CD1: Presenting Unusual Antigens to Unusual T Lymphocytes

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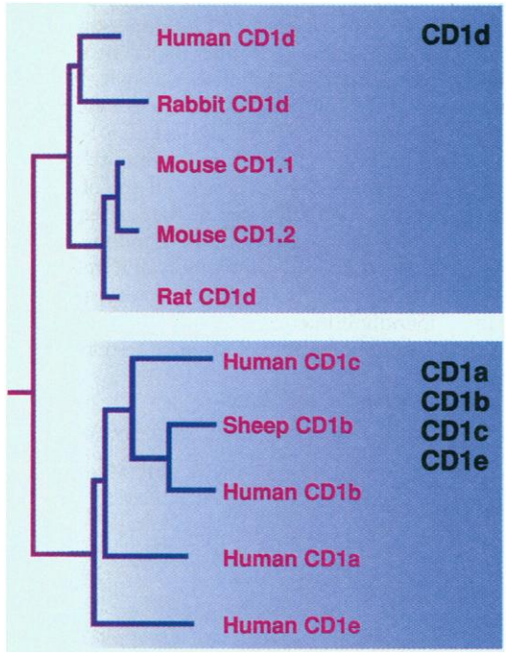
Fortuitously discovered in 1979 as the first human leukocyte antigens recognized by a monoclonal antibody (*1*), CD1 proteins are distant cousins of major histocompatibility complex (MHC) molecules that are encoded outside the MHC region. Insights into the function of CD1 proteins in the immune system have been eagerly awaited, because they interact with an unusual type of T cell, those with a $CD4^-8^-$ phenotype (*2*), and because their sequence indicates unique evolutionary features (*3*). Unlike classical MHC molecules, which present peptide fragments of antigens to most T lymphocytes, CD1 proteins are not polymorphic but comprise different isotypes (CD1a, b, c, d, and e) that are conserved in several mammalian species (*3*) (see figure). A series of reports now reveals some unexpected aspects of the biochemistry and cell biology of CD1 and suggests that CD1 has novel functions in the immune system.

The first remarkable discovery is that CD1b seems to present lipid rather than peptide antigen to T cells. In purifying the mycobacterial antigen recognized by a CD1b-restricted human T cell line, Beckman *et al.* (*4*) observed that the antigen was surprisingly resistant to protease digestion and that it copurified with the lipid fraction of the mycobacterial extract. The antigen turned out to be the mycolic acid from *Mycobacterium tuberculosis* cell wall (*4*). This unexpected finding has now been extended in a report in this issue by Sieling *et al.* (*5*) in which two M. *leprae*–specific, CD1b-restricted human T cell lines recognize lipoarabinomannan, a glycolipid from the mycobacterial cell wall, and do not cross-react with mycolic acid. Like peptide presentation by MHC class II molecules, the endosomal pathway appears to process these lipids (*2*, *5*). However, none of the MHC-encoded antigen-processing molecules, DMA-DMB or TAP, is required for lipid presentation, raising the possibility that other molecules are used for CD1 trafficking and lipid antigen processing (*2*, *5*).

Direct binding of these unusual lipid antigens to CD1b remains to be demonstrated, but the specificity of the T cell lines for distinct lipids argues for co-recognition of CD1b and some fragment of the lipid antigens. Indeed, the unusual frequency of conserved hydrophobic residues in the "peptide-binding groove" of CD1 (*3*) raises the possibility that the lipid antigen also lies in the groove (*3*).

The second report in this issue of *Science* by Castano and co-workers (*6*) therefore comes as a surprise. Using the new technology of random peptide phage display libraries to screen peptide motifs that would bind to a soluble version of mouse CD1 (the homolog of human CD1d), the authors identified several dozen peptides. That these peptides are potentially relevant to the immune system is indicated by the finding that they can be used to elicit specific CD1-restricted $CD8^+$ killer cells. In addition, binding of these peptides is similar to that displayed by peptide binding to classical MHC molecules in that there is a clear conserved binding motif in the majority of these peptides, including aromatic residues at positions 1 and 7 and aliphatic residues in position 4.



Two classes of CD1 molecules. The distal regions comprising the α1 and α2 domains of CD1 molecules were used to construct a phylogeny tree based on amino acid sequence homology.

What, then, are the natural ligands of CD1 in vivo? Does human CD1b specialize in binding lipids whereas CD1d (the only isotype expressed by mouse and rats) binds peptides? The conservation of the α1 and α2 domains of different CD1 isotypes among species (*3*) (see figure) certainly argues for unique properties of at least two subfamilies of CD1 molecules—CD1d and CD1a, b, c, and e. However, further biochemical studies and determination of the crystal structures of both mouse CD1 and human CD1b are needed to clarify these issues.

