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THE CD1 SYSTEM: Antigen-Presenting Molecules for T Cell Recognition of Lipids and Glycolipids

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ABSTRACT

Recent studies have identified the CD1 family of proteins as novel antigen-presenting molecules encoded by genes located outside of the major histocompatibility complex. CD1 proteins are conserved in all mammalian species so far examined and are prominently expressed on cells involved in antigen presentation, which suggests a role in activation of cell-mediated immunity. This has now been confirmed by functional studies demonstrating the ability of CD1 proteins to restrict the antigen-specific responses of T cells in humans and mice. Identification of naturally occurring antigens presented by CD1 has revealed the surprising finding that these are predominantly a variety of foreign lipids and glycolipids, including several found prominently in the cell walls and membranes of pathogenic mycobacteria. Structural, biochemical, and biophysical studies support the view that CD1 proteins bind the hydrophobic alkyl portions of these antigens directly and position the polar or hydrophilic head groups of bound lipids and glycolipids for highly specific interactions with T cell antigen receptors. Presentation of antigens by CD1 proteins requires uptake and intracellular processing by antigen presenting cells, and evidence exists for cellular pathways leading to the presentation of both exogenous and endogenous lipid antigens. T cells recognizing antigens presented by CD1 have a range of functional activities that suggest they

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are likely to mediate an important component of antimicrobial immunity and may also contribute to autoimmunity and host responses against neoplastic cells.

INTRODUCTION

T cells occupy a central position in the generation of cell-mediated immune responses and are of key importance to the maintenance of immunological memory. The appreciation that T cells recognize peptide fragments of protein antigens bound to class I or class II antigen-presenting molecules encoded by the major histocompatibility complex (MHC) has been one of the key discoveries leading to the development of our current understanding of the cell-mediated immune response (1, 2). In fact, this paradigm has become so dominant that it is now difficult to conceive that T cell recognition of anything other than MHC/peptide complexes could play a significant role in specific cell-mediated immune responses. Nevertheless, it is now clear that other mechanisms exist by which specific T cell recognition of antigens that are chemically distinct from peptides may occur. This had long been suggested by a variety of incompletely characterized models of T cell responses to infectious agents (3–11) and has, in recent years, acquired a more solid foundation from detailed studies of human TCR $\gamma\delta$ + T cells that recognize small nonpeptide molecules of various microbes (12, 13).

Perhaps the most clearly established paradigm of nonpeptide antigen recognition by T cells has come from studies of the CD1 system of MHC class I-like proteins. These evolutionarily conserved proteins are now recognized as comprising a family of antigen-presenting molecules with unusually hydrophobic ligand binding grooves that are capable of presenting nonpeptide lipid and glycolipid antigens to T cells. These findings have enlarged the paradigm for generation of specific cell-mediated responses through T cell recognition of a broad and ubiquitous class of molecules that were not previously known to be T cell antigens. Here we review (*a*) the recent developments in this emerging field that have begun to clarify the molecular and cellular mechanisms enabling the presentation of lipid antigens by CD1 proteins and (*b*) their subsequent effects on the T cell response.

THE CD1 SYSTEM OF MHC-RELATED PROTEINS

Historical Perspective: Discovery of CD1 Proteins and Genes

The discovery of CD1 and its designation as the first cluster of differentiation (CD) has a special significance in the history of immunology research. The CD nomenclature, introduced at the First International Workshop on Human

Leukocyte Differentiation Antigens, groups or clusters monoclonal antibodies with similar reactivity, as judged by immunofluorescence and other techniques (14). One of the first monoclonal antibodies made against a human cell-surface antigen, shortly after the introduction of the hybridoma technique by Milstein and colleagues, was specific for the protein that is now known as CD1a (15). This antibody initially defined the first cluster of differentiation markers, thus marking the start of the continuing effort to systematically define and classify the differentiation antigens of human leukocytes. Subsequently, isolation of additional monoclonal antibodies subdivided the CD1 cluster into a group of distinct serologic and biochemical entities, indicating that human CD1 was actually a family of related proteins (16–18). With the pioneering molecular studies also from the laboratory of Milstein that defined the genes for these proteins, it was definitively shown that CD1 was in fact a family of related proteins encoded by separate closely linked genes (19–21). These studies, along with early biochemical analyses, revealed the MHC-related structure and tissue distribution of CD1 proteins and pointed the way to cellular studies that eventually revealed their function as a novel class of antigen-presenting molecules for T cell responses.

Genomic Organization and Evolutionary Diversification of CD1

The human CD1 family is encoded by five nonpolymorphic and closely linked genes located on chromosome 1 and are, therefore, unlinked to the MHC on chromosome 6 (22, 23). These show an intron/exon structure similar to MHC class I genes (21) and encode polypeptides with significant homology to both MHC class I and II proteins (19, 20, 24). Of the five CD1 genes in the human genome, four (the *CD1A*, *-B*, *-C*, and *-D* genes) are known to be expressed as proteins (21, 25, 26). These proteins represent distinct CD1 isoforms, which in humans are designated CD1a, -b, -c, and -d. The fifth human CD1 gene, designated *CD1E*, lacks obvious pseudogene features and has been shown to be transcribed (27), but no protein product (i.e. a CD1e protein) has yet been identified. CD1 genes and proteins have been studied in several other mammalian species besides humans, including ungulates [cows and sheep (28–35)], lagomorphs [rabbits and guinea pigs (36, 37; K LeClair, personal communication)], and rodents [mice and rats (38–43)]. In all mammals so far examined, CD1 genes and proteins have been found, although the differences in the size and complexity of the CD1 families of different mammals are striking (Table 1).

A separation of the known CD1 genes and proteins into two groups, now generally referred to as group I and group II CD1, was first proposed by Calabi and colleagues based mainly on homology of nucleotide and amino acid sequences (44). As described in subsequent sections of this review, this division of CD1 molecules into groups I and II now also appears to be supported by

Table 1 Size and complexity of CD1 gene families in various mammals

Species	Total CD1 genes[a]	Number of genes for indicated isoform				
		CD1A	*CD1B*	*CD1C*	*CD1D*	*CD1E*
Human	5	1	1	1	1	1
Mouse	2	0	0	0	2	0
Rat	1	0	0	0	1	0
Guinea pig	~10	?[b]	≥5	≥3	?	≥1
Rabbit	~8	?	≥1	?	≥1	?
Sheep	~7	?	≥3	?	?	?

[a]The number of genes per haploid genome is shown. This has been estimated based on genomic Southern blotting for rabbit (36), sheep (35), and guinea pig (K LeClair, personal communication).

[b]?, Indicates that there is insufficient data to determine the presence or absence of the indicated isoform.

a variety of studies on the expression and function of the two groups of CD1 proteins. Group I CD1 includes the products of the human *CD1A*, *-B*, and *-C* genes and their homologues in other mammals. These are the classic CD1 antigens first identified as differentiation antigens that are expressed on immature cortical thymocytes and subsequently shut off during the process of T cell maturation (45–49). Group II CD1 is currently defined as the products of the human *CD1D* gene and its close homologues in other species. These include all the CD1 proteins expressed in mice and rats and also one of the two forms of rabbit CD1 identified by cDNA cloning.

A comparison of the CD1 loci in humans and mice reveals the marked difference in the CD1 families of these species (Figure 1), which is an important point given the central role that the mouse currently plays as an animal model in immunology research. The available data indicate that the genomes of all strains of mice examined lack group I CD1 genes, although they have maintained at least two group II CD1 genes (38, 50, 51). The two CD1 genes in the mouse genome are extremely similar to each other (approximately 90–95% sequence identity in all domains) and clearly represent a relatively recent duplication event. The gene referred to as *CD1D1* (or *MCD1.1*) is now established to be expressed and functional (52, 53). In contrast, current data suggest that in at least one strain of mouse (C57BL/6), a frame shift mutation extinguishes expression of *CD1D2* (54), and it remains unclear whether this gene gives rise to a functional protein in other strains.

The finding that group I CD1 proteins are absent from mice and rats, whereas group II is present in these and probably most or all other mammals, raises questions about the different functions and relative importance of the two groups. It is currently unknown whether group I CD1 genes were once present and then

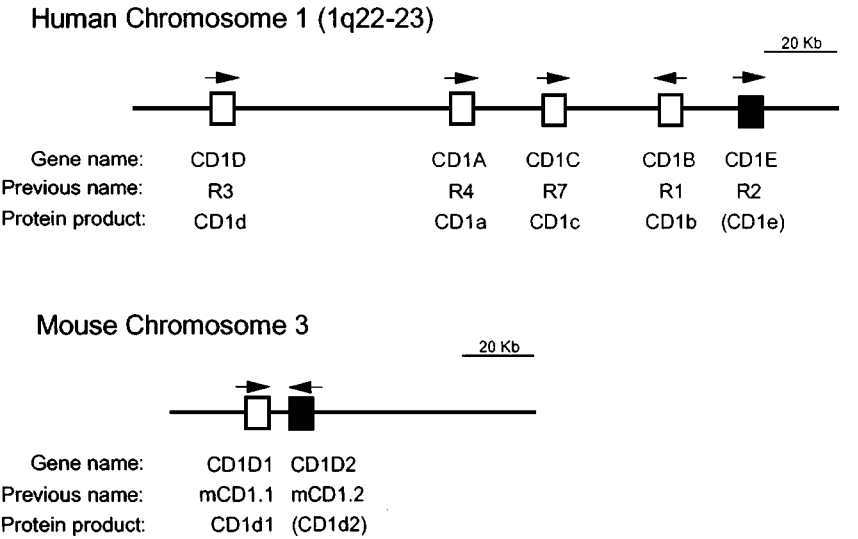

Figure 1 Genomic maps of the human and mouse CD1 loci. (*Open boxes*) Genes for which the protein product is known to be expressed in vivo; (*shaded boxes*) genes that are known to be transcriptionally active but not yet established to give rise to a protein product in vivo; (*arrows*) the direction of transcription. Previous names for genes refer to the original nomenclature of Milstein and colleagues (see text for references). (Reprinted with permission from Reference 57.)

deleted from the forerunners of modern mice and rats, or whether group II CD1 is the precursor of the group I genes and failed to diversify in rodents. However, the presence of both groups in rabbits, which are believed to be closely related to rodents in terms of evolutionary origin (55), strongly suggests that the former explanation is more likely. Furthermore, the finding that the murine CD1 genes are located near the boundary point of an area of chromosomal synteny between mice and humans, presumably the breakpoint of an ancient translocation event, provides circumstantial evidence that previously present group I CD1 genes may have been deleted in rodents (56). In either case, at least two explanations could account for the finding that the absence of group I CD1 genes is tolerated by mice and rats. One possiblilty is that the environmental factors (presumably specific pathogens) that are responsible for selecting the maintenance or development of group I CD1 are not relevant to mice and rats. Alternatively, the function of group I CD1 may have been replaced or compensated for by other mechanisms that have evolved in rodents. It is important to note that the absence of group I CD1 in mice means that in many cases it may be difficult to make direct extrapolations from this animal to humans. This ultimately may mandate the use of other animals (e.g. guinea pigs or nonhuman primates) in

order to acquire a broader and more accurate understanding of the role of the CD1 system in human immune responses.

Evolutionary Relation of CD1 to the MHC

The presence of at least one CD1 gene in all mammalian species studied to date indicates that this family is evolutionarily ancient and must predate the extensive radiation of mammalian species that is predicted to have occurred approximately 60–80 million years ago (Figure 2). Also supporting the view that CD1 is an ancient lineage of antigen-presenting molecules is the observation that CD1 proteins show comparable levels of similarity and divergence at the amino acid and nucleic acid sequence level to both MHC class I and class II proteins (20, 57). This similar level of homology to both families of MHC proteins implies that CD1 may have diverged from a primordial ancestral antigen-presenting molecule at a point close to the divergence of the precursors of modern MHC class I and class II molecules. Because both MHC class I and class II genes are known to exist in species as ancient as cartilaginous fish (58–60), one might expect to find CD1 genes also present in most vertebrate species. This possibility remains untested, as no studies establishing either the

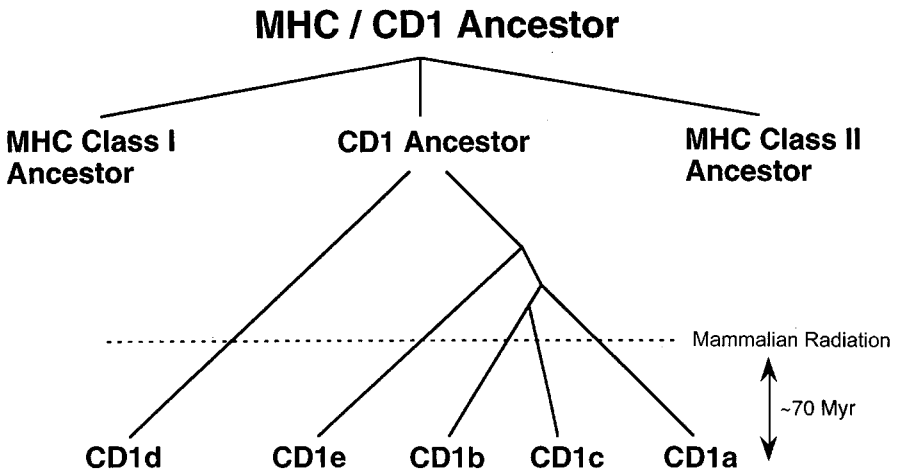

Figure 2 Hypothetical evolutionary tree for CD1 and major histocompatibility complex (MHC) class I and class II genes, based on nucleotide and amino acid sequence homologies. CD1 is proposed to have diverged from the common ancestral gene at a distant point in time close to the point at which the separate MHC class I and II lineages diverged. The presence of the same CD1 isoforms in a variety of mammals indicates that the subsequent diversification of CD1 into distinct isoforms (i.e. CD1a, -b, -c, -d, and -e) must have occurred prior to the extensive radiation of mammalian species that occurred between 60 and 80 million years (Myr) ago. (Adapted from Reference 149.)

presence or absence of CD1 genes or proteins in vertebrate species more ancient than mammals have been reported. Another feature that bears emphasis is the striking lack of polymorphism among CD1 heavy chains in different outbred individuals (61, 62), which suggests that CD1 proteins are not subject to the same evolutionary forces responsible for the extensive allelic polymorphism of classical MHC class I and II loci.

