GANGLIOTETRAOSYLCERAMIDE IS A MAJOR GLYCOLIPID OF EPITHELIAL CELLS OF MOUSE SMALL INTESTINE

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1. Introduction

Gangliotetraosylceramide (formula in fig.2) was first characterized as a core structure of brain gangliosides [1]. Much attention has been focused on this glycolipid as a cell surface marker. In the mouse it was shown both immunologically [2] and chemically [3] that gangliotetraosylceramide was present in mature splenic thymus-derived (T) lymphocytes but absent from most other lymphocytes. The level was 10–20-times that of thymus cells or neonatal splenic T cells [3], defining this glycolipid as a differentiation antigen [3,4]. Furthermore, in mouse mutants which lack natural killer cell-mediated cytotoxicity, which is normally present in mature cells, the level of gangliotetraosylceramide was low [3]. Antibodies to gangliotetraosylceramide abolished the natural killer activity [5–7] and enhanced the growth of lymphoma in vivo [7]. In the rat this glycolipid has been identified not only on T lymphocytes but on macrophages and eosinophilic cells as well [8,9].

In peripheral blood of man lymphocytes of 16 patients with acute lymphoblastic leukemia stained positively with anti-gangliotetraosylceramide antibodies while 20 normal persons or patients with other disorders were negative [9]. Autoantibodies to this glycolipid were found in human thyroid disorders [10] and in systemic lupus erythematosus disease [11].

In [12] on mass spectrometric analysis of small intestinal glycolipids of several animal species gangliotetraosylceramide was proposed as a major glycolipid of mouse small intestine. Here, we substantiate this and demonstrate the localization of this glycolipid to the epithelial cell layer.

2. Materials and methods

Fresh small intestine from 19 mice of strain C57/BL-J was scraped gently several times with a spoon in the cold. The mucosa scrapings (epithelial cells) and the residues after scraping were worked up for non-acid glycolipids mainly as in [13]. Gangliotetraosylceramide, a major compound of mouse small intestine [12], was purified from whole small intestine in acetylated form on a silicic acid column. The pure glycolipid (fig.1A) was analysed for carbohydrate [14] and subjected to permethylation [15] and reduction [16]. These derivatives were analysed by direct inlet mass spectrometry [12] and after degradation by gas chromatography [17]. The reduced derivative was also characterized by NMR spectroscopy [18–20]. A reference of gangliotetraosylceramide was prepared from human brain gangliosides by mild acid hydrolysis of the sialic acid [1].

For comparison non-acid glycolipids were also prepared from stomach and large intestine of the same animals.

3. Results and discussion

Fig.1 shows a thin-layer chromatogram of non-acid glycolipids of the mucosa scraping of small intestine (B, total 5.8 mg), the residue after scraping (C, total 11.3 mg), stomach tissue (D, total 4.2 mg), and large intestinal tissue (E, total 8.3 mg). The epithelial cells of small intestine (B) contained mostly glucosylceramide (see [12]) and a glycolipid migrating in the 6-sugar interval and earlier proposed to be gangliotetraosylceramide [12]. The identity of this latter glycolipid was confirmed as follows after isolation.

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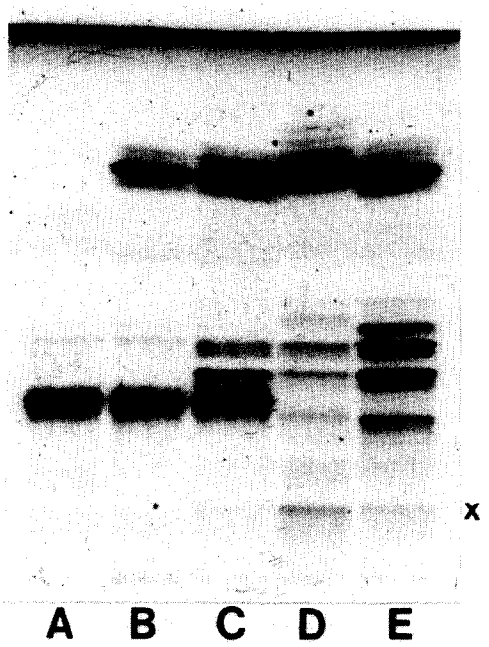

