CD1d ANTIGEN PRESENTATION – LIPIDS ARE THE KEY

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T lymphocytes recognize peptide antigens presented by MHC class I and II molecules, but they can also detect lipid-containing antigens in the context of CD1 molecules. CD1d is a member of CD1 family, a glycoprotein structurally resembling MHC I molecule. CD1d presents a large variety of endogenous and exogenous lipid antigens to a specific subset of innate-like T lymphocytes, iNKT cells. Through the cytokines secreted by iNKT cells, the outcome of the immune response against a variety of pathogens can be shaped. This review focuses on CD1d intracellular trafficking, antigen presentation and recognition by iNKT cells.

Keywords: CD1d, antigen presentation, iNKT cells.

INTRODUCTION

The Cluster of Differentiation 1 (CD1) family comprises type I integral membrane proteins structurally resembling major histocompatibility class I molecules (MHC I), including the presence of a shared subunit, β_2-microglobulin (β_2m). The physiological role of CD1 molecules is to present lipid-containing antigens to specialized T cells and to participate in the immune defense along with MHC molecules. The family members are divided into two groups based on amino acid sequence homology: group I, comprising CD1a, b and c, group II comprising CD1d. A third CD1 species, CD1e, is intermediate between the two groups. These proteins are expressed in mammals and humans, with CD1d being the only isoform present in mice and rats (1-3). CD1d is expressed on most monocytes, macrophages, dendritic cells (DC), B cells, thymocytes and certain non-lymphoid cells (intestinal epithelial cells, parenchymal cells and vascular smooth muscle cells) (4-6), and its expression can be regulated by different pathways (*e.g.*, Toll-like receptors (TLR) ligands, cytokines, viral infection) (7-16).

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NATURAL KILLER T CELLS (NKT CELLS)

MHC molecules present peptide antigens to T lymphocytes. However, pathogens also contain lipids that have been shown to generate a pool of antigens that can be recognized by the cells of the immune system. The biological role of CD1d molecules is to present endogenous and exogenous lipid-containing antigens to a particular lymphocyte subset, namely NKT cells. During infections bacterial lipids are recognized; however, when lipopolysaccharide-containing bacteria such as Salmonella infect the cells, recognition of endogenous lipid antigens (17) rather than bacterial lipids (18) occurs.

There are two types of CD1d-reactive NKT cells: type I or invariant (iNKT) and type II or non-invariant. These cells express the T cell receptor (TCR) in addition to receptors characteristic to natural killer (NK) cells, hence the name. iNKT cells express a semi-invariant TCR composed in mice of Vα14Jα18 rearrangement usually paired with Vβ8.2, Vβ7 and Vβ2 and in humans of Vα24JαQ paired with Vβ11 (19-21). The iNKT cells have been shown to exhibit species cross-reactivity, e.g. they can recognize antigens presented in the context of human or mouse CD1d molecules (22). They arise in the thymus from bone-marrow derived precursors where they undergo positive and negative selection (23-25). The early development of NKT and MHC-restricted T cells is similar but the differentiation between them occurs at the CD4+ CD8+ double positive (DP) stage (26). NKT cells are selected by DP thymocytes expressing CD1d (24, 27). The nature of the endogenous ligand involved in iNKT development is under debate. Some studies have pointed towards isoglobotrihexosyl ceramide (iGb3) as the selecting ligand (28) because mice lacking the lysosomal enzyme involved in degradation of the iGb3 precursor iGb4 exhibit impaired NKT cell development. More recent studies have shown that NKT cell development is also severely affected in mice with defects in other lysosomal enzymes involved in glycosphingolipid catabolism or lacking a specific cholesterol transporter (29-32), the common feature being alterations in lipid trafficking and lysosomal degradation. Moreover, iNKT cell development and function is normal in mice deficient in iGb3-synthase (33), and no iGb3 could be detected in mouse thymus or dendritic cells or any tissue, human or mouse, except the mouse dorsal root ganglion (34). However, more recently iGb3 has been identified in human (35) and mouse (36) thymus with more sensitive techniques.

