CD1a, CD1b, and CD1c in Immunity Against Mycobacteria

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Abstract The CD1 system is composed of five types of human CD1 proteins, CD1a, CD1b, CD1c, CD1d, and CD1e, and their mammalian orthologs. Each type of CD1 protein has a distinct antigen binding groove and shows differing patterns of expression within cells and in different tissues. Here we review the molecular mechanisms by which CD1a, CD1b, and CD1c capture distinct classes of self- and mycobacterial antigens. We discuss how CD1-restricted T cells participate in the immune response, emphasizing new evidence for mycobacterial recognition in vivo in human and non-human models.

1 Introduction

The discoverers of the CD1 locus originally separated CD1 proteins into group 1 (CD1a, CD1b, and CD1c) and group 2 (CD1d), based on amino acid sequence homology [1]. The cellular expression pattern of CD1a, CD1b, CD1c, and CD1d also supports grouping into group 1 and group 2 CD1 because the expression of group 1 CD1 molecules is limited to CD4 and CD8 double-positive thymocytes and professional antigen presenting cells, while group 2 CD1 has a more extensive

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distribution pattern that also includes non-hematopoietic cells. Group 1 proteins are more likely to be constitutively expressed on cells, and recent studies show inverse expression of group 1 and group 2 protein surface density when encountering bacterial stimuli [2].

Published research on CD1 function strongly emphasizes the role of NKT cells in mice, which are defined by the expression of a nearly invariant T cell receptor (TCR) and recognition of CD1d. Through germline deletion of murine CD1d or the invariant TCR, as well as the use of CD1d tetramers, invariant NKT cells have been shown to function in mycobacterial, bacterial, fungal and protozoal infections, as well as mouse models of cancer, atherosclerosis, diabetes, multiple sclerosis, and fat maintenance [3, 4]. Much of this research has emphasized the invariant nature of CD1d-restricted TCRs and a stereotyped function relating to rapid secretion of cytokines, especially Interferon-γ. However, invariant NKT cells are only part of the CD1d system, and CD1d-restricted responses are in turn only part of the much larger system of CD1-reactive T cells that respond to human CD1a, CD1b, and CD1c, as well as their mammalian orthologs. Antigen presentation by CD1a, CD1b, and CD1c is recently gaining a broader interest [5]. Because mice lack group 1 CD1 proteins, studies of CD1a, CD1b and CD1c use human T cells in vitro or ex vivo, non-murine animal models, or human CD1 proteins expressed in mice, and they are now revealing the functions of T cells that normally respond to CD1a, CD1b, and CD1c. It has become clear that many of the most well known features of invariant NKT cells, including strict TCR conservation, liver homing, α-galactosylceramide specificity, and an Interferon-γ dominated immunophenotype, are not broadly shared among the larger CD1-restricted repertoire.

Here we emphasize that the group 1 CD1 versus group 2 CD1 classification lumps CD1a, CD1b, and CD1c together in ways that obscure the distinctiveness of the individual group 1 CD1 isoforms, each of which has different patterns of expression and mechanism of antigen presentation. Here we review the data on the separate functions of the CD1a, CD1b, and CD1c isoforms and describe how different or similar they are, with an emphasis on their roles in recognizing foreign, mycobacterial antigens during infection. Further, we review CD1 genes and function among non-human mammals. Both functional and evolutionary data support the idea that CD1a, CD1b, and CD1c are remarkably different from each other.

2 Cellular Expression and Intracellular Trafficking of CD1

Human CD1a, CD1b, and CD1c vary in size of their antigen binding grooves, patterns of expression across different tissues and cell types [6], and subcellular trafficking patterns, leading to presentation of different types of antigens presented in different tissues. Whereas CD1d is abundantly expressed in the liver and gastrointestinal tract, CD1a proteins are expressed in the skin at high density on the surface of Langerhans cells. Accordingly, CD1d-restricted invariant NKT cells home to and accumulate to large numbers in the liver, whereas CD1a autoreactive T cells home to

the skin [7]. CD1b expression in the periphery is limited to the surface of dendritic cells and its inducible appearance on monocytes, and under those conditions CD1a and CD1c are also expressed. In addition, CD1c is expressed on marginal zone B cells in lymph nodes and peripheral blood. These clearly different, inducible, or constitutive patterns of each type of CD1 protein represent the first line of evidence that each CD1 gene likely plays a distinct role in immune response.

