Biochimica et Biophysica Acta 1840 (2014) 667–680

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

CD1d favors MHC neighborhood, GM_1 ganglioside proximity and low detergent sensitive membrane regions on the surface of B lymphocytes

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ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 15 October 2013
Accepted 18 October 2013
Available online 26 October 2013

Keywords:
CD1d
MHC
Rafts
FRET
Methyl-β-cyclodextrin
Simvastatin

ABSTRACT

Background: Cluster of differentiation 1 (CD1) represents a family of proteins which is involved in lipid-based antigen presentation. Primarily, antigen presenting cells, like B cells, express CD1 proteins. Here, we examined the cell-surface distribution of CD1d, a subtype of CD1 receptors, on B lymphocytes.
Methods: Fluorescence labeling methods, including fluorescence resonance energy transfer (FRET), were employed to investigate plasma membrane features of CD1d receptors.
Results: High FRET efficiency was observed between CD1d and MHC I heavy chain (MHC I-HC), β_2-microglobulin (β_2m) and MHC II proteins in the plasma membrane. In addition, overexpression of CD1d reduced the expression of MHC II and increased the expression of MHC I-HC and β_2m proteins on the cell-surface. Surprisingly, β_2m dependent CD1d isoform constituted only ~15% of the total membrane CD1d proteins. Treatment of B cells with methyl-β-cyclodextrin (MβCD) / simvastatin caused protein rearrangement; however, FRET demonstrated only minimal effect of these chemicals on the association between CD1d and GM_1 ganglioside on cell-surface. Likewise, a modest effect was only observed in a co-culture assay between MβCD/simvastatin treated C1R–CD1d cells and invariant natural killer T cells on measuring secreted cytokines (IFNγ and IL4). Furthermore, CD1d rich regions were highly sensitive to low concentration of Triton X-100. Physical proximity between CD1d, MHC and GM_1 molecules was also detected in the plasma membrane.
Conclusions: An intricate relationship between CD1d, MHC, and lipid species was found on the membrane of human B cells.
General significance: Organization of CD1d on the plasma membrane might be critical for its biological functions.

1. Introduction

Antigen presentation is a defining mechanism in the adaptive immune system. While several decades of information are available about peptide antigen presentation, through major histocompatibility complex (MHC) molecules, in the activation of conventional T cells, similar roles of lipids in antigen presentation have gained significant attention beginning only in the last couple of decades [1–3]. The non-polymorphic transmembrane glycoproteins known as Cluster of Differentiation (CD1) were found to present these non-peptide antigens to a distinct subset of T cells [3,4]. In fact, these CD1 molecules, encoded on human chromosome 1, resemble MHC I heavy chain (MHC I-HC) in physical structure [3,5–7] and MHC II molecules in function, mainly endosomal surveillance [6,8], thus possessing an admixture of features which have led to the hypothesis that these molecules have diverged from a common ancestral gene. Interestingly, MHC I and CD1d also share the chaperones, calnexin and calreticulin, which are important for their appropriate folding [9,10]. In human, CD1 a, -b and -c molecules are placed in group I CD1 while CD1d is kept separately in group II [7,10,11]. CD1d- restricted natural killer T (NKT) cells release copious amount of both Th1 and Th2 type cytokines and chemokines upon engagement with CD1d receptors, underscoring the roles of CD1d in immunoregulation [12,13]. Most earlier studies demonstrated the trafficking behavior of CD1 from the plasma membrane to the intracellular endosomal membrane compartment and vice versa [4,6,14–21]; these studies provided evidence that CD1 molecules sample antigens in the endocytic system but the membrane organization of these receptors which could influence such biological behaviors of these molecules has not been explored in detail yet. Furthermore, owing to the broad role played by CD1d in B lymphocytes, which came into prominence recently due to its role in sustaining antibody responses [22,23] and for the maintenance of invariant natural killer T

