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Review

Antigen presentation by CD1 molecules and the generation of lipid-specific T cell immunity

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Abstract. It is now well demonstrated that the repertoire of T cells includes not only cells that recognize specific MHC-presented peptide antigens, but also cells that recognize specific self and foreign lipid antigens. This T cell recognition of lipid antigens is mediated by a family of conserved MHC class I-like cell surface glycoproteins known as CD1 molecules. These are specialized antigen-presenting molecules that directly bind a wide variety of lipids and present them for T cell recognition at the surface of antigen-presenting cells. Distinct populations of T cells exist that recognize CD1-presented lipids of microbial, environmental or self origin, and these T cells participate in immune responses associated with infectious, neoplastic, autoimmune and allergic diseases. Here we review the current knowledge of the biology of the CD1 system, including the structure, biosynthesis and trafficking of CD1 molecules, the structures of defined lipid antigens and the types of functional responses mediated by T cells specific for CD1-presented lipids.

Keywords. CD1, lipid, antigen presentation, glycolipid, galactosylceramide, phospholipid, T cell, adaptive immunity.

Introduction

The immune system generates a large and diverse population of T cells bearing variable T cell antigen receptors (TCRs), through somatic rearrangement and thymic selection. This provides a huge T cell repertoire which allows recognition of many distinct antigens and enables specific adaptive immune responses to be mounted against a wide variety of pathogens. It is well known that most T cells recognize antigenic peptides presented by the highly polymorphic class I and class II MHC (major histocompatibility complex) molecules. More recently, it has also become well established that a significant subset of T cells is reactive not to peptides presented by MHC molecules, but rather to lipids and glycolipids presented by a related family of antigen-presenting molecules called CD1. The CD1 molecules are MHC class I -like glycoproteins with a limited polymorphism that are specialized for the binding and presentation of lipids and glycolipids to T cells. Several distinct but closely related forms of these molecules exist in most mammals, and despite their minimal allelic polymorphism, they have the capacity to bind and present a wide range of different lipid structures derived from a variety of pathogens and even from host tissues. As for MHC presentation

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Table 1. Number and diversity of CD1 genes in various species.

Species	Group 1 *CD1A*	*CD1B*	*CD1C*	Group 2 *CD1D*	Group 3 *CD1E*	Other	References
Human	1	1	1	1	1	0	[1, 4–6, 13]
Mouse	0	0	0	2	0	0	[13,19]
Rat	0	0	0	1	0	0	[14,15,19]
Guinea pig	ND	≥4	≥3	ND	1	ND	[8,11]
Rabbit	2	1	ND	1	1	ND	[7,10,13]
Sheep	ND	≥3	ND	1	1	ND	[9, 12, 16]
Cattle	1	≥4	ND	2Ψ	1	ND	[17]
Pig	1 + 1Ψ	1	1Ψ	1	1	ND	[18]
Chicken	0	0	0	0	0	2	[20–22]

ND, not detected in studies reported to date (possibly absent).Ψ, pseudogene.

pathways which use a variety of cellular components for protein degradation and peptide generation, the CD1 molecules are also dependent on specialized cellular pathways that use accessory proteins involved in lipid and glycolipid metabolism such as lipid transfer proteins and lysosomal glycosidases.

This well-conserved CD1 system allows potential surveillance of both self and non-self lipid of either intra- or extracellular origin. Rapid progress in this area has been achieved recently through a wide variety of studies in both humans and experimental animal models. These include highly detailed crystallographic studies of the structures of CD1/lipid complexes, and detailed analyses of the enlarging repertoire of microbial, self and environmental lipid and glycolipid antigens found to be presented by CD1 molecules to T cells. Here we review the current understanding of how CD1 molecules function in the presentation of lipid and glycolipid antigens, and the functional significance of this novel system for antigen recognition in host immunity.

Genetics and evolutionary conservation of CD1 molecules

Five CD1 genes have been identified in humans, which map to a single locus on chromosome 1 that is unlinked to the MHC locus on chromosome 6 (Table 1) [1]. These genes encode a family of distinct CD1 proteins that are referred to as CD1 isoforms. According to sequence homology, patterns of expression and functional attributes, CD1 isoforms are classified into at least two groups. These are referred to as group 1 CD1 molecules (CD1a, CD1b and CD1c in humans) and group 2 CD1 molecules (comprising only CD1d in humans) [2]. A fifth human CD1 molecule known as CD1e is not clearly classified yet as a member of one of these two groups, and it has some unique features which suggest that it may actually be more appropriately classified as a separate third group. For example, unlike other CD1 molecules, human CD1e was found to be expressed only intracellularly in human dendritic cells (DCs) [3], and does not appear to perform antigen presentation *per se*, although it is involved in antigen processing (discussed below).

Human CD1 genes do not show the extensive allelic variation that is characteristic of MHC genes and are often referred to as nonpolymorphic. However, some minor allelic variants of group 1 and group 2 CD1 genes have been found in population studies [4], and six *CD1E* alleles have also been identified [5,6]. Multiple CD1 isoforms are generally found in other mammalian species (Table 1) [7–18]. Muroid rodents (mouse and rat) do not have group 1 and group 3 CD1 genes, and genomic analysis suggests that these genes were lost during an ancient chromosomal translocation event in an immediate ancestor of these species [19]. In contrast, the bovine genome contains multiple genes encoding group 1 CD1 molecules and CD1e, but lacks any functional group 2 CD1 genes [17].

Two CD1 genes were very recently reported in chicken, and were mapped to a chromosomal location that is closely linked with the avian MHC locus. This suggests that CD1 genes are evolutionarily ancient and likely arose following the duplication of genes in the ancestral MHC locus [20–22]. It is not yet clear whether CD1 genes exist in more primitive species than birds, and further studies will be needed to determine whether lower vertebrates such as fish, amphibians and reptiles also possess a CD1 antigen-presenting system. Nevertheless, the finding that the CD1 system predates the evolution of mammals, and has been conserved through a very long evolutionary distance, indicates that they perform an important function in the immune systems of present-day mammals and possibly other vertebrates.

CD1 molecule biogenesis and trafficking

Biogenesis of CD1 molecules

CD1 molecules have a structure very comparable to MHC class I molecules, as they are formed by the noncovalent association of a CD1 transmembrane α chain with β2-microglobulin (β2-m) [23]. Following their synthesis in the rough endoplasmic reticulum (ER), CD1 proteins undergo a maturation process similar to that of MHC class I molecules. Newly translocated CD1 α chains are glycosylated by ER-resident glycosidases, and their correct folding is assisted by interaction with chaperone proteins such as calnexin, calcireticulin and ERp57 (Fig. 1) [24–26]. This is followed by association of β2-m with the CD1 α chain, which contributes to stabilization of the complex. The association of human CD1b α chain with β2-m appears to be absolutely required for subsequent maturation and trafficking to the cell surface [24]. In the case of human CD1d, β2-m association also occurs in the ER and is dependent on or stabilized by the glycosylation of a particular asparagine (N)-linked glycosylation site on CD1d [27]. However, it has also been found that a β2-m free form of CD1d, with immature N-linked glycans, can leave the ER and traffic to the cell surface [28]. Similarly, a β2-m free form of mouse CD1d has been also reported and was able to activate CD1d-restricted T cells [29].

All CD1 molecules have a relatively large and complex hydrophobic ligand binding site (often referred to as an antigen-binding groove, by analogy with the peptide-binding grooves of MHC class I and II molecules). These hydrophobic sites eventually become occupied by the specific self or foreign lipids that are presented by CD1 molecules on the cell surface, although these are often acquired only after the molecules have trafficked to the cell surface or endocytic compartments. In the ER, newly synthesized CD1 molecules have been found to be loaded with endogenous cellular lipids that might act as chaperons to stabilize their antigen-binding grooves and prevent them from collapsing. Some of the potential endogenous lipid ligands that associate with newly synthesized CD1 molecules in the ER have been identified in biochemical studies (Fig. 2). These include glycosylphosphatidylinositol (GPI) [30,31] or phosphatidylinositol (PI) [30–32], which associate with human CD1b, and with human or mouse CD1d. Other studies of CD1 molecules secreted from cells revealed that mouse CD1d and human CD1b were loaded with phosphatidylcholine (PC) together with a hydrophobic spacer [33,34]. The spacer was identified as a palmitic acid for mouse CD1d [34], and an incompletely identified aliphatic lipid of 41–44 carbons length for human CD1b [33]. The loading of lipids in the ER has been proposed to require activity of lipid transfer proteins that can interact with ER resident lipids such as PI, GPI and PC [35]. The microsomal triglyceride transfer protein (MTP) localizes in the ER, associates with mouse CD1d and can transfer phosphatidylethanolamine (PE, Fig. 2) into mouse CD1d *in vitro* [36]. In addition, abrogation of MTP expression in mouse and human antigen-presenting cells renders them less efficient for CD1d-mediated presentation to CD1d-restricted T cells [36,37].

Trafficking of CD1 molecules

After their initial synthesis and assembly in the ER, the CD1 molecules migrate through the Golgi network and, with the exception of CD1e, reach the cell surface. They can be re-internalized by entering the endocytic pathway, but here the trafficking of the various CD1 isoforms diverges, depending chiefly on the particular amino acid sequences of their cytoplasmic tails (Fig. 1) [38]. For example, human CD1a has a short cytoplasmic tail and can be internalized in sorting endosomes, then transit to recycling endosomes where it is transiently retained, at least in Langerhans cells [39]. This molecule appears to recycle between these endosomal compartments and the cell surface, where it is stabilized upon binding to exogenous glycolipids [40]. In contrast, the cytoplasmic tails of human CD1b, CD1c and human or mouse CD1d contain tyrosine-based motifs of the general sequence YXXZ (Y is tyrosine, X any amino acid and Z a bulky hydrophobic amino acid), which associate with AP-2 adaptor protein complexes and direct internalization to a variety of endosomal compartments via clathrin-coated pits [41–43]. The YXXZ motifs of mouse CD1d and human CD1b tails also interact strongly with AP-3 adaptor protein complexes, which further directs these molecules deeper into the endocytic network to lysosomes and multivesicular or multi-laminar bodies known as MHC class II compartments (MIIC) [42–46]. An alternative route allows mouse and human CD1d to reach the MIIC compartment by association with the invariant chain (Ii) or with Ii/MHC class II complexes [36,47,48]. Human CD1e appears to have a unique trafficking pattern, which leads to its accumulation in the Golgi network of immature DCs [3,49]. Upon DC maturation, CD1e migrates via sorting endosomes to late endosomes and lysosomes, where it is cleaved into a stable soluble form (Fig. 1) [3].