The subcellular trafficking of each type of CD1 molecule to differing steady-state distribution among endocytic compartments is mainly determined by motifs in the CD1 cytoplasmic tail, which bind to adaptor protein complexes [8–10]. Whereas CD1a lacks AP binding sequences and is found on the cell surface and sorting endosomes, human CD1c and CD1d bind to AP2, which mediates recycling and antigen capture in endosomes. Mouse CD1d and human CD1b bind both AP2 and AP3, driving their trafficking and steady-state accumulation in late endosomes and lysosomes. Therefore, human and mouse CD1 proteins differ and can be ranked as to the extent of penetration into the endosomal compartment with huCD1a < huCD1c < huCD1d < huCD1b and muCD1d. These differing trafficking patterns influence their function in T cell activation because endocytic compartments differ in their antigenic content. Also, the presence of endosomal lipid transfer proteins and low pH promote antigen loading onto CD1, yet highly acidic compartments can degrade some antigens so that they are not recognized [11–16]. These data provide clear evidence that each type of CD1 protein differs with regard to the type of cell on which it is expressed, and the cellular sub-compartment to which it most strongly localizes.

3 Antigen Presentation by CD1a, CD1b, and CD1c

Complementing another review on CD1d in this same edition, this review focuses on the group 1 CD1 proteins, CD1a, CD1b, and CD1c. Even though CD1a, CD1b, and CD1c are able to present mammalian self-lipids and lipids from several types of bacteria, mycobacterial lipids are the focus of this section. Here we review mycobacterial antigens, which were the first described antigens for the CD1 system [17] and for which the most information is available, including ex vivo studies of human patients. The molecular interactions of each type of CD1 groove with differing antigens are considered separately.

3.1 CD1a

Of all the human group 1 CD1 molecules, CD1a has the smallest antigen binding groove ($\sim 1,350$ Å^3) and apparently the least stringent conditions for loading antigens [18, 19]. Upon *de novo* synthesis, all CD1 molecules appear on the cell surface and subsequently undergo multiple rounds of internalization via the

Fig. 1 *Sources of CD1a-, CD1b-, and CD1c-presented antigens.* Group 1 CD1-expressing antigen presenting cells internalize mycobacterial lipids via the endocytic route in the form of bacteria or bacterial debris, debris of infected cells, as free lipids, or lipid-protein complexes. Lipids are loaded into the CD1 molecules in the early or late endocytic compartments to which CD1 isoforms differentially traffic. The structures of representative mycobacterial antigens that are presented are shown. R is glucose in glucose monomycolate, glycerol in glycerol monomycolate, or hydrogen in mycolic acid

endocytic route. In contrast to all other human CD1 proteins, human CD1a lacks discernable AP binding motifs and only cycles through early endosomes, bypassing late endosomes, before returning to the cell surface (Fig. 1) [14, 20]. CD1a may bind its antigens in the endoplasmic reticulum in the case of endogenous phospholipids, but also egresses to the cell surface and recycles through early endosomes, where it has access to exogenously added antigens and serum lipids. However, CD1a is not known to have requirements for low pH to capture antigens, and there is no clear evidence that early endosomes provide antigen loading factors, like activated saposins, which are preferentially located and activated in late endosomes and lysosomes. As contrasted with other CD1 proteins, which have mechanisms for preferential antigen capture in endosomes, these findings suggest that CD1a may have the most promiscuous mechanisms for binding endogenous lipids or exogenous lipids encountered at or near the cell surface.

The potential of CD1a to contribute to anti-mycobacterial immunity is demonstrated by its ability to present dideoxymycobactin (Fig. 1), a precursor molecule in the biosynthesis of mycobactin [21, 22]. The mycobactin siderophores are

essential in mycobacterial iron acquisition and survival of the bacterium in vivo [23, 24]. Mycobactins and related molecules have peptidic backbones that are synthesized by non-ribosomal protein synthases. The crystal structure of CD1a bound to a mycobactin shows that the acyl chain is bound deeply within the A' pocket in the antigen binding groove of the CD1a molecule, and the peptidic part is folded into a U-shaped conformation, so that it descends into the C' pocket with its' termini near the predicted plane of TCR contact. In contrast to the extended conformation of MHC I-presented peptides, for which the center of the peptide provides the key surface for TCRs, the structure of CD1a-dideoxymycobactin complexes predicts that only the termini of peptides are presented to the TCR [25]. However, unlike for CD1b- and CD1c-restricted anti-mycobacterial responses, which have been directly observed among polyclonal T cells ex vivo, published evidence for dideoxymycobactin recognition is limited to detailed studies of one clone so that broader studies of polyclonal responses ex vivo are needed.

