Biochimica et Biophysica Acta 1851 (2015) 98–106

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Distinct selectivity of gangliosides required for CD4$^+$ T and CD8$^+$ T cell activation☆

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ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 3 June 2014
Accepted 13 July 2014
Available online 3 September 2014

Keywords:
Gangliosides
Lipid rafts
T cell activation
Asthma

ABSTRACT

T cells compose a crucial part of the immune system and require activation. The first step of T cell activation is triggered by the movement of one of their surface molecules, known as T cell receptor, into localized regions of cell membrane known as lipid rafts. Molecules called gangliosides are known to be major components of lipid rafts, but their role in T-cell activation remains to be elucidated. This review summarizes recent findings that different types of T cells require distinct ganglioside types for the activation. Control of ganglioside expression would offer a strategy targeting for specific T-cell subpopulations to treat immune diseases. This article is part of a Special Issue entitled Linking transcription to physiology in lipodomics.

1. Introduction

The initial events of T-cell activation involve movement of the T-cell receptor into specialized membrane microdomains known as lipid rafts. The term "lipid rafts" was introduced by Kai Simons and Elina Ikonen, on the basis of the close association of sphingolipids and cholesterol in the detergent resistant complex of signaling molecules present in the membrane microdomains [1]. In T lymphocytes, lipid rafts are implicated in signaling from T-cell antigen receptor (TCR) and in localization and function of proteins residing proximal to the receptor, such as coreceptors CD4 and CD8, Src family kinases Lck and Fyn, transmembrane adaptor linker for activation of T cells (LAT), and protein kinase Cθ [2–8].

A growing body of evidence implicates that glycosphingolipid (GSL) heterogeneity contributes to membrane protein compartmentalization in resting and activated T cells. Gangliosides, sialic acid (SA)-containing glycosphingolipids (GSLs) associated with lipid rafts, are thought to be involved in T-cell activation. For example, following TCR clustering induced by anti-CD3 and anti-CD28 antibodies, polarization of GM1a ganglioside occurs in CD4$^+$ T cells but not in CD8$^+$ T cells [9]. In polarized human T cells, ganglioside GM3- and GM1a-enriched raft domains segregate to the leading edge and uropod, respectively, resulting in asymmetric and ganglioside-specific redistribution [10]. Additionally, GM1a expression levels are different among cell types or stages of cellular development [11], and GM1a expression in certain cell types is much lower than that of other gangliosides [12]. Thus, to understand the role of lipid rafts in the differentiation, maturation, and activation of CD4$^+$ T and CD8$^+$ T cells in vivo, it was necessary to understand the ganglioside composition in each respective T-cell subset. We analyzed the expression of gangliosides during T-cell differentiation by LC–MS/MS and investigated whether the activation of individual T-cell subsets requires distinct species of gangliosides (Fig. 1) [13]. We used two kinds of gene-targeted mice, one carrying disrupted GM3 synthase (*ST3GAL5*), and so lacking GM3-derived gangliosides (a- and b-series gangliosides) [13], and the other with an altered GA/GM2/GD2 synthase (*B4GALNT1*) and expressing only GM3 and GD3 gangliosides while lacking the o-series [14], and found distinct and dramatic changes in ganglioside profiles among primary thymocytes and resting CD4$^+$ T and CD8$^+$ T cells. In particular, CD4$^+$ T cells preferentially express a-series gangliosides, whereas CD8$^+$ T cells express very high levels of o-series gangliosides. Likewise, TCR-dependent activation of CD4$^+$ T cells selectively requires a-series gangliosides, and the activation of CD8$^+$ T cells, o-series gangliosides and not a-series gangliosides. Distinct expression patterns of gangliosides in CD4$^+$ T and CD8$^+$ T cells in unique functional lipid rafts may define immune functions in each T-cell subset. We propose that the

Abbreviations: ST3Gal5, GM3 synthase; B4Galnt1, GM2/GD2 synthase; GSLs, glycosphingolipids; SA, sialic acid; CTx-B, cholera toxin B subunit

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http://dx.doi.org/10.1016/j.bbalip.2014.07.013
1388-1981/

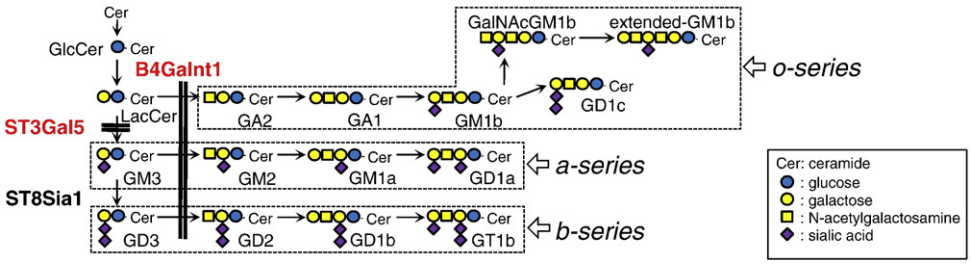

Fig. 1. Ganglio-series glycosphingolipids are synthesized from ceramide and are divided into o-, a-, and b-series species.

repertoire selection from immature thymocytes to mature T-cell subsets is accompanied by selective GSL expression in individual T-cell subsets. This GSL selection process may be indispensable in the formation of distinct and functional lipid rafts in mature T cells.