The homology of CD1 proteins to MHC class I heavy chains, although significant, is not extremely high (57, 63). Both CD1 and MHC class I proteins have three extracellular domains of similar size, designated $\alpha 1$, $\alpha 2$, and $\alpha 3$. In the most membrane distal $\alpha 1$ domains, only very minimal if any homology can be detected between CD1 and MHC class I at the amino acid or nucleic acid sequence level. In the $\alpha 2$ and $\alpha 3$ domains, the homology with the corresponding MHC class I domains rises to as much as approximately 35% at the amino acid sequence level, depending on the particular CD1 and MHC class I molecules that are compared. This is far less than the homology between the products of different MHC class I loci (e.g. HLA-A, B, or C and nonclassical MHC class I molecules such as HLA-E, F, and G), which are typically 70% homologous or more (64, 65). In contrast, this is much closer to the level of similarity observed between MHC class I and the intestinal immunoglobulin receptor FcRn, a protein encoded outside of the MHC that is known to have an MHC class I–like three-dimensional structure (66).

Another important point to consider is the relationship between the different CD1 isoforms (i.e. CD1a, -b,-c, -d, and -e). It has been consistently found that in comparing the sequences of CD1 genes and proteins of nonhuman species with those of human CD1 family members, it is possible to identify each nonhuman sequence as a clear homologue of one of the human isoforms (57). For example, CD1 sequences have been identified in several species (sheep, guinea pig, rabbit) that are clearly direct homologues of human CD1b (35, 36). This is evident from the observation that these sequences of nonhuman species are more closely related to human CD1b than the latter is to other human CD1 isoforms (i.e. CD1a, -c, -d, or -e). This evolutionary preservation of distinct isoforms of CD1 is very different from what is generally observed for MHC-encoded molecules, for which distinct interspecies homologues of individual MHC class I and II loci can not readily be identified. This feature of CD1 suggests that the different CD1 isoforms may have evolved specialized functions early in the course of mammalian evolution, thus resulting in strong selection for the preservation of their structure during the subsequent divergence of different species.

CD1 Protein Structure

CD1 genes encode polypeptides with a predicted molecular mass of approximately 33,000, although the presence of three or more N-linked glycans

generally raises their observed mass into a range between 41,000 and 55,000 (16, 26, 62, 67–69). All CD1 proteins studied to date are expressed on the surface of cells as type I transmembrane proteins that associate noncovalently with β2-microglobulin (β2-m). In most cases, this association appears to be necessary for efficient folding and surface expression of the CD1 protein (70, 71), although some possible exceptions to this rule have been noted (72–74). By analogy with MHC class I, the polypeptides encoded by CD1 genes are typically referred to as CD1 heavy chains.

Given the limited but significant homology between CD1 and MHC class I heavy chains, as well as the similar overall domain organization and β2-m association, it was predicted that CD1 proteins could adopt a folded structure similar to that of MHC class I proteins (44, 57, 75). This prediction was recently shown to be correct with the solution of the three-dimensional structure of mouse CD1d1 by X-ray crystallography (76), which revealed a remarkable similarity in overall shape to MHC class I proteins (Figure 3—See color section, p. C-0, at back of book). The membrane distal α1 and α2 domains of CD1d1 were found to adopt the typical antigen-binding superdomain structure found in all MHC class I and II molecules, consisting of two antiparallel stretches of α-helices overlying a floor of β-pleated sheet. As in all MHC class I structures, the two membrane distal domains of the heavy chain are supported by an immunoglobulin-like α3 domain and its associated β2-m subunit.

The most unique and potentially revealing aspect of the CD1d1 structure relates to its putative ligand binding groove. Similar to both classes of MHC-encoded antigen-presenting molecules, this consists of an opening between the two rows of α-helices that descends into a cavity within the core of this portion of the protein. However, in CD1d1 this cavity is much deeper than the peptide binding grooves of MHC class I or II. Instead of having a series of six to nine small pockets to accommodate individual amino acid side chains, as in MHC molecules, the CD1 groove is more accurately described as having just two large pockets, designated A′ and F′. Most notably, the interior of the CD1 groove is formed mainly by hydrophobic amino acid residues. This creates a surface that is almost entirely of neutral electrostatic potential and has little or no potential for hydrogen bonding or other polar interactions. The groove in CD1d1 appears to be closed at either end and is covered over much of its length, such that it may be accessible only through a narrow entrance extending from the center of the groove to the center of the F′ pocket. These features strongly suggest that the groove of CD1d1 would not be likely to interact with its ligands in the same way that peptide binding to MHC molecules is known to occur, and they also indicate that CD1d1 would most likely bind very hydrophobic ligands. Structures of other CD1 proteins are not yet available, but molecular modeling of some of these (e.g. human CD1b and -c) suggest features similar to

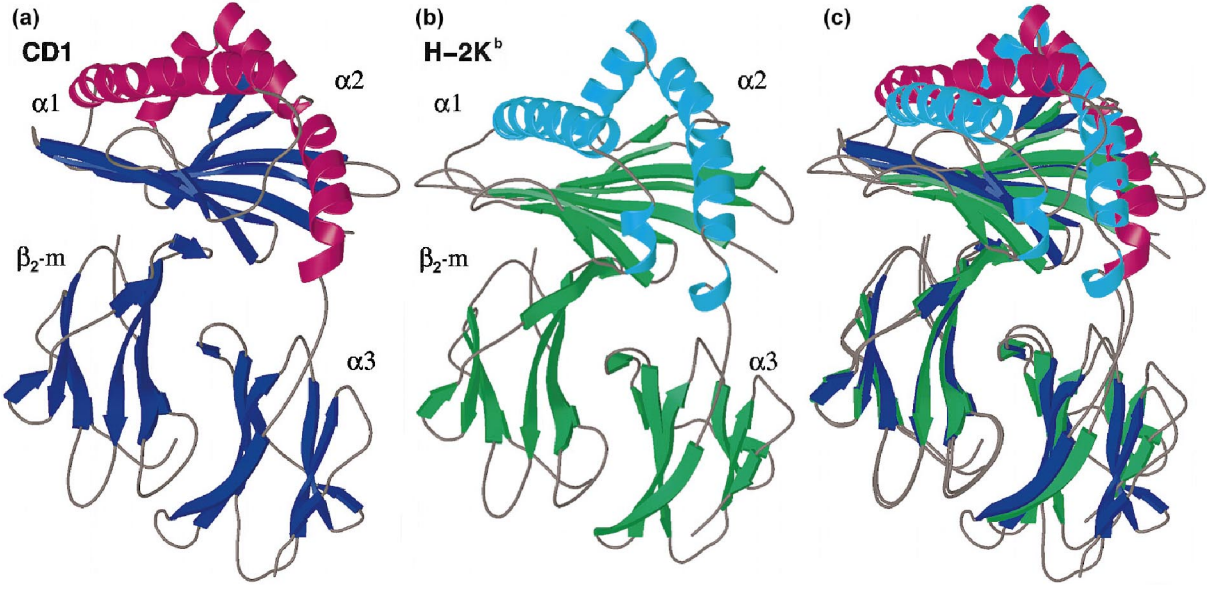

Figure 3 Comparison of the crystal structures of CD1 and MHC class I. (*Left*) Backbone ribbon diagram of mouse CD1d1: (*red*) α-helices; (*blue*) β-strands; and (*brown*) loops. (*Center*) Ribbon diagram of the mouse MHC class I molecule H-2K^b: (*cyan*) α-helices; (*green*) β-strands; and (brown) loops. (*Right*) Superposition using alignment of β2m-domains highlights some of the differences between CD1d1 and H-2K^b. Note in particular the shifting of the α-helices in the α1 and α2 domains. This produces a deeper and more voluminous groove in CD1d1, which is narrower at its entrance compared with H-2K^b. The color scheme is the same as in the *left* and *center panels*. (Reprinted with permission from Reference **?**.)

those observed for murine CD1d1 (M Degano, B Segelke, IA Wilson, personal communication).

Cellular Expression and Tissue Distribution of CD1 Proteins

The expression of CD1 proteins has been extensively studied in humans and rodents. The group I CD1 proteins in humans were first identified as differentiation markers expressed on immature cortical thymocytes. It is now well known that these proteins are also expressed on a variety of specialized antigen-presenting cells, especially dendritic cells present in both lymphoid and nonlymphoid tissues (57, 77). Group I CD1 proteins are also inducible in vitro on circulating human monocytes by exposure to granulocyte macrophage–colony stimulating factor (GM-CSF) (78, 79), which suggests that they might be up-regulated on tissue macrophages in many inflammatory lesions. A subset of human B lymphocytes also expresses the CD1c protein (80). This appears to be developmentally regulated and is much more prominent on circulating B cells of infants than adults (81, 82). In addition, CD1c expression has been shown to be up-regulated on marginal zone B cells of lymphoid follicles (83).

Information concerning the regulation and tissue expression of group II CD1 proteins (i.e. human CD1d and its homologues) indicates both similarities and differences compared with the human group I CD1 proteins. Several studies of human CD1d have found expression of these proteins by normal human gastrointestinal epithelia (25, 72, 84). In contrast, group I CD1 proteins appear not to be expressed at this site. The putative CD1d protein expressed by gut epithelia appears unusual in that a large fraction of it lacks glycosylation and appears to be expressed on the cell surface mainly without associated β2-m (72). One study in mice (85) and one in rats (86) also reported group II CD1 expression by the intestinal epithelium, but this has not been consistently found (73). The reason for the discrepancies remains unclear but could relate to the use of different monoclonal antibody reagents for detection.

It has also been shown that group II CD1 proteins are widely expressed on hematopoietic cells in both humans and mice (73, 87; M Exley, SA Porcelli, SP Balk, unpublished data). In both species, expression has been detected at high levels on a majority of thymocytes. The expression of group II CD1 (CD1d1) appears to be down-regulated during the thymic maturation process, but in mice this is not complete and substantial residual expression can still be detected on most mature mouse T cells (73, 87). Mouse CD1d1 is also expressed constitutively by most B cells and is particularly up-regulated on a population of splenic marginal zone B cells (87). Human CD1d has also been detected on a subset of circulating T and B lymphocytes and resting monocytes, although generally at rather low levels (M Exley, SA Porcelli, SP Balk, unpublished data).

Although studies in this area are ongoing, the overall impression at this point is that expression of group II CD1 is more strongly constitutive in mice than in humans, in which it may be regulated by inducible factors. Interestingly, CD1d is not up-regulated by GM-CSF treatment of human monocytes in vitro under the same conditions that strongly up-regulate group I CD1 expression (SA Porcelli, unpublished data), and cytokines or other factors that regulate the expression of group II CD1 proteins have not yet been discovered. These differences in cellular expression and regulation of groups I and II CD1 further support the classification of the CD1 proteins into these two categories. Expression of group II CD1 by dendritic cells has been less extensively studied than group I CD1 expression on this cell type. However, mouse splenic dendritic cells constitutively express CD1d1 (87), and preliminary studies indicate that isolated human epidermal dendritic cells (i.e. Langerhans cells) are uniformly positive at a low to moderate level for CD1d (M Sugita, SA Porcelli, unpublished data).

