Published in final edited form as:
Cell Mol Life Sci. 2014 August ; 71(16): 3069–3079. doi:10.1007/s00018-014-1603-6.

The CD1 size problem: lipid antigens, ligands, and scaffolds

Dalam Ly and **D. Branch Moody**
Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Smith Building, Room 538, One Jimmy Fund Way, Boston, MA 02115, USA

D. Branch Moody: bmoody@partners.org

Abstract

Whereas research on CD1d has emphasized a few glycosyl ceramides, the broader family of four human CD1 antigen-presenting molecules binds hundreds of distinct self-lipids. Individual lipid types bind within CD1 grooves in different ways, such that they partially fill the groove, match the groove volume, or protrude substantially from the groove. These differing modes of binding can now be connected to differing immunological functions, as individual lipids can act as stimulatory antigens, inhibitory ligands, or space-filling scaffolds. Because each type of CD1 protein folds to produce antigen-binding grooves with differing sizes and shapes, CD1a, CD1b, CD1c, CD1d, and CD1e have distinct mechanisms of capturing self-lipids and exchanging them for foreign lipids. The size discrepancy between endogeneous lipids and groove volume is most pronounced for CD1b. Recent studies show that the large CD1b cavity can simultaneously bind two self-lipids, the antigen, and its scaffold lipid, which can be exchanged for one large bacterial lipid. In this review, we will highlight recent studies showing how cells regulate lipid antigen loading and the roles CD1 groove structures have in control of the presentation of chemically diverse lipids to T cells.

Keywords

CD1; Lipid antigens; Spacers; Scaffolds; Structures

Introduction

Nearly two decades ago, CD1 proteins were shown to mediate T cell autoreactivity and present lipid antigens to T cells [1–4]. Since that time, crystal structures of T cell receptors (TCRs) bound to CD1-lipid complexes have established the basic model of lipid antigen recognition by T cells [5–14] (Fig. 1). The aliphatic hydrocarbon chains of lipids insert into the hydrophobic pockets of CD1 grooves, allowing the carbohydrate, peptide, phosphate, or sulfate moieties to protrude through a portal positioned between two α-helices to the surface of CD1, where they directly contact TCRs. However, within the constraints of this general mechanism, there is considerable diversity in the efficiency of antigen capture, stringency of antigen release from the groove, or even the number of lipids bound in the groove at any time. Here we review recent studies that show how CD1 proteins act in cells to capture

Correspondence to: D. Branch Moody, bmoody@partners.org.

surprisingly hundreds of self-lipid ligands or antigens. This review focuses on the problem of how non-polymorphic CD1 proteins, which are hollow cavities with a finite and defined volume, can capture lipids of diverse size and structure. For major histocompatibility class I proteins (MHC I), this basic function is accomplished through generation of nonamer peptides by proteases from precursor proteins of highly divergent size. In contrast, cells do not typically trim lipids so that they are of one size that match the volume of CD1 grooves. The aliphatic hydrocarbon chains of CD1-presented antigens are highly resistant to covalent cleavage [15], and the CD1 system relies mainly on expressing many types of CD1 proteins (CD1a, CD1b, CD1c, CD1d, CD1e) with differing groove volumes, combined with a combination of scaffolds, spacers, portals, and escape hatches to match ligand size to groove volume.

Structure of CD1

The first crystal structure of CD1 revealed a molecule resembling MHC class I [16]. All known CD1 antigen-presenting molecules consist of a heavy chain comprised of extracellular antigen-binding domain, a transmembrane domain, and an intracellular tail, which directs intracellular trafficking. The extracellular domain consists of an MHC fold with two anti-parallel α-helices (α1 and α2) flanking a floor composed of a six-strand α sheet. The α1 and α2 helices are supported by the α3 domain, which non-covalently interacts with β2-microglobulin (β2m) [16, 17]. CD1 proteins differ from the MHC peptide-binding grooves in at least two key aspects. The inner surface of the CD1 groove is largely lined by hydrophobic residues, and the α1 helices of CD1 proteins extend “upwards”, so that CD1 proteins have a larger vertical depth between the floor and the antigen entry portal.

For example, the first CD1 crystal structure showed that murine CD1d has two deep pockets named the A′ and F′, based on the analogous locations of the A and F pockets of MHC I [16]. All four types of human CD1 antigen-presenting molecules, CD1a, CD1b, CD1c, and CD1d, have now been crystallized [16, 18–21]. CD1b differs most substantially from the rest in that it is larger (~2,200 $Å^3$) and has two additional pockets, C′ and T′. The centrally located C′ pocket is named after the C pocket in MHC I, and the T′ pocket name is derived from the “tunnel” found only in the deepest point of the CD1b cavity. A crystal structure of human CD1b, which was the first solved structure bound to lipid ligands [18], showed that lipids are orientated such that hydrophobic alkyl chains are buried deep within the binding pockets of CD1, with exposure of hydrophilic head groups at the protein surface. Each CD1 structure reveals unique aspects of the antigen-binding grooves present in CD1a, CD1b, CD1c, and CD1d, which differ in volume and number of binding pockets (Fig. 2). Whereas all CD1 proteins have a main portal, located above the F′ pocket (F′ portal), CD1b and CD1c have additional portals (accessory portals) that are thought to allow large lipids to escape from the interior of the groove to the outer surface of the CD1 protein in ways that do not directly control TCR binding. The key points of structural divergence among individual CD1 isoforms are considered in turn.

