Trends in Immunology

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Series: The Biology of Antigen Presentation

Feature Review

Mechanisms and Consequences of Antigen Presentation by CD1

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The CD1 proteins are a family of non-polymorphic and MHC class I-related molecules that present lipid antigens to subsets of T lymphocytes with innate- or adaptive-like immune functions. Recent studies have provided new insight into the identity of immunogenic CD1 antigens and the mechanisms that control the generation and loading of these antigens onto CD1 molecules. Furthermore, substantial progress has been made in identifying CD1-restricted T cells and decoding the diverse immunological functions of distinct CD1-restricted T cell subsets. These findings shed new light on the contributions of the CD1 antigen-presentation pathway to normal health and to a diverse array of pathologies, and provide a new impetus for exploiting this fascinating recognition system for the development of vaccines and immunotherapies.

Antigen-Presentation Systems

Products encoded by the major histocompatibility complex (MHC) region of the vertebrate genome bind peptide fragments from pathogens and display them at the surface of antigen-presenting cells (APCs) for appraisal by T lymphocytes [1]. A hallmark of the classical MHC class I and class II proteins is their extensive polymorphism, which determines histocompatibility, controls host resistance to infection, and influences susceptibility to autoimmunity. In addition to the classical MHC class I products, many jawed vertebrates express non-polymorphic, MHC-related proteins with diverse immune functions [2]. Members of the CD1 family of MHC class I-related proteins present self- and foreign lipid antigens to T lymphocyte subsets whose functions are less well understood than conventional MHC-restricted T cells. Nevertheless, the CD1 antigen-presentation system provides new targets for the development of vaccines and immunotherapies against a variety of diseases. To accomplish this goal, it is crucially important to identify the antigens that are recognized by CD1-restricted T cells, to understand the pathways that control the generation and loading of these antigens onto CD1 molecules, and to clarify the molecular basis for lipid antigen recognition by CD1-restricted T cell receptors (TCRs). Recent studies have provided important insight into the mechanisms involved in the generation of immunogenic CD1 antigens, and this is invaluable for understanding the functions of this antigen-presentation system in health and disease as well as for exploiting this system for vaccine development and therapeutic purposes.

General Themes in the CD1 Antigen-Presentation System

CD1 Genes, Proteins, and Evolution

CD1 proteins were originally identified as **β_2-microglobulin** (β_2m; see Glossary) associated heavy chains encoded in a locus on human chromosome 1 [3,4]. This region encodes five CD1

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The CD1–lipid presentation system allows the immune system to sense alterations in lipid homeostasis, and complements the classical MHC–peptide presentation system. There are remarkable similarities and surprising differences in the way that TCRs engage CD1–lipid versus MHC–peptide complexes.

Group 1 CD1 proteins (CD1a–c) present a variety of endogenous, mycobacterial, and potentially other bacterial lipids to T cells. CD1b-restricted T cells include subsets expressing germline-encoded TCRs.

Group 2 CD1 proteins (CD1d) present diverse endogenous and exogenous lipid antigens to subsets of natural killer T (NKT) cells expressing semi-invariant, biased, or diverse TCRs. α-Linked glycosylceramides have emerged as major endogenous ligands that control the functions of invariant NKT cells.

Significant progress has been made towards the development of lipid-based vaccines and immunotherapies.

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isoforms (CD1a–e) that, based on sequence homology, were classified into group 1 (CD1a–c) and group 2 (CD1d) proteins, whereas CD1e was considered to be an intermediate isoform, sometimes referred to as group 3 [5]. Group 1 and 2 CD1 proteins are expressed at the cell surface and function as antigen-presenting molecules, whereas CD1e is only expressed intracellularly and is involved in processing and editing lipids for presentation by the other human CD1 isoforms. Another distinguishing feature is that group 1 CD1 proteins are expressed predominantly on **professional antigen-presenting cells**, whereas group 2 CD1 proteins are expressed more widely. In addition, expression of group 1 but not group 2 CD1 proteins is highly inducible by microbial products and cytokines. Each of the CD1 proteins is constitutively expressed on **cortical thymocytes**, and this expression is required for the intrathymic development of CD1d-restricted T cells [6] and most likely also for the selection of group 1 CD1-restricted T cells [7].

The CD1 antigen-presentation system predates the evolutionary split between mammals and reptiles [8]. The ancient origin of CD1, together with its evolutionary conservation among all mammalian species examined [9], suggests important functions for this antigen-presentation system during an immune response. The number of CD1 genes in mammals differs widely among species, with some mammals expressing >10 CD1 genes. Similarly to humans, several other mammals such as dogs, horses, and guinea pigs contain genes for all five CD1 isotypes, whereas mice only encode CD1d protein. The absence of group 1 CD1 genes in mice has complicated the functional analysis of this group of CD1 proteins, and this has been partially overcome by studying **humanized mice** [10,11].

CD1 Structure, Antigens, and TCR Interaction

Crystal structures of CD1 molecules have revealed an overall resemblance to MHC class I but with two key differences [1,8,12]: (i) the CD1 inner surface is lined with hydrophobic residues, and (ii) the α-helices of CD1 are extended further away from the floor of the cleft, resulting in a deeper antigen-binding groove (Figure 1A). The size of the antigen-binding groove differs substantially among distinct CD1 isoforms in the following order: CD1a < CD1d < CD1c < CD1e < CD1b (Table 1). Similarly to the **specificity pockets** (labeled A–F) of classical MHC class I molecules, all CD1 molecules contain two antigen-binding pockets, called A′ and F′ (Figure 1). In addition, CD1b contains a C′ pocket similar to the C pocket of MHC class I, as well as an additional T′ or 'tunnel' pocket.

CD1 molecules can bind to a variety of self- and foreign lipid antigens, but only a fraction of these activate T cells [8,13,14]. Examples of CD1 antigens are shown in Figure 2. Some specific lipids such as sulfatide (3′-sulfated β1-D-galactosylceramide), an endogenous glycolipid highly expressed in neuronal cells, can promiscuously bind to all CD1 isotypes [15]. Sulfatide-reactive T cell lines restricted by all group 1 and 2 CD1 isotypes have been identified (Table 1). The diversity of CD1 antigens includes lipids, glycolipids, phospholipids, lipopeptides, oils, and even non-lipid molecules. Crystal structures have revealed that lipids are oriented such that hydrophobic alkyl chains are buried deep within the antigen-binding pockets and the hydrophilic headgroups are solvent-exposed (Figure 1) [1,8,12]. Because of their differing volumes and shapes, the antigen-binding grooves of distinct CD1 isoforms can accommodate lipids containing alkyl chains of variable lengths. Interestingly, CD1b and CD1c can even bind to lipids with alkyl chains that are longer than the calculated size of their respective antigen-binding grooves. To accommodate long alkyl chains, one end of the lipid protrudes out of the groove via accessory **portals**. Such a detour ensures that the long alkyl chain does not interfere with TCR interactions. Conversely, CD1 molecules often present lipids with alkyl chains that are much shorter than the predicted size of the antigen-binding groove, which would leave extra space in the pockets, possibly causing structural problems. Analyses of CD1 complexes bound to short-chain lipids revealed that the unoccupied pockets were filled with spacer lipids that provide