iNKT cells are abundant in the thymus, spleen, liver, bone marrow, less abundant in lymph nodes and rarely found in sites such as mucosa. They normally express activation markers suggestive of a mature state and most of the Vα24 NKT cells derived from cord blood cells have an activated phenotype (37). NKT cells exert a critical influence on a variety of immune responses and pathologic conditions and have the ability to rapidly secrete cytokines within a few hours after stimulation by recognition of CD1d-antigen complexes. The rapid activation of NKT cells is more characteristic of innate immunity than adaptive immunity, and

provides a first line of defense against pathogens that invade the organism. Therefore, iNKT cells are often considered to form a bridge between innate and adaptive immunity. Through the cytokines they secrete these cells can influence the adaptive immune response according to the nature of the pathogen (5, 6, 38, 39).

It has been shown that NKT cells participate in the immune response against infections involving bacteria or viruses, in rejection of a variety of tumors, and in autoimmune diseases. They also activate various immune system cells such as DCs, B and T lymphocytes and NK cells. However, activation of NKT cells is not always favorable for the organism. For example, activation of CD1d-restricted NKT cells exacerbates atherosclerosis, induces the development of allergen-induced airway hyper-reactivity in asthma and can inhibit anti-tumor activity (6, 39, 40).

Type II CD1d-restricted non-invariant NKT cells do not express the semi-invariant TCR and do not recognize lipids typically recognized by iNKT cells (2, 41-44). However, these cells can recognize CD1d loaded with endogenous ligands, such as sulfatide (45) and they secrete more IL-4 than γ-IFN (46).

CD1d INTRACELLULAR TRAFFICKING

CD1d is a transmembrane protein with a fold similar to MHC I heavy chain. CD1d mainly exists in complexes in which the heavy chain non-covalently associates with β2m. The heavy chain is a glycoprotein with 4 (human) or 5 (mouse) Asn-linked glycans. The heavy chain is translocated into the ER where it acquires N-linked glycans, folds with the assistance of ER chaperones, and before β2m association. The heavy chain interacts simultaneously in a glucose-trimming dependent manner with calnexin, calreticulin and the oxidoreductase ERp57, and the association is coupled to intrachain disulfide bond formation (47). After association with β2m, CD1d leaves the ER, proceeds through the secretory pathway where post-translational modifications occur (*i.e.*, N-glycan processing), and reaches the plasma membrane (47-49). Association with β2m assists transport but may not be an absolute prerequisite for ER exit, as free CD1d heavy chains can be detected at the plasma membrane of intestinal epithelial cells (50). From the plasma membrane CD1d is internalized through specific interactions of tyrosine-based sorting sequences within the cytoplasmic tail with adaptor protein (AP) complexes, and recycles between the plasma membrane and the endocytic system (49). Both human and mouse molecules are located in early endosomes as a result of AP-2 interaction (51, 52). Both can also be detected in late endosomes and lysosomes; in the case of mouse CD1d this results from an AP-3 interaction (51). Mutants of CD1d lacking functional cytoplasmic tail endocytic motifs are predominantly localized at the plasma membrane and cannot present antigens that require endosomal trafficking of CD1d molecules (49{Chiu, 1999 #40, 53-59). Another route to the endocytic pathway involves an interaction with the invariant

chain (Ii) and MHC class II molecules, and localization in the late endosomes or lysosomes in the absence of an AP-3 interaction mediated by this interaction has been shown for both mouse and human CD1d molecules (49, 53, 60). This may result in the acquisition of different lipid antigens located within the different intracellular compartments. CD1d molecules associated with Ii and/or MHC class II molecules may also be transported directly from the Golgi apparatus to the lysosomes without necessarily trafficking via the plasma membrane (Fig. 1).

