

Invited Mini Review

The effect of intracellular trafficking of CD1d on the formation of TCR repertoire of NKT cells

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CD1 molecules belong to non-polymorphic MHC class I-like proteins and present lipid antigens to T cells. Five different CD1 genes (CD1a-e) have been identified and classified into two groups. Group 1 include CD1a-c and present pathogenic lipid antigens to $\alpha\beta$ T cells reminiscent of peptide antigen presentation by MHC-I molecules. CD1d is the only member of Group 2 and presents foreign and self lipid antigens to a specialized subset of $\alpha\beta$ T cells, NKT cells. NKT cells are involved in diverse immune responses through prompt and massive production of cytokines. CD1d-dependent NKT cells are categorized upon the usage of their T cell receptors. A major subtype of NKT cells (type I) is invariant NKT cells which utilize invariant V α 14-J α 18 TCR alpha chain in mouse. The remaining NKT cells (type II) utilize diverse TCR alpha chains. Engineered CD1d molecules with modified intracellular trafficking produce either type I or type II NKT cell-defects suggesting the lipid antigens for each subtypes of NKT cells are processed/generated in different intracellular compartments. Since the usage of TCR by a T cell is the result of antigen-driven selection, the intracellular metabolic pathways of lipid antigen are a key in forming the functional NKT cell repertoire. [BMB Reports 2014; 47(5): 241-248]

INTRODUCTION

Major histocompatibility complex class I (MHC-I) and MHC-II molecules present peptide antigens to CD8⁺ T cells and CD4⁺ T cells, respectively. Pathogen derived protein antigens synthesized in the intracellular compartment are loaded as a short peptide on the MHC-I molecules in the endoplasmic reticulum (ER) before MHC-I presents the peptide antigens to CD8⁺ $\alpha\beta$ T cells (1). Extracellular-derived pathogenic peptides are loaded on MHC-II molecules in the MHC-II compartment (MIIC) such

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as lysosomes before they are presented to CD4⁺ $\alpha\beta$ T cells (2).

Mammalian immune systems can recognize not only protein antigens but also lipid antigens presented by CD1 molecules. CD1 molecules are MHC-I-like proteins that are non-covalently associated with β 2 microglobulin (β 2m) (3). Based on sequence analysis, CD1 proteins can be classified into two groups in human. Group 1 comprises CD1a, CD1b, CD1c, and CD1e, group 2 CD1d (4). CD1e not expressed on the cell surface also can be independently classified as group 3 (5). Each CD1 molecule presents specific lipid antigens derived from foreign microorganism and/or self lipid antigens. For example, CD1a presents didehydroxymycobactin of mycobacteria (6). CD1b presents mycolic acids, phosphatidylinositol mannosides of mycobacteria and self antigen monosialotetrahexosylganglioside (GM1), the prototype ganglioside (7-9). CD1c presents mannosyl- β 1-phosphomycoketides of mycobacteria (10, 11). Lastly, CD1d present marine sponge-derived α -galactosylceramide (α -GalCer) and self lipid antigen, disialoganglioside GD3 (12, 13).

CD1a-c proteins are recognized by diverse conventional $\alpha\beta$ T cells. By contrast, CD1d proteins are recognized by a specialized subset of $\alpha\beta$ T cells, NKT cells (14). Unlike human, mouse CD1 proteins only contain group 2 (CD1d) (5). Due to the lack of group 1 CD1 genes in mouse, the biology of CD1 molecules including antigen presentation mechanism is less well studied in group 1 CD1 molecules. Here, we review recent progresses on lipid antigen presentation by CD1d.

CD1D STRUCTURE

CD1d is highly expressed in antigen presenting cells (APCs) such as dendritic cells (DCs), macrophages and B cells. APCs engulf exogenous pathogens such as mycobacterium and CD1d encounters glycolipid antigens of engulfed pathogens at endocytic vesicles such as late endosome and lysosome and loads glycolipid antigens through lysosomal machinery (15, 16).

The overall structure of CD1 molecules (isoform a-e) is similar to MHC-I molecules. As the case of MHC-I, CD1 molecules consist of α 1, α 2 and α 3 domains. Each α 1 and α 2 domain contains an α -helical structure positioned antiparallelly each on top of a β -pleated sheet and form an antigen binding region. The α 3 domain connects antigen binding region to the

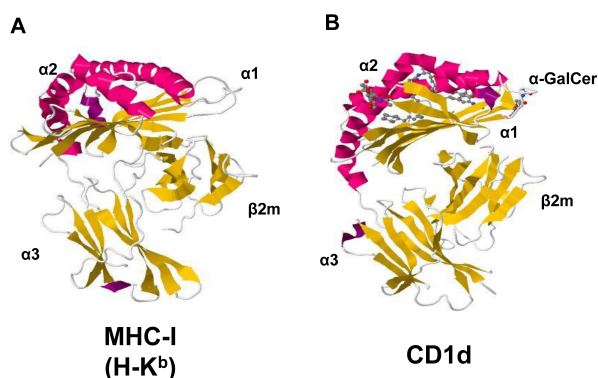


Fig. 1. The structures of MHC class I and CD1d. The side views of MHC-I (A) and CD1d (B) molecules. $\alpha 1$ and $\alpha 2$ domains consisting two alpha helices on top of the molecules (magenta) and a beta-plated sheet (yellow) beneath the alpha helices form an antigen binding region, which is facing toward T cell receptors. $\beta 2m$ -associated $\alpha 3$ domain is attached on the cell surface. PDB accession numbers are 2VAA (MHC I) and 1ZT4 (CD1d).

