 6. GD₂ was a major ganglioside of cells from all seven patients with T-cell disease. In contrast to thymus, in which 97% of the gangliosides was GM₁ and 3% was GD₂, T-cell lymphoblasts contained from 2 to 77.7% GM₁ and from 22 to 86% GD₂, with the median ganglioside content of 37% GM₁ and 63% GD₂. Non-T, non-B ALL, as indicated above, did not contain detectable GD₂, but rather appeared to contain SPG as a major ganglioside.

Gangliosides of T-Cell Leukemia Cell Lines. Gangliosides were extracted from two cultured ALL cell lines, CCRF-HSB2 and RPMI 8402. Unlike the results from patient-derived T-lymphoblasts, these cells did not contain increased levels of GD₂ relative to GM₁ (Fig. 7 and Table 2). Rather, GD₂ was only a minor ganglioside in HSB2 and was not detectable in 8402. These cell lines contained high levels of three other gangliosides, but not in red blood cells (Fig. 1) and cochromatographed with a purified GD₂ standard (Fig. 2). Further characterization of the nature of the gangliosides of T-malignant lymphoblasts followed isolation of the upper and lower doublet gangliosides from purified total ganglioside by preparative TLC.
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Fig. 3. Purified gangliosides of T-cell ALL. Thin-layer chromatograms of isolated individual gangliosides of T-cell ALL together with purified standards. Separations were on silica gel G HPTLC plates, utilizing solvent system A (A), solvent system B (B), and solvent system C (C). Purified ganglioside standards are indicated: T1, purified lower band ganglioside; Tu, purified upper band ganglioside. Ganglioside sialic acid (3 nmol) was spotted in each lane.

DISCUSSION

The results show that childhood T-cell acute lymphoblastic malignancies consistently expressed GD3, as the major ganglio-
GD3 in Malignant T-Lymphoblasts

The purified leukemia ganglioside was identified as GD3 by similar Rf values to authentic GD3 in three solvent systems, and by the products of neuraminidase treatment. GD3 has been observed in both childhood and adult leukemias in other laboratories. In particular, chronic lymphocytic leukemia blasts were shown by Goff et al. to also contain predominantly GM3 and GD3 (9), unlike chronic myelogenous leukemia cells (30). In two of three T-cell ALL patients, and one acute myeloblastic leukemia patient studied by Westrick et al. (10), a double-band ganglioside was visualized on TLC plates between IV3 NeuAc-nLeOse4Cer (SPG) and VI3 NeuAc-nLeOse6Cer, but this ganglioside was not identified as GD3 in this paper. Among T-cell malignancies, GD3 may not be restricted to childhood disease: a ganglioside which migrated in thin-layer chromatograms in the expected GD3 region compared to a human brain ganglioside standard was observed in lipid extracts of blasts from two adult T-cell leukemia patients (31). GD3 has been identified by anti-GD3 antibody immunostaining in several preparations of acute and chronic lymphocytic leukemia cells (11), although the cell surface antigen phenotypes were not further subclassified immunologically. In our study of the gangliosides of ten childhood lymphoblastic malignancies, GD3 was expressed in all seven T-cell malignancies, whereas no GD3 was found in the three non-T, non-B leukemias which were studied. Although very low levels of GD3 that are detectable only by sensitive anti-GD3 but not resorcinol staining may be present in non-T, non-B ALL, the results suggest that large quantitative differences in GD3 levels may distinguish these subtypes of childhood lymphoblastic malignancies.

A major ganglioside of the non-T, non-B (CALLA−) blasts, besides GM3, was a ganglioside tentatively identified as SPG. SPG was not found exclusively in these non-T blasts, since low amounts were also observed in two of seven T-ALL cells. Westrick et al. (10) observed in one non-T, non-B lymphoblastic leukemia the expression of high SPG by TLC analysis. SPG was also observed as one of two major gangliosides of acute myelomonoblastic leukemia (32).

Unlike the results obtained with freshly isolated patient T-cell leukemic lymphoblasts, two cultured T-ALL cell lines did not contain a high relative concentration of GD3. Rather, only trace amounts were observed, in contrast to high levels of three other gangliosides which were not found in the patient samples. Several explanations are possible for this observation. One is that these two leukemias were established from two patients with rare T-cell leukemias that can be established in culture and coincidently contained this unique ganglioside pattern. It is well known that blasts from T-cell ALL are very difficult to establish in culture (33). Since this ganglioside pattern was observed in both cultured T-ALL lines, a relationship between establishment of these cells in culture and the ganglioside content is suggested. Secondly, it is possible that the ganglioside composition of T-cell leukemia blasts is altered during establishment of the cells in culture. In this regard, the ganglioside composition of cells in culture is dependent upon the culture stage (34). Third, these cultured leukemias may represent a stage of leukemogenesis that is different from any stage of T-cell lymphoid malignancy in our current patient population, although this is unlikely due to the relatively large number of T-cell malignancies studied. Saito et al. (35), have tested various cultured
GD\textsubscript{3} IN MALIGNANT T-LYMPHOBLASTS

Fig. 7. Gangliosides of T-ALL cell lines. Thin-layer chromatograms of gangliosides of two T-ALL cell lines compared to primary T-cell ALL cells. Gangliosides from CCRF-HSB2 cells (HSB2) or RPMI 8402 (8402) cells were separated on silica gel G HPTLC plates, and developed in either solvent system A (A) or solvent system B (B). T, cells from a T-cell ALL patient; St, standards (a, GM\textsubscript{3}; b, GM\textsubscript{2}; c, SPG; d, GM\textsubscript{1}; e, GD\textsubscript{3}; f, CD\textsubscript{a}).

Table 2 Gangliosides of T-ALL cell lines

<table>
<thead>
<tr>
<th>Ganglioside region</th>
<th>HSB2</th>
<th>RPMI 8402</th>
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<tr>
<td>GM\textsubscript{3}</td>
<td>10.5</td>
<td>5.6</td>
</tr>
<tr>
<td>GD\textsubscript{3}</td>
<td>4.5</td>
<td>ND*</td>
</tr>
<tr>
<td>GM\textsubscript{2}</td>
<td>42.7</td>
<td>30.6</td>
</tr>
<tr>
<td>GM\textsubscript{1}</td>
<td>18.6</td>
<td>25.6</td>
</tr>
<tr>
<td>GD\textsubscript{a}</td>
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<td>38.0</td>
</tr>
<tr>
<td>Other</td>
<td>3.3</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not detected.

human leukemic cultured cells for ganglioside content. Varied patterns were observed for the four T-cell lines tested, from simple to complex, but GD\textsubscript{3} does not appear to be present in these lines according to their densitometric tracings. It is clear from our results that ganglioside patterns of T-cell lines are distinctly different from that of freshly obtained, uncultured cells, and therefore these lines may not necessarily be useful tools for obtaining information on specific glycolipid markers in T-cell lymphoblastic malignancies. We are currently utilizing an anti-GD\textsubscript{3} antibody to investigate the surface expression of GD\textsubscript{3} in large numbers of patients with childhood lymphoblastic malignancies.

We conclude that the immunological subclassification of childhood lymphoid malignancies appears to correlate with the ganglioside composition, particularly the expression of GD\textsubscript{3}. Since GD\textsubscript{3} is increased in T-cell malignancies relative to normal thymocytes, GD\textsubscript{3} may be a leukemia/lymphoma-specific alteration rather than an alteration related to normal T-lymphocyte ontogeny, although the possibility that GD\textsubscript{3} is expressed in very early stem cells of normal bone marrow and again in these malignancies cannot be discounted.

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