The distinct trafficking patterns of the various CD1 isoforms results in different subcellular localization patterns. Thus, at steady state CD1a is found mainly at the cell surface and in sorting endosomes, whereas CD1c and human CD1d molecules accumulate

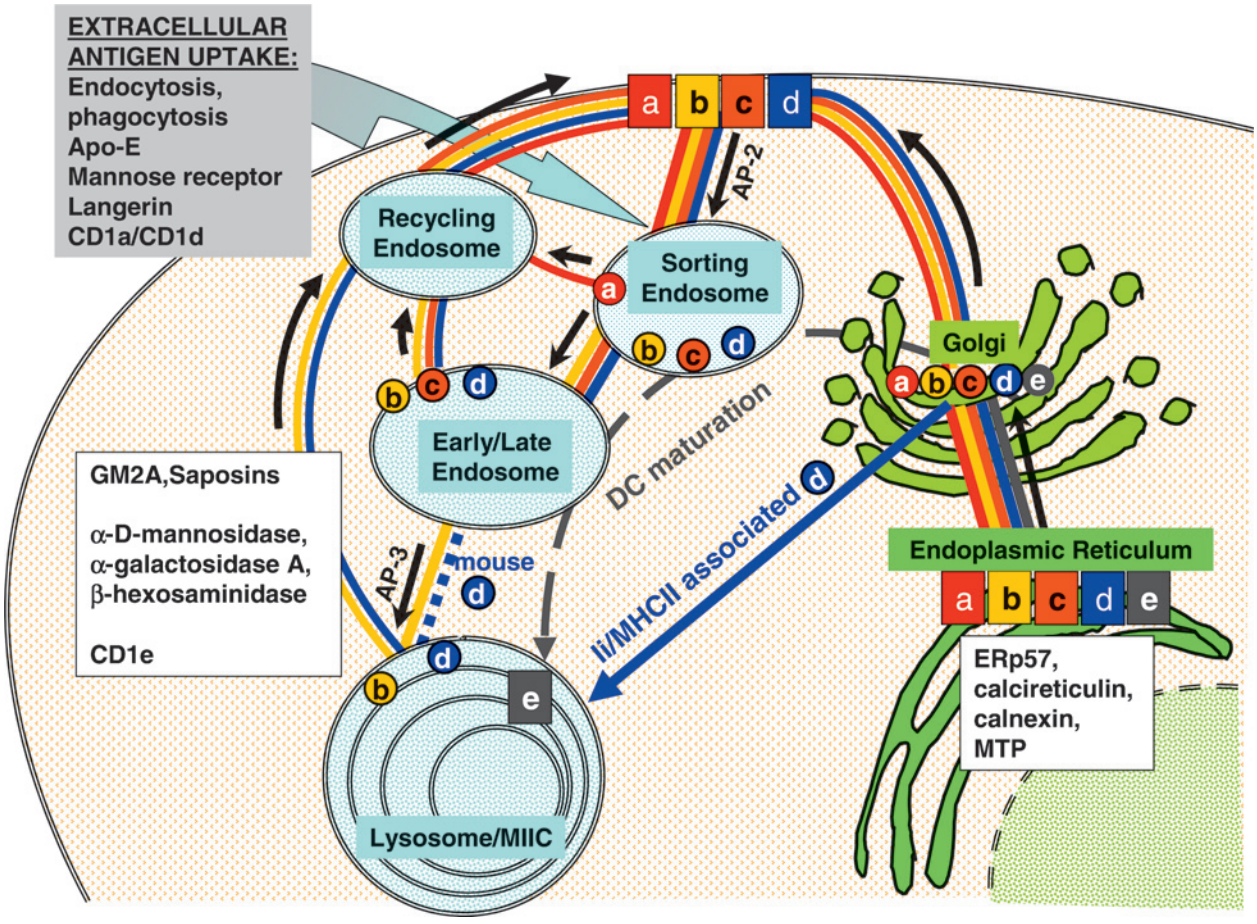

Figure 1. Intracellular acquisition of lipids and trafficking of CD1 molecules for cell surface presentation. The trafficking of the various human and mouse CD1 isoforms is depicted with colored symbols and lines, with CD1a in red, CD1b in yellow, CD1c in orange, CD1d in blue and CD1e in dark gray. The CD1 molecules are indicated in the ER or at the cell surface by colored squares containing the letter of the relevant CD1 isoform, or by similarly colored circles at sites of transient localization. Black arrows indicate the predominant direction of CD1 molecule movement along each pathway. Factors involved in CD1 assembly and in intracellular antigen processing or loading of antigens onto CD1 molecules are depicted in white boxes (right box: ER-associated factors, left box: endo-/lysosomal-associated factors). Factors involved in antigen capture and uptake are shown in the gray box. CD1 molecules are folded and assembled in the ER with the assistance of chaperone proteins and MTP, before they traffic to the cell surface through the Golgi network. CD1e is an exception as it accumulates in the Golgi and is routed to lysosomes via sorting and late endosomes, upon dendritic cell maturation. Other CD1 isoforms are re-internalized in endosomal pathways, depending on their cytoplasmic tail motifs, and are re-expressed at cell surface for presentation. CD1a has a short tail and is directly routed back to cell surface via recycling endosomes. CD1b, CD1c and mouse/human CD1d recirculate to endosomes in a process involving AP-2 binding. CD1b and mouse CD1d can traffic deeper as a result of stronger associations of their cytoplasmic tails with AP-3, which results in accumulation in lysosomal/MHC class II (MIIC) compartments. An alternative route has also been described for CD1d, which involves trafficking directly from the Golgi to lysosomes as a result of physical interaction with the invariant Ii or with Ii/MHC class II complexes. Extracellular antigens are acquired by endocytosis and phagocytosis, which may involve specific uptake receptors such as the macrophage mannose receptor, langerin and the Apo-E receptor. CD1a and human CD1d are suggested to mediate cell surface binding of pollen grains, facilitating their capture and internalization. Antigens directed to the endosomal route can then be processed by glycosidases resident in endosomes and lysosomes, with the possible assistance of CD1e. At the same locations, loading and exchange of lipids onto CD1 proteins are assisted by resident lipid transfer proteins GM2-A and saposins.

strongly in early/late endosomes, and CD1b and mouse CD1d are particularly prominent in lysosomes and MIIC compartments. This variation in trafficking patterns and subcellular localization of different CD1 isoforms suggests a significant degree of functional specialization. In humans and other species that possess multiple different CD1 isoforms, this may be an important adaptation that allows the CD1 system to efficiently carry out surveillance of exogenous and endogenous lipids that accumulate or originate in different intracellular compartments.

Antigen acquisition and processing for CD1 presentation

Given that CD1 molecules are prominently localized to endocytic compartments through their specialized

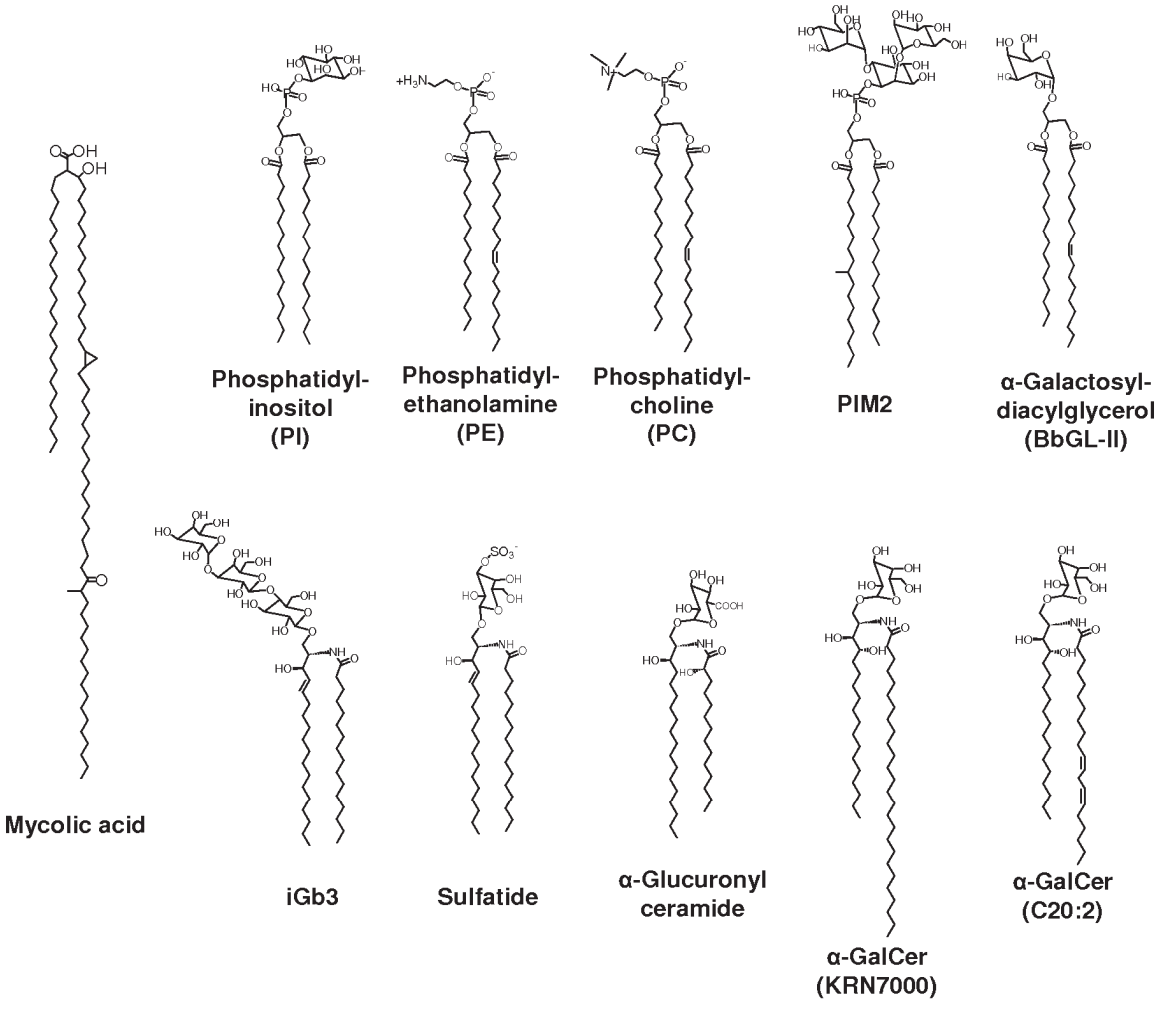

Figure 2. Structure of representative lipid ligands of CD1 molecules. The structure of some CD1 ligands is presented, including examples of glycerolipids (top panel), sphingolipids (bottom panel) and mycolic acid (left). PI, PE, PC, iGb3 and sulfatide are self-derived lipids, whereas αGalCer compounds are synthetic. Mycolic acid and PIM2 are derived from mycobacteria. Note that PIM4, PIM6 or lipoarabinomannan (LAM) (not illustrated) are structurally related to PIM2, but have larger and more complex carbohydrate moieties. Glucose monomycolate (GMM) corresponds to mycolic acid esterified to the 6-position of a single glucosyl residue. The α-glucuronylceramide and α-galactosyldiacylglycerol (BbGL-IIc) are derived from specific bacterial pathogens (*Sphingomonas* or related species, and *Borrelia burgdorferi*, respectively).

trafficking properties, it is apparent that they have the potential to capture and present exogenous lipid antigens that are taken up by antigen-presenting cells (APCs) (Fig. 1). This property of CD1 molecules is strongly analogous to MHC class II molecules, and involves a variety of antigen uptake and processing steps that also is reminiscent of previous studies on the MHC class II pathway for presentation of exogenous peptide antigens.

Antigen uptake or capture

Endocytosis is the most obvious way of capturing exogenous lipid antigens for presentation by CD1 molecules. The phagocytosis of apoptotic bodies was found to facilitate the cross-presentation of *Mycobacterium tuberculosis* (Mtb)-infected cells by non-infected bystander DC, and this included the presentation of specific lipid antigens by CD1 molecules [50]. Apolipoprotein E (Apo-E), which is secreted by APCs, was found to mediate uptake and facilitate CD1-dependent presentation of Mtb-derived lipids or glycolipids dissolved in serum [51]. Lipids associated

with Apo-E were actively engulfed by APC as a result of interactions with lipoprotein receptors, and this led to their efficient delivery to endosomal compartments. Receptor-mediated capture of exogenous glycolipid antigen for presentation by CD1 is also achieved by the macrophage mannose receptor [52]. It can deliver lipoarabinomannan (LAM), a large and complex glycolipid produced by mycobacterial pathogens, to endocytic compartments and facilitate its subsequent presentation by CD1 to specific T cells. It was also suggested that langerin (CD207), expressed by Langerhans cells, is involved in capturing antigens derived from *Mycobacterium leprae* and facilitating their presentation by CD1a [53]. Surface CD1 molecules themselves might also be involved in antigen capture, as the specific blockade of CD1a and CD1d inhibits pollen grain adhesion to DC [54].

Processing of glycolipid antigens and loading onto CD1 molecules

Endosomal/lysosomal glycosidases have been found to be involved in the trimming of glycans to modify glycolipid antigens (Fig. 1). For example, treatment of the mycobacterial glycolipid lipomannan with purified α-D-mannosidase was shown to decrease the ability of this antigen to be subsequently recognized by CD1b-restricted T cells [55]. This suggests that mannosylated glycolipids might undergo intracellular enzymatic processing prior to presentation by CD1 molecules, although such a processing step has not yet been demonstrated to occur within APCs. Such processing was actually also shown in experiments using model synthetic glycolipid antigens, in which α-digalactosylceramide [Gal(α1→2)αGalCer] was shown to be degraded by lysosomal α-galactosidase A activity into a monogalactosylated form (αGalCer, Fig. 2) that is effectively presented to a major subset of CD1d-restricted T cells [56].