Glossary

Adjuvant: chemical substance used to induce an innate immune response, and then enhances and directs adaptive immune responses to the vaccine antigen.
Anergic: an immune cell property characterized by unresponsiveness to antigen resulting from cell-intrinsic tolerance induction.
Chaperone: in antigen processing, chaperones facilitate the assembly and transport of MHC proteins. They include products involved in general ER quality-control and those with specific functions in MHC class I-, MHC class II-, or CD1-restricted antigen presentation.
Cortical thymocytes: thymocytes are hematopoietic precursor cells to the T cell lineage in the thymus. Thymocytes in the cortical area of the thymus, the outer layer of this organ, are the most immature and express CD1.
Double-negative cells: T cells that lack CD4 and CD8 coreceptor expression.
Hepatic stellate cells: these cells, also known as perisinusoidal or Ito cells, store vitamin A in the liver, become activated during liver injury, and play a major role in liver fibrosis.
Humanized mice: mice carrying functional human genes, cells, tissues or organs.
Langerhans cells: dendritic cells that uniquely express langerin (CD207) and reside in the epidermis of the skin and some mucosal epithelia.
Lectin: a protein that binds particular sugars.
Marginal zone B cells: a subset of non-circulating B cells with innate-like properties that reside within the marginal zone–the region between the white (lymphoid) and the red (non-lymphoid) pulp–of the spleen.
Microbiota: the variety of symbiotic, commensal and parasitic microorganisms that are normally associated with metazoans.
β_2-Microglobulin: the invariant soluble component of all MHC class I and many MHC class I-related molecules such as the CD1 proteins. It provides stability to MHC class I and class I-like structures.
MHC class II-associated invariant chain: this transmembrane protein, also known as the MHC class II γ chain or CD74, is a chaperone that binds within the groove of MHC class

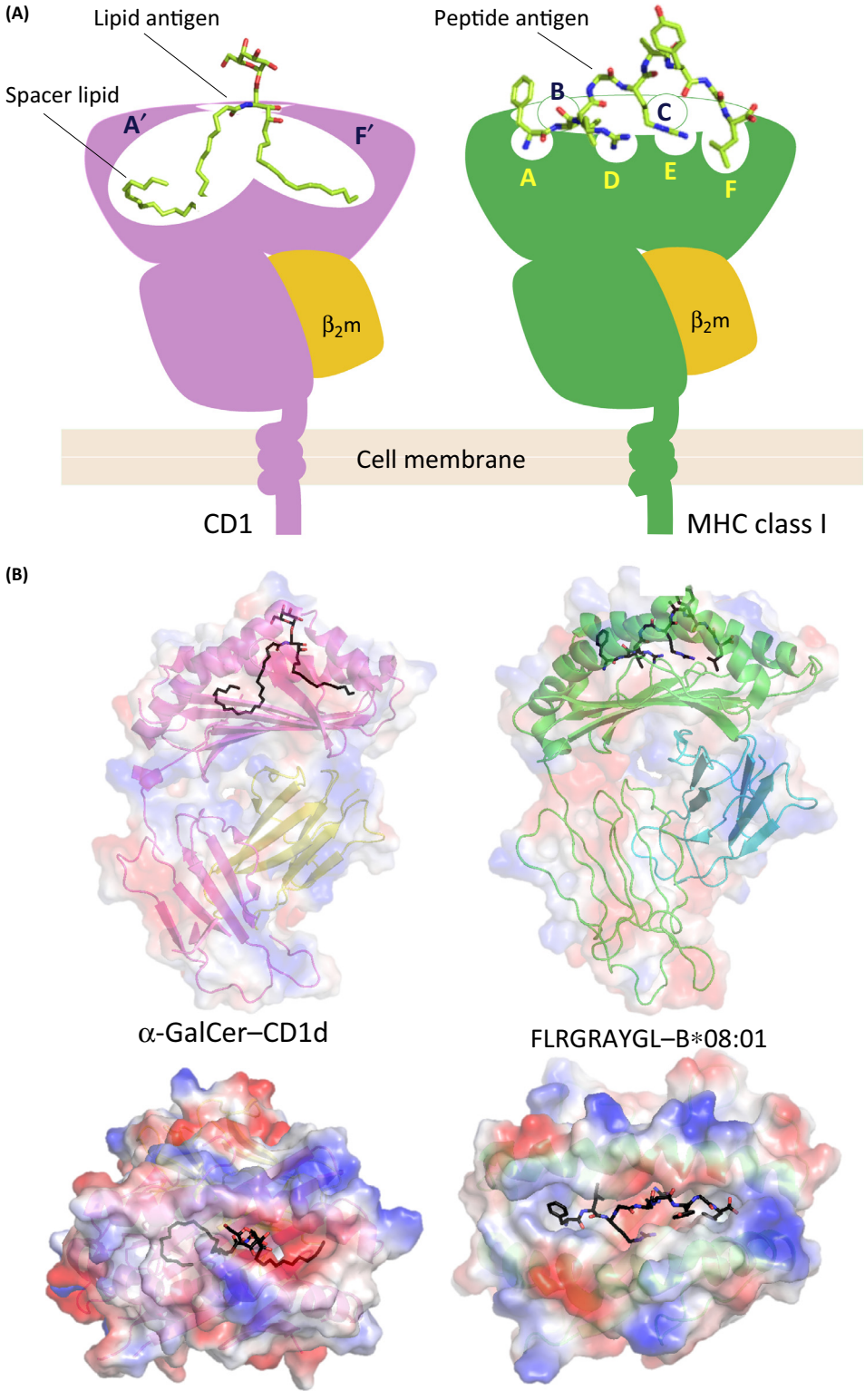

Figure 1. Comparative Anatomy of the α-GalCer–CD1d and Peptide–MHC Class I Ternary Structures. (A) Schematic view showing the key features of CD1 and MHC class I proteins. The specificity pockets are labeled A′ and F′ for CD1 and A–F for MHC class I molecules. Note that CD1b contains additional pockets (C′ and T′; not depicted). The CD1 groove is occupied by a lipid antigen and the MHC class I groove is occupied by a peptide antigen. In addition to an antigenic

(Figure legend continued on the bottom of the next page.)

Il molecules, facilitates class II transit to endolysosomal compartments, and prevents peptide binding until its release and exchange with antigenic peptides.

Pathobiont: any organism that under normal circumstances lives as a symbiont with its host but under some situations can cause disease.

Phospholipase: any of the four enzymes that hydrolyze specific ester bonds in phospholipids.

Portal: for CD1 proteins, portals are open areas of the antigen-binding groove where portions of the antigen can project out of the groove.

Professional antigen-presenting cells: a group of antigen-presenting cells that include dendritic cells, macrophages, and B cells, and which can activate naïve T cells.

Specificity pockets: for the classical MHC class I and II proteins, specificity pockets in the antigen-binding groove interact with conserved residues of the bound peptide. CD1 proteins similarly contain specificity pockets that interact with conserved portions of the bound lipid.

Tetramers: in T cell biology, fluorescent-labeled, tetrameric forms of MHC or CD1 proteins loaded with specific antigens are employed to identify antigen-specific T cells.

structural stability to the groove (Figure 1A, left) [16,17]. Thus, with the assistance of multiple antigen-binding pockets, accessory portals, and spacer lipids, CD1 molecules can diversify the size and range of lipids they present to T cells.

The crystal structures of CD1–lipid complexes have revealed striking similarities and important differences with MHC–peptide complexes that have crucial implications for TCR recognition [1,8,12]. Whereas amino acid side-chains of the peptide are solvent-exposed and available for TCR interactions along the MHC groove, the region above the A′ pocket at the left side of the CD1 groove (referred to as the A′ roof) is closed, leaving only the polar moiety of the lipid to protrude out of CD1 via the F′ portal at the right side of the groove, thereby making the headgroup available for interactions with the TCR (Figure 1). Crystallographic studies of TCR/MHC–peptide complexes have revealed a diagonal or sometimes near-orthogonal binding mode with a common docking topology along the center of the groove in which the TCRα chain is positioned over one α-helix (from the α_2 domain of MHC class I or the β_1 domain of MHC class II) and the TCRβ chain is positioned over the second α-helix (from the α_1 domain of MHC class I or the α_1 domain of MHC class II). A similar topology has been observed in several TCR/CD1–lipid complexes, although the semi-invariant TCR expressed by a subset of CD1d-restricted T cells is oriented parallel to the axis of the groove (Figure 3) [1,8]. In addition, owing to the asymmetric nature of the CD1 groove, TCRs may shift either to the left side or to the right side of the groove, resulting in wide variation in the extent of interactions with the protruding lipid headgroup.

