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Gangliosides in Acute Myeloid Leukaemia (AML) and Non-Hodgkin's Lymphoma (NHL)

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Abstract—*Gangliosides of human acute myelomonocytic leukaemia (AMMoL), acute monocytic leukaemia (AMoL) and lymphoblastoid (pre-B) lymphoma cells were analysed by overpressured thin-layer chromatography (OPTLC) followed by scanning densitometry. AMMoL cells were found to contain four gangliosides, viz.* GM_3 (47.2%), GM_1 (31.8%), GD_{1a} (7.5%) *and an unidentified compound migrating between* GM_1 *and* GD_{1a} (13.5%). *In AMoL cells, six components were identified* (GM_3:51.3%, GM_1:13.4%, GD_3:7.8%, GD_{1a}:4.7%, GD_{1b}:6%, *and an unidentified compound migrating between* GM_2 *and* GM_1). *The total gangliosides extracted from pre-B lymphoma cells of the hand-mirror variant were composed of* 10 *species* (GM_3:50.8%, GM_2:11.2%, GM_1:7.8%, GD_3:2.3%, GD_{1a}:1.1%, GD_{1b}:2.2%, GT_1:1.6%, *and three unidentified components with chromatographic mobilities between* GM_2 *and* GD_{1a}).

Additional studies must still be performed to clarify the question as to whether ganglioside GD_3 *represent a qualitative glycolipid marker for AMoL and pre-B lymphoblastic lymphoma.*

INTRODUCTION

GANGLIOSIDES are a class of acid glycosphingolipids (GSLs). A ganglioside molecule is composed of a hydrophilic sialosyloligosaccharide headgroup and a hydrophobic ceramide portion which consists of sphingosine and fatty acid. They are membrane components and are located nearly exclusively in the outer part of the plasma membrane bilayer. A number of cellular functions are evidently closely associated with, or are directly dependent on membrane carbohydrate [1–3]. Differentiation as well as malignant transformation of cells is accompanied by changes in the composition of membrane GSLs [4,5]. Of particular interest is the recent finding that human leucocytes from healthy donors and from patients with various types of leukaemia differentially express the ganglioside GD_3, as examined by chemical analysis [6–8] or by immunostaining [9].

With the recently developed OPTLC, the present work was undertaken to study variations in the ganglioside composition of AMMoL, AMoL and pre-B lymphoblastic lymphoma cells, in comparison with normal leucocytes. To the best of our knowledge, this is the first report on gangliosides in NHL.

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MATERIALS AND METHODS

Patients

Four patients with AMoL, one with AMMoL and one with lymphoblastic lymphoma presenting in a leukaemic phase were included in the study. These patients had white blood cell (WBC) counts in the peripheral blood ranging from 15 to $168 \times 10^9/l$ with 96–100% blasts. The NHL patient had a clinical picture characterised by systemic symptoms (fever, general malaise), splenic and bone marrow involvement, and the appearance of a large number ($114 \times 10^9/l$) of hand-mirror lymphocytes both in peripheral blood and bone marrow. The circulating lymphoma cells were immunologically identified as pre-B cells. The samples were taken by leukapheresis or as anticoagulated blood prior to initiation of chemotherapy.

Cell separation procedures

Contaminating red blood cells (RBC) were eliminated by dextran sedimentation and osmotic lysis, while platelets were removed by differential centrifugation [8]. The WBC/RBC ratio of the purified cell preparations was approx. 50 : 1, whereas the WBC/platelet ratio was greater than 4 :1.

Isolation of gangliosides

Lipid extraction was carried out with chloroform : methanol (C : M) mixtures, 2 : 1, 1 : 1, 1 : 2 (v/v). Gangliosides were separated from the

total lipid extract by DEAE Sephadex A-25 column chromatography combined with reversed-phase high-performance liquid chromatography (RP–HPLC). The fractionation of sulphatides and gangliosides, and further intraclass separation of gangliosides were performed by OPTLC with two-step gradient development, using each of the following eluents. C : M (7 : 3) and C : M : 0.025 M aqueous KCl (55 : 36 : 9), as described in our previous publication [10]. The identification of the isolated individual gangliosides was based on the chromatographic migration, by comparison with known standards. Relative proportions of ganglioside species were determined by densitometric scanning of the chromatograms, and expressed as percentage of the total gangliosides extracted from the preparation. All values were determined from at least three parallel measurements.

RESULTS

Since the designations GM_3, GM_2, GM_1, GD_3, etc. were based only on TLC mobility as compared to standard brain gangliosides, identification of gangliosides separated on TLC is only tentative. There is the possibility that gangliosides with lacto- and globo-series core structure may also be represented in these bands.