Fig.1. Thin-layer chromatogram of non-acid glycolipids of mucosa scraping of mouse small intestine (B, 20 μg), residue after scraping of mouse small intestine (C, 20 μg), mouse stomach tissue (D, 40 μg), mouse large intestine (E, 40 μg) and purified gangliotetraosylceramide of mouse small intestine (A). A Merck HPTLC precoated Si 60 plate was used and solvent was chloroform–methanol–water 60:35:8 (by vol.). Anisaldehyde was used for detection. The band marked x was a dialysis bag contaminant.

The overall composition (lipophilic part and number of sugars) and sugar sequence were obtained by mass spectrometry of intact permethylated and permethylated-reduced derivatives [12]. Ions for the correct sequence are indicated at the ring formula of fig.2, with underlined figures for masses obtained from the reduced derivative (see also [12]). The series of peaks at *m/e* 1185–1297 was evidence for 16–24 carbon hydroxy fatty acids with 22, 23 and 24 carbon species in dominance, as shown by the relative intensity of peaks. Phytosphingosine was the major long-chain base. Degradation of the non-derivatized glycolipid and gas–liquid chromatography showed the presence of Glc, Gal and GalNAc but the absence of GlcNAc. Fig.2 shows gas chromatograms after degradation of the permethylated derivative (top) and the reduced derivative [17], results in agreement with the top formula of fig.2. The anomerity was finally established by NMR spectroscopy of the reduced derivative (fig.2). The identity of the 4 signals was concluded