transmembrane segment which is followed by a short cytoplasmic tail region. The $\alpha 3$ domain is also associated non-covalently with $\beta 2m$ (Fig. 1) (17). Although the overall structures of CD1 molecules are quite similar to that of MHC-I molecules, the antigen binding grooves of two molecules shows substantial differences. While MHC-I molecules have relatively shallow and long antigen binding groove along with horizontal axis, CD1 molecules have more deeper and voluminous antigen binding groove (17). Although the antigen binding grooves of CD1 isoforms have similar hydrophobic nature, their fine architectures are slightly different in accommodating distinct glycolipid antigens (17). The antigen binding groove of CD1d consists of two pockets: the A' pocket and the F' pocket with a narrow entrance at the junction between these two pockets. The deep and hydrophobic pockets of CD1d can accommodate alkyl chains of glycolipid antigens and the hydrophilic sugar in the head group protrudes toward approaching TCRs of NKT cells (17). The crystal structure of the TCR- α -GalCer-CD1d ternary complex shows the contact of the head group of α -GalCer and the CD1 α -helices with the TCR of NKT cells as predicted (18). While TCRs of conventional T cells in most of the structure so far solved, approach diagonally or orthogonally to the central top of peptide-MHC plane and produce substantial area of footprint, the TCRs of NKT cells approach parallel to the long axis of the antigen binding groove of CD1d and towards one end of CD1d molecule (18).

NKT CELLS

CD1d-restricted T cells express T cell markers along with canonical NK cell markers on the cell surface, thus they are called as NKT cells. In mouse, NKT cells represent $\sim 1\%$ of the T cell population in most lymphatic tissues including peripheral

blood, spleen, thymus and lymph nodes but NKT cells represent up to 30% of T cells in the liver (15, 16). The expression of LFA-1 and CXCR6 on NKT cells seems important for the striking levels of accumulation of NKT cells in the liver (19-21).

NKT cells can produce a large quantity of cytokines promptly upon recognition of glycolipid antigens presented by CD1d. The major cytokines, including IFN- γ , IL-4, IL-10, IL-13, IL-21, GM-CSF and IL-17 secreted by NKT cells have been identified so far (16). Cytokines secreted by NKT cells can transactivate other lymphocytes such as DCs, NK cells, T cells and B cells. Thus NKT cells can bridge innate immunity and adaptive immunity (16).

CD1d-restricted NKT cells consist of type I and type II NKT cells depending on the usage of TCR α chains. Type I NKT cells utilize an invariant V $\alpha 14$ -J $\alpha 18$ TCR alpha chain in mouse (V $\alpha 24$ -J $\alpha 18$ in human) coupled mostly with V $\beta 2$, 7 and 8 (V $\beta 11$ in human), hence they are called invariant NKT (iNKT) cells (16). While type I NKT cells occupy about 80% of NKT cells, the remaining 20% is type II NKT cells which utilize diverse TCR V α and TCR V β chains. Both type I and type II NKT cells show autoreactivity against CD1d expressing cells, especially thymocytes (16). Although both types of NKT cells show autoreactivity, their antigen specificities are non-overlapping due to their different TCR usage. Type I NKT cells but not type II NKT cells recognize a well known prototype NKT cell antigen, α -GalCer and a self antigen isoglobotrihexosylceramide (iGb3) (15). Instead, type II NKT cells recognize sulfatide which is not recognized by type I NKT cells (22). Both type I and type II NKT cells have been known to cross-regulate with each other (23-25).

CD1D ANTIGEN

α -GalCer, a typical glycolipid antigen of type I NKT cells was derived from a marine sponge *Agelas mauritanicus* in an anti-cancer drug screening (13). The galactose residue is connected to sphingosine base through α -anomeric glycosidic linkage. Various derivatives of α -GalCer have been synthesized through the modification of acyl carbon chain and some of those derivatives induced either Th1 or Th2-biased responses of type I NKT cells. For example, α -C-galactosylceramide (α -C-GalCer) induces Th1-biased responses from type I NKT cells (26-29), while OCH and α -GalCer(C20:2) produce Th2-biased responses from type I NKT cells (30, 31) (Fig. 2). Although α -GalCer induces strong responses of type I NKT cells and has been a reference antigen for NKT cell research, it may not be considered as the natural antigen for NKT cells as it is not found in the body of mammals. In addition to the synthetic glycolipids, antigens derived from microbes have been shown to be presented by CD1d. Phosphatidylinositol mannosides derived from *Mycobacterium tuberculosis*, α -Galactosyl-diacylglycerol derived from *Borrelia burgdorferi*, lipophosphoglycan derived from *Leishmania donovani* and α -glucur-

