α1,4Galactosyltransferase activity and Gb3Cer expression in human leukaemia/lymphoma cell lines

CHERYL L.M. STULTS1*, ROBERT D. LARSEN2 and BRUCE A. MACHER1
1Department of Chemistry/Biochemistry, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132, USA
2Glycomed Incorporated, 860 Atlantic Ave., Alameda, CA 94501, USA

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We have used two methods to evaluate the level of expression of Gb3Cer in several human leukaemia/lymphoma cell lines representative of the myeloid (K562, KG-1, HL-60, and THP-1) and lymphoid (Reh, Daudi, Raji, RPMI 8226, CCRF-CEM, MOLT-4) lineages blocked at varied stages of differentiation. TLC immunostaining of glycolipid extracts with a monoclonal antibody, 12-101, and FACS analysis with the same antibody were used to demonstrate that the expression of Gb3Cer in neoplastic myeloid and lymphoid cells is both lineage and differentiation dependent. As a possible control point in the regulated expression of Gb3Cer we have investigated the first committed step in the synthesis of globo series glycosphingolipids that involves UDP-Gal:LacCer α(1,4)-galactosyltransferase (α1,4GalT). We present the first characterization of this enzyme in a human myeloid cell line using an ELISA-based assay, which was subsequently used to measure α1,4GalT activity in the human leukaemia/lymphoma cell lines. In general, there is a positive correlation between the levels of endogenous Gb3Cer and the level of the α1,4GalT activity. However, in two cases (KG-1 and CCRF-CEM) the level of enzyme activity did not correspond to the level of Gb3Cer expression.

Keywords: α-galactosyltransferase, globotriaosylceramide, human leukocytes, regulation of glycosphingolipid biosynthesis

Introduction

The glycosphingolipid, globotriaosylceramide (Gb3Cer) is of biological interest for many reasons. Expression of Gb3Cer is known to be elevated in various lymphomas [1, 2], leiomyosarcoma [3], seminoma [4] and familial dysautonomia [5]. Gb3Cer has been identified as a ligand for Shiga [6, 7] and Shiga-like [8, 9] toxins. Its presence in human milk [10] is thought to exert a protective effect against infantile diarrhoeas caused by these toxins. Gb3Cer has also been identified as the Pk blood group antigen [11]. This antigen is expressed on many types of human blood cells (erythrocytes [11], lymphocytes [12] and platelets [13]). Gb3Cer has been identified as a differentiation antigen for B lymphocytes (i.e. CD77, see ref. [14]), was proposed as a marker for apoptosis of germinal centre B-cells [15] and was recently shown to induce apoptosis in Burkitt lymphoma cells [16]. Thus Gb3Cer has been implicated as an important functional ligand in several biological processes.

Our interest in the expression of this compound centres around its presence or absence at various stages of leukocyte differentiation along the myeloid and lymphoid pathways. Previous studies have shown that immature myeloid cells [17] and all lymphoid cells [18-20] express globo series compounds. Therefore, it has been established that human leukocytes express different families of glycosphingolipids depending on their lineage and maturity. The regulatory events controlling the expression of these compounds in various populations of human leukocytes have not been determined.

Gb3Cer is synthesized in a stepwise fashion: Cer → GlcCer → LacCer → Gb3Cer. This glycosphingolipid is also a substrate for Gb3Cer synthesis. Therefore the level of expression of Gb3Cer may be controlled by one or more of the enzymes involved in its synthesis or turnover. The enzyme, UDP-Gal:galactose α(1,4)-
Galactosyltransferase ($\alpha 1,4\text{GalT}$), plays a key role in the biosynthetic pathway for glycosphingolipids. It catalyses the first committed step in the biosynthesis of globo series glycosphingolipids:

$$\text{Gal} \beta 1-4\text{Glc} \beta 1-1\text{Cer(LacCer)} + \text{UDP-Gal} \rightarrow \text{Gala}1-4\text{Gal} \beta 1-4\text{Glc} \beta 1-1\text{Cer (Gb3Cer)} + \text{UDP}$$

This galactosyltransferase has been partially purified from rat liver [21]. A recent report described the characteristics of a CF-54 extract of this enzyme from human placenta [22]. However, there is little information available regarding the characteristics of this enzyme in human leukocytes. We describe in detail in this report the characteristics of $\alpha 1,4\text{GalT}$ from a human monocytic leukemia (THP-1) cell homogenate.

