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All-*trans*-retinoic acid and CD38 ligation differentially regulate CD1d expression and α-galactosylceramide-induced immune responses

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ABSTRACT

The MHC class-I like molecule CD1d presents glycolipid antigens and thereby activates invariant natural killer-T (NKT) cells. However, little is understood regarding the regulation of its expression. All-*trans*-retinoic acid (RA) and CD38, which is itself a target of RA, both independently regulate the differentiation of antigen presenting cells. In the current study, we treated human THP-1 cells and murine splenic cells with RA, with and without antibody-mediated ligation of cell-surface CD38. Whereas a physiological concentration (20 nM) of RA alone rapidly and markedly increased CD1d protein in THP-1 cells, there was a marked synergy between RA and ligation of CD38 with antibody to CD38. Moreover, RA and CD38 ligation differentially regulated CD1d protein distribution between the cell surface and intracellular compartments, as, whereas RA mainly increased intracellular CD1d protein, ligation of CD38 increased CD1d protein both at the cell surface and intracellularly. By confocal microscopy, CD1d was located close to the plasma membrane but only partially overlapped with LAMP1, a late endosomes/lysosomal marker. Furthermore, RA and/or CD38 ligation increased splenocyte proliferation and differentiation after treatment with the CD1 ligand α-galactosylceramide (αGalCer), evidenced by an increase in the number of splenic dendritic cells, NKT cells, and germinal center plasmacytes. RA also differentially regulated αGalCer-induced cytokine expression, increasing IL-4 and decreasing IFNγ production by total spleen cells and the NKT cell population. Our results indicate a previously unknown mechanism in which RA and CD38 differentially yet cooperatively regulate CD1d expression and antigen-presenting function, which could be important for the enhancement of immunity.

Introduction

CD1d is a MHC class I like molecule expressed on various antigen presenting cells (APC), including dendritic cells, macrophages, and splenic B cells (Brigl and Brenner, 2004). CD1d processes and presents glycolipid antigens that in turn activate invariant NKT cells, which themselves are regulators of both innate and adaptive immune responses (Brennan et al., 2013; Rossjohn et al., 2012). The prototypic CD1d ligand, α-galactosylceramide (αGalCer), isolated from a marine sponge (Morita et al., 1995) as well as other glycolipids isolated from bacteria and mammalian cell components are recognized by CD1d and thereby elicit anti-bacterial, autoimmune, and anti-tumor responses (Chen and Ross, 2012; Mattner et al., 2005; Sriram et al., 2005; Terabe and Berzofsky, 2014; Zhou et al., 2004). As is also the case for other antigen-presenting molecules, such as MHC class I and II, CD1d expression is subject to regulation by cytokines and antigenic stimulation, and is regulated epigenetically through histone modification (Skold et al., 2005; Yang et al., 2012). The level of CD1d on APC is likely to affect their capacity for antigen presentation and therefore may determine the efficacy of NKT or T cell-mediated immune responses.

Retinoic acid is a quantitatively minor but very active metabolite of vitamin A. The requirement for RA as a regulator of innate and acquired immunity has been demonstrated in a variety of animal experiments and clinical settings (Cui et al., 2000; Glasziou and Mackerras, 1993; Ross et al., 2011). T cell, NK cell, and B cell functions all require RA (Chen and Ross, 2005; Seguin-Devaux et al., 2005; Zhao and Ross, 1995). RA potentiates T cell-dependent antibody production (DeCicco et al., 2001; Ma et al., 2005), regulates

Abbreviations: APC, antigen-presenting cell; αGalCer, α-galactosylceramide; DC, dendritic cell; LAMP1, lysosomal-associated membrane protein 1; NKT, natural killer T cell.

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NKT cell activity and cytokine production (Lee et al., 2012), and promotes the homing of T cells and B cells, which is necessary for immune protection in the gut (Hammerschmidt et al., 2011; Iwata et al., 2004). RA and other retinoids also induce differentiation and maturation of monocytic cells, implicating RA in the regulation of APC functions (Chen and Ross, 2004; Estey et al., 1999; Gaboli et al., 1998). Moreover, RA is a potent direct inducer of CD1d gene expression in monocytic cells, as we have shown previously (Chen and Ross, 2007), which could explain one way in which RA contributes to promoting the immune response.

CD38 is a type II membrane-associated glycoprotein that is expressed in almost all cells of the immune system. CD38 is most often used as a marker to define the developmental stage and differentiation status of cells (Donis-Hernandez et al., 2001; Reinis et al., 1997; Ridderstad and Tarlinton, 1998; Vences-Catalan and Santos-Argumedo, 2011). However, decades of research suggest that CD38 is not only a marker but also a dual-functional cell surface receptor and enzyme involved in cell signaling and intracellular calcium trafficking. CD38 has been shown to interact with the BCR, TCR/CD3 complex, CD16, and MHC class II molecules on various lymphoid and myeloid cells respectively, to facilitate the activation process as appropriate to these different types of cells (Deaglio et al., 2002; Lund et al., 1996; Morra et al., 1998; Zilber et al., 2005). Moreover, the intrinsic enzymatic activity of the CD38 extracellular domain functions in the metabolism of NAD^+, which is associated with calcium mobilization from intracellular storage and calcium-dependent intracellular signaling (Flora et al., 2004; Franco et al., 1998; Shubinsky and Schlesinger, 1997). RA has been shown to be a potent inducer of CD38 expression (Kishimoto et al., 1998; Mehta and Cheema, 1999), which occurs concomitantly with the cell differentiation process (Chen and Ross, 2004).

