Changes in Glycosphingolipid Composition during Differentiation of Human Leukemic Granulocytes in Chronic Myelogenous Leukemia Compared with in Vitro Granulocytic Differentiation of Human Promyelocytic Leukemia Cell Line HL-60

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INTRODUCTION

GSLs are chemically well-defined molecules residing in the cell surface membrane. According to their carbohydrate structure, they are classified into three major series, i.e., the globo-, lacto-, and ganglio- series (6). Acidic sialo-GSLs, gangliosides, have been thought to be located almost exclusively on the external side of the plasma membrane of mammalian cells (9), providing cell surface recognition sites with negative charges. They might play important physiological roles in specific interactions with various bioactive, exogenous factors and in cell-to-cell interactions (6), although they constitute only a small proportion of the cell surface glycoconjugates (9). Besides it should be noted that GSLs show dramatic changes associated with oncogenesis and ontogenesis in their composition and biosynthesis (6).

We have reported differentiation-associated changes of the ganglioside composition in mouse myeloid leukemia M1 cells (19) and the distinctive characteristics of the ganglioside-profiles in human leukemia-lymphoma cell lines which were considered to be blocked at certain stages of differentiation (18). Similar results were also reported by Rosenfelder et al. (15) for various leukemia-lymphoma cell lines. Human promyelocytic leukemia cell line HL-60 cells (3) could be induced to differentiate into both granulocytic mature cells (2, 4) and macrophage-like cells (16). Recently we reported the distinct expression of GSL profiles during the bipotential cell differentiation of HL-60 cells and demonstrated that the GSL profiles expressed depended not only on the maturation stage but also on the direction of differentiation (12). These results indicated that the GSL profiles were differentiation-associated phenotypes in leukemic cells.

CML is a neoplastic disease that results from the development of an abnormal hemopoietic stem cell which gives rise to progeny that have the Philadelphia chromosome. In CML, a large number of immature and mature granulocytic cells accumulate in the bone marrow and the peripheral blood. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 8/26/85.
The abbreviations used are: GSL, glycosphingolipid; CML, chronic myelogenous leukemia; RA, retinoic acid; C.M., chloroform/methanol; HPTLC, high-performance thin-layer chromatography; DMSO, dimethyl sulfoxide; CMH, ceramide monohexoside; CDH, ceramide dihexoside; LTC, lactosylceramide; CDH, ceramide monohexoside; PG, paragloboside; G, G1, G2, G3, G4, G5, G6, G7, G8, G9, G10; SNHC, sialosylparagloboside;thic granulocytic cells derived from human chronic myelogenous leukemia (CML) cases and were compared with those found in the in vitro granulocytic differentiation of the human promyelocytic leukemia HL-60 cell line. Two neutral GSLs, ceramide monohexoside and ceramide dihexoside, and two molecular species of gangliosides, one being the ganglio-series ganglioside NeuAc(a2-3)Gal(β1-4)Glc-Cer (G0) and the other being the lacto-series sialosylparagloboside, were predominant in the granulocytic cells at an early maturation stage. During the granulocytic differentiation of CML cells, the contents of ceramide dihexoside and paragloboside increased strikingly with a concomitant decrease in ceramide monohexoside, and the total amount of neutral GSLs increased to about three times as much as that of the most immature granulocytic cells, myeloblasts. On the other hand, lacto-series gangliosides, with longer sugar moieties increased with a concomitant decrease in ganglio-series ganglioside G0, and the ganglioside profile became more complex. The total content of ganglioside increased in parallel with the complexity of the ganglioside profile. Similar differentiation-associated changes were also found in GSL composition during the in vitro granulocytic differentiation of HL-60 cells. However, a marked difference between the differentiation-dependent change in the GSL composition of CML cells and that of HL-60 cells was observed for a ganglioside species which was found to be one of the major gangliosides in normal neutrophils: in the former, the ganglioside level increased up to the level in normal mature granulocytes as the cells differentiated; in contrast, it decreased significantly during granulocytic differentiation of the latter cells.