Similarly, the lysosomal hexosaminidase complex has been reported to be required for the degradation of isoglobotetrahexosylceramide (iGb4) into isoglobotrihexosylceramide (iGb3, Fig. 2), which can then be recognized as a natural self glycolipid ligand by mouse CD1d-restricted T cells [57]. This processing step may be critically involved in the thymic selection of a major subset of CD1d-restricted T cells, as mice deficient for the β-subunit of hexosaminidase B do not develop these T cells [57]. However, a recent study has shown that multiple other enzymatic deficiencies that lead to accumulation of lysosomal glycosphingolipids are also associated with severe impairment of CD1d-restricted T cell development and function, thus casting doubt on whether the effect of hexosaminidase deficiency is directly due to its impact on iGb3 synthesis [58]. Human CD1d-restricted T cells have been found to be reactive in vitro to exogenously added iGb3 [57], but it is not determined yet whether iGb3 can be syntheszied by human APCs or whether it is involved in thymic selection in humans. There is also some experimental evidence that lipophosphoglycan (LPG), a large glycolipid antigen produced by the protozoal parasite *Leishmania donovani*, requires an intracellular processing step to be recognized by a subset of CD1d-restricted T cells in mice [59].

CD1e was found to be required for trimming the mycobacterial glycolipid phosphatidylinositol hexamannoside (PIM6) into phosphatidylinositol dimannoside (PIM2, see Fig. 2) in order for this glycolipid to be successfully recognized by a human CD1b-restricted T cell line [60]. This raises the possibility that CD1e functions primarily by presenting lipids to lysosomal glycosidases, rather than by presenting lipid antigens directly to T cells, which would be consistent with the finding that CD1e is not expressed at the cell surface. Subsequently, lysosomal lipid transfer proteins such as saposins (discussed below) might favor the transfer of processed antigens from CD1e to other CD1 proteins that are ultimately destined for transport to the cell surface for T cell recognition.

Influence of pH

CD1a, CD1c and CD1d can load short lipids at neutral pH, but their loading can be enhanced at acidic pH [61]. In contrast, CD1b absolutely requires endosomal acidification to bind lipids with very long aliphatic chains such as the mycolic acids and mycolate-containing glycolipids of mycobacteria [39]. Low pH seems to influence the conformation of CD1b, and converts it into a state that is more permissive for the loading of lipids [61]. However, it has been observed that CD1b can still bind somewhat less large and hydrophobic glycolipids, such as gangliosides at neutral pH [62].

Lipid transfer proteins

Several different intracellular lipid transfer proteins assist the loading of CD1 proteins with their lipid ligands. As mentioned above, MTP is involved in lipid loading of nascent CD1 proteins in the ER [36,37]. The GM2 activator protein (GM2A) and saposins A–D are present in late endosomes and lysosomes, where they can load endogenous and exogenous lipids onto CD1d or CD1b proteins (Fig. 1) [63–65]. Recombinant GM2A and saposins are able to exchange lipids bound to mouse CD1d [65], and saposins are necessary for efficient intracellular generation of αGalCer/CD1d complexes by human APCs [63], although in some experimental systems it appears that αGalCer can also be directly loaded onto CD1d at the cell surface. Only saposin C was found to physi-

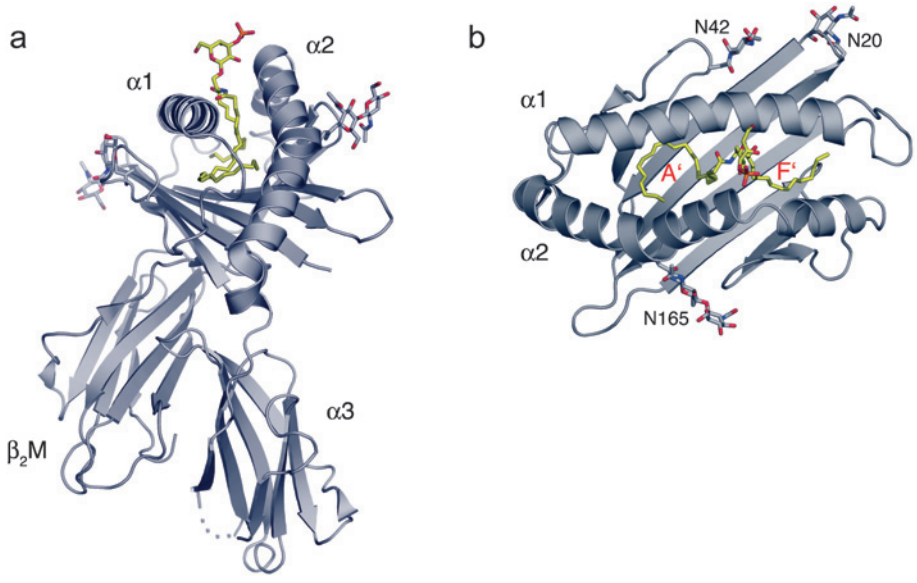

Figure 3. Structure of mouse CD1d with bound sulfatide. Schematic representation of the mouse CD1d/β2-microglobulin complex, shown in ribbon representation, with a bound sulfatide glycolipid as a stick representation. Atoms are depicted as follow: carbon, grey/yellow; oxygen, red; nitrogen, blue; sulfur, orange. (*a*) Side view of the complex showing the solvent-exposed sulfated galactose moiety protruding out of the mCD1d surface and β2-microglobulin noncovalently associated with the CD1d α3 domain. (*b*) Top view showing the α1 and α2 domains organized in two α-helices overlying the β-sheet floor to form the A' and F' lipid binding pockets. The sulfatide fatty acid is bound in the A' pocket, and the sphingosine backbone is bound in the F' pocket. N-linked glycosylations located on N20, N42 and N165 are included based on modeling. This figure is reproduced with permission from Zajonc *et al.*, *Journal of Experimental Medicine* 2005, 202: 1517–1526 Copyright 2005 The Rockefeller University Press [72].

cally interact with CD1b and mediate the loading of the mycobacterial lipid antigens LAM, mycolic acid and glucose monomycolate (GMM) [64].

CD1 protein structure and the molecular basis for lipid binding

Three-dimensional structures of CD1 molecules show a striking overall similarity in shape and domain organization to classical MHC class I proteins, including a similar noncovalent association of the β2-m subunit with the membrane proximal α3 domain of the larger transmembrane α chain (Fig. 3) [66]. As in MHC class I proteins, this association supports the α1 and α2 domains, which are folded in two α-helices positioned over a β-sheet floor, which together form the ligand binding and TCR contact area of the structure. However, the structure and chemical properties of the ligand binding sites of CD1 molecules is radically different from the peptide binding sites found in MHC-encoded antigen-presenting molecules. In all CD1 molecules, the membrane distal α1 and α2 domains contain a large and complex hydrophobic ligand binding site with a narrow portal entry. The structural basis for the binding of lipid antigens in the hydrophobic ligand binding site has been investigated in great detail through a series of elegant crystallographic studies of various CD1 proteins in complex with specific bound ligands. To date, human CD1a has been crystallized with its ligand binding site occupied by sulfatide [67] or with the synthetic lipopeptide didehydroxymycobactin [68]. The human CD1b structure has been investigated with bound PI or ganglioside GM2 [69], PC [33] and GMM [70]. The mouse CD1d structure has been reported in a form loaded with detergent [71] or loaded with sulfatide (Fig. 3 and [72]), PC [34], a variant of αGalCer with a short acyl chain [73], α-galacturonosylceramide [74] or PIM2 [75]. The human CD1d structure has been also solved empty or loaded with αGalCer [76].

The ligand binding sites of CD1a, as well as human and mouse CD1d, contain two pockets designated A' and F' that can accommodate aliphatic lipid tails. CD1b contains an additional pocket (C') and an inner tunnel (T') which connects the distal ends of the A' and F' pockets together. This connection makes a long A'T'F' 'super-channel' that can bind impressively long alkyl chains, such as those present in mycobacterial mycolic acids (Fig. 2) [66]. CD1c and CD1e have not been crystallized yet, but the presence of conserved amino acid residues predicts that they have an overall structure comparable to CD1a or CD1d, with two lipid binding pockets in their hydrophobic ligand binding sites [66].

Another common feature between CD1 molecules is their ability to bind the aliphatic tails of lipids and glycolipids in an orientation that allows the polar head groups of these ligands to remain solvent exposed at the α-helical surface of the CD1 proteins, where they are accessible for direct contact with residues of cognate TCRs. Important amino acid residues of CD1 α-helices, localized at the portal leading into the hydrophobic ligand binding site, can interact with the polar head groups of glycolipids. Interactions occur especially through hydrogen bonding to stabilize the binding and conformation of the glycolipid when bound to CD1 into a complex that is recognized by specific T cells [33,34,66,73–76].

Interestingly, recent reports revealed that CD1 molecules can be physiologically loaded with a glycolipid together with spacer lipids, in a manner that fully occupies the antigen binding pockets [33,34,74]. This was previously observed in CD1b structures, which were solved using recombinant CD1b folded with PI or GM2, and contained a detergent molecule that filled the portions of the hydrophobic ligand binding site that was not occupied by the lipid or glycolipid antigen [69]. The spacer lipids correspond to aliphatic chains and have been identified as a C14 or C16 fatty acid [73,74], and an unknown lipid of 41–44 carbons in length [33].

CD1-expressing cells and regulation of CD1 expression

Human group 1 and group 2 CD1 proteins are prominently expressed by cortical thymocytes and by cells of the myeloid lineage, especially DC [77]. A subset of B cells was also found to express CD1c [78,79]. Human and mouse CD1d have a broader distribution and are expressed at detectable levels on most cells of hematopoietic origin, with the highest levels usually observed on leukocytes such as dendritic cells, B cells or monocytes [80,81]. Human CD1d expression can also be identified in non-professional APC such as intestinal and bile duct epithelial cells [82,83], keratinocytes [84], hair follicles [85] and peripheral nerve Schwann cells [86]. In mouse, CD1d expression has been documented on hepatocytes [87]. Expression in additional cell types has been suggested by an immunohistochemical survey of multiple tissues using anti-mouse CD1d antibodies that are cross-reactive to human CD1d [88], but the validity of these other potential sites of CD1d expression has not yet been confirmed.

The level of CD1 protein expression can be modulated in certain circumstances. For example, group 1 CD1 proteins are upregulated by Gramulocyte-macrophage colony-stimulating factor (GM-CSF) and during differentiation of monocytes to DC [77], and CD1d expression on DCs is increased by inflammatory cytokines such as interferon (IFN)β [89] and IFNγ [90], or by Toll-like receptor ligands [91,92]. Activation of peroxisome proliferator activator receptor γ (PPARγ) was found to be involved in upregulation of CD1d during DC maturation [93,94]. CD1d expression can be also downmodulated by immunoregulatory cytokines such as interleukin (IL)-10 [95] and transforming growth factor (TGFβ) [96]. In addition, infectious agents, including human immunodeficiency virus (HIV) [97,98], herpes simplex virus [99] and *Leishmania donovani* [100], can downregulate CD1d expression of infected cells.

CD1-restricted T cell subsets and their cognate lipid antigens

Lipid reactive CD1-restricted T cells have been found to be heterogenous in terms of phenotype, and include T cell subsets expressing an αβ or γδ TCR, and they can have either a $CD4^+$, $CD8^+$ or $CD4^-CD8^-$ [i.e., "double negative" (DN)] phenotype. The TCRs of group 1 CD1-restricted T cells are highly diverse, whereas many mouse and human group 2 CD1-restricted T cells are found to have TCRs with an invariant TCR α-chain and much less diversity than the TCRs of MHC-restricted T cells (see below). Many CD1-restricted T cells have been shown to possess weak or moderate reactivity against normal cells, even from a syngeneic source, suggesting that these T cells have a relatively high level of inherent autoreactivity. The self and foreign lipid ligands recognized by various CD1-restricted T cell subsets have been partially defined in many cases, and are summarized in the sections that follow.