One may wonder whether the raison d'être of CD1 is solely to allow T cells to recognize lipids or peptides with particular motifs. Although targeting of bacterial cell wall lipids and glycolipids makes evolutionary sense—they are conserved components of mycobacteria and other prokaryotic pathogens, which are nowhere to be found in eukaryotes (*7*)—recognition of mycobacterial peptides is already performed by the classical MHC molecules. They can be presented by conventional MHC class II molecules to elicit responses by classical $CD4^+$ helper cells. Can the additional and redundant recognition of bacterial lipids impart enough evolutionary advantage to justify the conservation of CD1?

There are indeed several hints that the unique function of CD1 may also be related to the special properties of the T cells that interact with it. Sieling *et al.* (*5*) report that CD1b is abundantly expressed in the lesions of patients with the self-healing form of leprosy [where interferon-γ (IFN-γ)–secreting T helper 1 (T_H1) $CD4^+$ cells are recruited] but not with the lepromatous form (where T_H2 $CD4^+$ cells predominate), and suggest that CD1b expression may determine the T_H1/T_H2 set of specialized cytokines produced in response to M. *leprae*. The CD1-specific cells identified so far in humans consist mainly of cells with the unusual $CD4^-8^-$ phenotype (*2*, *8*). In addition, many of the human CD1-restricted cell lines seem to be autoreactive, in that they recognize $CD1^+$ cells without foreign antigen. This autoreactive aspect of CD1-specific cells has been reported mainly but not exclusively for CD1a, c, and d (*2*, *8*, *9*).

This is where the mouse system may provide more information than humans. CD1-specific T cells have been identified in normal unimmunized mice (*10*, *11*) and appear also to be autoreactive. Although they include cells with the unusual $CD4^-8^-$ phenotype, a majority are $CD4^+$ cells. The absence of $CD8^+$ cells is explained by the fact that persistence of the CD8 coreceptor imparts negative selection (*12*), presumably because of the high avidity of their T cell receptors (TCRs) for CD1. Indeed, a promi-

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nent subset of these CD1-specific cells uses an invariant TCRα chain in conjunction with a restricted set of TCR V_β families (*13*), and a human equivalent was found that also includes $CD4^-8^-$ cells with a homologous invariant TCRα chain (*8*, *13*, *14*). During their differentiation in the mouse thymus, such cells acquire several unique properties: First, they express natural killer cell receptors (*12*, *15*), and second, they display unusual cytokine secretion functions (*16*). In particular, the $CD4^+$ subset exhibits the ability to secrete large amounts of interleukin-4 (IL-4) upon primary stimulation, within an hour of TCR engagement (*17*). It is likely therefore that activation of these cells in vivo, through their explosive release of IL-4, will promote the differentiation of T_H2 responses.

How is the unique functional differentiation of CD1-restricted cells imparted? Although all CD1 isotypes are constitutively expressed in the thymus (presumably for positive selection of CD1-restricted cells), they are mainly expressed by cortical thymocytes and not by epithelial cells (*18*), the classical cell type that mediates positive selection of most T cells. Whether the phenotype imparted in the thymus to CD1-restricted cells is related to the pattern of expression of CD1 or to high-avidity interactions with CD1 remains to be investigated.

These unusual features of CD1 and CD1-specific T cells, and their conservation in different species suggest a novel role for CD1. T cell responses to antigens do not automatically follow TCR engagement, but in fact require second (co-stimulatory) signals (*19*, *20*). These signals have been postulated to be provided or induced by foreign, adjuvant-like signals (*7*) and internal, "danger" signals (*21*). In fact, the logic of this two-signal system has recently been extended to the suggestion that the immune system may not really discriminate between self and nonself, but between dangerous and harmless entities (*21*). Similarly, external and internal clues are also expected to function in determining whether immune responses are humoral (T_H2) or cell-mediated (T_H1). It is tempting to speculate that the CD1 gene family has evolved and been conserved to participate in these types of decisions. Mycobacterial lipids are an ideal foreign target; indeed, mycolic acid is an active principle of one of the most powerful adjuvants (Freund's complete adjuvant) (*22*). Early production of cytokines by CD1b-restricted, mycobacterial-specific T cells may therefore jump-start the immune response or, as postulated by Sieling *et al.* (*5*), determine its T_H1 differentiation. On the other hand, CD1d alone or associated with self peptides may constitute an internal signal that, upon induction in the target tissue, promotes humoral responses through the early secretion of IL-4 by CD1d-specific $CD4^+$ lymphocytes. Thus, CD1 and CD1-specific T cells may carry a form of "innate" immunity, focused on a restricted set of pathogens and on self antigens that are expressed upon cell activation. Like natural killer cells, their early activation and release of specialized sets of cytokines may also profoundly influence the differentiation of the upcoming "adaptive" immune response (*23*).

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