Our current studies began with knowledge that RA is a regulator of both CD1d gene expression (Chen and Ross, 2007), and of CD38 expression and monocytic cell differentiation (Chen and Ross, 2004; Chen et al., 2002; Fedele et al., 2004). We hypothesized that RA together with stimulation of CD38 might drive CD1d expression and function to a higher level than by either stimulus alone. We first observed that although RA alone was a potent regulator of CD1d protein expression in THP-1 cells, the combination of RA and CD38 ligation acted synergistically to up-regulate CD1d protein accumulation. We therefore conducted further experiments to test whether RA together with engagement of CD38 enhanced αGalCer-stimulated splenocyte activation. Together, these results provide evidence for a new retinoid-CD38-inducible pathway of CD1d/αGalCer-mediated antigen presentation, which may have important implications for the immune response of NKT and B cells to lipid antigens.

Materials and methods

Cell culture and treatments

THP-1 monocytic cells were propagated in RPMI-1640 medium supplemented with 10% FBS and 5×10^{-5} M β-mercaptoethanol (Invitrogen, Grand Island, NY), as described in (Chen and Ross, 2004). During each experiment, THP-1 cells were cultured in medium with 3% FBS to reduce exposure to RA and growth factors which may be present in serum while providing sufficient serum for cell maintenance (Chen and Ross, 2004).

Spleen cells were prepared from 8 week-old female Balb/c or C57BL/6 mice (Charles River, Wilmington, MA) that were fed a standard diet and maintained in plastic cages in temperature and humidity controlled rooms with 12-h light/dark housing. Spleens were dissociated on a wire mesh and mononuclear cells were isolated by Histopaque gradient centrifugation (Sigma, St. Louis, MO). All-*trans*-RA (Sigma) was dissolved as a stock in absolute ethanol and diluted prior to each experiment so that the final concentration in the cell culture medium was 20 nM, a physiological concentration similar to that in plasma (Eckhoff and Nau, 1990). αGalCer (Alexis Biochemicals, San Diego, CA) and βGalCer (Sigma) as a negative control (Morshed et al., 2009) were prepared in PBS with 0.5% Tween 20 and used at a final concentration of 100 ng/ml in cell cultures. THP-1 cells were treated with 1 μg/ml of anti-human CD38 antibody (AT1, Santa Cruz Biotechnology, Santa Cruz, CA), and mouse cells with 1 μg/ml of anti-mouse CD38 antibody (NIMR-5, Southern Biotech, Birmingham, AL).

Western blot analysis

THP1 cells were cultured in 6-well plates (2×10^6 cells/2 ml per well) with RA (20 nM) and/or α-CD38 (1 μg/ml) for various times. At the time of harvesting, cells were washed with PBS and the cell pellets were lysed in RIPA buffer (follow the instruction from Santa Cruz) with the presence of 0.1 mM Sodium orthovanadate and complete proteinase inhibitor cocktail (Roche laboratory). Fifty micrograms of cellular protein was applied to SDS-PAGE and subjected to Western blot analysis as described previously (Chen et al., 2002). An anti-CD1d monoclonal antibody (C3D5, Santa Cruz Biotechnology) was used to detect the CD1d protein, followed by incubation with an anti-mouse antibody conjugated with horseradish peroxidase (Sigma) and chemiluminescence detection (Pierce Biotechnology, Inc., Rockford, IL).

Flow cytometry and confocal microscopic analysis

To determine the relative amount of cell surface CD1d protein, THP-1 cells after culture were incubated with anti-CD1d antibody for 30 min at 4° C, then incubated with Alexa 488-conjugated secondary antibody (Invitrogen Corp., Carlsbad, CA) for another 30 min. The fluorescence output was analyzed by flow cytometry using isotype-matched control antibody for background subtraction. Intracellular CD1d staining was performed by first fixing and permeabilizing cells according to the manufacturer's instructions (BD Biosciences, San Jose, CA), and then the primary antibody was incubated with cells overnight at 4° C, followed by addition of fluorochrome-conjugated secondary antibody for an additional 30 min at 4° C. Lysosomal-associated membrane protein 1 (LAMP1), a marker of the late endosome/lysosome compartment, was detected using a rabbit anti-LAMP1 antibody (H-228, Santa Cruz) plus Alexa 568-conjugated secondary anti-rabbit antibody. The cells were then washed and subjected to analysis by flow cytometry (BD Accuri C6 flow cytometer) and confocal microscopy (Olympus Fluoview 1000). Flow cytometric and confocal microscopy data were analyzed by FlowJo (Tree Star, Ashland, OR) and Flowview software (Olympus optical company, Olympus America, Melville, NY), respectively.

To determine the cell cycle activity, THP-1 cells cultured for 24 h were subjected to propidium iodide staining (Chen and Ross, 2004), and analyzed by flow cytometer to determine the cell cycle distribution of the cells.

To trace cell proliferation, splenocytes were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE, 10 nM, Life technologies, Grand Island, NY) for 30 min and followed by 4 days of culture, following protocols described in Chen and Ross (2005). Cells were then stained with fluorescent antibodies, including CD11c, CD3, CD49b, CD19, CD23 and CD138, all from BD Biosciences. Details regarding the combination of fluorescent-labeled antibodies for each experiment are described in the figure legends.

For NKT cell cytokine staining, spleen cells were cultured in the absence of Golgi Plug (Brefeldin A, BD Biosciences, San Diego, CA) for 4 days. Cells were first stained with TCR Vβ-FITC antibody