Glycolipid Presentation by CD1

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T cells recognize lipid antigens as complexes associated with CD1 antigen-presenting molecules. The biophysical properties of lipids impose unique mechanisms for lipid delivery, cell internalization, membrane trafficking, processing of large glycolipid antigens and loading of CD1 molecules. T cells specific for lipid antigens participate in the regulation of immune response, protection during infections and pathogenesis of autoimmune diseases.

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Introduction

Antigen recognition by T lymphocytes differs from that of B cells. B cells utilize soluble or membrane-bound antibodies, which interact with shapes present on the surface of recognized antigens. Instead, T cells recognize complexes formed by small antigenic fragments associated with dedicated antigen-presenting molecules. Antigen-presenting molecules bind small peptides or glycolipids which can derive from partial degradation of large glycolipids. Molecules encoded in the major histocompatibility complex (MHC) present peptide antigens, whereas molecules belonging to the CD1 family present lipid antigens.

The T-cell receptor (TCR) for antigen establishes a cognate interaction with residues provided by both the antigen-presenting molecule and the antigen. This allows the recognition of unique shapes for each complex, thus defining the antigen-specificity of the TCR. T cells recognizing peptide–MHC and glycolipids–CD1 complexes use the TCR heterodimer composed of the α and β chains, although some exceptions have been reported showing rare TCR $\gamma\delta$ cells capable of recognizing CD1-expressing antigen-presenting cells (APCs).

The immunogenicity of the antigens stimulating T cells is determined by a variety of biochemical, cellular and molecular properties, which all together contribute to the mechanisms of antigen presentation. Although similar rules apply to the presentation of both peptidic and lipid antigens, the unique biophysical and biochemical properties of these different antigen classes involve different mechanisms for presentation to T cells. Solubility of lipid antigens, the mechanisms of lipid extracellular transport and cell uptake, their trafficking in membranes of different cellular organelles and the molecular requirements for their processing and loading on CD1 molecules all participate in the determination of lipid immunogenicity. Finally, the unique structures of CD1 molecules and their capacity to bind lipids with defined structures, shapes the repertoire of immunogenic lipid antigens.

CD1 Genes and Molecules

CD1 molecules are evolutionary conserved antigen-presenting glycoproteins characterized by unusual hydrophobic pockets, which allow binding of lipid, glycolipid and lipopeptide molecules that stimulate specific T cells.

In humans, five CD1 genes, called *CD1A*, *CD1B*, *CD1C*, *CD1D* and *CD1E* encoding protein molecules CD1a, CD1b, CD1c, CD1d and CD1e, respectively, are present on chromosome 1 (band 1q22-23) in a locus paralogous to the MHC locus. According to their nucleotide and amino acid sequences homology, CD1 proteins are currently grouped into group 1, including CD1a, CD1b and CD1c, and in group 2 which includes only CD1d.

CD1 genes show an intron/exon structure similar to that of MHC class I molecules. In comparison with MHC genes, which are characterized by a large number of polymorphic alleles, CD1 genes are almost nonpolymorphic. Only *CD1E* gene has been found polymorphic with six alleles identified; two of them frequently found in the population.

CD1 genes are present, although with variations in number, in all mammalian species studied so far. In addition, chicken and other avian species also express CD1-like genes, indicating an ancient origin for the CD1 gene family. Mouse, an important animal species for immunological investigations, has two CD1 genes, *cd1d1* and *cd1d2*, with *cd1d1* giving raise to a cell surface protein.

The existence of at least one CD1 gene in all mammalian species studied so far indicates that the CD1 family is evolutionary conserved and predates the mammalian radiation. Moreover, the homology of CD1 proteins, comparable to both MHC class I and II proteins, suggests that CD1 is an old family diverged from a primordial common precursor, thus also indicating that it is an ancient lineage of antigen-presenting molecules.

The structure of CD1 molecules

CD1 proteins have a predicted molecular mass of approximately 33 000 Da. Upon addition of three or more *N*-linked glycans they reach a mass of 41 000–55 000 Da. All CD1 molecules form heterodimers together with β2-microglobulin (β2-m) and, with the exception of CD1e which remains intracellular, are expressed on the cell surface. Crystal analyses of CD1a, CD1b and CD1d molecules have revealed a three dimensional structure resembling that of classical MHC class I molecules. CD1 proteins are organized into three extracellular domains, a transmembrane region and a cytoplasmic tail. The membrane distal α1 and α2 domains adopt a conformation similar to that of other antigen-presenting molecules, consisting of two antiparallel α-helices overlying a floor of β-plated sheet. These two distal domains are supported by the α3 domain that interacts with β2-m.

