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CD1 assembly and the formation of CD1–antigen complexes

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The CD1 antigen presentation system presents lipid antigens to effector T cells, which have diverse roles in antimicrobial responses, antitumor immunity and in regulating the balance between tolerance and autoimmunity. The trafficking of CD1 molecules and lipid antigens facilitates their intersection and binding in specific intracellular compartments. Recent studies have now identified unexpected accessory molecules that are critical to CD1 assembly and lipid loading. The atomic structures of CD1–antigen complexes have defined both the orientation of polar headgroups between the α1 and α2 helices of CD1 and the manner in which distinct CD1 isoforms bind a range of lipids that have different lengths and numbers of hydrocarbon chains.

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Abbreviations

αGalCer	α-galactosylceramide
β_2m	beta-2 microglobulin
AP	adaptor protein
DC	dendritic cell
DDM	didehydroxymycobactin
ER	endoplasmic reticulum
GMM	glucose monomycolate
Ii	invariant chain
LAM	lipoarabinomannan
MTP	microsomal triglyceride transfer protein
NKT	natural killer T
PtdIns	phosphatidylinositol
SAP	sphingolipid activator protein
TCR	T cell receptor

Introduction

CD1 molecules are glycoproteins composed of a heavy chain noncovalently linked to beta-2 microglobulin (β_2m), similar to MHC class I. Unlike MHC class I, however, CD1 molecules have evolved hydrophobic channels for binding hydrocarbon alkyl chains, and have cytoplasmic tails that target them to distinct endocytic compartments. CD1a traffics through the early endocytic system, especially the recycling compartment; CD1c and human CD1d (hCD1d) traffic extensively throughout the endocytic system, whereas CD1b and murine CD1d (mCD1d) traffic deeply in the endocytic system and predominantly localize in late endosomes and lysosomes [1–3]. The broad ability of CD1 to survey endocytic compartments is functionally analogous to the different modes of traffic and localization of MHC class I and II molecules, which are tailored to survey and acquire peptide antigens either from the cytosol or the late endosomes and lysosomes, respectively.

CD1a, CD1b and CD1c (group 1) molecules present microbial fatty acids, glycolipids, phospholipids and lipopeptide antigens by anchoring the alkyl chain(s) of the ligand within their hydrophobic binding grooves, positioning the polar headgroup or hydrophilic cap of the bound ligand at or near the opening of the groove [3]. This mechanism exposes the headgroup of the ligand at the surface of the CD1 molecule where, together with parts of the surface of the CD1 heavy chain, it can be recognized by the T-cell receptor (TCR). CD1d (Group 2) molecules are believed to mainly present self-lipid antigens, including sphingolipids and diacylglycerols, and unusual α-ceramides have been used pharmacologically to examine the function of CD1d-restricted T cells. CD1d binds self-lipids by the same mechanism that group 1 CD1 molecules bind exogenous lipids. A majority of CD1-restricted T cells express αβ TCRs. CD1a-, CD1b- and CD1c-restricted TCRs reveal extensive germline gene usage and marked junctional diversity. CD1d-restricted T cells include both those with an invariant TCRα (Vα14–Jα18 in mice, Vα24Jα18 in humans) and those with extensive TCR α and β diversity.

Recent studies of CD1 assembly and antigen loading have resulted in the identification of unexpected accessory molecules that contribute to CD1 function. This article reviews these recent advances, as well as new insights into CD1–antigen interactions gained from the atomic structures of CD1 with natural antigens. For a