CD1 Antigen-Processing Pathways

The assembly of CD1–lipid antigen complexes is initiated in the lumen of the endoplasmic reticulum (ER) by association of CD1 heavy chains with a variety of **chaperones** such as calnexin, calreticulin, and the thiol oxidoreductase ERp57 (Figure 4), which assist in folding and assembly [5,18,19]. CD1 heavy chains then bind to β_2m as well as to a variety of endogenous ER lipids, possibly with the assistance of spacer lipids, that together function as chaperones to stabilize the CD1 molecule. This association is reminiscent of the binding of newly synthesized MHC class II molecules with the **MHC class II-associated invariant chain** in the ER. Loading of such ER-resident lipids onto newly synthesized CD1 molecules is facilitated by microsomal transfer protein (MTP), an ER-resident lipid-transfer protein (LTP) that is also known for its capacity to facilitate the assembly of very low density lipoproteins (VLDL) and chylomicrons. The CD1–lipid complexes subsequently egress to the plasma membrane, followed by internalization and entry into endosomes. CD1b–d proteins contain a tyrosine-based sorting motif that permits their binding with the adaptor protein complex 2 (AP2), which facilitates entry into a variety of endosomal compartments. Human CD1b and mouse CD1d (but not human CD1d) also contain AP3 sorting motifs that facilitate the entry of these CD1 isoforms into lysosomes. CD1a, which lacks such sorting motifs, exhibits AP-independent recycling to early endosomes. In this manner, different CD1 isoforms can sample the lipid antigens that may be present in distinct intracellular compartments (Figure 4), potentially eliciting specific and non-redundant T cell responses. The ER-derived lipid cargo on these internalized and differentially sorted CD1 proteins may then be replaced with other endogenous or exogenous lipids. Lipid transport to these compartments is facilitated by lipoproteins, lipoprotein receptors, and **lectin** receptors

lipid, the CD1 groove may also contain a spacer lipid that fills up extra space in the antigen-binding pockets to stabilize the CD1 structure. (B) The structures of human α-GalCer–CD1d (top left) and peptide (Epstein–Barr virus-derived FLRGRAYGL)–HLA-B*08:01 (top right) complexes showing the electrostatic surfaces of the heavy chain and β_2m. Note the depth of the CD1d antigen-binding groove that is accessed by the ligand through a narrow portal. By contrast, the peptide antigen-binding groove is relatively shallow and is accessed by a larger opening. These same structures were turned 90° toward the reader to show the solvent-exposed polar galactose headgroup of α-GalCer (bottom left) and the roughly alternating amino acid side-chains of the MHC-bound peptide (bottom right). Structures were generated by PyMol using the protein database IDs 3HUJ (left) [118] and 3SJV (right) [119]. Abbreviation: β_2m, β_2-microglobulin.

Table 1. Salient Features of the CD1 Antigen-Presentation System[a]

CD1 isoform	Groove volume	TCR	TCR variability: T cell subset designation	Antigens recognized	Refs
CD1a	1.35 nm^3	αβ	Diverse	Sulfatide, PE, PI, PC, lyso-PC, fatty acids, squalene, wax esters, dideoxymycobactin	[27,28,30,107]
CD1b	2.20 nm^3	αβ	Diverse	Sulfatide, mycolic acid, glycerol monomycolate, diacylated sulfoglycolipids	[107–110]
		αβ	Semi-invariant: GEM T	Glucose monomycolate	[35]
		αβ	Biased: LDN5-like T	Glucose monomycolate	[36]
CD1c	1.78 nm^3	αβ	Diverse	Phosphomycoketide, mannosyl-Phosphomycoketide, lipopeptides	[41,40]
		γδ	Diverse	Sulfatide, lyso-PC, phosphomycoketide, mannosyl-phosphomycoketide	[43,107]
CD1d	1.65 nm^3	Type I NKT or iNKT cells			
		αβ	Semi-invariant: Vα14 (mouse) or Vα24 (human) NKT	α-GalCer, β-GalCer, α-GlcCer, α-GalACer, α-GalDAG, α-GlcDAG, cholesteryl α-glucoside, asperamide B, GD3, iGb3, PE, PI, PC, lyso-PC, lysopeptidophosphoglycan	[50–59,74,77,78,111–115]
		αβ	Semi-invariant: Vα10 NKT	α-GalCer, α-GlcCer, α-GlcADAG	[81]
		Type II NKT, dNKT, or vNKT cells			
		αβ	Biased: sulfatide-reactive dNKT	Sulfatide, β-GlcCer, β-GalCer	[62,107,116]
		αβ	Diverse: 'atypical' dNKT	α-GalCer	[97]
		αβ	Diverse: non-sulfatide-reactive dNKT	PG, PI, cardiolipin, lyso-PE non-lipid small molecules (e.g., PPBF), peptides (synthetic, ovalbumin-derived, collagen-derived)	[60,61,63,98,99,102]
		TCRγδ$^+$ NKT cells			
		γδ	Biased	Cardiolipin	[117]
CD1e	2.00 nm^3	–		Sulfatide, PI, dimannosylated PI, diacylated sulfoglycolipids, hemi-bis(monoacylglycero)-phosphate	[105,106]

[a]Abbreviations: dNKT, diverse NKT; GalCer, galactosylceramide; GalACer, galacturonosylceramide; GalADAG, galacturonosyldiacylglycerol; GalDAG, galactosyldiacylglycerol; GD3, ganglioside D3; GEM, germline-encoded mycolyl-specific; GlcCer, glucosylceramide; iGb3, isoglobotrihexosylceramide; iNKT, invariant NKT; NKT, natural killer T; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PPBF, phenyl 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonate; vNKT, variant NKT.