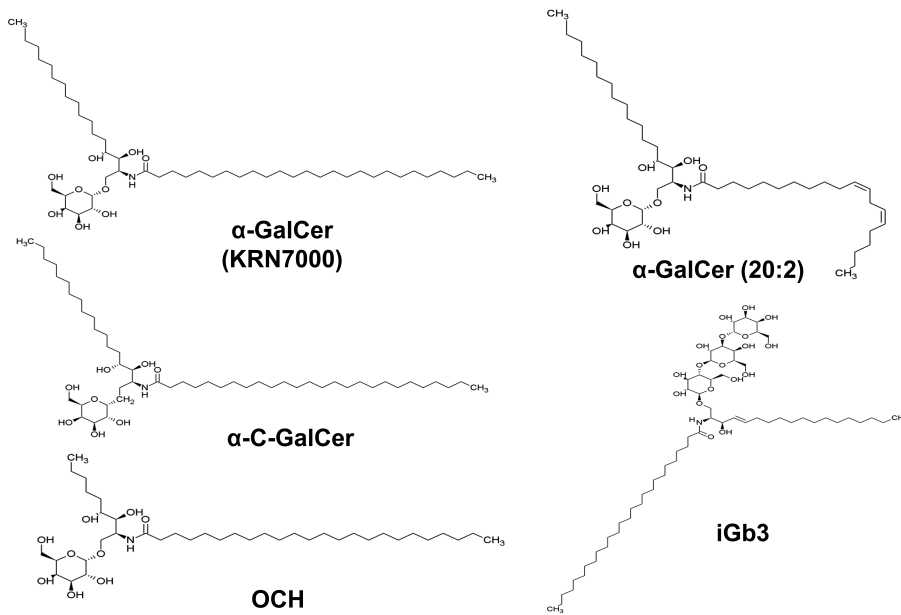


Fig. 2. The chemical structures of CD1d-specific glycolipid antigens. The structures of α -galactosylceramide (α -GalCer), α -C-galactosylceramide (α -C-GalCer), an α -GalCer with truncated sphingosine chain (OCH), an α -GalCer with unsaturated carbons in acyl chain (α -GalCer (C20:2)), and isoglobotrihexosylceramide (iGb3).

onosylceramide derived from *Sphingomonas spp.* are presented by CD1d (32-35). An enormous effort has been invested to date in trying to find self antigens of NKT cells as NKT cells clearly showed autoreactivity against syngenic cells, especially thymocytes without the addition of exogenous antigens (36-39). iGb3 was the first identified self glycolipid antigen and was considered to be involved in the development of NKT cells (40). The frequency of NKT cells in the lysosomal glycosphingolipid degrading enzyme β -hexosaminidase b subunit knock-out (Hexb^{-/-}) mice, deficient of β -hexosaminidase involved in the biosynthesis of iGb3, was dramatically reduced in the thymus and spleen (40). However, iGb3 synthase-deficient mice still maintained normal development of NKT cells (41). Regardless of such controversy, it is clear that iGb3 can be loaded on CD1d of antigen presenting cells and can stimulate type I NKT cells in vitro (33, 40).

Other self glycolipid antigens for NKT cells such as phosphatidylinositol, phosphatidylglycerol, and phosphatidylethanolamine have been eluted from endoplasmic reticulum (ER) using recombinant CD1d (42). Ganglioside GD3, a major antigen for autoantibodies was isolated from human melanoma. Human melanoma cell or GD3-pulsed dendritic cells stimulated NKT cells in a CD1d-dependent manner (12). According to a recent research, blocking of a biosynthetic pathway for peroxisome-derived self glycolipids affected the normal development of NKT cells (43). An analogue of peroxisome-derived self glycolipid, synthetic ether-bonded plasmalogen C16-lysophosphatidylethanolamine (pLPE) has been shown to be presented by CD1d and stimulate type I NKT cells (43). Sulfatide, a component of myelin, is a glycosphingolipid that has sulfated β -linked galactose headgroup. Initially, sulfatide was

known to be presented by CD1a, b, and c (44) but it has been evident that sulfatide is presented by CD1d and stimulate type II NKT cells (22, 45, 46).

CD1D PRESENTATION PATHWAY

CD1d assembly

The biosynthesis and surface expression pathways of CD1d are mostly common with those of conventional MHC-I molecules. Newly synthesized CD1d molecule has signal sequence directing the molecule to the lumen of ER and thus, CD1d is immediately glycosylated in the ER (47). For proper folding, ER-directed nascent CD1d is retained in the ER by the lectin-like ER chaperone calnexin, which assists in protein folding and promotes assembly with β 2m. Calreticulin, another lectin-like ER chaperone, replaces calnexin and make complex with a thiol oxidoreductase ERp57 (47-49). In case of conventional MHC-I molecules, calreticulin and ERp57 complexed with tapasin and are connected to transporters associated with antigen processing (TAP) 1 and TAP2 to form peptide loading complex (PLC). In case of CD1d, however, an ER-resident lipid transfer protein (MTP) is involved in the presentation of lipid antigen to CD1d (50). Although the detailed action mechanism is not clear, MTP, involved in proper assembly and secretion of very low density lipoprotein (VLDL), probably mediate the loading of phospholipids to CD1d in the ER. Stabilized-CD1d/ β 2m/lipid complex moves to the cell surface through trans-Golgi network (TGN) (14).

CD1d endocytic pathway

CD1d exit to the cell surface through TGN after being loaded

with self lipid antigen in the ER. The NKT cells which recognize ER-loaded self lipid antigens do not respond against α -GalCer although they have TCR V α and V β usage similar with those of type I NKT cells (42). The presentation of self lipid antigens on CD1d seems to be mediated by MTP as APCs with defective MTP function has a defect in CD1d antigen presentation ability (50). Also the treatment of MTP inhibitor during fetal thymic organ culture (FTOC) almost completely reduced the number of developing NKT cells (51).

CD1d molecules which arrived on the cell surface are internalized into the early endosome by interacting with adaptor protein-2 (AP-2) and move to late endosome or lysosome by the mediation of AP-3 (52-54). There, the lipid antigen loaded in the ER is replaced by lysosomal-derived lipid antigens. Here, saposin is known to be involved in the replacement process (55-58). Lysosomal antigen-loaded CD1d molecules are recycled to the cell surface and present the antigen to type I NKT cells. Tyrosine motif on the cytoplasmic tail of CD1d is important in the interaction with AP-2 and AP-3, which mediate the endocytic pathway and lysosomal trafficking of CD1d (52, 53, 59). APCs with cytoplasmic tail-deleted CD1d (CD1d-TD) were reported to have defective antigen presentation to type I NKT cells (60-63). CD1d-TD showed a defect in the presentation of disaccharide glycolipid antigens and also a defect in optimal presentation of α -GalCer (63-65). CD1d knock-out mouse reconstituted with CD1d-TD also showed dramatically reduced number of type I NKT cells (62). However, the removal of cytoplasmic tail of CD1d did not affect the development of type II NKT cells (62). Type I NKT cell-specific defect in antigen presentation of CD1d-TD was considered to be a problem originated from the inability of its trafficking to the endolysosomal compartment where it replaces ER antigens with type NKT cell-specific lysosomal antigens.