CD1 AND T CELL RECOGNITION

The discovery that human T cells recognize CD1 and mediate typical cell-mediated immune functions constitutes the first solid evidence that CD1 gene products play an immunological role. The MHC class I–like structure of CD1 proteins and their prominent expression on antigen-presenting cells provided the initial stimulus to investigate the role of these molecules in T cell activation and antigen presentation. The first direct evidence implicating CD1 in T cell function was provided by human circulating $CD4^{-}CD8^{-}$ T cell clones expressing either $\alpha\beta$ or $\gamma\delta$ TCRs that lysed tumor cells expressing specific isoforms of human CD1 (i.e. CD1a or CD1c) (88). This finding was subsequently confirmed for circulating $\gamma\delta$ T cells (89), and similar findings were also reported for human intestinal intraepithelial lymphocyte lines (90). Several years after these initial findings in the human system were reported, studies of mice also demonstrated CD1-reactive T cells, both in the residual $CD4^{+}$ population of MHC class II–deficient mice (74) and in the $NK1^{+}$ T cell fraction of normal mice (52). Interestingly, all the CD1-reactive T cells demonstrated in these initial studies were responsive in the absence of any deliberately added foreign antigen, which suggests that such responses represent a form of T cell autoreactivity that is inherent in the normal lymphocyte pool.

A second significant step was made with the derivation of human T cell lines that responded to *Mycobacterium tuberculosis* antigens in a CD1b-restricted fashion (78). The antigen-specific responses of these T cells were absolutely dependent on CD1b expression and could be demonstrated to be independent of MHC class I and II expression by the antigen-presenting cells. The existence of CD1b-restricted T cells specific for mycobacteria was subsequently

confirmed in other studies (91–93) and extended to show similar T cell responses restricted by CD1c (94) and CD1a (JP Rosat, MB Brenner, personal communication). Although the first examples of CD1-restricted T cells were phenotypically $CD4^-8^-$ or double negative (DN) T cells, it is now apparent that CD1 recognition or restriction is more broadly distributed among T cell subsets. In particular, CD1-restricted T cells specific for mycobacterial antigens within the $CD8^+$ subset have been clearly demonstrated (95; JP Rosat, MB Brenner, personal communication). These T cells express the CD8 $\alpha\beta$ heterodimers classically found on MHC class I–restricted cytolytic T lymphocytes (CTL). It is currently unclear what role if any the CD8 molecule plays in human CD1-restricted T cell recognition of antigen. However, several studies indicate that mouse CD1 interacts with CD8 molecules (115, 152, 153). Recent studies suggest the existence of human $CD4^+$ T cells responsive to mycobacterial antigens presented by CD1 proteins (P Sieling, RL Modlin, unpublished data), and many mouse T cells reactive with CD1 are also $CD4^+$ (74, 96). Thus, the CD1 family may be involved in the function of T cells found within all the major phenotypic subsets as currently defined.

The recognition of CD1 or CD1-restricted microbial antigens by T cells shows all of the hallmarks of immune recognition mediated by antigen-specific, clonotypic TCRs, and this has now been definitively established by TCR gene transfer studies. Thus, the transfection of cloned TCRα and TCRβ cDNAs isolated from CD1-restricted, mycobacteria-specific T cell lines into Jurkat cells conferred both CD1 restriction and antigen specificity on the resulting transfectants (E Grant, MB Brenner, personal communication). In limited studies carried out to date, the TCRs of human mycobacteria-specific, CD1-restricted T cells have been found to be formed from a variety of different germline V and J segments, and to encode substantial junctional diversity (E Grant, MB Brenner, personal communication). Thus, antigen recognition through this pathway, as for the MHC-dependent pathways, involves a range of clonally diverse receptors and may, therefore, mediate recognition of a wide array of potential foreign antigens.

CD1-Restricted NK T Cells

Most knowledge about the function of CD1 in mice has come from studies of a unique subset of T cells often referred to as NK T cells, so called because of their expression of cell-surface proteins previously associated mainly with the natural killer (NK) cell lineage (96, 97). Several lines of investigation over the past 10 years have revealed a range of unusual properties for this specialized population of T cells in mice. Perhaps foremost among these is their expression of an invariant TCRα chain (Vα14-Jα281 with no N region additions or deletions) and limited TCRβ chain repertoire (98), thus endowing them with an antigen

receptor repertoire of limited diversity. In addition, these T cells are unusual in their ability to secrete large amounts of interleukin-4 (IL-4) and other cytokines rapidly upon TCR engagement (99, 100). The development of mouse NK T cells has been shown to be dependent on expression of CD1d1 because these cells fail to develop in mice that have had either the *CD1D1* gene (53) or both mouse CD1 genes (101, 102) inactivated by targeted gene disruption. Consistent with this finding, isolated mouse NK T cells and NK T cell hybridomas appear to be universally reactive to mouse CD1 proteins in studies carried out in vitro (52).

The functions of NK T cells in the immune response remain unclear, but many intriguing observations have been made that suggest they are an important component of the immune system. Numerically, they represent a major fraction of the T cell compartment, accounting for 20–30% of the T cells in the liver and bone marrow and up to 1% of splenocytes, amounting to a total number of about one million T cells in each of these tissues in mice (96). Given that these cells have limited TCR variability and therefore are likely to be responding to a narrow spectrum of ligands, a population of this size is likely to give rise to substantial responses. The rapid production of IL-4 by NK T cells has implicated them in the early programming of immune responses, in some cases leading to outcomes associated with Th2 responses that are important for antibody production and immunity to extracellular parasites (103). However, in other cases their activation has been associated with outcomes more typical of a Th1-type inflammatory response (104). Other lines of investigation have recently shown a profound role of mouse NK T cells in IL-12–induced tumor rejection (105) and also a potential role in regulating autoimmunity (106, 154).

Also consistent with the hypothesis that NK T cells perform a critical role in immunity is the marked evolutionary conservation of this subset between mice and humans. Thus, recent studies have identified human T cells expressing an invariant Vα24-JαQ TCRα chain highly homologous to that expressed by murine NK T cells and coexpressing a variety of NK cell-associated markers (107–109). These human NK T cells also produce high levels of both IL-4 and interferon-γ upon activation and are almost universally reactive with the human CD1d protein expressed in various cell types by transfection (107). As in mice, their function remains unknown, although recent studies have linked reductions in their numbers and alterations in their cytokine secretion patterns to progression of human autoimmune disorders (110, 111).

The issue of how the NK T cell populations in humans and mice relate to other CD1-restricted T cell subsets, such as those reactive with mycobacterial antigens, remains to be resolved. The relatively fixed TCR structure of NK T cells stands in marked distinction to the diverse TCR repertoire observed for CD1-restricted mycobacteria-specific T cells in humans. Moreover, emerging

data suggest that even in the murine system, CD1 restriction extends to populations of T cells that do not express the canonical Vα14-Jα281 invariant TCRα chain used by most or all NK T cells (54, 74; SM Behar, MB Brenner, personal communication). This finding, along with the extensive data now available from studies of human CD1-restricted T cells, strongly suggests that development and activation of the NK T cell system is likely to be only one aspect of the function of CD1 in the murine model.

Identification of CD1-Presented Antigens

Perhaps the most striking feature of the CD1 system is the chemical identity of the antigens it presents to T cells (Figure 4). Direct purification of the antigen recognized by the prototype CD1b-restricted *M. tuberculosis*–specific human T cell line DN1 revealed this to be a subset of mycolic acids, a heterogeneous group of branched- and long-chain fatty acids unique to mycobacteria and a restricted group of related bacterial species (112). This finding suggested the remarkable conclusion that CD1 proteins could be antigen-presenting molecules that have evolved the ability to present nonpeptide lipid antigens to T cells. Subsequent studies confirmed and extended this finding, showing CD1b-restricted T cell recognition of structurally defined glycolipids (91, 113). It has also now been established that both human CD1c (94; DB Moody, SA Porcelli, unpublished data) and CD1a (JP Rosat, MB Brenner, personal communication) can similarly present lipid antigens of mycobacteria to T cells.

Comparison of the structures of the known and proposed CD1-presented lipid antigens suggests a structural motif that is common to all these compounds (Figure 4). This consists of a hydrophobic portion composed of branched or dual acyl chain function, which is covalently coupled to a hydrophilic cap formed by the polar or charged groups of the lipid and its associated carbohydrates. Several studies have provided insight into how the structural features of CD1-presented lipid and glycolipid antigens correlate with their ability to be presented to and recognized by T cells. Initial studies of the *M. tuberculosis* mycolic acids presented by human CD1b demonstrated that recognition of this lipid was completely blocked when the carboxylate was derivatized with a bulky bromophenacylbromide group (112). This suggested that the hydrophilic end of this lipid participated in the specific interaction of this antigen with either the CD1b protein or with the TCR of the responding T cells.

Subsequently, these studies were extended by an analysis of two *Mycobacterium leprae*–reactive, CD1b-restricted T cell lines recognizing a major structurally defined mycobacterial cell wall constituent known as lipoarabinomannan (LAM) (91). This molecule belongs to the family of glycosylphosphatidyl inositols and is composed of a hydrophobic lipid-containing phosphatidyl inositol group attached to a large and complex hydrophilic heteropolysaccharide (114).

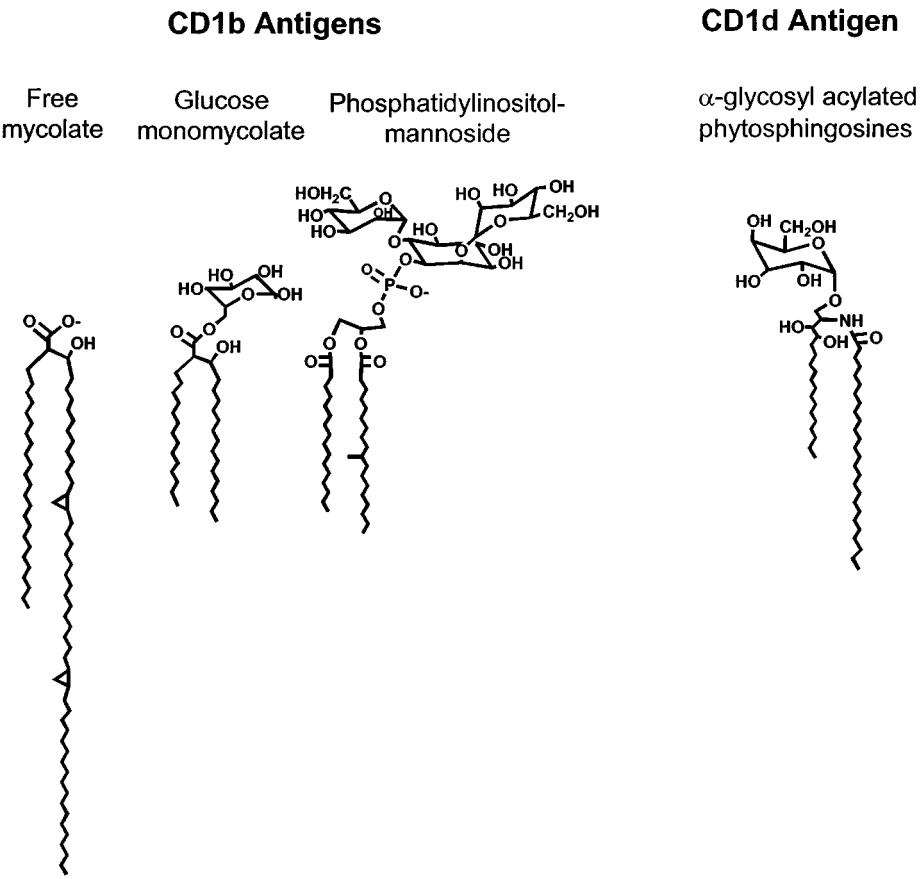

Figure 4 Structures of CD1b-presented antigens. (*Left*) Representative members of three different classes of CD1b-presented antigens. Each of these antigens is a naturally occurring glycolipid component of mycobacterial cell walls. (*Right*) The CD1d antigen is a synthetic glycolipid with a ceramide-like lipid structure that is recognized by most or all mouse and human CD1d-restricted NK T cells. Note that all these antigens have a common general structure composed of a hydrophilic head group and two aliphatic tails, thus defining one proposed structural motif for a class of CD1-presented antigens.

Studies of these T cell lines showed that the acyl chains of LAM were absolutely necessary for presentation of LAM to T cells because removal of these by alkaline hydrolysis resulted in loss of activity. A requirement for some but not the entire carbohydrate portion of LAM was also demonstrated for recognition of this compound by CD1b-restricted T cells. In this case, one T cell line required the presence of virtually the entire carbohydrate region for optimal recognition, whereas the second T cell line recognized a subunit of LAM with

a smaller carbohydrate backbone composed of the inositol group with two to six covalently linked mannose groups (PIM_{2-6}). These results provided further support for the hypothesis that the hydrophilic (in this case carbohydrate) portion of the antigen was involved in specific TCR-mediated recognition, while also confirming the necessity of the hydrophobic lipid moiety for presention by CD1.