Type I CD1d-restricted NKT cells

Type I NKT cells (also referred to as Vα14 invariant NKT cells in mice, and Vα24 invariant NKT cells in humans) are unusual αβ T cells that express a semi-invariant TCR and potently recognize α-galactosylceramide (αGalCer, Fig. 2) presented by CD1d [101,102]. These cells express an invariant TCRα chain, which is formed by precise rearrangement of the germline sequences of Vα24 with Jα18 in humans, or Vα14 with Jα18 in mice. These invariant TCRα chains pair with TCRβ chains that also have somewhat restricted diversity, generally encoded by rearrangement of the Vβ11 gene segment in humans, or Vβ8, Vβ7 or Vβ2 in mice, with a variety of Jβ segments and substantial diversity in the joining region. Some human αGalCer/CD1d specific T cells, lacking the

Vα24 TCR segment, have been also described [103]. Type I NKT cells uniformly show an effector/memory phenotype and have innate-like proinflammatory and immunoregulatory properties [104]. Upon antigen recognition they can rapidly produce Th1 [IFNγ, tumor necrosis factor α (TNFα)] and Th2 (IL-4, IL-10, IL-13 and IL-5) cytokines, transactivate other effector lymphocytes (T, B and NK cells) and trigger DC maturation. Anti-tumor activities of type I NKT cells have been described in mice *in vivo,* especially after specific activation with αGalCer [105]. The identity of natural lipids presented to and recognized by type I NKT cells is not completely known, but an unexpected variety of synthetic and natural lipids that can potentially activate this subset of CD1d-restricted T cells has been identified recently.

Synthetic type I NKT cell antigens

α-Galactosylceramide (αGalCer, Fig. 2) remained for long time the model antigen to study the reactivity of type I NKT cells. It is a synthetic glycolipid, and is fundamentally different in structure from the glycosphingolipids of mammals that generally contain only β-anomeric linkages of the proximal sugar to the ceramide. While not a physiologically relevant antigen, synthetic αGalCer is a strong agonist of type I NKT cells and has been proposed as a possible therapeutic immunomodulator for treatment of autoimmune diseases or cancer [106]. In addition, recombinant CD1d tetramers complexes loaded with αGalCer bind with high sensitivity and specificity to virtually all type I NKT cells, which has enabled the production of extremely useful reagents for the identification and isolation of these cells [102].

New αGalCer analogs are the subject of intensive research that seeks to identify compounds that can specifically induce either anti-inflammatory or proinflammatory responses from type I NKT cells. For example, tail-truncated analogs of αGalCer, such as the compound designated OCH which has a shortened sphingosine chain or the C20:2 analog with a shortened fatty acyl moiety (Fig. 2), induce a striking polarization of the cytokine secretion by type I NKT cells [107,108]. These αGalCer analogs induce the preferential secretion of Th2 cytokines by type I NKT cells, resulting in mainly IL-4 release with little and short-lived IFNγ secretion, due to a lack of NK cell transactivation [108]. Another compound known as α-C-galactosylceramide, which is a C-glycoside analog of αGalCer, has the opposite effect and induces a predominantly Th1-type response from type I NKT cells *in vivo*, and provides superior anti-metastatic or anti-malarial compared protection to αGalCer [109,110].

Natural mammalian lipid antigens recognized by type I NKT cells

Type I NKT cells have frequently been observed to have significant levels of reactivity to CD1d molecules on the surfaces of normal host cells and tumor cells. This appears to be a type of regulated self-reactivity that can be augmented by exposure to microbial products such as lipopolysaccharide through effects mediated by IL-12 [111]. This reactivity is presumed to involve recognition of self lipid ligands bound to CD1d, and the precise identity of the lipid antigens involved in such reactivity has been an area of great interest. There is some data suggesting that common cellular phospholipids may be relevant ligands that trigger self-reactivity of type I NKT cells. As discussed earlier, several phospholipids have been shown to associate with CD1 molecules after their synthesis in mammalian cells, and it has been found that PE and PI (Fig. 2) can be stimulatory to some but not all murine type I NKT cell hybridomas [112,113]. The degree of activation by PE was dependent upon the presence of unsaturations and the length of acyl chains [113]. Little is known about the effector functions induced by recognition of these self phospholipids, since only responses of type I NKT hybridomas which are limited to IL-2 production have been studied [112,113].

Several self glycosphingolipids have been identified as potential type I NKT cell antigens. One study found that the ganglioside GD3 can activate a subset of murine type I NKT cells *in vivo* in a CD1d-dependent manner [114]. Interestingly, GD3 induced IL-4 secretion from NKT cells, but not IFNγ secretion [114]. The reactivity of human type I NKT cells to GD3 remains to be determined. β-Galactosylceramides, which are produced by normal mammalian cells, were previously thought not to be recognized by type I NKT cells. However, recent studies show that mouse type I NKT cells can respond to high doses of βGalCer with a particular acyl chain length [115,116]. This antigen induced proliferation and TCR downregulation of type I NKT cells, but no cytokine secretion or activation of bystander NK cells [115,116]. Isoglobotrihexosylceramide (iGb3, Fig. 2) has been identified as an endogenous antigen that is recognized by most or all αGalCer-reactive murine and human type I NKT cells [57]. This mammalian glycolipid induced proliferation of human type I NKT cells *in vitro* that was similar to that resulting from αGalCer stimulation, and induced comparable secretion of IFNγ and IL-4. However, iGb3-loaded CD1d tetramers do not bind detectably to type I NKT cells. This antigen has been proposed as the conserved and endogenous glycolipid involved in thymic selection and in the activation of type I NKT cells in the periphery during bacterial infection [117].

Natural microbial antigens recognized by type I NKT cells

Phosphatidylinositol tetramannoside (PIM4), a glycolipid produced by mycobacteria, was the first bacterial type I NKT cell antigen identified [118]. Only a fraction of the type I NKT cells, in both human and mouse, have been found to recognize PIM4 purified from BCG. The PIM4-specific subset of NKT cells was shown to produce IFNγ but little or no IL-4, and displayed cytolytic activity [118]. However, these results could not be reproduced in another study using synthetic PIM4, raising questions about the true identity of the natural BCG-derived NKT cell ligand [119].

Lipophosphoglycan derived from *Leishmania donovani* (LPG, also known as LD1S) has been recently reported as a pathogen-derived antigen for murine type I NKT cells [59]. Like PIM4, only a fraction of the murine type I NKT cells recognized LD1S. The LD1S specific subset of NKT cells produces IFNγ but no detectable IL-4 [59].

α-Glucuronosylceramides have a structure very close to αGalCer, as they correspond to 6'-carboxylated α-glycosylceramides (Fig. 2) expressed by particular bacteria of the *Sphingomonas* [120] or *Ehrlichia* genuses. It was found that a fraction of type I NKT cells react to α-glucuronosylceramides in mice [117,121], but all human NKT cell lines tested recognize such antigens [117,121,122]. These antigens induce strong IFNγ and IL-4 release in both human and murine NKT cells [117,121,122]. Similarly, α-galactosyldiacylglycerol (BbGL-IIc, Fig. 2), derived from *Borrelia burgdorferi*, which is the causative agent of Lyme disease, was found to be recognized in a fraction of mouse type I NKT cells, and also by a human type I NKT cell line. This antigen preferentially stimulated IFNγ rather than IL-4 [119].

Type II or non-invariant NKT cells

Type II NKT cells are also specific for antigens presented by CD1d, but they express TCRs with highly diverse structures similar to those of conventional T cells, and are generally not reactive with αGalCer [101]. They are poorly characterized because the antigens that they recognize remain nearly unknown, and there currently exist no specific reagents that allow them to be precisely identified and isolated. However, type II NKT cells specific for sulfatides (sulfated and self-derived β-galactosylceramides, Fig. 2), have been described in mice [72,123], These have the ability to secrete IFNγ and IL-4, and their specific activation *in vivo* by sulfatide injection prevented the onset of experimental autoimmune encephalomyelitis [123] or type 1 diabetes in susceptible mice [124]. The existence of corresponding sulfatide-specific CD1d-restricted T cells in human has not yet been reported. Mouse type II NKT cells with unknown specificity and a $CD4^+$ phenotype can exert a suppressive role on anti-tumor responses [125]. This occurs via their production of IL-13, which in turn induces TGFβ release from myeloid suppressor cells and subsequent inhibition of tumor-reactive $CD8^+$ T cells [126,127]. The first human type II NKT cells identified were reactive to phenylpentamethylbenzofuran sulfonate (PPBF), a synthetic hydrophobic but nonlipidic molecule that is presented by CD1d [128]. Little is known about the functional response induced by PPBF, as only proliferation and IL-2 release by PPBF-responsive T cells has been demonstrated so far. A few human clones reactive to pollen-derived PE or PC presented by CD1d have been recently reported [54]. Most of these produced IL-4 when stimulated *in vitro*, although some of them also secreted IFNγ, IL-10 or TGFβ [54]. Other human type II NKT cells with unknown antigen specificity have been isolated and shown to have a Th1 polarization with IFNγ production in subjects with chronic viral hepatitis [129,130], whereas type II NKT cells with a Th2 polarization (IL-4 or IL-13 production) appear to be frequent in normal bone marrow [131], or in patients with ulcerative colitis [132]. Like type I NKT cells, subsets of type II NKT cells thus have the ability to produce Th1 or Th2 cytokines or both.

Group 1 CD1-reactive αβ T cells

The reactivity of MHC-unrestricted human T cells to mycobacterial cell-wall extract was primarily found to be dependent on antigen presentation by group 1 CD1 molecules, in particular CD1b in the first examples described [133]. The molecular identity of mycobacterial lipids presented by CD1b and recognized by T cells was then investigated, which led to identification of mycolic acid (Fig. 2) [134] and its monoglucosylated derivative GMM [135] as relevant CD1b-presented antigens. Interestingly, the synthesis of GMM by mycobacteria requires glucose uptake from the host, indicating that GMM synthesis is a marker for infectious mycobacteria that have the capacity to grow within a mammalian host [136]. Mycobacterial phosphatidylinositols (PIs) linked to carbohydrate structures of varying complexity can also be presented by CD1b, including lipoarabinomannan (LAM) [55], and mannosylated PI such as PIM2 (Fig. 2) [60] or PIM6 [55]. Another class of glycolipid presented by CD1b is diacylated sulfoglycolipids [137]. CD1c molecules also present glycolipids derived from the cell wall of mycobacteria, including a family of β1-mannosyl isoprenoid lipids that are now referred to as mycoketides (MPD) [138,139]. Human T cells have also been identified that specifically recognize dide-

hydroxymycobactin, a mycobacterial lipopeptide presented by CD1a [140].

In general, group 1 CD1-restricted T cells reactive to mycobacterial lipids have been found *in vitro* to display functionalities consistent with a role in antibacterial immunity. These include IFNγ secretion [55,137,141–143], cytolytic activity [133–135,137–139,144] and in some cases the ability to mediate bactericidal activity through the secretion of the antimicrobial peptide granulysin [145]. Most of these cells show a $CD8^+$ or DN (CD4/CD8 double negative) phenotype, but some Th1-polarized CD1-restricted $CD4^+$ cells have been found in leprosy lesions [142]. Mycobacteria-reactive T cells were found to cross-react to multiple mycobacterial species, and are believed to recognize conserved lipid structures shared by these organisms [143]. Specific group 1 CD1-restricted T cell responses against mycobacterial lipids are more easily detectable in human subjects who have a clinical history of mycobacterial infection, or in BCG-immunized and PPD^+ healthy donors [137,138,141,143]. This suggests that these T cells are expanded by previous antigen exposure, and most likely represent a type of long-lived memory T cell. Studies using guinea pigs as an animal model for group 1 CD1-restricted T cell responses support this notion, as immunization of naïve guinea pigs with purified mycobacterial lipids induces specific and long-lived CD1-dependent T cell responses [144]. Guinea pigs immunized in this manner and challenged with virulent *Mycobacterium tuberculosis* show improved control of infection and a milder lung pathology when compared to naïve animals [146], which is similar to the level of protective immunity induced by BCG immunization. Although it remains to be definitively proven that the protection elicited in this model is directly linked to CD1-restricted vaccine-elicited T cell responses, this is a plausible hypothesis that deserves further detailed study.