CD1d molecules have several positive charged amino acids

in between transmembrane region and short cytoplasmic tail. Recently, it has been shown that when three consecutive arginines of this motif in murine CD1d were replaced with non-charged alanines (CD1d-RA), antigen presentation ability of CD1d-RA to type I NKT cells was severely reduced either for endogenous self lipid antigen and exogenous lipid antigens even though CD1d molecules were highly accumulated in the endolysosomal (lamp-1⁺) compartments. Moreover, CD1d-RA also had a defect in antigen presentation to type II NKT cells (66).

Strikingly however, the removal of both arginine motif and cytoplasmic tail of CD1d (CD1d-RATD) showed normal trafficking of CD1d molecules to the lamp-1⁺ endolysosomal compartment (Fig. 3). Since CD1d-TD obviously has a defect in recycling CD1d from cell surface to endosomal compartment, the finding of CD1d-RATD in the endolysosomal compartment suggest the existence of a yet unidentified direct trafficking route of CD1d from ER to lysosomal compartment other than the internalization from cell surface. In that sense, this direct trafficking seems reminiscent of that of MHC-II, where it is a direct traffic from Golgi apparatus to endosomal compartment by the guidance of invariant chain (Ii). Indeed, similar trafficking was observed when MHC-II or Ii was overexpressed with CD1d-TD (60, 67). However, CD1d-RATD traffic to lamp-1⁺ compartment regardless of the existence of MHC-II or Ii (66). Moreover, CD1d-TD trafficked to lamp-1⁺ compartment by overexpressed MHC-II or Ii showed recovered antigen presentation to type I NKT cells compared to CD1d-TD (60, 67). However, the antigen presentation ability of CD1d-RATD to type I NKT cells was not recovered even though CD1d molecules were highly accumulated in lamp-1⁺ compartment (66).

Although lamp-1⁺ compartment is considered as late endosome and lysosome, there is a possibility that the lamp-1⁺ compartment where CD1d internalized from cell surface (type I lysosome) and the lamp-1⁺ compartment where CD1d traffic directly from ER or TGN regardless (type II lysosome) are not

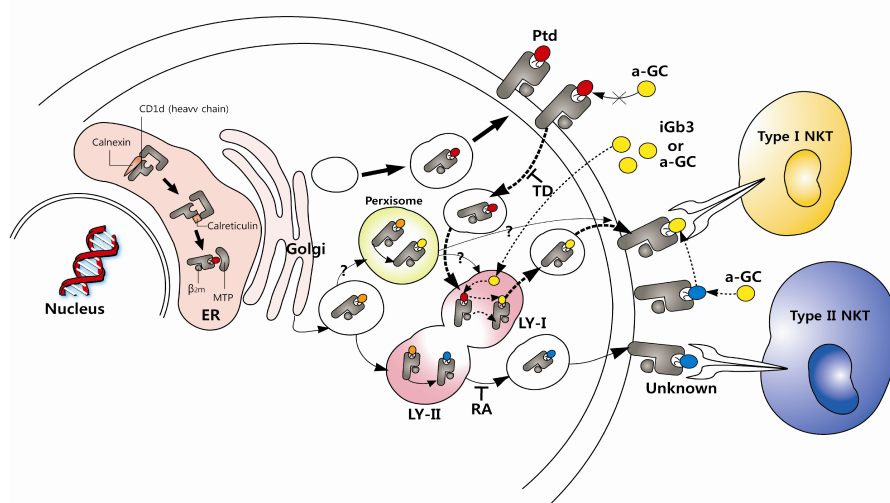


Fig. 3. Model for the assembly, trafficking and antigen presentation pathway of CD1d. CD1d molecules assembled in the ER bind with ER glycolipid and traffic to the extracellular surface through TGN and then they are internalized into type I lysosomal compartments (LY-I) where they replace antigens with type I NKT cell-specific lysosomal antigens. Some CD1d molecules may traffic directly from ER/Golgi to a distinct lysosomal compartment (LY-II) where they are loaded with type II NKT cell-specific glycolipid antigens. The tri-arginine motif may facilitate the exit of antigen-loaded CD1d molecules from lysosomal compartment to the extracellular surface for the interaction with NKT cells.

within the same compartment. Interestingly, the presence of NKT cell antigens not only on the ER or lysosome but also in peroxosome has been reported (43). Although there is no direct evidence showing the trafficking of CD1d directly to peroxosome, it is possible that CD1d acquires lipid antigens directly or indirectly from other compartment not belong to ER or lysosome. Considering both, it is possible that CD1d in the type I lysosomal compartment recycled from the cell surface pick up glycolipid antigens for type I NKT cells while CD1d trafficked to type II lysosomal compartment directly from ER/Golgi apparatus pick up antigens for type II NKT cells. The arginine motif of CD1d probably then functions to modulate upward recycling of CD1d from lysosomal compartments to the cell surface. The finding of these additional trafficking routes of CD1d may open the basis to understand how CD1d can differentially present lipid antigens to the different types of NKT cells (Fig. 3).