CD1a also activates T cells in the absence of exogenous antigen, suggesting that self-antigens for CD1a exist [7, 26–29]. The antigens recognized by these clones are unknown, but most likely represent cellular lipids. Using purified or commercially available lipids to prime human T cells ex vivo, it is possible to detect responses against molecules like gangliosides and sulfatides [30] and unknown ligands possibly originating from serum [18]. The very high expression of CD1a by Langerhans cells and the recognition of CD1a in the absence of exogenously added antigen by T cell clones and polyclonal T cell populations with skin homing phenotype strongly suggests that there is a relevant physiologic function of CD1a in the skin, independent from a role in antimycobacterial immunity, possibly related to skin homeostasis [7]. For CD1d-restricted NKT cells the phenomenon of self-reactivity and reactivity to exogenously added foreign antigens is well described, including the physiologic relevance of this dual reactivity [31]. It seems that CD1a represents a comparable situation in that foreign bacterial ligands certainly exist and are capable of stimulating the immune system in a CD1a-dependent manner, but self-antigens also importantly contribute to its normal function.

3.2 CD1b

Among all CD1 isoforms, the size and shape of the CD1b groove stands out as having the largest volume (~ 2.200 Å^3). The CD1b groove is nearly twice as large as the CD1a groove, and compared to other CD1 isoforms, CD1b binds correspondingly larger lipids, including mycolyl lipids and sulfoglycolipids that are up to C80 in length [32–37]. Because the volume of the groove is estimated to accommodate optimally C76 [38] and the fact that mycobacterial mycolyl lipids are amongst the longest hydrocarbon chains known in biology, CD1b likely plays a biological role in presenting particularly large foreign lipids. The crystal structure of human CD1b bound to glucose monomycolate shows that the very long

meromycolate chain is twisted through the combined length of the A', T', and F' pockets, which are oriented end to end, creating a superchannel in the CD1b molecule. The shorter α-branch lies in the C' pocket [32]. The large volume of the CD1b groove may represent an evolved adaptation by which CD1b particularly focuses on presentation of foreign lipids. Supporting this speculation, CD1b autoreactive T cells are rarely observed among studies of autoreactive T cell populations or clones [7, 26]. Also, ~C76–C80 lipids that are commonly found in mycobacteria are generally absent in mammalian cells and were not detected in studies that broadly measured cellular CD1b ligands in eluents [39].

Considering all antigens known for all CD1 isoforms, CD1b shows the greatest flexibility for capture of lipids of different size, ranging from C12 to C80, including glucose monomycolate [37], glycerol monomycolate [36], free mycolic acid [33], diacylated sulfoglycolipids [40] and phosphatidylinositol mannosides (Fig. 1) [41, 42]. Long chain self-lipids in the range of C80 are not yet known and may not exist, begging the question of how smaller ~C40 self-lipids can bind to CD1b.

This question has been recently solved by showing that deeply seated scaffold lipids and antigenic lipids simultaneously bind to CD1b, so that their combined length fills the complete antigen binding groove of CD1b [34, 39, 43]. Crystallographic and functional studies now show that small self-antigens, like phosphatidyl ethanolamines with approximately ~C40 alkyl chains, are seated in the 'upper' region of the groove so that their head groups protrude for TCR contact. These antigens bind simultaneously with ~C32–40 scaffold lipids. Scaffold lipids derive their name from their deep seating within the 'bottom' of the groove, where provide upward support to position antigens near the A' portal for TCR contact from the 'bottom' of the groove to support antigens. Scaffold lipids were originally suggested to exist based on co-crystallization of detergents in the groove [43], and naturally occurring spacer lipids have been recently identified as diacylglycerols and deoxyceramides [39, 43, 44]. It has been known for more than a decade that CD1b can present glucose mycolate antigens with either C80 or C32 tails, and the latter finding is likely explained by binding a diacylglycerol spacer beneath the short chain mycolic acid [37, 39].