The antigen-binding groove of CD1 molecules has unique structural features. In comparison with MHC groove which binds the peptide in a superficial cavity, CD1 groove binds lipids deeply. Moreover, the CD1 groove is composed of almost independent pockets delimited by hydrophobic amino acids. The pockets merge in a small portal allowing insertion of the alkyl tails of lipid antigens. This structural feature permits binding of the hydrophobic part of lipid antigens and display of their polar moieties outside the pocket. In different CD1 molecules lipid-binding pockets differ in number, length and total volume, thus allowing selective binding of lipids differing in the number and length of alkyl chains. CD1b is characterized by the presence of three pockets (called A′, C′ and F′) that merge in the portal and a fourth pocket, called T, that connects the A′ and F′ pockets. This intricate structure allows binding of very long alkyl chains which occupy the entire A′, T and F′ pockets. The T pocket is closed in other CD1 molecules, which therefore cannot bind antigens with very long alkyl chains.

CD1a is characterized by an F′ pocket which is almost open on the CD1 surface. This allows ready binding of lipids in the absence of partial CD1a denaturation and without the help of lipid transfer proteins (LTP). Finally, an open F′ pocket also permits the protrusion of the terminal part of lipid tails, which might interact with the TCR, and thus increasing cognate interaction with TCR.

Recycling of CD1 molecules

CD1 molecules also differ in their capacity to traffic through intracellular compartments. Upon internalization, CD1a recycles through early endosomes (EE) and does not reach late endosomes/lysosomes (LE/Ly). CD1b and CD1d recycle through LE/Ly, while CD1c recycles through both EE and LE. CD1e remains intracellular and does not reach the cell surface. The recycling capacity of CD1 molecules is determined by association with adaptor proteins (AP), such as AP-2 that directs CD1b and CD1d in clathrin-coated endosomal compartments and AP-3 that directs these two CD1 molecules to LE/Ly (Briken *et al*., 2002). The presence of tyrosine motifs in the cytoplasmic part of both CD1b and CD1d is responsible for association with AP-3. Importantly, differential recycling allows CD1 molecules to bind different lipid antigens accumulating in different endosomes. Another important outcome is that antigen loading of CD1b and CD1d, but not of CD1a, occurs in the lumen of LE/Ly, which have acidic pH. In these conditions, CD1 proteins partially unfold and this facilitates exchange of lipid antigens. Finally, in LE/Ly, but not in EE, resident LTP, such as saposins and GM-2 activator protein bind lipid antigens and participate in the loading of CD1 molecules.

In some instances recycling of CD1 molecules is affected by pathogens. Herpes simplex virus type 1 prevents surface reappearance of recycling and novel CD1d molecules by redistributing CD1d to the lysosomal limiting membrane. Such topological alteration results in altered stimulation of invariant Natural Killer T (iNKT) cells, suggesting that this might be an important immune evasion strategy.

iNKT cells represent a population which utilizes a semi-invariant TCR (Vα-Jα without junctional diversity), is characterized by the $CD4^+$, $CD4^-CD8^-$, or $CD8\alpha^+$ phenotype in humans, and very often express the NKG2D (Ly49 in mice) and CD161 (NK1.1 in mice) markers. iNKT cells are abundant in liver (~20% of T cells), bone marrow (~3%), spleen (~2%) and thymus (~0.2%) in mice, whereas are less frequent among human circulating T cells (~0.1%). This population recognizes selfantigens (isoGb3 and other unknown endogenous ligands) and microbial glycolipids (α-galacturonosylceramide and α-glucuronosylceramide from *Sphingomonas* species, α-galactosyldiacylglycerol from *Borrelia burgdorferi* and unknown ligand(s) from *Ehrlichia muris*). iNKT cells release large amounts of cytokines immediately after antigen-recognition and are important protagonists of immunoregulation.

The Structure and Origin of Lipid Antigens

Several lipid antigens of microbial or self-origin have been identified. A large number of immunogenic microbial lipids are present in the cell wall of *Mycobacterium tuberculosis*. Some of these lipids both stimulate specific T cells and trigger innate receptors, thus stimulating both acquired and innate responses.

Microbial lipid antigens

Representative immunogenic lipids are illustrated in **Figure 1**.

