GLYCOSPHINGOLIPIDS IN MURINE LYMPHOCYTES FROM THYMUS AND SPONTANEOUS AND X-RAY INDUCED THYMOMAS**

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Lymphocytes from spontaneous thymoma in AKR mice and from X-ray induced thymoma in C57Bl/6 mice showed elevated levels (by 50% and 100%, respectively) of lipid-bound sialic acid as compared with lymphocytes from normal thymuses used as controls. Some ganglioside fractions in thymomas were elevated 4-6-fold over those in normal thymuses while other fractions decreased or disappeared.

Neutral glycosphingolipid (NGSL) content in lymphocytes from thymomas was also changed. Thin-layer chromatography of NGSLs showed that the fractions migrating as ceramide monohexoside (CMH), dihexoside (CDH) and below globoside standards were increased, respectively, 2-3-fold, 3-6-fold and 2-fold in both types of thymomas.

Methylation and gas-liquid chromatography analysis confirmed the presence of CMH, CDH and globoside in NGSLs isolated from X-ray induced thymoma.

Growing interest in glycosphingolipids, both neutral and sialic acid-containing gangliosides, results from their possible involvement in membrane-mediated phenomena (Critchley et al., 1979; Brady & Fishman, 1979; Schwarting & Summers, 1980; Hakomori, 1981). Changes in glycosphingolipid content and composition were observed in transformed cell lines (Fishman & Brady, 1975; Hakomori, 1975) and cancerous tissues (Morré et al., 1979; Hakomori & Kannagi, 1983). Moreover, a number of glycosphingolipids have been recognized as tumour markers (Tai et al., 1983; Hakomori & Kannagi, 1983).

** This work was supported by contract No. 21007 from the Polish National Cancer Programme PR-6.
In spontaneous thymoma which occurs in AKR mice an increase of LBSA\(^1\) content over that in normal thymus has been observed (Lengle, 1979). In this report we compare ganglioside and neutral glycosphingolipid content and composition in lymphocytes from spontaneous and X-ray induced thymomas in AKR and C57Bl/6 mice, respectively, with those from normal thymuses.

**MATERIALS AND METHODS**

*Glycosphingolipids.* CMH, CDH, CTH and globoside were isolated from human erythrocyte lipid extract kindly given by Dr. A. Gardas, Medical Center for Postgraduate Education, Warszawa. Ganglioside GM3 from canine erythrocytes and gangliosides GM1, HDlb, and GTlb from bovine brain were isolated and purified as described by Mullin et al. (1978) and Pacuszka et al. (1978). Gangliosides GM2 and GD2 were prepared from gangliosides GM1 and GDlb, respectively, after removal of terminal galactose with rat liver \(\beta\)-galactosidase (Cumar et al., 1971).

*Chemicals.* Silica gel 60 and silica gel 60 precoated plates were purchased from E. Merck (Darmstadt, F.R.G.); Bio-SilA, 200 - 400 mesh, from Calbiochem; \(N\)-acytelyneuraminic acid, from Serva (Heidelberg, F.R.G.).

*Mice.* Mice of C57Bl/6 and AKR strains were fed standard diet ad lib. Healthy animals of two age groups: young, 2 weeks old, and adult, 3 months old, were used as controls. AKR mice developed thymomas spontaneously after about 6 months. In C57Bl/6 mice thymomas were induced by X-ray irradiation according to Kaplan & Brown (1952). Thymic lymphocytes were isolated from normal thymuses or thymomas by the procedure of Lengle (1979). About 90% of the isolated cells were viable as evaluated by Trypan blue exclusion.

*Isolation and purification of glycosphingolipids.* Glycosphingolipids were extracted from cells or tissues with methanol and chloroform as described by Svennerholm & Fredman (1980) with a minor modification: initial homogenization of lymphocytes or thymoma tissue was performed in 0.05 M-EDTA, pH 5.0, at 4°C. After the addition of organic solvents the extracts were made slightly alkaline with ammonium hydroxide.

Gangliosides separated by Folch partition (Folch et al., 1957) were purified on DEAE-Sephadex and Bio-SilA columns (Yu & Ledeen, 1972).

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\(^1\) Abbreviations used: Cer, ceramide; CMH, ceramide monohexoside, Glec \(\rightarrow\) 1Cer; CDH, ceramide dihexoside. Gall \(\rightarrow\) 4Glec \(\rightarrow\) 1Cer; CTH, ceramide trihexoside. Gall \(\rightarrow\) 4Gall \(\rightarrow\) 4Glec \(\rightarrow\) 1Cer; Gl, globoside, GalNAc \(\rightarrow\) 3Gall \(\rightarrow\) 4Gall \(\rightarrow\) 4Glec \(\rightarrow\) 1Cer; Gal, d-galactose; Glec, d-glucose; GalNAc, \(\rightarrow\) N-acetylgalactosamine; LBSA, lipid-bound sialic acid; NeuAc, \(\rightarrow\) N-acetylneuraminic acid, sialic acid; NGSL, neutral glycosphingolipid; glc, gasliquid chromatography; tlc, thin-layer chromatography. Ganglioside nomenclature of Svennerholm (1963) was used.
Complex NGSLs present in trace amounts in the Folch upper phase were not analysed in the present study. NGSLs present in the Folch lower phase were treated with NaOH, dialysed and purified on Bio-SilA columns (Pacuszka et al., 1981).