CONCLUSION

Two decades since the discovery of NKT cells, NKT cells have drawn extensive interest because they were believed to involve in many immunological situations as immuno-regulatory cells. NKT cells can promptly produce a large amount of key cytokines such as IFN- γ and IL-4 upon recognition of glycolipid antigens. The cytokines produced by NKT cells can modulate various ongoing immune responses such as microbe infection, autoimmune diseases and cancer rejection. The involvement of NKT cells in these immunological conditions can produce either beneficial or detrimental effects depending on the involved subtypes of NKT cells. For example, there are evidences that the activation of NKT cells alleviate autoimmune disease diabetes while worsening another autoimmune disease, rheumatoid arthritis, (68-70). Although rich evidences support antitumor reactivity of type I NKT cells, type II NKT cells seem to suppress tumor-specific cytotoxic T cell responses (71). These conflicting activities of NKT cells are probably dependent on the lipid antigens they recognize at the site of inflammation as shown by the α -GalCer and a derivative of α -GalCer, OCH drive NKT cells into Th1 and Th2 cytokine production, respectively. By these reasons, the effect of NKT cell-mediated immunotherapies will be greatly affected by the type of different glycolipid antigens. Based on the facts that several different intracellular antigen-loading compartments for CD1d such as ER, TGN, endosome and lysosome and obvious differentiation of the antigen loading compartments for type I and type II NKT cells, the revealing of intracellular trafficking routes of CD1d and the antigen loading compartments for different antigens is essential for immunotherapies using NKT cells.

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REFERENCES

1. Rock, K. L., York, I. A., Saric, T. and Goldberg, A. L. (2002) Protein degradation and the generation of MHC class I-presented peptides. *Adv. Immunol.* **80**, 1-70.
2. Hiltbold, E. M. and Roche, P. A. (2002) Trafficking of MHC class II molecules in the late secretory pathway. *Curr. Opin. Immunol.* **14**, 30-35.
3. Porcelli, S. A. (1995) The CD1 family: a third lineage of antigen-presenting molecules. *Adv. Immunol.* **59**, 1-98.
4. Calabi, F., Jarvis, J. M., Martin, L. and Milstein, C. (1989) Two classes of CD1 genes. *Eur. J. Immunol.* **19**, 285-292.
5. de la Salle, H., Mariotti, S., Angenieux, C., Gilleron, M., Garcia-Alles, L. F., Malm, D., Berg, T., Paoletti, S., Maitre, B., Mourey, L., Salamero, J., Cazenave, J. P., Hanau, D., Mori, L., Puzo, G. and De Libero, G. (2005) Assistance of microbial glycolipid antigen processing by CD1e. *Science* **310**, 1321-1324.
6. Moody, D. B., Young, D. C., Cheng, T. Y., Rosat, J. P., Roura-Mir, C., O'Connor, P. B., Zajonc, D. M., Walz, A., Miller, M. J., Levery, S. B., Wilson, I. A., Costello, C. E. and Brenner, M. B. (2004) T cell activation by lipopeptide antigens. *Science* **303**, 527-531.
7. Beckman, E. M., Porcelli, S. A., Morita, C. T., Behar, S. M., Furlong, S. T. and Brenner, M. B. (1994) Recognition of a lipid antigen by CD1-restricted alpha beta+ T cells. *Nature* **372**, 691-694.
8. Sieling, P. A., Chatterjee, D., Porcelli, S. A., Prigozy, T. I., Mazzaccaro, R. J., Soriano, T., Bloom, B. R., Brenner, M. B., Kronenberg, M., Brennan, P. J. and Modlin, R. L. (1995) CD1-restricted T cell recognition of microbial lipoglycan antigens. *Science* **269**, 227-230.
9. Shamshiev, A., Donda, A., Prigozy, T. I., Mori, L., Chigorno, V., Benedict, C. A., Kappos, L., Sonnino, S., Kronenberg, M. and De Libero, G. (2000) The alphabeta T cell response to self-glycolipids shows a novel mechanism of CD1b loading and a requirement for complex oligosaccharides. *Immunity* **13**, 255-264.
10. Moody, D. B., Ulrichs, T., Muhlecker, W., Young, D. C., Gurcha, S. S., Grant, E., Rosat, J. P., Brenner, M. B., Costello, C. E., Besra, G. S. and Porcelli, S. A. (2000) CD1c-mediated T-cell recognition of isoprenoid glycolipids in *Mycobacterium tuberculosis* infection. *Nature* **404**, 884-888.
11. Matsunaga, I., Bhatt, A., Young, D. C., Cheng, T. Y., Eyles, S. J., Besra, G. S., Briken, V., Porcelli, S. A., Costello, C. E., Jacobs, W. R., Jr. and Moody, D. B. (2004) *Mycobacterium tuberculosis* pks12 produces a novel polypeptide presented by CD1c to T cells. *J. Exp. Med.* **200**, 1559-1569.
12. Wu, D. Y., Segal, N. H., Sidobre, S., Kronenberg, M. and Chapman, P. B. (2003) Cross-presentation of disialoganglioside GD3 to natural killer T cells. *J. Exp. Med.* **198**, 173-181.
13. Kawano, T., Cui, J., Koezuka, Y., Taura, I., Kaneko, Y.,