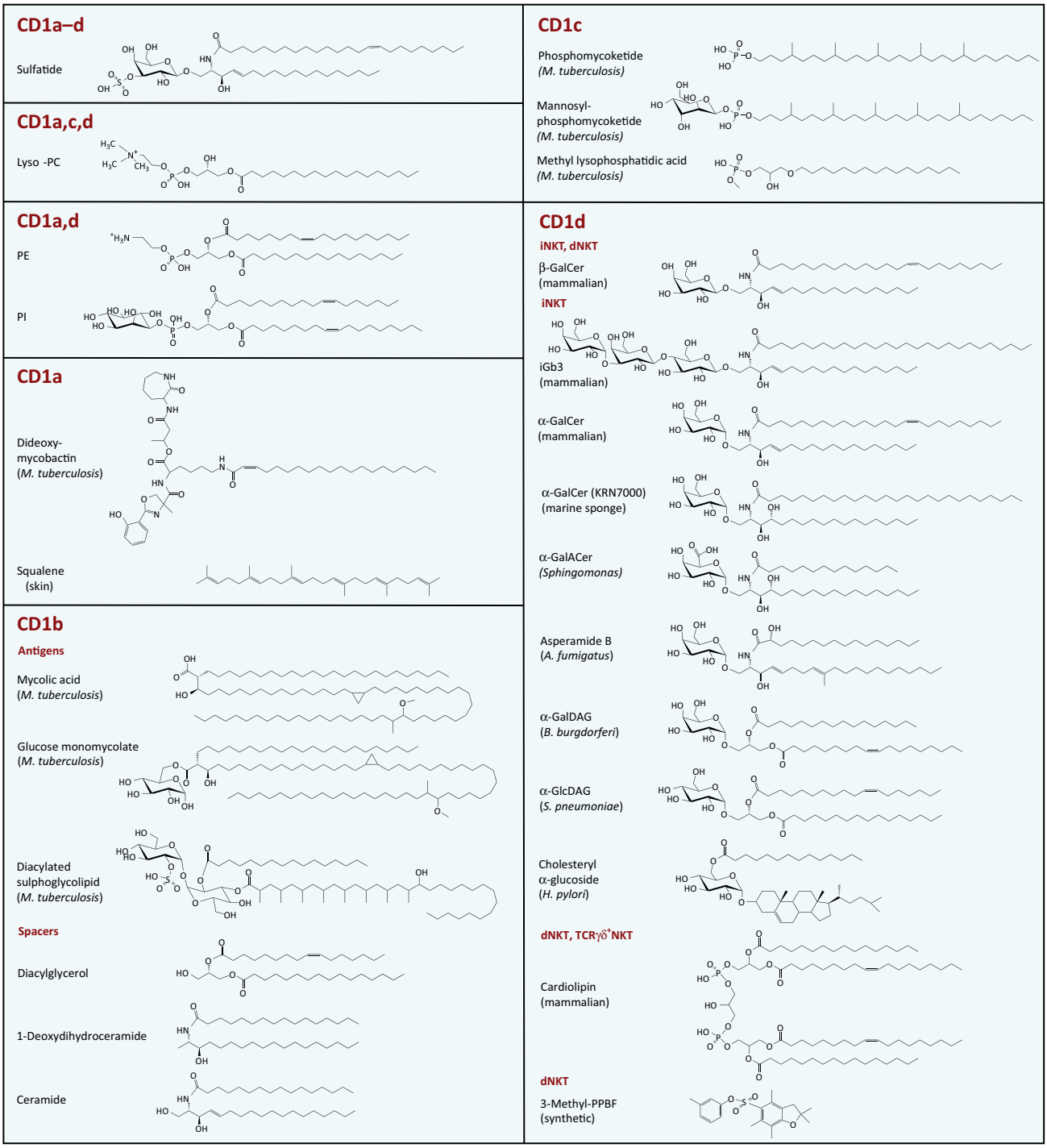

Figure 2. Select Antigens and Spacer Lipids of the CD1 Antigen-Presentation System. Structures are shown of select endogenous and exogenous antigens presented by distinct CD1 isotypes. Spacer lipids bound to CD1b, the only CD1 isotype where such lipids have been molecularly characterized thus far, are also depicted. For CD1d, antigens recognized by key subsets of CD1d-restricted T cells are shown. Note that endogenous, ER-derived phospholipids such as PE and PI, while recognized by some CD1-restricted T cells, also function as chaperone lipids to stabilize the CD1 groove and facilitate egress to the cell surface. Abbreviations: *A. fumigatus*, *Aspergillus fumigatus*; *B. burgdorferi*, *Borrelia burgdorferi*; dNKT, diverse NKT; ER, endoplasmic reticulum; GalACer, galacturonosylceramide; GalCer, galactosylceramide; GalDAG, galactosyldiacylglycerol; GlcDAG, glucosyldiacylglycerol; *H. pylori*, *Helicobacter pylori*; iGb3, isoglobotrihexosylceramide; iNKT, invariant NKT; *M. tuberculosis*, *Mycobacterium tuberculosis*; NKT, natural killer T; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PPBF, phenyl 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonate; *S. pneumoniae*, *Streptococcus pneumoniae*.

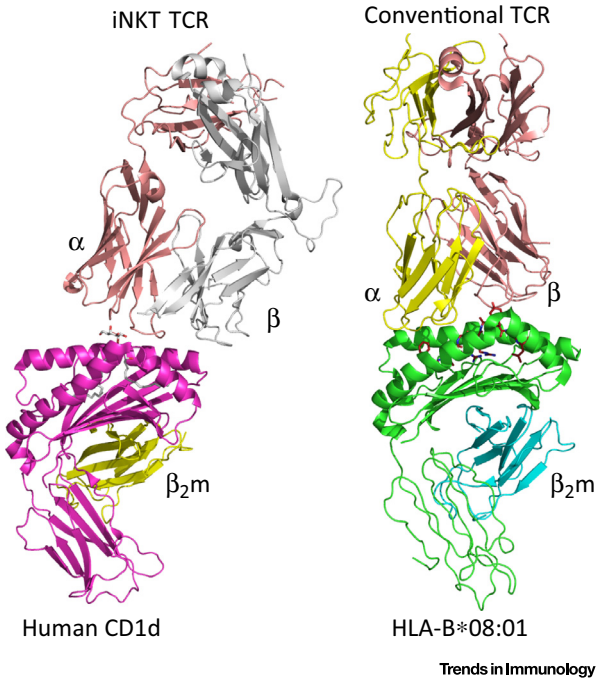

Figure 3. Comparative Anatomy of the Semi-Invariant NKT Cell TCR/α-GalCer–CD1d and Conventional TCR/peptide–MHC Class I Ternary Structures. The interactions between human iNKTCR and its ligand, α-GalCer–CD1d (left), as well as the interactions between a conventional human TCR and its antigen, peptide (as in Figure 1B)–HLA-B*08:01 (right), are shown. Structures were generated by PyMol using the protein database IDs 3HUJ (left) [118] and 3SJV (right) [119]. Abbreviations: α, TCRα; β, TCRβ; β_2m, β_2-microglobulin; HLA, human leukocyte antigen; iNKT, invariant NKT cell; MHC, major histocompatibility complex; NK, natural killer; TCR, T cell receptor.

[20] (Figure 4). In addition, some CD1 isoforms, especially CD1a, can exchange lipids at the cell surface. While many lipids do not require any additional processing for their association with CD1 proteins, some antigens require trimming by intracellular or extracellular carbohydrate hydrolases or **phospholipases**. Lipid exchange in endosomal compartments, which may or may not involve removal of spacer lipids, is facilitated by CD1e (in human but not mouse) or a variety of LTPs, including several saposins and the GM2 activator protein [21].

CD1-Restricted T Cells

The analysis of CD1-restricted T cells has been greatly facilitated by the generation of CD1–lipid **tetramers**, which are now available for all group 1 and 2 CD1 isoforms [13]. A general theme in CD1-restricted T cell responses is a propensity for autoreactivity, which has been observed for each of the CD1 isoforms [5,13]. CD1-restricted T cells may express αβ or γδ TCRs (Table 1), and include $CD4^+$, $CD8^+$ and **double-negative cells** [5,13,22]. Most studies on group 1 CD1-restricted T cells have focused on TCRs reactive with self- or mycobacterial antigens [5,13]. T cells with reactivity against mycobacterial antigens are enriched in tuberculosis patients, and many of these cells have cytotoxic properties. With the exception of some CD1b-restricted T cells, group 1 CD1-restricted $TCR\alpha\beta^+$ T cells express diverse TCRs and exhibit adaptive-like effector functions similar to those of conventional MHC-restricted T cells [5,13]. The majority of CD1d-restricted T cells express natural killer (NK) cell surface markers such as NK1.1 (in mice) and are referred to as natural killer T (NKT) cells [23,24] (Table 1). One subset of NKT cells, known as type I NKT cells or invariant NKT (iNKT) cells, express TCRα chains that are germline encoded, whereas a second subset of NKT cells, known as type II NKT cells, diverse NKT (dNKT) cells, or variant NKT cells, express relatively diverse TCRs. In this review article we will refer to these subsets as iNKT and dNKT cells. NKT cells recognize a variety of endogenous and exogenous lipid antigens, and

