PLASMA MEMBRANE GLYCOSPHINGOLIPIDS (GSLs) OF THE HUMAN LYMPHOBLASTOID CELL-LINE BRI 8 AND DIFFERENCES BETWEEN THE GSLs OF BRI 8 CELLS AND THOSE OF PERIPHERAL LYMPHOCYTES

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SUMMARY: Plasma membrane of the human lymphoblastoid cell-line BRI 8 contained about tenfold (expressed relative to per mg of protein) more hematoside and about three fold more GL_2, GL_3 and GL_4, but less GL_1 than the whole cell homogenate. Human peripheral blood lymphocytes contained small quantities of hematoside but were two to three times more rich in neutral GSLs than BRI 8 cells. GL_3 was the major GSL of BRI 8 cells cultured in media supplemented with piglet or human serum. This GLS was not detected in human peripheral lymphocytes which contained mostly GL_2. The results suggest that growth of human lymphocytes causes activation of enzymes which mediate the elongation of the GL_2 oligosaccharide chain.

The composition of the plasma membrane glycosphingolipid (GSL) as well as the distribution of GSLs in the mammalian cell show greater organ and species specificity than any of the other classes of lipids (1-9). In cultures, cell to cell contact and cell transformation also influence GSL composition (10). In our previous work we have shown a possible relation between cell proliferation and GSL metabolism (11). Increasing knowledge of these aspects may help in understanding the role of GSL in membrane functions. This study presents analysis of GSL of whole human lymphoblastoid BRI 8 cells and of their plasma membranes. The GSL composition of BRI 8 cells is also compared with that of human peripheral lymphocytes.

Abbreviations: GL_1: Monohexosyl ceramide, GL_2: Dihexosyl ceramide
GL_3: Trihexosyl ceramide, GL_4: Globoside
GLS: Glycosphingolipid

MATERIALS AND METHODS

Human lymphoblastoid cells (BRI 8) were cultured in RPM1 1640 medium containing 10% (v/v) of either piglet (6-7 month old) serum or human serum (Flow Laboratories Ltd., Irvine, Ayrshire, U.K.) and were harvested during logarithmic phase of growth (generation time about 18 h). Cells grown in the presence of piglet serum were purchased from Searle Diagnostics High Wycombe, Bucks., U.K. The cells were homogenized by pumping a suspension at a controlled pressure through a small orifice occupied by a spring-loaded ball bearing (12). Plasma membrane was isolated from the cell homogenate and tested for purity as described previously (12). Human peripheral lymphocytes were isolated from whole blood of healthy donors stored for transfusion purposes, by centrifugation in a Ficoll-Hypaque density gradient (13). Total lipids were extracted with chloroform-methanol 2:1 v/v and partitioned with 0.1M KCl (14). GSLs of the lower chloroform phase were isolated, purified and separated into classes by silicic acid column chromatography, mild alkaline hydrolysis and preparative thin layer chromatography (11,15,16). Quantitative determination was performed by gas liquid chromatographic analysis of the trimethylsilyl derivatives of the O-methyl glycosides obtained by acid methanolysis; mannitol was employed as an internal standard (11,15,17). Acidic GSLs were isolated from the upper water phase by using the following procedure. The water phase was dried, saponified, dialysed, dried and then chromatographed on a DEAE-Sephadex column (18). The acidic fraction recovered from the column was taken down to two ml volume. This was then extracted and partitioned according to Folch et al (14), the lower chloroform phase as deduced by thin layer chromatographic analysis and colour reactions contained henatoside which was estimated by gas liquid chromatography of the sialic acid residue (19). The upper water phase was dialysed, dried and tested for brain type gangliosides by thin layer chromatography (9).

RESULTS

Table I summarizes the data on the lipid content of human lymphoblastoid BRI 8 cells cultured in the presence of piglet serum and their plasma membrane. The plasma membrane contained approximately 3 times as much neutral GSL per mg protein as the whole cell homogenate. Hematoside (N-acetyl derivative) was present almost exclusively in the plasma membrane. Larger gangliosides were not detected in any of the preparations. Data for whole BRI 8 cells grown in human serum and for human peripheral lymphocytes are also shown in Table I. It can be seen that BRI 8 cells cultured in human serum had a somewhat higher content of neutral GSL than cells cultured in piglet serum, whereas most

interestingly human peripheral lymphocytes were richer in neutral GSL than BRI 8 cells cultured either in piglet or human serum. Major differences were also observed in hematoside content, human lymphoblastoid contained considerable quantities of this small ganglioside as compared to blood lymphocytes where only traces were detected as compared to their neutral GSLs.

The four neutral GSLs usually present in extraneural tissues have been detected in the lower chloroform phase obtained from homogenates of BRI 8 cells grown in human and piglet serum, and of the plasma membrane of BRI 8 cells cultured in piglet serum. Their percentage composition is shown in Table II. Plasma membrane of BRI 8 cells cultured in piglet serum contained much less monohexosyl ceramide than the cell homogenate. However, more marked differences were observed when the neutral GSLs of the

TABLE I LIPID COMPOSITION OF HUMAN LYMPHOBLASTOID CELLS (BRI 8) AND HUMAN PERIPHERAL BLOOD LYMPHOCYTES

		BRI 8 CELLS			PERIPHERAL LYMPHOCYTES	
		PLASMA MEMBRANES	CELL HOMOGENATE			
Lipid fraction						
TRIGLYCERIDES	µg/mg protein	130*	61*	40**	14	30
CHOLESTEROL	" "	192	65	42	100	100
GLYCOSPHINGOLIPIDS (Chloroform phase)	" "	22	6	10	21	25
ACIDIC FRACTION (Hematoside)	" "	8	0.9	1.3	0.05	N.D.
PHOSPHOLIPIDS	" "	400	240	216	241	360
CHOLESTEROL/ PHOSPHOLIPIDS	molar ratio	0.94	0.53	0.38	0.75	0.56

* Cultured in RPM1 1640 medium plus 10% piglet serum.
**Cultured in RPM1 1640 medium plus 10% human serum.
N.D. Not detected <0.05 µg/mg protein

two cell types were compared. It can be seen that GL_3 which as it was expected (16) was not detected in human peripheral lymphocytes constituted a major GSL of human lymphoblastoid cells, indicating pronounced changes occuring during cell growth.