CD1a

CD1a crystal structures have been solved as liganded complexes with the self-antigen, sulfatide, and the mycobacterial lipopeptide, dideoxymycobactin. These structures show that CD1a has the smallest binding groove among human CD1 isoforms, with a volume of ~1,350 $Å^3$ [19, 22]. CD1a has two binding pockets, the A′ and F′, and no known accessory portals, although a small gap in the lateral wall of the F′ pocket might allow lipids to protrude laterally so that they do not remain beneath and do not traverse the plane of TCR contact. As is the case for most CD1 proteins, the A′ pocket of CD1a is separated from the outer surface of the protein by a roof-like structure above the A′ pocket, known as the A′ roof (Fig. 2). The roof is formed by interdomain contacts between the α1 and α2 helices and likely limits direct access of antigens to the A′ pocket. Instead, the region just above the F′ pocket is open to solvent, so antigens likely access the groove above the F′ pocket, through the F′ portal (Fig. 2) [19]. Whereas the A′ pocket of other CD1 isoforms connect directly with other pockets, the A′ pocket of CD1a curves around a central pole in the A′ pocket and then abruptly terminates deep inside the groove at a molecular barrier formed by Val28. As such, the A′ pocket of CD1a likely acts as a “molecular ruler” for selective binding of alkyl chains of discrete length [22], a prediction confirmed by preferential presentation of dideoxymycobactins with the optimal chain length (~C20), which matches the volume of this pocket [23].

CD1b

Several CD1b-lipid complexes have been reported [18, 24–26]. Amongst all CD1 isoforms, CD1b has the largest binding groove (~2,200 $Å^3$) [18]. The striking structural feature of the CD1b groove is its large volume comprised of four interconnected pockets (Fig. 2). Only CD1b is known to have a deeply buried T′ tunnel, a laterally oriented pocket, which connects the vertically oriented A′ and F′ pockets across the bottom of the groove to form a A′T′F′ super channel [18]. The A′T′F′ superchannel allows CD1b to bind extremely long meromycolate branches found in three types of CD1b-presented mycolyl lipids antigens: free mycolic acid, glucose monomycolate, and glycerol monomycolate [3, 27, 28]. Other CD1 isoforms lack this long channel and are not known to present long-chain mycolyl lipids. Additionally, it has been observed that an accessory portal, known as the C′ portal, connects the inner pocket of CD1b to the outer surface [18].

CD1c

CD1c has two hydrophobic antigen-binding pockets, A′ and F′, which create an antigen-binding volume capacity of ~1,780 $Å^3$ [20]. This structure has been solved in complex with mannosyl phosphomycoketide antigens, which have a series of five methyl branches in the alkyl chains [20, 29]. This branched lipid is positioned in the toroidal A′ pocket of CD1c with the all (S) branches pointed outward toward the larger concave surface, as the lipid descends downward into the groove. This mechanism provides a precise fit for CD1c with two known mycoketide lipids [30]. The independent identification of mannosyl-phosphomycoketide and phosphomycoketide as two branched lipids that function as CD1c

presented antigens suggests that the mycoketide backbone might be specialized to bind to CD1c.

Additionally, CD1c presents a lipopeptide with one alkyl chain [31]. Thus, the three known foreign or exogenous antigens for the CD1c system all have one alkyl chain. The A′ pocket is largely continuous with the F′ pocket, but opens to the exterior through an accessory portal known as the D′ portal, located under the α1 helix [20]. Unique amongst the CD1 isoforms, the F′ pocket of CD1c is the most exposed to solvent, potentially allowing for more promiscuous lipid occupancy and raising the possibility that antigens with lipid tails that are larger than those present in currently known antigens might also be presented by CD1c [32]. The crystallized CD1-lipid complex shows that the groove simultaneously binds two lipids. The A′ pocket captures the mycoketide backbone, and the F′ pocket contains a C12 hydrocarbon chain, which might be a detergent that was incidentally captured during crystallization and fills the space not occupied by the larger mycoketide lipid [20]. Because all three of the known CD1c antigens have only one alkyl chain that is smaller than the groove volume, spacer lipids might influence CD1c's antigen-presenting function in ways that are analogous to their proven role in CD1b, as discussed below.