The group 1 CD1 system thus seems to be particularly efficient for presentation of mycobacterial derived antigens to αβ T cells, leading to potentially protective memory responses against pathogens such as *M. tuberculosis* and *M. leprae,* but their direct role in infection remains to be fully demonstrated. Currently, there are very few data available concerning the involvement of group 1 CD1-restricted T cells in protective immune responses against other types of bacterial pathogens. However, there have been isolated reports of group 1 CD1-restricted T cells reactive to *Haemophilus influenza, Escherichia coli or Yersinia enterocolitica* lipid extracts [147,148]. Thus, generation of host immunity by T cells responding to bacterial lipids presented by this pathway may not be limited only to mycobacterial infections.

In addition to presentation of bacterial lipids, there is evidence that group 1 CD1 molecules play a significant role in presentation of environmental and self lipid antigens. For example, human clones reactive to pollen-derived PC presented mainly by CD1a have recently been reported [54], and have been postulated to be involved in seasonal allergic symptoms as a result of their production of IL-4 or other cytokines [54]. Intriguingly, some group 1 CD1-restricted T cell clones selected for reactivity to microbial lipids were found to be weakly reactive to unidentified self-derived lipids, and these secreted a combination of Th1- and Th2-associated cytokines [148,149]. Reactivity to various well-characterized self glycosphingolipids presented by group 1 CD1 molecules has also been well documented. For example, the glycolipid sulfatide can be recognized by T cells restricted by either CD1a, -b or -c, and these cells can produce either Th1 or Th2 cytokines [150]. T cells reactive to the ganglioside GM1 presented by CD1b have been identified with a Th1 cytokine profile, and their frequency appears elevated in the blood of patients with multiple sclerosis [151]. Because GM1 is a component of the myelin, it was suggested that these cells might contribute to pathogenesis of this demyelinating disease [151]. CD1a- or CD1c- reactive T cells have also been suggested to be involved in the pathogenesis of autoimmune thyroid diseases [79].

Lipid reactive γδ T cells

Human γδ T cells expressing Vδ2 TCR segments can show reactivity to microbial derived isopentenyl pyrophosphate [152], and such cells also display reactivity to tumor cells [153,154]. However, the reactivity of these cells with such small and negatively charged alkyl phosphates has been found not to be dependent on presentation by CD1 molecules. In contrast, CD1c-reactive γδ T cells expressing Vδ1 TCR segments have been identified. These cells are activated by CD1c-expressing APCs without requiring the deliberate addition of any exogenous antigen, and the putative self lipid ligands recognized by these T cells remain to be established. The few examples of these T cells so far studied produce mainly Th1 cytokines, possess cytolytic activity [155,156] and also trigger dendritic cell maturation [157]. In addition, human γδ T cells expressing TCRs using either the Vδ1 or Vδ2 were recently found to be reactive to pollen-derived PE presented by CD1d [158]. These cells could be identified in patients allergic to cypress pollen, but not in non-allergic individuals. Their ability to secrete IL-4 and provide helper activity for immunoglobulin E production suggest that they might be involved in generating or sustaining allergic disease [158].

Conclusion

The CD1 system appears to play an important role in various immune responses by mobilizing specific αβ and γδ T cell subsets that respond to lipid antigens. This function is analogous and complementary to that of the MHC class I and II antigen-presenting molecules, which present only antigenic peptides to T cells. A significant role for group 2 CD1 (CD1d) in immunoregulation is strongly implied by multiple animal models, and observations made recently with human T cell lines suggest that this function might also extend to group 1 CD1 molecules. Another new notion is the fact that lipid-reactive CD1-restricted T cells can cross-react with multiple distinct antigens, a phenomenon particularly evident for CD1d-restricted type I NKT cells and which has been suggested for group 1 CD1-restricted T cells.

It is also interesting to note that when CD1-presented lipid antigens are considered according to the quality of functional responses that they induce, self-derived mammalian lipids tend to induce more of a Th2 polarization of specific T cells, whereas pathogen-derived antigens preferentially induce Th1 polarization and cytolytic activity. In this manner, lipid-reactive T cells could exert a variety of diverse functions, behaving like pro-inflammatory cells in response to pathogen-derived glycolipids or as anti-inflammatory cells in response to self-derived glycolipids. In the case of type I NKT cells, this may provide an explanation for the widely divergent functions that have been associated with these T cells depending on the model or the disease studied [104,106].

Finally, the presentation of lipids to T cells might not be limited solely to CD1-dependent pathways, as other potential nonclassical antigen-presenting molecules like MR-1 or F1-ATPase/Apo A-I might also be involved in this process. Preliminary suggestive evidence for this has recently emerged, as one study reported that MR-1 can mediate presentation of a synthetic α-mannosylceramide to a subset of unconventional T cells with semi-invariant TCRs containing Vα19 [159]. Similarly, F1-ATPase/Apo A-I was found to promote tumor cell recognition by Vδ2 T cells [160], but it remains unknown whether it is involved in the presentation of lipids such as the prenyl pyrophosphates that are recognized by this T cell subset and produced by the active mevalonate pathway in some tumor cells. Further work to gain a more complete understanding of CD1 and other potential mechanisms involved in the T cell recognition of lipid antigens might ultimately lead to important new approaches to prevention and therapy of many infectious, neoplastic, inflammatory and autoimmune disorders.

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1 Yu, C. Y. and Milstein, C. (1989) A physical map linking the five CD1 human thymocyte differentiation antigen genes. *EMBO J.* 8, 3727 – 3732.
2 Dutronc, Y., Porcelli, S. A. (2002) The CD1 family and T cell recognition of lipid antigens. *Tissue Antigens* 60, 337 – 353.
3 Angenieux, C., Fraisier, V., Maitre, B., Racine, V., van der, W. N., Fricker, D., Proamer, F., Sachse, M., Cazenave, J. P., Peters, P. et al. (2005) The cellular pathway of CD1e in immature and maturing dendritic cells. *Traffic* 6, 286 – 302.
4 Han, M., Hannick, L. I., DiBrino, M. and Robinson, M. A. (1999) Polymorphism of human CD1 genes. *Tissue Antigens* 54, 122 – 127.
5 Mirones, I., Oteo, M., Parra-Cuadrado, J. F. and Martinez-Naves, E. (2000) Identification of two novel human CD1E alleles. *Tissue Antigens* 56, 159 – 161.
6 Tamouza, R., Sghiri, R., Ramasawmy, R., Neonato, M. G., Mombo, L. E., Poirier, J. C., Schaeffer, V., Fortier, C., Labie, D., Girot, R. et al. (2002) Two novel CD1 E alleles identified in black African individuals. *Tissue Antigens* 59, 417 – 420.
7 Calabi, F., Belt, K. T., Yu, C. Y., Bradbury, A., Mandy, W. J. and Milstein, C. (1989) The rabbit CD1 and the evolutionary conservation of the CD1 gene family. *Immunogenetics* 30, 370 – 377.
8 Dascher, C. C., Hiromatsu, K., Naylor, J. W., Brauer, P. P., Brown, K. A., Storey, J. R., Behar, S. M., Kawasaki, E. S., Porcelli, S. A., Brenner, M. B. et al. (1999) Conservation of a CD1 multigene family in the guinea pig. *J. Immunol.* 163, 5478 – 5488.
9 Ferguson, E. D., Dutia, B. M., Hein, W. R. and Hopkins, J. (1996) The sheep CD1 gene family contains at least four CD1B homologues. *Immunogenetics* 44, 86 – 96.
10 Hayes, S. M., Knight, K. L. (2001) Group 1 CD1 genes in rabbit. *J. Immunol.* 166, 403 – 410.
11 Hiromatsu, K., Dascher, C. C., Sugita, M., Gingrich-Baker, C., Behar, S. M., LeClair, K. P., Brenner, M. B. and Porcelli, S. A. (2002) Characterization of guinea-pig group 1 CD1 proteins. *Immunology* 106, 159 – 172.
12 Hopkins, J., Dutia, B. M. and Rhind, S. M. (2000) Sheep CD1 genes and proteins. *Vet. Immunol. Immunopathol.* 73, 3 – 14.
13 Hughes, A. L. (1991) Evolutionary origin and diversification of the mammalian CD1 antigen genes. *Mol. Biol. Evol.* 8, 185 – 201.
14 Katabami, S., Matsuura, A., Chen, H. Z., Imai, K. and Kikuchi, K. (1998) Structural organization of rat CD1 typifies evolutionarily conserved CD1D class genes. *Immunogenetics* 48, 22 – 31.
15 Matsuura, A., Hashimoto, Y., Kinebuchi, M., Kasai, K., Ichimiya, S., Katabami, S., Chen, H., Shimizu, T. and Kikuchi, K. (1997) Rat CD1 antigen: structure, expression and function. *Transplant. Proc.* 29, 1705 – 1706.
16 Rhind, S. M., Hopkins, J. and Dutia, B. M. (1999) Amino-terminal sequencing of sheep CD1 antigens and identification of a sheep CD1D gene. *Immunogenetics* 49, 225 – 230.
17 Van, R., I, Koets, A. P., Im, J. S., Piebes, D., Reddington, F., Besra, G. S., Porcelli, S. A., van Eden, W. and Rutten, V. P. (2006) The bovine CD1 family contains group 1 CD1 proteins, but no functional CD1d. *J. Immunol.* 176, 4888 – 4893.
18 Eguchi-Ogawa, T., Morozumi, T., Tanaka, M., Shinkai, H., Okumura, N., Suzuki, K., Awata, T. and Uenishi, H. (2007) Analysis of the genomic structure of the porcine CD1 gene cluster. *Genomics* 89, 248 – 261.
19 Dascher, C. C., Brenner, M. B. (2003) Evolutionary constraints on CD1 structure: insights from comparative genomic analysis. *Trends Immunol.* 24, 412 – 418.
20 Maruoka, T., Tanabe, H., Chiba, M. and Kasahara, M. (2005) Chicken CD1 genes are located in the MHC: CD1 and endothelial protein C receptor genes constitute a distinct

