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Research paper

Comparison of detection methods for cell surface globotriaosylceramide

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ABSTRACT

The cell surface-expressed glycosphingolipid (GSL), globotriaosylceramide (Gb_3), is becoming increasingly important and is widely studied in the areas of verotoxin (VT)-mediated cytotoxicity, human immunodeficiency virus (HIV) infection, immunology and cancer. However, despite its diverse roles and implications, an optimized detection method for cell surface Gb_3 has not been determined. GSLs are differentially organized in the plasma membrane which can affect their availability for protein binding. To examine various detection methods for cell surface Gb_3, we compared four reagents for use in flow cytometry analysis. A natural ligand (VT1B) and three different monoclonal antibodies (mAbs) were optimized and tested on various human cell lines for Gb_3 detection. A differential detection pattern of cell surface Gb_3 expression, which was influenced by the choice of reagent, was observed. Two mAb were found to be suboptimal. However, two other methods were found to be useful as defined by their high percentage of positivity and mean fluorescence intensity (MFI) values. Rat IgM anti-Gb_3 mAb (clone 38–13) using phycoerythrin-conjugated secondary antibody was found to be the most specific detection method while the use of VT1B conjugated to Alexa488 fluorochrome was found to be the most sensitive; showing a rare crossreactivity only when Gb_4 expression was highly elevated. The findings of this study demonstrate the variability in detection of Gb_3 depending on the reagent and cell target used and emphasize the importance of selecting an optimal methodology in studies for the detection of cell surface expression of Gb_3.

1. Introduction

Globotriaosylceramide-galα1-4 galβ1-4 glc ceramide (Gb_3) is a neutral, globo-series glycosphingolipid (GSL) found in cell membranes. It is also known as CD77 and P^k, reflecting its roles as a differentiation antigen at a restricted stage of B cell development and as a blood group antigen in erythrocytes (Marcus et al., 1981; Naiki and Marcus, 1974; Wiels et al., 1981). As such, expression of Gb_3 in humans has been restricted to certain cells, including erythrocytes and B lymphocytes (Fyfe et al., 1987; Gregory et al., 1987; Schwartz-Albiez et al., 1990; Spitalnik and Spitalnik, 1995). In human cell lines, Gb_3 is

Abbreviations: Alexa488, fluorescent dye derived from 3,6-diaminoxanthenium-4,5-disulfate;EBV, Epstein–Barr virus;FITC, fluorescein isothiocyanate;FSC, forward scatter;Gb_3, globotriaosylceramide;GSL, glycosphingolipid; HIV, human immunodeficiency virus;HUS, hemolytic uremic syndrome;IFN, interferon;mAb, monoclonal antibody;MFI, mean fluorescence intensity;MHC, major histocompatibility complex;PBMC, peripheral blood mononuclear cells; PE, phycoerythrin;RBL, rhamnose-binding lectin;SSC, side scatter;TLC, thin layer chromatography;VT, verotoxin;VT1B, verotoxin subunit B.

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characteristically expressed on Burkitt lymphoma-derived cells and on kidney epithelium and kidney-derived cell lines (Hughes et al., 2002; Obrig et al., 1993; Wiels et al., 1981). Other lymphocytic cell lines, such as megakaryoblastic leukemia cells, are also known to express Gb_3 (Furukawa et al., 2002).

Gb_3 functions as a receptor for verotoxins (VT), also known as Shiga toxins, produced by *Escherichia coli* O157 strain and *Shigella dysenteriea* bacteria (Cohen et al., 1990; Jacewicz et al., 1986; Lindberg et al., 1987; Lingwood et al., 1987; Lingwood, 1996; Waddell et al., 1988). Gb_3 is proven to be involved in the pathogenesis of these bacterial infections as VT-binding to Gb_3 mediates cellular toxicity (Ling et al., 1998; Sandvig et al., 1992; Taguchi et al., 1998; Tetaud et al., 2003). The resulting cell death can cause gastrointestinal diseases and it may lead to hemolytic uremic syndrome (HUS) (Johannes and Romer, 2010; Kaneko et al., 2001; Karmali et al., 1985; Okuda et al., 2006). In addition, increased expression of Gb_3 is documented in a variety of cancers, such as colon, breast, and testicular tumors, as well as in invasive meningioma (Falguieres et al., 2008; Johansson et al., 2009; Ohyama et al., 1990; Salhia et al., 2002). Since Gb_3 can initiate apoptosis via VT or monoclonal antibody (mAb) binding (Mori et al., 2000; Tetaud et al., 2003), the cell surface expression of Gb_3 has been tested as anti-cancer treatments (Arab et al., 1999; Salhia et al., 2002; Engedal et al., 2010; Falguieres et al., 2008; Frankel et al., 2000;). Cell surface expression of Gb_3 has also been implicated in B cell activation, immune effector mechanisms in endothelium, and major histocompatibility complex (MHC) class II peptide binding functions (George et al., 2001; Schwartz-Albiez et al., 1990; Zemunik et al., 2004). Furthermore, the role of Gb_3 is documented for the signal transduction of interferon (INF)-alpha-mediated growth inhibition, antiviral activity and adhesion pathways (Jackson et al., 2001; Khine and Lingwood, 2000; Maloney et al., 1999). Recently, Gb_3 has been implicated in innate immunity by cooperating with rhamnose-binding lectin (RBL) to enhance phagocytosis (Watanabe et al., 2009).