CD1d

Human and mouse CD1d represent the most extensively studied CD1 proteins, which have been crystallized with numerous self- and exogenous antigens, including the high affinity superagonists, α-galactosylceramides [5, 6, 8–11, 16, 33, 34]. CD1d contains A′ and F′ pockets, which provide total cavity volume of ~1,650 $Å^3$, and no accessory portals are known (Fig. 2). The sphingosine chain of α-galactosylceramides binds within the F′ pocket with the fatty acyl tail occupying the A′ pocket, thereby positioning the galactosyl head group on the surface. For a detailed description of CD1d and its ligands, see recent reviews for additional reading [35–37].

CD1e

CD1e is the only isoform that is not expressed on the cell surface, but instead traffics exclusively within the endolysosomal network, where it is cleaved to become a soluble protein. In contrast to the antigen-presenting function of other CD1 proteins, CD1e functions as a lipid-binding protein [38]. The crystal structure of CD1e shows a wide, solvent-exposed antigen-binding domain (Fig. 2) with A′ and F′ pockets not clearly separated from one another [21]. This groove has a volume of ~2,000 $Å^3$, and has a proposed in role in lipid transfer to CD1 antigen-presenting molecules [21, 38].

Accessory portals

In some [18] but not all [26] crystal structures, CD1b has been observed to have an accessory portal known as the C′ portal because it directly connects the interior of the C′ pocket to the outer surface of CD1b. Unlike the F′ portal, which is adjacent to the plane of TCR contact, the C′ portal is located beneath the α1 helix, distant from the site of TCR contact (Fig. 2). Therefore, even if present only transiently, this portal might represent a structural modification to allow the termini of particularly long mycolates to protrude

slightly from the groove in ways that promote binding of lipids without affecting TCR contact [15]. Similarly, CD1c has at least one accessory portal known as the D′ portal located under the α1 helix, which exposes the A′ pocket to the outer surface of CD1c [20]. In crystal structures, it has not been possible to directly observe lipid protrusion to the outer surface of CD1 through these portals. The lack of electron density observed outside the portal does not suggest that lipids do not protrude, but instead more likely reflects the fact that such lipids would not take on an ordered structure. Supporting this general idea, sulfatides added to CD1a to form liganded crystal structures had longer alkyl chains than those observed as ordered density within the groove, a finding that was interpreted as likely lipid protrusion as unordered structures through the lateral wall of the F′ pocket [19]. Also, the CD1b groove is predicted to have the capacity for C72–76 lipids, yet it actually binds C80–86 glucose mycolates, a finding interpreted as lipids likely protruding through the C′ portal [15]. In summary, the interior grooves of CD1a, CD1c, and CD1d are similar in size, but differ in the nature of the accessory portals, which likely represent isoform-specific adaptations to carry lipids of differing chain length.

The size problem for CD1b

In the MHC system, cellular and viral proteins greatly exceed the volume of MHC I and II. Therefore, cellular processing of proteins into peptides of 8 to ~30 amino acids is nearly universally required for MHC binding and recognition. In contrast, the lipids eluted from CD1a [39], CD1c, and CD1d proteins [40, 41] show a good size match of most known ligands to the ~1,700 $Å^3$ volume. Therefore, the simplest model for CD1 antigen presentation is that cells produce ligands that naturally approximate the size of the grooves that capture them, and so the aliphatic alkyl chains of lipids do not need to universally undergo antigen-processing reactions. Also, this simple model predicts that CD1 binds lipids with one-to-one stoichiometry. Although deglycosylation reactions that modify head-groups of sphingolipids and mycoketides are known [30, 42], the natural size match of the naturally occurring alkyl chains to CD1 groove volume, along with data that such antigens can be loaded onto CD1 proteins in APC-free systems, strongly support a working model that trimming of alkyl chains is not a usual antecedent to lipid-antigen loading.

However, the natural antigens for CD1b range in length from C30 to C80, and so include antigens that would not fully occupy the groove, which is estimated to hold C72–76 lipids, as well as those, like C80 mycolates, which apparently exceed the groove volume. Studies of long-chain mycolates bound to CD1b directly ruled out lipid trimming, suggesting that they bind in an intact form [43]. At the other end of the length range, most cellular lipids, which form the pool of self-lipid antigens, have a combined lipid length of about C32–C42, which is substantially smaller than the CD1b groove [44]. In fact, no common self-lipids approximate the expected C72 lipid length that fits within the CD1b groove, so there is a basic size mismatch between endogenous ligands and the groove volume of CD1b. Therefore, the question arises: what are the natural endogenous self-lipids for CD1b, and how could they fill this large groove volume? The problem of size was experimentally confirmed and highlighted by a study by Huang and colleagues that assess the average mass of ligands eluted from CD1a, CD1b, CD1c, and CD1d lipids [44]. Although CD1b has a larger volume, it bound a range of endogenous ligands that was similar in mass compared to

ligands bound by CD1 proteins with smaller grooves. This study confirmed the size mismatch and showed that it is true for most or all ligands bound to CD1b.