Mycobacterial mycolic acid has a α-branched β-hydroxy long-chain fatty acid and binds to CD1b, which is the only

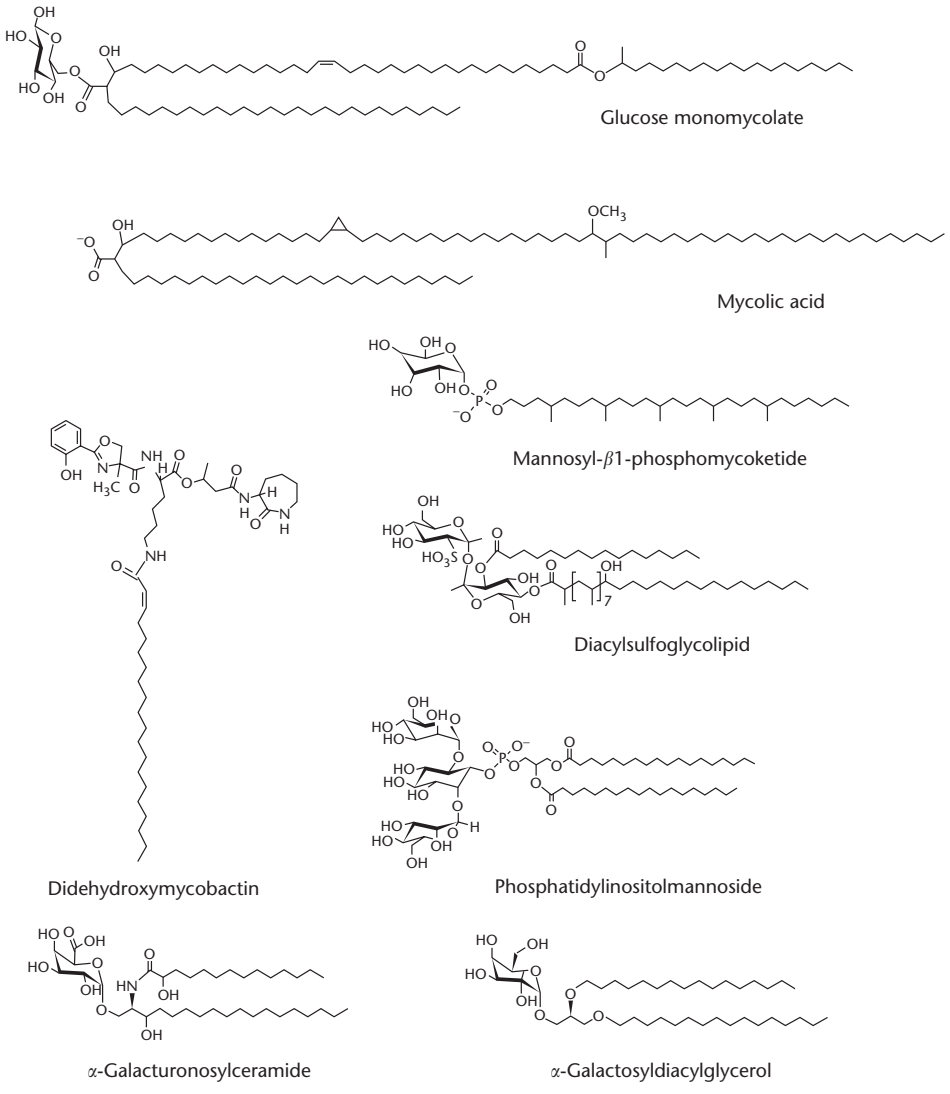

Figure 1 Structure of bacterial-lipid antigens. Most of microbial T-cell stimulatory lipid and lipopeptidic antigens identified derive from *Mycobacterium tuberculosis* and they are presented by group 1 CD1 molecules. α-Galacturonosylceramide and α-galactosyldiacylglycerol are instead produced by Sphingomonas species and *Borrelia burgdorferi*.

CD1 molecule having the appropriate network of hydrophobic pockets capable of allocating such long molecules. Glucose monomycolate (GMM), another mycobacterial lipid antigen, is composed of mycolic acid attached to a single glucose. Two other immunogenic lipids are phosphatidylinositolmannoside (PIM) and lipoarabinomannan (LAM). Both have a phosphatidylinositol component but have different sugar moieties. PIMs contain 2–6 mannoses and, in some cases, have 2–4 acyl chains. In contrast, LAM contains a very large sugar part, which requires partial degradation in order to bind CD1 molecules and become immunogenic.

Sulfoglycolipids are embedded in the cell wall of virulent mycobacteria and are composed of a trehalose attached to up to four short acyl chains. Sulfatide with only two acyl chains is highly immunogenic.

