Crystal Structure of Mouse CD1d Bound to the Self Ligand Phosphatidylcholine: A Molecular Basis for NKT Cell Activation

Barbara Giabbai, Stèphane Sidobre, M. D. Max Crispin, Yovan Sanchez-Ruìz, Angela Bachi, Mitchell Kronenberg, Ian A. Wilson and Massimo Degano

J Immunol 2005; 175:977-984; doi: 10.4049/jimmunol.175.2.977
http://www.jimmunol.org/content/175/2/977

References
This article cites 53 articles, 22 of which you can access for free at:
http://www.jimmunol.org/content/175/2/977.full#ref-list-1

Subscription
Information about subscribing to J Immunol is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Crystal Structure of Mouse CD1d Bound to the Self Ligand Phosphatidylcholine: A Molecular Basis for NKT Cell Activation

Barbara Giabbai,* Stéphane Sidobre,† M. D. Max Crispin,‡ Yovan Sanchez-Ruiz,§ Angela Bachi,§ Mitchell Kronenberg,† Ian A. Wilson,‡ and Massimo Degano*‡

NKT cells are immunoregulatory lymphocytes whose activation is triggered by the recognition of lipid Ags in the context of the CD1d molecules by the TCR. In this study we present the crystal structure to 2.8 Å of mouse CD1d bound to phosphatidylcholine. The interactions between the ligand acyl chains and the CD1d molecule define the structural and chemical requirements for the binding of lipid Ags to CD1d. The orientation of the polar headgroup toward the C terminus of the α helix provides a rationale for the structural basis for the observed α chain bias in invariant NKT cells. The contribution of the ligand to the protein surface suggests a likely mode of recognition of lipid Ags by the NKT cell TCR. The Journal of Immunology, 2005, 175: 977–984.

A third lineage of Ag-presenting molecules, CD1 proteins are able to bind lipids or glycolipids on the surface of professional APCs. CD1-bound lipid Ags are presented for recognition by T cells via a specific TCR-mediated interaction (1). CD1 molecules share a highly similar tertiary fold with class I MHC molecules, but the CD1 binding cavity is modified to create a deeper, narrower cleft lined by hydrophobic residues, which reflects the unique Ag binding specificity of these molecules (2).

CD1 molecules can be classified into two groups based on sequence similarity. Human CD1a, -b, -c, and -e as well as orthologous molecules in other species can be included in group I (3), and human and murine CD1d and their orthologs belong to group II (4). This distinction is paralleled by a clearly different functional role in vivo. All group I CD1 proteins present microbial lipids and lipoglycans (5–7) as well as self glycosphingolipids to T lymphocytes (8). Group I CD1-restricted T cells include double-negative T cells and group II CD1d molecules by the TCR of iNKT cells, a subset of T lymphocytes expressing NK receptors (11). Most NKT cells in mice are activated through the engagement of their TCR with a CD1d-ligand complex (12). The precise nature of this ligand is still unknown, but NKT cells are considered to be self-reactive, and therefore, this ligand is thought to be an autologous lipid Ag. The glycosphingolipid α-galactosyl ceramide (αGalCer), when bound to CD1d, strongly activates NKT cells. Because this compound was derived from a marine sponge, and because it has an α anomeric linkage of the hexose sugar to the ceramide lipid, which is rare in nature, αGalCer is not considered to be the natural ligand for NKT cells. This compound is a potent TCR agonist (13), however, and it has been instrumental in defining the characteristics and physiological role of these cells.