- Motoki, K., Ueno, H., Nakagawa, R., Sato, H., Kondo, E., Koseki, H. and Taniguchi, M. (1997) CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* **278**, 1626-1629.
14. Brigl, M. and Brenner, M. B. (2004) CD1: antigen presentation and T cell function. *Annu. Rev. Immunol.* **22**, 817-890.
 15. Barral, D. C. and Brenner, M. B. (2007) CD1 antigen presentation: how it works. *Nat. Rev. Immunol.* **7**, 929-941.
 16. Bendelac, A., Savage, P. B. and Teyton, L. (2007) The biology of NKT cells. *Annu. Rev. Immunol.* **25**, 297-336.
 17. Zeng, Z., Castano, A. R., Segelke, B. W., Stura, E. A., Peterson, P. A. and Wilson, I. A. (1997) Crystal structure of mouse CD1: An MHC-like fold with a large hydrophobic binding groove. *Science* **277**, 339-345.
 18. Borg, N. A., Wun, K. S., Kjer-Nielsen, L., Wilce, M. C., Pellizzi, D. G., Koh, R., Besra, G. S., Bharadwaj, M., Godfrey, D. I., McCluskey, J. and Rossjohn, J. (2007) CD1d-lipid-antigen recognition by the semi-invariant NKT T-cell receptor. *Nature* **448**, 44-49.
 19. Geissmann, F., Cameron, T. O., Sidobre, S., Manlongat, N., Kronenberg, M., Briskin, M. J., Dustin, M. L. and Littman, D. R. (2005) Intravascular immune surveillance by CXCR6+ NKT cells patrolling liver sinusoids. *PLoS Biol.* **3**, e113.
 20. Thomas, S. Y., Scanlon, S. T., Griewank, K. G., Constantinides, M. G., Savage, A. K., Barr, K. A., Meng, F., Luster, A. D. and Bendelac, A. (2011) PLZF induces an intravascular surveillance program mediated by long-lived LFA-1-ICAM-1 interactions. *J. Exp. Med.* **208**, 1179-1188.
 21. Ohteki, T., Maki, C., Koyasu, S., Mak, T. W. and Ohashi, P. S. (1999) Cutting edge: LFA-1 is required for liver NK1.1+TCR alpha beta+ cell development: evidence that liver NK1.1+TCR alpha beta+ cells originate from multiple pathways. *J. Immunol.* **162**, 3753-3756.
 22. Blomqvist, M., Rhost, S., Teneberg, S., Lofbom, L., Osterbye, T., Brigl, M., Mansson, J. E. and Cardell, S. L. (2009) Multiple tissue-specific isoforms of sulfatide activate CD1d-restricted type II NKT cells. *Eur. J. Immunol.* **39**, 1726-1735.
 23. Zhang, G., Nie, H., Yang, J., Ding, X., Huang, Y., Yu, H., Li, R., Yuan, Z. and Hu, S. (2011) Sulfatide-activated type II NKT cells prevent allergic airway inflammation by inhibiting type I NKT cell function in a mouse model of asthma. *Am. J. Physiol. Lung Cell Mol. Physiol.* **301**, L975-984.
 24. Halder, R. C., Aguilera, C., Maricic, I. and Kumar, V. (2007) Type II NKT cell-mediated anergy induction in type I NKT cells prevents inflammatory liver disease. *J. Clin. Invest.* **117**, 2302-2312.
 25. Ambrosino, E., Terabe, M., Halder, R. C., Peng, J., Takaku, S., Miyake, S., Yamamura, T., Kumar, V. and Berzofsky, J. A. (2007) Cross-regulation between type I and type II NKT cells in regulating tumor immunity: a new immunoregulatory axis. *J. Immunol.* **179**, 5126-5136.
 26. Lu, X., Song, L., Metelitsa, L. S. and Bittman, R. (2006) Synthesis and evaluation of an alpha-C-galactosylceramide analogue that induces Th1-biased responses in human natural killer T cells. *ChemBiochem* **7**, 1750-1756.
 27. Wipf, P. and Pierce, J. G. (2006) Expedient synthesis of the alpha-C-glycoside analogue of the immunostimulant galactosylceramide (KRN7000). *Org. Lett.* **8**, 3375-3378.
 28. Yang, G., Schmieg, J., Tsuji, M. and Franck, R. W. (2004) The C-glycoside analogue of the immunostimulant alpha-galactosylceramide (KRN7000): synthesis and striking enhancement of activity. *Angew. Chem. Int. Ed. Engl.* **43**, 3818-3822.
 29. Schmieg, J., Yang, G., Franck, R. W. and Tsuji, M. (2003) Superior protection against malaria and melanoma metastases by a C-glycoside analogue of the natural killer T cell ligand alpha-Galactosylceramide. *J. Exp. Med.* **198**, 1631-1641.
 30. Oki, S., Tomi, C., Yamamura, T. and Miyake, S. (2005) Preferential T(h)2 polarization by OCH is supported by incompetent NKT cell induction of CD40L and following production of inflammatory cytokines by bystander cells in vivo. *Int. Immunol.* **17**, 1619-1629.
 31. Yu, K. O., Im, J. S., Molano, A., Dutronc, Y., Illarionov, P. A., Forestier, C., Fujiwara, N., Arias, I., Miyake, S., Yamamura, T., Chang, Y. T., Besra, G. S. and Porcelli, S. A. (2005) Modulation of CD1d-restricted NKT cell responses by using N-acyl variants of alpha-galactosylceramides. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 3383-3388.
 32. Kinjo, Y., Wu, D., Kim, G., Xing, G. W., Poles, M. A., Ho, D. D., Tsuji, M., Kawahara, K., Wong, C. H. and Kronenberg, M. (2005) Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* **434**, 520-525.
 33. Mattner, J., Debord, K. L., Ismail, N., Goff, R. D., Cantu, C., 3rd, Zhou, D., Saint-Mezard, P., Wang, V., Gao, Y., Yin, N., Hoebe, K., Schneewind, O., Walker, D., Beutler, B., Teyton, L., Savage, P. B. and Bendelac, A. (2005) Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* **434**, 525-529.
 34. Kinjo, Y., Tupin, E., Wu, D., Fujio, M., Garcia-Navarro, R., Benhnia, M. R., Zajonc, D. M., Ben-Menachem, G., Ainge, G. D., Painter, G. F., Khurana, A., Hoebe, K., Behar, S. M., Beutler, B., Wilson, I. A., Tsuji, M., Sellati, T. J., Wong, C. H. and Kronenberg, M. (2006) Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat. Immunol.* **7**, 978-986.
 35. Amprey, J. L., Im, J. S., Turco, S. J., Murray, H. W., Illarionov, P. A., Besra, G. S., Porcelli, S. A. and Spath, G. F. (2004) A subset of liver NK T cells is activated during *Leishmania donovani* infection by CD1d-bound lipophosphoglycan. *J. Exp. Med.* **200**, 895-904.
 36. Bendelac, A. (1995) Positive selection of mouse NK1+ T cells by CD1-expressing cortical thymocytes. *J. Exp. Med.* **182**, 2091-2096.
 37. Bendelac, A. (1995) CD1: presenting unusual antigens to unusual T lymphocytes. *Science* **269**, 185-186.
 38. Exley, M., Garcia, J., Balk, S. P. and Porcelli, S. (1997) Requirements for CD1d recognition by human invariant Valpha24+ CD4-CD8- T cells. *J. Exp. Med.* **186**, 109-120.
 39. Bendelac, A., Killeen, N., Littman, D. R. and Schwartz, R. H. (1994) A subset of CD4+ thymocytes selected by MHC class I molecules. *Science* **263**, 1774-1778.
 40. Zhou, D., Mattner, J., Cantu, C., 3rd, Schrantz, N., Yin, N., Gao, Y., Sagiv, Y., Hudspeth, K., Wu, Y. P.,