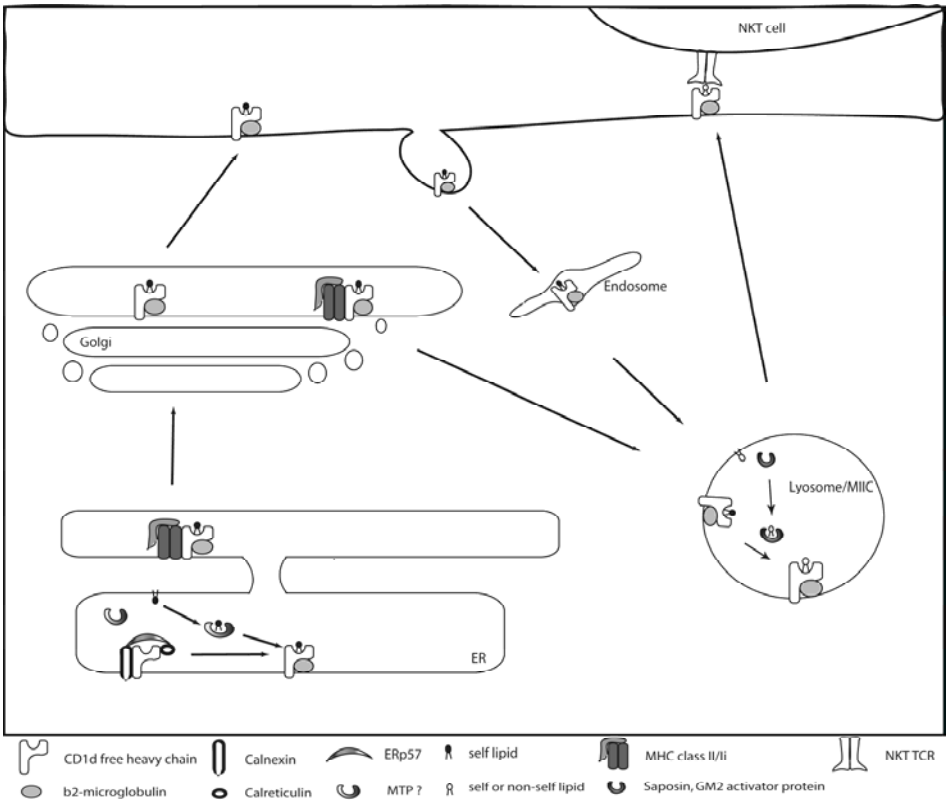


Fig. 1. – CD1d intracellular trafficking and antigen acquisition.

CD1d is synthesized in the ER and interacts with calnexin, calreticulin and ERp57. After the heavy chain exits the chaperone cycle it interacts with β2m and possibly acquires self-lipids with the help of MTP. After exiting the ER, CD1d-β2m complex reaches plasma membrane after passing through the secretory pathway where additional post-translational modifications occur. From the plasma membrane the complex is internalized in an AP2 (and AP3 for mouse CD1d)-dependent manner and reaches the lysosomes/MIIc compartments. Within the endocytic pathway the lipid from the binding site can be exchanged with a self or non-self one with the help of lipid transfer proteins such as saposins and GM2A. The antigen-CD1d-β2m complex traffics to the plasma membrane where it can undergo recycling in the endocytic pathway or recognition by the TCR of the NKT cells. Another pathway for reaching the lysosomes/MIIC compartments is through the interaction with MHC II/Ii complex that isinitiated in the ER.

ENDOGENOUS AND EXOGENOUS CD1d ANTIGENS

Although the capacity of CD1d to load and present non-lipidic small molecules has been reported (61), in the main CD1d antigens are lipid derived. A vast array of antigens presented by CD1d has been identified by different approaches, such as direct identification of eluted lipids from CD1d or by the capacity of individual lipids to stimulate invariant and non-invariant NKT cells. CD1d has been shown to present autologous lipids, such as phosphatidyl inositol (PI), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and phosphatidylglycerol (PG), to NKT cells (62-64). Glycosyl phosphatidyl inositol (GPI) anchors have been proposed as natural ligands for CD1d (65, 66), but other studies have shown that the autoreactivity of a broad panel of invariant and non-invariant NKT cells is not affected by the absence of GPI in antigen presenting cells (67). Ganglioside GD3, a glycolipid abundantly expressed in tumors of neuroectodermal origin such as melanoma, sarcoma, neuroblastoma, but poorly or not at all in normal tissues, can be presented to NKT cells by murine CD1d (68). Immunization of mice with GD3 induces the development of two iNKT cells populations, one of which can recognize GM3, another ganglioside present in tumors of neuroectodermal origin (69).