subfamily of class-I-like genes that predates the emergence of mammals. *Immunogenetics* 57, 590 – 600.
21 Miller, M. M., Wang, C., Parisini, E., Coletta, R. D., Goto, R. M., Lee, S. Y., Barral, D. C., Townes, M., Roura-Mir, C., Ford, H. L., Brenner, M. B. and Dascher, C. C. (2005) Characterization of two avian MHC-like genes reveals an ancient origin of the CD1 family. *Proc. Natl. Acad. Sci. U.S.A* 102, 8674 – 8679.
22 Salomonsen, J., Sorensen, M. R., Marston, D. A., Rogers, S. L., Collen, T., van Hateren, A., Smith, A. L., Beal, R. K., Skjodt, K. and Kaufman, J. (2005) Two CD1 genes map to the chicken MHC, indicating that CD1 genes are ancient and likely to have been present in the primordial MHC. *Proc. Natl. Acad. Sci. U.S.A* 102, 8668 – 8673.
23 Hava, D. L., Brigl, M., van den Elzen, P., Zajonc, D. M., Wilson, I. A. and Brenner, M. B. (2005) CD1 assembly and the formation of CD1-antigen complexes. *Current Opinion in Immunology* 17, 88 – 94.
24 Sugita, M., Porcelli, S. A. and Brenner, M. B. (1997) Assembly and retention of CD1b heavy chains in the endoplasmic reticulum. *J. Immunol.* 159, 2358 – 2365.
25 Huttinger, R., Staffler, G., Majdic, O. and Stockinger, H. (1999) Analysis of the early biogenesis of CD1b: involvement of the chaperones calnexin and calreticulin, the proteasome and beta(2)-microglobulin. *Int. Immunol.* 11, 1615 – 1623.
26 Kang, S. J., Cresswell, P. (2002) Calnexin, calreticulin, and ERp57 cooperate in disulfide bond formation in human CD1d heavy chain. *J. Biol. Chem.* 277, 44838 – 44844.
27 Paduraru, C., Spiridon, L., Yuan, W., Bricard, G., Valencia, X., Porcelli, S. A., Illarionov, P. A., Besra, G. S., Petrescu, S. M., Petrescu, A. J. and Cresswell, P. (2006) An N-linked glycan modulates the interaction between the CD1d heavy chain and beta2-microglobulin. *J. Biol. Chem.* 281, 40369 – 40378.
28 Kim, H. S., Garcia, J., Exley, M., Johnson, K. W., Balk, S. P. and Blumberg, R. S. (1999) Biochemical characterization of CD1d expression in the absence of beta2-microglobulin. *J. Biol. Chem.* 274, 9289 – 9295.
29 Amano, M., Baumgarth, N., Dick, M. D., Brossay, L., Kronenberg, M., Herzenberg, L. A. and Strober, S. (1998) CD1 expression defines subsets of follicular and marginal zone B cells in the spleen: beta 2-microglobulin-dependent and independent forms. *J. Immunol.* 161, 1710 – 1717.
30 Joyce, S., Woods, A. S., Yewdell, J. W., Bennink, J. R., De Silva, A. D., Boesteanu, A., Balk, S. P., Cotter, R. J. and Brutkiewicz, R. R. (1998) Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol. *Science* 279, 1541 – 1544.
31 Park, J. J., Kang, S. J., De Silva, A. D., Stanic, A. K., Casorati, G., Hachey, D. L., Cresswell, P. and Joyce, S. (2004) Lipid-protein interactions: biosynthetic assembly of CD1 with lipids in the endoplasmic reticulum is evolutionarily conserved. *Proc. Natl. Acad. Sci. USA* 101, 1022 – 1026.
32 De Silva, A. D., Park, J. J., Matsuki, N., Stanic, A. K., Brutkiewicz, R. R., Medof, M. E. and Joyce, S. (2002) Lipid protein interactions: the assembly of CD1d1 with cellular phospholipids occurs in the endoplasmic reticulum. *J. Immunol.* 168, 723 – 733.
33 Garcia-Alles, L. F., Versluis, K., Maveyraud, L., Vallina, A. T., Sansano, S., Bello, N. F., Gober, H. J., Guillet, V., de la Salle, H., Puzo, G. et al. (2006) Endogenous phosphatidylcholine and a long spacer ligand stabilize the lipid-binding groove of CD1b. *EMBO J.* 25, 3684 – 3692.
34 Giabbai, B., Sidobre, S., Crispin, M. D., Sanchez-Ruiz, Y., Bachi, A., Kronenberg, M., Wilson, I. A. and Degano, M. (2005) Crystal structure of mouse CD1d bound to the self ligand phosphatidylcholine: a molecular basis for NKT cell activation. *J. Immunol.* 175, 977 – 984.
35 van Meer, G. (2005) Cellular lipidomics. *EMBO J.* 24, 3159 – 3165.
36 Brozovic, S., Nagaishi, T., Yoshida, M., Betz, S., Salas, A., Chen, D., Kaser, A., Glickman, J., Kuo, T., Little, A. et al. (2004) CD1d function is regulated by microsomal triglyceride transfer protein. *Nat. Med.* 10, 535 – 539.
37 Dougan, S. K., Salas, A., Rava, P., Agyemang, A., Kaser, A., Morrison, J., Khurana, A., Kronenberg, M., Johnson, C., Exley, M. et al. (2005) Microsomal triglyceride transfer protein lipidation and control of CD1d on antigen-presenting cells. *J. Exp. Med.* 202, 529 – 539.
38 Sugita, M., Cernadas, M. and Brenner, M. B. (2004) New insights into pathways for CD1-mediated antigen presentation. *Curr. Opin. Immunol.* 16, 90 – 95.
39 Sugita, M., Grant, E. P., van Donselaar, E., Hsu, V. W., Rogers, R. A., Peters, P. J. and Brenner, M. B. (1999) Separate pathways for antigen presentation by CD1 molecules. *Immunity* 11, 743 – 752.
40 Manolova, V., Kistowska, M., Paoletti, S., Baltariu, G. M., Bausinger, H., Hanau, D., Mori, L. and De Libero, G. (2006) Functional CD1a is stabilized by exogenous lipids. *Eur. J. Immunol.* 36, 1083 – 1092.
41 Briken, V., Jackman, R. M., Watts, G. F., Rogers, R. A. and Porcelli, S. A. (2000) Human CD1b and CD1c isoforms survey different intracellular compartments for the presentation of microbial lipid antigens. *J. Exp. Med.* 192, 281 – 288.
42 Briken, V., Jackman, R. M., Dasgupta, S., Hoening, S. and Porcelli, S. A. (2002) Intracellular trafficking pathway of newly synthesized CD1b molecules. *EMBO J.* 21, 825 – 834.
43 Lawton, A. P., Prigozy, T. I., Brossay, L., Pei, B., Khurana, A., Martin, D., Zhu, T., Spate, K., Ozga, M., Honing, S. et al. (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.
44 Cernadas, M., Sugita, M., van der, W. 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.
45 Elewaut, D., Lawton, A. P., Nagarajan, N. A., Maverakis, E., Khurana, A., Honing, S., Benedict, C. A., Sercarz, E., Bakke, O., Kronenberg, M. et al. (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.
46 Sugita, M., Jackman, R. M., van Donselaar, E., Behar, S. M., Rogers, R. A., Peters, P. J., Brenner, M. B. and Porcelli, S. A. (1996) Cytoplasmic tail-dependent localization of CD1b antigen-presenting molecules to MIICs. *Science* 273, 349 – 352.
47 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.
48 Kang, S. J., Cresswell, P. (2002) Regulation of intracellular trafficking of human CD1d by association with MHC class II molecules. *EMBO J.* 21, 1650 – 1660.
49 Angenieux, C., Salamero, J., Fricker, D., Cazenave, J. P., Goud, B., Hanau, D. and de la Salle H. (2000) Characterization of CD1e, a third type of CD1 molecule expressed in dendritic cells. *J. Biol. Chem.* 275, 37757 – 37764.
50 Schaible, U. E., Winau, F., Sieling, P. A., Fischer, K., Collins, H. L., Hagens, K., Modlin, R. L., Brinkmann, V. and Kaufmann, S. H. (2003) Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. *Nat. Med.* 9, 1039 – 1046.
51 van den Elyen, P., Garg, S., Leon, L., Brigl, M., Leadbetter, E. A., Gumperz, J. E., Dascher, C. C., Cheng, T. Y., Sacks, F. M., Illarionov, P. A. et al. (2005) Apolipoprotein-mediated pathways of lipid antigen presentation. *Nature* 437, 906 – 910.
52 Prigozy, T. I., Sieling, P. A., Clemens, D., Stewart, P. L., Behar, S. M., Porcelli, S. A., Brenner, M. B., Modlin, R. L. and Kronenberg, M. (1997) The mannose receptor delivers

lipoglycan antigens to endosomes for presentation to T cells by CD1b molecules. *Immunity* 6, 187 – 197.
53 Hunger, R. E., Sieling, P. A., Ochoa, M. T., Sugaya, M., Burdick, A. E., Rea, T. H., Brennan, P. J., Belisle, J. T., Blauvelt, A., Porcelli, S. A. et al. (2004) Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells. *J.Clin.Invest* 113, 701 – 708.
54 Agea, E., Russano, A., Bistoni, O., Mannucci, R., Nicoletti, I., Corazzi, L., Postle, A. D., De Libero, G., Porcelli, S. A. and Spinozzi, F. (2005) Human CD1-restricted T cell recognition of lipids from pollens. *J. Exp. Med.* 202, 295 – 308.
55 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. et al. (1995) CD1-restricted T cell recognition of microbial lipoglycan antigens. *Science* 269, 227 – 230.
56 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.
57 Zhou, D., Mattner, J., Cantu, C., III, Schrantz, N., Yin, N., Gao, Y., Sagiv, Y., Hudspeth, K., Wu, Y. P., Yamashita, T. et al. (2004) Lysosomal glycosphingolipid recognition by NKT cells. *Science* 306, 1786 – 1789.
58 Gadola, S. D., Silk, J. D., Jeans, A., Illarionov, P. A., Salio, M., Besra, G. S., Dwek, R., Butters, T. D., Platt, F. M. and Cerundolo, V. (2006) Impaired selection of invariant natural killer T cells in diverse mouse models of glycosphingolipid lysosomal storage diseases. *J. Exp. Med.* 203, 2293 – 2303.
59 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.
60 de la Salle, H., Mariotti, S., Angenieux, C., Gilleron, M., Garcia-Alles, L. F., Malm, D., Berg, T., Paoletti, S., Maitre, B., Mourey, L. et al. (2005) Assistance of microbial glycolipid antigen processing by CD1e. *Science* 310, 1321 – 1324.
61 Im, J. S., Yu, K. O., Illarionov, P. A., LeClair, K. P., Storey, J. R., Kennedy, M. W., Besra, G. S. and Porcelli, S. A. (2004) Direct measurement of antigen binding properties of CD1 proteins using fluorescent lipid probes. *J.Biol.Chem.* 279, 299 – 310.
62 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.
63 Kang, S. J., Cresswell, P. (2004) Saposins facilitate CD1d-restricted presentation of an exogenous lipid antigen to T cells. *Nat. Immunol.* 5, 175 – 181.
64 Winau, F., Schwierzeck, V., Hurwitz, R., Remmel, N., Sieling, P. A., Modlin, R. L., Porcelli, S. A., Brinkmann, V., Sugita, M., Sandhoff, K., Kaufmann, S. H. et al. (2004) Saposin C is required for lipid presentation by human CD1b. *Nat.Immunol.* 5, 169 – 174.
65 Zhou, D., Cantu, C., III, Sagiv, Y., Schrantz, N., Kulkarni, A. B., Qi, X., Mahuran, D. J., Morales, C. R., Grabowski, G. A., Benlagha, K. et al. (2004) Editing of CD1d-bound lipid antigens by endosomal lipid transfer proteins. *Science* 303, 523 – 527.
66 Moody, D. B., Zajonc, D. M. and Wilson, I. A. (2005) Anatomy of CD1-lipid antigen complexes. *Nat. Rev. Immunol.* 5, 387 – 399.
67 Zajonc, D. M., Elsliger, M. A., Teyton, L. and Wilson, I. A. (2003) Crystal structure of CD1a in complex with a sulfatide self antigen at a resolution of 2.15 A. *Nat.Immunol.* 4, 808 – 815.
68 Zajonc, D. M., Crispin, M. D. M., Bowden, T. A., Young, D. C., Cheng, T. Y., Hu, J. D., Costello, C. E., Rudd, P. M., Dwek, R. A., Miller, M. J. et al. (2005) Molecular mechanism of lipopeptide presentation by CD1a. *Immunity* 22, 209 – 219.
69 Gadola, S. D., Zaccai, N. R., Harlos, K., Shepherd, D., Castro-Palomino, J. C., Ritter, G., Schmidt, R. R., Jones, E. Y. and Cerundolo, V. (2002) Structure of human CD1b with bound ligands at 2.3 A, a maze for alkyl chains. *Nat. Immunol.* 3, 721 – 726.
70 Batuwangala, T., Shepherd, D., Gadola, S. D., Gibson, K. J., Zaccai, N. R., Fersht, A. R., Besra, G. S., Cerundolo, V. and Jones, E. Y. (2004) The crystal structure of human CD1b with a bound bacterial glycolipid. *J. Immunol.* 172, 2382 – 2388.
71 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.
72 Zajonc, D. M., Maricic, I., Wu, D., Halder, R., Roy, K., Wong, C. H., Kumar, V. and Wilson, I. A. (2005) Structural basis for CD1d presentation of a sulfatide derived from myelin and its implications for autoimmunity. *J. Exp. Med.* 202, 1517 – 1526.
73 Zajonc, D. M., Cantu, C., Mattner, J., Zhou, D. P., Savage, P. B., Bendelac, A., Wilson, I. A. and Teyton, L. (2005) Structure and function of a potent agonist for the semi-invariant natural killer T cell receptor. *Nat. Immunol.* 6, 810 – 818.
74 Wu, D., Zajonc, D. M., Fujio, M., Sullivan, B. A., Kinjo, Y., Kronenberg, M., Wilson, I. A. and Wong, C. H. (2006) Design of natural killer T cell activators: structure and function of a microbial glycosphingolipid bound to mouse CD1d. *Proc. Nat. Acad. Sci.* USA 103, 3972 – 3977.
75 Zajonc, D. M., Ainge, G. D., Painter, G. F., Severn, W. B. and Wilson, I. A. (2006) Structural characterization of mycobacterial phosphatidylinositol mannoside binding to mouse CD1d. *J. Immunol.* 177, 4577 – 4583.
76 Koch, M., Stronge, V. S., Shepherd, D., Gadola, S. D., Mathew, B., Ritter, G., Fersht, A. R., Besra, G. S., Schmidt, R. R., Jones, E. Y. et al. (2005) The crystal structure of human CD1d with and without alpha-galactosylceramide. *Nat. Immunol.* 6, 819 – 826.
77 Moody, D. B. (2006) TLR gateways to CD1 function. *Nat. Immunol.* 7, 811 – 817.
78 Small, T. N., Knowles, R. W., Keever, C., Kernan, N. A., Collins, N., O'Reilly, R. J., Dupont, B. and Flomenberg, N. (1987) M241 (CD1) expression on B lymphocytes. *J. Immunol.* 138, 2864 – 2868.
79 Roura-Mir, C., Catalfamo, M., Cheng, T. Y., Marqusee, E., Besra, G. S., Jaraquemada, D. and Moody, D. B. (2005) CD1a and CD1c activate intrathyroidal T cells during Graves' disease and Hashimoto's thyroiditis. *J. Immunol.* 174, 3773 – 3780.
80 Exley, M., Garcia, J., Wilson, S. B., Spada, F., Gerdes, D., Tahir, S. M., Patton, K. T., Blumberg, R. S., Porcelli, S., Chott, A. et al. (2000) CD1d structure and regulation on human thymocytes, peripheral blood T cells, B cells and monocytes. *Immunology* 100, 37 – 47.
81 Gerlini, G., Hefti, H. P., Kleinhans, M., Nickoloff, B. J., Burg, G. and Nestle, F. O. (2001) Cd1d is expressed on dermal dendritic cells and monocyte-derived dendritic cells. *J. Invest Dermatol.* 117, 576 – 582.
82 Blumberg, R. S., Terhorst, C., Bleicher, P., McDermott, F. V., Allan, C. H., Landau, S. B., Trier, J. S. and Balk, S. P. (1991) Expression of a nonpolymorphic MHC class I-like molecule, CD1D, by human intestinal epithelial cells. *J. Immunol.* 147, 2518 – 2524.
83 Tsuneyama, K., Yasoshima, M., Harada, K., Hiramatsu, K., Gershwin, M. E. and Nakanuma, Y. (1998) Increased CD1d expression on small bile duct epithelium and epithelioid granuloma in livers in primary biliary cirrhosis. *Hepatology* 28, 620 – 623.
84 Bonish, B., Jullien, D., Dutronc, Y., Huang, B. B., Modlin, R., Spada, F. M., Porcelli, S. A. and Nickoloff, B. J. (2000) Over-