On the chromatogram, a set of most prominent double spots at the position of GM_3 was revealed in all samples (Figs. 1A, 2A, 3A). AMMoL cells had a second most prominent spot with a TLC mobility similar to GM_1, a well visible simplet which co-chromatographed with GD_{1a}, and some additional faint bands with TLC mobilities between GM_1 and GD_{1a} (Fig. 1A). AMoL cells showed a conspicuous band with a slightly lower mobility than GM_1, but faster than GM_2. These cells also displayed a set of spots with the same TLC mobility as GM_1 and some weakly resorcinol-positive spots with relative TLC properties similar to GD_3, GD_{1a} and GD_{1b} (Fig. 2A). The circulating pre-B cells from the patient with NHL had a complex ganglioside pattern, with at least eight visible resorcinol-positive spots. The most abundant species was GM_3, and gangliosides with relative TLC mobilities similar to GM_2, GM_1, GD_3 and GD_1 were apparent. Two sets of spots, one migrating between GM_2 and GM_1, and the other between GM_1 and GD_3 were also visible.

On the densitograms (Figs. 1B, 2B, 3B), the actual peaks of gangliosides were readily identifiable.

The relative distributions of individual gangliosides isolated from different cell types are given in Table 1. GD_3 represented appreciable proportions of AMoL (6–9.5%) and lymphoma gangliosides (2.3%), but it could not be detected in extracts from normal leucocytes. In comparison with normal granulocytes, AMMoL and AMoL cells contained GM_3 at proportions about 6 times more (47.2-52.6% vs. 8.3%), whereas gangliosides with more complex structures than GD_3 4–8 times less (10.7–21% vs. 83.2%) than granulocytes. Less polar components also accounted for 80.1% of the total gangliosides extracted from pre-B lymphoma cells, a proportion more than 2-fold higher than that of normal lymphocytes (37.7%).

DISCUSSION

The marked enrichment of GM_3 in leukaemic cells, as compared to normal leucocytes, noted in the present study is in accord with previous pub-

Table 1. Ganglioside profiles of acute myeloid leukaemia and lymphoblastoid (pre-B) lymphoma cells

Cell type	No. of cases tested	Ganglioside composition, % of the total							
		GM_3	GM_2	GM_1	GD_3	GD_{1a}	GD_{1b}	GM_1-GD_1*	GT_1
AMoL	4								
Range		50.0–52.6	0.†	10.4–16.4	6.0–9.5	4.5–4.9	5.8–6.3	0.‡	0.
Mean		51.3		13.4	7.8	4.7	6.0	0.	0.
AMMoL	1	47.2	0.	31.8	0.	7.5	0.	13.5	0.
Lymphoma	1	50.8	11.2§	7.8‖	2.3	1.1	2.2	0.	1.6
Granulocyte	3	8.3	Trace	8.6	0.	13.5	13.0	51.2	5.5
Lymphocyte	4	37.7	6.1	7.5	0.	6.0	13.6	29.2	0.

*Unidentified compounds migrating between GM_1 and GD_1.
†An unidentified compound (range: 10.9–22.8%, mean: 16.8%) migrating between GM_2 and GM_1.
‡< 0.1%.
§An unidentified compound (10.3%) with a lower chromatographic mobility than GM_2.
‖Two unidentified compounds (7.4% and 5.5%) migrating slower than GM_1.

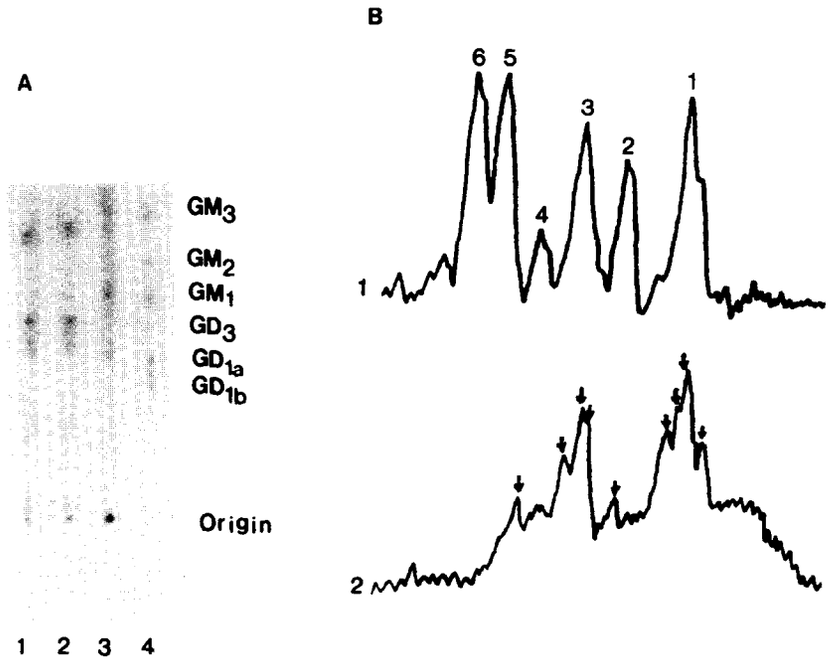

Fig. 1. *Thin-layer chromatogram* (*A*) *and densitogram* (*B*) *of the total gangliosides from AMMoL cells.*