Spacers and scaffolds

A theoretical solution to this problem would be that CD1a, CD1c, and CD1d typically bind self-lipids with 1–1 stoichiometry, but CD1b might bind two lipids at once. If portals allow concomitant capture of lipid antigen and a spacer lipid, then their combined length might match the groove volume. Direct evidence for such spacer lipids derived from early studies of CD1b, which bound phosphatidylinositol or GM2 ganglioside, whose lipid tails occupied only the A′ and C′ pockets. The T′ and F′ pockets were occupied by two electron densities of about ~C16 in length, which were assumed to be detergent molecules used during the protein refolding [18]. This observation led to the hypothesis that such artificial spacer lipids might have a naturally occurring equivalent that might normally fill partially empty grooves, starting the search for natural spacer lipids.

Several crystal structures of CD1 in complex with lipid antigens have revealed the presence of densities interpreted as aliphatic hydrocarbon chains within the binding pockets, which do not correspond to the alkyl chains of the antigens that protrude “upwards” through the F′ portal. In agreement with the earlier observations of Gadola et al. [18], studies have shown that naturally occurring spacer lipids were present when native refolding conditions were used to crystallize CD1d with short-chain α-galactosylceramide [45, 46] and CD1b with phosphatidylcholine [26]. Electron density measurements, as well as detection by native mass spectrometry revealed natural 16–40 carbon length “spacers” that serve to fill the remainder of the unoccupied CD1 pocket [26, 45, 46]. Additionally, lipid spacers have often been found in structures of CD1 in complex with monoacyl antigens. Structures of dideoxymycobactins in complex with CD1a and mannosyl-phosphomycoketide with CD1c (Fig. 3) identify small hydrocarbon chains that likely correspond to spacer lipids within the F′ pocket [20, 22]. Similarly lyso-phosphatidylcholine, in complex with CD1d, identifies a hydrocarbon chain in the unoccupied A′ pocket [8]. Dynamic modeling of CD1 molecules in lipid-bound and lipid-free states indicates that spacers may act to stabilize CD1 complexes from collapse. Unliganded CD1 proteins show collapse of hydrophobic pockets in the absence of lipids, resulting in closure of the helices. Surprisingly, the CD1a-binding cavity was interpreted as remaining preserved in the unliganded state [47].

Two studies independently identified the natural spacer lipids in CD1b as diacylglycerols and deoxyceramides [25, 44]. Natural sulfoglycolipids are a family of mycobacterial polyketide lipids that contain a heterogeneous mixture of fatty acids and polyketides linked to sulfotrehalose [48]. Sulfoglycolipid in complex with CD1b was found to release diacylglycerol in mass spectrometry. Further, the eluted spacer lipid corresponded in size to an electron density within the T′ and F′ pockets, which was positioned below the sulfoglycolipid (Fig. 3) [25]. The binding of sulfoglycolipids to CD1b results in a conformational change near the F′ pocket, allowing amino acids glutamate 80 within the α1 helix and tyrosine 151 within the α2 helix to interact. This interaction is thought to prevent the egress of diacylglycerol from the F′ pocket, thus stabilizing the CD1b-lipid complex [25].

A separate lipidomics study of all CD1b ligands found particularly hydrophobic lipids eluting selectively from CD1b, and identified diacylglycerol and deoxyceramides [44]. Further, adding diacylglycerols during the loading of CD1b-presented antigens resulted in increased T cell recognition of a short-chain form of glucose monomycolate, but abrogated recognition of long-chain glucose monomycolate. Thus, adding one large lipid, or instead adding two lipids whose total alkane chain length approximates the volume of the CD1b groove, leads to improved antigen recognition. Thus, the naturally occurring spacer lipids have an actual function in augmenting antigen recognition. Because crystal structures suggest that the natural spacer lipids are located in the T′ tunnel “beneath the antigen” the term “scaffold” emphasizes their upward lifting function [44]. Such scaffold lipids enhance recognition and so contrast with the side-by-side orientation of spacer lipids observed in CD1c, whose function, if any, on antigen recognition is unknown [20]. It is currently unknown what percentage of CD1b proteins are initially loaded with one or two lipids [44]. However, the relatively small average mass of all lipids eluted from CD1b relative to its large groove volume suggests that a large percentage of the initially formed CD1b-lipid complexes contain two or more lipid ligands [44]. With the broad range of structurally diverse ligands encountered in the endoplasmic reticulum and endosome, we speculate that scaffolding lipids might act to satisfy the energetic requirements for expelling water from hydrophobic surfaces that line the binding grooves of CD1. Stabilization of CD1 may influence antigen presentation. Studies using short-tail CD1 antigens have demonstrated that lipid length of the antigen will influence CD1-restricted T cell recognition glycolipid analogs [49, 50]. Though spacer lipids fill the remainder of unoccupied binding grooves, short-chain antigens may be less stable in vivo due to their inability to stably load within detergent-insoluble lipid rafts [51, 52].