Thus, CD1b has evolved specific mechanisms for sampling antigens greatly vary in length. A general structural formula of mycolyl lipids is provided in Fig. 1. CD1b also presents big non-mycolyl lipids, such as mycobacterial diacylated sulfoglycolipids (Fig. 1) and phosphatidylinositol mannosides, which have both been reported to bind CD1b and activate T cell clones [35, 40, 41]. Last, CD1b is known to present the ganglioside self-lipids GM1, GalNac GD1a, GD1b, and GQ1b [45].

For the presentation of long chain glucose monomycolate and mycolic acid, as well as diacylated sulfoglycolipid, trafficking of CD1b to low pH lysosomal compartments is necessary for antigen loading (Fig. 1) [13, 17, 40, 41, 46]. The low pH causes release of interdomain tethers that are present at neutral pH and normally connect the α1 helix across to the α2 helix and to a loop located at the end of the groove. Acidic pH at levels found in lysosomes protonates negatively charged residues that from ionic bonds within tethers, so interdomain contacts located immediately adjacent to the antigen entry portal are dissolved, providing

greater access to the groove, as well as unloading of prebound self-lipids [47]. Because antigens with small alkyl chains would be less affected by this proposed dilation of the antigen entry portal, this mechanism might explain how smaller self-antigens are initially captured in the secretory pathway at neutral pH, whereas larger mycobacterial antigens strictly require low pH found in endosomes.

In addition, late endosomes and lysosomes are likely enriched for exogenous or foreign antigens by the action of the mannose receptor, DC-SIGN, Langerin, LDL, or scavenger receptor-mediated endocytosis of bacteria and bacterial debris [48–52]. CD1b expressing cells that are infected with mycobacteria present lipids to T cells, but whether antigens can gain direct access from the bacterium that resides in the cytoplasm or phagolysosome to the lysosome where CD1b loads its antigens, or whether antigens need to be shed and endocytosed has not yet been solved. Supporting the notion that CD1b is of specific relevance to the immune responses against antigens with long alkyl chains, CD1b-mediated T cell responses have not only been detected among T cell clones, but also polyclonal CD1b-restricted T cell responses from infected humans and bovines against mycolic acid, glucose monomycolate, glycerol monomycolate, and sulfoglycolipid have been described by independent research groups and will be discussed in more detail in Sect. 4 [36, 40, 53–56].

3.3 CD1c

CD1c shows interaction with both $\alpha\beta$ or $\gamma\delta$ T cells [28, 57]. CD1c molecules broadly survey the endocytic compartments, trafficking into early and late endosomes, an observation suggesting CD1c may have a larger antigen sampling pool than other isoforms [58, 59]. A role for CD1c in mediating T cell responses against *M. tuberculosis* was first demonstrated by the successful derivation of human T cell clones recognizing mycobacterial lipid extracts [60, 61]. Several CD1c-restricted T cell lines have been successfully derived against mycobacterial lipid extracts and patients infected with *M. tuberculosis* have CD1c-restricted immune responses [61].

Identification of the antigenic compounds that are recognized by these T cell clones revealed that CD1c presents structurally related branched chain lipids to clones and polyclonal T cells, the first description of which defined a new class of mycobacterial lipid, mycoketides (Fig. 1). Using the CD1c-restricted T cell line CD8-1, mannosyl phosphomycoketide was identified from mycobacterial lipid extracts. Mycoketides were found in lipid extracts of various medically important mycobacterial species, including *M. avium*, *M. bovis* BCG, and *M. tuberculosis* [61, 62]. The methyl branching pattern of mycoketides is important in positioning the lipid within CD1c for optimal T cell activation. More specifically, comparisons of synthetic mycoketide analogs demonstrate that optimal T cell activation occur with all five methyl branches with S-stereocenters [63]. In addition, T cell clones can show specificity for the presence and linkage of carbohydrate moieties within dolichyl or mycoketide phosphoglycolipids [61, 63].