Isogloboside iGb3 was shown to stimulate iNKT cells and was proposed to play a major role in iNKT cell selection in the thymus (28); however, subsequent studies have challenged this hypothesis (33, 34). A recent paper shows the presence of iGb3 and its precursor iGb4 in B16F10 mouse melanoma cells. Presentation of iGb3 by CD1d in mice injected with melanoma cells induces a four-fold reduction in tumor growth because of activation of iNKT cells. The presentation of iGb3 is inhibited by GM3, the ganglioside synthesized by these melanoma cells (70).

Sulfatide, an abundant glycolipid in brain tissue, represents a target in multiple sclerosis. Sulfatide is a promiscuous ligand presented by CD1 molecules (71, 72) to specific T cells that respond in a Th1 (IFN-γ) or Th2 (IL-4) cytokine pattern. CD1d presents sulfatide to non-iNKT cells and induces a Th1 type cytokine profile (71). A second group of endogenous ligands for CD1d has been recently identified. These are lysophospholipids that are generated by the action of phospholipases on the parental phospholipids. Lyso-PC has been shown to activate an iNKT cell subset that is expanded in myeloma patients (73, 74). Moreover, lysosulfatide, the metabolic product of sulfatide, can be loaded and presented by CD1d to type II non-invariant NKT cells along with other sulfatide isoforms (45).

α-GalCer is a synthetic glycosphingolipid synthesized using as template the agelasphins, which are CD1d ligands discovered in during a screen for anti-tumor agents derived from the marine sponge *Agelas mauritianus*. α-GalCer is the most studied ligand for CD1d and stimulant for invariant NKT cells. It has been shown that α-GalCer specifically binds to mouse and human CD1d molecules and is presented to iNKT cells (22, 75, 76). Activation of NKT cells through presentation

of α-GalCer and other ligands by CD1d molecules results in NKT cell proliferation, rapid secretion of a variety of Th1 (IFNγ) and Th2 (IL-4) type cytokines and gain of cytolytic activity (6, 38, 77).) α-GalCer has an 18 carbon sphingosine base and a 26 carbon acyl chain. X-ray crystallography studies have revealed that the lipid portion of α-GalCer fits tightly in the antigen binding groove of the CD1d molecule (78). Substitutions of the galactose ring hydroxyls with various groups have revealed the importance of hydrogen bonds involved in the interaction of the polar head group of the lipid with CD1d, which is reflected in the stability and antigenicity of the complex. By varying the length, saturation and composition of the two lipid chains, insights into lipid binding in the A' and F' channels (see below) of CD1d have been obtained. Studies of the antigenicity of α-GalCer have allowed the development of other antigens that could have better clinical applications because of a more prolonged and polarized Th response, as well as the capacity to avoid the induction of NKT cell anergy. Nonglycosidic lipid ligands, e.g. analogues of α-GalCer in which the alcohol groups at C5 and/or C6 of the galactose ring were absent, have also been shown to bind to CD1d and stimulate NKT cells (79).