The great majority of αGalCer-reactive NKT (iNKT) cells bear a semi-invariant TCR (invTCR) (11) with a conserved α-chain rearrangement (Vα24Jα18 in humans; the homologous Vα14Jα18 in mice) and a limited use of VB gene segments (Vβ11 in humans; largely Vβ8.2 in mice) (14). Other ligands that can be bound to CD1d include cellular phospholipids, such as phosphatidylinositol (PI) that act as chaperones in stabilizing the hydrophobic CD1d cavity (15). These compounds could represent an inactive form of a ligand that becomes highly antigenic during inflammation and could then trigger NKT cell activation, but to date it has not been possible to activate most NKT cells with phospholipids (15–17). A notable exception is represented by the 24.8.A inKNT cell hybridoma that is activated by the self phospholipid phosphatidylethanolamine (PE) containing a polyunsaturated acyl chain (18).

NKT cells are a paradigm of T regulatory lymphocytes, able to secrete large amounts of cytokines, such as IL-4, IL-10, or IFN-γ, within minutes of TCR ligation (19). IFN-γ secretion by NKT cells can dramatically potentiate the rejection of tumors (20). Administration of αGalCer to diabetes-prone NOD mice blocked the onset of autoimmunity, either by stimulating the synthesis of Th2 cytokines such as IL-4 or by causing hyporesponsiveness of auto-reactive T cells (21). Hence, NKT cells can act as rheostats of the immune response.

Although the central role of CD1d/iNKT cell pathway in the modulation of several types of immune responses has been established, the molecular and structural details of lipid Ag binding to CD1d and the activation of the TCR of iNKT cells remain elusive.

*Biocrystallography Unit and †Mass Spectrometry Unit, DIBIT San Raffaele Scientific Institute, Milan, Italy; ‡Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92121; and §Department of Molecular Biology and Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037

Received for publication March 21, 2005. Accepted for publication May 2, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Human Frontier Science Program Grant RG0168/2000-M (to M.K. and M.D.); National Institutes of Health Grants AI45053 (to M.K.), CA58896 (to I.A.W.), and GM62116 (to M.K. and I.A.W.); and grants from the Italian Multiple Sclerosis Foundation (to M.D.) and the Italian Foundation for Cancer Research (to M.D.).

2 Coordinates and structure factors of the CD1d/PC complex have been deposited in the Protein Data Bank (www.rcsb.org), accession code 1ZBH.

3 Address correspondence and reprint requests to Dr. Massimo Degano, Biocrystallography Unit, DIBIT Scientific Institute San Raffaele, via Olgettina 58, 20132 Milan, Italy. E-mail address: degano.massimo@hsr.it

4 Abbreviations used in this paper: αGalCer, α-galactosyl ceramide; h, human; HPTLC, high performance TLC; iNKT, αGalCer-reactive NKT; invTCR, semi-invariant TCR; m, murine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIS, precursor ion scanning; rmsd, root mean square deviation.

Copyright © 2005 by The American Association of Immunologists, Inc.
Crystal structures of human CD1b (hCD1b) bound to PI or the GM2 ganglioside showed a complex series of tunnels able to bind the hydrophobic acyl chains of ligands (22) and solved the conundrum of how long chains, such as those present in mycolic acids, could be accommodated in the CD1 binding cavity (23). The structure of an hCD1a/sulfatide complex showed how the CD1 binding groove is tailored in different isoforms to bind specific lipidic ligands, and how the hydrophobic acyl chains could also be part of the TCR recognition surface (24). These structural differences between CD1 molecules are clearly encoded by the primary structure that delineates various frameworks for the selection of Ags. Thus, a detailed knowledge of the atomic interactions involved in ligand binding to CD1 proteins provides further insight into the structural basis for lipidic Ag recognition by NKT cells. Thus, we determined the crystal structure of murine CD1d (mCD1d) bound to a protectant solution containing 100 mM Tris (pH 7.4), 200 mM (NH₄)₂SO₄, crystals appeared after 3 wk at 25°C. Crystals were transferred to a cryo-radiation laboratory using the oscillation method at a wavelength of 1.0 Å. The ligand complex shows a different arrangement of hydrophobic tunnels compared with both hCD1a and hCD1b, and a distinct orientation of the Ag polar headgroup. The contribution of the Ag to the receptor recognition surface underlines how CD1d-restricted TCRs must sample highly localized structural changes to activate NKT cells.

