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CD1: Antigen Presentation and T Cell Function

Manfred Brigl and Michael B. Brenner
Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115;
email: mbrigl@rics.bwh.harvard.edu, mbrenner@rics.bwh.harvard.edu

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■ **Abstract** This review summarizes the major features of CD1 genes and proteins, the patterns of intracellular trafficking of CD1 molecules, and how they sample different intracellular compartments for self- and foreign lipids. We describe how lipid antigens bind to CD1 molecules with their alkyl chains buried in hydrophobic pockets and expose their polar lipid headgroup whose fine structure is recognized by the TCR of CD1-restricted T cells. CD1-restricted T cells carry out effector, helper, and adjuvant-like functions and interact with other cell types including macrophages, dendritic cells, NK cells, T cells, and B cells, thereby contributing to both innate and adaptive immune responses. Insights gained from mice and humans now delineate the extensive range of diseases in which CD1-restricted T cells play important roles and reveal differences in the role of CD1a, CD1b, and CD1c in contrast to CD1d. Invariant TCRα chains, self-lipid reactivity, and rapid effector responses empower a subset of CD1d-restricted T cells (NKT cells) to have unique effector functions without counterpart among MHC-restricted T cells. This review describes the function of CD1-restricted T cells in antimicrobial responses, antitumor immunity, and in regulating the balance between tolerance and autoimmunity.

CD1 GENES

CD1 molecules were first identified using monoclonal antibodies that bound to human thymocytes (1), and were designated Cluster of Differentiation 1 (CD1) based upon their leukocyte staining characteristics at the First International Workshop on Human Leukocyte Differentiation Antigens (2).[1] CD1 genes have an intron/exon

[1]Abbreviations used: ACAID, anterior chamber-associated immune deviation; AHR, allergen-induced airway hyperreactivity; APC, antigen-presenting cell; ANS, 1-anilinonapthalene-8-sulfonic acid; bp, base pairs; CD1, Cluster of Differentiation 1; DC, dendritic cell; DN, double negative; DTH, delayed type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; EMCV-D, diabetogenic encephalomyocarditis virus; ER, endoplasmic reticulum; GM-CSF, granulocyte/macrophage colony-stimulating factor; GPI, glycosylphosphatidylinositol; HEV, high endothelial venule; HSV-1/-2, herpes simplex virus type 1 and 2; IEL, intestinal intraepithelial lymphocytes; IFN-γ, interferon-γ;

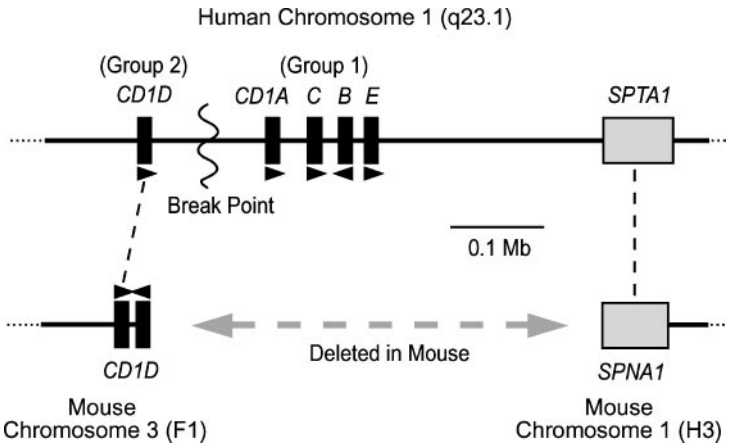

Figure 1 A genomic map of the human and mouse CD1 locus and the syntenic regions between human chromosome 1 (*upper part*) and mouse chromosomes 3 and 1 (*lower part*) indicates a 750,000 bp gap in synteny between the two species. This suggests that a chromosomal translocation may have led to the deletion of CD1A, B, and C in rodents. CD1 genes are depicted as black boxes, and the direction of transcription is indicated by arrowheads. Orthologous genes are connected by dashed lines. The hypothetical break point (*wavy line*) for the ancestral mouse species is arbitrarily placed between the group 1 and group 2 gene clusters within the human CD1 locus.

structure comparable to that of major histocompatibility complex (MHC) class I genes and encode type 1 integral membrane proteins consisting of $\alpha 1$, $\alpha 2$, and $\alpha 3$ domains, similar to MHC class I molecules (3, 4). The $\alpha 3$ domains are the most homologous among CD1 family members (71%–88%), showing clear but limited homology (21%) to MHC class I $\alpha 3$ domains (5). The CD1 genes are not human orthologs of murine Tla genes nor non-classical MHC-encoded class I genes, but rather form a distinct locus of tightly clustered genes located in a 170,000 bp region of chromosome 1q23.1 in human and on chromosome 3 in mouse, regions that are unlinked to the mammalian MHC (6–9) (Figures 1 and 2).

The five CD1 genes that have been identified in man are designated CD1A, CD1B, CD1C, CD1D, and CD1E, corresponding to five CD1 proteins: CD1a, CD1b, CD1c, CD1d, and CD1e (3, 5, 6, 10). We adopt the use of upper case letters for genes and lower case for proteins. The nomenclature used during active identification of the CD1 genes made assignments based on the nominal size of

LAM, lipoarabinomannan; LPS, lipopolysaccharide; MCA, methylchlorantrene; MDL, mature DC lysomomes; MHC, major histocompatibility complex; NK, natural killer; NKT, Natural Killer T cell; ODN, oligodinucleotide; PIM, phosphatidylinositol mannoside; RSV, respiratory syncytial virus; SLE, systemic lupus erythematosus; PPD, purified protein derivative; TCR, T cell receptor; TNF-α, tumor necrosis factor-α; TRAIL, TNF-related apoptosis-inducing ligand.

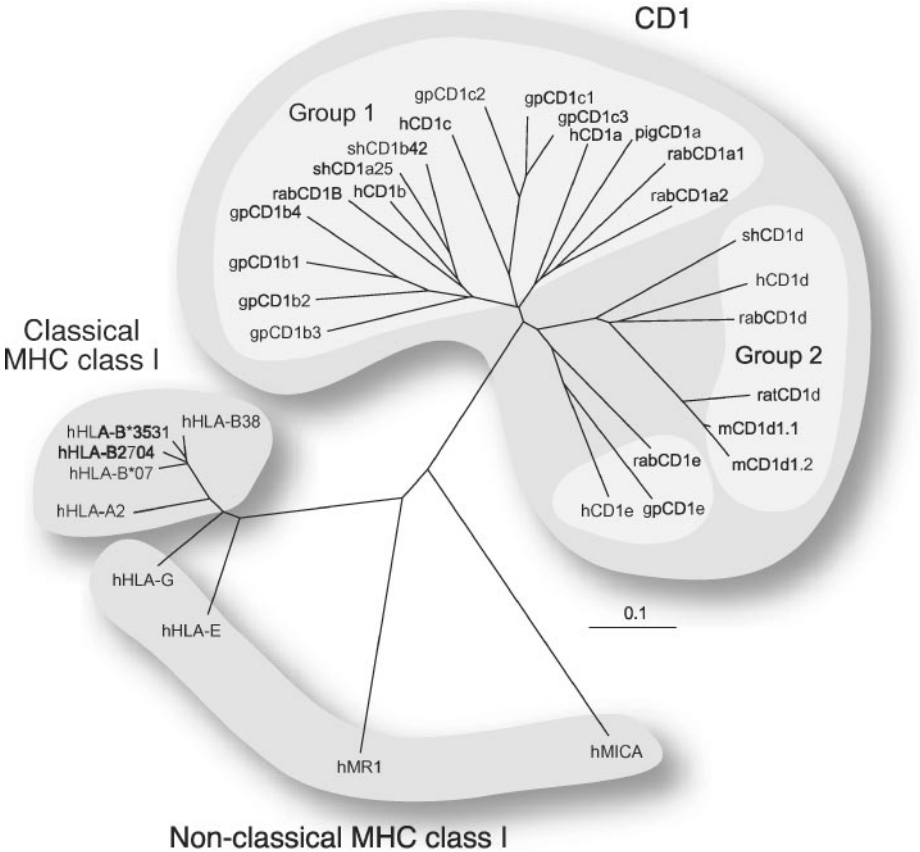

Figure 2 Similarity and proposed evolutionary relationship between known CD1, MHC class I, and non-classical MCH class I protein sequences. All known full-length CD1 proteins from humans, mice, and other mammals (h, human; m, mouse; gp, guinea pig; rab, rabbit; sh, sheep; rat; pig) and examples of classical and non-classical human MHC class I proteins are shown (15). The unrooted dendrogram was created using the Neighbor-Joining method of Satori and Nei.

their genomic EcoRI fragments and is now replaced by their CD designations (R4 = CD1A, R1 = CD1B, R7 = CD1C, R3 = CD1D, and R2 = CD1E). Based on sequence identities in the $\alpha 1$ and $\alpha 2$ domains, the CD1 isoforms were separated into two sets, the CD1a, b, and c set (group 1) and the CD1d set (group 2) and CD1e intermediate (10) (Figure 2).

Mice only contain CD1D orthologs. Two highly homologous CD1D genes (CD1D1 and CD1D2) are located on chromosome 3 in tail-to-tail orientation ~6 kb apart (11, 12) (Figure 1). The existence of two CD1D genes in mice is likely the result of a relatively recent duplication event. Rats contain only one CD1 gene that is an ortholog of the human CD1D (13, 14). A chromosomal break event is now

commonly believed to be the reason for the absence of group 1 CD1 from rodents (9, 11, 15) (Figure 1).

Unlike the characteristic polymorphism of MHC class I and II genes, allelic variation of CD1 genes is extremely limited (16, 17). Studies of 110 (16) or 166 (18) unrelated donors from various ethnic groups revealed polymorphisms in CD1A, B, C, D, and E genes. The nucleotide substitutions in CD1B and CD1C were silent, whereas two CD1A, two CD1E, and CD1D alleles resulted in amino acid changes. Two alleles of CD1A, designated CD1A*01 and CD1A*02, have sequence differences that are predicted to be outside of the antigen binding groove, and no differences in expression or antibody reactivity were noted (18, 19). These alleles occur with different frequencies in ethnic populations (17). CD1E alleles, CD1E*01 and CD1E*02, were noted and could potentially influence ligand binding (16, 20, 21). However, to date the few studies carried out found no correlation between CD1 allelic polymorphisms and mycobacterial infection (22; C. Dascher, unpublished data).

The reasons for the overall lack of polymorphism of CD1 alleles are a matter of speculation. One possibility is that the opportunity for variation in the structure of lipid tails of microbial species may be markedly less than the potential for variation in the sequence of microbial antigenic peptides. Single nucleotide changes can readily alter peptide anchor residues needed for peptide binding to MHC alleles. On the other hand, lipid tails are synthesized by multiple enzymatic steps, and the modifications that microorganisms might make are limited by the structural constraints needed for lipid organization in microbial membranes and cell walls. Thus, if the lipid tails of antigens binding CD1 are more constrained in their potential structural diversity, correspondingly little polymorphism of CD1 grooves might be needed to accommodate their binding.

Besides humans and rodents, CD1 genes have now been reported in sheep (23, 24), cow (25), rabbit (26, 27), guinea pig (28), and rhesus macaque (29), suggesting their preservation as a gene family in evolution (Figure 2). Whereas humans express a single example of CD1A, B, C, D, and E, these isoforms are expanded or deleted in particular species. Rabbits express two CD1A genes and one CD1E gene (27) as well as CD1B and CD1D orthologs (26). Guinea pigs also possess an extended family of CD1 genes (28). More than 11 potential CD1 genes were identified by cross-hybridization to a conserved $\alpha 3$ probe, and among these 11 genes were 4 CD1B orthologs and 3 CD1C orthologs, but no genes cross-hybridizing with CD1A or CD1D could be found. Many of the guinea pig CD1B and C genes contain tyrosine-based sorting motifs in their cytoplasmic tails. Interestingly, one of the guinea pig CD1B orthologs, CD1B3, lacked the typical tyrosine-based sorting motif that in human CD1b mediates binding to the AP3 adaptor protein complex and localization in late endosomes and lysosomes (30), and instead displayed a pattern of intracellular trafficking like that of human CD1a (31). Given that guinea pig CD1A genes have not yet been found, evolutionary pressure may have resulted in modifying a CD1B ortholog into a gene that codes for a CD1 molecule that can traffic through and sample the early endosome recycling pathway like human CD1a (15, 31). Evolutionary pressure to develop CD1 isoforms that sample

relevant endosomal compartments may also account for the fact that murine CD1d associates with the adaptor protein AP3 that mediates its localization to lysosomes (32, 33). Although mice lack CD1b, they have evolved CD1d to traffic along the endosomal route followed by CD1b in other species. These examples have led to a "traffic hypothesis" of CD1 evolution: Each species evolves CD1 isoforms that follow distinct intracellular trafficking routes enabling the sampling of antigens, even if it is not the same isoform in each species (15, 31).

CD1 PROTEINS

CD1 polypeptides are expressed as heterodimers composed of the CD1 heavy chain noncovalently paired with β_2-microglobulin. CD1 heavy chains have a M_r of 49, 45, and 43 kDa for CD1a, b, and c, respectively, with the differences mainly accounted for by the number of N-linked glycan additions, since the peptide backbones of each are similar (~33 kDa) (34–38). Most CD1d protein complexes are expressed on the cell surface as a 49 kD heavy chain associated with β_2-microglobulin (39, 40). In general, association with β_2-microglobulin appears to be necessary for cell surface expression of various CD1 isoforms, as shown by lack of surface localization in β_2-microglobulin-deficient cells (38, 41–43). The ratios of β_2-microglobulin associated with CD1a, b, and c are not always similar, suggesting that in some cases the CD1 heterodimers may be more prone to dissociate than the MHC class I/β_2-microglobulin complexes (34, 36). Moreover, the expression of human and murine CD1d at the cell surface without β_2-microglobulin has been observed (44). The fully glycosylated CD1d heavy chains were associated with β_2-microglobulin, but a subset of β_2-microglobulin-free CD1d molecules either contain only immature high mannose (endoglycosidase H-sensitive) N-linked glycans or completely lack glycan additions (45). Both forms may occur on the same cell types. In the case of intestinal epithelial cells, the form lacking glycosylation was restricted to the apical surface (46). The functional significance of the β_2-microglobulin-free forms of CD1d is not known.

CD1 mRNA splicing complexity occurs and may generate secreted forms of CD1 proteins (47). A secreted form of CD1a results from unspliced transcripts, and intrathymic splicing results in a secreted CD1c product. CD1e mRNA that lack transmembrane domains or that lack α1 or α2 domains as a result of alternative splicing also have been observed and may correspond to protein products found in Golgi or late endosomal compartments, or lead to secreted products (48).

CD1 ATOMIC STRUCTURES

CD1d

The atomic structures have now been determined for murine CD1d and human CD1a and b. The CD1d structure revealed the striking similarity in secondary, tertiary, and quaternary structure of CD1 to MHC class I in which the α-chain

folds into three domains, $\alpha 1$, $\alpha 2$, and $\alpha 3$ associated noncovalently with β_2-microglobulin (49). A putative antigen-binding superdomain is composed of the $\alpha 1$ and $\alpha 2$ helices that sit atop and traverse an eight-stranded antiparallel β-pleated sheet platform (Figure 3*a*,*b*). Compared to MHC class I, the $\alpha 2$ helix is kinked upward and the $\alpha 1$ helix is raised 4–6 Å higher above the β-sheet, resulting in a deeper groove. In addition, the β-strand platform displays substantially greater curvature and is more bowl shaped compared with MHC class I or class II platforms. The relative positions of the $\alpha 1$ and $\alpha 2$ helices are closer together along their longitudinal axes, resulting in a narrower groove (14 Å) compared with MHC class I or II.

Despite the overall similarity in domain organization, striking differences between CD1d and MHC class I and II are found in the topography and molecular surfaces of the antigen-binding grooves. For CD1d, the overall volume and surface area of the groove is larger than any MHC class I molecule, and the smaller pockets characteristic of MHC molecules are coalesced into two large pockets, designated A′ and F′ (49) (Figure 3*c*). The CD1d groove is closed at both ends but is accessible at the top of the molecule through a narrow opening extending from the center of the groove to a point over the center of the F′ pocket. Few amino acid sidechains that line the groove of CD1d are capable of hydrogen bonding. As a result, the likelihood of forming an extensive hydrogen bonding network at the ends of a peptide (as in class I) or along the sides of the longitudinal axis of the groove (as in class II) is not apparent in CD1d.

CD1b

The structure of ligand-bound CD1b complexes [ganglioside GM2/CD1b and phosphatidylinositol (PI)/CD1b] showed the lipid tails to be buried in hydrophobic channels and the polar head of the lipid ligand oriented at the surface of the molecule between the CD1 α-helices (50) (Figure 3). A space-filling surface view illustrates how only the polar head of the glycolipid is exposed at the CD1b surface (Figure 3*d*). The amount of surface-exposed hydrophilic lipid head group covers only a small surface of the CD1 molecule, compared with MHC class I, where the presented peptide covers the central plane, and MHC class II, where the peptide extends over the whole surface (Figure 3*d*). Compared with CD1d, unexpected differences were noted in the antigen-binding domain of CD1b. Instead of the single opening for the ligand-binding groove providing access to the two large A′ and F′ pockets, the CD1b structure revealed a network of four hydrophobic channels (designated A′, C′, F′, and T′) in the $\alpha 1$ and $\alpha 2$ superdomain, each of which contained a hydrocarbon chain of 11–22 carbons in length (50) (Figure 3*c*). Three of these channels, A′, C′, and F′, communicate with the top surface of the CD1b molecule through openings between the $\alpha 1$ and $\alpha 2$ helices and are interconnected at their other termini with a fourth distinct channel, designated the T′ tunnel. Furthermore, the C′ channel extends to a portal opening under the $\alpha 2$ helix and may allow egress of longer chains out through its under-the-helix portal. These remarkable structural features suggest a mechanism by which the hydrophobic binding capacity of the

channels may be utilized to accommodate lipids containing several hydrocarbon tails or long hydrocarbon chains that sequentially traverse interconnected channels or exit the C′ portal under the $\alpha 2$ helix (Figure 3*c*).