When the GSL composition of the neutrophils obtained from CML cells, which were apparently normal as to morphology, stimulus-induced membrane potential changes, and superoxide-producing capacity, was compared with that of normal neutrophils, an obvious difference was observed between them, especially with regard to ganglioside G0, the amount of ganglioside G0 in the former was about one-sixth of that in the latter. This difference indicates some alterations in the cell membrane structure of neutrophils of CML origin.

Changes in glycosphingolipid (GSL) composition during differentiation of human leukemic granulocytes were investigated qualitatively and quantitatively in immature and mature granulocytic cells derived from human chronic myelogenous leukemia (CML) cases and were compared with those found in the in vitro granulocytic differentiation of the human promyelocytic leukemia HL-60 cell line. Two neutral GSLs, ceramide monohexoside and ceramide dihexoside, and two molecular species of gangliosides, one being the ganglio-series ganglioside NeuAc(a2-3)Gal(β1-4)Glc-Cer (G0) and the other being the lacto-series sialosylparagloboside, were predominant in the granulocytic cells at an early maturation stage. During the granulocytic differentiation of CML cells, the contents of ceramide dihexoside and paragloboside increased strikingly with a concomitant decrease in ceramide monohexoside, and the total amount of neutral GSLs increased to about three times as much as that of the most immature granulocytic cells, myeloblasts. On the other hand, lacto-series gangliosides, with longer sugar moieties increased with a concomitant decrease in ganglio-series ganglioside G0, and the ganglioside profile became more complex. The total content of ganglioside increased in parallel with the complexity of the ganglioside profile. Similar differentiation-associated changes were also found in GSL composition during the in vitro granulocytic differentiation of HL-60 cells. However, a marked difference between the differentiation-dependent change in the GSL composition of CML cells and that of HL-60 cells was observed for a ganglioside species which was found to be one of the major gangliosides in normal neutrophils: in the former, the ganglioside level increased up to the level in normal mature granulocytes as the cells differentiated; in contrast, it decreased significantly during granulocytic differentiation of the latter cells.

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peripheral blood, and granulocytes at all stages of development are present (17). In the present study, we investigated changes in the composition of GSLs during cell differentiation of human granulocytes in vivo, using human leukemic granulocytes at various stages of maturation obtained from CML patients, and compared them with those found during the in vitro granulocytic differentiation of HL-60 cells. We report that, as the granulocytic cells attained maturity, CDH and PG markedly increased, and ganglio-series ganglioside GM3 decreased with a concomitant increase in lacto-series gangliosides with longer sugar moieties and in the total amount of gangliosides in either CML cells or HL-60 cells, but a marked difference between CML cells and HL-60 cells was observed in the differentiation-associated change of a ganglioside molecular species which was one of the major gangliosides in normal neutrophils. We also demonstrated that the ganglioside composition of CML neutrophils was obviously different from that of normal neutrophils, although the former cells appeared to be almost the same morphologically and functionally as the latter cells.

MATERIALS AND METHODS

Preparation of Cells. Granulocytes at various stages of maturation were prepared from heparinized peripheral blood from five patients with CML, three in the chronic phase and two in the myeloid blastic crisis stage, by the Conray-Ficoll method (Conray from Mallinkrodt, Inc., St. Louis, MO; Ficoll from Pharmacia Fine Chemicals, Inc., Piscataway, NJ), as described previously (10). Immature and mature granulocytes were separated into three fractions, i.e., the interface layer, the Conray-Ficoll layer, and the bottom fraction. Contaminating erythrocytes in the bottom fraction were removed by hypotonic lysis. Normal human neutrophils were also prepared by the Conray-Ficoll method followed by hypotonic lysis to remove contaminating erythrocytes. The final neutrophil preparations consisted of more than 98% neutrophils of which 15% were banded neutrophils and 85% were segmented neutrophils, as determined morphologically.

Cell Culture. Human promyelocytic leukemia cell line HL-60 cells were grown in Falcon No. 3024 tissue culture flasks (Becton Dickinson Laboratory, Oxnard, CA) in RPMI 1640 (Flow Laboratories, McLean, VA) supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum (Flow Laboratories, Stanmore, New South Wales, Australia), penicillin (100 IU/ml), and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere of 5% carbon dioxide.