A more complete and detailed demonstration of the relation between the chemical structure of the antigen and its recognition by CD1-restricted T cells has come from studies using the CD1b-presented glycolipid antigen glucose monomycolate (GMM) (113). T cells reactive with a naturally occurring form of GMM containing a C80 wax-ester mycolate could also recognize a fully synthetic GMM containing a simple C32 mycolate, indicating that the fine structure of the lipid moiety was not critical to the presentation and recognition of this glycolipid. In contrast, elimination of the branched structure of the lipid moiety led to complete loss of recognition, which suggests that the dual alkyl chain motif was required. Most notably, the T cell response to GMM could be shown to be extremely specific for the precise stereochemistry of the polar head group, as substituting mannose or galactose for glucose led to a complete loss of T cell recognition. These findings, together with recent information on the structure of CD1 proteins, suggest a straightforward mechanism for lipid and glycolipid presentation by CD1 proteins, which is discussed in detail below.

Studies of antigen presentation by mouse CD1d molecules have also identified a number of specific ligands that bind to CD1 and are presented to murine T cells. In contrast to the lipids and glycolipids consistently found in studies seeking natural microbial antigens produced by human CD1 proteins, initial studies in the murine system sought and identified synthetic peptide ligands for recombinant mouse CD1d1 by using a powerful random peptide library screening technique (115). This yielded a collection of peptides with high CD1d1 binding affinity ($K_D \sim 10^{-7}$ M) that were quite hydrophobic and appeared to contain a motif with three anchor positions occupied by aromatic or bulky hydrophobic amino acids. CD1-restricted T cells specific for these peptides could be demonstrated, which suggests that these findings were of immunological significance. Also raising the possibility of peptide antigen presentation by mouse CD1, one report has recently appeared in which a DNA vaccination approach appeared to give rise to mouse CD1d1-restricted T cells specific for the protein antigen ovalbumin (116). Together, these results raised the interesting possibility that mouse CD1d1, and possibly other CD1 proteins, might maintain at least some potential for interacting with peptides in a manner that leads to their presentation to T cells.

However, the possibility that mouse CD1d1 plays a major role as a peptide-presenting molecule now seems less appealing in light of several other findings favoring the hypothesis that the antigens bound and presented by mouse CD1

proteins, as for their human counterparts, are predominantly lipids and glycolipids. Thus, mouse CD1-restricted NK1.1$^+$ T cells have recently been shown to recognize synthetic ceramide-containing glycolipid antigens (117, 154a, 158). Reactivity to these glycolipids is also consistently seen for human CD1d-restricted NK T cell clones (155, 156). These synthetic compounds have an unusual structure, consisting of an α-anomeric hexose sugar (glucose or galactose) linked to an acylphytosphingosine moiety. Lipids with this structure are currently known to occur naturally only in marine sponges, but it is possible that they represent structural analogues of lipid antigens that occur in relevant pathogens or in abnormal tissues.

It has also been demonstrated in one study that immunopurified mouse CD1d1 contains associated lipids, whereas no associated peptides could be detected (118). In fact, structural studies of acid-eluted ligands from mouse CD1d1 have identified cellular glycosylphosphatidyl inositols (GPI) as possibly the major bound endogenous ligands of this protein. The possible significance of this result is emphasized by the fact that the structure of mammalian GPI is extremely similar to that of the mycobacterial antigens LAM and PIM, GPIs identified in earlier studies of human CD1b-presented antigens (91). An interesting possibility to consider is that structural variations in the endogenous GPIs or other lipids presented by CD1d1 on different cell types could account for the patterns of tissue-specific recognition of CD1d1 observed for mouse NK T cell hybridomas (54, 119).

UPTAKE AND PROCESSING PATHWAYS OF CD1-PRESENTED ANTIGENS

The identification of CD1 as a system for the presentation of lipid antigens raises numerous fundamental questions about how such antigens are taken up and processed by antigen-presenting cells. In all cases so far studied, CD1-restricted T cell recognition of nonpeptide antigens could be shown to require uptake and delivery to an intracellular compartment in APCs (78, 91). Furthermore, like MHC class II presentation, the presentation of lipid antigens by human CD1b (78, 91) and by murine CD1d1 (117) is inhibited by agents that prevent endosomal acidification (e.g. chloroquine and concanamycin A), indicating a crucial endosomal step in the pathway. However, the peptide transporter complex TAP-1/2, which is required for assembly and stable expression of MHC class I proteins, is not required either for expression of CD1 proteins (120, 121) or for their antigen-presenting function (78, 94). Likewise, HLA-DM complexes, which are required for efficient antigen presentation by MHC class II, are not necessary for normal expression and function of human CD1b or CD1c proteins (78, 94). These findings indicate that the intracellular pathways

involved in protein assembly and antigen processing are in some ways distinct between CD1 and MHC class I and II, as might be anticipated from the marked differences in the properties of the antigens presented by these systems.

Current evidence suggests that either exogenous (i.e. taken up by phagocytosis or endocytosis) or endogenous (i.e. produced by pathogens living within an APC) lipid antigens can enter the CD1 antigen processing route and be presented to CD1-restricted T cells. The mechanism by which exogenous lipid antigens are taken up by APCs has been studied in detail only for LAM, which represents a large and heavily glycosylated CD1b-presented glycolipid antigen (122). It has been clearly demonstrated that the macrophage mannose receptor (MR) is involved in the uptake of LAM, probably through its ability to bind the mannose core of this mycobacterial glycolipid. The presentation of LAM by monocyte-derived $CD1^+$ APCs has been shown to be dependent on MR uptake of the antigen because presentation is completely blocked when this process is competitively inhibited by soluble mannan and by antibodies to the MR. Furthermore, $CD1b^+$ transfectant cell lines that lack expression of MR are unable to present LAM (SA Porcelli, unpublished data), although they are capable of taking up and presenting other lipid and glycolipid antigens (78, 94, 113). Overall, these results suggest that the uptake of relatively small and predominantly hydrophobic antigens (such as mycolic acids and GMM) may not involve interaction with specific receptors on APCs, whereas the much larger and more hydrophilic glycolipids such as LAM may require specific receptor-mediated uptake for presentation.

Uptake of CD1-presented lipid antigens by APCs leads to their delivery to endosomes, and the requirement for endosomal acidification in CD1b-restricted presentation suggests that association of lipid antigens with CD1 most likely occurs in an acidic endosomal compartment. This hypothesis is strongly supported by the finding that the human CD1b protein localizes prominently in a variety of acidic endosomal compartments in APCs, including those in which MHC class II molecules are known to be loaded with peptides (122, 123). These MHC class II–containing compartments (MIICs) are lipid-rich late endosomes with either a multilammellar or multivesicular membrane arrangement. Localization of CD1b to MIICs and other endocytic compartments is mediated by a targeting motif (YXXZ, where Y is tyrosine, X is any amino acid, and Z is a bulky hydrophobic residue) in the short cytoplasmic tail of this protein (123). Similar results have been reported for mouse CD1d1 (119). Sequences corresponding to the targeting motif interact with at least two different adapter protein complexes (AP-1 and AP-2), which direct proteins bearing the motif into clathrin-coated pits and vesicles (124). In the case of human CD1b, cytoplasmic tail-mediated endosomal targeting has been shown to be required for efficient presentation of exogenous lipid antigens to CD1b-restricted T cells

(125). A YXXZ motif is also present in most other CD1 proteins, with one clear exception being human CD1a, which appears not to traffic to MIICs and may be loaded with antigens at a different site (M Sugita, MB Brenner, personal communication).

The lipid-rich composition of the MIICs may provide an ideal site in which to concentrate foreign lipid antigens, such as those presented by CD1b. In fact, one CD1b-presented antigen, LAM, has been shown to colocalize with CD1b at this site (122). The acidic pH (∼4.0) of MIICs also may be important, as this promotes a conformational change in CD1b that increases accessibility to the hydrophobic interior of the protein and facilitates binding of lipid ligands by purified CD1b in vitro (126). In addition, MIICs contain a wide array of degradative enzymes, which may be involved in the trimming of large glycan components of some CD1-presented antigens, such as those found in LAM. Currently, little is known about the potential of antigen-presenting cells to modify the covalent structure of bacterial lipid antigens. However, one recent study has shown that trehalose-6,6'-dimycolate, a relatively small mycobacterial cell wall glycolipid that contains both mycolic acid and GMM as components of its structure, is not processed by APCs into components that can be recognized by mycolic acid or GMM-specific CD1b-restricted T cells (127). This suggests that for some bacterial lipid antigens, APCs may not possess enzymes capable of cleaving their covalent bonds, and that intracellular antigen processing may reflect only the requirements for loading intact lipid antigens or their precursors onto CD1. In contrast, for LAM, which has an extremely bulky polysaccharide component, it has been proposed that enzymatic processing to reduce the antigen to a smaller core structure most likely is a component of the intracellular processing (91, 122).

In addition to the exogenous pathway of antigen loading for CD1b, there is an endogenous pathway as well. This has been demonstrated by infecting APCs in vitro with virulent *M. tuberculosis* (95, 125). Both DN and $CD8^+$ T cell lines were able to recognize and lyse *M. tuberculosis*–infected targets in a CD1b restricted manner. Effective antigen presentation in this system also depended on the trafficking of CD1b to endosomes because deletion of the cytoplasmic tail YXXZ targeting sequence markedly diminished the recognition of infected cells by all CD1b-restricted T cell lines tested. Although the details of this pathway for endogenous lipids are not yet known, it seems unlikely that antigen is loaded into CD1b molecules in the same endocytic vesicles in which the live mycobacteria reside because these compartments do not acidify normally (128, 129). It is more likely that secreted or shed lipid antigens of *M. tuberculosis* are able to translocate to other subcellular compartments that do not harbor bacilli and are therefore able to acidify. In support of this hypothesis, immunoelectron microscopy of *M. tuberculosis*–infected macrophages indicates that

LAM is present in endosomal structures that do not contain *M. tuberculosis* organisms (129).

Mechanism of Lipid Antigen Interaction with CD1 Proteins

Together with the demonstration that naturally occurring CD1-presented antigens are lipids, the structure of the putative CD1 ligand-binding grooves points strongly to a molecular model for how antigens interact with these proteins in a way that leads to specific recognition by the antigen receptors of T cells (113, 126, 130). Although unlikely to bind peptides in the manner of MHC class I and II molecules, the putative antigen-binding groove demonstrated in mouse CD1d1, and predicted to be present in other CD1 proteins, is well suited to bind the twin acyl tails of the known CD1b-presented antigens (76). This mechanism of binding would mimic that which has been shown for nonspecific lipid transport proteins, which bury the hydrophobic tail of their ligands in an electrostatically neutral binding pocket lined by hydrophobic residues (131). In this way, a relatively nonspecific interaction of the lipid with the hydrophobic surface of the CD1 groove could provide most of the binding energy needed to generate a stable interaction between the antigen and the CD1 protein.

The anchoring of lipid antigens into the CD1 groove through hydrophobic interactions would also be predicted to orient amphipathic lipid and glycolipid antigens such that their polar head groups would be positioned outside the ligand-binding groove or near the groove entrance where several hydrophilic residues are present (76). Such positioning of the antigen would in theory allow the hydrophilic head groups and specific residues on the α-helical face of the CD1 protein to interact directly with TCRs, leading to the highly specific recognition that has been observed for CD1-restricted T cells. This proposed mechanism of antigen interaction is strongly supported by findings on the structural basis for CD1-restricted T cell recognition of LAM and PIM (91), and especially by studies of the glycolipid GMM that showed exquisite fine specificity for the carbohydrate but not the lipid component of this antigen (113). Also arguing strongly for this mechanism is the finding that recently obtained crystals of recombinant mouse CD1d1 protein contain a distinct electron density buried in the hydrophobic groove. Although the origin of this material is uncertain, its structure appears most consistent with that of a bound lipid (BW Segelke, AR Castaño, EA Stura, PA Peterson, IA Wilson, personal communication).

It has also proven possible to directly observe the interaction of human CD1b with three of its known ligands, the mycobacterial glycolipid antigens LAM, PIM, and GMM, using evanescent wave sensor and surface plasmon resonance measurements (126). Binding of intact LAM and GMM was shown to be detectable only at acidic pH, and for LAM the optimal pH was determined

to be pH 4.0. In contrast, an interaction between deacylated LAM and CD1b could not be detected, consistent with the proposal that CD1b directly binds the acyl functions of this antigen. At acid pH, the CD1b-LAM equilibrium binding constant (K_D) was determined to be 3.2×10^{-8} M, which indicates an affinity similar to those determined for interactions between immunogenic peptides and MHC class I molecules (132, 133). Binding of PIM and GMM could be shown to have affinities approximately one order of magnitude less than that measured for LAM, and all three of these glycolipids could be shown to bind to the same or closely adjacent sites on CD1b by competition studies. Another study using different techniques has examined the binding affinity between a soluble form of mouse CD1d1 and phosphatidylinositol, the proposed lipid anchor of the major cellular ligand of CD1d1 (118). This revealed a K_D at neutral pH of approximately 4×10^{-7} M, which is extremely similar to that measured at acid pH for PIM and GMM binding to CD1b. Whether this reflects a true difference in the pH requirement for lipid ligand binding by different CD1 proteins will require further detailed investigation.