CD1a

The structure of the human CD1a antigen-binding groove revealed two large hydrophobic pockets extending out from the center of the groove and running between the $\alpha 1$ and $\alpha 2$ helices (51) (Figure 3*c*). This hydrophobic cavity pattern was more like CD1d than the four-channel structure of CD1b. The CD1a cocrystal with a sphingolipid, 3′-sulfated β-D-galactosylceramide, showed that the two hydrocarbon binding regions each accommodated one alkyl chain (Figure 3*c*). The A′ pocket runs deep into the CD1a groove, with its terminus clearly defined as it ends in a semicircular curve. It perfectly accommodated the 18 carbons of the sphingosine base. In contrast, the F′ pocket is long and straight and widened as it approached the surface of CD1a between the helices. The sulfogalactosyl polar head of the antigen was positioned at the entrance to the F′ pocket with both the sulfate and the galactose moieties anchored by hydrogen bonding to polar sidechains in the $\alpha-$helical groove at the junction of the A′ and F′ pockets (51) (Figure 3*c*). This positioned the polar lipid headgroup to partially protrude from the surface of the CD1a binding groove for TCR recognition, while at the same time being nestled closely in the groove (Figure 3*d*). Thus, for CD1a, the intersection of the A′ and F′ pockets contains polar residues that can interact with and provide specificity for binding the polar moieties of amphipathic lipids. Moreover, unlike the CD1b structure in which the acyl chains were enclosed in the hydrophobic channels, in the CD1a structure the amide-linked acyl chain was exposed and protruded at the TCR recognition surface, making van der Waals contacts with hydrophobic amino acid sidechains of the F′ pocket (Figure 3*c*,*d*).

Comparison of CD1d, CD1b, and CD1a

Of the three known structures, the CD1b groove is the largest, with an estimated total volume of 2200 Å compared with that of CD1a (1300 Å) or CD1d (1650 Å). Moreover, the four interconnected channels of CD1b might accommodate up to 76 carbons whereas the two channels of CD1a are predicted to accommodate 36 carbons (49–51). Of the binding channels, the A′ pockets are more conserved among the isoforms than are the other channels (Figure 3*c*). The T′ tunnel was observed only in CD1b as it runs across the top of the β-strand platform and is made possible by the small side chains Gly 98 and Gly 116. In the CD1a and CD1d structures, larger sidechains block a separate T′ tunnel (51) (Figure 3*c*). The C′ channel of CD1b also does not occur in CD1a or CD1d, as bulky hydrophobic residues block any possible C′ portals (Figure 3*c*). Thus the ligand-binding capacities and the structural organization of the hydrophobic channels are distinct for CD1a, b, and d and may allow binding of an array of structurally diverse lipid-containing antigens.

ANTIGENS

Foreign Microbial Lipid and Lipopeptide Antigens

Identification of the first antigen presented by a CD1 molecule to T cells indicated that the antigens were lipids (52). Thus far, the foreign antigens presented by CD1 molecules that have been characterized include a range of diverse lipids found in the cell walls of mycobacteria. Mycolic acid, an α-branched β-hydroxy long chain fatty acid (52), glucose-monomycolate (GMM), which consists of mycolic acid esterified to a single glucose moiety (53), phosphatidylinositol mannoside (PIM) and lipoarabinomannan (LAM), which both consist of a phosphatidylinositol anchor linked to additional glycans (54), are all presented by CD1b molecules (Figure 4). A CD1c-restricted T cell line was shown to recognize mycobacterial mannosyl-β-1-phosphoisoprenoid, a glycophospholipid with only a single short lipid tail (55) (Figure 4). CD1a-restricted T cells have been recently shown to recognize a lipopeptide (didehydroxymycobactin) from *Mycobacterium tuberculosis*, defining a new biochemical class of antigens for CD1-restricted T cells (56) (Figure 4). Thus far, microbial lipid and lipopeptide antigens presented by CD1 have been confirmed only for CD1a, b, and c. There have been conflicting reports as to whether murine CD1d-restricted T cells recognize glycosylphosphatidylinositol (GPI)-anchored glycoproteins from *Plasmodium* or *Trypanosoma* spp. in vitro or affect the IgG response to GPI-linked proteins in vivo (57–61). There is no apparent reason why CD1d molecules could not also present foreign lipid antigens. However, at present, foreign antigen presentation has been limited to CD1a, b, and c.

Self-Lipid Antigens

CD1-restricted T cells can be stimulated by exposure to $CD1^+$ antigen-presenting cells (APCs) in the absence of foreign antigens, resulting in their activation as measured by proliferation, cytokine secretion, or cytolysis. This was first observed for CD1 group 1–restricted T cells (62) and later for CD1d-restricted T cells (63, 64). Examples of CD1-presented self-lipids that occur in normal mammalian tissues include ubiquitous phospholipids, such as phosphatidylinositol, phosphatidylethanolamine, and phosphatidylglycerol, which can be recognized by murine CD1d-restricted T cell hybridomas (65) (Figure 4). Cellular phosphatidylinositol and GPI have been eluted from CD1d proteins, indicating that these phospholipids may be naturally loaded into CD1d (66, 67). However, recognition of self-GPIs that results in activation of CD1d-restricted T cells has not been demonstrated to date, and $CD1d^+$ APCs lacking the ability to synthesize GPIs retain the ability to stimulate autoreactive CD1d-restricted T cell hybridomas (58, 67). Therefore, it is not yet clear whether GPIs are self-antigens that activate CD1d-restricted T cells. Several self-sphingolipids have been found to activate human or murine CD1-restricted T cells. Sphingolipids containing complex oligosaccharides, such as the ganglioside GM1 which is mainly present in neural tissues, can be presented by CD1b to T cells (68). The ganglioside GD3, which constitutes

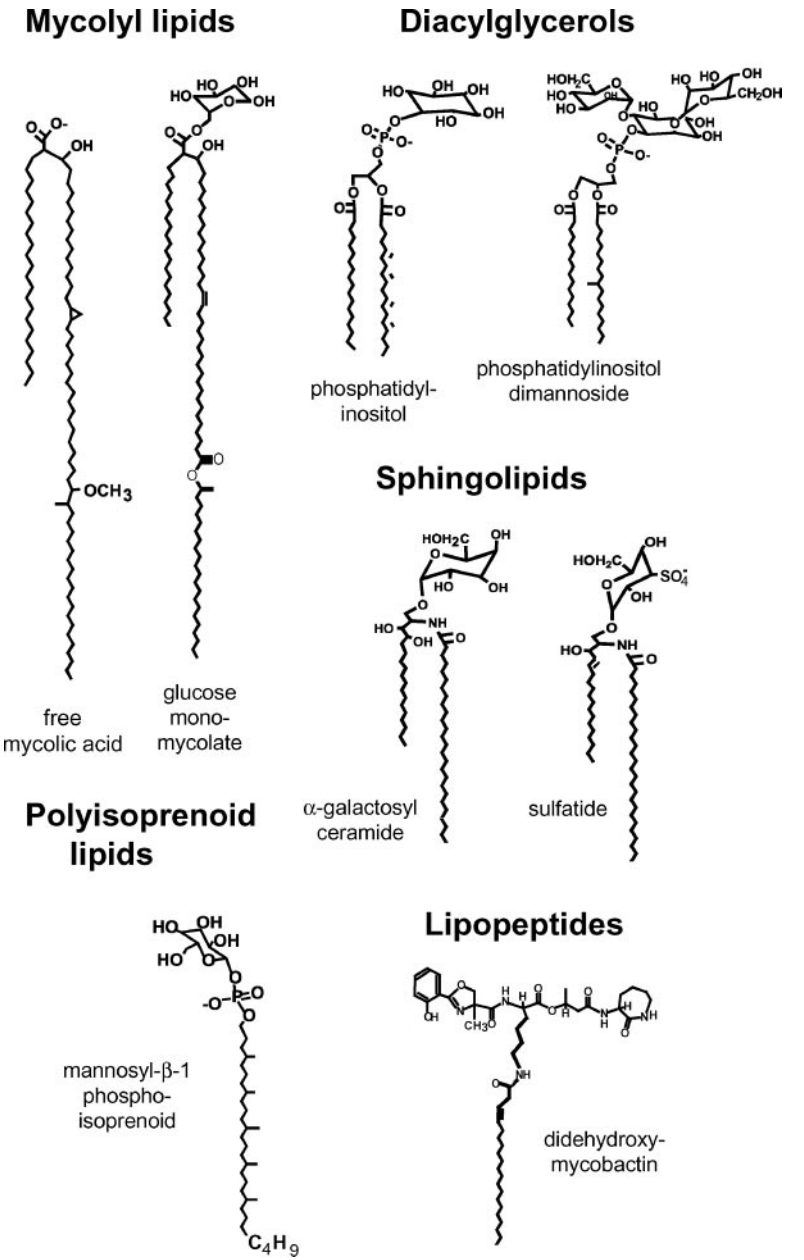

Figure 4 Examples of antigens presented by CD1. Structures of CD1-presented antigens are grouped according to lipid classes. Phosphatidylinositol mannoside (PIM) containing two mannosyl residues is depicted, but antigenic PIMs typically have additional mannosyl residues.

a major fraction of the glycolipids of tumors of neuroectodermal origin, such as melanomas, and which also occurs in normal mammalian tissues (69), was recognized by murine CD1d-restricted T cells (70). Sulfatide, another sphingolipid and main constituent of mammalian brain lipids, is a sulfate ester of β-D-galactosylceramide and could be recognized by CD1a-, CD1b-, or CD1c-restricted T cells, indicating that the same self-lipid antigen can, in some cases, be presented by different CD1 molecules (71) (Figure 4). It is not known whether self-lipid antigens are constitutively expressed at the cell surface or in intracellular membranes, or if alterations in synthesis, processing, or trafficking control their stimulatory capacity. The frequent demonstration of self-reactivity, however, highlights this as a characteristic feature of many CD1-restricted T cells.

α-Galactosylceramide (αGalCer)

Most mouse and human CD1d-restricted T cells that use TCRα-invariant TCRs recognize α-galactosylceramide (αGalCer), a glycosphingolipid found in marine sponges (72) (Figure 4). The αGalCer structure resembles mammalian ceramides in that it contains a sphinosine-like base, an amide-linked acyl chain, and an O-linked pyranose. However, the anomeric carbon of the sugar is in the α-linkage to the oxygen, whereas in mammals, the corresponding linkage is of the β-anomeric type. αGalCer has no known physiological function in mammalian immunity but has been widely used as a pharmacological agent to study activation of CD1d-restricted T cells.

FUNCTIONAL ANALYSIS OF LIPID BINDING TO CD1

Biophysical analyses of LAM binding to CD1b revealed an equilibrium binding constant ($K_D = k_{diss}/k_{ass}$) of 3.2×10^{-8} M using evanescence wave sensor analyses and a K_D of 5.3×10^{-8} M calculated by Scatchard analysis, values that are in a range similar to those calculated for high-affinity peptide binding to MHC molecules (73). Separately, a K_D of 6.6×10^{-7} M was calculated using surface plasmon resonance for binding of a phosphatidylinositol mannoside (PIM2, with two mannose and two C:18 moieties) to CD1b. Since CD1b is likely to be loaded in acidic endosomal compartments (30, 74, 75), pH-dependent binding of LAM and glucose monomycolate to CD1b was examined and found to occur at pH 4. This correlated with partial unfolding of the α-helical segments of CD1b as measured by circular dichroism and with greater binding of a hydrophobic environment-sensitive fluorescent probe, 1-anilino-napthalene-8-sulfonic acid (ANS) (73). The studies above utilized immobilized soluble human CD1b with lipids in the fluid phase. Because lipids are expected to associate into micelles in aqueous solutions, this may have unknown biophysical effects on antigen-loading analysis. Using another approach, biotinylated lipids were immobilized and soluble CD1d molecules in the fluid phase were examined for their binding characteristics using surface

plasmon resonance (76). These studies revealed a K_D of 3.4×10^{-7} M for the binding of soluble murine CD1d to immobilized acyl-biotin-substituted αGalCer (76). In contrast, studies using an isoelectric focusing equilibrium binding assay and isothermal titration calorimetry showed a K_D in the range of 1–9.7×10^{-6} M for the binding of soluble CD1d to αGalCer (77).

TCR SPECIFICITY IN RECOGNITION OF CD1-ANTIGEN COMPLEXES

TCRs of CD1a-, b-, and c-Restricted T Cells

Molecular cloning and transfection of cDNAs encoding the TCR$\alpha\beta$ or $\gamma\delta$ chains isolated from CD1a-, b-, and c-restricted glycolipid antigen-specific T cells confirmed that T cell recognition of lipid antigens was mediated by the TCR (78–80). The sequences of the $\alpha\beta$ TCRs that recognize CD1a-, b-, and c-presented microbial lipid antigens revealed diverse TCRα and β chain rearrangements and junctional diversity including evidence for D segment usage and N-nucleotide additions at the V-D and J joining ends (78). Hence, in primary structure, the $\alpha\beta$ TCRs that recognize CD1a, b, and c are indistinguishable from those that recognize MHC class I or II complexes with peptides. Similar to TCR recognition of peptide-MHC complexes, a role for the CDR3 loop sequences of the TCR in recognition of specific CD1-lipid antigen complexes was confirmed by a combination of TCR mutagenesis and transfection (81).

TCRs of CD1d-Restricted T Cells

A remarkable feature of many human and murine T cells that recognize CD1d molecules is their use of an invariantly rearranged TCRα chain. In mice, the Vα14 gene segment is rearranged in a germline configuration with Jα18 (formerly called Jα281), and this Vα14Jα18 TCRα chain is paired predominantly with TCRβ chains that use Vβ8, Vβ7, or Vβ2 gene segments (82, 83). Human CD1d-restricted T cells use Vα24 gene segments invariantly rearranged with Jα18 (formerly JαQ), which are highly homologous to the murine Vα14 and Jα18 gene segments, respectively (83–85). The human Vα24Jα18 invariant TCRα chain is preferentially paired with Vβ11, which is homolgous to murine Vβ8. In both humans and mice, Vα24$^+$ and Vα14$^+$ invariant TCRα chains, respectively, have been observed that contain noncoding nucleotide substitutions near the V/J junctional region, presumably as a result of trimming followed by N-region additions (64, 82, 83). CD1d-restricted T cells using Vα24Jα18$^+$ and Vα14Jα18$^+$ TCRs with single amino acid substitutions at the end of the V gene segment, a region corresponding to the beginning of the CDR3 loop, have also been found, but the significance of this limited sequence variation is not known (83, 86). Hence, it appears that the invariant, germline-configured TCRα chains of CD1d-restricted T cells are generated by the same mechanisms that give rise to diverse TCRs, but that there

is strong selection pressure to maintain the germline amino acid sequence. The VDJ junctional regions of the TCRβ chains of TCRα-invariant CD1d-restricted T cells are extremely diverse, with little evidence of biased J chain usage or of conserved amino acid motifs (83, 87). By analyzing Vβ CDR3 sequences from individual CD1d-restricted TCRα-invariant T cell clones in mice, a clonal size of 5–10 T cells per clone was estimated, which is similar to that of naïve conventional T cells, suggesting that CD1d-restricted T cells in previously unchallenged mice are not clonally expanded (88, 89).

Most murine and human TCRα-invariant CD1d-restricted T cells recognize αGalCer (72, 90, 91) and stain with αGalCer-loaded CD1d tetramers (92–95). However, thus far, no single microbial or self-antigen has been found with similar antigenic capacity for TCRα-invariant CD1d-restricted T cells. Tail-deleted murine CD1d molecules, which lack the tyrosine-based endosomal targeting motif and traffic differently than normal CD1d, do not stimulate Vα14$^+$ CD1d-restricted hybridomas, suggesting that these T cells recognize self-lipids loaded in endosomal compartments (96, 97). In contrast, CD1d-recognition by Vα24$^+$/Vβ11$^+$ CD1d-restricted T cell clones was not dependent on the endosomal targeting motif of human CD1d (64). Interestingly, CD1d-restricted T cells reactive with the ganglioside GD3, which have been generated in mice by immunization with a human melanoma cell line, comprised a subpopulation of αGalCer/CD1d-tetramer$^+$ T cells, suggesting that the TCRβ chain may influence the antigen-specificity of Vα14$^+$ TCRα-invariant TCRs (70). Other studies also concluded that the TCRβ chain can influence the binding of Vα14Jα18$^+$ TCRs to CD1d/antigen complexes (98, 99).

CD1d-restricted T cells that use diverse Vα gene segments, in contrast to invariant TCRα chains, have been identified in both humans and mice (97, 100–104). Sequencing of the TCRs of a large panel of Vα14$^-$ CD1d-restricted T cell hybridomas derived from MHC class II–deficient mice showed that they contained expanded T cell populations that use Vα3.2Jα9 or Vα8 TCRα chains paired preferentially with Vβ8 TCRβ chains (105). Other analyses have identified additional Vα14$^-$ CD1d-restricted T cells that do not use Vα3.2Jα9 or Vα8 TCRα chains, but these studies also found frequent use of genes from the Vβ8 gene family (106). Given the lack of specific markers for Vα14$^-$ CD1d-restricted T cells, this population is less studied and its relative frequency remains controversial (100, 103, 105). But Vα14$^-$ CD1d-restricted T cells are clearly not rare and may prove to be numerically substantial and functionally important. Human CD1d-restricted T cell lines using TCRs other than Vα24/Vβ11 have been isolated from bone marrow and livers of hepatitis C–infected patients (107, 108) and Vα24$^-$ T cells that stain with αGalCer/CD1d-tetramers have been observed in peripheral blood of humans (104). Further, murine T cells using Vγ4$^+$ $\gamma\delta$ TCRs recognized myocytes infected with coxsackie virus B3 in a CD1d-dependent manner (109).

In mice, the Vα14$^-$ CD1d-restricted T cells resemble the Vα14-invariant subset in demonstrating autoreactivity to CD1d$^+$ APCs; however, Vα14$^-$ CD1d-restricted T cell hybridomas were able to respond to tail-deleted CD1d molecules, in contrast

to Vα14$^+$ CD1d-restricted T cell hybridomas that did not recognize such CD1d molecules (96, 97). The Vα14-invariant CD1d-restricted T cells tested thus far did not respond to αGalCer (65, 110). Thus, Vα14$^+$ and Vα14$^-$ CD1d-restricted T cells in mice appear to have different antigen specificities.

TCR-Recognition of CD1-Antigen Complexes

Although cocrystals of CD1-antigen complexes with the TCRs that recognize them have not yet been solved, optimized molecular modeling and mutagenesis of CD1b and CD1d α1- and α2-helical residues predict possible TCR contacts and suggest a diagonal orientation of the molecular footprint of the TCR across the longitudinal axis of the CD1 α-helices, similar to that observed for TCR-MHC complexes (78, 111, 112). Surface plasmon resonance has revealed a relatively high-affinity binding of soluble Vα14$^+$ TCR to the αGalCer/CD1d complex, compared with that of $\alpha\beta$ TCRs with MHC/peptide complexes (77, 113).