Spacers are likely displaced when large exogenous lipids are encountered (Fig. 4). A highly consistent observation in the CD1b system is that C32 glucose monomycolate can be loaded on CD1b proteins at the cell surface, whereas C80 glucose monomycolate antigens have stringent requirements for exchange in the low-pH environment of lysosomes [15, 43]. We speculate that the smaller C32 antigen might exchange for the antigen located in the “upper” region of the groove (Fig. 4, blue), whereas loading the C80 antigen likely requires unloading of the more deeply positioned scaffold lipids as well (Fig. 4, red and blue). Supporting this hypothesis, spacers are not detected in the structure of CD1b in complex with long-chain glucose monomycolate (Fig. 3), which fully accommodate the CD1b-binding channel [24]. Detailed mechanisms of lipid exchange and spacer displacement are not completely understood, but the reversible denaturation of CD1b at low pH promotes antigen loss from the groove and renders CD1b proteins able to bind large lipids [53]. Thus, several predictions of the scaffold loss hypothesis have been experimentally verified.

CD1 proteins load lipids in distinct compartments

CD1 proteins are assembled in the endoplasmic reticulum in association with chaperones calnexin, calreticulin, and with the thiol oxidoreductase ERp57, which facilitate the folding and formation of disulfide bonds within the glycoprotein [54]. Once assembled and matured, CD1 associates with β2m and traffics through the *trans*-Golgi network towards the plasma membrane [55]. CD1 proteins are subsequently internalized and enter the endosomal

pathway. It is here that CD1 isoforms diverge and traffic into different endosomal compartments based on the amino acid sorting motifs of their cytoplasmic tails. CD1b, CD1c, and CD1d are targeted to the endosomal network by their expression of a tyrosine-based sorting motifs, YXXZ, where Y is tyrosine, X is any amino acid, and Z, is a bulky hydrophobic amino acid. This tail motif binds the adaptor protein complex 2 (AP2) to direct internalization to a variety of endosomal compartments via clathrin-coated pits [56]. CD1a does not contain any sorting motifs and is internalized through an AP-independent pathway, similar to MHC class I [57]. Human CD1b and mouse CD1d have tail motifs that mediate additional interactions with adaptor protein complex 3 (AP3), driving these antigen-presenting molecules into lysosomes. These interactions predict the observed localization of each CD1 isoform within the endolysosomal pathway. CD1a associates with the GTPases ADP-ribosylation factor 6, Ras-related protein Rab-22A, and ADP-ribosylation factor-like protein 13B, which regulate endocytic recycling traffic [58]. Lacking an endosomal localization motif, CD1a is mainly seen at the surface at steady state, whereas CD1b, CD1c, and CD1d substantially enter lysosomal-associated membrane protein 1 expressing compartments [59–61]. CD1e never reaches the cell surface, but traffics within the *trans*-Golgi network of immature dendritic cells and upon maturation reaches the late endosome lysosomal compartments where it is cleaved into its active form [38]. The isoform-specific trafficking patterns allow CD1 to sample and survey the endogenous and exogenous lipid contents that arrive from different cellular compartments.

ER assembly and lipid association

Throughout CD1 assembly and recycling, CD1 proteins sample and survey lipids from the ER and endosomal compartments [56]. Whereas many studies of trafficking focus on late events in endosomal recycling, the initial capture of self-ligands in the endoplasmic reticulum and egress to the surface are biologically important events that lead to the capture of self-lipids, so are emphasized here. The first endogenous lipids eluted from murine CD1d included phosphatidylinositol (PI) and phosphatidylinositol-glycans, which were assembled with CD1d in the ER [62, 63]. Whereas early studies suggested that phosphatidylinositol containing lipids might dominate the spectrum of endogenous CD1 ligands, later studies clearly show that the cellular ligands of CD1 proteins are diverse, and include neutral lipids, sphingolipids, and phospholipids [44].

Elution of secreted CD1d has shown that all major family members of glycerophospholipids and sphingolipids were found associated with CD1d [41, 64, 65]. The variety of glycerophospholipid head groups included unmodified phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) and previously identified PI. Sphingolipid species included sphingomyelin (SM), glycosphingolipids (GSL), and higher-order GM ganglio-sides [41, 64]. Diacyl species were predominant, but lyso-phospholipids, and tetra-acyl cardiolipins were also found. Further, analysis also revealed the presence of peroxisome-derived plasmalogen lipids [41, 65, 66]. In fact, under homeostatic conditions, CD1 molecules accumulate the most abundant lipids from both secretory and endosomal compartments [65]. For example, CD1d engineered with an ER retention signal is predominantly found associated with phosphatidylcholine, the most abundant lipid in the cell. In addition to

phosphatidylcholine, CD1d that have access to secretory and endosomal compartments was found associated with sphingomyelin and lysophospholipids, dominant lipids within the *trans*-Golgi network and lysosomes, respectively [65]. Thus, it seems that CD1 is capable of associating promiscuously with a variety of endogenous lipids. In contrast to invariant chain model in which one type of peptide predominates in the initially formed MHC class II complex, dozens [41] or hundreds [44] of hydrophobic lipids act to stabilize the initial CD1 formed in cells.