Other biophysical studies carried out in vitro have provided further insight into the mechanism by which lipid antigens are loaded onto CD1b, demonstrating that the properties of the CD1b protein are dramatically altered by acidic pH to facilitate its direct interaction with hydrophobic ligands (126). Circular dichroism analysis of CD1b indicates reversible unfolding of the α-helical portions of the molecule at acid pH. This may lead to marked changes in the accessibility of the hydrophobic ligand-binding groove in CD1b because the α-helical portions of the protein form the walls and outlet of the groove. Indeed, for human CD1b it appears that the unfolding of the α-helices in the presence of low pH exposes a hydrophobic binding site, as detected by the enhanced binding and emission of the fluorescent probe 1-anilo-naphthalene-8-sulfonic acid (126).

Collectively, these data are consistent with a model in which the hydrophobic ligand-binding groove of CD1b becomes exposed in the acid milieu of the endosome, thus allowing the direct binding of the hydrophobic portions of lipid and glycolipid antigens present at this intracellular site. This mode of antigen binding would bury the hydrophobic alkyl chain component within the core of the CD1 protein and leave the hydrophilic or charged cap of the antigen exposed at the opening of the groove where it may make direct contacts with the TCRs of specific T cells. This model thus accounts for the requirement for a hydrophobic alkyl component in all of the CD1-presented antigens so far studied and explains the relative lack of specificity of T cells for this portion of the antigen. In addition, it also makes clear the reason for the exquisite specificity for structural features of the polar and hydrophilic ends of these antigens (113).

THE ROLE OF CD1-RESTRICTED T CELLS IN MICROBIAL IMMUNITY

Investigation of human leprosy has provided strong evidence for involvement of the CD1-restricted T cells in host immune response to infection. Leprosy provides an ideal model to study the role of human T cell subsets in host defense against microbial pathogens because infection by the causative bacillus, *M. leprae*, results in disease manifestations that encompass an immunological spectrum (134). At one pole of the spectrum are patients with tuberculoid leprosy, who are able to restrict the growth of the pathogen. These individuals mount strong cell-mediated immune responses to *M. leprae*, resulting in a generally benign clinical state characterized by relatively few skin lesions containing low numbers of bacilli. In contrast, at the opposite pole are patients with lepromatous leprosy who are unable to contain the infection. These individuals have poor cell-mediated immunity against the pathogen and, consequently, have widespread lesions with an enormous bacterial burden. In a study of skin biopsy specimens from human leprosy patients, CD1a, -b, and -c expression was found to be up-regulated on mature $CD83^+$ dendritic cells infiltrating dermal granulomas (159). The frequency of $CD1^+$ cells correlated with the level of cell-mediated immunity to *M. leprae*, being tenfold more abundant in the granulomas of patients with the immunologically responsive tuberculoid form of the disease compared with the unresponsive lepromatous form. The prominence of $CD1^+$ cells in tuberculoid lesions is likely influenced by the local cytokine environment and may directly reflect the high expression of GM-CSF, a key differentiation factor for dendritic cells, in these lesions (135, 136).

In contrast, the low frequency of $CD1^+$ cells in lepromatous lesions correlates with low levels of GM-CSF in these lesions and may also be due to directly inhibitory factors in the local cytokine milieu. For example, IL-10 is strongly expressed in lepromatous lesions (135, 136) and is likely to be a key inhibitor of the CD1 system. This cytokine inhibits GM-CSF secretion by antigen-stimulated peripheral blood mononuclear cells (137) and also inhibits the CD1 expression normally induced by GM-CSF on monocytes (138). Because administration of GM-CSF to lepromatous leprosy patients results in the infiltration of $CD1a^+$ cells into skin lesions (139), it may be possible to develop immunotherapeutic strategies to up-regulate this antigen presentation pathway. The bacilli themselves may also influence CD1 expression, as infection of $CD1^+$ antigen-presenting cells with virulent mycobacteria causes a down-regulation of CD1 expression, but not of MHC class I or II expression (160). This effect required infection of the cells with live mycobacteria because heat-killing of the bacteria completely abrogated the effect and was associated with decreased

the steady state CD1 mRNA levels. The down-regulation of CD1 expression by mycobacteria may represent a novel immune evasion mechanism for this pathogen. The finding that mycobacteria have apparently targeted this pathway as part of their strategy in evading the host immune response suggests that the CD1-dependent T cell response is a significant component of host resistance to these pathogens.

Consistent with the prominent expression of CD1 in leprosy lesions, it has also proven possible to derive CD1-restricted T cells from the tissue lesions and blood of patients with mycobacterial infections. Initially, a CD1b-restricted, *M. leprae*–specific T cell line was derived from a cutaneous lesion of a subject infected with *M. leprae* (91). In addition, *M. tuberculosis*–reactive T cells that recognize lipid antigens of *M. tuberculosis* in the context of human CD1 molecules have been derived form normal individuals (78, 94), patients with tuberculosis (95), and patients coinfected with *M. tuberculosis* and HIV (93). There is currently little information about the precise frequency of CD1-restricted T cells in the T cell repertoire, and what the details are of how these T cells may expand and whether they persist following antigenic challenges are major questions that have not yet been addressed.

Several different mechanisms are apparent by which CD1-restricted T cells may contribute to protective immunity to microbial pathogens. Host defense against intracellular pathogens, such as mycobacteria, is thought to involve two major effector T cell pathways. First, mycobacteria-specific group I CD1-restricted T cells release high levels of interferon γ and low levels of IL-4 (93), typical of the Th1 pattern of cytokines required for activation of macrophage-mediated killing of intracellular pathogens and development of effective cell-mediated immunity against such organisms (91). Second, mycobacteria-reactive CD1-restricted T cells typically show a high degree of cytolytic activity in vitro against antigen-pulsed $CD1^+$ mononuclear phagocytes (95, 127), and they also recognize and lyse $CD1^+$ targets infected with live virulent *M. tuberculosis* bacilli (95, 125). Lysis of chronically or productively infected macrophages would be expected to contribute to host defense either by directly killing the bacteria or by disbursing the pathogen and thereby allowing freshly recruited macrophages to take up and destroy it (140, 141). The apoptotic death of mononuclear phagocytes harboring bacteria could limit the reservoir of host cells for the pathogen and also increase the ratio between Th1 cytokine producing T cells and infected cells. Infected macrophages that have undergone apoptosis can also be rapidly ingested by dendritic cells, which may facilitate the generation of additional CTL to combat the infection (142).

Studies of the mechanisms by which CD1-restricted *M. tuberculosis*–specific T cells lyse mycobacteria-infected target cells have revealed an interesting dichotomy in the lytic pathways used by different phenotypic subsets of CTLs

(95). The cytotoxicity of $CD4^{-}CD8^{-}$ CD1-restricted T cells was mediated by Fas/FasL interaction because anti-Fas and anti-FasL antibodies could block cytotoxicity. In contrast, target cell lysis by $CD8^{+}$ T cell clones (either CD1-restricted or MHC class I-restricted) was not inhibited by anti-Fas or anti-FasL antibodies but could be blocked by depletion of cytotoxic granules by strontium pretreatment. Lysis of infected target cells by $CD4^{-}CD8^{-}$ T cells had no effect on the viability of intracellular *M. tuberculosis* bacilli, whereas CD1-restricted $CD8^{+}$ T cells killed approximately 50% of the intracellular bacteria during an 18-h coincubation with infected cells. Recent data link this direct antimicrobial effect of $CD8^{+}$ CTL (including those that are either CD1 or MHC class II restricted) to their expression of granulysin (157), a protein component of CTL granules (143, 144). The reason for the existence of two distinct subsets of CTL that use different mechanisms to kill infected cells is not yet clear, but it is likely that these contribute in different ways to host defense against intracellular infection.

The Potential Range of CD1-Presented Lipid and Glycolipid Antigens

At present, nearly all of the known naturally occurring CD1-presented foreign antigens have been isolated from mycobacteria. However, it seems likely that T cell recognition through the CD1 system will extend to a much wider range of microbial pathogens and may also include antigens of host cell origin. It is in fact already known that human CD1b-restricted T cells can recognize glycosylated mycolates produced by organisms that are phylogenetically related to the mycobacteria, including rhodococci and nocardia (DB Moody, SA Porcelli, unpublished data). Based on the putative motif of dual alkyl chains linked to a polar cap that has been proposed for CD1b-presented antigens, a variety of other more widespread potential targets for CD1-restricted T cells can be postulated (Figure 5). These include the lipoteichoic acids of gram-positive organisms and also components or precursors of the ubiquitous lipopolysaccharides of gram-negative bacteria (145).

The basic dual alkyl chain motif is also found in the abundant capsular polysaccharides of virulent gram-negative bacilli such as *Haemophilus influenzae* and *Neisseria meningitidis*, which are major targets of protective antibody responses against these organisms. Although such responses have generally been thought to be T cell independent, it has recently been suggested that CD1-restricted T cells could be involved (146). This possibility is supported by the expression of CD1 proteins by B cells, as shown first in humans (80) and more recently in mice (73, 87). Other complex pathogens, such as protozoal or multicellular parasites, are also known to harbor a range of unique lipids that could hypothetically function as CD1-presented antigens. Although a few

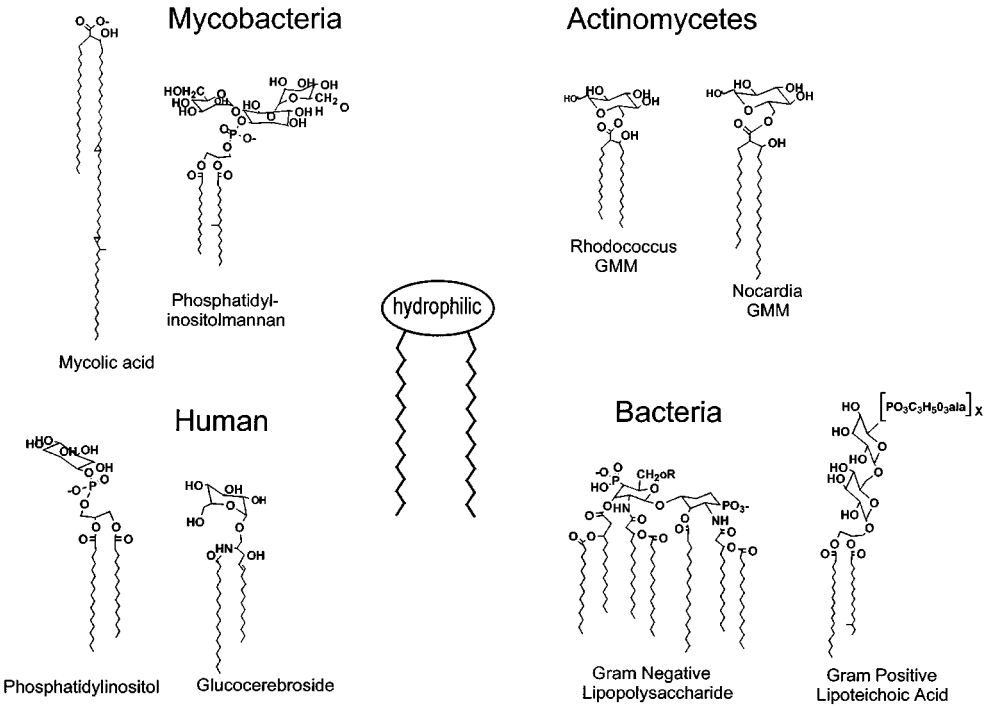

Figure 5 Some examples of the potential range of CD1-presented antigens are depicted, based on the proposed CD1b antigen motif illustrated schematically in the center of the diagram. The glycolipids illustrated for mycobacteria and actinomycetes are known to be recognized by CD1b-restricted T cells (91, 112; DB Moody, SA Porcelli, unpublished data). The glycolipids illustrated below have not yet been shown to be recognized by CD1-restricted T cells. These are examples of potential self antigens found in normal human tissues that conform to the CD1b antigen motif and common glycolipids of gram-negative (lipopolysaccharide) and gram-positive (lipoteichoic acid) bacteria that may also contain the motif within their structures. GMM, Glucose monomycolate.

examples, such as the lipophosphoglycans of *Leishmania* species (147), have been partially explored in terms of their structure and antigenicity, this remains for the most part a largely uncharted territory in the search for molecular targets of immune recognition. We anticipate future studies delineating involvement of CD1-restricted T cells in these and other infectious diseases.