CD1-restricted TCRs that recognize foreign antigens can distinguish even small changes in the structure of the hydrophilic head group of the antigens. For example, studies of mycobacterial mycolic acid antigens presented by CD1b reveal that changes to any of the hydrophilic functions of the fatty acids or their attached glycans alter recognition. For example, substitutions of the carboxylic acid (52, 81), the type of the esterified sugar moiety (79), the stereochemical arrangement of the glycan and its linkage to the acyl chain (53, 79), or changes in the hydrophilic R-group attached to the main acyl chain (81) all determine the specificity of recognition by the TCR. TCRα-invariant CD1d-restricted T cells recognize either glucose or galactose in the α-anomeric linkage, but mannose (a stereoisomer of glucose and galactose) in this linkage is not recognized, and neither are a variety of other related sugar head groups (72, 91). In addition, the α-anomeric linkage is absolutely essential for recognition since the β-anomeric forms of these ceramide-like antigens are not recognized. Specificity for the sugar head group was also shown by the finding that an αGalCer containing an additional galactose [αGal(1–2)GalCer] was not recognized by TCRα-invariant CD1d-restricted T cells, unless cleaved in lysosomes to generate αGalCer (114). These studies emphasize that foreign, self-, and synthetic lipid antigen recognition by the TCR of CD1-restricted T cells involves fine specificity in distinguishing the hydrophilic moieties of the amphipathic lipid antigens.

Functional studies have also examined the bioactivity of CD1-presented lipids with differences in the acyl chains. αGalCer molecules with acyl chains of 26 carbons were optimal for stimulating CD1d-restricted T cell responses, whereas acyl chains even two carbons shorter were significantly less active. Similarly, the optimum sphingosine base was C_{18}, and again, those tails that were even a few carbons shorter displayed significantly less bioactivity (72, 115). CD1c-restricted T cells reactive with mycobacterial mannosyl-β-1-phosphoisoprenoid also recognize structurally related semisynthetic mannosyl-β-1-phosphodolichols (MPD) that are similar to phosphodolichols found in eukaryotes and prokaryotes (55).

Strong T cell responses were seen with semisynthetic MPDs containing C_{55} dolichols typically found in protozoan pathogens, but responses to MPDs containing long C_{95} dolichols, which can be found in eukaryotic cells and human tissues, were very weak or absent (55). In these studies of ceramide and dolichol antigens, it was not possible to distinguish changes in TCR recognition of the CD1/lipid complexes from the effect of hydrocarbon length on direct binding to CD1 or from influences resulting from trafficking and delivery of the glycolipids to relevant antigen-loading compartments, since these studies were carried out in live cells rather than using immobilized molecules. For example, differences in recognition of phosphatidylethanolamine by a CD1d-restricted T cell hybridoma due to differences in the saturation of its alkyl chains correlated with the ability of lipid binding to CD1d (116). Another study using glucose monomycolate glycolipid antigens of varying hydrocarbon tail lengths concluded that longer alkyl chain containing antigens were delivered to late endosomes whereas shorter ones were loaded at the cell surface (117).

ASSEMBLY AND INTRACELLULAR TRAFFICKING OF CD1 MOLECULES

The intracellular trafficking of antigen-presenting molecules is central to their ability to intersect and bind antigens that have entered cells and then deliver them to the cell surface where they can be recognized by T cells. For MHC class I and class II molecules, the trafficking routes and loading compartments are essential for sampling cytosolic and endosomal antigens, respectively (118, 119) (Figure 5*a*). The intracellular trafficking routes followed by CD1 molecules are distinct from those of their MHC counterparts.

CD1 Assembly

CD1 heavy chains are translocated into the ER where N-linked glycans are attached, interactions with ER chaperones occur, and association with β_2-microglobulin takes place (38, 43, 120). Studies on CD1d revealed that it associates in the ER with both calnexin and calreticulin and the thiol oxidoreductase ERp57 in a manner dependent on glucose trimming of its N-linked glycans, a process coupled to disulfide bond formation in the CD1d heavy chain (120). Similarly, the β_2-microglobulin-free CD1b heavy chain was found to associate with calnexin and calreticulin (38, 43). Engineered forms of CD1d tagged with a KDEL sequence that were retained in the ER bound phosphatidylinositol, suggesting that lipid ligand binding to CD1d can occur in the ER (67). Based on pulse chase experiments, it was determined that newly synthesized CD1b and CD1d molecules are then rapidly delivered from the Golgi to the plasma membrane, presumably along the secretory pathway (121, 122) (Figure 5*b*,*c*,*d*).

CD1a, b, and c Trafficking

After reaching the plasma membrane, CD1b molecules appear to follow a major pathway of internalization into clathrin-coated pits (74, 122, 123) (Figure 5*b*). Deletion of the tail of CD1b results in failure to localize in late endosomes and lysosomes, leads to its accumulation on the plasma membrane, and compromises its capacity to present foreign antigens (74, 124). Adaptor proteins mediate cargo-selective transport by interacting with tyrosine-based sorting motifs or dileucine

Figure 5 Intracellular trafficking of CD1 and MHC class I and II molecules. (*a*) MHC class I and II intracellular trafficking. MHC class I and II molecules are assembled in the endoplasmic reticulum (ER). MHC class I molecules associate with β_2-microglobulin (β_2m), acquire peptides delivered from the cytosol and follow the secretory route to the plasma membrane. MHC class II molecules assemble into invariant chain (Ii) complexes in the ER that prevent peptide binding and direct their trafficking to endosomal compartments. In lysosomes, the Ii is cleaved, peptides are loaded and MHC class II molecules traffic to the cell surface. These pathways allow peptide sampling from the cytosol and endosomal compartments by MHC class I and class II, respectively. Nuc, nucleus; HC, heavy chain; G, Golgi; TGN, trans Golgi network; TAP, transporter associated with antigen processing. (*b*) CD1a and CD1b intracellular trafficking. Newly synthesized CD1 molecules assemble with β_2-microglobulin in the ER, acquire self-lipids (*open circle with zigzag tail*), and traffic to the plasma membrane along the secretory route. Cell surface CD1b molecules interact with adaptor protein AP2 and are internalized in clathrin-coated pits. CD1a molecules are internalized by an unknown mechanism. CD1a is then sorted into recycling endosomes and traffics back to the plasma membrane in an AFR6-dependent manner. It is largely excluded from lysosomes. In contrast, CD1b is sorted to late endosomes and directed to lysosomes by binding the adaptor protein AP3. CD1a and CD1b then acquire distinct self- or foreign lipid antigens (*filled circle and zigzag tail*) in the recycling endosomes or lysosomes, respectively. (*c*) CD1c and CD1d intracellular trafficking. Assembly is as above for CD1a and b. However, CD1c broadly traffics in both early recycling endosomes as well as in late endosomes and lysosomes. CD1d traffics mainly in early and late endosomes but not in recycling endosomes and only partially localizes in lysosomes. Neither CD1c nor CD1d associates with the adaptor protein AP3. CD1c and CD1d then acquire distinct self- or foreign lipid antigens (*filled circle and zigzag tail*) in the intracellular compartments shown. (*d*) Murine CD1d intracellular trafficking. Newly synthesized CD1d molecules may or may not assemble with β_2-microglobulin in the ER, acquire self-lipids (*open circle with zigzag tail*), and traffic to the plasma membrane along the secretory route. A subset of CD1d molecules associate in MHC class II/Ii chain complexes and may be directed to endosomal compartments from the trans Golgi network (TGN). CD1d molecules are internalized from the plasma membrane and traffic through the early and late endosomal compartment and are delivered to lysosomes in an AP3-dependent manner. CD1d then acquires distinct self-lipid antigens (*filled circle and zigzag tail*) in late endosomal/lysosomal compartments.

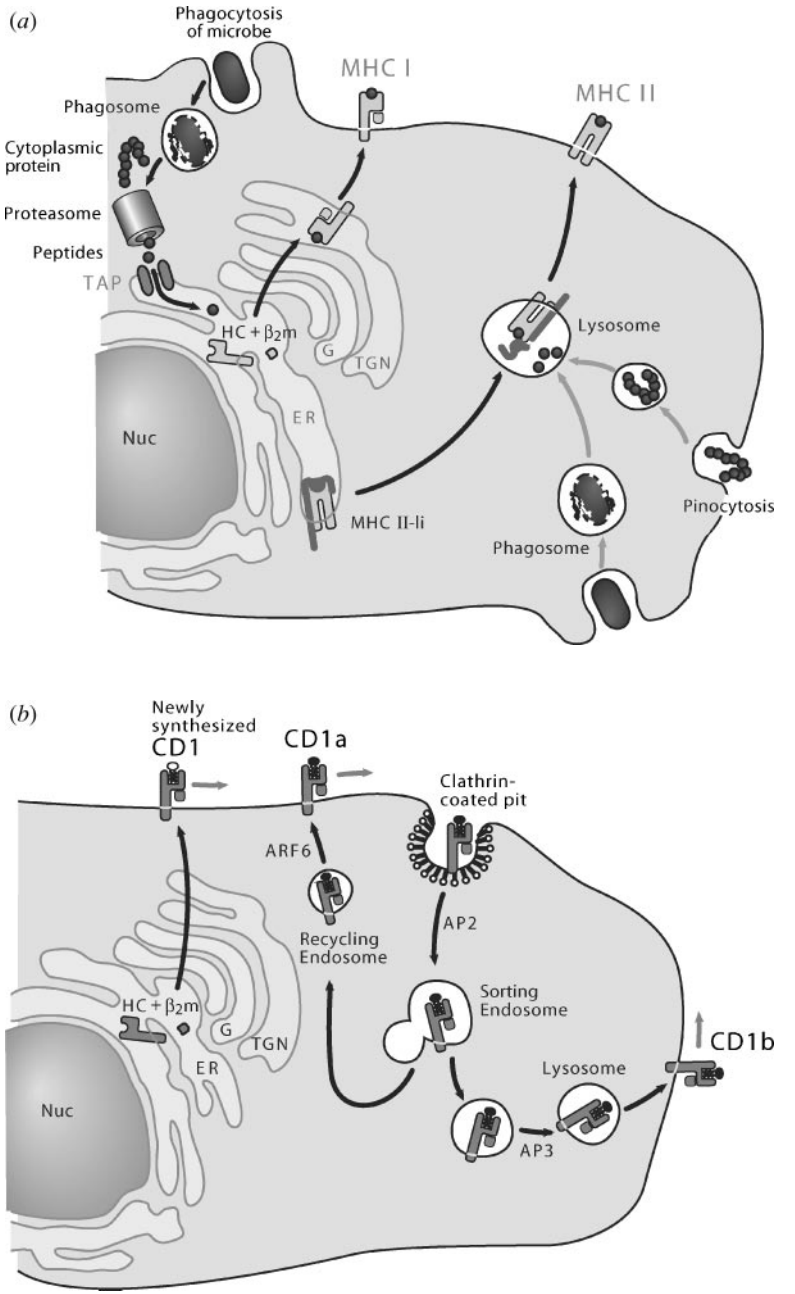

Figure 5 (*Continued*)

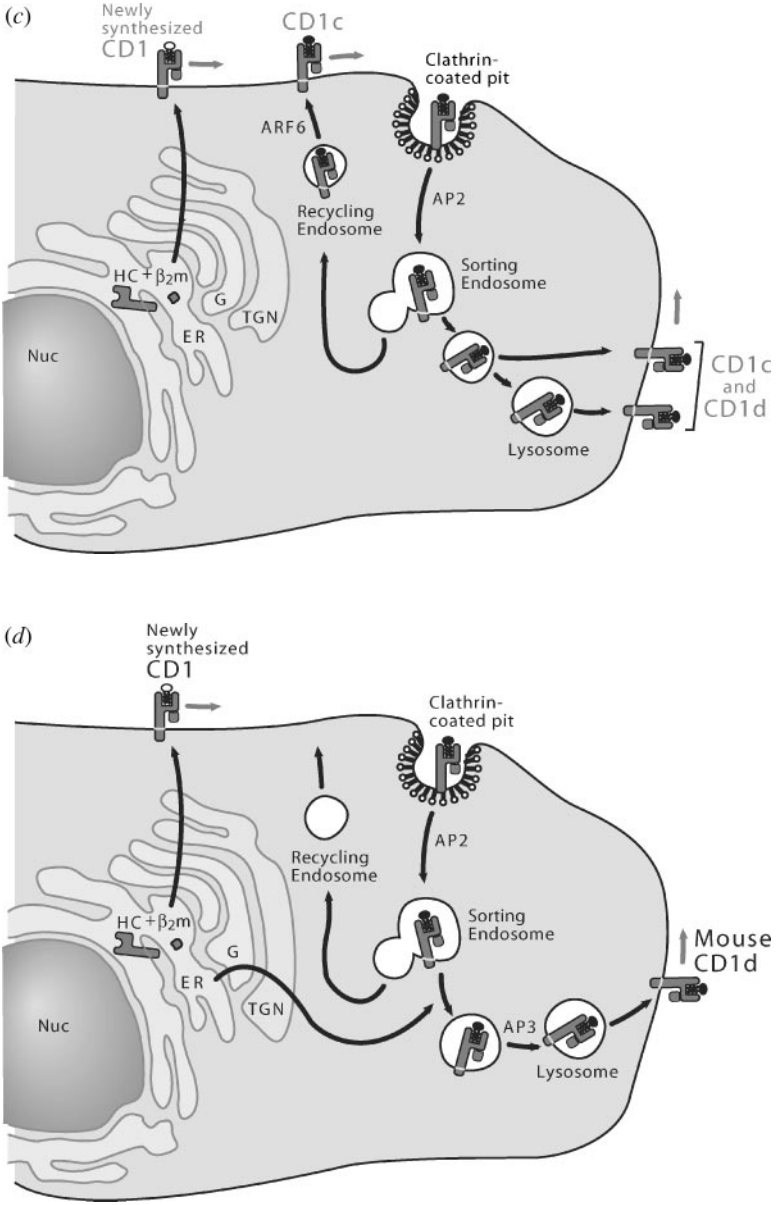

Figure 5 (*Continued*)

motifs in the cytoplasmic tails of integral membrane proteins. The internalization of CD1b and its subsequent delivery to deep endocytic compartments is determined by tyrosine-based sorting motifs in its cytoplasmic tail (YXXZ, where Y = tyrosine, X = spacer, and Z = bulky hydrophobic residue). Binding to the adaptor protein AP2 was demonstrated using surface plasmon resonance (122). The mechanism responsible for delivery to lysosomes, the main steady-state intracellular location of CD1b (74, 125) (Figure 5*b*), was shown to be the preferential association of the CD1b cytoplasmic tail tyrosine-based sorting motif with another adaptor protein, AP3, based on yeast two-hybrid assays (30) and surface plasmon resonance measurements (122). In AP3-deficient cells, CD1b was mislocated to the plasma membrane and early endosomes, failed to localize to lysosomes and failed to present CD1b-restricted glycolipid antigens, whereas localization and the antigen-presenting function of other CD1 molecules were not affected (30).

Delivery of antigens to lysosomal compartments may be essential for efficient loading and presentation by CD1b. In the case of LAM, the antigen is taken up by the macrophage mannose receptor (MR) at the cell surface and subsequently delivered to the lysosomes of dendritic cells (DCs) where it colocalized with CD1b (75). ^{14}C-labeled GMM with long lipid tails (C_{80}) was also shown to be taken up by cells and delivered to the lysosomes where it colocalized with CD1b (117). In contrast, short-chain (C_{32}) GMM was less efficiently presented by cells and was found to localize less efficiently to lysosomes. Instead, it appeared that the C_{32} GMM was able to load into CD1b on the plasma membrane (117).

In contrast to the steady-state intracellular localization of CD1b in lysosomes, CD1a was found to be largely excluded from lysosomes and instead localized to early recycling endosomes, using an ARF6-dependent pathway (125) (Figure 5*b*). Consistent with the differences in intracellular trafficking of CD1a and CD1b, antigen presentation by CD1b was critically dependent on vesicular acidification, whereas presentation by CD1a was independent of acidification blockade (125). Studies in human Langerhans cells confirm CD1a trafficking in Rab 11$^+$ recycling endosomes and in Birbeck granules (126).

Thus, the intracellular trafficking routes followed by CD1a and CD1b represent largely separate localization in early recycling endosomes and lysosomes, respectively (Figure 5*b*). In contrast to their largely nonoverlapping and restricted steady-state localization, the intracellular localization of CD1c partly overlapped both CD1a and CD1b, suggesting that it might broadly survey throughout the endocytic system (127, 128) (Figure 5*c*).

CD1d Trafficking

The intracellular localization of human CD1d was partly in LAMP-1$^+$ late endosomes and lysosomes; however, unlike CD1b whose localization in lysosomes was nearly absolute, a substantial fraction of human CD1d molecules did not colocalize with LAMP-1 and appeared to be in more proximal endosomal compartments (30) (Figure 5*c*). The less efficient localization of human CD1c and CD1d to

lysosomes, compared with CD1b, was likely based on the inability of the cytoplasmic tail tyrosine-based sorting motif of CD1c and CD1d to bind AP3 (30).

After assembly in the ER, murine CD1d is rapidly transported to the plasma membrane with a time course consistent with trafficking along the secretory route (121). Internalization from the plasma membrane is dependent on the CD1d cytoplasmic tail, following which traffic through early and late endosomes to lysosomes occurs (121) (Figure 5*d*). The steady-state localization of murine CD1d revealed a pattern of abundant colocalization with LAMP-1 (97, 129). The CD1d cytoplasmic tail tyrosine-based sorting motif controlled its localization to LAMP-1^+ compartments (97, 129). Like the role of AP3 in the selective delivery of human CD1b to lysosomes (30), it was shown using yeast two-hybrid binding and surface plasmon resonance binding that the murine CD1d cytoplasmic tail also mediates binding to AP3, which controls its delivery to lysosomes (32, 33). The striking localization of murine CD1d in lysosomes proved to be essential for antigen presentation. For example, a cytoplasmic tail-truncated CD1d that does not traffic through lysosomes fails to stimulate Vα14$^+$ CD1d-restricted T cells, but is still capable of stimulating Vα14$^-$ CD1d-reactive T cells (97, 129). Further, AP3-deficient Pearl, Mocha, and AP-3b1^{LN} mice displayed altered CD1d trafficking and a marked reduction in Vα14$^+$ CD1d-restricted T cells in thymus, spleen, and liver (32, 33). These studies suggest that CD1d acquires the self-lipid antigens that stimulate Vα14$^+$ CD1d-restricted T cells in lysosomes.