**Thin-layer chromatography.** Glycosphingolipids separated by Folch partition were analysed on silica gel 60 precoated plates.

Gangliosides were developed with chloroform/methanol/0.25% aqueous CaCl₂, 60:35:8, by vol. (solvent system A) or n-propanol/0.08% aqueous CaCl₂, 76:24, v/v (solvent system B). Sialic-acid containing bands were stained with resorcinol and quantitated densitometrically (Fishman et al., 1979).

NGSLs were developed with lower phase of the chloroform/methanol/water (65:30:8, by vol.) mixture (solvent system C), stained with orcinol and quantitated densitometrically.

For sugar analysis NGSLs separated by tlc were visualized with iodine vapours, scraped from silica gel plates, scrapings eluted with chloroform/methanol/water (1:1:0.1, by vol.) and dried under a stream of nitrogen.

**Paper chromatography.** NGSL fractions isolated by the above procedure were hydrolysed with 2 m-HCl for 2 h at 100°C (Kościelak et al., 1973). Liberated sugars were analysed by paper chromatography with ethyl acetate/pyridine/water (10:4:3, by vol.) solvent system.

**Gas-liquid chromatography.** Glycosphingolipids isolated from X-ray induced thymoma (C57Bl/6, 15 g wet tissue of pooled thymomas) were purified by Folch partition, saponification with NaOH and Bio-SilA chromatography, and separated into individual fractions by tlc. Monosaccharide constituents in these fractions were analysed by glc after conversion into alditol acetates or permethylated alditol acetates (Kościelak et al., 1976; Zdebska et al., 1983).

**Colorimetric assay.** LBSA was determined by the resorcinol method (Svennerholm & Fredman, 1980). Absorbance was read at 620 nm.

**RESULTS AND DISCUSSION**

We have confirmed previous observations of Lengle (1979) that lymphocytes from spontaneous thymoma in AKR mice contain more LBSA than lymphocytes from normal thymus, yet in our experiments this increase did not exceed 50% (Table 1). We have extended these studies to the X-ray induced thymoma in C57Bl/6 mice. In this case lymphocytes isolated from thymoma had by about 100% more LBSA than lymphocytes from normal thymuses (Table 1).

All lymphocyte samples gave complex ganglioside patterns with at least twelve separate bands (Plate 1). This agrees with the observations of
Schwarting & Gajewski (1983) who studied gangliosides in normal murine thymus. Although the overall increase in LBSA level in lymphocytes from thymoma did not exceed 100% over that in lymphocytes from normal thymuses, some ganglioside fractions in thymoma were elevated severalfold (4-6 times) whereas other diminished or disappeared (Plate 1).

Table 1

Levels of lipid-bound sialic acid in murine lymphocytes from normal thymuses and thymomas

LBSA levels were determined after purification of ganglioside-containing lipid extract through Folch partition followed by DEAE-Sephadex and Bio-SilA column chromatography.

<table>
<thead>
<tr>
<th>Source of lymphocytes</th>
<th>Number of lymphocytes used (×10⁻⁹)</th>
<th>LBSA (nmol NeuAc/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57Bl/6 adult</td>
<td>4.5</td>
<td>20.7</td>
</tr>
<tr>
<td>C57Bl/6 adult</td>
<td>12.7</td>
<td>16.4</td>
</tr>
<tr>
<td>C57Bl/6 young</td>
<td>3.5</td>
<td>25.8</td>
</tr>
<tr>
<td>C57Bl/6 young</td>
<td>7.0</td>
<td>19.4</td>
</tr>
<tr>
<td>C57Bl/6 thymoma</td>
<td>3.2</td>
<td>50.3</td>
</tr>
<tr>
<td>C57Bl/6 thymoma</td>
<td>3.2</td>
<td>50.3</td>
</tr>
<tr>
<td>AKR adult</td>
<td>5.3</td>
<td>21.7</td>
</tr>
<tr>
<td>AKR adult</td>
<td>5.6</td>
<td>21.1</td>
</tr>
<tr>
<td>AKR young</td>
<td>3.6</td>
<td>23.9</td>
</tr>
<tr>
<td>AKR young</td>
<td>8.6</td>
<td>16.5</td>
</tr>
<tr>
<td>AKR thymoma</td>
<td>5.6</td>
<td>28.6</td>
</tr>
<tr>
<td>AKR thymoma</td>
<td>3.6</td>
<td>33.1</td>
</tr>
</tbody>
</table>