Materials and Methods

Protein production and purification

Murine CD1d was produced in stably transfected Drosophila melanogaster S2 cells following previously described protocols (25). The protein was purified using a combination of immobilized metal affinity, size exclusion, and anion exchange chromatography. Purity levels were judged to be >95% from Coomasie Blue-stained denaturing SDS-PAGE gels. The protein concentration was estimated using the bicinchoninic acid assay.

High performance TLC (HPTLC) and mass spectrometric analysis

One hundred micrograms of pure recombinant mCD1d was extracted twice with a 1/1 mixture of methanol and chloroform in Eppendorf tubes. Organic phases were concentrated to 10 μl under a flow of nitrogen gas and spotted on HPTLC silica plates. Chromatograms were developed in glass tanks with a 25/25/100/0.02 solvent mixture of ethyl acetate, 1-propanol, chloroform, methanol, and sodium chloride. All plates were prerun in the same solvent mixture. Plates were dried, dipped in a 1% (v/v) solution of phosphomolybdic acid in ethanol, and heated for 10 min at 100°C. The standards used were a mixture of total myelin lipids from bovine cauda equina and commercially available PC or phosphatidylserine. For mass spectrometric analysis, developed plates were reversibly stained using iodide vapors. The ligand band was scraped off with a scalpel and extracted overnight in chloroform-methanol (3/1, v/v). The organic phase was injected in a QSTAR Pulsar quadrupole time-of-flight mass spectrometer (MDS Sciex) equipped with a nano-electrospray source for precursor ion scanning (PIS) experiments. PIS of the 184.075 m/z observed in residual Fo-Fc correlation coefficient of 0.53. The structure was rebuilt by extensive manual adjustment using the program O (28) in a prime-and-switch likelihood-modified electron density map calculated with RESOLVE (29). Difference electron density maps showed unambiguously the presence of carbohydrates at three N-linked glycosylation sites. A frame shift affecting the terminal residues (273–279) of the heavy chain was apparent in the electron density maps and was correctly modeled in the mCD1d/PC complex. The structure was subjected to cycles of maximum likelihood positional and isotropic temperature factor refinement, as implemented in REFMAC5 (30), followed by manual model rebuilding. Sigmaa-weighted (31) and shake-omit maps (32) calculated with (2Fo-Fc, Fo-ρc) coefficients were simultaneously inspected to avoid model bias. Shake-omit maps were calculated after omitting stretches of 10 residues per protruding the model coordinates to a final root mean square deviation (rmsd) of 0.5 Å, occasionally followed by five cycles of refinement. Only adjustments that resulted in decrease in Rfree and did not lead to divergence between Rfree and Rref were considered successful. The initial electron density maps exhibited weak positive difference density inside the mCD1d cavities that allowed the inclusion of short acyl chains to the model. The residual density improved in quality after careful refinement of the protein structure. Inclusion of C24/C12 PC in the model improved the refinement statistics by 4% (both Rfree and Rref) and accounted for all the residual electron density in the binding cavity. Modeling of a shorter acyl chain in the A’ pocket (for instance, with a C18/C12 PC molecule) did not satisfy all residual density and resulted in higher R values after refinement. At later stages of refinement, one glycerol molecule was included in the model as well as 26 water molecules that displayed density >4σ in residual Fo-Fc maps and resulted in 2Fo-Fc, density >1.2σ after refinement using ARP/wARP (33).

Model analysis

Model quality was judged using the programs O and OOPS2 (34). Protein-ligand contacts for the mCD1d/PC, hCD1d-H (PDB code 1GZP), and hCD1b-GM2 (1GZQ) complex structures were analyzed using the program CONTACTSYM (26). The rmsd values were calculated using the program LIGPLOT (35). Molecular and solvent-accessible surfaces were calculated using the program MSMS (35). Surface clefts and binding site volume calculations were evaluated using the program SURFNET (36), defined as volumes accessible to 1.6, but not to 6.0 Å, radius spheres.