For the induction of granulocytic differentiation of HL-60 cells, the cells were seeded at an initial concentration of 2.0 x 10⁶ cells/ml and incubated with 1 μM RA (trans-vitamin A acid; Sigma Chemical Co., St. Louis, MO). For morphological assessment of granulocytic differentiation-induced HL-60 cells, cytospin slide preparations were prepared with a Shandon cytopsin centrifuge (Shandon Southern Products, Ltd., Astmoor, Runcorn, Cheshire, United Kingdom) and stained with Wright-Giemsa staining solution. Differential cell counting was performed under a light microscope with 200-400 stained cells for each experimental point.

Lipid Extraction. For lipid analyses, the cells were collected, washed twice with phosphate-buffered saline (pH 7.4), lyophilized, and then kept at -80°C until use. Total lipids were extracted from the lyophilized materials, equivalent to about 2-3 x 10⁶ viable cells, with C:M (1:1, v/v) containing 5% (v/v) water and then with C:M (2:1, v/v) and C:M (1:2, v/v) successively (2 h for each extraction at 37°C). The extracts were combined and separated into the acidic and neutral lipid fractions by column chromatography on DEA-Sephadex A-25, acetate form (Phar macia), essentially as described by Ando et al. (1).

Analysis of Neutral GSLs. Neutral GSLs were purified from the neutral lipid fraction according to the acetylation method described by Saito and Hakomori (20). They were separated by HPTLC on precoated Silica Gel 60 plates (E. Merck, Darmstadt, Federal Republic of Germany) with a solvent system of C:M:water (65:25:4, v/v/v), sprayed with orcinol:H₂SO₄ reagent, and visualized by heating of the plate at 100°C. Quantitation was carried out by scanning HPTLC chromatograms with a dual wavelength TLC scanner (Shimadzu CS-910) at 540 nm. The hexose content of the total neutral GSLs was determined by the anthrone:H₂SO₄ method (26) with galactose as a standard.

Analysis of Gangliosides. The acidic lipid fraction was subjected to mild alkaline treatment to hydrolyze contaminating phospholipids and then desalted by gel filtration on Sephadex G-50. The recovered ganglioside fraction was separated on a HPTLC plate with a solvent system of C:M:0.5% CaCl₂ (55:45:10, v/v/v). The gangliosides were sprayed with resorcinol:HCl reagent, visualized by heating the plate at 95°C, and determined quantitatively by the densitometric scanning method, as described previously (19). Lipid-bound sialic acid in the total ganglioside fraction was determined by the resorcinol:HCl method as modified by Suzuki (21).

RESULTS

Fractionation of CML Cells. Immature and mature granulocytes from peripheral blood of CML patients either in the chronic phase or in the myeloid blastic crisis stage were separated into three fractions according to the stage of differentiation. Fraction 1 was obtained from the interface layer, Fraction 2 was from the Conray-Ficoll layer, and Fraction 3 was from the bottom. The percentages of myeloid cells recovered in each fraction were as follows: Fraction 1, 29%; Fraction 2, 32.8%; and Fraction 3, 38.1%, in the chronic phase; and Fraction 1, 68.6%; Fraction 2, 17.0%; and Fraction 3, 14.4%, in the blastic crisis stage. The result of differential counting for each fraction is shown in Table 1. The main constituents of Fraction 1 in the chronic phase were myeloblasts, promyelocytes and myelocytes; those of Fraction 2 were myelocytes and metamyelocytes; and those of Fraction 3 were banded and segmented neutrophils. Most of the myeloblasts in the myeloid blastic crisis stage were recovered in Fraction 1, and the residual, more mature cells were recovered in either Fraction 2 or Fraction 3. In parallel with the morphological maturation, functional maturation as shown by indications such as the significant increases in stimulus-induced membrane potential changes, superoxide-producing capacity, and phagocytic activity was observed as reported previously (10) (data not shown).