Intracellular trafficking and antigen presentation by CD1d have been found to be influenced by MHC class II molecules, the invariant chain, cathepsin S, and cathepsin L proteases. Murine CD1d was noted to coimmunoprecipitate with the invariant chain (121), and human CD1d was noted to coimmunoprecipitate with MHC class II complexes (130). These findings suggest that at least for a fraction of CD1d molecules, their trafficking to endosomal compartments could be influenced by MHC class II/invariant chain interactions. This was evident in invariant chain–deficient mice where B cells and DCs, which normally express invariant chain, revealed increased surface expression of CD1d compared to wild-type mice, whereas cells that do not express invariant chain, like thymocytes, did not have altered surface expression (121). Invariant chain–deficient DCs were inefficient at stimulating self-reactive Vα14$^+$ CD1d-restricted T cells (121). However, the lack of invariant chain did not alter the percentage or total number of Vα14$^+$ CD1d-restricted T cells in the thymus, spleen, or liver and did not alter the ability of splenocytes to present αGalCer or Gal(α1–2)αGalCer (33).

Mice lacking cathepsin S, an endosomal protease critical in invariant chain processing, revealed altered trafficking of CD1d, with striking accumulation in endosomes colocalized with invariant chain (131). The antigen-presenting function of CD1d by APCs from cathepsin S–deficient (catS$^{-/-}$) mice was abnormal, with a reduced capacity to stimulate autoreactive hybridomas directly or to stimulate fresh CD1d-restricted T cells with αGalCer. In vivo, catS$^{-/-}$ mice failed to select or expand normal numbers of CD1d-restricted T cells (131). Cathepsin L, another lysosomal cysteine protease that can participate in invariant chain cleavage,

was found to influence CD1d-restricted T cell recognition and development (132). Unlike cathepsin S, which appeared to alter CD1d trafficking, cathepsin L–deficient ($catL^{-/-}$) mice did not show altered intracellular CD1d localization or cell surface expression. Yet, $catL^{-/-}$ mice lacked the $V\alpha14^+$ CD1d-restricted T cells, while maintaining normal numbers of other CD1d-restricted T cells, such as the $V\alpha3.2^+$ subset. $CatL^{-/-}$ thymocytes expressing CD1d failed to stimulate autoreactive $V\alpha14^+$ CD1d-restricted T cell hybridomas, whereas $V\alpha3.2^+$ CD1d-restricted T cell hybridomas were responsive. The effect appeared to be limited to thymocytes, in that neither $catL^{-/-}$ nor $catS^{-/-}$ (in contrast to the above study) splenocytes showed alteration in their ability to stimulate any CD1d-restricted T cell population (132). These studies emphasize that cathepsin L plays a critical role in the presentation of self-ligands by CD1d in thymocytes that are recognized by the $V\alpha14^+$ subset of CD1d-restricted T cells (132).

Thus, the association of CD1d with MHC class II/invariant chain complexes and a role for endosomal proteases that may affect invariant chain processing reveal the involvement of molecules typically thought to participate only in antigen presentation by MHC class II. However, whether the effects by which these molecules influence CD1d intracellular trafficking and antigen-presenting function are direct or whether they affect other proteins that more directly impact antigen presentation by CD1d is not known.

CELLULAR EXPRESSION OF CD1a, b, AND c AND CD1a-, b-, AND c-RESTRICTED T CELLS

Expression of CD1a, b, and c on Antigen-Presenting Cells

The most striking expression of CD1 molecules is on dendritic cells and other professional APCs. CD1a, b, and c are widely used as DC markers in humans. Langerhans cells express CD1a as a key marker and also express CD1c, but appear to lack expression of CD1b (Figure 6). Freshly isolated immature and mature dermal Langerhans cells efficiently presented antigens to CD1a-restricted T cells (133). Dermal DCs and interdigitating DCs in lymph nodes express CD1b (134). Expression of CD1c is characteristic of human DC populations. However, CD1c is unique among group 1 CD1 antigens in its expression on subsets of B cells. CD1c is expressed in lymph node mantle zones and germinal centers (134), in marginal zone B cells of spleen (135), and on a subpopulation of circulating B cells in fetal and adult human peripheral blood (136, 137).

The expression of CD1 isoforms on DCs has also been determined in studies using in vitro differentiation systems. $CD34^+$ hematopoietic progenitor cells isolated from either umbilical cord blood or bone marrow cultured with granulocyte/macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-α (TNF-α) develop into two separate DC lineages (Figure 6). One subset is $CD1a^+$ and lacks expression of CD14. As is characteristic of Langerhans cells in the

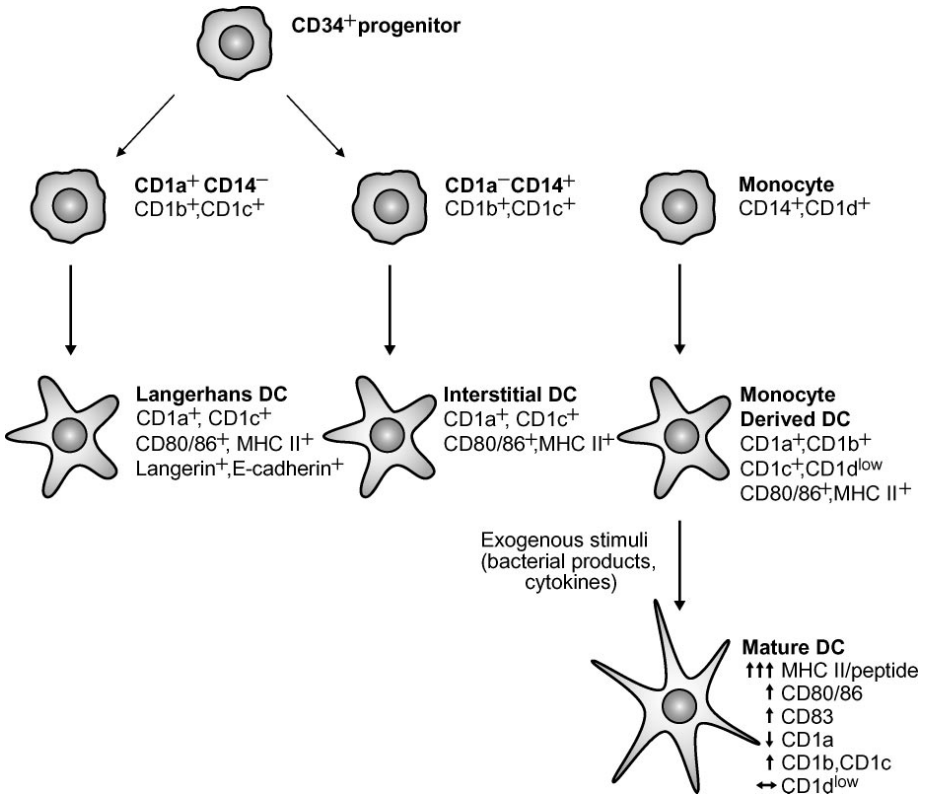

Figure 6 CD1 expression during myeloid DC ontogeny. $CD34^+$ hematopoietic progenitor- and blood monocyte-derived DCs acquire CD1 expression early in their development. The DC precursor intermediates derived from $CD34^+$ progenitors can be distinguished by the absence or expression of CD1a, and both populations develop expression of CD1b and CD1c. These precursors further differentiate into two $CD1a^+$ DC populations with distinct features. The DCs that differentiate from the $CD1a^+$ precursors acquire Langerin, E-cadherin, and Birbeck granules, which are characteristic features of Langerhans cells. The DCs that develop from the $CD1a^-CD14^+$ precursors acquire CD1a expression and phenotypic characteristics of interstitial DCs, including CD9, CD68, and factor XIIIa. Blood monocytes, which express CD1d, give rise to DCs that acquire high levels of expression of the CD1a, b, and c isoforms. After activation with exogenous stimuli such as TNF-α, expression of CD1b and CD1c on monocyte-derived DCs increases slightly, whereas CD1a expression decreases. This is in contrast to MHC class II, which is strikingly redistributed to the cell surface from its intracellular pool upon activation.

epidermis, these precursors develop into DCs that express the Langerin protein, E-cadherin, Birbeck granules, CD1a, and CD1c, but not CD1b (138, 139) (Figure 6). Another subset of $CD34^+$ progenitors develops into DCs that pass through a $CD14^+CD1a^-$ intermediary population. These precursors develop into DCs that express CD1a, b, and c and lack Langerin, E-cadherin, and Birbeck granules and that appear to correspond to lymph node interdigitating DCs and DCs in many nonlymphoid tissues (140) (Figure 6).

Human blood monocytes also can be induced to differentiate into DCs by culture with GM-CSF and interleukin-4 (IL-4) (141, 142) (Figure 6). Culture in the presence of fetal calf serum enhances CD1a, b, and c expression after monocyte culture with GM-CSF, whereas human serum inhibits expression of CD1a on these in vitro cultured DCs (143–145). The monocyte-derived $CD1a^+$ and $CD1a^-$ DC subpopulations may have different functional capabilities (146).

CD1 Trafficking During DC Maturation

Recent studies show that CD1 molecules do not follow the same course as MHC class II molecules during DC maturation (123, 147) (Figure 6). In immature DCs, both CD1b and MHC class II are located in multilamellar lysosomes, but CD1b is found on the limiting membrane, whereas MHC class II is located in the internal membranes (74, 123). During DC maturation, the surface levels of CD1b and c either remain constant or increase only slightly, whereas surface levels of MHC class II increase strikingly (123, 147). The expression of CD1a molecules typically decreases and CD1d levels remain barely detectable. During DC maturation, unraveling of multilamellar lysosomes and tubulation of multivesicular bodies are part of the process that results in delivery of MHC class II proteins to the plasma membrane (123, 148–150). During the first hours after DC maturation is initiated, MHC class II molecules and CD1b and c molecules were found to segregate from one another and localize in different intracellular vesicles (123). After maturation has occurred, CD1b and c molecules maintained their prematuration steady-state distribution, whereas MHC class II molecules redistributed to the plasma membrane. The intracellular lysosomes containing CD1b and c were markedly altered compared with lysosomes in immature DCs. These new lysosomes (called mature DC lysomomes or MDL) lack the multilamellar structure of immature DCs and appear as electron-dense single-membrane vesicles that still contain CD63 and LAMP1, but are nearly devoid of MHC class II (123). The localization of CD1 molecules during DC maturation appears to result from their redelivery to lysosomes via continued active internalization from the plasma membrane, since the internalization rate for CD1b from the plasma membrane does not change during DC maturation, whereas internalization is markedly reduced for MHC class II (123). Interestingly, glycolipid antigens were efficiently presented to CD1-restricted T cells by immature DCs, whereas MHC class II-restricted responses occurred more efficiently when stimulated by mature DCs (147). The ability of CD1 antigen-presentation to function in immature DCs likely relates to

the fact that CD1 trafficking to present antigens at the cell surface is not dependent on DC maturation in the same manner as for MHC class II molecules (123, 147).

CD1a-, b-, and c-Restricted T Cells in Antimicrobial Immunity

Using mycobacterial species, a large number of CD1a-, b-, or c-reactive T cell lines and clones were generated, all of which recognized lipid antigens. The first *M. tuberculosis*–specific T cells were restricted by CD1b and were double negative ($CD4^-CD8^-$) (141). Subsequently, CD1-restricted T cells expressing CD8 (151) and CD4 (152) were found, making it clear that CD1-restricted T cells are a component in all of the major phenotypic groups previously thought to be MHC class I– or class II–restricted.

Expression of CD1a, b, and c by dendritic cells in infectious lesions has been studied and correlates with an effective immune response in leprosy lesions (153, 154). T cell clones and lines that recognize microbial lipid antigens presented by CD1a, b, and c have been isolated from the blood of healthy donors and from tuberculoid leprosy lesions, suggesting that such T cells are part of the normal peripheral T cell repertoire and can be recruited to sites of inflammation (52–55, 141, 151, 152, 155, 156). Most of the CD1-restricted microbial antigen-specific T cells described so far recognize lipid antigens from mycobacteria, but two CD1-restricted T cell lines recognizing antigens from *Haemophilus influenza* type B have been described, suggesting that CD1a, b, and c molecules may present antigens from a wider variety of microorganisms to T cells (157). The effector capabilities of the mycobacteria-specific T cell clones were all of the T_H1 type that produce high levels of interferon-γ (IFN-γ) and TNF-α (151, 152). Most are potent cytotoxic T cells that contain perforin, and both $CD8^+$ and $CD4^+$ CD1-restricted T cells showed direct microbicidal activity attributed to granulysin (151, 158–160).

Antigen-specific CD1b-restricted human T cells were able to kill macrophages infected with *M. tuberculosis* in a CD1b-dependent manner (159). CD1a, b, and c molecules have been shown to localize in phagosomes of some DCs infected with mycobacteria, suggesting that infected DCs may also be able to stimulate CD1-restricted T cell responses (161). However, the expression of CD1 molecules by DCs was found to be downregulated following infection of monocyte-derived DCs with live *M. tuberculosis*, but not following phagocytosis of heat-killed bacteria (162, 163). This resulted in complete loss of CD1a, b, and c from the cell surface by 48 h after infection in vitro and was associated with the corresponding disappearance of mRNA for all three CD1 isoforms (162). *M. bovis* bacillus Calmette Guerin (BCG) infection of blood monocytes was also found to diminish the upregulation of CD1b that would otherwise be induced by culture in the presence of GM-CSF (164). Phagocytosis of several types of heat-killed bacteria, but not of inert particles, by the THP-1 macrophage cell line resulted in down-modulation of CD1b expression on the cell surface (165). These findings suggest that down-regulation of CD1 antigen-presenting molecules may represent a microbial evasion strategy. One host mechanism that may overcome these problems is the delivery of

mycobacterial antigens to uninfected DCs via apoptotic bodies that are released from macrophages infected with *M. tuberculosis* (166). This mechanism may provide a means of delivering microbial antigens to professional APCs that are capable of efficiently stimulating CD1-restricted T cell responses.

Responses to *M. tuberculosis* isoprenoid lipids, such as mannosyl-β-1-phosphodolichols, were observed in patients recently infected with *M. tuberculosis* but not in normal controls (55). CD1-restricted T cell responses to lipid antigens were found to be increased in individuals who recently converted to PPD (purified protein derivative) skin test positive following contact with infected individuals or in individuals with active tuberculosis who started antibiotic therapy, compared with normal uninfected individuals. The predominant proliferative and IFN-γ producing responses were in the $CD4^+$ T cell pool of peripheral blood leukocytes (167). Additionally, the major $CD8^+$ T cell response occurring after *M. bovis* BCG immunization was CD1-restricted (168). *M. tuberculosis*-specific CD1-restricted responses are also seen in guinea pigs, a species that has several CD1b and CD1c isoforms (169). Vaccination of guinea pigs with mycobacterial lipids has a protective effect against infection with virulent *M. tuberculosis* resulting in both reduced bacterial burden and pulmonary pathology, which suggests that CD1-restricted T cells may also contribute to protective memory responses in appropriate animal models (170).

Together, these findings show that CD1-mediated presentation of microbial antigens occurs during microbial infection and that CD1-restricted T cells contribute to T_H1 biased cell-mediated antimicrobial responses (Figure 7). They may

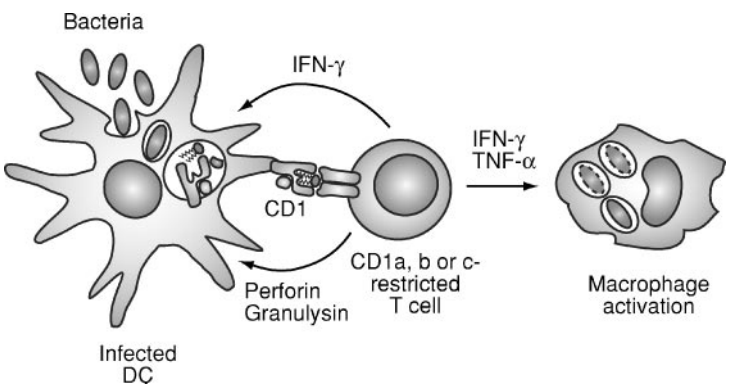

Figure 7 CD1a-, CD1b-, and CD1c-restricted T cells in antimicrobial immunity. DCs that are infected with intracellular bacteria present foreign bacterial lipid antigens on the cell surface bound to CD1 molecules. CD1-restricted T cells that are specific for the foreign microbial lipids are stimulated to carry out effector functions, including the secretion of cytolytic granules containing perforin and granulysin, which lyse the infected cells and have direct antimicrobial effects, respectively, and the production of IFN-γ and TNF-α, which activate the microbicidal functions of macrophages.

respond earlier to foreign antigens than MHC-restricted T cells by recognizing antigens presented by immature DCs and then participate as part of the memory response that follows infection (147, 171–173).

In addition to recognizing foreign microbial lipid antigens as described above, many CD1-restricted T cells appear to recognize CD1a, b, or c in the absence of foreign lipid antigens. Such CD1 self-reactive T cell clones express either $\alpha\beta$ or $\gamma\delta$ TCRs, are cytolytic, and secrete mainly IFN-γ and other T_H1-type cytokines (62, 80, 172, 174). CD1a, b, c, and d self-reactive T cell clones induced immature monocyte-derived DC maturation in vitro and were critical in determining the IL-12-producing capacity of DCs (172, 175). T cell factors appear to be important in developing critical IL-12 production by DCs, but MHC-restricted foreign antigen-specific T cells are rare at the initiation of a new immune response. Thus, CD1 self-reactive T cells may play a critical role in providing DC instruction at early points in the immune response and could thereby influence the subsequent adaptive CD1 and MHC-restricted foreign antigen-specific T cell response. CD1 self-reactive T cells might bridge the gap between immediate innate and delayed adaptive immune responses (80, 176).

Expression of CD1a, b, and c in Noninfectious Lesions

CD1 expressing APCs have also been observed in a variety of chronic inflammatory conditions associated with autoimmune diseases. Expression of CD1a and CD1b was observed on $CD68^+$ endoneurial macrophages in acute and chronic inflammatory demyelinating polyneuropathies and in vasculitic neuropathies, conditions characterized by T cell infiltration of peripheral nerves (177, 178). In the central nervous system, CD1b was expressed in chronic acute plaques of multiple sclerosis lesions, especially on astrocytes (179). In a guinea pig model of experimental autoimmune encephalomyelitis (EAE), CD1b and CD1c expression was detected on astrocytes as well as infiltrating cells in affected lesions (180). Interestingly, CD1-restricted T cells specific for gangliosides and sulfatide, lipids that are enriched in neural tissues, have been isolated from individuals with multiple sclerosis, suggesting that such T cells may participate in the pathogenesis of organ-specific autoimmune diseases (68, 71).