Abbreviations: MHC, major histocompatibility complex; CD1, cluster of differentiation 1; NKT cells, natural killer T cells; iNKT, invariant natural killer T cells; β_2m, β2-microglobulin; MβCD, methyl-β-cyclodextrin; FRET, fluorescence resonance energy transfer; NCS, newborn calf serum; Mabs, monoclonal antibodies; TfR, transferrin receptor; FCET, flow-cytometric FRET; CTxB, cholera toxin subunit B; FCDR test, flow cytometric detergent resistance test; TX100, Triton X-100; DRMs, Detergent resistant membrane regions; PBMC, peripheral-blood mononuclear cells; α-gal, α-galactosylceramide; APC, Antigen presenting cell

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http://dx.doi.org/10.1016/j.bbagen.2013.10.030

(iNKT) cells [24], we focused our studies on this molecule, which is also the only CD1 isoform expressed by mouse [7,25]. Two isoforms of CD1d have been observed on the surface of cells: one that is physically bound to β2-microglobulin (β_2m) and the other that is independently present without β_2m [11,26]. Hereon, we will address these isoforms as β_2m-dependent and β_2m-independent CD1d proteins. Biochemical studies also illustrated that CD1d is in association with MHC II molecules in the plasma membrane [25] and with invariant chain in membrane-bound intracellular compartments [27]. These observations suggested that these molecules might be in close vicinity of each other and most probably in some specific domains like lipid-rafts. In a murine cell line system, CD1d was distinctly found to be located in the lipid-rafts and the disruption of lipid-rafts by methyl-β-cyclodextrin (MβCD) was found to interfere with the NKT cell activational responses [28–31]. Furthermore, we reported the co-existence of MHC I and MHC II in nanodomains of various types of cells using fluorescence resonance energy transfer (FRET) [32–36] and electron microscopy [33]. Since, co-immunoprecipitation was mainly used to detect protein–protein interactions in the case of CD1d, even for the membrane fractions, which can present artefactual interactions and is difficult to reproduce, we thought to utilize FRET, which can demonstrate physiological and dynamic interactions and is a better approach to investigate the distribution of CD1d proteins in the plasma membrane. FRET is very sensitive to changes in distance because the rate of energy transfer is inversely proportional to the sixth power of the distance separating the donor and acceptor fluorophores. This sensitivity to distance makes FRET a useful tool for investigating protein–protein interactions within the range of 1–10 nm. In this study, we demonstrate that CD1d proteins are indeed proximal to MHC proteins and are present in detergent sensitive domains of the membrane in lymphocytes. Surprisingly, β_2m dependent CD1d comprised only a small fraction of CD1d proteins on the membrane (~15%). We also noted that CD1d rich regions were mildly affected by cholesterol modulation, but were significantly altered by low concentration of Triton X-100 (TX100). Likewise, a co-culture assay between C1R–CD1d and iNKT cells also demonstrated the partial dependence of CD1d rich regions on cholesterol. In summary, CD1d seems to have an intricate relationship with MHC proteins and membrane lipids, and it also can apparently form larger protein–protein or protein–lipid complexes in the membrane of C1R–CD1d cells.

2. Materials and methods

2.1. Cell lines

The HLA-C expressing mutant B lymphoid cell line, C1R [37], stably expressing full-length CD1d was used in this study. The characterization of this cell line, including transfection details has been described elsewhere [38]. To obtain cells with uniform CD1d expression, the transfected C1R cells were magnetically sorted using 27.1.9 CD1d antibody and pan anti-mouse secondary antibody conjugated magnetic Dynabeads (Life Technologies/Invitrogen, Budapest, Hungary) following the manufacturer's instructions. Once sorted, the cells were referred to as C1R–CD1d. The cells were grown in RPMI media containing 10% newborn calf serum (NCS), with the inclusion of drug G418 at 300 μg/ml concentration unless stated otherwise.