Mycobacterial lipids with one lipid tail may also form stimulatory complexes with CD1 molecules. Mannosyl-β-1-phosphomycoketide is composed of one saturated alkane with methyl branches on every fourth carbon. All these mycobacterial lipids are presented by group I CD1 molecules and induce adaptive responses after priming of specific T cells.

Other bacterial lipids, which instead stimulate invariant NKT cells, are produced by *Sphingomonas* species, by *Ehrlichia muris* and by *Borrelia burgdorferi* (Kinjo *et al*., 2006). These lipids comprise α-glucuronosylceramide, α-galacturonosylceramide and 1,2-diacyl-3-*O*-α-galactosyl-*sn*-glycerol,

which share an α glycosidic bond linked to a ceramide or to a diacylglycerol. Importantly, all these bacteria do not produce lipopolysaccharide, and therefore activation of iNKT cells by these compounds might represent an evolutionarily selected mechanism for stimulating pro-inflammatory and protective responses.

The lipoglycan of *Leishmania donovani* also forms stable complexes with CD1d and activates iNKT cells. Whether other parasites produce lipoglycan antigens presented by CD1 molecules is not known.

Self lipid antigens

Self glycosphingolipids (GSL) and phosphoglycerolipids bind to CD1 molecules and stimulate autoreactive T cells (**Figure 2**). Glycolipids are composed of an oligosaccharide linked to a ceramide. Ceramides are amides of fatty acids with di- or tri-hydroxy bases, sphingosine being the most common in mammals. The acyl group of ceramides is variable and is generally a saturated or monounsaturated long-chain fatty acid.

Among GSL, sulfatide and gangliosides that are abundant in myelin sheets in the brain, are highly immunogenic (Shamshiev *et al*., 2000). Sulfatide binds to all human CD1 molecules and also to mouse CD1d, showing the capacity of different CD1 molecules to present an overlapping lipid repertoire. The gangliosides GM1, GD1a, GD1b, GT1b and GQ1b are presented by CD1b molecules and induce expansion of autoreactive T cells in patients with multiple sclerosis, an inflammatory disease of the brain characterized by expansion of autoimmune T cells. The ganglioside GD3 is instead enriched in tumours of neuroectodermal origin and may also stimulate specific T cells having an antitumour function.

Phosphoglycerolipids are composed of a phosphorylated diacylglycerol modified with one nitrogenous base, inositol or two glycerol molecules. Phosphatidylinositol, phosphatidylethanolamine and phosphatidylglycerol are presented by CD1a and CD1d molecules and stimulate human T cells participating to allergic reactions. These lipids also bind to CD1d and stimulate rare mouse NKT cells.

Lipoproteins may also bind to CD1 molecules and stimulate specific T cells (Moody *et al*., 2004). Didehydroxymycobactin is the precursor of the mycobacterial siderophore mycobactin, binds to CD1a and is recognized by its amino acids. As bacteria contain many different lipoproteins, it is likely that other lipopeptides induce CD1-restricted T-cell responses.

Finally, some nonpeptide nonlipid molecules also stimulate CD1-restricted T cells. These compounds are composed of aromatic hydrocarbon rings with sulfur groups and a general structure resembling that of pentamethyldihydrobenzofuran sulfonate. The details of their CD1 binding and TCR interaction are not known.

Importantly, the structure of the lipid tail directly influences TCR recognition of self lipids. This is likely associated with the formation of complexes in which the exact position of the hydrophilic moieties change according to the length and saturation of alkyl chains.

Delivery and Internalization of Extracellular Lipids

Because of their hydrophobicity, lipids circulate associated with lipid-binding proteins or are transported within membranes. The delivery of extracellular lipid antigens to APC is mediated by lipoproteins (van den Elzen *et al*., 2005) (**Figure 3**). Apolipoprotein E (ApoE), a component of high-density (HDL) and very low-density (VLDL) lipoprotein particles mediates binding to the specific receptors: low-density lipoprotein receptor (LDLR) and the LDL receptor related protein 1 (also called CD91). Upon binding, lipoprotein–ApoE complexes are internalized and reach EE. In intracellular compartments with acidic pH, ApoE dissociates from VLDL, then associates with HDL and is secreted. During this exchange, intracellular lipids may become incorporated into nascent HDL particles and thus are released in the extracellular space. This has been found important in the case of transfer of mycobacterial lipid antigens synthesized by *M. tuberculosis* infecting macrophages. With this mechanism the microbial lipids become available to other APC thus allowing their cross-presentation. Lipoprotein-specific receptors deliver lipids into clathrin-coated pits, which allow traffic through early recycling endosomes and in LE/Ly. Extracellular lipids associated with plasma membrane can be internalized by two mechanisms. Lipids such as sulfatide and sphingomyelin are internalized through clathrin-coated vesicles, then reach LE/Ly and are degraded. Glycosphingolipids such as gangliosides, globosides and lactosylceramide are instead internalized through clathrin-independent mechanisms, in some instances involving caveolae. All these lipids reach early recycling endosomes. However, caveolae-derived lipids form independent membrane domains and thus do not mix with lipids derived from clathrin-coated pits. Whether separation into independent domains affects CD1 loading is not known.