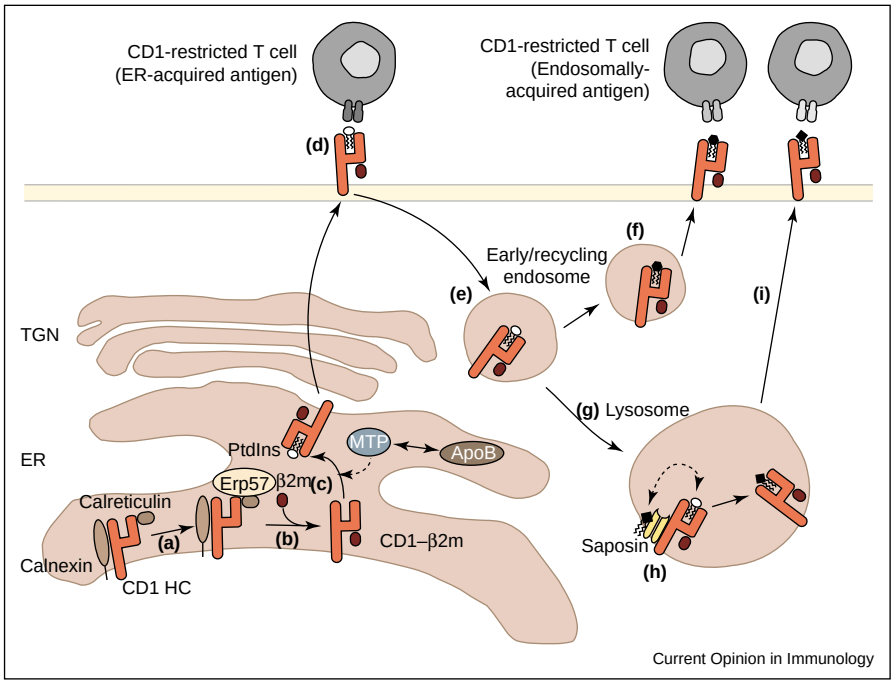
discussion on the functions of CD1-restricted T cells, the reader should refer to several recent reviews [3,47–49].

CD1 assembly, trafficking and antigen sampling

Following translocation into the endoplasmic reticulum (ER), CD1d forms a ternary complex with calnexin and calreticulin. This complex, in turn, recruits the thiol oxidoreductase Erp57, which catalyzes disulfide bond formation in the CD1d heavy chain [4–6]. Fully oxidized CD1d disassociates from the complex, associates with β_2m and enters the secretory pathway (Figure 1). CD1b and CD1d differ in their strict requirement for β_2m association before exiting the ER: CD1b heavy chains are confined to the ER in β_2m-deficient cells [4], whereas a portion of CD1d heavy chains that are not associated with β_2m can exit the ER and reach the cell surface [7–10]. The exit of MHC class I and II molecules from the ER normally requires occupation of their peptide-binding groove. In the case of MHC class I, peptide fragments derived from proteasome processing are loaded through mechanisms involving transporter associated with antigen processing (TAP) and tapasin. In the case of MHC class II molecules, the αβ chains form a nonameric complex with invariant chain (Ii) in the ER, which serves the dual purpose of blocking the peptide-binding groove and targeting the complex to the endocytic pathway. Similar quality control mechanisms for CD1 have not been described; however, it has been postulated that self-lipids are loaded into CD1 during assembly in the ER to occupy the hydrophobic antigen-binding groove during traffic through the secretory and endocytic system. Such a function could be performed by phosphatidylinositol (PtdIns), which associates with CD1b, as well as mouse and human CD1d, in the ER [11,12]. It is not yet clear if the loading of PtdIns into CD1 is an essential step in the assembly and folding of CD1 before exit from the ER.

Following synthesis and assembly in the ER, the majority of newly synthesized CD1b and CD1d molecules are transported to the plasma membrane at a rate indicating direct transport from the Golgi to the cell surface without first entering the endocytic system [6,13,14]. From the surface, CD1 is re-internalized and each isoform differentially traffics through the endocytic system, where it is able to exchange previously loaded self-lipids for self-lipids or microbial lipids present in endosomal compartments. CD1b and mCD1d are specifically targeted to

Figure 1



CD1 assembly and traffic. **(a)** In the ER, newly synthesized CD1 HC forms a complex with calnexin and calreticulin to recruit Erp57. Following oxidation, the complex dissociates and the CD1 heavy chain (CD1 HC) is free to associate with both β_2m **(b)** and endogenous lipids, such as PtdIns, which occupy the CD1 antigen-binding groove **(c)**. In this model, lipid loading is depicted as the terminal event before exiting the ER; however, this has not been experimentally determined. The transfer of lipids to CD1 might involve MTP, which also functions in the lipidation of ApoB. From the plasma membrane **(d)**, CD1 isoforms traffic to different endocytic compartments where they can acquire self-lipid **(e)** or microbial **(f)** antigens. In lysosomes **(g)**, the formation of CD1–antigen complexes involves saposins, which associate with both the lysosomal membrane and CD1 **(h)**. Following the acquisition of lipids, CD1 traffics to the plasma membrane and where lipid antigens are recognized by CD1-restricted T cells **(i)**. TGN, trans-Golgi network.

