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Review

Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction

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ABSTRACT

Lactosylceramide (LacCer), a neutral glycosphingolipid, is abundantly expressed on human neutrophils, and specifically recognizes several pathogenic microorganisms. LacCer forms membrane microdomains coupled with the Src family kinase Lyn on the plasma membrane, and ligand binding to LacCer activates Lyn, resulting in neutrophil functions. In contrast, neutrophilic differentiated HL-60 cells do not have Lyn-associated LacCer-enriched microdomains and lack LacCer-mediated functions. In neutrophil plasma membranes, the very long fatty acid C24:0 and C24:1 chains are the main components of LacCer, whereas plasma membrane of D-HL-60 cells mainly includes C16-LacCer species. Here, we suggest that LacCer species containing very long fatty acid chains are indispensable for the association of Lyn with LacCer-enriched microdomains and LacCer-mediated functions.

1. Introduction

Cellular membranes, which are indispensable components of cells, consist of several kinds of lipids and proteins. Although the fluid mosaic model of membrane structure [1] proposes that transmembrane proteins "float in a sea of lipids" on cellular membranes, the composition of plasma membranes is more complex than expected. Localized regions of cellular membranes have been found to have specific molecular compositions and physical properties that differ from the rest of the membrane. Membrane microdomains, also known as lipid rafts, are membrane lipid domains rich in glycosphingolipids (GSLs) and cholesterol; moreover, they contain GPI-anchored proteins and membrane-anchored signaling molecules and are associated with the cytoskeleton [2,3]. Because of their physicochemical characteristics, GSLs tend to form clusters with cholesterol on plasma membranes [4]. For example, immunoelectron microscopy in the absence of organic solvents or using SDS-treated freeze-fracture replicas has shown that GSLs form clusters 40–50 nm in diameter within the plasma membrane, and that some of these clusters are closely associated with signal transducer molecules such as Lyn, a member of the Src family of tyrosine kinases [5,6]. In contrast, the average size of GPI-anchored protein clusters on plasma membranes ranges from 5 nm to 300 nm, depending on fixation and measuring methods [7]. The ceramide structure of each GSL is highly variable [8], whereas GPI-anchored proteins contain C18 and/or C16 fatty acid chains. Moreover, the sn-2 fatty acid chains of GPI-anchored proteins are C18:0 fatty acid chains, which are critical for the integration of GPI-anchored proteins into membrane microdomains [9]. Importantly, the presence of a C24:0 or C24:1 fatty acid chain in GSLs has been shown to be necessary for the functional connection with a Src family kinase in membrane microdomains [6,10]. In contrast, GPI-anchored receptor clusters transiently recruit Src family kinases for temporary cluster immobilization and activation [11,12]. These observations suggest that the organization and signal transduction mechanisms of GSL-enriched microdomains differ from those of GPI-anchored protein-enriched microdomains. In this review, we focus on the organization and characteristics of GSL-enriched microdomains, especially lactosylceramide (LacCer)-enriched microdomains, and the mechanisms by which lactosylceramide-enriched microdomains mediate cell functions.

2. Characteristics of GSL-enriched microdomains

GSLs are membrane components consisting of hydrophobic ceramide and hydrophilic sugar moieties [13]. More than 400 species of GSLs have been identified, based on their sugar chain structure [14]. Furthermore, the ceramide structure of each GSL is also highly variable [8]. GSL metabolism and composition are specifically altered during the proliferation and differentiation of various types

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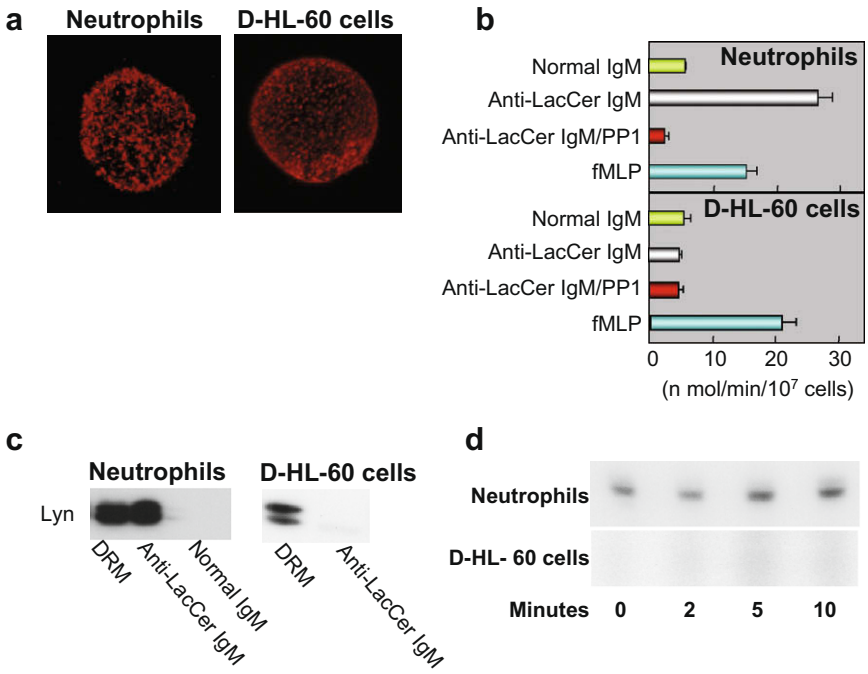