The human and piglet serum used for the culture of BRI 8 cells were analysed for GSL composition as described in Methods. The major neutral components were GL_2 for human serum and GL_3 (40%), GL_2 (30%), GL_4 (18%), GL_1 (12%) expressed in per cent weight for piglet serum. It is obvious that there is no apparent relationship between GSL composition of human and piglet serum and the major GSLs components (GL_2, GL_3) of the cells, which were similar irrespectively of the serum used in the culture medium. However the possibility that the GL_4 found in the BRI 8 cells cultured in piglet serum originate from the culture medium needs further investigation.

TABLE II GLYCOSPHINGOLIPID COMPOSITION OF PLASMA MEMBRANE AND WHOLE CELL HOMOGENATE OF HUMAN LYMPHOBLASTOID BRI 8 CELLS AND WHOLE CELL HOMOGENATE OF HUMAN PERIPHERAL LYMPHOCYTES (PER CENT WEIGHT)

GLYCOSPHINGOLIPID	BRI 8 CELLS		PERIPHERAL LYMPHOCYTES	
	PLASMA MEMBRANE	CELL HOMOGENATE	I	II
GL_1	0.7	9.1* 3.9**	4.5	7.9
GL_2	49.2	40.1 27.4	90.5	87.7
GL_3	41.0	34.5 68.2	N.D.	N.D.
Globoside	9.1	16.3 0.5	5.0	4.4

Glycosphingolipids of lower chloroform phase were separated by preparative thin layer chromatography on silica gel H plates, quantitation was performed by gas liquid chromatography analysis of the sugars. A mean M.W. of 300 for fatty acids was used for the calculation of weight

* BRI 8 cells cultured in piglet serum
**BRI 8 cells cultured in human serum
N.D. Not detected <0.5%

DISCUSSION

The presence of GSL has been shown in the plasma membranes of erythrocytes (1), rat liver (2,3), intestinal microvilus (8), pig lymphocytes (9) and several lines of cultured fibroblasts (4-7). The present findings showing a selective localization of GSLs in the plasma membranes of human lymphoblastoid cells are in accordance with results obtained from the analysis of the GSLs of most of the above tissues. The GSL pattern of the plasma membrane is usually similar to that of the cell homogenate except for mouse fibroblasts (L cells) in which neutral GSLs were not detected at the cell surface (4). In the present investigation quantitative differences in the distribution of GSLs have been demonstrated between the homogenate of human lymphoblastoid BRI 8 cells and the isolated plasma membrane. Thus, GL_1 was almost absent from the plasma membrane, while hematoside was major component only of the plasma membranes.

The most striking findingsof this study were the differences between the GSL composition of BRI 8 cells and peripheral lymphocytes. These differences concerned the hematoside content which was increased about 20-fold in BRI 8 cells, and GL_3 which was not detected in peripheral lymphocytes. The very low content of hematoside found in human peripheral lymphocytes,as compared with BRI 8 cells and its almost exclusive location at the plasma membrane of these cells,may indicate that one of the major functions of this small ganglioside is related with processes which take place at the cell surface during cell growth. In agreement with the above finding,hematoside has been detected in considerable quantities in proliferating tissues of the pig and not in blood lymphocytes (11),as well as in PHA-stimulated human lymphocytes where active biosynthesis of hematoside has been shown (20)

and similar concentrations to those estimated here for human lymphoblastoid cells have been reported (21).

The results presented here, show a dramatic increase of GL_3 in human lymphoblastoid BRI 8 cells grown in media containing either piglet or human serum as compared to peripheral human lymphocytes. The GSL composition of the sera used for cell growth (see present results and 22,23) excludes the possibility that GL_3 originate from the culture medium. Therefore unless the differences in hematoside and neutral GSL content and composition of BRI 8 human lymphoblastoid cells and peripheral blood lymphocytes, are peculiar to BRI 8 cells and in this respect more cell lines have to be examined, then they rather should be related to cell growth than to virus transformation. Support to this is offered by the fact that BRI 8 cells which represent a particular stage in the lymphocyte cell growth, show no evidence of virus infection apart from DNA hybridisation.

This is further substantiated by the fact that the appearance of GL_3 and hematoside suggesting the activation of the enzymatic systems needed for the elongation of the oligosaccharide chain of GL_2, is in contrast with changes reported for other virally transformed cells where simplification of GSLs due to blocked synthesis has been reported (10, 24-32). Therefore it seems most probable that BRI 8 human lymphoblastoid cells do not show characteristic of malignant transformation with respect to GSL metabolism. This is advantageous in comparing GSL content of lymphoblastoid cells with those of peripheral lymphocytes. In this respect the increase content GSL found in the latter cells are confirmative of a previous report (11) suggesting a relation of GSL metabolism and proliferation of non malignant human cells.

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