Multiple studies have identified exogenous antigens presented by CD1d to NKT cells. Glycosphingolipids containing α-glucuronic (GSL-1) or α-galacturonic acid (GSL-1' or GalA-GSL) from *Sphingomonas* and *Ehrlichia* species (Gram negative, lipopolysaccharide negative bacteria) are presented by CD1d to Vα14 and Vα24 NKT cells *in vivo* and *in vitro* (17, 80, 81). CD1d presents α-glucuronosyl ceramide from the cell wall of the lipopolysaccharide negative *Sphingomonas paucimobilis* to Vα14 NKT cells along with a glycosphingolipid with a cyclopropyl ring containing C21:0 instead of the sphingosine chain and a weaker antigen GSL-4A, a glycolipid with an α-glucuronosyl ceramide core, but with additional sugar residues (82, 83). Phosphatidyl inositol mannoside (PIM), a lipid located in the inner membrane of *Mycobacteria*, is presented by CD1d to iNKT cells inducing a Th1 response (84). The α-galactosyl glycerols from *Borrelia burgdorferi* are loaded onto CD1d and presented to iNKT cells (85). PC and PE from cypress grains can be presented in a CD1a and CD1d-dependent manner participating in the allergic response against cypress pollen (62, 86).

CD1d ANTIGEN LOADING

The main role of CD1d is to bind and present endogenous and exogenous lipid-containing antigens to specialized T cells. One of the main questions in the field is what molecules facilitate lipid antigen acquisition, as these antigens have to be mobilized from the membranes, maintained in a hydrophilic milieu and possibly enzymatically processed. Antigen acquisition can occur at many steps during the transit of CD1d-β2m complex through the cell. This begins in the ER, shown by the detection of PC and small amounts of PE in association with a soluble

ER-retained form of CD1d expressed in mammalian cells (87). Microsomal triglyceride transfer protein (MTP), an ER resident protein involved in the formation of apolipoprotein B (apoB)-containing chylomicrons and very-low-density lipoproteins in hepatocytes and intestinal epithelial cells, has been shown to transfer triglycerides, cholesterol, PC and PI onto apoB (88). Initial studies have suggested that MTP can also transfer lipids onto CD1d in the ER (Fig. 1). A physical association between MTP and CD1d was detected, the absence of MTP induced reduced CD1d plasma membrane expression and exogenous lipid presentation, and MTP could facilitate PE loading onto CD1d *in vit*ro (89, 90). However, more recent data suggest a more distal effect of MTP (91, 92). In the absence of MTP, CD1d was shown to accumulate in late endosomes/lysosomes and the recognition of endogenous ligand-CD1d complex by three non-invariant autoreactive NKT cell hybridomas was unaffected (92).

By eluting the lipids bound by soluble secreted forms of human and mouse CD1d it has been shown that lipid binding can also occur throughout the secretory pathway. Glycerophospholipids and sphingolipids associate with CD1d. Glycosphingolipids such as GD1a, GM1a, GM2 and GA2 have been eluted from soluble mouse CD1d expressed in monocyte/macrophage cells (93). Sphingomyelin and PC were the predominant lipids bound to soluble human CD1d expressed in B cell-like cells (87), while another report identified a larger array of lipids that also included some unexpected antigens such as plasmalogens, cardiolipins and lysolipids (94). It has been suggested that an unidentified lipid transfer protein might promote lipid binding/exchange in the Golgi apparatus and/or some of the lipids might undergo processing within the secretory pathway (94).

Lipid loading also occurs in the endocytic system where both endogenous and exogenous lipid antigens reside. Exogenous lipids reach the endocytic system by bulk flow and receptor-mediated endocytosis, and can undergo processing and loading onto CD1 molecules. The lysosomal glycoprotein Niemann-Pick Type C1 (NPC-1) may be involved in the lysosomal transport of the antigens as in its absence the presentation of exogenous glycolipids by mouse CD1d is altered (29). Apolipoprotein E has been shown to deliver exogenous antigens in the endocytic system of DCs (95) and B cells (96) facilitating presentation by CD1d. Particulate CD1d antigen uptake and presentation was enhanced by targeting the B cell receptor with specific molecules such as hen egg lysozyme (97). Elution of lipids from membrane CD1d engineered to contain a cleavage site between the ectodomain and the transmembrane domain has revealed PC, sphingomyelin, lysolipids and glycosphingolipids as endogenous ligands that are believed to be loaded onto CD1d during recycling in the endocytic system (87, 93).