Membrane Trafficking of Lipid Antigens

Lipids traffic into membranes according to their biophysical properties, to interaction with other membrane components and also to the capacity of associating with lipid-transfer proteins and lipid translocators. Two aspects of lipid membrane topology are important for lipid presentation: (i) the localization in the exoplasmic leaflet of membranes, i.e. on the same side where the lipid-binding domains of CD1 molecules are located and

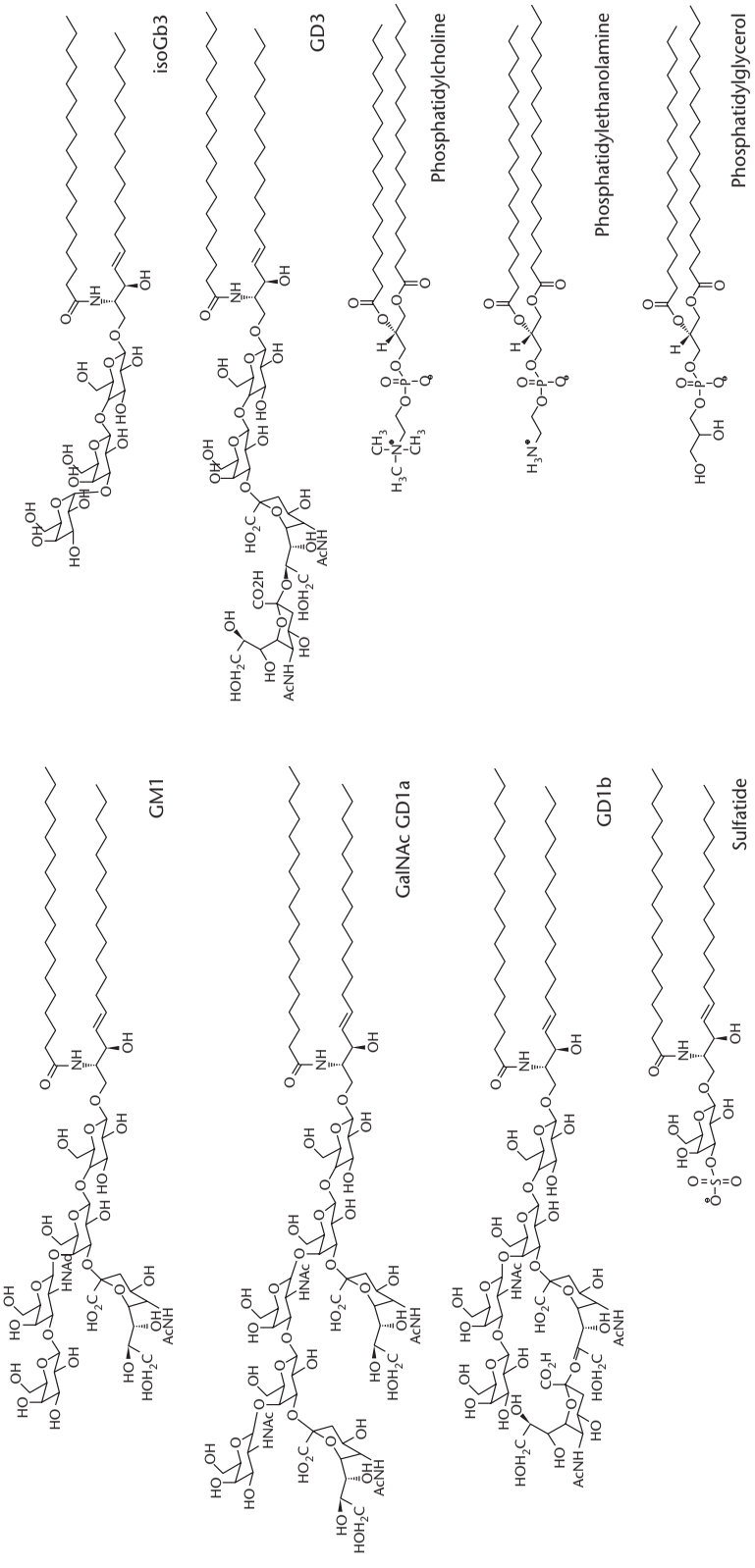

Figure 2 Structure of self-lipid antigens. The T-cell stimulatory self-lipid antigens so far described are glycosphingolipids and phosphoglycerolipids.