Several studies have shown that there is a direct correlation between the level of $\alpha 1,4\text{GalT}$ activity and the expression of Gb3Cer in human tissues. Decreased levels of globo series glycosphingolipid expression and $\alpha 1,4\text{GalT}$ activity were found [23] after chemically induced differentiation of TERA-2 cells. Other studies with fibroblasts [24] and lymphoid cells [1, 25] have shown that the level of Gb3Cer expression directly correlates with the level of $\alpha 1,4\text{GalT}$ activity in those cells. However, in two studies [26, 27] it was shown that the level of Gb3Cer expression did not correlate with the level of $\alpha 1,4\text{GalT}$ activity. We have investigated whether or not Gb3Cer expression correlates with $\alpha 1,4\text{GalT}$ activity in human leukocytes in the current study.

Recent studies [1, 2, 25, 26] on the expression of Gb3Cer in human leukocytes have focused only on cells of the lymphoid lineage. In one case [2] only glycosphingolipid extracts from B-cell neoplasms were analysed. In two cases [1, 28] both glycosphingolipid extracts and cell surface expression of this glycosphingolipid were examined. In only one of the latter cases [1] was the $\alpha 1,4\text{GalT}$ activity also measured. Thus a more complete study was necessary to begin to understand what factors regulate expression of Gb3Cer in human leukocytes.

We present here the results of such a study using a series of human leukaemia/lymphoma cell lines, which are representative of various stages of leukocyte (myeloid and lymphoid) differentiation. We have examined Gb3Cer levels in glycosphingolipid extracts and on the cell surface and measured the activity of $\alpha 1,4\text{GalT}$ for each of the cell lines. TLC immunostaining, FACS analysis, and a microtitre ELISA-based enzyme assay were used to show that there is a positive correlation between the expression of Gb3Cer and $\alpha 1,4\text{GalT}$ activity. Minor quantitative variations were found between Gb3Cer expression levels and enzyme activity. Interestingly, we found that this correlation did not hold for one of the T-cell lines (CCRF-CEM) and one of the myeloid cell lines (KG-1).

### Materials and methods

#### Materials

Neutral glycosphingolipids were prepared from human myeloid cells [17], as previously described. The cell lines were obtained from ATCC (Rockville, MD) or the cell culture facility at University of California, San Francisco, CA. UDP-galactose, $\gamma$-galactonolactone, CDP-choline, bovine serum albumin (BSA), irrelevant IgM antibody (MOPC-104E) and p-nitrophenylphosphate (Sigma 104 phosphatase substrate) were purchased from Sigma (St Louis, MO). Sodium cacodylate was purchased from Aldrich (Milwaukee, WI). The monoclonal antibody, 12-101 [29], was purchased from Accurate Chemical & Scientific (Westbury, NY). R-Phycoerythrin conjugated goat anti-mouse IgM anti sera $(F(ab')_2)$ was obtained from TAGO (Burlingame, CA). Biotin-conjugated goat anti-mouse IgM, biotinylated alkaline phosphatase, and the TLC immunostain substrate were obtained from Vector Laboratories (Burlingame, CA) as part of the Alkaline Phosphatase Standard Vectastain ABC Kit. UDP-$[^{14}\text{C}]$Gal (257 mCi mmol$^{-1}$) and $[^{14}\text{C}]$Gal (55.7 mCi mmol$^{-1}$) were obtained from American Radiolabelled Chemicals, Inc. (St. Louis, MO) and Amersham (Arlington Heights, IL), respectively. Hydrofluor liquid scintillation cocktail was obtained from National Diagnostics (Manville, NJ). Protein assay reagent was obtained from Bio-Rad (Richmond, CA). All other reagents were of the highest grade available.

#### Cell culture

The cell lines used (classified according to Saito [30]) were: K562, myeloid stem cell; KG-1, myeloblast; HL-60, promyelocyte; Reh, lymphoid stem cell; CCRF-CEM, T-blast I; MOLT-4, T-blast II; Daudi and Raji, B-blast I; and RPMI 8226, plasma cell. The human monocyte cell line, THP-1 [31], was also used. All cell lines were grown in suspension at 37 °C in a humidified atmosphere with 5% CO$_2$. The medium was RPMI 1640 supplemented with 20% (KG-1 and RPMI 8226) or 10% (all others) fetal calf serum and 1% penicillin/streptomycin.

#### Cell homogenates

Homogenates from each cell line were prepared for use in the enzyme assay. Cells were collected and washed three times with 50 mM cacodylate buffer (pH 6.7) and finally suspended in the same buffer (2–6 mg protein per ml). Aliquots of each suspension were used fresh or centrifuged and stored as pellets at −70 °C. Frozen cell pellets were thawed and resuspended in 50 mM cacodylate buffer, pH 6.7 to a protein concentration of 2–6 mg ml$^{-1}$. The suspensions were kept at 0°C and homogenized with an Omni 5000 homogenizer. These crude preparations were used as the source of $\alpha 1,4\text{GalT}$...