Importantly, Gb_3 has been suggested to be involved in the mechanism of human immunodeficiency virus (HIV) pathogenesis (Viard et al., 2004). The accumulation of Gb_3 in peripheral blood mononuclear cells (PBMCs), as well as subsequent appearance of anti-Gb_3 antibodies, was observed in HIV-1 infected patients (Fantini et al., 1998). Initial studies reported Gb_3 as a facilitator for the virus/host cell fusion (Puri et al., 1998; Puri et al., 1999). However, recent studies have reported the cell surface expression of Gb_3 to act as a natural resistance factor against HIV infection (Lund et al., 2005; Lund et al., 2009). Use of pharmacologic agents to modulate Gb_3 expression, as well as utilizing synthetic analogues of Gb_3, has confirmed the HIV protective effect of Gb_3 (Harrison et al., 2010; Lund et al., 2006; Ramkumar et al., 2009).

Because of the growing implications of cell surface Gb_3 expression in a variety of diseases and its potential as a predictor of susceptibility to HIV infection, it becomes important to determine the best method for detection of cell surface-expressed Gb_3. However, methods for detecting the cell surface expression of Gb_3 have not been well documented or compared. Therefore, in the study reported herein, we have used flow cytometry to compare the relative ability to detect cell surface Gb_3 expression using different, commercially available mAbs and a natural, nontoxic Gb_3 ligand conjugated to a fluorescent dye. Our findings show a considerable differential between the various detection methods for Gb_3 expressed on different cell types and indicate appropriate reagents to be used for the highest sensitivity and reproducibility for use in cell surface Gb_3 detection.

2. Material and methods

2.1. Cells

The human T-cell line, Jurkat clone E6.1, was obtained from the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program (Rockville, MD). The human monocyte cell line, THP-1, was obtained from the American Type Culture Collection (ATCC; Manassas, VA), and the Gb_3-low THP-1 cell line was produced by culturing THP-1 cells in the presence of VT. Two of the Burkitt lymphoma-derived cell lines, Raji and Ramos, were obtained from the NIH AIDS Research and Reference Reagent Program, while Daudi and Namalwa were from ATCC. The human cervical cancer cell line, HeLa (clone 6C), and the human embryonic kidney cell line, 293, were from the NIH AIDS Research and Reference Reagent Program. The human breast cancer cell line, MCF-7 and the human colon epithelial cancer cell line, Caco-2, were provided by Dr. T. Jin (University of Toronto, Toronto, ON). The human B lymphoblast cell line, DB, was a gift from Dr. S. Neron (Hema-Qubec, Quebec, QC).

2.2. Cell culture

Jurkat E6.1, THP-1, Gb_3-low THP-1, Daudi, Raji and HeLa cells were cultured in RPMI1640 medium (Invitrogen Canada, Burlington, ON) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 10 μM gentamicin. Namalwa and DB cells were grown in RPMI1640 medium (Invitrogen) supplemented with 7.5% FBS, 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 10 mM HEPES, 1 mM sodium pyruvate and 10 μM gentamicin. Ramos cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM; Invitrogen) supplemented with 10% FBS, 2 mM L-glutamine and 10 μM gentamicin. Caco-2, 293 and MCF-7 cells were grown in Eagle's Minimum Essential Medium (EMEM; Sigma-Aldrich Canada, Oakville, ON) supplemented with 10% FBS, 2 mM L-glutamine and 10 μM gentamicin. The suspension cell lines (Jurkat E6.1, THP-1, Gb_3-low THP-1, Daudi, Raji, Ramos, Namalwa, and DB) were maintained at a density of 500,000 to 2,000,000 cells/ml. The adherent cell lines (HeLa, Caco-2, 293 and MCF-7) were grown as monolayer cultures. All cell lines were incubated in a fully humidified atmosphere containing 5% CO_2 at 37 °C.

2.3. Reagents

Four reagents, including a ligand and three mAbs, were used for flow cytometry analysis. The ligand was purified verotoxin 1-derived B subunit conjugated with Alexa Fluor488 (VT1B-Alexa488) as described previously (Head et al., 1991; Khine and Lingwood, 1994; Ramotar et al., 1990). The three mAbs to Gb_3 were fluorescein isothiocyanate (FITC)-labeled or unlabeled mouse IgM (clone 5B5; BD Biosciences, Mississauga, ON), rat IgM (clone 38–13; AbD Serotec, Raleigh, NC) and mouse IgG2b (clone BGR23; Seikagaku, Tokyo, Japan). Secondary antibodies were all phycoerythrin (PE)-labeled. Mouse anti-rat IgM (BD Biosciences) was used for clone 38–13 anti-Gb_3 antibody, while