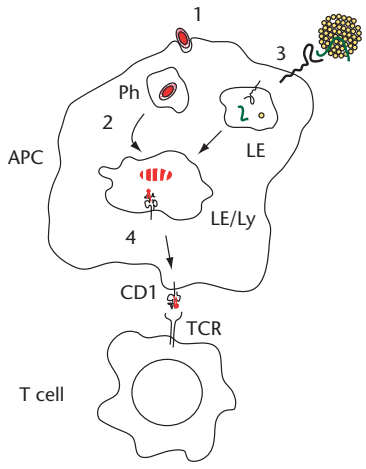

Figure 3 Antigen presentation of lipids to T cells. Bacterial cells are phagocytosed (1) by antigen-presenting cells (APC) and accumulate in phagosomes (Ph) which fuse with lysosomes (2). Lipoproteins are internalized by specific receptors (3) into late endosomes (LE) where lipoprotein particles dissociate and lipids traffic to late endosomes/lysosomes (LE/Ly). In LE/Ly large lipids are further processed and associate with CD1 antigen-presenting molecules (4). CD1–lipid antigen complexes are then displayed on the APC cell surface where recognition by specific T cells trough their T-cell receptor (TCR) occurs.

(ii) the colocalization in endosomal organelles where CD1 molecules are present and become loaded with lipid antigens. Lipids continuously move across membranes. Neutral lipids and acidic lipids with charges neutralized by protonation move rapidly between leaflets, whereas lipids with charged headgroups move slowly. As most glycolipids have charged headgroups their spontaneous movement across leaflets is minimal and require the assistance of translocases, which actively participate in lipid transport between the bilayers with an ATP-dependent process. Several transporters of the ABC family are involved in lipid transport across membranes. They facilitate lipid secretion into bile, translocation into organelles such as the Golgi and peroxisomes or egression out of the cell. Other non-ABC transporters are NPC1 and MLN64, which facilitate egression of lipids from lysosomes to cytoplasm. NPC2 protein is instead involved in exchange and in the degradation of lipids within lysosomes. Deficiency of NPC proteins induces development of an abnormal iNKT TCR repertoire and a marked reduction of the number of iNKT cells.

Lipid association with LTP is another relevant mechanism contributing to lipid trafficking. A major part of lipid traffic between organelles is mediated by cytosolic and lumenal LTP, which bind lipids in a hydrophobic pocket with a stoichiometry of 1:1 and usually assume two different conformations. One permits lipid extraction from membranes, whereas the other allows transport within the cytoplasm or cellular organelles. Most LTP have domains involved in accurate membrane targeting, which confer targeting specificity. Although there is no study addressing the role of cytosolic LTP in lipid antigen presentation, it is likely that some of these proteins have a role in transferring lipid antigens in the compartments where CD1 molecules are loaded.

The lipid biophysical features also influence trafficking and presentation to T cells. Lipids with long and saturated chains readily form liquid-ordered microdomains and pack more closely than lipids having unsaturated chains. These features increase membrane viscosity, thus decreasing the rate of lipid movement into membranes and promoting traffic to deep endosomal compartments. The immunological consequence of this preferential trafficking into LE/Ly is that lipids with long alkyl chains become associated with CD1 molecules such as CD1b, which also recycles in LE/Ly. This has been shown to be the case of glucosyl-monomycolate, a mycobacterial lipid containing 80 carbons, which accumulates in LE/Ly (Moody *et al.*, 2002). A synthetic analogue containing only 32 carbons is three times less abundant in LE/Ly and is less stimulatory than the natural lipid.

Processing of Lipid Antigens

Upon docking on the CD1-lipid antigen complexes, the TCR establishes direct interactions with amino acid residues provided by the two α helices of CD1 molecules and also with hydrophilic residues contributed by the lipid antigen. This allows enough energy contacts that result in T cell activation. Some glycolipid antigens, mostly of bacterial origin, have large carbohydrate moieties, which prevent the TCR–CD1 interaction. Therefore, these lipid antigens require partial degradation to become immunogenic. Like proteins, glycolipids are processed within LE/Ly. Using response to synthetic antigens as models, the hydrolases α-galactosidase and hexosaminidase B were shown to generate immunogenic molecules after degradation of nonstimulatory analogues bearing larger glycosidic moieties. α-Galactosidase removes the terminal galactose from Gal-(α1-2)-Gal-α-ceramide, thus generating the, strong agonist α-galactosylceramide, which activates iNKT cells. Similarly, hexosaminidase B digests the synthetic GalNac-(β1-4)-Gal-α-ceramide glycolipid to α-galactosylceramide. The role of these enzymes in processing natural antigens has not been confirmed yet.