expression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells. *J. Immunol.* 165, 4076 – 4085.

85 Adly, M. A., Assaf, H. A. and Hussein, M. (2005) Expression of CD1d in human scalp skin and hair follicles: hair cycle related alterations. *J. Clin. Pathol.* 58, 1278 – 1282.

86 Im, J. S., Tapinos, N., Chae, G. T., Illarionov, P. A., Besra, G. S., DeVries, G. H., Modlin, R. L., Sieling, P. A., Rambukkana, A. and Porcelli, S. A. (2006) Expression of CD1d molecules by human schwann cells and potential interactions with immunoregulatory invariant NK T cells. *J. Immunol.* 177, 5226 – 5235.

87 Mandal, M., Chen, X. R., Alegre, M. L., Chiu, N. M., Chen, Y. H., Castano, A. R. and Wang, C. R. (1998) Tissue distribution, regulation and intracellular localization of murine CD1 molecules. *Mol. Immunol.* 35, 525 – 536.

88 Canchis, P. W., Bhan, A. K., Landau, S. B., Yang, L., Balk, S. P. and Blumberg, R. S. (1993) Tissue distribution of the non-polymorphic major histocompatibility complex class I-like molecule, CD1d. *Immunology* 80, 561 – 565.

89 Raghuraman, G., Geng, Y. and Wang, C. R. (2006) IFN-beta-mediated up-regulation of CD1d in bacteria-infected APCs. *J. Immunol.* 177, 7841 – 7848.

90 Colgan, S. P., Morales, V. M., Madara, J. L., Polischuk, J. E., Balk, S. P. and Blumberg, R. S. (1996) IFN-gamma modulates CD1d surface expression on intestinal epithelia. *Am. J. Physiol* 271, C276-C283.

91 Roura-Mir, C., Wang, L., Cheng, T. Y., Matsunaga, I., Dascher, C. C., Peng, S. L., Fenton, M. J., Kirschning, C. and Moody, D. B. (2005) Mycobacterium tuberculosis regulates CD1 antigen presentation pathways through TLR-2 *J. Immunol.* 175, 1758 – 1766.

92 Skold, M., Xiong, X., Illarionov, P. A., Besra, G. S. and Behar, S. M. (2005) Interplay of cytokines and microbial signals in regulation of CD1d expression and NKT cell activation. *J. Immunol.* 175, 3584 – 3593.

93 Szatmari, I., Gogolak, P., Im, J. S., Dezso, B., Rajnavolgyi, E. and Nagy, L. (2004) Activation of PPARgamma specifies a dendritic cell subtype capable of enhanced induction of iNKT cell expansion. *Immunity* 21, 95 – 106.

94 Szatmari, I., Pap, A., Ruhl, R., Ma, J. X., Illarionov, P. A., Besra, G. S., Rajnavolgyi, E., Dezso, B. and Nagy, L. (2006) PPARgamma controls CD1d expression by turning on retinoic acid synthesis in developing human dendritic cells. *J. Exp. Med.* 203, 2351 – 2362.

95 Gerlini, G., Tun-Kyi, A., Dudli, C., Burg, G., Pimpinelli, N. and Nestle, F. O. (2004) Metastatic melanoma secreted IL-10 down-regulates CD1 molecules on dendritic cells in metastatic tumor lesions. *Am. J. Pathol.* 165, 1853 – 1863.

96 Ronger-Savle, S., Valladeau, J., Claudy, A., Schmitt, D., Peguet-Navarro, J., Dezutter-Dambuyant, C., Thomas, L. and Jullien, D. (2005) TGFbeta inhibits CD1d expression on dendritic cells. *J. Invest Dermatol.* 124, 116 – 118.

97 Chen, N., McCarthy, C., Drakesmith, H., Li, D., Cerundolo, V., McMichael, A. J., Screaton, G. R. and Xu, X. N. (2006) HIV-1 down-regulates the expression of CD1d via Nef. *Eur. J. Immunol.* 36, 278 – 286.

98 Cho, S., Knox, K. S., Kohli, L. M., He, J. J., Exley, M. A., Wilson, S. B. and Brutkiewicz, R. R. (2005) Impaired cell surface expression of human CD1d by the formation of an HIV-1 Nef/CD1d complex. *Virology* 337, 242 – 252.

99 Yuan, W., Dasgupta, A. and Cresswell, P. (2006) Herpes simplex virus evades natural killer T cell recognition by suppressing CD1d recycling. *Nat. Immunol.* 7, 835 – 842.

100 Amprey, J. L., Spath, G. F. and Porcelli, S. A. (2004) Inhibition of CD1 expression in human dendritic cells during intracellular infection with Leishmania donovani. *Infect. Immun.* 72, 589 – 592.

101 Godfrey, D. I., MacDonald, H. R., Kronenberg, M., Smyth, M. J. and Van Kaer, L. (2004) NKT cells: what's in a name? *Nat. Rev. Immunol.* 4, 231 – 237.

102 Gumperz, J. E. (2004) Antigen specificity of semi-invariant CD1d-restricted T cell receptors: the best of both worlds? *Immunol. Cell Biol.* 82, 285 – 294.

103 Gadola, S. D., Dulphy, N., Salio, M. and Cerundolo, V. (2002) Valpha24-JalphaQ-independent, CD1d-restricted recognition of alpha-galactosylceramide by human CD4(+) and CD8alphabeta(+) T lymphocytes. *J. Immunol.* 168, 5514 – 5520.

104 Godfrey, D. I., Kronenberg, M. (2004) Going both ways: immune regulation via CD1d-dependent NKT cells. *J.Clin.-Invest* 114, 1379 – 1388.

105 Swann, J., Crowe, N. Y., Hayakawa, Y., Godfrey, D. I. and Smyth, M. J. (2004) Regulation of antitumour immunity by CD1d-restricted NKT cells. *Immunol. Cell Biol.* 82, 323 – 331.

106 Yu, K. O., Porcelli, S. A. (2005) The diverse functions of CD1d-restricted NKT cells and their potential for immunotherapy. *Immunol. Lett.* 100, 42 – 55.

107 Miyamoto, K., Miyake, S. and Yamamura, T. (2001) A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. *Nature* 413, 531 – 534.

108 Yu, K. O., Im, J. S., Molano, A., Dutronc, Y., Illarionov, P. A., Forestier, C., Fujiwara, N., Arias, I., Miyake, S., Yamamura, T. et al. (2005) Modulation of CD1d-restricted NKT cell responses by using N-acyl variants of alpha-galactosylceramides. *Proc. Natl. Acad. Sci.USA* 102, 3383 – 3388.

109 Fujii, S., Shimizu, K., Hemmi, H., Fukui, M., Bonito, A. J., Chen, G., Franck, R. W., Tsuji, M. and Steinman, R. M. (2006) Glycolipid alpha-C-galactosylceramide is a distinct inducer of dendritic cell function during innate and adaptive immune responses of mice. *Proc. Natl. Acad. Sci.USA* 103, 11252 – 11257.

110 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.

111 Brigl, M., Bry, L., Kent, S. C., Gumperz, J. E. and Brenner, M. B. (2003) Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nat. Immunol.* 4, 1230 – 1237.

112 Gumperz, J. E., Roy, C., Makowska, A., Lum, D., Sugita, M., Podrebarac, T., Koezuka, Y., Porcelli, S. A., Cardell, S., Brenner, M. B. et al. (2000) Murine CD1d-restricted T cell recognition of cellular lipids. *Immunity* 12, 211 – 221.

113 Rauch, J., Gumperz, J., Robinson, C., Skold, M., Roy, C., Young, D. C., Lafleur, M., Moody, D. B., Brenner, M. B., Costello, C. E. et al. (2003) Structural features of the acyl chain determine self-phospholipid antigen recognition by a CD1d-restricted invariant NKT (iNKT) cell. *J. Biol. Chem.* 278, 47508 – 47515.

114 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.

115 Ortaldo, J. R., Young, H. A., Winkler-Pickett, R. T., Bere, E. W., Jr., Murphy, W. J. and Wiltrout, R. H. (2004) Dissociation of NKT stimulation, cytokine induction, and NK activation in vivo by the use of distinct TCR-binding ceramides. *J. Immunol.* 172, 943 – 953.

116 Parekh, V. V., Singh, A. K., Wilson, M. T., Olivares-Villagomez, D., Bezbradica, J. S., Inazawa, H., Ehara, H., Sakai, T., Serizawa, I., Wu, L. et al. (2004) Quantitative and qualitative differences in the in vivo response of NKT cells to distinct alpha- and beta-anomeric glycolipids. *J. Immunol.* 173, 3693 – 3706.

117 Mattner, J., Debord, K. L., Ismail, N., Goff, R. D., Cantu, C., Zhou, D. P., Saint-Mezard, P., Wang, V., Gao, Y., Yin, N. et al. (2005) Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 434, 525 – 529.