- Yamashita, T., Teneberg, S., Wang, D., Proia, R. L., Lavery, S. B., Savage, P. B., Teyton, L. and Bendelac, A. (2004) Lysosomal glycosphingolipid recognition by NKT cells. *Science* **306**, 1786-1789.
41. Porubsky, S., Speak, A. O., Luckow, B., Cerundolo, V., Platt, F. M. and Grone, H. J. (2007) Normal development and function of invariant natural killer T cells in mice with isoglobotrihexosylceramide (iGb3) deficiency. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 5977-5982.
 42. Gumperz, J. E., Roy, C., Makowska, A., Lum, D., Sugita, M., Podrebarac, T., Koezuka, Y., Porcelli, S. A., Cardell, S., Brenner, M. B. and Behar, S. M. (2000) Murine CD1d-restricted T cell recognition of cellular lipids. *Immunity* **12**, 211-221.
 43. Facciotti, F., Ramanjaneyulu, G. S., Lepore, M., Sansano, S., Cavallari, M., Kistowska, M., Forss-Petter, S., Ni, G., Colone, A., Singhal, A., Berger, J., Xia, C., Mori, L. and De Libero, G. (2012) Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus. *Nat. Immunol.* **13**, 474-480.
 44. Shamshiev, A., Gober, H. J., Donda, A., Mazon, Z., Mori, L. and De Libero, G. (2002) Presentation of the same glycolipid by different CD1 molecules. *J. Exp. Med.* **195**, 1013-1021.
 45. Jahng, A., Maricic, I., Aguilera, C., Cardell, S., Halder, R. C. and Kumar, V. (2004) Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide. *J. Exp. Med.* **199**, 947-957.
 46. Arrenberg, P., Halder, R., Dai, Y., Maricic, I. and Kumar, V. (2010) Oligoclonality and innate-like features in the TCR repertoire of type II NKT cells reactive to a beta-linked self-glycolipid. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 10984-10989.
 47. Kang, S. J. and Cresswell, P. (2002) Calnexin, calreticulin, and ERp57 cooperate in disulfide bond formation in human CD1d heavy chain. *J. Biol. Chem.* **277**, 44838-44844.
 48. Hughes, E. A. and Cresswell, P. (1998) The thiol oxidoreductase ERp57 is a component of the MHC class I peptide-loading complex. *Current biology: CB* **8**, 709-712.
 49. Morrice, N. A. and Powis, S. J. (1998) A role for the thiol-dependent reductase ERp57 in the assembly of MHC class I molecules. *Current biology: CB* **8**, 713-716.
 50. Dougan, S. K., Salas, A., Rava, P., Agyemang, A., Kaser, A., Morrison, J., Khurana, A., Kronenberg, M., Johnson, C., Exley, M., Hussain, M. M. and Blumberg, R. S. (2005) Microsomal triglyceride transfer protein lipidation and control of CD1d on antigen-presenting cells. *J. Exp. Med.* **202**, 529-539.
 51. Brozovic, S., Nagaishi, T., Yoshida, M., Betz, S., Salas, A., Chen, D., Kaser, A., Glickman, J., Kuo, T., Little, A., Morrison, J., Corazza, N., Kim, J. Y., Colgan, S. P., Young, S. G., Exley, M. and Blumberg, R. S. (2004) CD1d function is regulated by microsomal triglyceride transfer protein. *Nat. Med.* **10**, 535-539.
 52. Cernadas, M., Sugita, M., van der Wel, N., Cao, X., Gumperz, J. E., Maltsev, S., Besra, G. S., Behar, S. M., Peters, P. J. and Brenner, M. B. (2003) Lysosomal localization of murine CD1d mediated by AP-3 is necessary for NK T cell development. *J. Immunol.* **171**, 4149-4155.
 53. Elewaut, D., Lawton, A. P., Nagarajan, N. A., Maverakis, E., Khurana, A., Honing, S., Benedict, C. A., Sercarz, E., Bakke, O., Kronenberg, M. and Prigozy, T. I. (2003) The adaptor protein AP-3 is required for CD1d-mediated antigen presentation of glycosphingolipids and development of Valpha14i NKT cells. *J. Exp. Med.* **198**, 1133-1146.
 54. Lawton, A. P., Prigozy, T. I., Brossay, L., Pei, B., Khurana, A., Martin, D., Zhu, T., Spate, K., Ozga, M., Honing, S., Bakke, O. and Kronenberg, M. (2005) The mouse CD1d cytoplasmic tail mediates CD1d trafficking and antigen presentation by adaptor protein 3-dependent and -independent mechanisms. *J. Immunol.* **174**, 3179-3186.
 55. Salio, M., Ghadbane, H., Dushek, O., Shepherd, D., Cypen, J., Gileadi, U., Aichinger, M. C., Napolitani, G., Qi, X., van der Merwe, P. A., Wojno, J., Veerapen, N., Cox, L. R., Besra, G. S., Yuan, W., Cresswell, P. and Cerundolo, V. (2013) Saposins modulate human invariant Natural Killer T cells self-reactivity and facilitate lipid exchange with CD1d molecules during antigen presentation. *Proc. Natl. Acad. Sci. U. S. A.* **110**, E4753-4761.
 56. Kang, S. J. and Cresswell, P. (2004) Saposins facilitate CD1d-restricted presentation of an exogenous lipid antigen to T cells. *Nat. Immunol.* **5**, 175-181.
 57. Yuan, W., Qi, X., Tsang, P., Kang, S. J., Illarionov, P. A., Besra, G. S., Gumperz, J. and Cresswell, P. (2007) Saposin B is the dominant saposin that facilitates lipid binding to human CD1d molecules. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 5551-5556.
 58. Zhou, D., Cantu, C., 3rd, Sagiv, Y., Schrantz, N., Kulkarni, A. B., Qi, X., Mahuran, D. J., Morales, C. R., Grabowski, G. A., Benlagha, K., Savage, P., Bendelac, A. and Teyton, L. (2004) Editing of CD1d-bound lipid antigens by endosomal lipid transfer proteins. *Science* **303**, 523-527.
 59. Sugita, M., Cao, X., Watts, G. F., Rogers, R. A., Bonifacino, J. S. and Brenner, M. B. (2002) Failure of trafficking and antigen presentation by CD1 in AP-3-deficient cells. *Immunity* **16**, 697-706.
 60. Jayawardena-Wolf, J., Benlagha, K., Chiu, Y. H., Mehr, R. and Bendelac, A. (2001) CD1d endosomal trafficking is independently regulated by an intrinsic CD1d-encoded tyrosine motif and by the invariant chain. *Immunity* **15**, 897-908.
 61. Chiu, Y. H., Jayawardena, J., Weiss, A., Lee, D., Park, S. H., Dautry-Varsat, A. and Bendelac, A. (1999) Distinct subsets of CD1d-restricted T cells recognize self-antigens loaded in different cellular compartments. *J. Exp. Med.* **189**, 103-110.
 62. Chiu, Y. H., Park, S. H., Benlagha, K., Forestier, C., Jayawardena-Wolf, J., Savage, P. B., Teyton, L. and Bendelac, A. (2002) Multiple defects in antigen presentation and T cell development by mice expressing cytoplasmic tail-truncated CD1d. *Nat. Immunol.* **3**, 55-60.
 63. Prigozy, T. I., Naidenko, O., Qasba, P., Elewaut, D., Brossay, L., Khurana, A., Natori, T., Koezuka, Y., Kulkarni, A. and Kronenberg, M. (2001) Glycolipid antigen processing for presentation by CD1d molecules. *Science* **291**, 664-667.
 64. Bai, L., Sagiv, Y., Liu, Y., Freigang, S., Yu, K. O., Teyton, L., Porcelli, S. A., Savage, P. B. and Bendelac, A. (2009) Lysosomal recycling terminates CD1d-mediated presentation of short and polyunsaturated variants of the NKT