The possibility that lipids produced by self tissues could also act as CD1-presented antigens has implications for autoimmunity and tumor immunity. Multiple examples of T cells that recognize CD1 proteins in the absence of deliberately added foreign antigens, and therefore appear to be autoreactive T cells, have been isolated from both humans (78, 89, 90, 94) and mice (52, 54, 74, 119).

The recent finding that cellular glycolipids can associate with CD1 proteins has strengthened the view that such T cells may in fact be responsive to self-lipid ligands bound to CD1 (118). This implies that mechanisms must exist to maintain tolerance for CD1-restricted T cells, presumably at least in part through positive and negative selection steps that are analogous to those that take place in the thymus for conventional MHC-restricted T cells. Ceramide-containing self glycolipids such as glucocerebroside and other gangliosides are similar in their overall structure to some of the known bacterial and synthetic lipid antigens recognized by human and mouse CD1-restricted T cells. The possibility that these could become altered or dysregulated in inflammatory diseases affecting lipid-rich tissues, such as multiple sclerosis, remains an attractive mechanism for activating CD1-restricted T cells in such lesions. In addition, many known tumor antigens are glycolipid-associated carbohydrate epitopes including gangliosides and other ceramide-containing structures (148), and this could represent another situation in which the cell-mediated immune response might be targeted to nonpeptide antigen recognition by CD1.

CONCLUDING REMARKS

The discovery of nonpeptide lipid and glycolipid antigen recognition by CD1-restricted T cells defines a new paradigm for immune recognition and provides a novel mechanism for host responses to infection. The existence of this MHC-independent pathway for T cell activation may substantially expand the immune repertoire and could have important implications for many aspects of cell-mediated immunity. It seems likely that CD1 has evolved as part of the unique adaptation of the immune system to its task of combating a myriad of microbial pathogens. The implications of lipid antigen presentation to T cells are potentially broad and may extend to antimicrobial and anti-tumor immunity as well as to immunoregulation and autoimmunity. One immediate practical consideration for this new insight into antigen presentation resides in the area of vaccine development. Much effort is being devoted to the development of protein subunit vaccines for bacterial and parasitic diseases, which may vary in effectiveness according to the MHC haplotype of the individual and the ability of the microbe to modulate the particular epitopes targeted. Because CD1 molecules are nonpolymorphic, the nonpeptide antigens they present may offer particular advantages as vaccine subunits. In addition, CD1-presented lipid antigens appear to be critical components of microbial organisms that can not be readily altered by random single-step mutations, and thus they represent relatively fixed targets. A next critical step will be to determine whether CD1-restricted T cell responses to nonpeptide antigens can contribute significantly to protective immune responses to microbial pathogens. Studies employing

CD1-restricted antigens as vaccines offer new promise in the fight against infectious disease and should also provide an integrated understanding of how CD1 participates in the immune response.

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Literature Cited

1. Pamer EG, Cresswell P. 1998. Mechanisms of MHC class I-restricted antigen processing. *Annu. Rev. Immunol.* 16:323–58
2. Cresswell P. 1994. Assembly, transport, and function of MHC class II molecules. *Annu. Rev. Immunol.* 12:259–93
3. Shapiro ME, Onderdonk AB, Kasper DL, Finberg RW. 1982. Cellular immunity to Bacteroides fragilis capsular polysaccharide. *J. Exp. Med.* 155:1188–97
4. Jackson S, Folks TM, Wetterskog DL, Kindt TJ. 1984. A rabbit helper T cell clone reactive against group-specific streptococcal carbohydrate. *J. Immunol.* 133:1553–57
5. Mitchell GF, Handman E. 1985. T-lymphocytes recognise *Leishmania* glycoconjugates. *Parasitol. Today* 1:61–63
6. Katsuki M, Kakimoto K, Kawata S, Kotani S, Koga T. 1987. Induction of delayed-type hypersensitivity by the T cell line specific to bacterial peptidoglycans. *J. Immunol.* 139:3570–72
7. Domer JE, Garner RE, Befidi-Mengue RN. 1989. Mannan as an antigen in cell-mediated immunity (CMI) assays and as a modulator of mannan-specific CMI. *Infect. Immun.* 57:693–700
8. Moll H, Mitchell GF, McConville MJ, Handman E. 1989. Evidence of T-cell recognition in mice of a purified lipophosphoglycan from *Leishmania major*. *Infect. Immun.* 57:3349–56
9. Baer H, Chaparas SD. 1964. Tuberculin reactivity of a carbohydrate component of unheated BCG culture filtrate. *Science* 146:245–47
10. Mehra V, Brennan PJ, Rada E, Convit J, Bloom BR. 1984. Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid. *Nature* 308:194–96
11. Nataraj C, Brown ML, Poston RM, Shawar SM, Rich RR, Fischer Lindau K, Kurlander R. 1996. H2-M3wt-restricted, *Listeria monocytogenes*-specific CD8 T cells recognize a novel, hydrophobic, protease-resistant, periodate-sensitive antigen. *Int. Immunol.* 8:367–78
12. Tanaka Y, Morita CT, Nieves E, Brenner MB, Bloom BR. 1995. Natural and

synthetic nonpeptide antigens recognized by human gamma/delta T cells. *Nature* 375:155–58
13. Constant P, Davodeau F, Peyrat M, Poquet Y, Puzo G, Bonneville M, Fournie J. 1994. Stimulation of human gamma delta T cells by nonpeptidic mycobacterial ligands. *Science* 264:267–70
14. Bernard A, Boumsell L, Hill C. 1984. Joint report of the First International Workshop on Human Leucocyte Antigens by the investigators of the participating laboratories. In *Leucocyte Typing—Human Leucocyte Differentiation Antigens Detected by Monoclonal Antibodies*, ed. A Bernard, L Boumsell, J Dausset, C Milstein, SF Schlossman, pp. 9–135. Berlin: Springer-Verlag
15. McMichael A, Pilch JR, Galfre G, Mason DY, Fabre JW, Milstein C. 1979. A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur. J. Immunol.* 9:205–10
16. Knowles RW, Bodmer WF. 1982. A monoclonal antibody recognising a human thymus leukemia-like antigen associated with β2-microglobulin. *Eur. J. Immunol.* 12:676–81
17. Favaloro EJ, Bradstock KF, Kamath S, Dowden G, Gillis D, George V. 1986. Characterization of a p43 human thymocyte antigen. *Dis. Markers* 4:261–70
18. Olive D, Dubreuil P, Mawas C. 1984. Two distinct TL-like molecular subsets defined by monoclonal antibodies on the surface of human thymocytes with different expression on leukemia lines. *Immunogenetics* 20:253–64
19. Calabi F, Milstein C. 1986. A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. *Nature* 323:540–43
20. Martin LH, Calabi F, Milstein C. 1986. Isolation of CD1 genes: a family of major histocompatibility complex-related differentiation antigens. *Proc. Natl. Acad. Sci. USA* 83:9154–58
21. Martin LH, Calabi F, Lefebvre F-A, Bilsland CAG, Milstein C. 1987. Structure and expression of the human thymocyte antigens CD1a, CD1b, and CD1c. *Proc. Natl. Acad. Sci. USA* 84:9189–93
22. Yu CY, Milstein C. 1989. A physical map linking the five CD1 human thymocyte differentiation antigen genes. *EMBO J.* 8:3727–32
23. Albertson DG, Fishpool R, Sherrington P, Nacheva E, Milstein C. 1988. Sensitive and high resolution in situ hybridization to human chromosomes using biotin labelled probes: assignment of the human thymocyte CD1 antigen genes to chromosome 1. *EMBO J.* 7:2801–5
24. Balk SP, Bleicher PA, Terhorst C. 1989. Isolation and characterization of a cDNA and gene coding for a fourth CD1 molecule. *Proc. Natl. Acad. Sci. USA* 86:252–56
25. Blumberg RS, Terhorst C, Bleicher P, McDermott FV, Allan CH, Landau SB, Trier JS, Balk SP. 1991. Expression of a nonpolymorphic MHC class I-like molecule, CD1D, by human intestinal epithelial cells. *J. Immunol.* 147:2518–24
26. Bilsland CAG, Milstein C. 1991. The identification of the β2-microglobulin binding antigen encoded by the human *CD1D* gene. *Eur. J. Immunol.* 21:71–78
27. Calabi F, Bilsland CAG, Yu CY, Bradbury A, Belt KT, Martin LH, Milstein C. 1989. Recent progress in the molecular study of CD1. See Ref. 150, pp. 254–58
28. MacHugh ND, Bensaid A, Davis WC, Howard CJ, Parsons KR, Jones B, Kaushal A. 1988. Characterization of a bovine thymic differentiation antigen analogous to CD1 in the human. *Scand. J. Immunol.* 27:541–47
29. Parsons KR, MacHugh ND. 1991. Individual antigens of cattle. Bovine CD1 (BoCD1). *Vet. Immunol. Immunopathol.* 27:37–41
30. Mackay CR, Maddox JF, Gogolin-Ewens KJ, Brandon MR. 1985. Characterization of two sheep lymphocyte differentiation antigens, SBU-T1 and SBU-T6. *Immunology* 55:729–37
31. Dutia BM, Hopkins J. 1991. Analysis of the CD1 cluster in sheep. *Vet. Immunol. Immunopathol.* 27:189–94
32. Hopkins J, Dutia BM. 1991. Workshop studies on the ovine CD1 homologue. *Vet. Immunol. Immunopathol.* 27:97–99
33. Hopkins J, Dutia BM, Bujdoso R, McConnell I. 1989. In vivo modulation of CD1 and MHC class II expression by sheep afferent lymph dendritic cells. Comparison of primary and secondary immune responses. *J. Exp. Med.* 170:1303–18
34. Rhind SM, Dutia BM, Howard CJ, Hopkins J. 1996. Discrimination of two subsets of CD1 molecules in the sheep. *Vet. Immunol. Immunopathol.* 52:265–70
35. Ferguson ED, Dutia BM, Hein WR, Hopkins J. 1996. The sheep CD1 gene family contains at least four CD1B homologues. *Immunogenetics* 44:86–96
36. Calabi F, Belt KT, Yu CY, Bradbury A, Mandy WJ, Milstein C. 1989. The rabbit CD1 and the evolutionary conservation

of the CD1 gene family. *Immunogenetics* 30:370–77
37. Wang CR, Chen G-H, Mandy WJ. 1987. Identification of a rabbit class I-like thymocyte-specific antigen. *J. Immunol.* 138:3352–59
38. Bradbury A, Belt KT, Neri TM, Milstein C, Calabi F. 1988. Mouse CD1 is distinct from and co-exists with TL in the same thymus. *EMBO J.* 7:3081–86
39. Balk SP, Bleicher PA, Terhorst C. 1991. Isolation and expresion of cDNA encoding the murine homologues of CD1. *J. Immunol.* 146:768–74
40. Ichimiya S, Matsuura A, Takayama S, Kikuchi K. 1993. Molecular cloning of a cDNA encoding the rat homologue of CD1. *Transplant. Proc.* 25:2773–74
41. Ichimiya S, Kikuchi K, Matsuura A. 1994. Structural analysis of the rat homologue of CD1. Evidence for evolutionary conservation of the CD1D class and widespread transcription by rat cells. *J. Immunol.* 153:1112–23
42. Kasai K, Matsuura A, Kikuchi K, Hashimoto Y, Ichimiya S. 1997. Localization of rat CD1 transcripts and protein in rat tissues: an analysis of rat CD1 expression by in situ hybridization and immunohistochemistry. *Clin. Exp. Immunol.* 109:317–22
43. Matsuura A, Hashimoto Y, Kinebuchi M, Kasai K, Ichimiya S, Katabami S, Chen H, Shimizu T, Kikuchi K. 1997. Rat CD1 antigen: structure, expression and function. *Transplant. Proc.* 29:1705–6
44. Calabi F, Jarvis JM, Martin L, Milstein C. 1989. Two classes of CD1 genes. *Eur. J. Immunol.* 19:285–92
45. Lanier L, Allison JP, Phillips JH. 1986. Correlation of cell surface antigen expression on human thymocytes by multicolor flow cytometric analysis: implications for differentiation. *J. Immunol.* 137:2501–7
46. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF. 1980. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. *Proc. Natl. Acad. Sci. USA* 77:1588–92
47. Lopez-Botet M, Moretta L. 1985. Functional characterization of human thymocytes: a limiting dilution analysis of precursors with proliferative and cytolytic activites. *J. Immunol.* 134:2299–304
48. Bhan AK, Reinherz EL, Sibrand P, McCluskey RT, Schlossman SF. 1980. Location of T cell and major histocompatibility complex antigens in the human thymus. *J Exp. Med.* 152:771–82
49. Blue ML, Levine H, Daley JF, Branton KRJ, Schlossman SF. 1989. Expression of CD1 and class I MHC antigens by human thymocytes. *J. Immunol.* 142:2714–20
50. Bradbury A, Calabi F, Milstein C. 1990. Expression of CD1 in the mouse thymus. *Eur. J. Immunol.* 20:1831–36
51. Balk SP, Bleicher PA, Terhorst C. 1991. Isolation and expression of cDNA encoding the murine homologues of CD1. *J. Immunol.* 146:768–74
52. Bendelac A, Lantz O, Quimby ME, Yewdell JW, Bennink JR, Brutkiewicz RR. 1995. CD1 recognition by mouse NK1+ T lymphocytes. *Science* 268: 863–65
53. Mendiratta SK, Martin WD, Hong S, Boesteanu A, Joyce S, Van Kaer L. 1997. CD1d1 mutant mice are deficient in natural T cells that promptly produce IL-4. *Immunity* 6:469–77
54. Park SH, Roark JH, Bendelac A. 1998. Tissue-specific recognition of mouse CD1 molecules. *J. Immunol.* 160:3128–34
55. Novacek MJ. 1997. Mammalian evolution: an early record bristling with evidence. *Curr. Biol.* 7:R489–91
56. Moseley WS, Watson ML, Kingsmore SF, Seldin MF. 1989. CD1 defines conserved linkage group border between human chromosomes 1 and mouse chromosomes 1 and 3. *Immunogenetics* 30:378–82
57. Porcelli S. 1995. The CD1 family: a third lineage of antigen presenting molecules. *Adv. Immunol.* 59:1–98
58. Kasahara M, Mckinney EC, Flajnik MF, Ishibashi T. 1993. The evolutionary origin of the major histocompatibility complex: polymorphism of class II α chain genes in the cartilaginous fish. *Eur. J. Immunol.* 23:2160–65
59. Bartl S, Weissman IL. 1994. Isolation and characterization of major histocompatibility complex class IIB genes from the nurse shark. *Proc. Natl. Acad. Sci. USA* 91:262–66
60. Okamura K, Ototake M, Nakanishi T, Kurosawa Y, Hashimoto K. 1997. The most primitive vertebrates with jaws possess highly polymorphic MHC class I genes comparable to those of humans. *Immunity* 7:777–90
61. van Agthoven A, Terhorst C. 1982. Further biochemical characterization of the human thymocyte differentiation antigen T6. *J. Immunol.* 128:426–32