118 Fischer, K., Scotet, E., Niemeyer, M., Koebernick, H., Zerrahn, J., Maillet, S., Hurwitz, R., Kursar, M., Bonneville, M., Kaufmann, S. H. et al. (2004) Mycobacterial phosphati-

dylinositol mannoside is a natural antigen for CD1d-restricted T cells. *Proc.Natl. Acad. Sci.USA* 101, 10685 – 10690.
119 Kinjo, Y., Tupin, E., Wu, D., Fujio, M., Garcia-Navarro, R., Benhnia, M. R. E. I., Zajonc, D. M., Ben Menachem, G., Ainge, G. D., Painter, G. F. et al. (2006) Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat. Immunol.* 7, 978 – 986.
120 Kawahara, K., Moll, H., Knirel, Y. A., Seydel, U. and Zahringer, U. (2000) Structural analysis of two glycosphingolipids from the lipopolysaccharide-lacking bacterium Sphingomonas capsulata. *Eur.J.Biochem.* 267, 1837 – 1846.
121 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.
122 Wu, D., Xing, G. W., Poles, M. A., Horowitz, A., Kinjo, Y., Sullivan, B., Bodmer-Narkevitch, V., Plettenburg, O., Kronenberg, M., Tsuji, M. et al. (2005) Bacterial glycolipids and analogs as antigens for CD1d-restricted NKT cells. *Proc. Natl. Acad. Sci.USA* 102, 1351 – 1356.
123 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.
124 Buschard, K., Hanspers, K., Fredman, P. and Reich, E. P. (2001) Treatment with sulfatide or its precursor, galactosylceramide, prevents diabetes in NOD mice. *Autoimmunity* 34, 9 – 17.
125 Terabe, M., Swann, J., Ambrosino, E., Sinha, P., Takaku, S., Hayakawa, Y., Godfrey, D. I., Ostrand-Rosenberg, S., Smyth, M. J. and Berzofsky, J. A. (2005) A nonclassical non-Valpha14Jalpha18 CD1d-restricted (type II) NKT cell is sufficient for down-regulation of tumor immunosurveillance. *J. Exp. Med.* 202, 1627 – 1633.
126 Terabe, M., Matsui, S., Noben-Trauth, N., Chen, H., Watson, C., Donaldson, D. D., Carbone, D. P., Paul, W. E. and Berzofsky, J. A. (2000) NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat. Immunol.* 1, 515 – 520.
127 Terabe, M., Matsui, S., Park, J. M., Mamura, M., Noben-Trauth, N., Donaldson, D. D., Chen, W., Wahl, S. M., Ledbetter, S., Pratt, B. et al. (2003) Transforming growth factor-beta production and myeloid cells are an effector mechanism through which CD1d-restricted T cells block cytotoxic T lymphocyte-mediated tumor immunosurveillance: abrogation prevents tumor recurrence. *J. Exp. Med.* 198, 1741 – 1752.
128 Van, R., I, Young, D. C., Im, J. S., Levery, S. B., Illarionov, P. A., Besra, G. S., Porcelli, S. A., Gumperz, J., Cheng, T. Y. and Moody, D. B. (2004) CD1d-restricted T cell activation by nonlipidic small molecules. *Proc. Natl. Acad. Sci.USA* 101, 13578 – 13583.
129 Durante-Mangoni, E., Wang, R., Shaulov, A., He, Q., Nasser, I., Afdhal, N., Koziel, M. J. and Exley, M. A. (2004) Hepatic CD1d expression in hepatitis C virus infection and recognition by resident proinflammatory CD1d-reactive T cells. *J. Immunol.* 173, 2159 – 2166.
130 Exley, M. A., He, Q., Cheng, O., Wang, R. J., Cheney, C. P., Balk, S. P. and Koziel, M. J. (2002) Cutting edge: compartmentalization of Th1-like noninvariant CD1d-reactive T cells in hepatitis C virus-infected liver. *J. Immunol.* 168, 1519 – 1523.
131 Exley, M. A., Tahir, S. M., Cheng, O., Shaulov, A., Joyce, R., Avigan, D., Sackstein, R. and Balk, S. P. (2001) A major fraction of human bone marrow lymphocytes are Th2-like CD1d-reactive T cells that can suppress mixed lymphocyte responses. *J. Immunol.* 167, 5531 – 5534.
132 Fuss, I. J., Heller, F., Boirivant, M., Leon, F., Yoshida, M., Fichtner-Feigl, S., Yang, Z., Exley, M., Kitani, A., Blumberg, R. S. et al. (2004) Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J. Clin. Invest* 113, 1490 – 1497.
133 Porcelli, S., Morita, C. T. and Brenner, M. B. (1992) CD1b restricts the response of human CD4 – 8- T lymphocytes to a microbial antigen. *Nature* 360, 593 – 597.
134 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.
135 Moody, D. B., Reinhold, B. B., Guy, M. R., Beckman, E. M., Frederique, D. E., Furlong, S. T., Ye, S., Reinhold, V. N., Sieling, P. A., Modlin, R. L. et al. (1997) Structural requirements for glycolipid antigen recognition by CD1b-restricted T cells. *Science* 278, 283 – 286.
136 Moody, D. B., Guy, M. R., Grant, E., Cheng, T. Y., Brenner, M. B., Besra, G. S. and Porcelli, S. A. (2000) CD1b-mediated T cell recognition of a glycolipid antigen generated from mycobacterial lipid and host carbohydrate during infection. *J. Exp. Med.* 192, 965 – 976.
137 Gilleron, M., Stenger, S., Mazorra, Z., Wittke, F., Mariotti, S., Bohmer, G., Prandi, J., Mori, L., Puzo, G. and De Libero, G. (2004) Diacylated sulfoglycolipids are novel mycobacterial antigens stimulating CD1-restricted T cells during infection with Mycobacterium tuberculosis. *J. Exp. Med.* 199, 649 – 659.
138 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. et al. (2000) CD1c-mediated T-cell recognition of isoprenoid glycolipids in Mycobacterium tuberculosis infection. *Nature* 404, 884 – 888.
139 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. et al. (2004) Mycobacterium tuberculosis pks12 produces a novel polyketide presented by CD1c to T cells. *J. Exp. Med.* 200, 1559 – 1569.
140 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. et al. (2004) T cell activation by lipopeptide antigens. *Science* 303, 527 – 531.
141 Kawashima, T., Norose, Y., Watanabe, Y., Enomoto, Y., Narazaki, H., Watari, E., Tanaka, S., Takahashi, H., Yano, I., Brenner, M. B. et al. (2003) Cutting edge: major CD8 T cell response to live bacillus Calmette-Guerin is mediated by CD1 molecules. *J. Immunol.* 170, 5345 – 5348.
142 Sieling, P. A., Ochoa, M. T., Jullien, D., Leslie, D. S., Sabet, S., Rosat, J. P., Burdick, A. E., Rea, T. H., Brenner, M. B., Porcelli, S. A. et al. (2000) Evidence for human CD4+ T cells in the CD1-restricted repertoire: derivation of mycobacteria-reactive T cells from leprosy lesions. *J. Immunol.* 164, 4790 – 4796.
143 Sieling, P. A., Torrelles, J. B., Stenger, S., Chung, W., Burdick, A. E., Rea, T. H., Brennan, P. J., Belisle, J. T., Porcelli, S. A. and Modlin, R. L. (2005) The human CD1-restricted T cell repertoire is limited to cross-reactive antigens: implications for host responses against immunologically related pathogens. *J. Immunol.* 174, 2637 – 2644.
144 Hiromatsu, K., Dascher, C. C., LeClair, K. P., Sugita, M., Furlong, S. T., Brenner, M. B. and Porcelli, S. A. (2002) Induction of CD1-restricted immune responses in guinea pigs by immunization with mycobacterial lipid antigens. *J. Immunol.* 169, 330 – 339.
145 Stenger, S., Hanson, D. A., Teitelbaum, R., Dewan, P., Niazi, K. R., Froelich, C. J., Ganz, T., Thoma-Uszynski, S., Melian, A., Bogdan, C. et al. (1998) An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science* 282, 121 – 125.
146 Dascher, C. C., Hiromatsu, K., Xiong, X., Morehouse, C., Watts, G., Liu, G., McMurray, D. N., LeClair, K. P., Porcelli, S. A. and Brenner, M. B. (2003) Immunization with a mycobacterial lipid vaccine improves pulmonary pathology in the guinea pig model of tuberculosis. *Int. Immunol.* 15, 915 – 925.
147 Fairhurst, R. M., Wang, C. X., Sieling, P. A., Modlin, R. L. and Braun, J. (1998) CD1 presents antigens from a gram-

negative bacterium, Haemophilus influenzae type B. *Infect. Immun.* 66, 3523 – 3526.
148 Vincent, M. S., Xiong, X., Grant, E. P., Peng, W. and Brenner, M. B. (2005) CD1a-, b-, and c-restricted TCRs recognize both self and foreign antigens. *J. Immunol.* 175, 6344 – 6351.
149 Porcelli, S., Brenner, M. B., Greenstein, J. L., Balk, S. P., Terhorst, C. and Bleicher, P. A. (1989) Recognition of cluster of differentiation 1 antigens by human CD4-CD8-cytolytic T lymphocytes. *Nature* 341, 447 – 450.
150 Shamshiev, A., Gober, H. J., Donda, A., Mazorra, Z., Mori, L. and De Libero, G. (2002) Presentation of the same glycolipid by different CD1 molecules. *J. Exp. Med.* 195, 1013 – 1021.
151 Shamshiev, A., Donda, A., Carena, I., Mori, L., Kappos, L. and De Libero, G. (1999) Self glycolipids as T-cell autoantigens. *Eur. J. Immunol.* 29, 1667 – 1675.
152 Tanaka, Y., Morita, C. T., Tanaka, Y., Nieves, E., Brenner, M. B. and Bloom, B. R. (1995) Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. *Nature* 375, 155 – 158.
153 Bukowski, J. F., Morita, C. T., Tanaka, Y., Bloom, B. R., Brenner, M. B. and Band, H. (1995) V gamma 2V delta 2 TCR-dependent recognition of non-peptide antigens and Daudi cells analyzed by TCR gene transfer. *J. Immunol.* 154, 998 – 1006.
154 Gober, H. J., Kistowska, M., Angman, L., Jeno, P., Mori, L. and De Libero, G. (2003) Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J. Exp. Med.* 197, 163 – 168.
155 Faure, F., Jitsukawa, S., Miossec, C. and Hercend, T. (1990) CD1c as a target recognition structure for human T lymphocytes: analysis with peripheral blood gamma/delta cells. *Eur. J. Immunol.* 20, 703 – 706.
156 Spada, F. M., Grant, E. P., Peters, P. J., Sugita, M., Melian, A., Leslie, D. S., Lee, H. K., van Donselaar, E., Hanson, D. A., Krensky, A. M.et al. (2000) Self-recognition of CD1 by gamma/delta T cells: implications for innate immunity. *J. Exp. Med.* 191, 937 – 948.
157 Leslie, D. S., Vincent, M. S., Spada, F. M., Das, H., Sugita, M., Morita, C. T. and Brenner, M. B. (2002) CD1-mediated gamma/delta T cell maturation of dendritic cells. *J. Exp. Med.* 196, 1575 – 1584.
158 Russano, A. M., Agea, E., Corazzi, L., Postle, A. D., De Libero, G., Porcelli, S., de Benedictis, F. M. and Spinozzi, F. (2006) Recognition of pollen-derived phosphatidyl-ethanolamine by human CD1d-restricted gamma delta T cells. *J. Allergy Clin. Immunol.* 117, 1178 – 1184.
159 Okamoto, N., Kanie, O., Huang, Y. Y., Fujii, R., Watanabe, H. and Shimamura, M. (2005) Synthetic alpha-mannosyl ceramide as a potent stimulant for an NKT cell repertoire bearing the invariant Valpha19-Jalpha26 TCR alpha chain. *Chem. Biol.* 12, 677 – 683.
160 Scotet, E., Martinez, L. O., Grant, E., Barbaras, R., Jeno, P., Guiraud, M., Monsarrat, B., Saulquin, X., Maillet, S., Esteve, J. P. et al. (2005) Tumor recognition following Vgamma9V-delta2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein A-I. *Immunity* 22, 71 – 80.