Differentiation-dependent Changes in GSL Composition of CML Cells. Neutral GSLs of immature and mature granulocytes derived from peripheral blood of CML patients were composed of CMH, CDH, LTC, and PG (Figs. 1 and 2). CDH was shown to be the main component of neutral GSLs, and its level was decreased in HL-60 cells, but a marked difference between CML cells and HL-60 cells was observed in the differentiation-associated change of a ganglioside molecular species which was one of the major gangliosides in normal neutrophils. We also demonstrated that the ganglioside composition of CML neutrophils was obviously different from that of normal neutrophils, although the former cells appeared to be almost the same morphologically and functionally as the latter cells.

Table 1

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mbl</th>
<th>Pro</th>
<th>Myel</th>
<th>Meta</th>
<th>Band</th>
<th>Seg</th>
<th>Lym</th>
<th>Mono</th>
<th>Baso</th>
<th>Eos</th>
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</thead>
<tbody>
<tr>
<td>Fraction 1</td>
<td>13.5</td>
<td>43.0</td>
<td>39.5</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>0</td>
<td>5.5</td>
<td>65.5</td>
<td>19.0</td>
<td>6.0</td>
<td>1.0</td>
<td>0</td>
<td>1.0</td>
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<tr>
<td>Fraction 3</td>
<td>0</td>
<td>0</td>
<td>11.5</td>
<td>7.5</td>
<td>37.5</td>
<td>39.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Mbl, myeloblasts; Pro, promyelocytes; Myel, myelocytes; Meta, metamyelocytes; Band, banded neutrophils; Seg, segmented neutrophils; Lym, lymphocytes; Mono, monocytes; Baso, basophils; Eos, eosinophils.
be the major constituent of the neutral GSL composition of CML granulocytic cells, whether they were immature or mature. The most immature granulocytic cells, myeloblasts, recovered in Fraction 1 in the CML myeloid blastic crisis stage contained more CMH, less CDH, and much less of the more polar neutral GSLs with a longer sugar chain such as PG when compared with the more mature granulocytic cells from CML patients in the chronic phase (Chart 1). Consequently the CMH:CDH ratio on a weight basis in myeloblasts from CML blastic crisis cells was remarkably higher than that in more mature CML cells. The total content of neutral GSLs in the myeloblasts was found to be less than one-third of that in more mature granulocytic cells recovered in Fractions 1, 2, and 3 of the chronic phase CML cells for which the total neutral GSL value was rather constant. However, the CMH content and the CMH:CDH ratio slightly decreased depending on the stage of differentiation, with the CDH content remaining rather constant, in the chronic phase CML cell fractions (Chart 1).

Gangliosides of immature and mature granulocytic cells obtained from CML patients were composed of doublets of GM3; SPG; an unidentified ganglioside species, Gx, which migrated close to G0 in HPTLC in the neutral solvent system; SNHC; and some unidentified gangliosides with longer sugar moieties which migrated to near the origin on HPTLC (Fig. 2). No ganglioside molecular species were found to be characteristic of a particular stage of granulocytic differentiation in CML, and the ganglioside composition changed in a differentiation-dependent manner. G0 and SPG were the two major components in the most immature myeloid cells, myeloblasts, recovered in Fraction 1 of the CML cells in the myeloid blastic crisis stage, and lacto-series gangliosides with longer sugar chains were minor constituents (Fig. 2). In contrast, the ganglioside profile was found to be more complex in more mature granulocytes from CML cells in the chronic phase (Fig. 2). As the cells attained maturity, the amounts of G0 and the lacto-series gangliosides with longer

Fig. 1. HPTLC chromatogram of neutral GSLs in CML granulocytic cells fractionated according to maturation stage. The neutral GSL fractions corresponding to 5 x 10⁷ cells were separated on HPTLC plates as described in "Materials and Methods."