Results

PC is bound to recombinant mCD1d

Recombinant class I MHC molecules produced in insect cells are not devoid of Ag in the binding groove; rather, they bind a mixture of peptides from the growth medium (37). Furthermore, we previously characterized murine NKT cell hybridomas that reacted to soluble mCD1d without addition of exogenous Ag (38). To determine whether a specific ligand was bound to the recombinant protein, we performed an organic extraction of purified mCD1d, followed by HPTLC analysis. The major component of the organic phase had retention factors substantially identical with PC standards, both from a commercial source and in purified myelin lipids. Minor amounts of PE and sphingomyelin were observed (Fig. 1). The ligand was also not detected in the insect cell growth medium or in class I MHC molecules produced in the same expression system (not shown). To further characterize the nature of the ligand bound to mCD1d, we performed mass spectrometric analysis on the HPTLC-purified material (39, 40). We identified seven major molecular species of PC with total fatty acid compositions consistent with C36:2, C34:1, and C34:2 bound to mCD1d. Thus, PCs with different acyl chain compositions are the main molecular...
species bound to insect cell-produced mCD1d. PC shares a common structural framework with the epitome of group II CD1 ligands, αGalCer (Fig. 1). PE is also bound to recombinant mCD1d, consistent with previous studies showing its weak agonist activity toward iNKT cell hybridomas (18).

Crystal structure of mCD1d bound to PC

We crystallized mCD1d in complex with a mixture of Drosophila-derived PCs and solved its structure to 2.8 Å resolution (Table I). The electron density maps improved during refinement and facilitated the interpretation of a PC molecule with fatty acid chains of C24 and C12 composition (Fig. 2). The inclusion of the long fatty acid chain was guided by the residual electron density observed in the binding cavity, which already was very strong within the A' and F' pockets and at the gloceryl moiety from the initial stages of refinement. Modeling of a shorter fatty acid, such as C18, resulted in clear residual Fo-Fc density at the 3σ level inside the binding pocket and higher crystallographic R values. A C24 fatty acid is extremely rare in insect phospholipids, because it is largely found in sphingolipids (41). Nevertheless, the modeled Ag shows the possible binding mode for both short- and long-chain fatty acids in the binding pocket.

The overall structure of mCD1d bound to PC (Fig. 3) is substantially similar to the previously deposited structure (2) where no ligand was included (rmsd, 0.67 Å for all Ca, 1.72 Å for all atoms in the Ag binding domain). The electron density clearly shows the presence of three N-linked glycans in the mCD1d/PC complex. Remarkably, the Asn165 site shows complete density for a hexasaccharide due to its involvement in lattice contacts with two symmetry-related mCD1d molecules in the crystal.

Interaction of lipid Ags with mCD1d

The PC molecule is bound to mCD1d with the acyl chains inserted in the A' and F' pockets, and the hydrophilic phosphocholine extending from the central opening of the cleft toward the protein surface. The A' pocket of mCD1d is occupied by the longer acyl chain of PC linked to position 2 of glycerol that is typically extended by unsaturated chains. The chain adopts a curved conformation pivoting around a pole formed by residues Cys12 and Phe70 (Fig. 4). The conformation of the chain suggests that fatty acids bearing up to four double bonds, such as arachidonic acid, could optimally fit the C-shaped A' pocket. No unsaturation was modeled in the bound Ag due to the intermediate resolution of the crystallographic data. However, the conformation of the fatty acid suggests that double bonds present at position 9, 12, or 15 would impose the correct curvature to the alkyl chain to optimally fit the A' pocket. The acyl chain is bound to the protein exclusively via van der Waals' interactions. However, the side chains of Gln14, Ser28, Thr37, and His38 extend in the A' pocket within 4.5 Å of the acyl chain and are poised for hydrogen bonding with donors or acceptors branching from the hydrocarbon chain. Hydroxyl groups at the C11, C12, or C17 position of the acyl chain could take advantage of these polar interactions and display higher binding affinities to mCD1d. The A' pocket also displays two small cavities branching from the main tunnel that could accommodate methyl substituents on the acyl chain. The volume of the A' cavity (530 Å3) of mCD1d can fit up to 28 methyl groups, and, similarly to other lipid-binding proteins, shorter acyl chains can be accommodated. The quality of the electron density, despite the heterogeneity of lipids bound to mCD1d, suggests that both long and short acyl chains bind to the A' pocket in the same conformation. These findings are consistent with biochemical data showing a broad specificity of group II CD1 proteins with respect to acyl chain length in modified αGalCer derivatives.