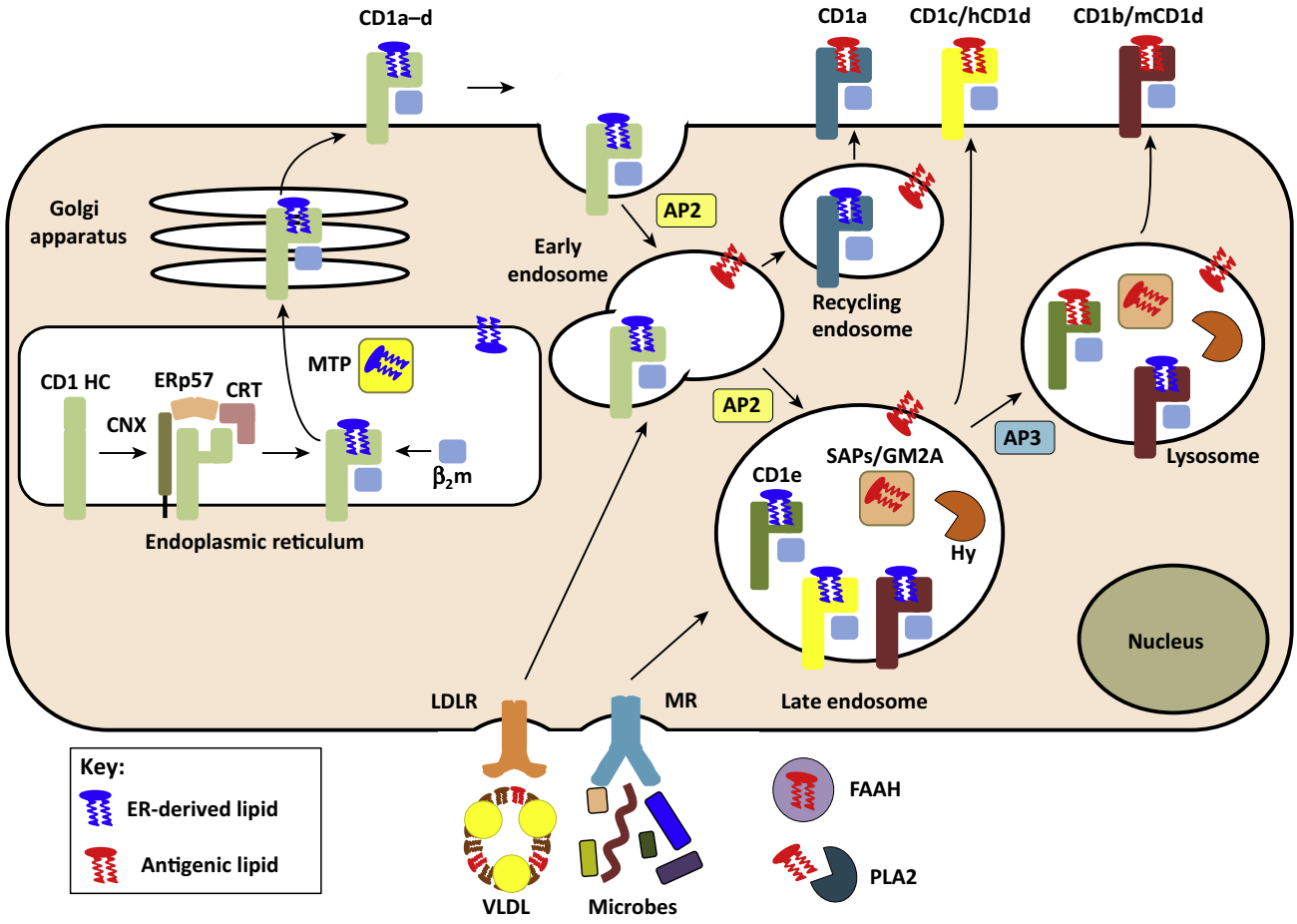

Figure 4. CD1-Restricted Antigen-Processing Pathways. Newly synthesized CD1 heavy chains (generic CD1 heavy chains are indicated in light green whereas individual heavy chains are indicated in other colors) in the ER are stabilized by a variety of chaperones (CNX, ERp57, and CRT) and assemble with β_2m and ER-derived chaperone lipids that are loaded onto CD1 with the assistance of the lipid-transfer protein (LTP) MTP. CD1–lipid complexes then transit to the cell surface, except for CD1e, which is directly transported to endosomal compartments, where it is cleaved into a soluble form. Following their arrival at the cell surface, CD1 proteins are internalized via their cytoplasmic, tyrosine-based sorting motifs that interact with AP2 (CD1b-d) and AP3 (CD1b and mCD1d) complexes, which permit CD1 proteins access to distinct intracellular compartments. CD1a, which lacks AP2 and AP3 sorting motifs, only accesses early endosomes in an AP-independent manner. ER-resident CD1d proteins may also gain access to endolysosomal compartments via an auxiliary pathway that involves the MHC class II-associated invariant chain (not depicted). In the endocytic system, CD1 proteins are loaded with endogenous or exogenous (e.g., microbial) lipid antigens, with the assistance of a variety of LTPs such as CD1e, SAPs, and GM2A. CD1 antigens may be delivered to these intracellular compartments via extracellular lipid-binding proteins such as VLDL and FAAH, followed by receptor-mediated uptake via a variety of receptors such as the mannose and LDL receptors. Some products also require processing into antigenic ligands via extracellular factors such as PLA2 or intracellular factors such as lipid or carbohydrate Hy. Following loading with lipid in intracellular compartments, CD1–lipid complexes recycle back to the cell surface. Note that most antigen-presenting CD1 isoforms can also be loaded with some antigens at the cell surface (not depicted). Abbreviations: AP, adaptor protein; CNX, calnexin; CRT, calreticulin; ER, endoplasmic reticulum; FAAH, fatty acid amide hydrolase; GM2A, GM2 activator; HC, heavy chain; hCD1d, human CD1d; Hy, hydrolase; LDLR, low-density lipoprotein receptor; mCD1d, mouse CD1d; MHC, major histocompatibility complex; MR, mannose receptor; β_2m, β_2-microglobulin; MTP, microsomal triglyceride protein; PLA2, phospholipase A2; SAP, saposin; VLDL, very low density lipoprotein.

exhibit innate-like effector functions, with complementary although sometimes opposing roles. One common feature of the CD1 antigen recognition system is that, in addition to TCRs with exquisite antigen-specificity, TCRs with limited or no antigen-specificity and a heavy bias towards the recognition of CD1 alone are frequently observed. The latter property is consistent with the finding that this recognition system frequently involves reactivity of individual TCRs against both self- and foreign antigens. In addition to TCR-mediated activation, CD1-restricted T cells are also highly responsive to innate cytokine signals, even in the absence of TCR engagement [24–26], in this respect behaving similarly to innate lymphoid cells.

CD1a

Like the other CD1 isotypes, CD1a is expressed by myeloid APCs, but is especially abundant on epidermal **Langerhans cells**, and is employed as a characteristic cell marker for this cell type in humans [5,13]. CD1a-restricted T cells have been identified that react with antigens derived from mycobacteria, pollen, and endogenous skin oils [27–29]. Although autoreactivity is a common feature of CD1-restricted T cells, this is particularly the case for CD1a-restricted T cells [30]. The latter finding is consistent with the capacity of the CD1a groove to accommodate a large variety of antigens [8]. CD1a has the smallest antigen-binding groove of all CD1 proteins and can accommodate not only lipids with polar headgroups, such as sulfatide, but also 'head-less' hydrophobic lipids such as skin-derived squalene, wax esters, and triacylglycerides [8,12,31]. CD1a-restricted T cells reactive with both types of lipid antigen have been identified. Because the TCRs reactive with lipids containing polar headgroups are usually very sensitive to head-group modifications, it has been suggested that such TCRs interact with both CD1a and lipid, consistent with the classical concept of dual recognition of both MHC and antigen by TCRs. This mode of recognition contrasts sharply with that of CD1a-restricted TCRs to lipids that lack polar headgroups. Crystallographic studies of an autoreactive TCR showed that it interacted with the A′ roof of CD1a in an asymmetric, left-sided manner but did not contact the lipid antigen in the groove [30]. It was proposed that in those cases the lipid largely plays a CD1a-stabilizing role without directly contributing to TCR interactions and that it is therefore not an antigen in the strict sense but rather a permissive ligand. Such a recognition mode, which is likely common among autoreactive CD1a-restricted T cells, might be able to accommodate a variety of lipids that can stabilize the CD1a groove and activate the TCR, provided that their headgroups do not interfere with the capacity of the TCR to dock onto the A′ roof of the CD1a groove. Indeed, permissive lipids for this autoreactive TCR include phospholipids, lysophosphatidylcholine (lyso-PC), and fatty acids that lack polar headgroups, whereas non-permissive lipids include sphingomyelin and sulfatide that contain polar headgroups [8,12,31].