CD1 bearing DCs have been noted in several other diseases. For example, DCs expressing CD1a and CD1c were noted in rheumatoid synovium, with preferential localization to the synovial lining (181). Slightly increased expression of CD1b and CD1c was observed in psoriasis lesions (182). CD1a bearing DCs were found in salivary glands of Sjögren's syndrome and correlated with the presence of lymphocytic infiltration (183). CD1a, b, c, and d were all noted in macrophage-derived lipid-laden foam cells in atherosclerotic plaques (184). In tumors found in humans, infiltrating DCs often express CD1 isoforms, suggesting that they may play a role in presenting tumor glycolipid antigens and could also be of prognostic value (185–187). CD1a was expressed by the main cells of histiocytosis X, a malignancy of Langerhans cells (188, 189), and in the cellular infiltrate of oral

lichen planus, a lesion considered to be precancerous (190). CD1b and CD1c expression was significantly increased in mycosis fungoides, a form of cutaneous T cell lymphoma (182). The expression of CD1 isoforms by B and T cell leukemia and lymphoma cells may be a potential target for immune recognition (191). However, CD1c and CD1d are two of the genes with the most profoundly depressed transcripts on chronic lymphocytic leukemia cells, suggesting that downregulation of CD1 molecules may represent a means of tumor immune evasion (192).

Together, these studies on the expression of CD1a, b, and c suggest that CD1-restricted T cells are likely to participate in infectious, inflammatory, autoimmune, and malignant conditions. Rather than having only a restricted role in mammalian immunity, all evidence points to CD1a-, b-, and c-restricted T cells participating like MHC-restricted T cells in an extensive array of host defense and immunopathologic processes.

CELLULAR EXPRESSION OF CD1d

CD1d Expression in Humans

In humans, CD1d expression on myeloid lineage cells appears to be regulated independently from CD1a, b, and c. Low levels of CD1d can be detected on most monocytes, and this expression decreases during culture with GM-CSF and IL-4, a condition that strongly induces CD1a, b, and c expression (193) (Figure 6). Maturation of monocyte-derived DCs with lipopolysaccharide (LPS), TNF-α, or CD40/CD40L interaction does not alter the expression of CD1d significantly (193, 194). Despite these low expression levels, monocytes, monocyte-derived immature DCs, mature DCs, and macrophages can potently stimulate the proliferation and cytokine secretion of Vα24$^+$ CD1d-restricted T cell clones when loaded with αGalCer (193). This suggests that CD1d may be present at functional levels on resting monocytes and tissue macrophages at all times, allowing CD1d and CD1d-dependent T cells to function at early points during host response to infection or other challenges. CD1d expression has also been detected by immunohistochemistry on dermal DCs, but not on Langerhans cells (195). In contrast to the low levels of surface expression of CD1d on myeloid lineage cells, many circulating and splenic human B cells express CD1d at substantial levels (194, 196). In human lymph node, mantle zone B cells were strongly CD1d$^+$ by immunohistochemical analysis, whereas CD1d$^+$ cells in germinal centers were rare (194). Expression of CD1d has also been reported at high levels on cortical thymocytes, but it is not detectable on resting mature T cells (194). However, after activation with PHA low levels of CD1d surface expression have been observed on human T cells (194), and CD1d proteins appear to accumulate intracellularly (197). Whether CD1d expression on activated human T cells has functional relevance is unknown.

In humans, CD1d is also expressed on epithelial cells, parenchymal cells, and vascular smooth muscle cells in nonlymphoid tissues including gut and liver (196, 198). Freshly isolated human intestinal epithelial cells were able to present

αGalCer to a murine CD1d-restricted T cell hybridoma, suggesting that intestinal epithelial cells may present lipid antigens to intestinal CD1d-restricted T cells (199). IFN-γ and heat shock protein 110 upregulated CD1d expression on intestinal epithelial cell lines, although this has not been shown for professional APCs (200, 201). Thus, in humans, CD1d expression is much broader than CD1a, b, and c expression, in that it is expressed on most monocytes, macrophages, DCs, and B cells, as well as on certain nonlymphoid cells. However, in contrast to CD1a, b, and c expression, CD1d expression is characteristically low and is not clearly upregulated on professional APCs during maturation.

CD1d Expression in Mice

Mice have two CD1 genes, CD1D1 and CD1D2. Expression of CD1d2 protein in mice has been reported only on thymocytes, and its expression was not sufficient for the development of CD1d-restricted T cells (202, 203). Thus, due to the limited expression of CD1d2, most of the CD1d expression in mice outside of the thymus seems to be expression of CD1d1.

In mice, CD1d is expressed on professional APCs, including splenic DCs, macrophages, and B cells (204–206). Similar to human CD1a, b, and c proteins, exposure to IL-4 and GM-CSF has been shown to increase surface expression of murine CD1d on bone marrow–derived macrophages and DCs, although only moderately (206, 207). IFN-γ did not upregulate CD1d expression on bone marrow–derived macrophages (206). Bone marrow–derived Flt3-ligand stimulated murine DCs cultured in the presence of LPS or IFN-α increased their surface expression of CD1d in parallel with MHC class II, CD80, CD86, and CD40 (207). Furthermore, increased CD1d expression has been reported on DCs in inflamed colonic lamina propria (208). Thus, in contrast to human CD1d, expression of murine CD1d on myeloid lineage cells appears to be upregulated by culture in GM-CSF/IL-4 and under inflammatory conditions. Splenic $CD21^{hi}CD23^{lo}IgM^{hi}IgD^{lo}$ marginal zone B cells appear to have the highest levels of CD1d expression among B cells in mice (205). In mice, CD1d has been reported to be expressed on immature and mature thymocytes and on peripheral T cells (205, 206). A subset of splenic $CD161^{-}$ $V\alpha14J\alpha18^{+}$ T cells has been shown to express high levels of CD1d, and these T cells were able to autopresent αGalCer (209). However, whether CD1d expression on peripheral murine T cells has a physiologic role is not known.

Expression of murine CD1d outside the lymphoid system has been observed in liver and on gastrointestinal epithelium (39, 204). A murine intestinal epithelial cell line presented αGalCer but not Gal(α1–2)αGalCer to a CD1d-restricted T cell hybridoma, suggesting that intestinal epithelial cells in mice may be able to present lipid antigens to intestinal CD1d-restricted T cells, but that antigen-processing may not be efficient (199). However, in situ hybridization techniques detected CD1d mRNA in intestinal tissue only in Paneth cells, but not in epithelial cells (210), and others also have not detected expression of CD1d in murine intestinal tissue by immunohistochemistry (204, 206) or western blot (211).

CD1d-RESTRICTED T CELLS

CD1d-restricted T cells have been shown to contribute to antimicrobial responses, antitumor immunity, and the balance between tolerance and autoimmunity. The divergent roles of CD1-restricted T cells in promoting both inflammatory and tolerogenic responses can be understood on the basis of their ability to promptly secrete cytokines and cytotoxic granules, and to regulate the function of DCs, NK cells, T cells, and B cells. The existence of functionally distinct subsets of CD1d-restricted T cells and modification of their effector functions by costimulatory pathways may explain the different outcomes following their activation.

Phenotype of CD1d-Restricted T Cells

CD1d-reactive T cells were initially identified among NK1.1$^+$ thymocytes from normal mice and CD4$^+$ T cells in MHC class II–deficient animals (63, 100), and subsequently shown to be present also in peripheral blood of humans (64). Many CD1d-restricted T cells in mice and humans coexpress CD161, a cell surface molecule usually observed on NK cells that corresponds to the NK1.1 antigen, and therefore are often referred to as Natural Killer T (NKT) cells. However, it has become clear that CD3$^+$CD161$^+$ T cells are heterogeneous and that many do not recognize CD1d molecules but instead recognize other restriction elements, including MHC class I and II, TL, Qa-1, and H2-M3 (171, 212, 213). In uninfected C57BL/6 mice, 20%–80% of CD161$^+$ T cells stained with αGalCer/CD1d tetramers, with spleen and bone marrow containing larger populations of NK1.1$^+$$\alpha$GalCer/CD1d tetramer$^-$ T cells than thymus and liver (92, 214). In humans, 20%–25% of T cells from PBMCs were CD161$^+$, but only less than 1% of the CD161$^+$ T cells are stained with αGalCer/CD1d tetramers (94). Furthermore, many αGalCer/CD1d-tetramer$^+$ T cells do not express CD161. Importantly, following activation, expression of the CD161 antigen on T cells can be upregulated on a majority of conventional T cells (215, 216) and downregulated on CD1d-restricted T cells (217, 218). Thus, CD1d-restricted T cells and CD161$^+$CD3$^+$ T cells (NKT cells) are frequently not identical T cell subpopulations, and therefore use of the term NKT cells has become confusing and inaccurate. It is preferable to refer to them as CD1d-restricted T cells or CD1d tetramer$^+$ T cells.

CD1d-restricted T cells in mice and humans express additional receptors commonly found on NK cells. CD1d-restricted T cells in C57BL/6 mice express intermediate levels of IL-2 receptor β (CD122), and expression of the inhibitory NK receptors Ly49A, C/I and Ly49G2 has been reported on a subset of CD161$^+$CD3$^+$ T cells (219, 220). Many Vα24$^+$/Vβ11$^+$ T cells in human PBMCs express CD122 (221). Expression of CD94, a C-type lectin, has been reported on approximately 50% of αGalCer/CD1d tetramer$^+$ T cells in human PBMCs (94), whereas the immunoglobulin superfamily killer inhibitory receptors (KIRs) CD158a/b appeared to be absent on human CD1d-restricted T cells (95).

A striking feature of most murine and human CD1d-restricted T cells is their expression of markers associated with recently activated or memory T cells. In mice, most CD1d-restricted T cells are $CD44^{hi}CD69^{int}CD45RB^{hi}CD62L^{lo}CCR7^{neg}$, and in humans, most CD1d-restricted T cells are $CD45RO^{+}CD45RA^{-}CD25^{+}CD62L^{-}CCR7^{-}$, but only 5%–15% express CD69 (221–224). Interestingly, CD1d-restricted T cells in human cord blood and in germ-free mice display this activated/memory surface phenotype, suggesting that previous exposure to foreign microbial antigens is not the reason for this phenotype and that stimulation by CD1d-presented self-ligands is likely to be sufficient (223–225). In addition, CD1d-restricted T cells in mice, and to a lesser extent in humans, express intermediate levels of TCR at the cell surface, which may be the consequence of continuous low-level TCR stimulation provided by recognition of self-antigens that are constitutively presented by CD1d.

CD1d-restricted T cells are usually $CD4^{+}$ or double negative (DN). In mice, 60%–90% of CD1d-restricted T cells have been reported to be $CD4^{+}$ and 10%–40% to be DN, and only very few appear to express $CD8\alpha$ or $CD8\beta$ (92, 93, 214). In humans, a mean of 50% of αGalCer/CD1d tetramer^{+} T cells are $CD4^{+}$, with high donor-to-donor variability, and $CD8\alpha$ expression is common, but only very few $CD8\beta^{+}$ CD1d-restricted T cells exist (<1% in unstimulated PBMCs) (94, 104, 226).

The surface phenotype of murine and human CD1d-restricted T cells that do not use $V\alpha14J\alpha18$ or $V\alpha24J\alpha18$ TCRs, respectively, has been difficult to determine on freshly isolated cells owing to the lack of specific reagents for their detection. Analysis of the surface phenotype of murine $V\alpha14J\alpha18^{-}$ CD1d-restricted T cell clones or T cells from mice with transgenic expression of such TCRs showed expression of markers of the NK lineage and of activation/memory markers similar to that of $V\alpha14J\alpha18^{+}$ CD1d-restricted T cells (102, 105, 227).

Development and Selection of CD1d-Restricted T Cells

Like MHC class I– and II–restricted T cells, most CD1d-restricted T cells appear to originate in the thymus (228–230). There also exists evidence for the additional origin of CD1d-restricted T cells in developing liver and bone marrow (231, 232). Recent studies using αGalCer/CD1d tetramers have identified $HSA^{low}CD44^{low}CD161^{-}$ $V\alpha14^{+}$ T cells as precursors of $V\alpha14^{+}CD161^{+}$ CD1d-restricted T cells (228–230). The ultimate precursors of $V\alpha14^{+}$ CD1d-restricted T cells appear to be $CD4^{+}CD8^{+}$ (DP) thymocytes that can also give rise to MHC class I– and II–restricted $CD8^{+}$ and $CD4^{+}$ T cells (228–230). CD1d-restricted T cells can first be detected in thymus, spleen, and liver 5–8 days after birth, and their numbers in spleen and liver reach a plateau at 4–6 weeks of age (230). Positive selection of TCRα-invariant CD1d-restricted T cells appears to be mediated by CD1d-expressing $CD4^{+}CD8^{+}$ DP immature cortical thymocytes and not by thymic epithelial cells (233). However, the structures of CD1d-presented ligands that are used for positive selection of CD1d-restricted T cells are not known.

Reduced numbers of αGalCer/CD1d tetramer$^+$ T cells have been observed in fetal thymic organ cultures exposed to αGalCer, in mice repeatedly injected with αGalCer from day 3 after birth on, and in transgenic mice overexpressing CD1d, suggesting that CD1d-restricted T cells undergo negative selection when engaged by high-avidity antigens or abundant self-antigens during development (234, 235). Negative selection could be mediated by bone marrow–derived dendritic cells overexpressing CD1d, but not by thymic epithelial cells (234). After completion of their TCRα and β chain rearrangement, CD1d-restricted T cells detected in thymus with αGalCer/CD1d tetramers do not yet appear to express NK receptors, such as CD161 or members of the Ly-49 inhibitory NK receptor family (228–230). Subsequently, NK receptor$^-$ CD4$^+$/DN thymic precursors have been shown to divide rapidly and to acquire expression of NK receptors. The delayed expression of inhibitory NK receptors on CD1d-restricted T cells during development has been proposed as an evolutionarily conserved mechanism to terminate their expansion and to control the autoreactivity of these cells (236). Immature thymic NK receptor$^-$αGalCer/CD1d tetramer$^+$ T cells appear to produce much more IL-4 than IFN-γ mRNA and protein, whereas in mature NK receptor$^+$ CD1d-restricted T cells this balance reverts in favor of IFN-γ (229, 230, 264). Export of most CD1d-restricted T cells from the thymus seems to occur before the expression of NK receptors, indicating that complete maturation of CD1d-restricted T cells occurs mainly post-thymically. Several gene mutations selectively disturb the development of CD161$^+$CD3$^+$ T cells or Vα14Jα18$^+$ CD1d-restricted T cells, but do not affect the development of conventional CD4$^+$ and CD8$^+$ T cells, indicating additional differences between the developmental pathways of T cells restricted by either CD1d or MHC class I and II. These gene mutations include the tyrosine kinases Fyn (237, 238) and Itk (239); the transcription factors Ets-1 (240), Irf-1 (241), and RelB (242, 243); IL-15 (244) and the cytokine receptors IL-15Rα (245), IL-2/IL-15Rβ but not IL-2 (246) and GM-CSFRβ (247); and membrane lymphotoxin (248, 249).

Tissue Distribution, Localization, and Recruitment of CD1d-Restricted T Cells

In mice, of the total lymphocytes per organ, αGalCer/CD1d tetramer$^+$ T cells comprise approximately 0.5%–1% in thymus, 1%–2% in spleen, 0.5% in lymph nodes, 10%–50% in liver, 0.5% in bone marrow, and 1% of intestinal intraepithelial lymphocytes (IEL) (92, 214). In humans, Vα24$^+$ CD1d-restricted T cells account for a mean of 0.2% of peripheral blood T cells, as determined by use of both Vα24/Vβ11 antibodies and αGalCer/CD1d tetramers (85, 94, 104). Their numbers in liver of humans also are lower than they are in liver of mice (250–252).

The chemokine receptor and homing molecule profile of many CD1d-restricted T cells in mice and humans is more like that of effector (memory) T cells than that of naïve T cells. In humans, for example, 50%–90% of Vα24$^+$/Vβ11$^+$

CD1d-restricted T cells expressed CCR5, CXCR3, and CXCR6, chemokine receptors associated with T_H1 responses and migration to sites of inflammation, and only about 20% of Vα24$^+$/Vβ11$^+$ CD1d-restricted T cells expressed CCR7 and almost no peripheral blood Vα24$^+$/Vβ11$^+$ CD1d-restricted T cells expressed CXCR5, both receptors required for migration into T and B cell zones of lymphoid organs (94, 95, 253, 254). In mice, most Vα14$^+$ CD1d-restricted T cells expressed CXCR3 and CXCR6 (255). Only murine NK1.1$^-$ Vα14$^+$ CD1d-restricted T cells that are considered to be immature and recently released from the thymus (229, 230), but not NK1.1$^+$ Vα14$^+$ CD1d-restricted T cells, migrated in response to the CCR7 ligand SLC/CCL21 (255). In mice, Vα14$^+$ CD1d-restricted T cells from spleen, but not liver, bone marrow, or blood, migrated in response to the CXCR5 ligand BCA-1/CXCL13, suggesting that these cells could be recruited to B cell–rich areas in the spleen and thus modulate the follicular B cell response. Interestingly, some chemokine receptors appear to be differentially expressed by CD4$^+$ and CD4$^-$ CD1d-restricted T cell subsets. For example, in humans, CCR4 was preferentially expressed by CD4$^+$ CD1d-restricted T cells, whereas the chemokine receptors CCR6 and CXCR6 were preferentially expressed on CD4$^-$ CD1d-restricted T cells, and CD4$^+$ and CD4$^-$ CD1d-restricted T cell subsets preferentially migrated in response to the CCR4 ligand TARC and the CCR6 ligand LARC, respectively (253, 254). Similarly, differences in the chemotactic response of CD4$^+$ and DN Vα14$^+$ CD1d-restricted T cells have been observed in mice (255). Thus, particular subsets of CD1d-restricted T cells with differential expression of chemokine receptors may be recruited to different sites. Up to 20% of human Vα24$^+$ CD1d-restricted T cells expressed cutaneous lymphocyte antigen (CLA), associated with homing to skin, and 30%–75% stained positive for the integrin $\alpha_4\beta_7$, associated with homing to gut (94, 253). In contrast, only a small proportion of CD1d-restricted T cells in humans and mice expressed high levels of L-selectin (CD62L) that would allow entry into secondary lymphoid organs via high endothelial venules (HEVs). Together, these features suggest that most CD1d-restricted T cells recirculate through peripheral tissues and enter lymph nodes most likely through the afferent lymphatics rather than through HEVs.

CD1d-restricted T cells can be recruited rapidly to inflamed and infected tissues in vivo. Injection of mycobacterial lipids into skin of mice leads to a granulomatous reaction that depends on Vα14Jα18$^+$ T cells (256–258). Vα14Jα18$^+$ T cells could be detected at the injection site within 6 h. The mechanism of recruitment of Vα14Jα18$^+$ T cells to these lipid-induced granulomas is unclear, although it has been shown that CD1d expression is not required and that Vα14Jα18$^+$ T cells can be recruited by subcutaneous injection of TNF-α alone (258). Vα14$^+$ T cells accumulated in the lungs of mice during pulmonary infection with *Cryptococcus neoformans*, and this recruitment was dependent on the CCR2 ligand MCP-1 (259). During anterior chamber associated immune deviation (ACAID), CD161$^+$ CD3$^+$ T cells are recruited to the spleen in a CXCR2- and MIP-2-dependent manner (260).