Acidic α-mannosidase is required for processing of mycobacterial PIM antigens (de la Salle *et al.*, 2005). These lipoglycans display up to six mannoses and four lipid tails. T cells recognize the di-mannosylated form, which is generated by digestion of PIM bearing more than two mannoses. Lipoarabinomannan is another mycobacterial antigen, which is also degraded within LE/Ly before becoming immunogenic. However, the hydrolases involved in its processing have not been identified yet.

A role for lipases in lipid antigen presentation has not been shown. For example, it is not clear whether PIM antigens with four acyl chains are processed to forms with less acyl chains and generate immunogenic complexes with CD1b or instead are not immunogenic at all.

The Function of CD1e

CD1e is the fifth member of human CD1 gene family. It has low homology with other CD1 proteins and therefore it is not included into CD1 Group 1, nor Group 2 families. CD1e is conserved in other mammalian species, and is expressed in dendritic cells (DC) without reaching the plasma membrane. In immature DC, CD1e accumulates in the Golgi compartment and upon DC maturation it translocates into LE/Ly where it is cleaved and becomes soluble. A structural model shows that CD1e has a large and single hydrophobic pocket, which might accommodate large lipid molecules. Soluble CD1e binds several lipid antigens, including self-glycosphingolipids and mycobacterial antigens. CD1e is required for appropriate processing of PIM antigens (de la Salle *et al.*, 2005) (**Figure 4**). Indeed, presentation of PIM_6 (the PIM form with six mannoses) necessitates the presence of CD1e. Furthermore, recombinant α-mannosidase digests PIM_6 to PIM_2 only in the presence of CD1e. Thus, CD1e acts as a chaperone facilitating the activity of α-mannosidase.

CD1e has two additional important features. Its α 1 helix does not contain charged amino acids, which instead are present on classical antigen-presenting molecules interacting with the TCR. The CD1e α 1 helix has a partial amphipatic structure, which might allow its direct insertion inside membranes. In this case it would behave as a liftase, which extracts antigens embedded within microbial and/or endosomal membranes. Finally, CD1e shows an unusually long and unique amino-terminal peptide, which is removed in LE/Ly. This peptide might close the hydrophobic pocket thus preventing binding of lipid molecules in the endoplasmic reticulum (ER) and Golgi, whereas it would not hinder binding of exogenous lipid molecules accumulating in LE/Ly.

The relevance of CD1e polymorphism is being appreciated. Patients homozygous for the CD1e 01 allele have increased risk to develop Guillain Barré syndrome, an autoimmune disease caused by the presence of antiganglioside antibodies, whereas subjects with a CD1e 01/02 genotype show decreased risk. The mechanism of these associations are not clear. Probably it reflects a different capacity of CD1e alleles to bind lipid antigens. Indeed, the two alleles differ in a single amino acid predicted to affect the structure of the hydrophobic pocket.

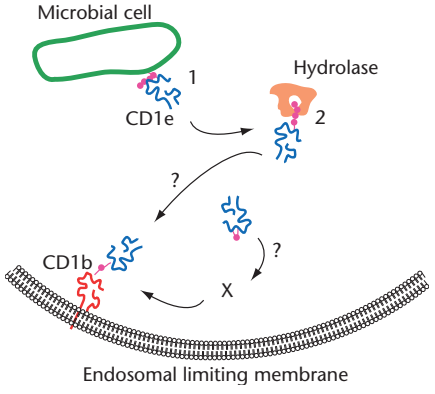

Figure 4 The function of CD1e. CD1e binds complex microbial lipid antigens (1) and offers them to hydrolases (2) thus facilitating the processing. Whether CD1e is also involved in loading other CD1 molecules such as CD1b is unknown (?).

CD1 Stability and Loading of Lipid Antigens

CD1 molecules are stabilized during their assembly by lipids, also called spacers, which are probably loaded in the ER. Mouse CD1d binds a molecule of palmytic acid in the A′ pocket. Instead, human CD1b associates with a long hydrophobic molecule, 41–44 carbon atoms long. The analyses of both CD1 crystals also showed the presence of an endogenous phosphatidylcholine molecule. Both spacers are likely to stabilize nascent CD1 molecules, but may also have the important function of inhibiting binding of other self lipids to avoid autoimmune responses. How the spacers are extracted in LE/Ly before binding lipid antigens with long alkyl tails is not known.