Similar to the events in the ER, lipid-containing antigens have to be extracted from membranes and transferred to CD1d molecules. Lipid transfer proteins (saposins and GM2 activator protein) have been shown to facilitate CD1d lipid loading. Saposins are small heat-stable lysosomal glycoproteins that serve as

co-factors in the lysosomal hydrolysis of sphingolipids. There are four saposins, A, B, C and D, generated from proteolysis of a common precursor named prosaposin. They are proposed to bind monomeric lipids and mobilize them from the bilayer into the aqueous phase where they can be processed by specific degradative enzymes. Defects in saposin function induce substrate accumulation in the lysosomes and therefore lysosomal storage disorders (5, 6).

Saposins have been shown to be involved in lipid loading onto CD1 molecules. They are required for intracellular binding of α-GalCer to CD1d molecules (98) and for presentation of isoglobotrihexosyl ceramide (iGb3) (28). *In vitro* studies demonstrated the capacity of saposins to mobilize lipids from the bilayer and to transfer them onto CD1 molecules. Saposin B has been shown to facilitate iGb3 and iGb4 loading onto CD1d (28), while A and C facilitate phosphatidyl serine (PS) and sulfatide loading in a pH-dependent manner (99). Saposin B has been shown to be the most efficient of the saposins in α-GalCer loading onto human CD1d (100). None of the studies demonstrated that saposins facilitate the removal of bound lipids from CD1d molecules, but ganglioside GM2 activator protein was shown to remove GT1b from CD1d antigen binding site and to assist loading of α-GalCer *in vitro* (99). Niemann-Pick type C2 (NPC-2) protein is a ubiquitously expressed lysosomal glycoprotein that binds and transfers cholesterol. The NPC-2 dimer can load the GM3 ganglioside and iGb3 onto CD1d and also release existing lipid from the binding site. However, its absence does not affect the presentation of all the ligands of CD1d suggesting that other molecules may be involved (30).

Certain lipids have been shown to bind directly to CD1d molecules at the plasma membrane. Variants of α-GalCer that have modifications in the sphingosine chain can directly load on plasma membrane CD1d without intracellular uptake. The resulting complexes localize outside lipid rafts and induce a biased Th2 type cytokine response from NKT cells (101). When they enter the endocytic system, the complexes are more unstable and dissociate in the acidic milieu of the endosomes/lysosomes, allowing rapid lipid exchange (102).

CD1d ATOMIC STRUCTURE, AND INTERACTION WITH THE INKT TCR

The first determined atomic structure of a member of the CD1 family was of mouse CD1d at a 2.8Å resolution in the absence of added ligand. Mouse CD1d is similar to MHC class I molecules in secondary, tertiary and quaternary structure even though the similarity at the level of amino acid sequence is not more than 25%. The CD1d heavy chain folds into three domains (α1, α2 and α3) and associates non-covalently with the soluble β2m protein. α3 and β2m domains have c-type Ig folds and the antigen binding groove is composed of α1 and α2 helices that sit atop and traverse a eight-stranded anti-parallel β sheet platform. The α1 and

α2 helices are closer together along their longitudinal axes, resulting in a narrower groove (14 Å) compared to MHC molecules. Unlike in MHC class I molecules, the α2 helix is kinked upward and the α1 helix is raised 4-6 Å higher above the platform, resulting in a deeper groove. The platform has a greater curvature and is more bowl shaped than in MHC class I or class II molecules. In contrast to MHC molecules, the antigen binding groove has only two pockets, which, by analogy to those in MHC class I molecules, are called A' and F'. The A' pocket is longer and wider than the F' pocket. The groove is closed at both ends and is accessible through a portal extending from the center of the groove to the center of the F' pocket. The amino acids lining the groove are mainly hydrophobic and only about 10 amino acid residues concentrated at the bottom of A' pocket and at the entrance to the groove participate in hydrogen bonds with atoms of the antigen (103).