Fig. 1. (a) Three-dimensional reconstructed images of LacCer on the plasma membranes of LacCer-loaded D-HL-60 cells. Neutrophils and D-HL-60 cells were stained with Alexa-594-conjugated anti-LacCer IgM T5A7. After washing, the cells were fixed and examined with a Zeiss LSM 510 confocal microscope equipped with a Plan-Apochromat ×100 oil DIC objective. The three-dimensional images were reconstructed using IMARIS software (Bitplane, Zurich, Switzerland). (b) Anti-LacCer antibody-induced superoxide generation in neutrophils but not DMSO-treated HL-60 cells. Neutrophils and DMSO-treated HL-60 cells were incubated at 37 °C for 30 min in 96 well plates coated with anti-LacCer IgM T5A7, anti-LacCer IgM T5A7 plus 10 μM PP1, or normal IgM. As a positive control for superoxide generation, we used cells incubated with 10^{-7} M fMLP in BSA-coated wells at 37 °C for 30 min. Superoxide production was calculated by measuring the superoxide-dismutase inhibited reduction of cytochrome c at 550 nm. Each bar shows the mean ± S.D. of 4 independent experiments. (c) Association of Lyn with LacCer in neutrophils but not DMSO-treated HL-60 cells. DRM fractions of neutrophils and DMSO-treated HL-60 cells were immunoprecipitated with the mouse anti-LacCer antibody Huly-m13 (Anti-LacCer IgM) or normal mouse IgM (Normal IgM). The immunoprecipitants were analyzed by SDS–PAGE/immunoblotting using rabbit anti-Lyn IgG. (d) T5A7-induced phosphorylation of Lyn in neutrophils. Neutrophils and DMSO-treated HL-60 cells were incubated in anti-LacCer IgM T5A7-coated 10-cm dishes at 37 °C for indicated periods. The kinase activity of Lyn was measured by an in vitro autophosphorylation assay using γ-^{32}P-ATP.

of cells [14,15]. These properties indicate that molecular varieties and expression patterns of GSLs may reflect the functions of those cells. Compared with GSLs that lack hydroxyl groups and can only accept hydrogen bonds, GSLs with hydroxyl groups are thought to have a greater ability to both donate and accept hydrogen bonds through the hydroxyl group of sphingosine and the acyl amide group [14], respectively. GSLs that lack hydroxyl groups within the ceramide moiety are also able to donate hydrogen bonds through the amine nitrogen. These physicochemical properties result in cis interactions of GSLs within the same membrane [16,17]. Moreover, lipids containing saturated alkyl chains with higher transition temperatures differ from those containing unsaturated chains with a lower transition temperature, sometimes below 0 °C, with the former organized in an ordered, less fluid, liquid phase. In general, the transition temperatures of GSLs are generally higher than those of other lipids [15]. Sphingolipids differ from phospholipids in that they contain long saturated acyl chains [18], allowing them to pack together more tightly. This may explain why the melting temperatures of sphingolipids are much higher than those of phospholipids. For example, in human neutrophils, the major molecular species of LacCer are composed of C16:0, C22:0, C24:0 and C24:1 acyl chains; with more than 50% of plasma membrane-derived LacCer composed of C24 or longer fatty acid chains [6]. The transition temperatures of LacCer are greater than 65 °C [19], indicating that LacCer cannot exist as a liquid in cells at physiological temperatures. Because of these physicochemical characteristics, GSLs exist as clusters on plasma membranes [4].

Membrane microdomains (lipid rafts) have been defined as "small (10–200 nm), heterogeneous, highly dynamic, cholesterol- and sphingolipid-enriched domains that compartmentalize cellular processes [20]. Membrane microdomains are dynamic structures constantly in the process of either association or dissociation. Thus, it is quite difficult to biochemically isolate these domains as they are from cells. Cholesterol is another important molecule in the phase behavior of membrane microdomains. Cholesterol is much smaller than GSLs and does not contain a long tail. The small cholesterol sterol-ring system and the ceramide moiety of GSLs are thought to interact via hydrogen bonds and hydrophobic van der Waal's interactions [21]. Furthermore, cis hydrophilic interactions among GSL headgroups promote the lateral association of GSLs and cholesterol. These interactions result in the separation of "GSL-enriched lipid domains" from other phospholipids in the cell membrane and the formation of distinct microdomains. These concepts are in agreement with biochemical findings, that cell membranes are not fully solublized by non-ionic detergents at low temperatures and that detergent-resistant membranes (DRMs) can be isolated as a low-density fraction [22]. DRMs are membrane fragments that can be biochemically isolated at low temperatures from cellular membranes using non-ionic detergents, such as Triton X-100 [22]. The DRM association of a molecule has been accepted widely as the biochemical definition of its being a microdomains-associating molecule, while there is no direct evidence that a molecule associated with the DRM resides primarily in microdomains within the membrane.

3. LacCer as a pattern recognition receptor

Phagocytosis is a major component of the innate immune response and is essential in the elimination of invading microorganisms [23,24]. Phagocytosis is initiated by the association of