CD1a is the only antigen-presenting CD1 isoform that lacks a tyrosine-based sorting motif for internalization into endosomes [5]. Its localization is therefore restricted to the cell surface and early endosomal compartments with a pH close to neutral. CD1a is relatively stable in the absence of bound lipid and can exchange lipids at the cell surface, without the need for additional accessory factors. Recent studies have provided evidence that the permissive free fatty acids that stabilize the CD1a groove for recognition by autoreactive CD1a-restricted TCRs are generated from common skin phospholipids via endogenous or exogenous phospholipase A2 (PLA2) action [28,31–33].

Among the CD1a-restricted T cells, the autoreactive T cells in the skin have been characterized most extensively [31]. These cells are present in the dermis of the skin and are thus physically separated from the CD1a-expressing Langerhans cells in the epidermis. These T cells exhibit autoreactivity against hydrophobic skin lipids such as squalene, fatty acids, and wax esters. These lipids are contained within skin sebum, which is produced by sebaceous glands and exits the skin via hair follicles to coat the outer layer of the epidermis. Thus, access of these lipids to CD1a-expressing Langerhans cells and CD1a-restricted T cells requires a skin breach via infection or injury. Recent studies have suggested that endogenous PLA2 secreted by skin cells may be involved in processing ubiquitous skin phospholipids into the natural oils recognized by CD1a-reactive T cells [33]. This possibility was suggested by a provocative study showing that PLA2 in insect venom, when introduced into the dermis of the skin, can convert phospholipids into CD1a-binding fatty acids that are capable of activating autoreactive CD1a-restricted T cells [32]. Consistent with this finding, CD1a-restricted T cells were expanded in individuals allergic to bee and wasp venom [34]. Autoreactive CD1a-restricted T cells produce the cytokines IFN-γ and IL-22 [28] which contribute to antimicrobial defenses, keratinocyte proliferation, and a variety of skin inflammatory diseases. In this manner, CD1a-reactive T cells

might be able to respond quickly to tissue injury, infectious agents, and venoms by promoting antimicrobial and inflammatory responses. Consequently, this pathway may be exploited for therapeutic purposes against a variety of diseases. In this context it is interesting that several skin **pathobionts**, including the fungal organism *Candida albicans* and the bacterial organism *Staphylococcus aureus*, secrete phospholipases, which may thus contribute to the generation of stimulatory ligands for CD1a-reactive T cells. In addition, some vaccine **adjuvants** contain squalene or other oils that may be able to activate CD1a-restricted T cells, thus potentially contributing to adjuvanticity. In addition to skin-derived self-lipids, CD1a-restricted T cells reactive with mycobacterial and pollen-derived lipids have also been identified [29], although their physiological function requires further study.

CD1b

Apart from thymocytes, CD1b is nearly exclusively expressed by dendritic cells (DCs). Autoreactive and pathogen-reactive CD1b-restricted T cells have been identified [5,13]. CD1b has the largest binding groove of all CD1 proteins and contains four interconnected pockets (A′, C′, F′, and T′). This permits CD1b to bind to lipids containing long alkyl chains such as mycobacterial mycolic acid, glucose monomycolate, and glycerol monomycolate [8,12]. In addition, shorter lipids such as mycobacterial sulfoglycolipids and a short-chain form of glucose monomycolate can be loaded onto CD1b in the presence of a spacer lipid, a diacylglycerol, or a deoxyceramide (see chemical structures in Figure 2) [16]. Such natural spacer lipids in CD1b push the groove upwards to enhance antigen recognition, and thus function as scaffolds to support the TCR/CD1b–lipid interface. This concept is consistent with the finding that long-chain glucose monomycolate requires the low pH environment of lysosomes for lipid exchange, whereas short-chain monomycolate can be loaded at the cell surface [8,12]. Presumably, the former type of lipid exchange involves release of either a large ER-derived lipid, or a short ER-derived lipid together with a spacer lipid, whereas the latter type of lipid exchange may involve release of only the short ER-derived lipid while the spacer lipid remains bound with CD1b.

Although most CD1b-restricted T cells express diverse TCRs, subsets of glucose monomycolate-reactive T cells expressing semi-invariant [termed germline-encoded mycolyl (GEM) lipid-reactive T cells] [35] or biased (termed LDN5-like T cells) [36] TCRs have been identified (Table 1). Among all CD1-restricted T cells that recognize mycobacterial antigens, CD1b-restricted T cells are most abundant [5]. Such cells are enriched in peripheral blood and at sites of infection in tuberculosis patients [37]. These cells exhibit cytotoxic activities and produce a variety of cytokines such as IL-2 and IFN-γ [37]. Recent studies with humanized transgenic animals expressing all human group 1 CD1 proteins together with a mycolic acid-specific CD1b-restricted TCR have provided the first convincing evidence for rapid and protective host immune responses mediated by the CD1 antigen-presentation system [38]. This rapid response to *Mycobacterium tuberculosis* infection suggests that this system can be targeted for the development of tuberculosis vaccines.

The recent analysis of a set of autoreactive CD1b-restricted T cells revealed reactivity against phosphatidylglycerol (PG) produced by mitochondria as well as by several different bacterial pathogens such as *Salmonella* and *Staphylococcus* spp. [39]. Thus, these cells exhibit mixed reactivity against self- and foreign antigens. These findings also provide evidence for a much broader reactivity of CD1-reactive T cells to bacterial pathogens, and suggest a role for these cells in settings such as metabolic diseases that are associated with mitochondrial stress.

CD1c

CD1c is abundantly expressed by myeloid DCs and B cells, especially **marginal zone B cells**. CD1c-restricted αβ and γδ T cells that react with endogenous sulfatides or cholesteryl esters, or with mycobacterial cell-wall antigens, have been identified [5,13,40,41]. Each of the exogenous

CD1c antigens identified–phosphomycoketide, mannosyl-phosphomycoketide, and a synthetic lipopeptide (acyl-12)–contain a single alkyl chain which is embedded in the A′ pocket [17,42]. In one of the CD1c-lipid co-crystals, the F′ pocket contained a C12-hydrocarbon chain likely derived from a detergent in the solution used during crystallization [17]. This finding suggests that spacer lipids are involved in the CD1c-mediated presentation of lipid antigens containing a single alkyl chain.

CD1c-reactive T cells have been identified in human blood, expand in patients with tuberculosis, and infiltrate organs affected by autoimmunity [5]. Although CD1c-restricted γδ T cells were identified over 25 years ago, the natural ligands of Vδ1$^+$ TCRs have only recently been identified as mycobacterial phosphomycoketides [43]. Strikingly, these γδ T cells also reacted with diverse lipids, including lyso-PC, sulfatide, and mannosyl-phosphomycoketide, which is consistent with a dominant role for TCR interactions with CD1c and with the concept of mixed self- and foreign antigen reactivity often observed in the CD1 system. Although the available evidence is highly suggestive of functions in infection and autoimmunity, this has not yet been conclusively demonstrated.