lysosomes through the interaction of a tyrosine-based motif in the cytoplasmic tail and the adaptor protein AP-3 [15–18]. Functionally, AP-3-dependent traffic of CD1b and mCD1d is essential for the presentation of antigens by both molecules [19–22]. Although endocytic trafficking is necessary for the presentation of many mCD1d-restricted antigens to Vα14$^+$ CD1d-restricted T cells, endocytic trafficking is dispensable for human Vα24$^+$ CD1d-restricted T cells and for murine NKT cells with diverse TCRs [20–23]. This suggests that CD1d can acquire and present lipid antigens from the secretory and endocytic systems, and from the plasma membrane.

Accessory molecules involved in CD1 antigen presentation

Efficient loading of lipids into CD1 molecules requires an exchange of lipids between membrane bilayers and the CD1 binding groove. Insight into the loading of endogenous self-lipids in the ER has been gained by the identification of a role for microsomal triglyceride transfer protein (MTP) in CD1d function [24•]. MTP is an ER-resident protein that is able to transfer lipids between vesicles, and is involved in the assembly and secretion of apolipoprotein B (ApoB) [25]. Using mouse hepatocytes, Brozovic and colleagues [24•] demonstrated that conditional deletion of MTP alters CD1d trafficking, decreases CD1d surface expression and abrogates the presentation of CD1d antigens to both diverse and Vα14$^+$ invariant CD1d-restricted T cells. Given these phenotypes and the role of MTP in the lipidation of ApoB, it is attractive to hypothesize that MTP is involved in transferring lipids into the CD1d binding groove in the ER; however, this mechanism, together with a role for MTP in CD1a, CD1b and CD1c traffic and presentation, has not yet been investigated.

An understanding of lipid transfer to CD1 in endocytic compartments has been advanced by reports implicating the involvement of sphingolipid activator proteins (SAPs) [26•–28•]. SAPs are small cysteine-rich proteins that have an essential role in the degradation of glycosphingolipids in lysosomes [29]. Four SAPs (saposins A–D) are derived from the common precursor prosaposin, and saposin deficiency results in lysosomal storage disorders in mice and humans. Despite a high degree of homology, and the fact that they share the general feature of associating with membranes, the four saposins differ in their mechanism of action. For example, SAP-B forms complexes with glycolipids and is proposed to lift target glycolipids from membranes for processing [30], whereas SAP-C associates with degradative enzymes and is involved in their allosteric activation [29].

Lipid antigen presentation by CD1b and CD1d is diminished in cells isolated from prosaposin-deficient mice or patients [26•–28•]. Thymocytes from SAP-deficient mice fail to activate autoreactive invariant Vα14 NKT cells and, strikingly, SAP-deficient mice completely lack NKT cells, suggesting a role for saposins in loading lipids in the thymus that are responsible for positive selection [27•]. By contrast, studies with human CD1d and human prosaposin did not identify a role for saposins in the stimulation of autoreactive CD1d-restricted human T cells [28•]. This is possibly because of differences in the trafficking of mouse and human CD1d or because of differences in the lipids that these specific T cells recognize. In both mice and humans, only a minor role for saposins was noted in the presentation of α-galactosylceramide (αGalCer) to invariant NKT cells. By contrast, as saposins are known to be responsible for the activity of lysosomal hydrolases that act on sphingolipids, other groups have shown that they are necessary for the presentation of αGalGalCer, which requires removal of its terminal galactose for activity [27•,28•]. Similarly, CD1b-restricted presentation of structurally diverse mycobacterial glycolipids by saposin-deficient fibroblasts transfected with CD1d requires SAP-C [26•]. Hence, saposins influence the presentation of diverse glycolipids by both group 1 and group 2 CD1 isoforms.