The ER resident protein, microsomal transfer protein (MTP), which promotes the assembly and secretion of large apolipoprotein B lipoproteins, was also found to function in CD1 lipid association [67]. MTP facilitates the transfer of triacylglycerol and glycerophospholipids from sites of synthesis to the nascent apolipoprotein B polypeptide [68] and can also associate with CD1d to influence phospholipid association with CD1d in cell-free systems [69]. The role of microsomal transfer protein in CD1 lipid presentation is substantiated in patients with abetalipoproteinemia (ABP), an autosomal recessive disorder caused by mutations in microsomal transfer protein. Patients with abetalipoproteinemia have low levels of apolipoprotein B in the plasma, as well as APCs that have reduced ability to activate CD1-restricted T cells [70]. Microsomal transfer protein deficiency affects the presentation of secretory as well as endosomal antigens that require lysosomal loading. For example, self-reactive CD1-restricted T cells, as well as NKT cells recognizing the glycosphingolipid isoglobotrihexosylceramide 3 (iGb3), or CD1b-restricted T cells recognizing mycoyl lipids, have reduced activity when presented by microsomal transfer protein-deficient antigen-presenting cells [69–72]. The surprising finding that an ER protein may also affect endosomal loading of CD1 lipids could be explained by the observation that CD1 molecules from MTP-deficient antigen-presenting cells are more susceptible to lysosomal degradation [70] and show reduced endosomal recycling [71]. The distal effect of microsomal transfer protein deficiency suggests that microsomal transfer proteins play a role in maintaining stability of CD1 molecules, possibly by regulating lipid availability or by acting as a CD1 lipid “editor”, selecting for lipids only capable of stabilizing CD1 molecule during assembly and biosynthesis.

Regulation by CD1 structure and trafficking

With the capacity of CD1 molecules to bind a variety of structurally unrelated self-lipid ligands [41, 44], the biophysical properties of CD1 isoforms and their distinct trafficking into various endosomal compartments regulate availability of antigens for presentation to T cells. For example, the shallow antigen-binding groove, early endosomal trafficking, and the stability of the CD1a-binding cavity in the absence of ligand [47], all contribute to the general conclusion that CD1a may have the least stringent loading requirements amongst CD1 isoforms. Consistent with this hypothesis, CD1a binds shorter alkyl chains that load at neutral pH or on the cell surface [17, 73].

CD1b and CD1d traffic into late endosome/lysosomes where acidic pH and endosomal cofactors help regulate lipid availability and CD1 complex formation. The capacity of CD1b to bind very long chain mycoyl lipids of mycobacteria requires acid in vitro [15] and occurs more readily in cells with intact acidification mechanisms [43, 74]. Mechanistically, acid

functions to neutralize acidic residues that would normally use their positively charged state to bind anionic residues and tether the α1 and α2 helices of CD1b together. Low pH interrupts these charge–charge interactions to promote partial unfolding to allow lipid exchange [53]. Besides pH, acidic compartments also contain cofactors such as saposins, which can catalyze the formation of CD1 lipid complexes by directly binding and solubilizing lipids or by destabilizing cellular membranes, thereby making lipid ligands available for CD1 capture [75–78].

Similar to CD1b, several examples exists that indicate the requirement of CD1d to traffic into late endosome/lysosomes where pH-dependent co-factors are required to process CD1d ligands to antigens [42, 79, 80]. The unique properties of CD1c also allow for a level of lipid regulation. The A′ pocket of CD1c binds branched alkyl chain lipids such as mycoketides [29, 30], and its promiscuous trafficking patterns allow it to bypass lysosomal compartments to present lipopeptides that would otherwise be degraded in lysosomes [31]. Thus, lipid complex formation is both regulated by the physical properties of CD1 isoforms and its ability to traffic into various endosomal compartments that promote more permissive loading environments (Fig. 4).

Concluding thoughts

The molecular immunology and cell biology have advanced since the discovery of CD1 and lipid reactive T cells over the last 20 years, such that an integrated model of cellular lipid capture and display is now emerging (Fig. 4). Developments in crystallography have revealed isoform-specific CD1-binding grooves, and show how lipids bind within CD1 isoforms. New methods in mass spectrometry and high-resolution analysis have shown that not only amphi-pathic lipids bind within CD1, but that highly hydrophobic spacer lipids also play important roles in maintaining CD1 stability and regulation. The ability of CD1 to ubiquitously associate with the majority of cellular self-lipids suggests a potential role of self-lipids in maintaining lipid-reactive T cell homeostasis or T cell selection. Indeed, data has shown that self-antigen reactive CD1a-restricted T cells are very common amongst individuals and may have roles in skin homeostasis [81, 82]. Emerging tools such as humanized mice [83] CD1 transgenic mice [84], and human CD1 tetramers [30, 85–87] will shed light on the role of T cell recognition of lipids in vivo.