CD1d

In contrast to group 1 CD1 proteins, whose expression is limited to hematopoietic cells, CD1d is expressed more widely by both hematopoietic and non-hematopoietic cells, including thymocytes, professional APCs, hepatocytes, **hepatic stellate cells**, intestinal epithelial cells, and adipocytes, and is particularly abundant on marginal zone B cells [6,44]. CD1d can present a variety of glycosphingolipids, diacylglycerols, phospholipids, lipopeptides, ether lipids, non-lipid small molecules, and possibly even peptides to CD1d-restricted cells [8,13,24,31,45–48]. Exogenous antigens recognized by iNKT cells include lipids derived from ubiquitous environmental bacteria, pathogenic bacteria, fungi, commensal bacteria, and pollen. Much of our understanding of iNKT cell biology has been gleaned from studies with KRN7000, a synthetically optimized version of an α-galactosylceramide (α-GalCer), agelasphin 9b, derived from the marine sponge *Agelas mauritianus* [49,50]. Several microbes, including the commensal bacterium *Bacteroides fragilis* [51,52] and the fungal pathogen *Aspergillus fumigatus* [53], a common cause of airway hypersensitivity, contain iNKT cell-stimulating α-GalCers. These findings also suggest that agelasphin 9b is derived from commensal organisms associated with the marine sponge, rather than from the sponge itself. Other microbial iNKT cell agonists include α-glycuronosylceramides from *Sphingomonas* bacteria [54–56], diacylglycerols from *Streptococcus pneumoniae* [57] and the Lyme disease agent *Borrelia burgdorferi* [58], and cholesteryl α-glucosides from *Helicobacter pylori* [59], a common agent of stomach ulcers and gastric cancer. Endogenous antigens recognized by iNKT cells include α- and β-linked GalCers and glucosylceramides (GlcCers), isoglobotrihexosylceramide (iGb3), ganglioside D3 (GD3), ether-bonded lipids, and glycerophospholipids such as phosphatidylinositol (PI), phosphatidylethanolamine (PE), and lyso-PC [24]. Antigens recognized by subsets of dNKT cells include endogenous sulfatide, lysophospholipids, lyso-PC, β-glycosylceramides (β-GlyCers), and bacteria-derived PG, di-PG (cardiolipin), and PI [60–62]. In addition to lipid-reactivity, CD1d-restricted T cells have been identified that react with synthetic, non-lipidic phenyl pentamethyldihydrobenzofuran sulfonates (PPBF) [63]. While provocative, the functional significance of this type of reactivity remains unknown.

Crystal structures of human or mouse CD1d complexed with a variety of ligands have been analyzed [8,24]. The fatty-acyl chain of α-GalCer binds within the large A′ pocket of CD1d whereas the sphingosine chain fits within the F′ pocket, with the galactosyl head extending out of the groove [64,65] (Figure 1B, left). Other NKT cell antigens, including β-GlyCer, sulfatide, iGb3, phosphoglycerolipids, and microbial diacylglycerols, bind to CD1d in the same conserved manner. In the case of diacylglycerols, however, the acyl chains can bind to CD1d in two

different orientations, with the individual chains positioned within either the A′ or F′ pockets, which has a major impact on TCR recognition. The sugar headgroups of α-linked glycolipids that extend out of the CD1d groove adopt a similar orientation such that they are easily accessible by the TCR. In sharp contrast, the headgroups of the β-linked glycolipids project up and away from the CD1d groove. Co-crystals of TCR/CD1d–lipid complexes have revealed that, in sharp contrast to MHC- and group 1 CD1-restricted TCRs, the iNKT cell TCR (iNKTCR) docks parallel onto the CD1d groove, with most of the interface being dominated by the germline-encoded TCRα chain (Figure 3) [1,8,24,66]. To maintain this conserved footprint, the TCR was able to induce structural changes in both CD1d and the orientation of the ligand. For example, the iNKTCR interacted with CD1d–β-GlcCer and CD1d–iGb3 complexes by flattening the sugars that protrude out of the antigen-binding groove [67,68]. Interestingly, the structure of a dNKTCR complexed with CD1d–lysosulfatide revealed a diagonal footprint similar to that of TCR/MHC–peptide complexes, with lysosulfatide being recognized exclusively by the TCRβ chain [69], thus revealing features of recognition similar to iNKT cells and conventional peptide-reactive T cells.

Like the group 1 CD1 proteins, CD1d assembles in the ER where it is stabilized by chaperone lipids that have been identified as phospholipids, which are rarely recognized by NKT cells [19,24,60]. Association with ER-derived chaperone lipids is catalyzed by MTP. Following display at the cell surface, CD1d is internalized via its tyrosine-based sorting motif and enters endosomal and lysosomal compartments. While some autoreactive CD1d-restricted T cells recognize antigens that do not require CD1d internalization, and α-GalCer can be loaded directly onto CD1d at the cell surface, most CD1d antigens are acquired in endocytic compartments. An alternative, auxiliary pathway for CD1d to arrive in these intracellular structures is via association with the MHC class II-associated invariant chain [70]. In these intracellular compartments, CD1d binds to lipids with the assistance of LTPs, including saposins A-D, GM2 activator, Niemann–Pick type C2 protein, and thymocyte-derived cathepsin L. Delivery of antigens to these compartments may involve extracellular lipid-binding proteins such as apolipoprotein E-containing VLDL and fatty-acid amide hydrolase (FAAH), and receptor-mediated entry via lipid receptors such as the low-density lipoprotein receptor (LDLR) or lectin receptors such as the mannose receptor [20]. Several precursors to CD1d-presented antigens require processing by carbohydrate hydrolases or phospholipases. For example, in the case of CD1d-restricted T cells in the liver that respond to hepatitis B virus infection, antigenic ER lipids such as lyso-PE were generated from ubiquitous phospholipids via secretory PLA2 [71]. In addition, lysosomal PLA2 was shown to be required for the intrathymic development of iNKT cells and for the presentation of endogenous antigens by CD1d [72].

A topic of controversy in the NKT cell field that remains to be fully resolved is the identity of the natural self-antigen(s) that mediate the intrathymic development and peripheral functions of iNKT cells [24]. For many years it was thought that iNKT cells selectively react with α-linked glycosphingolipids, which was unanticipated in the context of the common belief that mammalian cells only produce β-linked glycosphingolipids. A first surprise was that cells deficient in β-GlcCer synthase were unable to activate autoreactive mouse iNKT cell hybridomas [73]. Subsequent studies provided evidence that β-GlyCer may be recognized by iNKT cells, which was supported by X-ray crystallographic studies [68], but the synthetic preparations used in these studies appeared to contain minute amounts of α-linked GlyCers. A second surprise was the identification of the endogenous, lysosomal β-linked glycosphingolipid iGb3 as a weak iNKT cell agonist [74]. However, human cells do not produce iGb3, and the lack of an iNKT cell phenotype in iGb3 synthase-deficient mice [75] raised doubt about the physiological relevance of iGb3 to iNKT cell function. The final surprise came when two research groups performed a variety of elegant biochemical and structural analyses of lipid preparations that were able to stimulate autoreactive iNKT cells [76–78]. These studies provided strong evidence for

α-GlcCers and α-GalCers–not thought to be present in mammalian cells–as natural iNKT cell ligands. The minute amounts of these antigens produced by mammals were likely overlooked in previous studies, but their low prevalence is consistent with the physiological functions of iNKT cells. The nature of the enzymatic activities involved in the generation of mammalian α-linked GlyCers and how these activities are controlled during normal and pathological conditions remain to be elucidated. While current evidence indicates that these α-GlyCers control the physiological functions of iNKT cells in the periphery, they do not appear to be required for the intrathymic development of these cells. Instead, one study provided evidence for the involvement of peroxisome-derived, ether-bonded lipids in the intrathymic development of iNKT cells [79]. If and how the requirement of lysosomal PLA2 activity for iNKT cell development [72] aligns with these findings remains to be determined.