The F' pocket is a linear, hydrophobic groove extending from the central opening of mCD1d toward the C-terminal end of the α1 helix (Fig. 4). The residues lining this pocket are exclusively hydrophobic, with no possibilities for hydrogen bonding interactions between ligand and protein. The electron density for the hydrocarbon chain observed extending from the C1 atom of glycerol in the F' pocket is considerably shorter (C12) than that found in the A' pocket and adopts a fully extended conformation. Further extension of the acyl chain in the cavity by more than four methylene groups is prevented by a wall of hydrophobic residues formed by...
Polar headgroup of mCD1d ligands interacts with protein surface residues

Although the volume and shape of the A' and F' pockets pose constraints on the length, degree of unsaturation, and substitutions of the hydrocarbon chains of lipidic Ags, they do not provide sufficient specificity to orient ligands when both acyl chains are shorter than 18 carbon atoms. Thus, the hydrogen bonding interactions between glycerol (or sphingosine of GalCer) and mCD1d molecules (42, 43). Moreover, the acyl chain in the F' pocket could potentially kink toward the protein surface through this opening, similar to that observed for CD1a- and CD1b-bound ligands (22, 24).

Polar headgroup of mCD1d ligands interacts with protein surface residues

Although the volume and shape of the A' and F' pockets pose constraints on the length, degree of unsaturation, and substitutions of the hydrocarbon chains of lipidic Ags, they do not provide sufficient specificity to orient ligands when both acyl chains are shorter than 18 carbon atoms. Thus, the hydrogen bonding interactions between glycerol (or sphingosine of αGalCer) and mCD1d contribute to the specific orientation of the hydrocarbon chains in the A' and F' pockets (Fig. 4). The glycerol moiety of PC is bound via hydrogen bonding interactions with the side chains of residue Ser76, Thr156, and an ordered water molecule (Fig. 4). A weak polar interaction is observed between the carboxyl oxygen of the C24 acyl chain and the main chain nitrogen of Tyr73 that probably contributes only marginally to the overall enthalpy of PC binding to mCD1d. The sphingosine backbone of glycosphingolipids could interact with the same protein residues through similar interactions. These hydrogen bonds are clearly important for the binding of Ags to mCD1d, because αGalCer derivatives without hydroxyl groups on the 3 and 4 carbons of the sphingosine or with altered stereochemistry at these positions are less efficient in stimulating iNKT cells, most likely because of either decreased binding affinity for CD1d or faster dissociation rates. Thus, the structural and biochemical data demonstrate how the hydrogen-bonding interactions between the polar moiety of the Ag and CD1d are crucial determinants for the biological activity of ligands, and how they enhance Ag selectivity.