NGSLs from all lymphocyte samples analysed migrated on silica gel plates in the region from CMH down to below globoside standards (Plate 2). A common feature of lymphocytes from both types of thymoma was elevation of NGSL in fractions migrating as CMH (2-3-fold), CDH (3-6-fold) and below globoside (2-fold), (Plate 2, Table 2). The NGSL fractions separated by tlc and eluted from silica gel scrapings were subjected to acid hydrolysis to liberate their sugar constituents, which were subsequently analysed by paper chromatography. For all lymphocyte samples the NGSL fraction migrating as CMH contained only glucose; this indicates that this glycosphingolipid fraction is composed solely of glucosylceramide. Other NGSL fractions migrating on tlc below globoside consisted of glucose, galactose, galactosamine and glucosamine. This is the first indication of the existence of glucosamine-containing glycosphingolipids in murine thymic lymphocytes, although such compounds are abundant in these cells from other species (Iwamori & Nagai, 1981).
Plate 1. Chromatographic patterns of gangliosides in murine lymphocytes from normal thymuses and thymomas. Lanes 1 through 2b contain gangliosides from lymphocytes of: 1, thymus of adult C57Bl/6 mice; 1a, thymus of young C57Bl/6 mice; 1b, X-ray induced thymoma in C57Bl/6 mice; 2, thymus of adult AKR mice; 2a, thymus of young AKR mice; 2b, spontaneous thymoma in AKR mice. Ganglioside fractions elevated 4-6-fold in thymomas are marked with +, whereas those decreased, with −. A. Solvent system A; B, solvent system B; Stds, standards. Both chromatograms were stained with resorcinol. For details see Materials and Methods.

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Plate 2. Chromatographic patterns of neutral glycosphingolipids from normal thymuses and thymomas. Lymphocyte samples used for isolation of NGSLs were the same as described in the legend to Plate 1. Each lane contains NGSLs isolated from $5 \times 10^9$ cells. Chromatogram developed with solvent system C and stained with orcinol (cf. Table 2).
Table 2

Content of neutral glycosphingolipids in murine lymphocytes from normal thymuses and thymomas

NGSLs were separated by tlc (see Fig. 2), stained with orcinol and quantitatively densitometrically (Fishman et al., 1979). Colour intensities of the separated NGSL bands were compared with those of known quantities of glucosylerceramide standard. Numbers in parentheses refer to the chromatographic pattern (Fig. 2).

<table>
<thead>
<tr>
<th>Source of lymphocytes</th>
<th>Hexoses (nmol glucose/10^9 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMH (1 and 1')</td>
</tr>
<tr>
<td>C57Bl/6 adult</td>
<td>61.1</td>
</tr>
<tr>
<td>C57Bl/6 adult</td>
<td>65.3</td>
</tr>
<tr>
<td>C57Bl/6 young</td>
<td>60.1</td>
</tr>
<tr>
<td>C57Bl/6 young</td>
<td>63.8</td>
</tr>
<tr>
<td>C57Bl/6 thymoma</td>
<td>141.7</td>
</tr>
<tr>
<td>C57Bl/6 thymoma</td>
<td>120.7</td>
</tr>
<tr>
<td>AKR adult</td>
<td>36.1</td>
</tr>
<tr>
<td>AKR adult</td>
<td>40.3</td>
</tr>
<tr>
<td>AKR young</td>
<td>43.3</td>
</tr>
<tr>
<td>AKR young</td>
<td>41.2</td>
</tr>
<tr>
<td>AKR thymoma</td>
<td>144.4</td>
</tr>
<tr>
<td>AKR thymoma</td>
<td>130.5</td>
</tr>
</tbody>
</table>

NGSLs isolated from 15 g (wet weight) of pooled thymomas (C57Bl/6) after purification, separation by tlc and conversion of their sugar residues into alditol acetates and methyl ethers of alditol acetates were analysed by glc. We have identified CMH and CDH, although in the latter compound the ratio of galactitol to glucitol was 1:1.5, resulting from a possible contamination with CMH. From a number of NGSL components which occur in thymoma we have identified only globoside. A NGSL with a chromatographic mobility similar to that of globoside was also detected by Schwarting & Summers (1980) in murine thymus lymphocytes. Moreover, globoside is a marker for alloantigen-activated murine T cell subpopulations (Gruner et al., 1981).

Accumulation of CMH has been observed in rat hepatoma (Walter et al., 1980), whereas increased amounts of CDH were found in human gastric and colonic carcinoma (Siddiqui et al., 1978; Pacuszka et al., 1981). The physiological significance of these changes remains unknown; however, they may prove of diagnostic value (Walter et al., 1980, Hakomori & Kannagi, 1983).
We are indebted to Professor Dr. Jerzy Kościelak for making accessible to us the gas chromatograph and all the standards and reagents necessary for glc analysis of glycosingolipids.

REFERENCES


Received 23 July, 1984;
revised 3 November, 1984