As already noted, NKT cells are CD1d-restricted T cells that coexpress lipid-reactive αβ (or γδ) TCRs together with NK cell markers [6,23,44,47,80]. iNKT cells express TCRs composed of an invariant TCRα chain (Vα14–Jα18 in mouse or Vα24–Jα18 in human) and a restricted set of TCRβ chains that react with α-GalCer. These cells are substantially more abundant in mice than humans, and their numbers vary widely among different human subjects. iNKT cells are most prevalent in liver and are also abundant in spleen, peripheral blood, bone marrow, thymus, and mucosal tissues in gut and lung. Apart from $V\alpha14^+$ NKT cells, an additional subset of α-GalCer-reactive NKT cells have been identified that express a unique Vα10–Jα50 TCRα chain in mice [81]. Strikingly, the latter cells exhibited greater reactivity to α-GlcCer and also reacted with mycobacterial α-glucuronosyldiacylglycerol (α-GlcADAG), suggesting important functions. iNKT cells express the innate master transcription factor promyelocytic leukemia zinc finger (PLZF), exhibit cytotoxic activities, and can produce a wide variety of cytokines. Subsets of iNKT cells with specialized effector functions similar to the diverse properties of adaptive $CD4^+$ helper T cell subsets have been identified, and these include NKT1 cells producing IFN-γ (and IL-4), NKT2 cells producing IL-4 and IL-13, NKT10 cells producing the immunosuppressive cytokine IL-10, NKT17 cells producing IL-17a, and follicular helper NKT cells producing IL-21 [24,82,83]. These iNKT cell subsets are selectively enriched in distinct organs and tissues. Consequently, iNKT cells can influence the functions of a variety of innate and adaptive immune cells. These cells rapidly elicit their broad effector functions following stimulation with α-GalCer and, instead of developing immune memory, become unresponsive to antigen restimulation because they acquire an **anergic** and regulatory phenotype [23,84,85]. The functions of iNKT cells span the entire range of the immune response, including host defense against pathogens, autoimmunity, tissue graft rejection, hypersensitivities, tumor immunity, and metabolic disease [5,6,23,24,44]. The role of iNKT cells in immune responses against pathogens is not limited to organisms containing natural iNKT cell antigens but extends to many organisms such as viruses that lack cognate iNKT cell antigens. For such pathogens, iNKT cells are activated in response to innate cytokine signals, in the presence or absence of TCR signaling via autoantigens [26]. In fact, one study provided evidence that the response of iNKT cells to pathogens is dominated by innate cytokines, even for those pathogens containing cognate iNKT cell antigens [86]. The role of CD1d-restricted T cell responses against pathogenic organisms is underscored by evasion mechanisms that are employed by multiple pathogens to subvert CD1d-restricted antigen presentation [87]. Studies over the past several years have further shown that iNKT cells influence the composition of the natural gut **microbiota** and, conversely, that members of the microbiota such as *Bacteroides fragilis* that contain iNKT cell antigens shape iNKT cell effector functions [51,88]. This dynamic interaction between iNKT cells and the microbiota may have major consequences for human health, raising the possibility of preventing the development of diseases such as asthma and inflammatory bowel disease by administering neonates with probiotics that influence iNKT cell function [89]. iNKT cell agonists such as α-GalCer have been extensively employed to explore the adjuvant and therapeutic activities of iNKT cells, with promising preclinical studies for some tumors, infectious agents, and autoimmune diseases, and

some encouraging findings with cancer patients [49,90,91]. Nevertheless, inducing biological responses of iNKT cells in humans has proved to be challenging, and the potential for generating adverse effects rather than protecting against disease remains an important concern.

In contrast to iNKT cells, dNKT cells do not typically react with α-GalCer and most of these cells express diverse TCRs [13,47,80]. Nevertheless, the subset of sulfatide-reactive dNKT cells contains populations with biased TCRs. Importantly, dNKT cells are more prevalent in humans than in mice. Like iNKT cells, dNKT cells express PLZF [92], exhibit innate-like functions, and can influence a wide variety of immune responses [47,93,94]. An interesting phenomenon is that dNKT cells often oppose the functions of iNKT cells [95]. For example, while iNKT cells exhibit natural immunity against some metastatic cancers, dNKT cells have a propensity to promote cancer growth [96]. Preclinical studies with sulfatide have provided promising results with several autoimmune diseases, raising the possibility of developing dNKT cell-based immunotherapies for human diseases.

Recently, a subset of human dNKT cells termed 'atypical NKT cells' expressing diverse α-GalCer-reactive TCRs has been identified [97]. While the functions of these cells remain unclear, crystal structures of two of these TCRs bound with CD1d–α-GalCer complexes revealed orthogonal binding over the A′ pocket of CD1d, contrasting sharply with the docking mode of iNKTCRs [97].

A recent provocative report [98] has renewed interest in the possibility that CD1d-restricted T cells can react with peptides, a notion that has been entertained for over 20 years. Early studies provided evidence that mouse CD1d can present a variety of hydrophobic synthetic peptides with the common motif [FW]-X-X-[ILM]-X-X-W to CD1d-restricted T cells [99]. Subsequent studies identified CD1d-restricted $CD8^+$ T cells reactive with peptides from the model antigen ovalbumin [100,101]. However, owing to difficulties in understanding the molecular basis of peptide recognition and the preponderance of lipid-reactivity among CD1d-restricted T cells, the issue of peptide-reactivity was largely put to rest. A more recent study reported CD1d-restricted recognition of a collagen-derived peptide by $CD4^+$ T cells [102], but its molecular basis was unclear. Until the recent study by Girardi *et al.* [98], the exact location of peptide binding to CD1d remained elusive. These investigators determined the crystal structure of the complex between mouse CD1d and the first peptide identified to bind CD1d, synthetic p99. This peptide adopts an α-helical conformation in the CD1d groove that orients the motif residues towards the bottom of the groove, in a manner consistent with its presentation to TCRs. Although the functions of peptide-reactive dNKT cells remain to be determined, it is striking that the CD1d peptide motif is contained within peptides derived from several viruses such as HIV [103], suggesting potential antiviral functions. In addition, studies with the CD1d-binding, collagen-derived peptide have provided evidence for potent immune-modulatory activities of peptide-reactive, CD1d-restricted T cells [102].

CD1e

CD1e is expressed by thymocytes and DCs. As already noted, CD1e is only expressed intracellularly and does not function as an antigen-presenting molecule [5]. Instead, membrane-anchored CD1e is transported to endolysosomal compartments where it is cleaved into soluble proteins (Figure 4). CD1e is a lipid-binding protein with a wide, solvent-exposed antigen-binding groove that includes contiguous A′ and F′ pockets [104]. CD1e modulates the presentation of endogenous and exogenous lipids by human CD1b, CD1c, and CD1d, and this has been proposed to reflect its capacity to accelerate the generation and dissociation of CD1–lipid complexes [105]. In addition, CD1e has been suggested to assist lysosomal α-mannosidase in the processing of mycobacterial lipids into antigenic CD1b ligands [106]. In this manner, CD1e might influence both lipid availability and the generation and persistence of CD1–lipid complexes.

Concluding Remarks and Future Perspectives

The studies reviewed here highlight the emergence of the CD1 antigen-presentation system as an important complement to the classical MHC antigen-presentation system in health and disease. Recent studies have provided new insight into this system by identifying novel cognate antigens and the factors involved in the generation and processing of antigens. These studies have also revived the idea that CD1d can present peptide antigens to T cells. We now understand in some depth how distinct antigens bind to individual CD1 isoforms, and this not only involves antigenic lipids but also lipids that function as chaperones, spacers, and scaffolds. TCRs engage CD1–antigen complexes with footprints that exhibit a surprising amount of diversity. While significant progress has been made regarding the effector functions and immunological properties of CD1-restricted T cells, many questions remain to be addressed (see Outstanding Questions) before this fascinating antigen-presentation system can be fully exploited for the development of vaccines and immune therapies.

Outstanding Questions

From a structural perspective, how do CD1 proteins bind to chemically diverse ligands, including lipids, non-lipidic small molecules, and even peptides? How can CD1-restricted TCRs engage such diverse CD1–antigen complexes?

What are the mechanisms that control the activities and functions of CD1-restricted T cells which exhibit mixed reactivity against self- and foreign antigens?

Does the range of microbial products recognized by group 1 CD1-restricted T cells extend much beyond mycobacterial cell-wall products?

Is the prevalence and function of group 1 CD1-restricted T cells influenced by environmental mycobacteria or commensal microorganisms?

How can one effectively target group 1 CD1-restricted T cells for vaccine development and immunotherapies? Studies with humanized mice and with guinea pigs that express all five CD1 isoforms would be revealing.

What are the physiological ligands that control the intrathymic development of CD1d-restricted NKT cells?

What are the mechanisms that control the enzymatic activities responsible for the generation of the natural, endogenous α-linked glycosylceramide ligands of iNKT cells?

What is the significance of peptide presentation by CD1d?

How can one selectively activate distinct iNKT cell subsets for therapeutic purposes?

Can one manipulate iNKT cell functions by administering probiotics to human neonates so as to lower disease susceptibility later in life?

How can the preclinical vaccine and therapeutic studies with NKT cell antigens be effectively translated to human subjects?

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