In light of the multiple mechanisms by which saposins are involved in sphingolipid metabolism, the specific manner in which they influence CD1 antigen presentation is not immediately clear. Winau and colleagues [26•], as well as Zhou and colleagues [27•], utilized *in vitro* systems to determine if saposins might provide a means by which lipids are transferred from membranes into CD1 molecules. First, SAP-C and, to a lesser extent, SAP-A were shown to facilitate the release of biotinylated lipoarabinomannan (LAM) from liposomes [26•]. Second, using native isoelectric focusing, Zhou *et al.* [27•] found that saposins can facilitate the exchange of phosphatidylserine from liposomes with acidic trisialoganglioside G_{T1b} bound to CD1d. Furthermore, each saposin has a different fine specificity for lipid–CD1 transfer, leading to a model in which saposins promote the loading, as well as the editing, of lipids on CD1 on the basis of their respective affinities for lipids [27•]. Investigations into the mechanistic role of SAPs in sphingolipid metabolism, together with the directed knockouts of saposins in cells without lysosomal disorders will help to define the precise role of saposins in CD1 antigen presentation.

Intersection and formation of antigenic CD1–antigen complexes

The formation of CD1–antigen complexes is governed by several factors. First, spatial constraints require the intersection of antigens with the appropriate CD1 isoform in endocytic compartments suitable for antigen processing and loading. Second, structural differences in the binding grooves of each CD1 isoform influence the selection and binding affinity of antigens.

Intersection of CD1 with antigen

Microbial antigens presented by CD1 can be acquired in different endocytic compartments. In dendritic cells

(DCs) infected with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG), CD1a, CD1b and CD1c each localize to a subset of mycobacterial phagosomes, raising the possibility that antigen processing and loading might occur in phagosomes [31]. Bacterial immune evasion tactics, such as the inhibition of phagosome maturation and acidification, however, might deter the generation of CD1–antigen complexes. Alternatively, CD1 might bind antigens that are released from bacterial phagosomes. Indeed, in both infected macrophages and DCs, mycobacterial glycolipids traffic from bacterial phagosomes through the endocytic system to compartments that might contain CD1 [31,32]. Thus, in infected DCs, different CD1 isoforms intersect with their respective antigens at several intracellular sites, creating multiple opportunities where antigens can be processed and/or loaded in the face of bacterial immune evasion.

Furthermore, at sites of infection, uninfected bystander cells can acquire and present foreign lipids. The uptake of lipid antigens by bystander cells can occur via surface receptors that bind lipid antigens and target them to $CD1^+$ endosomes. When provided to DCs, LAM was shown to bind to mannose receptor on the cell surface and was subsequently delivered to deep endosomal compartments where it colocalized with CD1b [33]. Additionally, DC-SIGN a recently described receptor for *M. tuberculosis* that also binds LAM, might serve a similar function [34–36]. Antigenic mycobacterial lipids are also transferred to uninfected bystander antigen-presenting cells, and such delivery can occur via apoptotic bodies that are released from dying macrophages infected with mycobacteria [31,37].