Fig. 2. HPTLC chromatogram and densitometric scanning pattern of gangliosides in CML granulocytic cells fractionated according to maturation stage. The ganglioside fractions equivalent to 2.6 μg lipid-bound sialic acid were separated on HPTLC plates, and the chromatograms were scanned with a dual wavelength thin-layer chromatography scanner as described in "Materials and Methods." For ganglioside G0, which was one of the major ganglioside components in normal human neutrophils, the Rf value was similar to that of the G0 doublet. Mbl, myeloblast.
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Chart 1. Differentiation-dependent changes in GSL composition of CML granulocytic cells. The total amounts of neutral GSLs (NGSLs) and gangliosides were determined by measuring lipid-bound hexose and lipid-bound sialic acid, respectively, as described in "Materials and Methods." Each GSL component was determined quantitatively by the densitometric scanning method. Each value represents the mean of duplicate quantitations for three separate experiments. Mbl, myeloblasts.

Table 2

<table>
<thead>
<tr>
<th>Morphology (%)</th>
<th>Phagocytic activity</th>
<th>Nitroblue tetrazolium reduction (%)</th>
</tr>
</thead>
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<tr>
<td>Mbl* Pro Myel Meta Band Seg</td>
<td>Mbl* Pro Myel Meta Band Seg</td>
<td>Mbl* Pro Myel Meta Band Seg</td>
</tr>
<tr>
<td>Control 4 89 7 0 0 0 12 4 5</td>
<td>Control 4 89 7 0 0 0 12 4 5</td>
<td>Control 4 89 7 0 0 0 12 4 5</td>
</tr>
<tr>
<td>RA 2 days 3 38 35 23 2 0 35 28.0</td>
<td>RA 2 days 3 38 35 23 2 0 35 28.0</td>
<td>RA 2 days 3 38 35 23 2 0 35 28.0</td>
</tr>
<tr>
<td>4 days 3 37 35 4 6 7 2 70 64.0</td>
<td>4 days 3 37 35 4 6 7 2 70 64.0</td>
<td>4 days 3 37 35 4 6 7 2 70 64.0</td>
</tr>
</tbody>
</table>

* Mbl, myeloblasts; Pro, promyelocytes; Myel, myelocytes; Meta, metamyelocytes; Band, banded neutrophils; Seg, segmented neutrophils.

Differentiation-dependent Change in GSL Composition in the In Vitro Granulocytic Differentiation of HL-60 Cells. When HL-60 cells were cultured in the presence of 1 nM RA, the cells morphologically and functionally differentiated into more mature granulocytes (Table 2). Neutral GSLs of HL-60 cells were composed of CMH, CDH, LTC, and PG, the major component being CDH (Fig. 3; Chart 2). Gangliosides of these cells consisted of doublets of GM3, SPG, Gx, and SNHC with other polar gangliosides as minor constituents (Fig. 4). The major components were GM3 and SPG, which were also the major components at the earliest differentiation stage in granulocytes obtained from the peripheral blood at the CML blastic crisis stage. When granulocytic differentiation was induced in HL-60 cells with RA, a characteristic differentiation-associated change in the GSL composition was observed, as demonstrated previously for the granulocytic differentiation induced by DMSO (12), without the appearance of new molecular species of GSL in the induced cells (Figs. 3 and 4). As observed for the differentiation-dependent change in the GSL composition of CML cells, remarkable increases in the contents of CDH and PG were observed, and lacto-series gangliosides with longer sugar chains such as SNHC increased with a concomitant decrease in ganglio-series ganglioside G.

Comparison of the GSL Compositions of CML Neutrophils and Granulocytic Differentiation-induced HL-60 Cells with That of Normal Human Neutrophils. Fig. 5 compares the results of HPTLC and the densitometric scanning patterns with regard to the ganglioside composition between neutrophils obtained

* NGSLs, neutral GSLs; 2d, 2 days; 4d, 4 days.
* CMH, CDH, LTC, PG, CMH, CDH, LTC, PG, CMH, CDH, LTC, PG
* 0 10 20 30 40
* 0 1 2 3 4
* Fig. 3. HPTLC chromatogram of neutral GSLs in HL-60 cells and their granulocytic differentiation-induced derivatives. The neutral GSL fractions corresponding to 5 × 10⁶ cells were separated on HPTLC plates as described in "Materials and Methods." 2d, 2 days; 4d, 4 days.
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Fig. 4. HPTLC chromatogram and densitometric scanning pattern of gangliosides in HL-60 cells and their granulocytic differentiation-induced derivatives. The ganglioside fractions equivalent to 2.0 μg lipid-bound sialic acid were separated on HPTLC plates followed by densitometric scanning as described in "Materials and Methods.”