2. Structures of ganglioside species in the T cell subsets

GSLs including gangliosides are built on a ceramide backbone comprising a long-chain amino alcohol, sphingosine, and an amide-linked fatty acid. GM3, the simplest of the "a-series" gangliosides, is synthesized by GM3 synthase (ST3Gal5), which catalyzes the transfer of SA to the nonreducing terminal galactose (Gal) of lactosylceramide (LacCer). GM3 is altered by GM2 synthase (B4Galnt1) to form GM2 and GD2, a downstream a-series gangliosides, or by GD3 synthase (ST8Sia1) to form GD3, the simplest of the "b-series" gangliosides. B4Galnt1 also elongates LacCer to form GA2, the simplest precursor GSL of the "o-series" ganglioside. Each branch of GSL biosynthesis is a committed pathway (Fig. 1), then competition between enzymes at a key branch point determines the relative expression levels of o-, a-, and b-series gangliosides.

Ganglioside expression in T cells was studied using biochemical analyses (TLC and HPLC) of whole T cell populations including cloned T cell lines and T cell blastocytes stimulated by concanavalin A. However, whole T cell populations are truly a "mixed population", therefore any of such results would be of limited value if we focus on characteristic features of specific T cell subsets. Previously, FACS analysis with monoclonal antibodies (mAbs) against several gangliosides demonstrated that mature peripheral $CD4^+$ T cells and $CD8^+$ T cells express different species of gangliosides [11,12]. These studies mainly demonstrated the predominance of GalNAc-GM1b in CD8T cells [11] and GD1c in CD4T cells [12]. We examined the structures of gangliosides in immature thymocytes, and the primary $CD4^+$ T cells and $CD8^+$ T cells isolated from cell mixtures prepared from lymph nodes and spleen by LC–MS/MS analysis [13]. Thymocytes consisting mainly of T, double positive (DP) cells, expressed the six distinct gangliosides, GM1a, GM1b, GD1b, GD1c, GalNAcGM1b, and extended-GM1b (Table 1). The presence of these gangliosides was common in all T cell subsets, but their expression levels are remarkably different in each subset (Fig. 2A). The expression of o-series gangliosides, GalNAcGM1b and extended-GM1b, was greatly enhanced by the differentiation from thymocytes (23.0 and 6.5%) to $CD4^+$ T (36.6 and 18.9%) and $CD8^+$ T (49.0 and 37.1%) cells. On the other hand, the expression of another o-series ganglioside GD1c was enhanced to double in $CD4^+$ T cells (20.3%) but greatly decreased in $CD8^+$ T cells (3.7%). GD1b was expressed in a considerable amount (30%) in thymocytes, but 6% in $CD4^+$ T and 0.8% in $CD8^+$ T cells. The expression of GM1b was maintained among T cell subsets, but GM1a was expressed in both thymocytes and $CD4^+$ T cells but only trace amounts in CD8T cells. We examined the expression of *ST3Gal5* and *B4Galnt1* genes among T cell subsets (Fig. 2B) [13]. *B4Galnt1* expression was markedly increased more than 100-folds in both $CD4^+$ T and $CD8^+$ T cells compared to thymocytes. *ST3Gal5* expression was increased to 180% in $CD4^+$ T cells and was decreased to 30% in $CD8^+$ T cells, compared to the expression in thymocytes. These gene expression patterns could partially explain the above mentioned distinct expression profile of gangliosides during the differentiation from immature thymocytes to the mature $CD4^+$ T and $CD8^+$ T cells. In the case of GD1c, Nakamura et al. [12] reported that $CD4^+$ T cells can be separated into GD1c-positive IL-2-producing Th1-like cells and GD1c-negative IL-4-producing Th2-like cells. These findings suggest that T cell subsets can be classified into the distinct functional subpopulations based on the differences of ganglioside expression profiles. Wang et al. [15,16] reported that cross-linking of GM1 on $CD4^+$ and $CD8^+$ effector T cells (Teff) by galectin-1 plays a significant role in autoimmune suppression through the modulation of Ca^{2+} influx via transient receptor potential channel 5 (TRPC5). They showed that resting $CD4^+$ Teff and $CD8^+$ Teff contain GM1 and GD1a as the major gangliosides detected by HPTLC with sialidase treatment and CTx-B binding, $CD8^+$ Teff expresses higher levels of GD1a than $CD4^+$ Teff, and both GM1 and GD1a are upregulated by the activation with anti-CD3 and anti-CD28. They did not discuss the possibility that GD1a assigned in their analysis might contain extended-GM1, but this possibility is supported by the following lines of evidence; extended-GM1 is sialidase-resistant, migrates like GD1a on HPTLC developed with chloroform–methanol–water containing $CaCl_2$ [17], exhibits CTx-B binding activity very similar to GM1 [17] and is detected in a higher amount in $CD8^+$ T cells than $CD4^+$ T cells [18]. We compared ganglioside profiles among thymocytes and peripheral $CD4^+$ T and

Table 1
Structures of ganglioside species found in mouse primary T cells.

Structure	Biosynthetic pathway [a]	Trivial name	Relative expression level (%)		
			Thy	CD4T	CD8T
Galβ1-3GalNAcβ1-4(SAα2-3)Glβ1-4Glcβ1-1′Cer	a-Series	GM1a	8.4	4.1	0.8
Galβ1-3GalNAcβ1-4(SAα2-8SAα2-3)Galβ1-4Glcβ1-1′Cer	b-Series	GD1b	29.9	5.7	0.8
SAα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1′Cer	o-Series	GM1b	18.9	14.4	8.6
SAα2-8SAα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1′Cer	o-Series	GD1c	13.3	20.3	3.7
GalNAcβ1-4(SAα2-3)Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1′Cer	o-Series	GalNAc-GM1b	23.0	36.6	49.0
Galβ1-4GalNAcβ1-4(SAα2-3)Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1′Cer	o-Series	Extended-GM1b	6.5	18.9	37.1

[a] Biosynthetic pathway of each series gangliosides is illustrated in Fig. 1.