Acknowledgments

This work was supported by the Burroughs Wellcome Fund for Translational Research, NIAID R01 AI04393 and R01 AR 048632.

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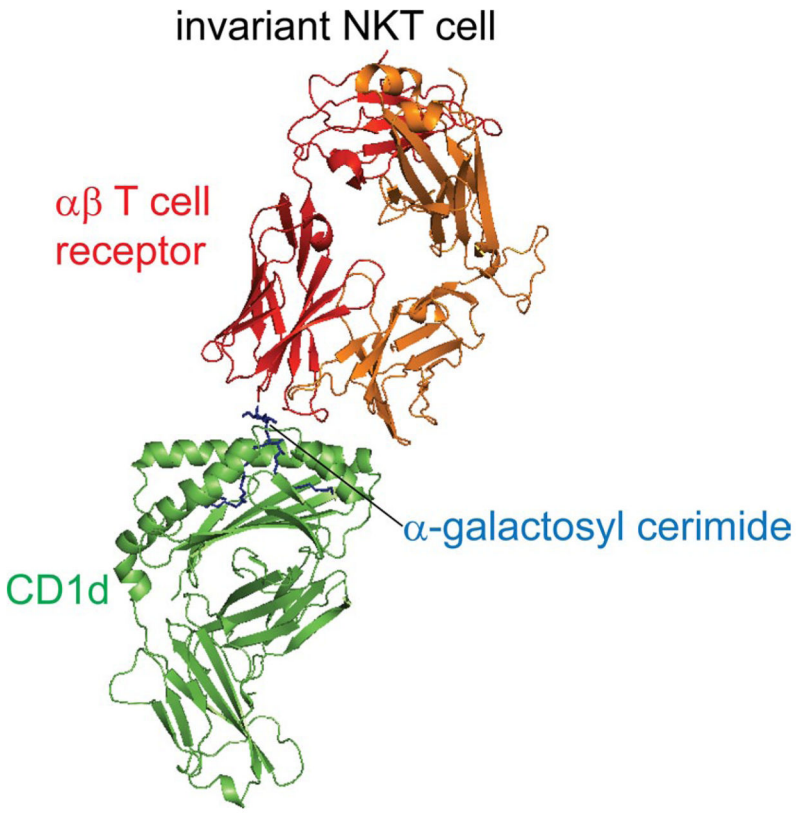

Fig. 1.

CD1d and TCR complexes. The invariant natural killer T cell TCR recognizes the ligand-CD1d complex (*green*) using predominantly the TCRα chain (*red*) [figure generated from RCSB protein data bank files 2PO6 (iNKT-CD1d)]

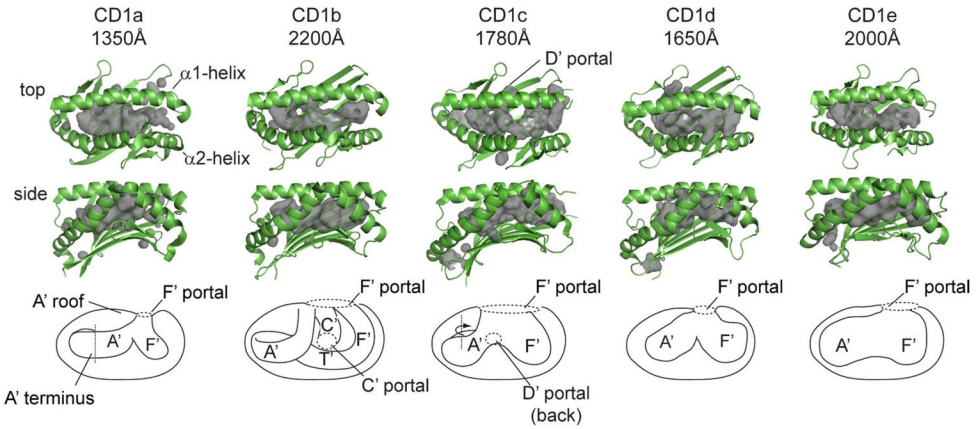

Fig. 2.