2.2. Antibodies

The following monoclonal antibodies (Mabs) were used: the pan-MHC-I W6/32 (recognizes MHC I-HC associated with β_2m, IgG2a) [39,40] , HC-10 (recognizes free MHC I-HC, IgG2a) [40–43], L368 (anti-β_2m, IgG1) [44], L243 (anti-MHC-DR, IgG2a) [45], OKT9 (anti-transferrin receptor (TfR), IgG1) [46], 51.1.3 (anti-CD1d, IgG2b) and 27.1.9 (anti-CD1d, IgG1) [47]. Antibodies were prepared from hybridoma supernatants according to the standard protocol by Sepharose A affinity chromatography. Antibodies were coupled to Alexa 546- and Alexa 647-succinimidyl ester dyes following the manufacturer's instructions (Molecular Probes, Invitrogen) and the dye to protein ratios were calculated based on the spectrophotometric absorbance values of the proteins. MEM75 antibody, against TfR [48] was a kind gift from Václav Horejsí (Institute of Molecular Genetics, Academy of Sciences, Prague, Czech Republic).

2.3. Cell labeling

For each sample, approximately one million freshly harvested cells were taken in 50 μl volume of PBS buffer (pH 7.4) containing 1 mg/ml BSA and 0.01% sodium azide. A saturating concentration of the dye conjugated antibodies was added to these cells, and the mixed suspension was incubated in a dark environment for 30 min on ice. After the incubation, these cells were washed twice with ice-cold PBS buffer in order to remove unbound antibodies. Finally, the cells were suspended in 1% formaldehyde solution, and they were kept at 4 °C until the measurements were performed in a flow cytometer or a confocal microscope.

2.4. Quantitation of membrane receptors

In order to determine the expression level of each of the receptors, QIFIKIT (Dako Cytomation, Glostrup, Denmark) was used according to the manufacturer's instructions. QIFIKIT is an indirect immunofluorescence based method of determining antigen density per cell by flow cytometry. This consists of a series of beads coated with known quantities of Mabs which emulate cells with defined antigen densities. Specimen cells were labeled with the primary mouse Mab at saturating concentration. Then, the cells were incubated, in parallel with the QIFIKIT beads, with FITC dye tagged polyclonal anti-mouse secondary $F(ab')_2$. Finally, a calibration curve was plotted between the fluorescence intensity of the individual bead populations against the number of Mab molecules on these beads. This curve was then used for determining the number of receptors on the specimen cells by interpolation. For our purpose, fluorescence intensities were measured on a FACSCalibur instrument using a 530 +/− 30 BP filter.

2.5. Confocal microscopy

A Zeiss LSM 510 confocal laser scanning microscope (Carl Zeiss AG, Jena, Germany) with a Plan-Apochromat 40× (NA = 1.2) water immersion objective was used to record the images. An optical slice (512 × 512-pixels) of 1.5 μm thickness, four-times averaged, was taken from the top of a cell for co-localization experiments. Simultaneously, a multitrack option of the microscope was used to acquire images to avoid any possible crosstalk between the detection channels.

2.6. Co-localization study of the receptors

The spatial proximity of membrane proteins was studied by image cross-correlation method at ~200 nm scale. For this purpose, cells were doubly or triply labeled with markers specific for distinct molecular species but tagged with spectrally different fluorophores. Images were acquired from the top of the cells as a horizontal optical slice by confocal microscope. A custom program written in LABVIEW platform was used for the computational analysis of the cross-correlation coefficient (C) from the image pairs [49,50]. For an image pair 'x' and 'y', the image cross-correlation coefficient was calculated by the following formula:

$$C = \frac{\sum_i \sum_j \left(x_{i,j} - \langle x \rangle\right)\left(y_{i,j} - \langle y \rangle\right)}{\sqrt{\sum_i \sum_j \left(x_{i,j} - \langle x \rangle\right)^2 \sum_i \sum_j \left(y_{i,j} - \langle y \rangle\right)^2}}$$