The PC moiety of PC is oriented toward the C terminus of helix α1, parallel to the direction of the acyl chain in the F' pocket (Fig. 3). The phosphate group is bound via a direct hydrogen bond to the side chain of Ser76 and a salt bridge interaction to the charged guanidinium group of Arg79. A highly ordered water molecule bridges the phosphate group of the Ag to the side chain of Asp80. The quaternary amino group of choline extends away from the protein surface without making specific contact. The headgroup of PC occupies a volume that corresponds to the P6 position of peptide Ags bound to class I MHC proteins (44). These results are in contrast to those observed for both CD1 isoforms studies performed to date. In the hCD1b structures in complex with GM2 ganglioside or PI, the polar moiety of the ligand has an opposite orientation and makes no specific contacts with the protein (22). In the hCD1a/sufatide structure, the headgroup is inserted more deeply in the F' pocket and sandwiched between the α1 and α2 helices. The lipid Ag headgroup orientation with respect to the Ag-presenting molecule also differs from that observed for glycopeptides bound to class I MHC molecules. Nevertheless, the high B values observed in all CD1/ligand crystal structures suggest a certain flexibility of the hydrophilic moiety of these lipid Ags.

Comparison of ligand binding modes in CD1 isoforms

The binding grooves of the various CD1 isoforms have clearly evolved to efficiently and selectively bind different subsets of lipid Ags (Fig. 5). The A' pocket of mCD1d shares highest similarity with hCD1b, but lacks the T' tunnel that allows the accommodation of extremely long chains, such as those found in mycolic
Structure of the mCD1d/PC complex.

A, Ribbon diagram of mCD1d/PC with helices in pink, β-strands in blue, and β₂-microglobulin in cyan. The bound ligand is represented as a space-filling model, with carbon atoms in yellow, oxygens in red, nitrogen in blue, and phosphorus in magenta. B, The Ag-binding domain of mCD1d bound to PC viewed from the TCR perspective. The N-linked carbohydrates covalently attached to mCD1d are shown in a ball-and-stick representation. The figures were generated using MOLSCRIPT (53) and RASTER3D (54).

Suggested modes for invTCR interaction with CD1d/ligand complexes

We analyzed the surface clefts of the murine class I MHC molecule Kb bound to the superagonist peptide SIYR (Fig. 6) (44). These pMHC clefts are completely occupied by the CDR1 and CDR3 loops of both the α₁- and β₁-chains of the 2C TCR upon formation of the TCR/MHC/peptide complex. The P4 peptide residue of the SIYR Ag is pinched between the CDR3α and CDR3β loops, whereas the P6 residue is accommodated between CDR3β and CDR1β. Similar results have been obtained with other class I MHCs bound to different peptides as well as class II MHC molecules (not shown). Thus, the spatial distribution of the clefts on the Ag-presenting molecules could provide hints about the positioning of the CD1d-reactive TCR. The phosphocholine headgroup in the mCD1d/PC complex is surrounded by two large clefts, similar to the Kβ/SIYR complex. The Ag headgroup is positioned similarly to the P6 residue of SIYR and, hence, is likely to interact with the CDR3β and CDR1β loops of the invTCR. This model is supported from differences in reactivity toward mCD1d-restricted glycolipids and phospholipids by invTCRs bearing different CDR3 regions or Vβ rearrangements (45). To fill the surface clefts of the mCD1d/ligand complex, the footprint of the invTCR could be shifted toward the N-terminal region of the α₁ helix. This difference in orientation together with either an upward displacement or a difference in the tilt angle of the invTCR would allow tighter binding interactions. The limited contribution of the headgroup to the total CD1d/Ag surface (110 Å²) (2) compared with the typical corresponding peptide/MHC surfaces (∼250 Å²) further underscores how the TCR must be able to sample fine changes in the antigenic surface and appropriately transduce these into lower kinetic off rates and tighter binding interactions.