CD1 isoforms each have unique-antigen binding groves and capacities. CD1 isoforms have differing groove architecture, as revealed by crystal structures of CD1 with ligands: CD1a with dideoxymycobactin, CD1b with C55 glucose monomycolate, CD1c with mannosyl-phosphomycoketide, CD1d with α-galactosyl-ceramide, and CD1e alone. CD1 structures are rendered in *green* with cavity surface highlighted in *gray* and schematic of cavity [figures were generated from RCSB protein data bank files for 1XZO (CD1a), 1UQS (CD1b), 3OV6 (CD1c), 2PO6 (CD1d), and 3S6C (CD1e)]

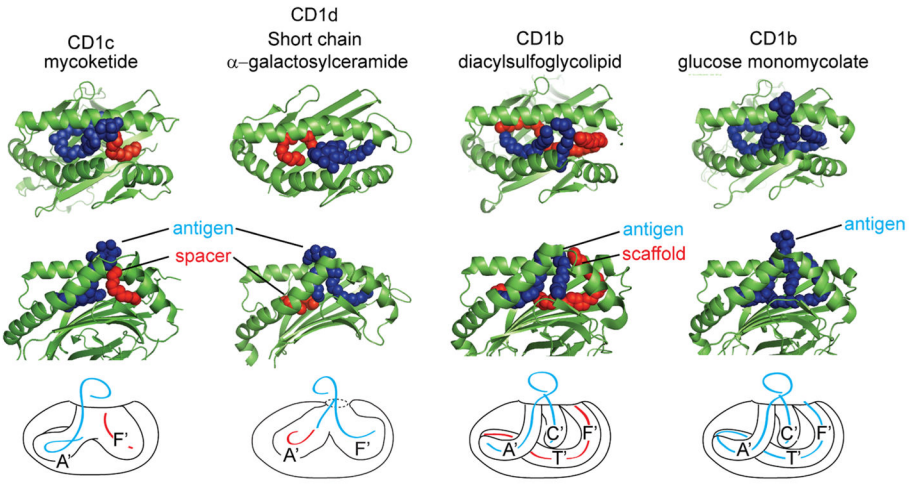

Fig. 3.

Hydrophobic spacer lipids can fill the empty cavity of CD1 isoforms as shown in the structure CD1c with mannosyl-phosphomycoketide (MPM) and CD1d with short-chain α-galactosylceramide (PBS-25). When higher affinity lipids are present in permissive environments, ligands and spacers must both be displaced during antigen exchange reactions. To illustrate this, CD1 is rendered with antigenic lipids in *blue* with hydrophobic spacer lipids in *red*, with schematic [figures were generated from RCSB protein data bank files 3OV6 (CD1c-MPM), 1Z5L (CD1d-PBS-25), 3T8X (CD1b-sulfoglycolipid), and 1UQS (CD1b-GMM)]

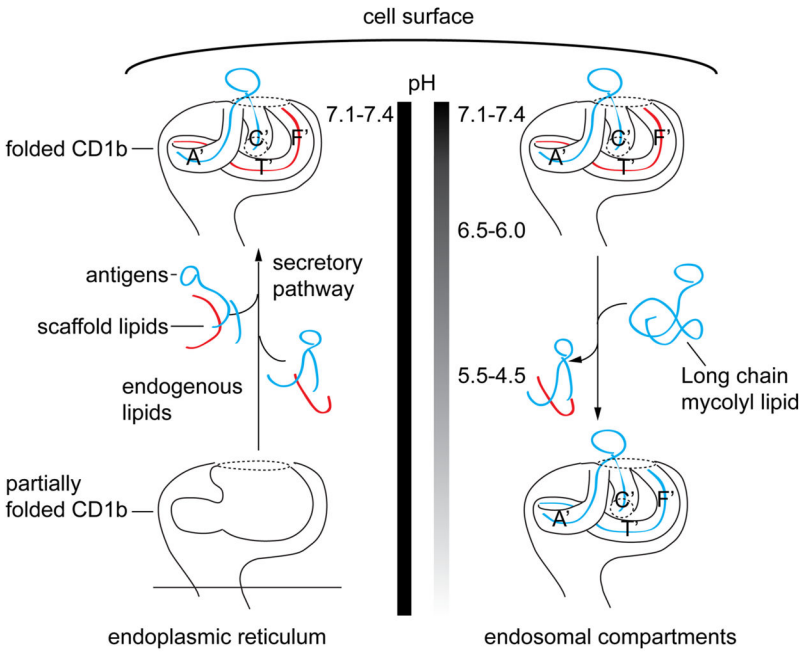

Fig. 4.

Endosomal recycling of CD1b and pH. CD1 is synthesized in the ER in the presence of a variety of lipid ligands, which help to stabilize the protein-lipid complex. CD1b then is shuttled to the cell surface within the secretory pathway, where further lipid exchange can take place with endogenous or exogenous lipids. CD1b is re-internalized and sorted based on isoform-specific sorting motifs into early endosomes or enter late endosome/lysosomes. Lipids entering with CD1b or from other endosomal compartments can be generated by endosomal co-factors. Acidic pH alters the physical properties of CD1b, which promotes lipid exchange in an editing process that allows lipids with higher affinity for CD1b to be loaded in late endosomes before recycling back to the cell surface. Whereas the ability of cell surface CD1b to bind only short-chain lipids has been long known, spacers now provide a candidate mechanism that explains why short-chain lipids have lower stringency loading requirements: exogenous short-chain lipids would only need to exchange with the superficially seated antigen on top, whereas long-chain lipids would require expulsion of antigens and deeply seated spacers