Activation and Effector Functions of CD1d-Restricted T Cells

CYTOKINE SECRETION AND CYTOTOXICITY In mice and humans, TCRα-invariant CD1d-restricted T cells secrete large amounts of IFN-γ, IL-4, IL-2, IL-5, IL-10, IL-13, GM-CSF, and TNF-α within minutes after TCR stimulation (94, 261, 262), a property that distinguishes these T cells from naïve MHC class I– and II–restricted T cells that acquire their ability to secrete cytokines during proliferation after primary stimulation. IL-4 and IFN-γ proteins could only be detected in activated, but not in resting, αGalCer/CD1d-tetramer$^+$ T cells (92). However, abundant mRNA transcripts for IL-4 and IFN-γ were detected in resting αGalCer/CD1d-tetramer$^+$ T cells (263, 264). Unlike conventional T cells, TCRα-invariant CD1d-restricted T cells activate IL-4 and IFN-γ transcription already during thymic development and populate the periphery with both cytokine loci previously modified by histone acetylation (264). This constitutive cytokine mRNA expression may allow the rapid cytokine production and secretion by TCRα-invariant CD1d-restricted T cells.

The events following activation of CD1d-restricted T cells by αGalCer involve TCR ligation by the CD1d/αGalCer complex and interactions of costimulatory molecules that lead to T cell activation, resulting in cytokine secretion and increased CD40L expression (265) (Figure 8). In mice, CD1d-restricted T cells constitutively express CD28, and its interaction with CD80 and CD86 expressed by APCs augmented both IFN-γ and IL-4 secretion by the T cells after TCR-mediated activation (262, 266). Through CD40/CD40L interaction and IFN-γ stimulation, DCs are then activated and secrete IL-12 (172, 265, 267) (Figure 8). Vα14Jα18$^+$ T cells showed a constitutively high expression of IL-12 receptor (IL-12R), and IL-12R expression on these cells increased in an IL-12- and IFN-γ-dependent manner (265, 275). Although IL-12Rβ2 and IL-4Rα expression on CD1d-restricted T cells and CD40 expression on APCs were not required for the immediate IL-4 and IFN-γ production by CD1d-restricted T cells following αGalCer stimulation (263), available IL-12 and IFN-γ can amplify the activation of CD1d-restricted T cells and augment their IFN-γ secretion (265–268) (Figure 8). Inhibitory NK receptors, such as Ly49A, C/I, and Ly49G2, expressed on CD161$^+$CD3$^+$ T cells appear to be capable of inhibiting TCR-mediated activation, suggesting that downregulatory mechanisms may counterbalance the activation of CD1d-restricted T cells (219, 220).

In addition to the prompt cytokine secretion, CD1d-restricted T cells are also potently cytolytic and release perforin and granzymes and express membrane-bound members of the TNF family [FasL or TNF-related apoptosis-inducing ligand (TRAIL)] (269–271). The cytotoxicity of CD1d-restricted T cells is augmented by IL-2 and IL-12 and is likely to be important in antimicrobial and antitumor immune responses (272, 273). Furthermore, human CD1d-restricted T cell clones expressed granulysin after activation with αGalCer, suggesting that they can contribute directly to bacterial killing (274).

T_H1/T_H2 BALANCE The in vivo responses of CD1d-restricted T cells may be influenced by a variety of local factors present during their activation. T_H1 responses

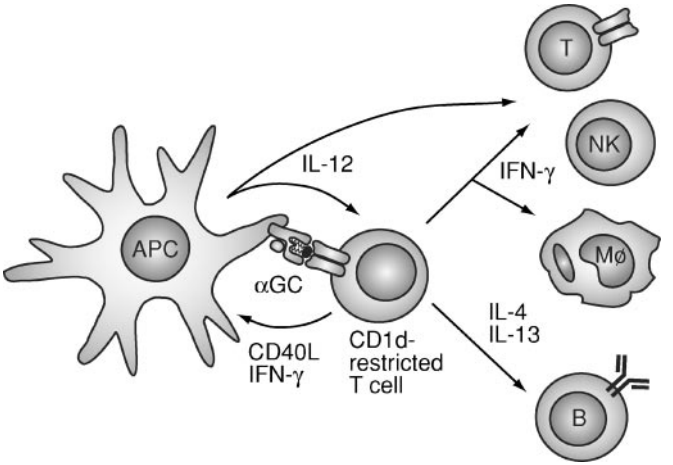

Figure 8 Effects of αGalCer recognition. αGalCer is taken up by APCs and presented to CD1d-restricted T cells. This antigenic stimulus potently elicits production of both T_H1 cytokines, such as IFN-γ, and T_H2 cytokines, such as IL-4 and IL-13, from CD1d-restricted T cells. Recognition of αGalCer induces CD1d-restricted T cells to secrete IFN-γ and upregulate CD40L, which stimulates the maturation of immature DCs and the production of IL-12 from DCs. IFN-γ and IL-12 activate NK cells and T cells, whereas T_H2 cytokines activate B cells. IFN-γ released by CD1d-restricted T cells, NK cells, and T cells activates macrophages. NK, NK cell; T, T cell; B, B cell; Mø, macrophage.

were favored by cytokines such as IL-12, which augmented IFN-γ production, or by signaling through CD161 on murine T cells, which induced IFN-γ secretion and polarized the cytokine response of activated CD1d-restricted T cells to T_H1 (275, 276). The T_H1/T_H2 bias of the cytokine response of CD1d-restricted T cells during cerebral malaria infection in mice was influenced by genes located in the NK complex, further indicating a role for NK receptors on cytokine production (277). T_H2-type cytokine responses were favored when CD1d-restricted T cells were activated in the presence of IL-7 or IL-18 (278–280). Neonatal, and to a lesser extent adult, $V\alpha24^+/V\beta11^+$ T cells differentiated preferentially into T_H1-type T cells when stimulated with PHA in the presence of monocyte-derived DCs. Alternatively, when stimulated by $CD4^+CD3^-CD11c^-$ plasmacytoid cell-derived DCs, they differentiated into T_H2-type T cells, suggesting that distinct subsets of DCs influence their cytokine production (281). An αGalCer derivative that lacks one alkyl chain also stimulated T_H2-biased cytokine secretion (282). In mice, administration of a single dose of αGalCer elicits IFN-γ and IL-4 secretion, whereas repeated αGalCer applications tend to induce a T_H2 bias in CD1d-restricted T cell cytokine secretion (263, 283, 284). In contrast, altering the antigen dose or slowly delivering antigen by an osmotic pump, manipulations that polarize naïve $CD4^+$

MHC class II–restricted T cells, did not alter the ratio of intracellular IFN-γ to IL-4 staining of CD1d-restricted T cells in response to αGalCer (263).

FUNCTIONALLY DISTINCT SUBSETS CD4 coreceptor expression on human CD1d-restricted T cells correlates with their effector functions. The $CD4^-$ subset produced mainly T_H1 cytokines and prominently expressed perforin after activation, whereas the $CD4^+$ subset potently produced both T_H1 and T_H2 cytokines (94, 95, 285). This suggests that different activities of CD1d-restricted T cells could result from activation of such functionally distinct subsets. Interestingly, as discussed above, analysis of $CD4^+$ and $CD4^-$ subsets of CD1d-restricted T cells revealed differences in their expression of chemokine receptors and in their chemotactic responses to chemokines. Thus, CD1d-restricted T cell subsets with distinct functional capabilities may be recruited to different sites.

INTERACTION WITH OTHER CELL TYPES One major attribute of CD1d-restricted T cells is their ability to stimulate many other cell types. After the initial activation of CD1d-restricted T cells, the IFN-γ and IL-2 that they secrete in combination with IL-12 produced by DCs can lead to activation of NK cells and memory $CD4^+$ and $CD8^+$ T cells (284, 286–289) (Figure 8). This activation seems to increase IFN-γ secretion and cytotoxicity by these cell types. IFN-γ from all these sources may contribute to the activation of macrophages (290) (Figure 8). IL-4 secretion, and likely CD40/CD40L interactions, activate B cells, which can lead to their proliferation and production of immunoglobulins (284, 286, 291, 292) (Figure 8). αGalCer-induced activation of CD1d-restricted T cells has many effects on DCs, including differentiation and maturation (172, 293, 293a), induction of IL-12 secretion (172, 265, 267), killing of DCs (294, 295), and release of myeloid progenitors from bone marrow to the periphery (296). The maturation of DCs as a consequence of αGalCer administration in mice led to an adjuvant-like induction of the $CD4^+$ and $CD8^+$ T cell response to a coadministered protein (293, 293a, 293b). This enhancement of the $CD8^+$ and $CD4^+$ T cell response required CD40 signaling but not signaling through the IFN-γ receptor, indicating that IFN-γ was not required for this adjuvant-like effect of αGalCer (293a).

FATE FOLLOWING ACTIVATION A number of studies have found that murine $CD161^+CD3^+$ T cells become undetectable in vivo soon after stimulation with anti-CD3 antibodies (297), αGalCer (298) or IL-12 (297), and during viral (299) and bacterial (300) infections. Subsequent analyses that used αGalCer/CD1d tetramers to more specifically detect CD1d-restricted T cells appeared to confirm their disappearance after stimulation with αGalCer in vivo (92, 301). A fraction of $CD161^+CD3^+$ T cells and αGalCer/CD1d $tetramer^+$ T cells express markers of cells undergoing apoptotic cell death following activation in vivo, suggesting that the disappearance of CD1d-restricted T cells is due to cell death. However, the extent to which this activation-induced cell death occurs is controversial (298, 301, 302). In contrast, other studies have found that rather than dying, CD1d-restricted

T cells persist and/or alter expression of their key surface markers. Downregulation of CD161 has been observed on $CD161^+CD3^+$ T cells after anti-CD3 stimulation in vitro, on αGalCer/CD1d tetramer$^+$ T cells after stimulation with αGalCer or IL-12, and during *Salmonella typhimurium* infection in vivo (217, 218, 303). CD1d-restricted T cells appear to lose αGalCer/CD1d tetramer staining gradually within hours after i.p. administration of αGalCer (218, 303a), suggesting that CD1d-restricted T cells, like MHC-restricted T cells, downregulate their TCR surface levels following activation. Using αGalCer/CD1d tetramer staining, CD1d-restricted T cells were observed to expand after αGalCer administration (218, 303a, 303b) and during infection with *Plasmodium berghei* (277). Following *S. typhimurium* infection, the total numbers of CD1d-restricted T cells in spleen and liver did not change significantly during the first three days of infection (218, 304). Thus, following activation, a fraction of CD1d-restricted T cells appears to undergo activation-induced cell death. However, a number of CD1d-restricted T cells seem to survive, downregulate their TCR and CD161, and appear capable of robust in vivo proliferation and expansion. The population dynamics of CD1d-restricted T cells at sites of infection or inflammation are therefore likely the result of a combinatin of cell death, proliferation, and recruitment.

CD1d-RESTRICTED T CELLS IN ANTIMICROBIAL IMMUNITY

CD1d-restricted T cells contribute to antimicrobial host responses in bacterial, parasitic, viral, and fungal infections. Depending on the infection studied, CD1d-restricted T cells have been found to control growth of microorganisms, influence antibody production and adaptive immune responses against them, and contribute to immunopathologic tissue injury.

Bacterial Infections

In *Pseudomonas aeruginosa* infection, CD1d-deficient animals and mice treated with anti-CD1d mAb had impaired bacterial clearance from their lungs and decreased numbers of neutrophils in the broncho-alveolar lavage during early infection (290). Decreased levels of MIP-2, a chemokine secreted by activated alveolar macrophages and able to recruit neutrophils into infected lungs, were noted in broncho-alveolar lavage of infected CD1d-deficient animals. Administration of αGalCer prior to *P. aeruginosa* infection improved the clearance of bacteria from the lungs through enhanced phagocytosis of bacteria by activated alveolar macrophages. Thus, CD1d-restricted T cells may contribute to bacterial clearance by activation of macrophages and recruitment of neutrophils (290). In a model of Lyme disease, mouse strains normally resistant to the spirochete *Borrelia burgdorferi* developed arthritis and had increased spirochete DNA in tissues when CD1d-restricted T cells were absent (305). Infected CD1d-deficient animals had enhanced production of spirochete-specific IgG_{2a} antibodies that are commonly induced in

disease-susceptible mice. Hence, CD1d-restricted T cells appear able to modulate B cell activation and antibody production during infection.

In a noninfectious model, injection of lipids from *M. tuberculosis* cell walls results in the rapid accumulation of Vα14Jα18$^+$ T cells in subcutaneous granulomas (256–258). CD1d expression has been observed in tuberculoid granulomas in humans (274), and Vα24Jα18 transcripts have been detected in T cell–reactive lepromatous lesions together with CD1d expression on CD83$^+$ cells (306). Together, these studies suggest that CD1d-restricted T cells also may contribute to antimycobacterial immunity. However, mice deficient in CD1d-restricted T cells do not show impaired protective immunity to respiratory or i.v. infection with *M. tuberculosis* or *M. bovis* BCG (307–311). In contrast, administration of anti-CD1d mAb impaired early immunity to *M. tuberculosis* infection (312), and in one study Jα18-deficient animals were marginally more susceptible to infection with *M. tuberculosis* (313). Following i.v. infection with *M. bovis* BCG, Jα18-deficient mice developed more granulomas that had signs of caseation and larger cellular infiltrates, suggesting that Vα14$^+$ CD1d-restricted T cells may play a regulatory role by limiting lymphocyte influx and tissue pathology (311). Thus, although not absolutely required for protective immunity, CD1d-restricted T cells appear to participate in the immune response to *M. tuberculosis* and *M. bovis* BCG infection.

Most examples suggest that CD1d-restricted T cells are beneficial in bacterial infection. However, anti-CD1d mAb blocking during infection with *Listeria monocytogenes* improved survival and the pathogen-specific T_H1 immune response (314). Activation of CD1d-restricted T cells during the immune response to microbial infection can also lead to increased tissue damage, as suggested by the finding that Jα18-deficient mice had less severe liver damage during *Salmonella choleraesuis* infection compared with controls (315).

Parasitic Infections

Jα18-deficient animals that lack TCRα-invariant CD1d-restricted T cells have increased parasite burden during the course of *Leishmania major* infection (316). A role for CD1d-restricted T cells in the humoral immune response to GPI-linked antigens from *Plasmodium* spp. and *Trypanosoma cruzi* has been observed in mice deficient in CD1d or the Jα18 gene, but these effects remain controversial and an influence on survival has not been found by all investigators (57–61, 317, 317a). The role of CD1d-restricted T cells during infection with a strain of *Plasmodium berghei* that causes cerebral malaria was dependent on the genetic background of infected mice. For example, CD1d-deficient BALB/c mice were more susceptible to infection and had more severe cerebral pathology than wild-type BALB/c mice, whereas CD1d-deficient and Jα18-deficient C57BL/6 mice were partially protected against disease compared with controls (277). This study showed further that the functional properties of CD1d-restricted T cells appear to vary according to the expression of loci of the NK complex, suggesting that NK receptors may

influence the cytokine response of CD1d-restricted T cells during infection (277). After inoculation with *Schistosoma mansoni* eggs, CD1d-deficient animals had a reduced protective T_H2 response and developed less marked fibrotic granuloma pathology (318). $CD4^+CD161^+$ T cells from MHC class II–deficient animals augmented the protective $CD8^+$ T cell response to *Toxoplasma gondii* infection after vaccination with attenuated *T. gondii*, suggesting that CD1d-restricted T cells can help in priming a pathogen-specific $CD8^+$ T cell response (319). Thus, for a variety of parasitic infections in mouse models, CD1d-restricted T cells have been implicated in protective T_H2 immunity and in modulating antiparasite antibody responses.

Viral Infections

Mice deficient in CD1d-restricted T cells were more susceptible to infection with herpes simplex virus type 1 and 2 (HSV-1/-2) (320, 321) and diabetogenic encephalomyocarditis virus (EMCV-D) (323). Following respiratory syncytial virus (RSV) infection, CD1d-deficient C57BL/6 mice developed more severe disease, whereas CD1d-deficient BALB/c and 129 $\times$ C57BL/6 mice were less ill compared with controls (322). In CD1d-deficient BALB/c mice, numbers of virus-specific $CD8^+$ MHC class I–restricted T cells were reduced, suggesting that CD1d-restricted T cells influence the adaptive immune response to RSV infection (322). In many infection models, the protective effect of CD1d-restricted T cells can be mediated by $V\alpha14^+$ TCRα-invariant CD1d-restricted T cells. A role for CD1d-restricted T cells using diverse TCRα chains, however, was observed in the immune response of mice to hepatitis B antigens and EMCV-D infection and in hepatits C–infected patients (108, 323, 324). In contrast to protective effects, a CD1d-mediated immune response appeared to be reponsible for the tissue pathology following infection with coxsackie virus B3 (109). Wild-type and Jα18-deficient, but not CD1d-deficient animals, developed myocarditis that was mediated by CD1d-restricted $V\gamma4^+$ T cells. Thus, CD1d-restricted T cells that use TCRα-invariant and diverse TCRs, as well as $\gamma\delta$TCRs, are implicated in reponses to viral infections.

Fungal Infections

Jα18-deficient animals had delayed clearance of *Cryptococcus neoformans* from lung and an impaired in vitro recall response and delayed type hypersensitivity reaction to cryptococcal antigen, suggesting that CD1d-restricted T cells contribute to the development of an adaptive immune response to this pathogen (259).