Another important ER-resident LTP is the microsomal triglyceride binding protein (MTP), required for surface expression of CD1d (Brozovic *et al.*, 2004). MTP binds to triglycerides and phospholipids and is involved in their transfer to ApoB lipoprotein during its assembly in the ER. Similarly, MTP might be involved in transfer of the spacer and phosphatidylcholine to nascent CD1d. It is unclear whether MTP is necessary for the assembly and maturation of other CD1 molecules.

Another important issue is the stability of CD1-antigen complexes in living cells. Comparison of complexes in which sulfatide binds to CD1a, CD1b and CD1c shows that the half life of CD1b and CD1c complexes is about 20 h, whereas the half life of CD1a complexes is longer, at 36 h. This difference might be ascribed to the different CD1 recycling route. Indeed, CD1b and CD1c recycle through LE/Ly in which low pH and presence of LTP facilitate exchange of lipid antigens, whereas CD1a recycles in EE, where lipid replacement remains more difficult. The *in vivo* functional implications of these important differences remain unclear.

Loading CD1 molecules with lipid antigens is not clearly understood. Lipids within membranes require liftases,

which extract them, and dedicated chaperones establishing close contact with CD1 molecules to which lipid antigens are transferred. LTP present in LE/Ly such as saposins and GM2A protein have been identified as important lipid chaperones required for CD1 loading. Saposins are four short LTP generated after partial digestion of the common precursor prosaposin. Saposins form homodimers, which upon assembly generate a hydrophobic pocket allowing lipid binding. GM2A has a sequence different from that of saposins and binds lipid by inserting them in a hydrophobic pocket. Although lipid binding is not specific, both saposins and GM2A show a marked selectivity in their lipid interaction. Therefore, these LTP are not redundant. They were first identified as involved in the pathogenesis of some lipidoses, diseases in which lack of lipid degradation leads to lipid accumulation and cell toxicity. This phenotype is recapitulated in prosaposin gene knockout mice, which show early degradation of neuronal cells and have several symptoms in common with lipidoses patients. These LTP behave as liftases on small vesicles present in endosomal compartments and identified as multivesicular bodies (MVB). Upon interaction with the negatively charged lipids bis-(monoacylglycero)-phosphate and phosphatidylinositol-3-phosphate that are enriched in MVB, these LTP extract lipids from these membranes and offer them to hydrolases. In the absence of these LTP, lipid degradation is impaired and their accumulation leads to cell death. The immunological role of LTP has been demonstrated in saposin-deficient mice, which have reduced numbers of iNKT cells and also show impaired antigen presentation (Zhou *et al*., 2004). Furthermore, saposin-deficient cell lines do not present CD1d- and CD1b-restricted antigens, thus showing the involvement of these LTO also in lipid antigen presentation. *In vitro*, saposins and GM2A facilitate CD1 loading with lipid antigens and also participate in CD1 lipid unloading. Therefore, they are important molecules required for lipid degradation as well as for lipid transfer. An important issue is whether the lipid binding selectivity of these LTP allows their involvement in loading of microbial antigens, which have hydrophobic structures very different from those of self lipids. Perhaps, the main function of saposins is to facilitate CD1 unloading with self lipids, thus allowing insertion of microbial lipids, which are conveyed by other chaperones. The possible participation of CD1e in loading CD1 molecules with microbial lipid antigens has not been disclosed.

Immunogenicity of CD1–Lipid Antigen Complexes

Cross-priming and cross-presentation occur with lipid antigens, similarly to that found with peptide antigens. Mice immunized with melanoma cells producing large amounts of GD3 ganglioside develop CD1d-restricted GD3-specific responses, as a consequence of GD3 release from melanoma cells and its acquisition by CD1d-positive APC. Cross-presentation of lipid antigens may occur by two main mechanisms. First, ingestion of apoptotic bodies leads to internalization of associated lipids. If lipid antigens produced by intracellular bacteria infecting apoptotic cells are delivered to APC during phagocytosis of apoptotic bodies, an immune response against microbial lipids can develop. This is relevant to generate immune response against *M. tuberculosis* lipids (Schaible *et al*., 2003). The second mechanism is by release of exosomes from living cells. Exosomes deliver associated lipids to APC, and thus allow delivery of lipid antigens from CD1-negative to CD1-positive APC.

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