CD1d has been crystallized as complexes with both endogenous and exogenous lipids, including phosphatidyl choline (PC) (104), sulfatide (71), PBS-25 (α-GalCer with a C8 fatty acid chain and a C18 sphingosine chain) (105), synthetic di-palmitoyl phosphatidylinositol dimannoside (PIM2) (106), iGb3 (107), GalA-GSL (108), α-GalCer (78), analogs of α-GalCer with phenyl rings and short hydrocarbon chains instead of the acyl chain (109). Generally, for glycosphingolipids the longer acyl chain of the antigen occupies the A' pocket, adopting a curved conformation around a central pole, while the shorter alkyl chain occupies the linear F' pocket (104). Substitutions of the acyl chain are also accommodated in the A' channel even if the total length of the chain is smaller than the alkyl one (109).The A' pocket can accommodate and form stable complexes with unsaturated acyl chains (up to 4 double bonds) with a *cis* conformation (110), as well as bulkier groups such as phenyl (109). The F' pocket is linear, hydrophobic, with no possibility for hydrogen bonding. The shorter hydrocarbon chain is fully extended in the F' pocket and partially accessible to the solvent, supporting previous data showing pH-independent loading of CD1d molecules (111) and the absence of a requirement for major conformational changes in CD1d to accommodate the antigen. When CD1d-PBS-25 complex was analyzed, a conformational change was revealed that involved the formation of a "roof" above the F' pocket and reducing the solvent-accessible surface of the antigen (105). Comparison of mouse CD1d-antigen conformations has shown large variation at the end of the F' pocket and it has been proposed that the flexibility in this channel correlates with NKT TCR affinity towards CD1d-antigen complexes, i.e. the shorter the sphingosine chain of the antigen, the more the flexibility of the CD1d-antigen complex is increased and less stable is the interaction with the TCR (109, 110). However, when the chains are not long enough to fully occupy the pockets, "spacer lipids" can help fill and stabilize it (105, 108, 109, 112).

The polar groups of the lipid antigens are exposed at the TCR recognition surface, but the orientation differs depending on the lipid. The orientation is stabilized by interactions with residues from the surface of CD1d protein and

sometimes with water molecules (71, 104, 105). However, more flexibility can be detected for some of the antigens (107), (108). If the polar head group is β-linked, then it is oriented perpendicular to the CD1d surface as is for PC, sulfatide and iGb3 (71, 104, 107). The α-linkage of the headgroup induces a different conformation, *e.g.* almost parallel to the CD1d surface, as is for PBS-25 and α-GalCer (78, 105).

The atomic structure of human CD1d with or without α-GalCer was determined at 3Å resolution. The crystallographic unit contained two molecules, one loaded with α-GalCer and the other with the ligand binding site lacking the lipid but stabilized by partial occlusion by the neighboring molecule. Overall, human and mouse CD1d have a similar 3D structure, with conserved amino acids involved in the interaction with the antigen (60.4% sequence identity in α1 domain and 62.4% in α2 domain) {Brossay, 1998 #73}(78, 103). The acyl chain of α-GalCer is perfectly accommodated in the A' channel, where it adopts a counterclockwise circular curve around the central pole. The sphingosine chain of α-GalCer resides in the F' channel. α-GalCer has a perfect fit in the antigen binding groove of human CD1d that explains the exceptionally high binding affinity displayed by this molecule (78).

The polar head group of α-GalCer has a single orientation in the 3D structure, lying almost parallel to CD1d surface and being exposed at the TCR recognition surface. The specific orientation is maintained by three hydrogen bonds at the junction of the two alkyl chains and the polar head group. Comparison of the structures of CD1d with or without α-GalCer indicates that "empty" CD1d adopts a more "open" conformation that enables lipid to bind, while preventing collapse of the channels in the absence of lipid by restricting the available space in the channels (78).

A recently published crystal structure of mouse CD1d in association with diacylglycerols from *Borrelia burgorferi* has shown a preference for the oleic chain to bind in the A' pocket, regardless of whether it is in sn-1 or sn-2 position. This alternative binding induces a 180° rotational difference between the positions of the two antigens in the antigen-binding groove that results in a different exposure of the polar head-group, probably explaining the difference in iNKT cell stimulating capacity (113).