Mechanism of the Antimicrobial Effect

Studies with CD1d- and Jα18-deficient mice demonstrate a role for CD1d-restricted T cells during the normal host response to microbial infection that can be mediated either directly by CD1d-restricted T cells themselves through both killing of infected cells and microbicidal effects, or indirectly through recruitment and

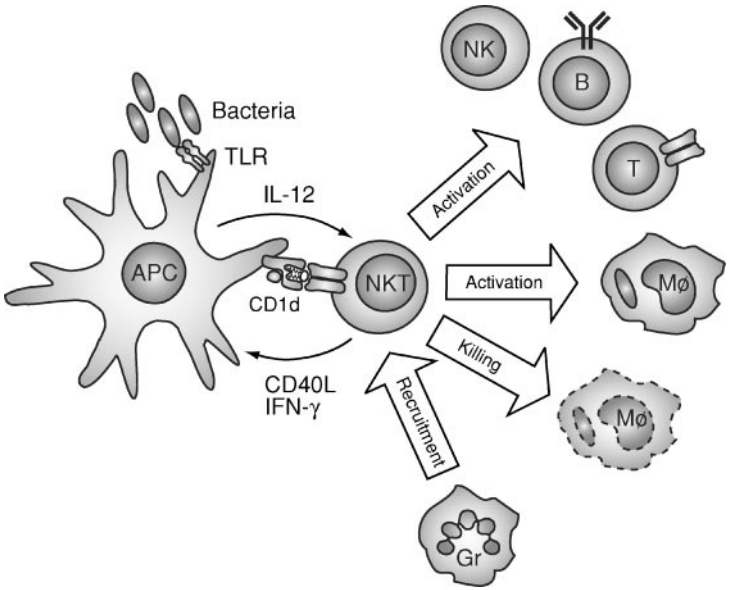

Figure 9 Role of CD1d-restricted T cells during microbial infection. Upon exposure to microorganisms, APCs become activated through Toll-like receptors (TLR) and secrete proinflammatory cytokines, such as IL-12. Signals provided by IL-12 amplify the weak TCR-mediated responses of CD1d-restricted T cells to self-lipid antigens presented by CD1d on the APCs, and lead to productive activation and cytokine secretion by CD1d-restricted T cells. This mechanism of activation may explain how TCRα-invariant CD1d-restricted T cells with their restricted TCR-specificity can become rapidly activated early in many different microbial infections. The early cytokine secretion by CD1d-restricted T cells during infection leads to activation of macrophages, NK cells, T cells, and B cells. Chemokines secreted by CD1d-restricted T cells contribute to the recruitment of other immune cells including granulocytes. Activated CD1d-restricted T cells can activate or kill macrophages directly. IFN-γ secretion and CD40L stimulation lead to maturation of immature DCs. TLR, Toll-like receptor; NKT, CD1d-restricted T cell; Gr, granulocyte; Mø, macrophage; NK, NK cell; T, T cell; B, B cell.

activation of cells of the innate immune system or through modulation of the adaptive immune response (Figure 9). A major unresolved question is whether CD1d-restricted T cells directly recognize foreign microbial antigens. An alternative mechanism has been proposed in which CD1d-restricted T cells can be activated by microbial products through stimulation by the inflammatory cytokine IL-12, secreted by DCs after exposure to bacterial products, in combination with recognition of CD1d-presented self-lipids (304) (Figure 9). This suggests that the activation of CD1d-restricted T cells during microbial infection may not require TCR-mediated recognition of foreign microbial antigens and would allow

their stimulation early and to virtually any infectious agent that stimulates IL-12 secretion.

Activation of CD1d-Restricted T Cells with αGalCer in Microbial Infection

In addition to their role during the natural course of infection, many studies have investigated the effects of pharmacological activation of CD1d-restricted T cells by αGalCer and have demonstrated dramatic protective effects against infection with *P. aeruginosa*, *M. tuberculosis*, *Plasmodium* spp., *T. cruzi*, hepatitis B, EMCV-D, RSV, mCMV, and *C. neoformans* (290, 322, 323, 325–330). As described earlier, the specific pharmacologic activation of CD1d-restricted T cells by αGalCer may improve host control of microbial infections by widespread immune activation (Figure 8). This "adjuvant" effect seems also to be reponsible for the improved efficacy of vaccines when administered together with αGalCer (331). Thus, activation of CD1d-restricted T cells by pharmacological compounds like αGalCer may have therapeutic potential in humans for the treatment of infectious diseases and the improvement of vaccine efficacy.

CD1d-RESTRICTED T CELLS IN ANTITUMOR IMMUNITY

CD1d-restricted T cells potently promote tumor rejection in murine models that use exogenously administered IL-12 or αGalCer as a stimulus, but they also contribute to the natural antitumor immune response in the absence of exogenous stimulation.

IL-12-Mediated Antitumor Immunity

Studies in Jα18-deficient animals and studies using adoptive transfer of IL-12-activated Vα14$^+$ T cells indicated that CD1d-restricted T cells were crucially important for the antitumor and antimetastatic activity of IL-12 (332, 333). However, the relative contribution of CD1d-restricted T cells and NK cells is controversial (303, 334–338). IL-12-induced antitumor immunity seems to be mediated by IFN-γ, secreted by CD1d-restricted T cells and NK cells, and perforin-mediated killing by NK cells. The antitumor effects mediated by IFN-γ appear to include direct inhibition of tumor growth and angiogenesis and activation of other effector cell types (339, 340). In addition, IFN-γ can function to upregulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on NK cells, thus allowing killing of TRAIL-sensitive tumor targets (341).

αGalCer-Mediated Antitumor Immunity

αGalCer was initially purified from marine sponges on the basis of its antitumor properties, and was later shown to be presented by CD1d to Vα14Jα18$^+$ T cells (72,

342). Antitumor effects of αGalCer have been observed against various tumors of different origins and their metastasis, including melanomas, colon, lung, prostate, breast and renal cell carcinomas, and lymphomas (341–344). As described earlier, activation of Vα14Jα18$^+$ CD1d-restricted T cells by αGalCer leads to initial IFN-γ secretion and CD40L upregulation on CD1d-restricted T cells, which subsequently promotes DC production of IL-12 (Figure 8). The secretion of IL-12 by DCs is required for the antitumor and antimetastatic effect of αGalCer, and together with the IFN-γ secreted by CD1d-restricted T cells, secondarily activates NK cells and CD8$^+$ cytotoxic T lymphocytes (CTLs) that function as direct antitumor effectors (302, 343–345). The antitumor activity of these effector cells is dependent on their IFN-γ secretion and TRAIL expression on NK cells, but, surprisingly, is independent of perforin and FasL expression (302, 341, 344, 345). Improved efficiency of tumor-rejection and a prolonged IFN-γ response were observed when DCs pulsed with αGalCer were used instead of αGalCer administration alone (301, 346). Thus, although activated Vα14Jα18$^+$ T cells display direct NK-like nonspecific tumor cell lysis in vitro (270), the in vivo antitumor and antimetastasis responses of CD1d-restricted T cells activated by αGalCer are mainly mediated by IFN-γ- and IL-12-dependent subsequent activation of NK cells and CD8$^+$ CTLs.

Natural Tumor Immunosurveillance

Studies using chemical mutagenesis with methylchlorantrene (MCA) suggest that CD1d-restricted T cells contribute to natural tumor immunosurveillance, in the absence of exogenous stimulation of Vα14Jα18$^+$ T cells by αGalCer or IL-12 (347). Natural host immunity against MCA-induced sarcoma required endogenous IL-12 production, early IFN-γ production by CD1d-restricted T cells, and IFN-γ expression by non-CD1d-restricted T cells, possibly NK cells and CD8$^+$ T cells (348). Tumor rejection was dependent on both NK cells and CD8$^+$ T cells and was perforin-dependent, whereas CD1d-restricted T cells were required but mediated their antitumor effects in a perforin-independent manner. This suggests that NK cells and CD8$^+$ T cells were the direct antitumor effectors (Figure 10).

Recognition of CD1d-presented antigens may be required for the natural antitumor activity of CD1d-restricted T cells, since adoptive transfer experiments showed that a CD1d-positive host environment was essential (349). Whether recognition of CD1d-presented self-antigens—unaltered or altered—by CD1d-restricted T cells is simply required in addition to other, CD1d-independent, tumor-induced "danger signals" (e.g., IL-12) or whether CD1d-restricted T cell activation is caused by the presentation of tumor-derived antigens in the context of CD1d remains to be determined. Recently, CD1d-mediated recognition of GD3, a ganglioside enriched in melanoma cells, has been reported and supports the latter possibility (70). Such tumor-derived lipid-antigens may activate CD1d-restricted T cells when presented by CD1d$^+$ APCs that have taken up tumor cell fragments (70) or by CD1d$^+$ tumors themselves.

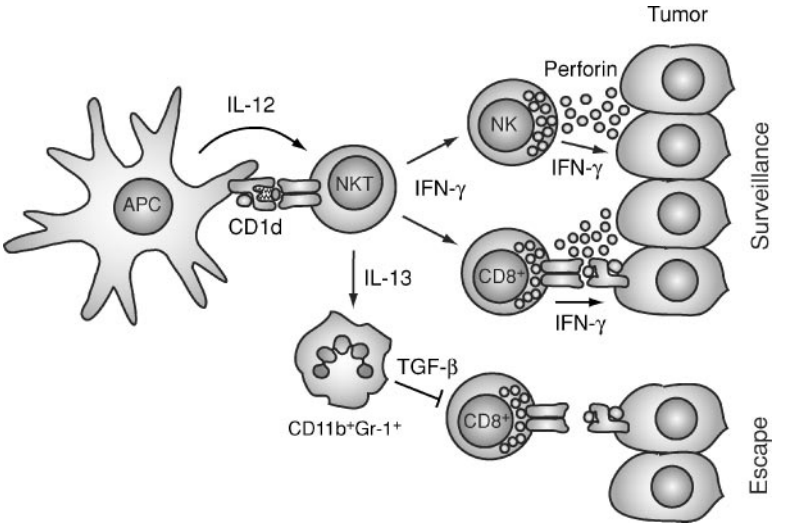

Figure 10 Model of CD1d-restricted T cell function in natural tumor immunosurveillance. CD1d-restricted T cells can mediate tumor rejection in the absence of exogenous stimulation through activation by endogenous or tumor-derived lipid antigens (*upper part of figure*). This tumor rejection depends on CD1d-recognition, IL-12, and IFN-γ, and the final antitumor effector cells are NK cells and $CD8^+$ CTLs that recognize tumor-derived peptide antigens. NK cells and CTLs kill tumor cells directly in a perforin-dependent manner and secrete IFN-γ, which has direct antitumor effects, including inhibition of tumor angiogenesis, and may activate other effector cells. CD1d-restricted T cells can also suppress antitumor immunity (*lower part of figure*). IL-13 produced by CD1d-restricted T cells acts on $CD11b^+Gr\text{-}1^+$ myeloid cells and induces the secretion of inhibitory cytokines, such as TGF-β, which in turn leads to inhibition of tumor-specific CTLs and suppression of antitumor immunity. NKT, CD1d-restricted T cell; NK, NK cell; $CD8^+$, tumor-specific $CD8^+$ T cell.

Cancer Vaccination

CD1d-restricted T cells have also been shown to participate in antitumor responses induced by cancer vaccination. Antitumor immunity against early tumor growth, induced by adoptive transfer of lymphocytes from draining lymph nodes of mice immunized with tumor-specific antigen, was abrogated in Jα18-deficient mice (350). Antitumor immunity stimulated by vaccination with irradiated melanoma cells engineered to secrete GM-CSF was abrogated in CD1d- and Jα18-deficient mice. DCs from immunized CD1d-deficient mice showed compromised maturation and impaired ability to stimulate T cells, suggesting that DCs may be critical for regulating vaccine-induced antitumor immunity (351).

Suppression of Antitumor Immunity

Paradoxically, CD1d-restricted T cells can also suppress natural antitumor responses. This was shown in a mouse tumor model in which the tumor regressed after initial growth because of a tumor-specific $CD8^+$ CTL response, but then recurred because of subsequent suppression of the CTL response mediated by CD1d-restricted T cells (352, 353). The suppression of the antitumor CTL response appears to require IL-13 production by CD1d-restricted T cells and subsequent IL-13-induced TGF-β secretion by $CD11b^+Gr\text{-}1^+$ myeloid cells (354) (Figure 10). Additional evidence for suppression of antitumor immune responses by CD1d-restricted T cells comes from studies in CD1d-deficient animals where CpG oligodinucleotide (ODN)-induced antitumor activity is enhanced compared with wild-type animals (355). Interestingly, the CpG-ODN-induced antitumor activity was present in CD1d-deficient but not in Jα18-deficient or wild-type animals, suggesting that CD1d-restricted T cells with TCRs other than the invariant Vα14J$\alpha18^+$ TCR were mediators of the suppressive effect. Also, a $CD4^+DX5^+$ NKT cell population induced by UV-irradiation that suppressed skin cancer development and delayed type hypersensitivity (DTH) responses has been described, although CD1d-restriction has so far only been demonstrated for DTH suppression but not for the antitumor function of these NKT cells (356). Thus, in natural tumor immunosurveillance CD1d-restricted T cells can promote or inhibit the development of protective antitumor responses (Figure 10).

Antitumor Immunity in Humans

Several studies document that human V$\alpha24^+$/V$\beta11^+$ T cells activated by stimulation with αGalCer can directly kill $CD1d^+$ tumor cells in vitro in a predominantly perforin- and granzyme B-dependent manner, with additional TNF-α-, FasL-, and TRAIL-mediated killing (191, 289, 357–361). Human CD1d-restricted T cells can also mediate their antitumor activity by activating NK cells, an effect that can be caused by IL-2 or IFN-γ secretion by CD1d-restricted T cells (289, 362). Moreover, human CD1d-restricted T cells stimulated with αGalCer can induce IL-12 secretion by DCs, which may contribute to their antitumor activity (172). Thus, human CD1d-restricted T cells in vitro display direct CD1d-dependent cytotoxicity against tumor cells and also can activate NK cells to kill tumor targets.

Numerical deficiencies of CD1d-restricted T cells, and in some cases their loss of IFN-γ production, have been reported for patients with solid tumors, multiple myeloma, or myelodysplastic syndrome, suggesting a correlation between advanced cancer and impaired CD1d-restricted T cell function (361, 363–367). A phase I clinical trial using i.v. αGalCer therapy to stimulate CD1d-restricted T cells in patients with solid tumors showed that αGalCer was well tolerated over a wide range of doses and cytokine responses were observed in several patients with relatively high pretreatment numbers of V$\alpha24^+$/V$\beta11^+$ T cells; however, no clinical responses were recorded (365).

Thus, CD1d-restricted T cells function in tumor immunity when activated by αGalCer or IL-12 and are capable of modulating natural tumor immunosurveillance. They may be directly cytolytic against tumor cells or influence the activation of cytolytic NK cells and $CD8^+$ CTLs, and their antitumor activity critically depends on IFN-γ. Recognition of tumor-derived glycolipids presented by CD1d may contribute to the activation of CD1d-restricted T cells in the absence of exogenous stimulation. Reconstitution and/or pharmacological activation of CD1d-restricted T cells with αGalCer may have therapeutic potential for certain human cancers.

CD1d-RESTRICTED T CELLS IN AUTOIMMUNITY, TOLERANCE, AND ALLERGY

CD1d-restricted T cells help maintain tolerance to self-antigens and thereby prevent autoimmune disease. On the other hand, they also can mediate tissue damage and play a pathogenic role in autoimmunity.

Type 1 Diabetes

Early reports using $CD161^+CD3^+$ or other surrogate markers for NKT cells have suggested numerical and functional deficiencies of CD1d-restricted T cells in nonobese diabetic (NOD) mice, a mouse strain that spontaneously develops a form of autoimmune diabetes that resembles human type 1 diabetes (368–371). More recent studies using CD1d-tetramers have confirmed a deficiency of CD1d-restricted T cells (214, 372). The genetic control of $V\alpha14^+$ CD1d-restricted T cell numbers in NOD mice was recently mapped to genomic regions implicated in conferring susceptibility to type 1 diabetes and lupus on mouse chromosomes 1 and 2 (373). An additional locus that controls $V\alpha14^+$ CD1d-restricted T cell numbers in mice was recently identified on chromosome 18 (373a). CD1d-deficient NOD mice had earlier diabetes onset, greater disease penetrance, and more severe disease, suggesting that the deficiency of CD1d-restricted T cells is linked to the development of diabetes in NOD mice (374–376). In addition, the development of diabetes in NOD mice was ameliorated by interventions aimed at correcting the CD1d-restricted T cell deficiency such as adoptive transfer of CD1d-restricted T cells and transgenic expression of the invariant $V\alpha14J\alpha18$ TCR (369, 371, 377, 378). The functional defects observed in the remaining NKT cell population in NOD mice were a reduced ability to produce IL-4 (368) and a defect in both proliferation and differentiation towards an IFN-γ-secreting phenotype upon TCR engagement and IL-12 stimulation (378a). However, the functional defects of CD1d-restricted T cells in NOD mice warrant reexamination with more specific reagents for the characterization of these cells. Protection from diabetes conferred by CD1d-restricted T cells was associated with a T_H2 shift within pancreatic islets, and IL-4 was implicated as a key mediator of the immunoregulation induced by CD1d-restricted T cells (377, 379). However, the mechanism of action and the

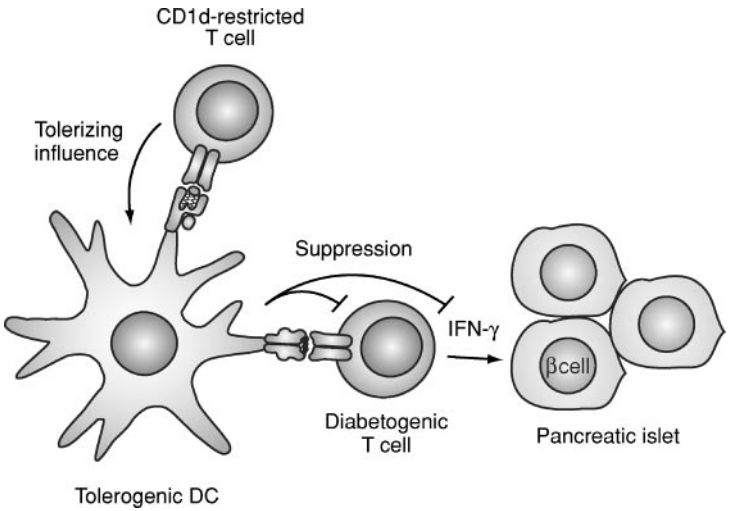

Figure 11 Model for the function of CD1d-restricted T cells in preventing type 1 diabetes. Activation of CD1d-restricted T cells leads to tolerizing DCs that suppress the autoantigen-specific T cell response, possibly by secretion of TGF-β or IL-10, thus preventing tissue damage. The mechanism of action and the target cells of IL-4 have not been determined.

target cells of IL-4 have not been determined. Interestingly, when naïve T cells expressing a transgenic TCR derived from a diabetogenic β cell-specific MHC class II–restricted T cell were stimulated with their auto-antigen in the presence of CD1d-restricted T cells, the initial activation of the β cell-specific T cells was not blocked, but both their production of IL-2 and IFN-γ and later proliferation were inhibited (380). The resulting β cell-specific T cells did not induce significant insulitis, and they were unable to destroy β cells. These findings suggest that CD1d-restricted T cells may prevent and ameliorate type 1 diabetes by preventing the differentiation of autoreactive T cells into effector cells (Figure 11). The inhibition of autoimmune inflammation by CD1d-restricted T cells may occur through the induction and/or recruitment of tolerogenic DCs (376) (Figure 11). In addition to a role in the natural course of type 1 diabetes, stimulation of CD1d-restricted T cells by administration of αGalCer in NOD mice prevented the onset and recurrence of diabetes and prolonged the survival of pancreatic islets transplanted into newly diabetic NOD mice (372, 374, 376, 381). αGalCer-induced protection from diabetes was associated with suppression of both T and B cell autoimmunity and IFN-γ production, and the generation of tolerogenic islet autoantigen-specific T cells with a protective cytokine production profile (372, 381). There are also reports of numeric and functional deficiencies of CD1d-restricted T cells in human type 1 diabetics (382, 383), but one study found no difference in αGalCer/CD1d tetramer$^+$ cells between diabetics and control donors (384), and another study

found evidence of increased numbers of Vα24$^+$/Vβ11$^+$ T cell in patients with type 1 diabetes (385).