Discussion

We have identified PC as the major glycolipid bound to mCD1d molecules produced in insect cells. The presence of this phospholipid indicates that loading of ligands into the CD1d groove can occur without any specialized proteins that are present only in APCs of vertebrate cells. Indeed, common self lipids may constitute the initial natural ligand repertoire for CD1 molecules. CD1d molecules can thus bind diverse phospholipids and glycolipids. The crystal structure of murine CD1d in complex with PC clarifies the chemical and structural requirements for ligand binding to group II CD1 molecules. The hydrophobic A’ and F’ pockets together with the absence of the long tunnel observed in the hCD1b structure pose a constraint on the length of both acyl chains of the bound Ags. Notably, the hydrocarbon chains of the PI and PC acids. The A’ pocket of hCD1a is limited in size by residue Val²⁸, resulting in a restricted A’ pocket volume. Despite these significant differences, this portion of the CD1 binding groove is relatively conserved in shape and position. The second acyl chain of lipid Ags is bound in completely different fashions in the three CD1 isoforms. In hCD1a, a lateral pocket that is not found in the other isoforms accommodates the acyl chain bound at the C1 position of glycerol. In hCD1b, the acyl chain of the sulfatide Ag extends into the F’ cavity with an S-shaped kink. The lipid chain notably rises toward the protein surface because of an underlying defined by residues Val¹⁹⁸, Leu¹¹⁶, Phe¹²⁶, Phe¹⁴⁴, and Val¹⁴⁷. The homologous residues of hCD1b and mCD1d are smaller in size and do not induce a similar protrusion in the molecular surface. A unique feature of mCD1d is the ridge caused by Leu¹⁰⁰, which prevents long acyl chains extending directly from the A’ to the F’ pocket and forces the hydrophilic glycerol moiety of PC toward the protein surface.
different conformations observed. The curved shape of the A
interface compared with hCD1b, thus accounting for the
opposite ends (Fig. 5). The interaction between the acyl chain of PC
ligands in these two structures access the A
cavity from the opposite ends (Fig. 5), as stick models. A, The mCD1d/PC; B, hCD1a/sulfatide; C, CD1h/PI/detergent complexes. Protein residues are in green. Ligand atoms are
colored as follows: carbon atoms in yellow, nitrogen in blue, oxygen in red, sulfur in green, and phosphorus in magenta. The figure was generated using
MSMS (35) and DINO (www.dino3d.org).

FIGURE 5. A comparison of the binding cavities of different CD1 iso-
forms. The molecular surfaces of the cavities in the different complex are
shown as semitransparent surfaces. The two views in each panel are related by
a 90° rotation about the vertical axis. The protein residues from each isoform
that define the structure and properties of the binding cavities are depicted. The
ligands are shown as stick models. A, The mCD1d/PC; B, hCD1a/sulfatide; C,
CD1h/PI/detergent complexes. Protein residues are in green. Ligand atoms are
colored as follows: carbon atoms in yellow, nitrogen in blue, oxygen in red,
sulfur in green, and phosphorus in magenta. The figure was generated using
MSMS (35) and DINO (www.dino3d.org).

FIGURE 6. Surface clefts suggest a mode for TCR/mCD1d interaction.
A, The molecular surface of H-2Kk in complex with the SIYR peptide
(PDB code 1B6J), with the ligand depicted as a ball-and-stick model. The
contribution to the surface of the MHC residues interacting with the 2C
TCR CDR1 and CDR2 loops of the α- and β-chains are highlighted in pink
and light blue, respectively. The positions of the CDR3 loops are indicated by
labels. The surface clefts are rendered as semitransparent orange sur-
faces. The 2C TCR CDR loops fill the clefts as indicated by the labels. B,
Molecular surface of mCD1d with PC as a stick model. The clefts of the
mCD1d/PC complex suggest that the CDR1 and three of the β-chains of
the invTCR could fill the rightmost cavities and discriminate the Ag. Res-
ides previously mapped by mutagenesis as important for Ag binding
recognized in high entropic stabilization. This model is apparently in contrast
with the interpretation of previous data showing how the αGalCer
analog AGL587 that bears a short acyl chain (four carbon atoms)
was unable to stimulate iNKT cells when bound to the Phe10Ala
A’ pocket mutant of mCD1d (38), although it could stimulate
iNKT cells when bound to wild-type CD1d. The Phe10 residue of
mCD1d contributes to the bottom of the curved A’ tunnel and
interacts via van der Waals’ interactions with the bound hydrocar-
bon chain. The results from the mutagenesis studies could reflect
a lower stability of the mutants compared with wild-type mCD1d.
The long acyl chain of αGalCer could provide ligand-induced sta-
bilization of the CD1d mutant and result in efficient presentation to
the invTCR.