The 3D structures of ternary complexes of αGalCer-human CD1d-human iNKT TCR and αGalCer-mouse CD1d-mouse iNKT TCR have been solved (114, 115), and reveal a "lock and key" model of interaction. Upon ligation, the iNKT TCR does not undergo appreciable conformational modifications (107, 114, 116). The TCR docks parallel to the CD1d antigen-binding groove as opposed to the diagonal docking of TCRs observed with MHC I-peptide complexes. The interface between the TCR and CD1d-α-GalCer is smaller than in TCR-pMHC and the TCR α chain makes more contacts with the CD1d-antigen than the β chain. The iNKT TCR docks on the C terminus of the antigen-binding groove using three out

of the six complementary-determining regions (CDRs). The contacts are made between residues in CDR1α, CDR 3α and CDR2β loops and both CD1d and α-GalCer, with CDR2β interacting exclusively with a stretch of amino acid residues in the α1 helix of CD1d (78, 114). The galactose residue of the ligand protrudes minimally from the surface of CD1d, sitting beneath CDR1α and neighboring CDR3α, and shifts a few Å upon TCR ligation. The 2', 3' and 4' hydroxyl groups, previously shown to be important for TCR recognition, make hydrogen bonds with the TCR, while the 6' hydroxyl group points toward the solvent allowing substitutions, such as carboxylation (117). Overall the structure shows a perfect fit between the invariant TCR and α-GalCer-CD1d complexes explaining the great antigenicity of α-GalCer (114), (115).

Further studies on the role of the CDRs in the recognition of antigen-CD1d complexes revealed a functional hot-spot that is entirely germline encoded at the TCR contact surface (118) and emphasized the importance of the CDR2β and 3β loops in governing iTCR affinity (119, 120). It has also been suggested that conformational modifications in CD1d structure upon antigen binding represent a major factor governing the interaction with TCR (119) and that the iNKT TCR functions as a pattern recognition receptor, e.g. the same TCR is able to recognize a diverse array of antigens (114, 118, 120).

VIRAL INTERFERENCE WITH CD1d ANTIGEN PRESENTATION

Because CD1 proteins are involved in the immune response to viruses, some viruses have developed mechanisms to impede antigen presentation by CD1 molecules. The Nef protein encoded by Human Immundeficiency virus (HIV) interacts with the cytoplasmic tail of CD1d, inducing a decrease in plasma membrane expression and diminishing the capacity of the infected cells to stimulate NKT cells (11). The interaction increases the rate of CD1d internalization from the plasma membrane and blocks CD1d traffic from the TGN to the cell surface (16). *In vitro*, addition of the gp120 protein of HIV to CD1d-expressing cells also induces CD1d down-regulation (15). Through the M protein, Vesicular Stomatitis Virus infection induces changes in the intracellular trafficking of CD1d and impedes lipid antigen presentation to NKT cells (14), (121). Herpes simplex virus-1 infection also down-regulates plasma membrane expression of CD1d by modifying its localization within the endocytic system (13), (122), and Karposi sarcoma-associated herpesvirus uses specific proteins to monoubiquitinate plasma membrane CD1d which increases its endocytosis and lysosomal degradation (12). Bacteria may also develop strategies to impair CD1d antigen presentation. For example, *Mycobacterium tuberculosis* infection down-regulates transcription of the CD1d gene, resulting in reduced CD1d expression in infected macrophages (123).

CONCLUDING REMARKS

Studies of CD1d, along with the other members of CD1 family, have revealed a new role for lipids in defense against pathogens. NKT cells, through the ability to rapidly respond to stimulation and the influence of the cytokines they secrete on other cells of the immune system, can dramatically modulate the immune response. Better understanding of NKT cells biology and CD1d antigen presentation may lead to the development of new therapies against multiple diseases.

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