- cell lipid antigen alphaGalCer. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 10254-10259.
65. Im, J. S., Arora, P., Bricard, G., Molano, A., Venkataswamy, M. M., Baine, I., Jerud, E. S., Goldberg, M. F., Baena, A., Yu, K. O., Ndonye, R. M., Howell, A. R., Yuan, W., Cresswell, P., Chang, Y. T., Illarionov, P. A., Besra, G. S. and Porcelli, S. A. (2009) Kinetics and cellular site of glycolipid loading control the outcome of natural killer T cell activation. *Immunity* **30**, 888-898.
66. Shin, J. H., Park, J. Y., Shin, Y. H., Lee, H., Park, Y. K., Jung, S. and Park, S. H. (2012) Mutation of a positively charged cytoplasmic motif within CD1d results in multiple defects in antigen presentation to NKT cells. *J. Immunol.* **188**, 2235-2243.
67. Kang, S. J. and Cresswell, P. (2002) Regulation of intracellular trafficking of human CD1d by association with MHC class II molecules. *EMBO J.* **21**, 1650-1660.
68. Hong, S., Wilson, M. T., Serizawa, I., Wu, L., Singh, N., Naidenko, O. V., Miura, T., Haba, T., Scherer, D. C., Wei, J., Kronenberg, M., Koezuka, Y. and Van Kaer, L. (2001) The natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat. Med.* **7**, 1052-1056.
69. Kim, H. Y., Kim, H. J., Min, H. S., Kim, S., Park, W. S., Park, S. H. and Chung, D. H. (2005) NKT cells promote antibody-induced joint inflammation by suppressing transforming growth factor beta1 production. *J. Exp. Med.* **201**, 41-47.
70. Jung, S., Shin, H. S., Hong, C., Lee, H., Park, Y. K., Shin, J. H., Hong, S., Lee, G. R. and Park, S. H. (2009) Natural killer T cells promote collagen-induced arthritis in DBA/1 mice. *Biochem. Biophys. Res. Commun.* **390**, 399-403.
71. Hong, C. and Park, S. H. (2007) Application of natural killer T cells in antitumor immunotherapy. *Crit. Rev. Immunol.* **27**, 511-525.