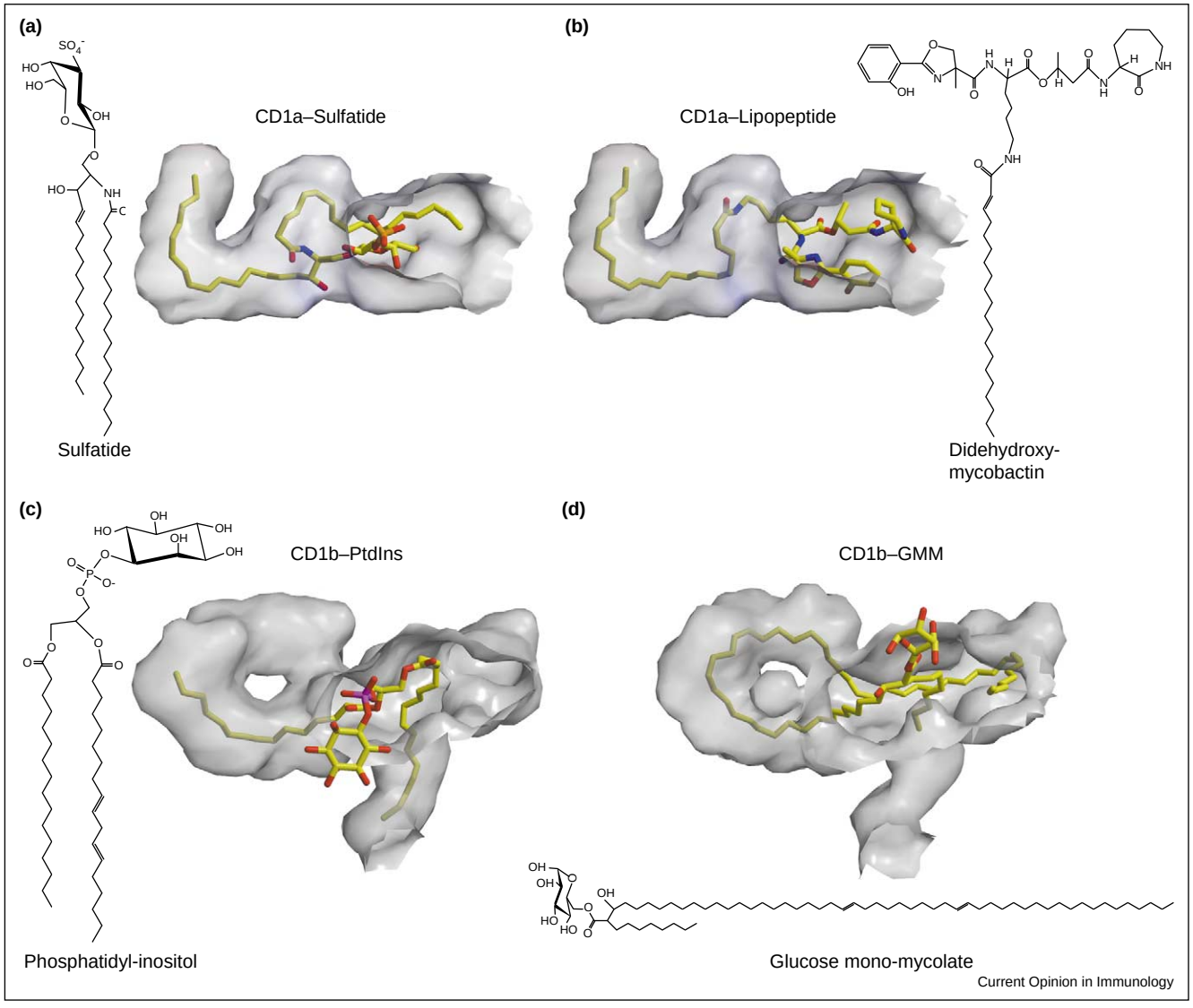
Whether taken up by receptor-mediated internalization or transferred from phagosomes, lipids can be sorted in the endocytic system on the basis of features of their hydrophobic tails [38]. This might provide a mechanism by which antigenic lipids are selectively distributed to subcellular compartments containing specific CD1 isoforms. Indeed, Moody *et al.* [39] have shown that mycobacterial glucose monomycolate (GMM) with long alkyl chains (C80), in comparison to GMM derivatives with shorter alkyl chains (C32), were preferentially delivered to late endosomes and lysosomes where the antigen could be loaded onto CD1b molecules [39]. Coupled with the finding that CD1d requires localization to lysosomes to acquire and present some self-antigens [14,20–22] it has become clear that the CD1 isoforms have evolved to traffic through and localize in various endocytic compartments where lipids of varying structure also traffic (Figure 1).