Fig. 5. HPTLC chromatogram and densitometric scanning pattern of gangliosides in CML neutrophils and normal neutrophils. The ganglioside fractions corresponding to 5×10^7 cells were separated on HPTLC plates, and the chromatograms were scanned as described in "Materials and Methods.” CML neutrophils were recovered in Fraction 3 of CML cells in the chronic phase.

from CML cases in the chronic phase and normal neutrophils. The CML neutrophils appeared to be normal in morphology and were almost the same as normal neutrophils in superoxide-producing capacity and stimulus-induced membrane depolarization as shown previously (10), but their ganglioside composition was evidently different from that of normal neutrophils. The total amount was significantly less, and the GM3 content was much less in the CML neutrophils (Table 3). However, the ganglioside profiles in the regions of lacto-series gangliosides with longer sugar moieties on HPTLC were similar for normal and CML neutrophils (Fig. 5). When the ganglioside composition of granulocytic differentiation-induced HL-60 cells was compared with that of normal neutrophils, a remarkable difference was observed between them (Table 3). In the induced HL-60 cells, the SPG
content was much higher with an extremely lower content of ganglioside G*. The contents of G* and SNHC were similar to those of normal neutrophils, and the total amount of gangliosides in the differentiated HL-60 cells was found to be almost the same as that in CML neutrophils. On the other hand, when the neutral GSL compositions were compared among CML neutrophils, granulocytic differentiation-induced HL-60 cells, and normal neutrophils, a remarkable difference was observed especially in the CDH content, and the difference in the total amount of neutral GSLs among them was mainly due to that in the CDH content (Table 3).

### DISCUSSION

We showed that the complexity of sugar moieties of gangliosides increases as granulocytic cells mature, using various myeloid leukemia cell lines which were considered to be blocked at certain stages of differentiation (18). Similar changes have been observed in neutral glycolipids (11) and in the carbohydrate structure of cell surface glycoproteins (5). Recently we demonstrated that the GSL profiles expressed depended not only on the differentiation stage but also on the differentiation direction in human promyelocytic leukemia HL-60 cells which were bipotent with respect to granulocytic and macrophage differentiation, indicating that the GSL profile was a differentiation-associated phenotype in human leukemic cells (12). We also showed that neutral GSLs and gangliosides with longer sugar moieties characteristically increased with concomitant decreases in CMH1 and G* during the granulocytic differentiation of HL-60 cells induced with DMSO (12). In the present study, we demonstrated differentiation-dependent changes in GSL composition at various stages of maturation in human leukemic granulocytes obtained from peripheral blood of CML patients and compared them with those observed during the in vitro granulocytic differentiation of HL-60 cells induced with RA.

A good coincidence of some of the differentiation-dependent changes in GSLs was observed between in vivo granulocytic differentiation of CML cells and in vitro granulocytic differentiation of HL-60 cells: CDH and PG remarkably increased; lacto-series gangliosides with longer sugar moieties increased with a concomitant decrease in ganglio-series ganglioside G*; and the ganglioside profile became more complex as granulocytic cells attained maturity. The total amount of gangliosides increased with the complexity of the profiles. These results seemed to be consistent with those obtained in our previous studies on a variety of human myeloid leukemia cell lines assigned to different differentiation stages by multiple surface marker analysis (18). Hildebrand et al. (7) also reported a striking increase of CDH in polymorphonuclear leukocytes during the maturation process seen on neutral GSL analysis of acute myeloid leukemic cells, unfractionated CML cells, and normal mature granulocytes. Recently Kannagi et al. (8) reported a sequential shift of glycolipid synthesis during differentiation of mouse myeloid leukemia M1 cells into macrophage-like cells. Our current results might imply a characteristic shift of ganglioside biosynthesis from that of ganglioside F* to that of lacto-series gangliosides during human granulocytic cell differentiation.