62. Amiot M, Dastot H, Fabbi M, Degos L, Bernard A, Boumsell L. 1988. Intermolecular complexes between three human CD1 molecules on normal thymus cells. *Immunogenetics* 27:187–95
63. Hughes AL. 1991. Evolutionary origin and diversification of the mammalian CD1 antigen genes. *Mol. Biol. Evol.* 8:185–201
64. Srivastava R, Lambert ME. 1991. Molecular organization and expression of class I HLA gene family. See Ref. 151, pp. 100–54
65. Parham P, Lawlor DA, Lomen CE, Ennis PD. 1989. Diversity and diversification of HLA-A,B,C alleles. *Proc. Natl. Acad. Sci. USA* 142:3937–50
66. Burmeister WP, Gastinel LN, Simister NE, Blum ML, Bjorkman PJ. 1994. Crystal structure at 2.2 Å resolution of the MHC-related neonatal Fc receptor. *Nature* 372:336–43
67. Lerch PG, van de Rijn M, Smart JE, Knowles RW, Terhorst C. 1986. Isolation and purification of the human thymocyte antigens T6 and M241. *Mol. Immunol.* 23:131–39
68. van de Rijn M, Lerch PG, Knowles RW, Terhorst C. 1983. The thymic differentiation markers T6 and M241 are two unusual MHC class I antigens. *J. Immunol.* 131:851–55
69. Mosser DD, Duchaine J, Martin LH. 1991. Biochemical and developmental characterization of the murine cluster of differentiation 1 antigen. *Immunology* 73:298–303
70. Sugita M, Porcelli SA, Brenner MB. 1997. Assembly and retention of CD1b heavy chains in the endoplasmic reticulum. *J. Immunol.* 159:2358–65
71. Bauer A, Huttinger R, Staffler G, Hansmann C, Schmidt W, Majdic O, Knapp W, Stockinger H. 1997. Analysis of the requirement for beta 2-microglobulin for expression and formation of human CD1 antigens. *Eur. J. Immunol.* 27:1366–73
72. Balk SP, Burke S, Polischuk JE, Frantz ME, Yang L, Porcelli S, Colgan SP, Blumberg RS. 1994. β2-Microglobulin-independent MHC class Ib molecule expressed by human intestinal epithelium. *Science* 265:259–62
73. Brossay L, Jullien D, Cardell S, Sydora BC, Burdin N, Modlin RL, Kronenberg M. 1997. Mouse CD1 is mainly expressed on hemopoietic-derived cells. *J. Immunol.* 159:1216–24
74. Cardell S, Tangri S, Chan S, Kronenberg M, Benoist C, Mathis D. 1995. CD1-restricted CD4+ T cells in major histocompatibility complex class II-deficient mice. *J. Exp. Med.* 182:993–1004
75. Longley J, Kraus J, Alonso M, Edelson R. 1989. Molecular cloning of CD1a (T6), a human epidermal dendritic cell marker related to class I MHC molecules. *J. Invest. Dermatol.* 92:628–31
76. Zeng Z, Castano AR, Segelke BW, Stura EA, Peterson PA, Wilson IA. 1997. Crystal structure of mouse CD1: an MHC-like fold with a large hydrophobic binding groove. *Science* 277:339–45
77. Cattoretti G, Berti E, Parravicini C, Buscaglia M, Cappio F, Caputo R, Cerri A, Crosti L, Delia D, Gaiera G, Polli N. 1989. Expression of CD1 molecules on dendritic cells: ontogeny, epitope analysis on normal and malignant cells, and tissue distribution. See Ref. 150, pp. 262–64
78. Porcelli S, Morita CT, Brenner MB. 1992. CD1b restricts the response of human CD4−8− T lymphocytes to a microbial antigen. *Nature* 360:593–97
79. Kasinrerk W, Baumruker T, Majdic O, Knapp W, Stockinger H. 1993. CD1 molecule expression on human monocytes induced by granulocyte-macrophage colony-stimulating factor. *J. Immunol.* 150:579–84
80. Small TN, Knowles RW, Keever C, Kernan NA, Collins N, O'Reilly RJ, Dupont B, Flomenberg N. 1987. M241 (CD1) expression on B lymphocytes. *J. Immunol.* 138:2864–68
81. Small TN, Keever C, Collins N, Dupont B, O'Reilly RJ, Flomenberg N. 1989. Characterization of B cells in severe combined immunodeficiency disease. *Hum. Immunol.* 25:181–93
82. Plebani A, Proserpio AR, Guarneri D, Buscaglia M, Cattoretti G. 1993. B and T lymphocyte subsets in fetal and cord blood: age-related modulation of CD1c expression. *Biol. Neonate* 63:1–7
83. Cattoretti G, Berti E, Mancuso A, D'Amato L, Schiro' R, Soligo D, Delia D. 1987. CD1: a MHC class I related family of antigens with widespread distribution on resting and activated cells. In *Leukocyte Typing III*, ed. A McMichael, PC Beverley, SP Cobbold, MJ Crumpton, W Gilks, FM Golch, N Hogg, M Horton, N Ling, ICM MacLennon, DY Mason, C Milstein, pp. 89–91. Oxford, UK: Oxford Univ. Press
84. Canchis PW, Bhan AK, Landau SB, Yang L, Balk SP, Blumberg RS. 1993. Tissue distribution of the nonpolymorphic major histocompatibility

complex class I-like molecule, CD1d. *Immunology* 80:561–65
85. Bleicher PA, Balk SP, Hagen SJ, Blumberg RS, Flotte TJ, Terhorst C. 1990. Expression of murine CD1 on gastrointestinal epithelium. *Science* 250:679–82
86. Burke S, Landau S, Green R, Tseng CC, Nattakom T, Canchis W, Yang L, Kaiserlian D, Gespach C, Balk S, Blumberg RS. 1994. Rat cluster of differentiation 1 molecule: expression on the surface of intestinal epithelial cells and hepatocytes. *Gastroenterology* 106:1143–49
87. Roark JH, Park SH, Jayawardena J, Kavita U, Shannon M, Bendelac A. 1998. CD1.1 expression by mouse antigen-presenting cells and marginal zone B cells. *J. Immunol.* 160:3121–27
88. Porcelli S, Brenner MB, Greenstein JL, Balk SP, Terhorst C, Bleicher PA. 1989. Recognition of cluster of differentiation 1 antigens by human CD4−CD8−cytolytic T lymphocytes. *Nature* 341:447–50
89. Faure F, Jitsukawa S, Miossec C, Hercend T. 1990. CD1c as a target recognition structure for human T lymphocytes: analysis with peripheral blood gamma/delta cells. *Eur. J. Immunol.* 20:703–6
90. Balk SP, Ebert EC, Blumenthal RL, McDermott FV, Wucherpfennig KW, Landau SB, Blumberg RS. 1991. Oligoclonal expansion and CD1 recognition by human intestinal intraepithelial lymphocytes. *Science* 253:1411–15
91. Sieling PA, Chatterjee D, Porcelli SA, Prigozy TI, Mazzaccaro RJ, Soriano T, Bloom BR, Brenner MB, Kronenberg M, Brennan PJ. 1995. CD1-restricted T cell recognition of microbial lipoglycan antigens. *Science* 269:227–30
92. Thomssen H, Ivanyi J, Espitia C, Arya A, Londei M. 1995. Human CD4−CD8−$\alpha\beta$+ T-cell receptor T cells recognize different mycobacteria strains in the context of CD1b. *Immunology* 85:33–40
93. Gong J, Stenger S, Zack JA, Jones BE, Bristol GC, Modlin RL, Morrissey PJ, Barnes PF. 1998. Isolation of mycobacterium-reactive CD1-restricted T cells from patients with human immunodeficiency virus infection. *J. Clin. Invest.* 101:383–89
94. Beckman EM, Melian A, Behar SM, Sieling PA, Chatterjee D, Furlong ST, Matsumoto R, Rosat JP, Modlin RL, Porcelli SA. 1996. CD1c restricts responses of mycobacteria-specific T cells. Evidence for antigen presentation by a second member of the human CD1 family. *J. Immunol.* 157:2795–803
95. Stenger S, Mazzaccaro RJ, Uyemura K, Cho S, Barnes PF, Rosat JP, Sette A, Brenner MB, Porcelli SA, Bloom BR, Modlin RL. 1997. Differential effects of cytolytic T cell subsets on intracellular infection. *Science* 276:1684–87
96. Bendelac A, Rivera MN, Park SH, Roark JH. 1997. Mouse CD1-specific NK1 T cells: development, specificity, and function. *Annu. Rev. Immunol.* 15:535–62
97. Bendelac A. 1998. Mouse NK1+ T cells. *Curr. Opin. Immunol.* 7:367–74
98. Lantz O, Bendelac A. 1994. An invariant T cell receptor α chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4−8− T cells in mice and humans. *J. Exp. Med.* 180:1097–106
99. Yoshimoto T, Paul WE. 1994. CD4^{pos} NK1.1^{pos} T cells promptly produced IL-4 in response to *in vivo* challenge with anti-CD3. *J. Exp. Med.* 179:1285–95
100. Hayakawa K, Lin BT, Hardy RR. 1992. Murine thymic CD4+ T cell subsets: a subset (Thy0) that secretes diverse cytokines and overexpresses the Vβ8 T cell receptor gene family. *J. Exp. Med.* 176:269–74
101. Smiley ST, Kaplan MH, Grusby MJ. 1997. Immunoglobulin E production in the absence of interleukin-4-secreting CD1-dependent cells. *Science* 275:977–79
102. Chen YH, Chiu NM, Mandal M, Wang N, Wang CR. 1997. Impaired NK1+ T cell development and early IL-4 production in CD1-deficient mice. *Immunity* 6:459–67
103. Yoshimoto T, Bendelac A, Watson C, Hu-Li J, Paul WE. 1995. Role of NK1.1 T cells in a TH2 response and in immunoglobulin E production. *Science* 270:1845–47
104. Denkers EY, Scharton-Kersten T, Barbieri S, Caspar P, Sher A. 1996. A role for CD4+ NK1.1+ T lymphocytes as major histocompatibility complex class II independent helper cells in the generation of CD8+ effector function against intracellular infection. *J. Exp. Med.* 184:131–39
105. Cui J, Shin T, Kawano T, Sato H, Kondo E, Toura I, Kaneko Y, Koseki H, Kanno M, Taniguchi M. 1997. Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors. *Science* 278:1623–26
106. Mieza MA, Itoh T, Cui JQ, Makino Y, Kawano T, Tsuchida K, Koike T,