Phospholipids, glycolipids, diacylglycerides, and sphingolipids
share a common chemical and structural framework that meets the
binding requirements of mCD1d. Indeed, PI binds to mCD1d, pos-
sibly acting as a chaperone for trafficking of the protein to the cell
surface (15). However, the exact nature of the physiological li-
gands bound to group II CD1 molecules during inflammation to
promote NKT cell activity is still elusive. The finding that PC
molecules differing in acyl chain composition can bind to the re-
combinant protein confirms that mCD1d molecules display a rel-
atively broad specificity for hydrocarbon chain length, similarly to
fatty acid-binding proteins and nonspecific lipid-binding proteins
(46, 47). The absolute predominance of van der Waals’ contacts
together with the paucity of hydrogen bonding partners within the
binding cavities impose a restraint only on the length of the ligand
acyl chain. Moreover, our results strongly suggest an entropy-
driven binding and imply that loading of Ag requires displacement
of highly homologous endogenous ligands. Hence, subtle modifi-
cations in the hydrogen bonding interactions between protein and
ligand could be major factors in shifting the binding equilibrium.
toward a specific Ag. Improved binding could derive, for instance, from a single hydroxylation either in the headgroup or on the acyl chains.

The structure of mCD1d bound to PC further defines the regions of the α1α2 domains responsible for TCR recognition. The hydrophobic portion of the Ag can interact with the surface residues of CD1d, effectively modifying the character of the surface presented for recognition to the TCR. Structure-based mutagenesis studies identified several residues of mCD1d that affect recognition of CD1αGalCer complexes by iNKT cells (38, 48). Together with the present structure, we can finely dissect the contributions of these mutated residues to interaction with Ag or the TCR. Residues Arg79, Asp80, and Asp153, shown to be important for the stimulation of iNKT cells, all contact the hydrophilic headgroup of the PC ligand. They, therefore, also could contribute to both the binding and orientation of αGalCer and other Ags for TCR recognition. Residue Ser76 affected the reactivity of only some NKT cell hybridomas and is likely to contribute less to the interactions with the galactose moiety of αGalCer. Compared with MHC-glycopeptide complexes, the PC headgroup points further away from the Ag-binding domain and is oriented toward the highpoint of helix α2. Thus, the structure of CD1d in complex with PC suggests a modified TCR recognition of Ag compared with both hCD1b and class I MHC-glycopeptide complexes.

The orientation of the PC polar headgroup toward the C terminus of the α1 helix also supports the sampling of the Ag by the footprint of the TCR β-chain. Hence, all available data suggest that the main role of the α-chain of the invTCR is in interacting largely, if not exclusively, with the mCD1d protein, and that of CDR1β and CDR3β is in encoding the fine Ag specificity. However, the highly diverse repertoire of CDR3β sequences in the αGalCer-reactive NKT cells in vivo supports a germline-mediated recognition of the endogenous Ag(s) driving the expansion of these cells via the CDR1β region (49). The exact definition of the TCR/CD1 interaction must await high resolution structural studies.

The structural aspects of lipid ligand binding to mCD1d derived from the present structure can now be explored in the search for novel synthetic glycolipid ligands to modulate iNKT cell function in experimental disease models (21, 50, 51). A glycosphingolipid termed OCH, for example, bears only a C4 sphingosine chain compared with αGalCer and can induce selective secretion of IL-4 by iNKT cells. This ligand has a potentially high relevance for the interaction must await high resolution structural studies. Science 277: 339–345.