CD1–lipid antigen complex atomic structures

The determination of the atomic structures of several CD1 isoforms, including CD1a bound with sulfatide and CD1b cocrystalized with a microbial antigen, provide a more complete understanding of the restrictions that their groove architecture places on ligand binding and affinity [40,41•,42,43•]. The CD1 antigen-binding groove consists of hydrophobic pockets that are well suited to bind hydrophobic lipid alkyl chains and orient the antigenic polar headgroup for TCR recognition. Human CD1a and mouse CD1d have similar binding grooves, consisting of two large pockets, A′ and F′, which each accommodate a single alkyl tail of a dialkyl lipid [40,41•]. The A′ pocket consists of a short, highly curved and narrow channel with a clear terminus [40,41•], which, for CD1a, restricts the alkyl chain length to C_{18}–C_{23}, analogous to a molecular ruler selecting lipids of a restricted length. The second alkyl chain is largely accommodated in shallower and wider F′ pocket (Figure 2) [41•]. This CD1a–sulfatide structure suggests that CD1d accommodates two-tailed antigens in a similar fashion, although an atomic structure of CD1d with bound antigen has not yet been published. Interestingly, in addition to αGalCer, OCH (an αGalCer analogue with a truncated sphingosine chain) and other analogues of αGalCer with truncated alkyl chains also are able to bind to CD1d and activate CD1d-restricted T cells [44,45], suggesting that binding of lipid antigens in a single pocket, leaving the second pocket unoccupied, might be permissive for antigen binding and T-cell stimulation. In the CD1a–sulfatide structure, the second pocket (F′) accommodates the polar headgroup, raising the likelihood that this might also occur with single-tailed lipid antigens [41•]. The recent determination of the molecular structure of didehydroxymycobactin (DDM), the first CD1a-restricted antigen to be identified, supports this model [46•]. In contrast to other known CD1-presented antigens, DDM is a lipopeptide consisting of a single alkyl chain and a short polar peptidic headgroup. In a predicted model of DDM and CD1a, the C_{20} alkyl chain occupies the A′ pocket (analogous to the sphingosine base of sulfatide) whereas the peptide backbone is positioned at the junction of the A′ and F′ pockets, making it available for interaction with the TCR (Figure 2) [46•]. This further supports the concept that, for CD1a (and by implication CD1d), lipid antigens with either one or two tails can be accommodated.

In contrast to CD1a and CD1d, the CD1b antigen-binding groove contains two additional channels (T′ and C′) that permit the binding of a wide array of lipids, including microbial lipids with very long alkyl chains (up to C_{80}) [42,43•]. The T′ tunnel links the A′ and F′ pockets to form a continuous channel that can allow a very long alkyl chain, such as the C_{49} alkyl chain of GMM, to be accommodated by consecutively traversing all three channels (Figure 2) [43•]. Furthermore, a C′ channel not only provides a fourth channel for binding hydrocarbon chains, but it uniquely ends in an open portal below the α2 helix that allows additional egress of structures too large to be contained in a closed channel [42,43•]. Thus, CD1b-presented antigens might have great flexibility in the

Figure 2



A comparison of the antigen binding grooves of CD1a and CD1b. Molecular surfaces are shown as transparent binding pockets with bound ligands for the CD1a–sulfatide **(a)** and CD1a–didehydroxy-mycobactin **(b)** complexes, and the CD1b-PtdIns **(c)** and CD1b–GMM **(d)** complexes. The binding pockets are shown from a top view, looking directly into the groove. The molecular surfaces were calculated in GRASP [50] from the respective protein without ligand. Aliphatic backbones are in yellow, nitrogen atoms in blue, oxygen atoms in red, sulfur atoms in orange and phosphor atoms in pink. This figure was prepared using the programs Molscript [51], GRASP and Raster 3D [52] using the Protein Data Bank (PDB) coordinates of CD1a (1ONQ), CD1b–PtdIns (1GZQ), CD1b–GMM (1UQS), as well as the energy minimized predicted model of the CD1a-didehydroxy mycobactin lipopeptide DDM838 bound to CD1a [46].

shape, number and length of hydrocarbon chains that can be accommodated according to the number of individual pockets, their combined capacity, and the existence in CD1b of an additional portal.

Summary

In this review, we have highlighted recent advances in the understanding of CD1 assembly, lipid antigen loading, and the structural interactions between CD1 and its antigens. The formation of stimulatory CD1–antigen complexes that are required for the generation of a CD1-restricted T-cell response is determined by multiple factors. It requires a spatial encounter between CD1 molecules and antigens in subcellular compartments or on the cell surface. At sites where CD1 and antigens intersect, candidate accessory molecules (MTP and saposin) influence CD1 antigen presentation and might prove to be lipid transfer proteins involved in CD1 loading. Furthermore, structural diversity in the antigen-binding groove of different CD1 isoforms enables the binding of a

wide array of lipids, including dialkyl self-lipids, long chain microbial lipids and newly identified single alkyl chain lipopeptides. Thus, the universe of lipid antigens presented by CD1 is expanding, and distinct mechanisms and machinery determine the trafficking and loading of CD1-lipid antigen complexes.

Future studies designed to reveal the nature of self and foreign antigens presented by CD1, identify the intracellular sites where antigens intersect with CD1, and define the precise mechanisms by which antigens are loaded into CD1 will provide critical insight into the formation of CD1–antigen complexes and the activation of CD1-restiricted T cells.

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- •• of outstanding interest

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