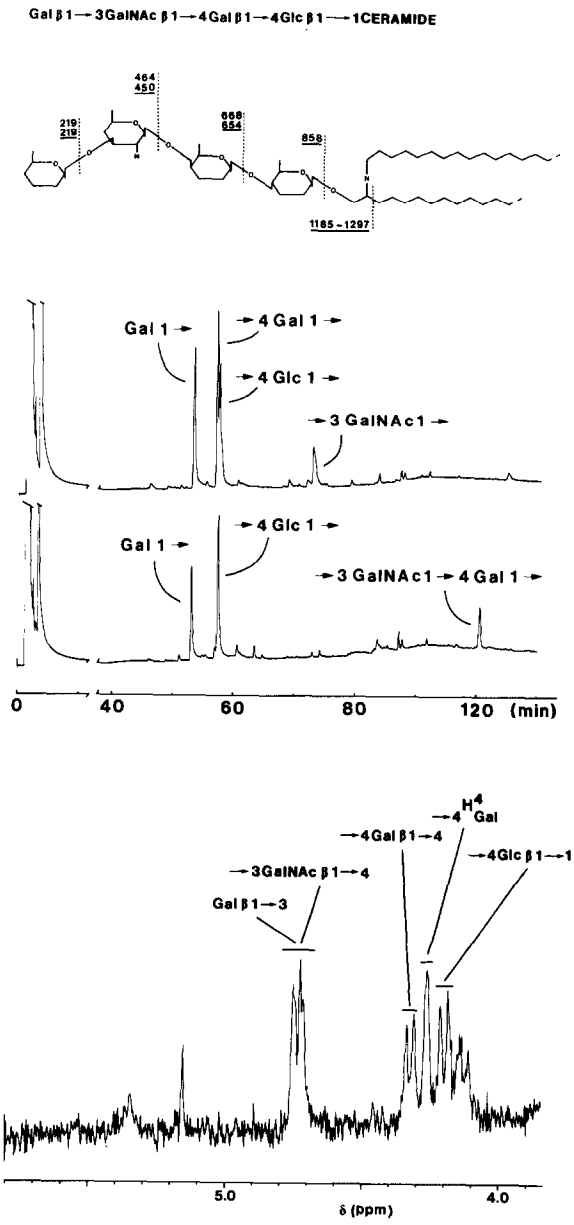

Fig.2. The top formula shows the structure of gangliotetraosylceramide concluded to be a major glycolipid of mouse small intestine (cf. fig.1). The simplified ring formula presents some fragments obtained from mass spectrometry of the permethylated (figures not underlined) and permethylated-reduced (figures underlined) derivative of purified glycolipid (see text and [12]). The 2 gas–liquid chromatograms were obtained after degradation of 250 μg permethylated (top) or 400 μg permethylated-reduced glycolipid. The conditions for analysis were about the same as those in [17]. The NMR spectrum (bottom) was recorded as in [18–20] from 1.5 mg permethylated-reduced glycolipid in 0.5 ml C^2HCl_3 and with 1000 pulses at 313 K.

from comparison with data from [18–20] and from spectra of permethylated and reduced derivatives of gangliotriaosyl- and gangliotetraosylceramide derived from brain gangliosides (unpublished).

As shown in fig.1, the 2 major glycolipids of the epithelial cells (B), glucosylceramide and gangliotetraosylceramide, are also major components of the residue after mucosa scraping (C). This is due to ineffective removal of epithelial cells by the gentle scraping, a large part probably being left mainly in the crypt (cf. [13]). The sharp band appearing just above gangliotetraosylceramide was concluded to be the Forssman glycolipid [12]. The band just in front of the latter was proposed to have the sequence hexosamine–hexose–hexose [12], although it migrated as a 4-sugar glycolipid. These 2 glycolipids being absent from sample B should derive from the non-epithelial residue. However, the ceramide of the triglycosylceramide was of the more hydroxylated type as for epithelial cell glycolipids in contrast to the Forssman glycolipid [12]. Therefore, the triglycosylceramide may be an epithelial cell component but localized to the crypt, which was probably not scraped off. Alternatively, and more likely, the band proposed to be the triglycosylceramide [12] may be a mixture of mainly globotetraosylceramide and the triglycosylceramide. Globotetraosylceramide which is expected to be a non-epithelial component [12] would then have sugar and fatty acid ions 30 mass units lower than those of corresponding species of the dominating gangliotetraosylceramide and may therefore have been overlooked [12]. Also, this band had a mobility identical with globoside of human erythrocyte. The triglycosylceramide would then be the band of (B) (epithelial part) moving as the faster of the two bands of globoside in (C) (residue).

It is of interest that the stomach tissue (D) and large intestine (E) of fig.1 apparently lacked gangliotetraosylceramide. Instead there was a more slow-moving band, which may be fucosylated gangliotetraosylceramide. The formation of this from gangliotetraosylceramide was shown to be induced in small intestine after conventionalization of germ-free mice [21,22]. A fucolipid was earlier detected by us but this was concluded to migrate as gangliotetraosylceramide [12].

The 3-sugar glycolipid of large intestine (E) being absent from small intestine (B,C) may be trihexosylceramide. In stomach (D) the monohexosylceramide (shown to be glucosylceramide on borate plates, see [12]) and some more slow-moving compounds apparently had a less hydroxylated ceramide than those of large intestine.

The finding of gangliotetraosylceramide as a major glycolipid of mouse small intestine [12,22] and localized to the epithelial cells may explain some findings referred to in section 1. In man it has been shown that Lewis activity of erythrocytes of Lewis genotypes appears first after birth as a result of uptake of Lewis glycolipids from plasma. The site of synthesis of these glycolipids has not been proved but small intestine is a probable tissue, where large amounts of Lewis glycolipids may exist in the epithelial cells [23]. Although the situation for gangliotetraosylceramide changes in T cells is not clear [4] there may be an uptake of intestine-derived glycolipid onto T cells which has left thymus [3]. It would therefore be of interest to see if mutant mice, which lack natural killer cell activity and have low levels of gangliotetraosylceramide in their splenic T cells [3], also have a lowered amount of this glycolipid in their intestine.

Immunofluorescence staining of mouse small intestine was used in [24] to show that gangliotetraosylceramide was exclusively localized to the brush border and basolateral membranes of epithelial cells and that Forssman glycolipid was present in the mesenchymal tissue. This result is in accordance with our conclusions based on direct chemical analysis after mucosa scraping.

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