A. *The plate* (10 × 10 cm *Merck HPTLC Si*60, *E. Merck, Darmstadt, F.R.G.*) *was developed with the solvent system chloroform* : *methanol* : 0.025 M *aqueous* KCl, 55 : 36 : 9 *(v/v/v), thereafter stained with resorcinol–HCl reagent at* 100°C *for* 15 min. *Sialic acid-containing spots were deep purple. Lanes* 1,2-*human plasma*; *Lane* 3- *AMMoL cells*; *Lane* 4-*standard mixture.*

B. *Note*: 1, *standard mixture*; 2, *AMMoL gangliosides. The small arrows outline the actual peaks, which were taken for integration. Tracing* (1): *peak* 1, GM_3; *peak* 2, GM_2; *peak* 3, GM_1; *peak* 4, GD_3; *peak* 5, GD_{1a}; *peak* 6, GD_{1b}. *Abbreviations of gangliosides follow the system of Svennerholm* [11].

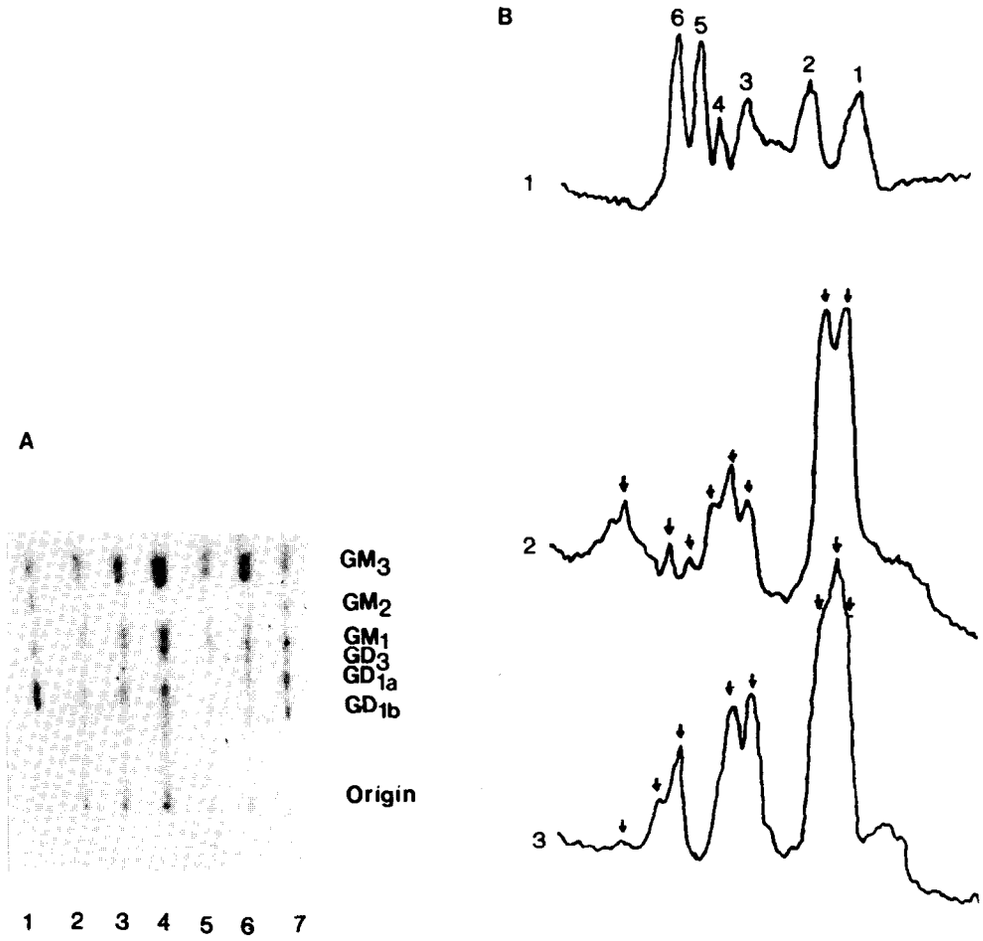

Fig. 2. *Thin-layer chromatogram* (A) *and densitogram* (B) *of the total gangliosides from AMoL cells.*
A. *Lanes* 1,7- *standard mixture; Lanes* 2,3,4,5,6- *AMoL cells.*
B. 1, *standard mixture; AMoL cells (lane* 6); 3, *AMoL cells (lane* 3).
See Fig. 1 *for explanations.*