In contrast to the preceding results, the change in ganglioside G*, which was found to be one of the major ganglioside molecules in normal neutrophils, detectable during in vitro granulocytic differentiation of HL-60 cells was evidently different from that found in in vivo granulocytic maturation of CML cells. The content of ganglioside G* increased up to the normal neutrophil level in the latter granulocytic differentiation, whereas it significantly decreased during the former granulocytic differentiation. When the GSL compositions of CML mature granulocytes (banded and segmented neutrophils) and granulocytic differentiation-induced HL-60 cells were compared with that of normal neutrophils, a clear difference was observed, especially in the gangliosides: (a) CML neutrophils and mature HL-60 cells contained 23 and 21.5%, respectively, less total gangliosides than did normal neutrophils; (b) the ganglioside G* content of CML neutrophils was approximately 6.5-fold less than that of normal neutrophils; (c) SPG was the most abundant component in mature HL-60 cells and its content was about 2.7-fold more than that in normal neutrophils; and (d) ganglioside G* was the most abundant component in both CML and normal neutrophils, while it was a minor component in mature HL-60 cells. Westrick et al. (24) reported that CML gangliosides differed from those of normal neutrophils in two respects, CML cells had approximately 4-fold less total gangliosides than did normal neutrophils and G* occupied a greater proportion of their gangliosides. Our present study showed that CML mature granulocytes had only about 20% less gangliosides than did normal neutrophils and that the ganglioside content of CML immature granulocytes was about 2-fold more than the value that Westrick et al. (24) reported for CML cells. Also in our study, G* was found to be dominant in the most immature granulocytic cells, myeloblasts, obtained from CML myeloid blastic crisis cells, and the ganglioside profile of CML neutrophils became more complex, with G* as a minor component. These discrepancies between the results obtained by us and by Westrick et al. (24) may be explained partly by the fact that we analyzed the ganglioside compositions of CML cells fractionated according to the stage of differentiation while they investigated gangliosides of a whole, unfractionated CML leukemia cell preparation.

We reported previously that the ganglioside composition of in vitro granulocytic differentiation in HL-60 cells induced with DMSO was apparently different from that of normal neutrophils, reflecting either some deficiency in the in vitro granulocytic
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differentiation or some leukemic properties of HL-60 cells (12). The difference in ganglioside composition observable between CML and normal neutrophils might also reflect some leukemic properties of CML cells. On the other hand, the difference in neutral GSL composition, especially the CDH content, among CML neutrophils, granulocytic differentiation-induced HL-60 cells, and normal neutrophils might be due chiefly to the difference in maturation of these cells.

Circulating mature granulocytes in patients with CML appear to be morphologically normal. Recently Kitagawa et al. (10) reported membrane potential changes, superoxide-producing capacity, and phagocytic activity in human leukemic granulocytes obtained from CML patients and demonstrated that the functional characteristics of CML mature granulocytes were similar to those of normal mature granulocytes. However, a few findings such as decreased alkaline phosphatase activity (14, 23), loss of granulocyte-specific alloantigen normally found in the mature granulocytes (13), and alterations in the polypeptide composition of whole cell lysates (27) have been reported as being alterations in CML neutrophils. It is noteworthy that the ganglioside composition of CML neutrophils obviously differed from that of normal neutrophils although CML neutrophils appeared to be morphologically and functionally normal. Some abnormality in the cell surface membrane of CML cells might be implied by the present results as to the ganglioside compositions since these acidic glycolipid molecules have been in general recognized to be exclusively localized in the cell surface membrane. Similar abnormalities in the cell membrane of CML cells have been described in relation to altered topography, the composition or mobility of membrane glycoproteins (22, 26). Such abnormalities in the cell surface membrane molecules of CML mature granulocytes might be partly implicated in antigenic alterations of the cells and their cell sociological behavior. We also found a clear difference in the ganglioside composition between erythrocytes obtained from CML patients and those obtained from a normal donor, reflecting the abnormal clonal origin. Structural analysis of the ganglioside Gs molecule and metabolic studies on GSLs during granulocytic differentiation are now in progress.

REFERENCES


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