Transplant Tolerance

CD1d-restricted T cells can contribute to transplant graft acceptance. Long-term survival of corneal allografts was impaired in Jα18-deficient compared with wild-type animals and correlated with reduced generation of allospecific T regulatory cells, whereas the initial rejection was comparable to that in wild-type animals (386). Induction of long-term acceptance of cardiac allografts induced by blockade of LFA-1/ICAM-1 or CD28/B7 interactions was dependent on Vα14Jα18$^+$ T cells (387), and immune tolerance to combined heart and bone marrow MHC-mismatched transplants after fractionated lymphoid irradiation failed to develop in CD1d-deficient animals (388). Vα14Jα18$^+$ T cells are also required for the acceptance of rat islet xenografts in mice treated with anti-CD4 antibody (389). Thus, CD1d-restricted T cells can function as immunosuppressive regulatory T cells that contribute to the induction of tolerance to transplant antigens.

Anterior Chamber-Associated Immune Deviation

Another example of a role for CD1d-restricted T cells in the induction of tolerance is the anterior chamber-associated immune deviation (ACAID), where systemic tolerance is induced to antigens introduced to the anterior chamber of the eye. Tolerance, as evidenced by a deficiency in the antigen-specific delayed-type hypersensitivity response at peripheral sites, was abrogated in CD1d-deficient mice (390). During tolerance induction, CD1d-restricted T cells were recruited to the spleen in a MIP-2-dependent manner, whereas the chemokine RANTES, secreted by CD1d-restricted T cells, was required for the recruitment of APCs and CD8$^+$ T cells to the spleen (260, 391, 392). IL-10, produced by CD1d-restricted T cells, was required for the induction of tolerance in ACAID, but the ultimate suppressor cell appears to be a non-CD1d-restricted antigen-specific CD8$^+$ T cell (393).

Experimental Autoimmune Encephalomyelitis

An important role for CD1d-restricted T cells has been shown in EAE, a mouse model for multiple sclerosis. SJL mice, which have the tendency to develop chronic EAE, show numerical deficiencies in CD161$^+$ T cells similar to those observed in NOD mice (394). During EAE, CD1d expression has been observed on microglia and on macrophages in the central nervous system (395). Transgenic expression of the Vα14Jα18$^+$ TCR in NOD mice, another mouse strain that is highly susceptible to EAE induction, protected mice from EAE and was associated with a striking inhibition of auto-antigen-specific IFN-γ secretion (396). This protection was independent of IL-4.

Studies of the EAE model using αGalCer administration confirmed the capacity of CD1d-restricted T cell activation to modulate disease. αGalCer administration can protect mice from EAE (397, 398), although this has not been observed

consistently (399). Activation of CD1d-restricted T cells by αGalCer in mice expressing a myelin-reactive transgenic TCR prevented EAE in an IL-4-dependent manner when αGalCer was administered prior to the activation of myelin-reactive T cells. However, when simultaneous activation of CD1d-reactive T cells occurred together with that of myelin-reactive T cells, αGalCer increased the severity of EAE due to an excessive T_H1 response (400). This suggests that the temporal relationship between the activation of CD1d-restricted T cells by αGalCer and disease-specific T cells is important in disease development. Manipulations that enhance the T_H2 bias of CD1d-restricted T cells strikingly reduce the development of EAE. For example, coadministration of blocking antibodies against CD86 together with αGalCer biased the αGalCer response to T_H2 and suppressed EAE (399). In addition, EAE was profoundly suppressed by the administration of a modified form of αGalCer, which lacks one alkyl chain, that selectively elicited IL-4 production by CD1d-restricted T cells (282). Thus, the ability of CD1d-restricted T cells that are activated by αGalCer to modulate the course of EAE in mice appears to depend on the balance of IL-4 versus IFN-γ secretion that is induced. In contrast, the natural protective effect of CD1d-restricted T cells during EAE seems to be mediated by inhibition of auto-antigen-specific IFN-γ secretion in an IL-4-independent manner.

Systemic Lupus Erythematosus

CD1d-reactive T cells expressing a Vα4.4$^+$/Vβ9$^+$ TCR were found to modulate a lupus-like syndrome in mice that was characterized by increased serum immunoglobulin concentrations, appearance of anti-double-stranded DNA antibodies, proteinuria, and immune complex glomerulonephritis, and that was dependent on IFN-γ (102). Transgenic T cells isolated from the spleen and those that were CD4$^+$ or CD8$^+$ from the bone marrow–induced disease. Interestingly, however, DN Vα4.4$^+$/Vβ9$^+$ TCR transgenic T cells from bone marrow dominantly suppressed the development of disease, suggesting that the microenvironment and/or coreceptor expression may influence the effector functions of autoreactive CD1d-restricted T cells. A CD1d-restricted T cell population with suppressor capabilities using diverse TCRs has also been isolated from human bone marrow (107). In addition, a pathogenic role for CD1d-restricted T cells in the development of systemic lupus erythematosus (SLE) is supported by experiments showing that administration of αGalCer to NZB/NZW mice, a strain that develops lupus spontaneously, exacerbated lupus disease activity as measured by earlier onset of proteinuria and increased mortality (401), by studies showing that transfer of activated Vα14$^+$ CD1d-restricted T cells in young NZB/NZW mice induced an autoimmune-like inflammation (402), and by studies showing that administration of anti-CD1d blocking antibodies ameliorated SLE and reduced the production of IgM and $IgG_{2a/2b}$anti-dsDNA antibodies in NZB/NZW mice (403, 401). Interestingly, CD1c-restricted autoreactive T cells from lupus patients stimulated antibody production by CD1c$^+$ B cells and induced isotype switching in vitro, suggesting that CD1-restricted T cells may also play a pathogenic role in the development of lupus in humans (406).

In contrast, MRL/lpr mice, another model for SLE, showed decreased numbers and functions of CD1d/αGalCer tetramer$^+$ T cells. Administration of αGalCer resulted in the expansion of CD1d/αGalCer tetramer$^+$ T cells and alleviated the inflammatory dermatitis (303b). CD1d deficiency exacerbated lupus nephritis and increased anti-dsDNA antibodies induced by the hydrocarbon oil pristane (405). These studies suggest that CD1d-restricted T cells can also play a protective role in the development of lupus. However, CD1d-deficient MRL/lpr mice did not develop lupus skin disease or nephritis differently from wild-type MRL/lpr mice (404).

Colitis and Hepatitis

Other examples showing the capability of CD1d-restricted T cells to either induce or suppress the development of inflammatory conditions come from studies of murine models of intestinal inflammation. In a model using oxazolone to induce colitis by intrarectal administration subsequent to skin sensitization, IL-13-producing CD1d-restricted T cells were required for the development of colitis (407). In contrast, in a murine model of colitis induced by dextran sodium sulfate, activation of CD1d-restricted T cells or adoptive transfer of αGalCer-activated CD1d-restricted T cells resulted in reduced colitis, indicating that CD1d-restricted T cells can also downregulate intestinal inflammation (408). TCRα-deficient mice develop spontaneous intestinal inflammation, a process that appears to be suppressed by B cells. B cells in mesenteric lymph nodes of TCRα-deficient mice showed strikingly increased CD1d-expression and CD1d/TCRα-double-deficient mice developed more severe colonic inflammation at later time points, suggesting that CD1d expression on B cells may be important for their regulatory function in this model (409). However, whether CD1d-restricted T cells are required for the downregulation of intestinal inflammation induced by these regulatory B cells is not known.

Autoimmune hepatitis induced by administration of concavalin A to mice depended on the presence of CD1d-restriced T cells (410). The cytotoxicity of CD1d-restricted T cells against hepatocytes induced by concavalin A required perforin and FasL expression on the activated T cells and could be augmented by an IL-4-dependent autocrine mechanism (411).

Allergic and Hypersensitivity Reactions

The ability to promptly produce large amounts of IL-4 upon primary stimulation and the ability to influence IgE production induced by anti-IgD antibodies in mice suggested that CD161$^+$ T cells may be important in allergic reactions (412). However, CD1d-restricted T cells were not required for IgE production in vivo after stimulation with anti-IgD mAbs (203, 413, 414), or in response to immunizations with ovalbumin or *Nippostrongylus brasiliensis* infection (415, 416), but appeared to be required, together with conventional CD4$^+$ T cells, for IL-18-induced IgE antibody production (280). Indeed, CD1d-deficient and Jα18-deficient mice displayed defects in the development of allergen-induced airway

hyperreactivity (AHR) (417, 418). However, the extent to which CD1d-restricted T cells influence airway eosinophilia and the development of allergen-specific IgE antibodies in this model remains controversial (417–419). Contact sensitivity is a T cell–dependent immune response to skin sensitization with reactive haptens that appears to require IgM antibodies produced by B-1 B cells to initiate T cell recruitment (419a). Contact sensitivity was defective in Jα18-deficient mice, and adoptive transfer experiments showed that it was dependent on IL-4 produced by Vα14$^+$ CD1d-restricted T cells (419b), showing that CD1d-restricted T cells are required for B cell activation and IgM antibody production in this form of hypersensitivity reaction.

SUMMARY

CD1 antigen presentation provides a pathway of T cell stimulation independent of MHC class I and II. Antigen presentation by CD1 molecules differs from that by MHC molecules by having distinct requirements for intracellular trafficking, processing, and loading of lipid antigens.

CD1-reactive T cells are found among the CD4, CD8, and DN T cell subsets previously assumed to be MHC-restricted. Among CD1-reactive T cells, several clearly different subpopulations exist, based on the CD1 isoform that they recognize, the diversity of their TCRs, and their effector functions. CD1a-, b-, and c-restricted T cells that recognize foreign microbial lipids appear to behave much like MHC-restricted T cells that recognize microbial protein antigens. They use clonally specific TCRs and may contribute to memory responses. In contrast, self-lipid reactive CD1-restricted T cells affect immune responses by mechanisms that lie outside the paradigm of foreign antigen-specific MHC-restricted adaptive immunity. The self-reactivity of CD1-restricted T cells may be regulated by the strength of the TCR signal received and by the microenvironment of cytokines and costimulation evoked by microbial interactions with APCs. In a variety of circumstances, early activation of CD1-restricted T cells occurs in advance of the MHC-restricted adaptive immune response. The rapid response of some CD1-restricted T cells (TCRα-invariant CD1d-restricted T cells, and $\gamma\delta$ T cells) may be activated at the same time as cells of the innate phase of the immune response. CD1-reactive T cells can stimulate DC differentiation and instruct DC maturation toward IL-12-producing inflammatory DCs, and they can potently activate NK cells, other T cells, and B cells to amplify the innate immune response and influence the subsequent adaptive immune response to infection. CD1-reactive T cells can mediate tumor rejection via IL-12 production, NK and T cell activation, or by direct cytolysis. Alternatively, they may help to suppress tumor rejection, prevent autoimmunity and generate tolerance. The mechanisms responsible for the tolerogenic actions of CD1-restricted T cells involve T_H2 cytokine secretion and CD1-dependent effects on other regulatory cells. Thus, divergent patterns of CD1-reactive T cells resulting in inflammatory and tolerogenic responses can be

understood on the basis of their potential to produce IFN-γ and to stimulate IL-12 production, or alternatively, to produce T_H2 cytokines and to generate tolerogenic DCs. Studies using αGalCer to activate CD1d-restricted T cells pharmacologically show their therapeutic potential in microbial immunity, antitumor immunity, and autoimmunity. Thus, CD1-reactive T cells participate in the full range of T cell responses previously thought to be mediated only by peptide-specific MHC-restricted T cells.

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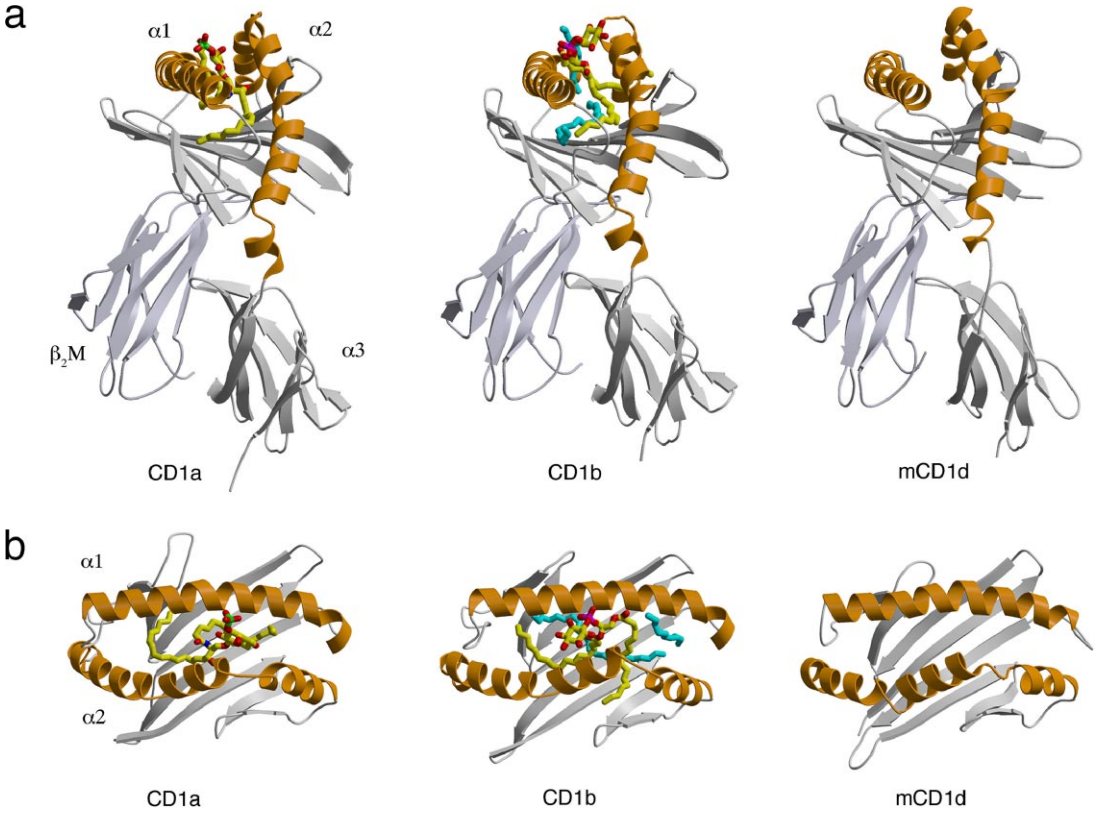

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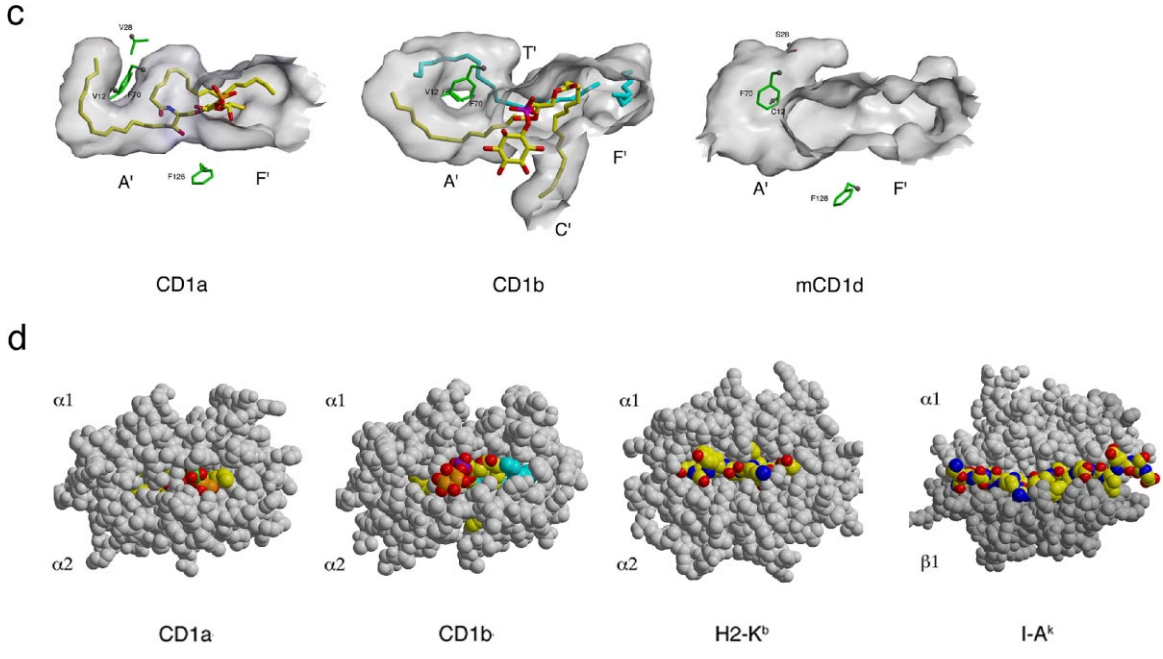

Figure 3 CD1 atomic structures. (*a*) Front and (*b*) top view of the CD1a-sulfatide and CD1b-phosphatidylinositol (PI) complexes and the mouse CD1d (mCD1d) structure without ligand. α1-α3 domains and β_2-microglobulin (β_2M) are shown in light gray ribbons, α1 and α2 helices in brown; yellow, carbon atoms of the ligands; red, oxygen; green, sulfur; blue, nitrogen; purple, phosphate; and cyan, detergent molecules. (*c*) Comparison of the antigen-binding grooves of CD1a, CD1b, and mCD1d. Molecular surfaces are shown as transparent binding pockets with or without bound ligand from a view directly into the groove, similar to (*b*). The A′ and F′ pockets, the C′ channel, and the T′ tunnel are labeled accordingly. Important protein residues are shown in green. (*d*) Space filling representations of the view as shown in (*b*) for CD1a and CD1b with bound ligand in comparison to MHC class I (H-2K^b) and MHC class II (I-A^k) with bound peptide. Atoms are shown as spheres. Gray, protein residues. Figures were prepared using Molscript, GRASP, and Raster3D software with the coordinates of CD1a (51) (1ONQ), CD1b (50) (1GZQ), mCD1d (49) (1CD1), H-2K^b (420) (2CKB), and I-A^k (421) (1D9K).

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