Shirai T, Yagita H, Matsuzawa A, Koseki H, Taniguchi M. 1996. Selective reduction of V alpha 14+ NK T cells associated with disease development in autoimmune-prone mice. *J. Immunol.* 156:4035–40

107. Exley M, Garcia J, Balk SP, Porcelli S. 1997. Requirements for CD1d recognition by human invariant Vα24+ CD4−CD8− T cells. *J. Exp. Med.* 186: 109–20
108. Davodeau F, Peyrat M-A, Necker A, Dominici R, Blanchard F, Leget C, Gaschet J, Costa P, Jacques Y, Godard A, Vié H, Poggi A, Romagné F, Bonneville M. 1997. Close phenotypic and functional similarities between human and murine $\alpha\beta$ T cells expressing invariant TCR α-chains. *J. Immunol.* 158:5603–11
109. Prussin C, Foster B. 1997. TCR Vα24 and Vβ11 coexpression defines a human NK1 T cell analog containing a unique Th0 subpopulation. *J. Immunol.* 159:5862–70
110. Sumida T, Sakamoto A, Murata H, Makino Y, Takahashi H, Yoshida S, Nishioka K, Iwamoto I, Taniguchi M. 1995. Selective reduction of T cells bearing invariant V alpha 24J alpha Q antigen receptor in patients with systemic sclerosis. *J. Exp. Med.* 182:1163–68
111. Wilson SB, Kent SC, Patton KT, Orban T, Jackson RA, Exley M, Porcelli S, Schatz DA, Atkinson MA, Balk SP, Strominger JL, Hafler DA. 1998. Extreme Th1 bias of invariant Vα24JαQ T cells in type 1 diabetes. *Nature* 391:177–81
112. Beckman EM, Porcelli SA, Morita CT, Behar SM, Furlong ST, Brenner MB. 1994. Recognition of a lipid antigen by CD1-restricted $\alpha\beta$+ T cells. *Nature* 372:691–94
113. Moody DB, Reinhold BB, Guy MR, Beckman EM, Frederique DE, Furlong ST, Ye S, Reinhold VN, Sieling PA, Modlin RL, Besra GS, Porcelli SA. 1997. Structural requirements for glycolipid antigen recognition by CD1b-restricted T cells. *Science* 278:283–86
114. Prinzis S, Chatterjee D, Brennan PJ. 1993. Structure and antigenicity of lipoarabinomannan from *Mycobacterium bovis* BCG. *J. Gen. Microbiol.* 139:2649–58
115. Castaño AR, Tangri S, Miller JE, Holcombe HR, Jackson MR, Huse WD, Kronenberg M, Peterson PA. 1995. Peptide binding and presentation by mouse CD1. *Science* 269:223–26
116. Lee DJ, Abeyratne A, Carson DA, Corr M. 1998. Induction of an antigen-specific, CD1-restricted cytotoxic T lymphocyte response in vivo. *J Exp. Med.* 187:433–38
117. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, Ueno H, Nakagawa R, Sato H, Kondo E, Koseki H, Taniguchi M. 1997. CD1d-restricted and TCR-mediated activation of Valpha14 NKT cells by glycosylceramides. *Science* 278:1626–29
118. Joyce S, Woods AS, Yewdell JW, Bennink JR, De Silva AD, Boesteanu A, Balk SP, Cotter RJ, Brutkiewicz RR. 1998. Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol. *Science* 279:1541–44
119. Brossay L, Tangri S, Bix M, Cardell S, Locksley R, Kronenberg M. 1998. Mouse CD1-autoreactive T cells have diverse patterns of reactivity to CD1+ targets. *J. Immunol.* 160:3681–88
120. Brutkiewicz RR, Bennink JR, Yewdell JW, Bendelac A. 1995. TAP-independent, beta 2-microglobulin-dependent surface expression of functional mouse CD1.1. *J. Exp. Med.* 182:1913–19
121. Hanau D, Fricker D, Bieber T, Esposito-Farese ME, Bausinger H, Cazenave JP, Donato L, Tongio MM, de la Salle H. 1994. CD1 expression is not affected by human peptide transporter deficiency. *Hum. Immunol.* 41:61–68
122. Prigozy TI, Sieling PA, Clemens D, Stewart PL, Behar SM, Porcelli SA, Brenner MB, Modlin RL, Kronenberg M. 1997. The mannose receptor delivers lipoglycan antigens to endosomes for presentation to T cells by CD1b molecules. *Immunity* 6:187–97
123. Sugita M, Jackman RM, van Donselaar E, Behar SM, Rogers RA, Peters PJ, Brenner MB, Porcelli SA. 1996. Cytoplasmic tail-dependent localization of CD1b antigen-presenting molecules to MIICs. *Science* 273:349–52
124. Marks M, Ohno H, Kirchhausen T, Bonifacino J. 1997. Protein sorting by tyrosine-based signals: adapting to the Ys and wherefores. *Trends Cell Biol.* 7:124–28
125. Jackman RM, Stenger S, Lee A, Moody DB, Rogers RA, Niazi KR, Sugita M, Modlin RL, Peters PJ, Porcelli SA. 1998. The tyrosine-containing cytoplasmic tail of CD1b is essential for its efficient presentation of bacterial lipid antigens. *Immunity* 8:341–51
126. Ernst WA, Maher J, Cho S, Niazi KR, Chatterjee D, Moody DB, Besra GS, Watanabe Y, Jensen PE, Porcelli SA, Kronenberg M, Modlin RL. 1998.

Molecular interaction of CD1b with lipoglycan antigens. *Immunity* 8:331–40
127. Moody DB, Reinhold BB, Reinhold VN, Besra GS, Porcelli SA. 1998. Uptake and processing of glycosylated mycolates for presentation to CD1b-restricted T cells. *Immunol. Lett.* In press
128. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG. 1994. Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 263:678–81
129. Xu S, Cooper A, Sturgill-Koszycki S, van Heyningen T, Chatterjee D, Orme I, Allen P, Russell DG. 1994. Intracellular trafficking in *Mycobacterium tuberculosis* and *Mycobacterium avium*-infected macrophages. *J. Immunol.* 153:2568–78
130. Porcelli SA, Segelke BW, Sugita M, Wilson IA, Brenner MB. 1998. The CD1 family of lipid antigen presenting molecules. *Immunol. Today* 19:362–68
131. Shin DH, Lee JY, Hwang KY, Kim KK, Suh SW. 1995. High-resolution crystal structure of the non-specific lipid-transfer protein from maize seedlings. *Structure* 3:189–99
132. Sette A, Sidney J, del Guercio MF, Southwood S, Ruppert J, Dahlberg C, Grey HM, Kubo RT. 1994. Peptide binding to the most frequent HLA-A class I alleles measured by quantitative molecular binding assays. *Mol. Immunol.* 31:813–22
133. Guercio M, Sidney J, Hermanson G, Perez C, Grey H, Kubo R, Sette A. 1995. Binding of a peptide antigen to multiple HLA alleles allows definition of an A2-like supertype. *J. Immunol.* 154:685–93
134. Ridley DS, Jopling WH. 1966. Classification of leprosy according to immunity. A five-group system. *Int. J. Lepr.* 34:255–73
135. Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom BR, Modlin RL. 1991. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science* 254:277–79
136. Yamamura M, Wang X-H, Ohmen JD, Uyemura K, Rea TH, Bloom BR, Modlin RL. 1992. Cytokine patterns of immunologically mediated tissue damage. *J. Immunol.* 149:1470–75
137. Sieling PA, Abrams JS, Yamamura M, Salgame P, Bloom BR, Rea TH, Modlin RL. 1993. Immunosuppressive roles for interleukin-10 and interleukin-4 in human infection: in vitro modulation of T cell responses in leprosy. *J. Immunol.* 150:5501–10
138. Thomssen H, Kahan M, Londei M. 1995. Differential effects of interleukin-10 on the expression of HLA class II and CD1 molecules induced by granulocyte/macrophage colony- stimulating factor/interleukin-4. *Eur. J. Immunol.* 25:2465–70
139. Kaplan G, Walsh G, Guido LS, Meyn P, Burkhardt RA, Abalos RM, Barker J, Frindt PA, Fajardo TT, Celona R, Cohn ZA. 1992. Novel responses of human skin to intradermal recombinant granulocyte/macrophage-colony-stimulating factor: Langerhans cell recruitment, keratinocyte growth, and enhanced wound healing. *J. Exp. Med.* 175:1717–28
140. Kaufmann SHE. 1988. CD8+ T lymphocytes in intracellular microbial infections. *Immunol. Today* 9:168–74
141. Kaufmann SHE. 1993. Immunity to intracellular bacteria. *Annu. Rev. Immunol.* 11:129–63
142. Albert ML, Sauter B, Bhardwaj N. 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 392:86–89
143. Pena SV, Hanson DA, Carr BA, Goralski TJ, Krensky AM. 1997. Processing, subcellular localization, and function of 519 (granulysin), a human late T cell activation molecule with homology to small, lytic, granule proteins. *J. Immunol.* 158:2680–88
144. Pena SV, Krensky AM. 1997. Granulysin, a new human cytolytic granule-associated protein with possible involvement in cell-mediated cytotoxicity. *Semin. Immunol.* 9:117–25
145. Ratledge C, Wilkinson SG. 1988. *Microbial Lipids*, Vol. 1. San Diego, CA: Academic. 963 pp.
146. Fairhurst RM, Wang CX, Sieling PA, Modlin RL, Braun J. 1998. CD1-restricted T cells and resistance to polysaccharide-encapsulated bacteria. *Immunol Today* 19:257–59
147. Turco SJ, Descoteaux A. 1992. The lipophosphoglycan of *Leishmania* parasites. *Annu. Rev. Microbiol.* 46:65–94
148. Hakomori S. 1985. Aberrant glycosylation in cancer cell membranes as focused on glycolipids: overview and perspectives. *Cancer Res.* 45:2405–14
149. Calabi F, Yu CY, Bilsland CAG, Milstein C. 1991. CD1: From structure to function. See Ref. 151, pp. 215–43
150. Knapp W, Dorkin B, Guilks WR, Reiber

EP, Schmitt RE, Stein H, von dem Borneed AEG, eds. 1989. *Leucocyte Typing IV.* Oxford: Oxford Univ. Press
151. Srivastava R, Ram BP, Tyle P, eds. 1991. *Immunogenetics of the Major Histocompatibility Complex.* New York: VCH Publ.
152. Teitell M, Holcombe HR, Brossay L, Hagenbaugh A, Jackson MJ, Pond L, Balk SP, Terhorst C, Peterson PA, Kronenberg M. 1997. Nonclassical behavior of the mouse CD1 class I-like molecule. *J. Immunol.* 158:2143–49
153. Bendelac A, Killeen N, Littman DR, Schwartz RH. 1994. A subset of CD4+ thymocytes selected by MHC class I molecules. *Science* 263:1774–78
154. Hammond KL, Poulton LD, Palmisano LJ, Silveira PA, Godfrey DI, Baxter AG. 1998. alpha/beta-T cell receptor (TCR)+CD4−CD8−(NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J. Exp. Med.* 187:1047–56
154a. Burdin N, Brossay L, Koezuka Y, Smiley ST, Grusby MJ, Gui M, Taniguchi M, Hayakawa K, Kronenberg M. 1998. Selective ability of mouse CD1 to present glycolipids: alpha-galactosylceramide specifically stimulates Valpha 14+ NK T lymphocytes. *J. Immunol.* 161:3271–81
155. Spada F, Koezuka Y, Porcelli SA. 1998. CD1d-restricted recognition of synthetic glycolipid antigens by human NK T cells. *J. Exp. Med.* 188:1529–34
156. Brossay L, Chioda MC, Burdin N, Koezuka Y, Casorati G, Dellabona P, Kronenberg M. 1998. CD1d mediated recognition of a alpha-galactosylceramide by Nk T cells is highly conserved through mammalian evolution. *J. Exp. Med.* 188: 1521–28
157. Stenger S, Hanson DA, Teitlebaum R, Dewan P, Niazi KR, Froelich CJ, Ganz T, Thoma-Uzynski S, Melian A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, Modlin RL. 1988. An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science.* 282:121–25
158. Brossay L, Naidenko O, Burdin N, Sakai T, Kronenberg M. Structural requirements for galactosylceramide recognition by CD1 restricted NK T cells. *J. Immunol.* In press
159. Sieling PA, Jullien D, Dahlem M, Tedder TF, Rea TH, Modlin RL, Porcelli SA. 1999. CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. *J. Immunol.* In press
160. Stenger S, Niazi KR, Modlin RL. 1998. Down-regulation of CD1 on antigen-presenting cells by infection with *Mycobacterium tuberculosis*. *J. Immunol.* 161:3582–88

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