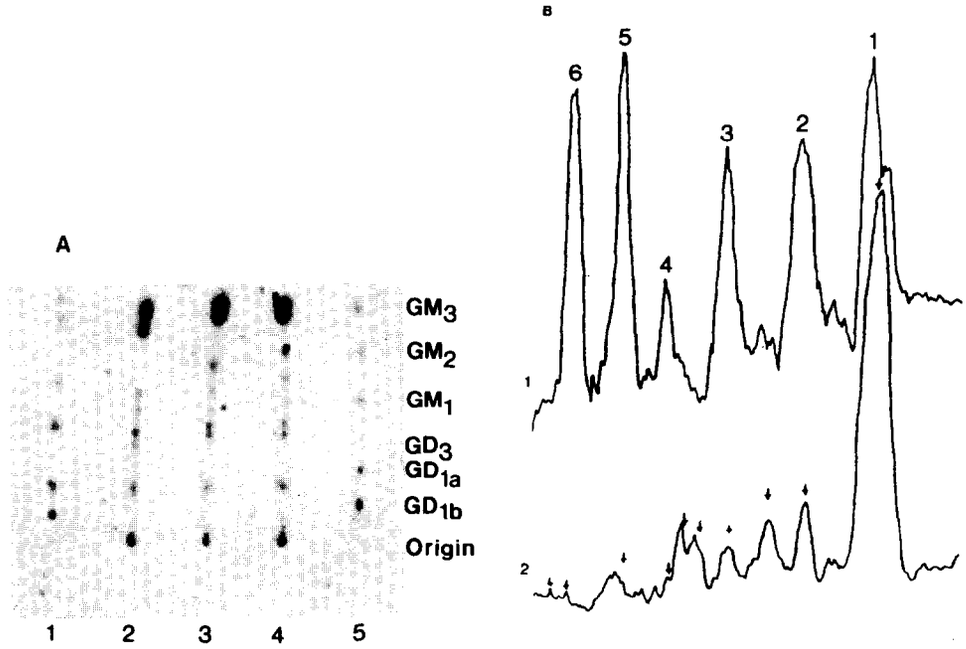

Fig. 3. *Thin-layer chromatogram* (A) *and densitogram* (B) *of the total gangliosides from circulating hand-mirror lymphocytes of the pre-B cell subtype.*
A. *Lanes* 1,5- *standard mixture; Lanes* 2,3,4- *circulating lymphoma cells.*
B. 1, *standard mixture*; 2, *circulating pre-B lymphoma cells.*
See Fig. 1 for explanations.

lications [6–9,12]. Thus, while a severe reduction in the chemical quantity of GM_3 has been demonstrated in various forms of sarcomas and adenocarcinomas as well as in experimentally transformed cells [4,5,13], a dramatic increase in the level of GM_3 seems to be a characteristic feature of glycolipid changes observed in malignancies of the haemopoietic system. Recent data suggest that the level of membrane GM_3 may modulate cell adhesion and cell growth behaviour [13].

Due to the lack of some ganglioside standards, we were not able to identify several compounds. A number of resorcinol-positive spots locating between GM_1 and GD_1 were observed in extracts from normal granulocytes (51.2%), lymphocytes (29.2%) and AMMoL cells (13.5%). It is likely that a part of these unidentified gangliosides was sialosylparagloboside (Spg), a compound behaving closely like GM_1 on TLC and being found in both normal and malignant leucocytes [7].

The non-Hodgkin's lymphomas are a heterogenous group of uncommon cancers — approx. 3% of all new cancers [14] — that share a common origin in the malignant transformation of the lymphocytes. There are a number of classification systems currently in use. The newer formulations are based on cell-surface marker analysis of the malignant lymphocytes. As far as we know, the present study describes the first analysis of gangliosides in NHL. Pre-B lymphoblastic lymphoma was found to be characterised by a complex ganglioside pattern (10 species) with some GD_3 (2.3%) and a large amount (80.1%) of less polar components.

Although the exact functional role of GD_3 is still to be determined, there is evidence of its biological role in serotonin binding [15]. Recently, GD_3 has been found to be associated with a cell-surface receptor molecule on cultured human melanoma cells, exposure of which to a monoclonal anti-GD_3-IgG-antibody (R-24) resulted in detachment of cells and blocking of cell growth [16], demonstrating a decisive role of this GSL in the growth of malignant melanoma cells. Conceivably, the expression of GD_3 on AML and pre-B lymphoblastic lymphoma cells (but not on normal leucocytes) could be explored for the production and application of specific anti-GD_3 antibodies to diagnostic and/or therapeutic purposes.

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