The crystal structure of CD1c in complex with all-S mannosyl-phosphomy-coketide reveals the second largest binding groove amongst all CD1 isoforms, at 1780 $Å^3$. CD1c has two hydrophobic binding pockets, called A' and F'. The CD1c A' pocket has a toroidal shape, which is large enough to accommodate the bulky alkyl chain of mycoketides and binds the branched lipid with a hand in glove fit. The complex also highlighted the importance of the all-S configuration of methyl branches, which allows for the favorable clockwise rotation of the lipid, an orientation that may not occur with stereorandom methyl branching [64]. Thus the lipid tail helps to orient interactions within the CD1c A' pocket to position the glycan headgroup for recognition by T cell. The short length (C30–36), saturation, and methyl branching of mycoketides differ from that seen in mammalian isoprenoid structures and may represent a pathogen-associated pattern that specifically binds to CD1c and supports recognition. Although less studied than CD1b-restricted T cell responses, CD1c-restricted T cells recognizing synthetic mycoketide homologs, mannosyl-phosphodolichols, have been observed to be expanded in number in the blood of human *M. tuberculosis* infected patients as compared to uninfected controls, providing evidence for an in vivo immune response to this phospholipid antigen during natural human infections [61].

4 Function of Group 1 CD1 In Vivo

The in vitro generated data discussed above suggest a possible function of group 1 CD1 in mycobacterial infection by providing proof of principle that mycobacterial lipids can be recognized by T cells and by identifying many such examples through the study of T cell clones. New data for presentation of mycobacterial lipids to polyclonal T cells in vivo or ex vivo provide direct evidence for a function of group 1 CD1 during mycobacterial infection in animal models and humans.

Guinea pigs (*Cavia porcellus*), a species in which group 1 CD1 is represented by multiple CD1b and CD1c orthologs, have been used in immunization experiments with *M. bovis* Bacille Calmette-Guérin (BCG) or with partially purified lipid extracts of *M. tuberculosis,* and were shown to develop strong proliferative and cytolytic T cell responses restricted by CD1b and CD1c [65, 66]. Guinea pigs are naturally highly sensitive to infection with *M. tuberculosis*, but immunization with *M. tuberculosis* lipids resulted in significantly smaller lung lesion size upon aerosol challenge with *M. tuberculosis* than vehicle only immunized animals. The level of protection was comparable to BCG vaccinated animals [67].

Cattle (*Bos taurus*) express multiple CD1a and CD1b proteins, but lack a gene for CD1c. Cattle are sensitive to natural infection with *M. bovis*, causing bovine tuberculosis that shares many clinical and pathological characteristics with human tuberculosis. Also, *M. avium paratuberculosis* causes bovine paratuberculosis, also known as Johne's disease. Polyclonal T cells of naturally infected animals have been stimulated with lipid antigens, and despite considerable overlap in lipid content, lipid extracts from *M. bovis* and *M. avium paratuberculosis* could

specifically stimulate T cells from cattle infected with *M. bovis* and *M. avium paratuberculosis* respectively, with very little crossreactivity [56]. The immunodominant lipid in *M. avium paratuberculosis* is glucose monomycolate [56]. In uninfected animals, immunization experiments with pure glucose monomycolate demonstrated that a CD1b-restricted T cell response against glucose monomycolate was generated and could be detected until 4 months after the last immunization, but no antibody response was induced [68].

Working with guinea pigs, cattle, or any other animal species that naturally expresses group 1 CD1 is much more cumbersome than working with mice. To create the possibility to study group 1 CD1 mediated immunity in mice, a mouse that is transgenic for the human group 1 CD1 molecules has been created [69]. The expression pattern of the individual human CD1 proteins in this mouse reflects the pattern in humans. This mouse gave rise to multiple T cell lines that were reactive to human CD1 proteins in the absence of exogenous antigens. Upon immunization with *M. tuberculosis* lipids or infection with *M. tuberculosis*, T cells that recognize *M. tuberculosis* lipids presented by human CD1 proteins could also be detected. An alternative way to create mouse models for human CD1 proteins is the humanized SCID mouse [70]. An important difference between the human CD1 transgenic mice and the humanized SCID mice is that the latter also has a human T cell compartment, while the human group 1 CD1 transgenic mouse uses its endogenous murine TCR gene segments.

Working with T cells from *M. tuberculosis* exposed human subjects provides the most physiologically relevant data on function of group 1 CD1 but in the past was mainly limited to studies of human clones in vitro. However analysis of fresh polyclonal T cells is feasible and is becoming more widespread with the recent invention of new tools. One approach is to stimulate ex vivo human T cells with group 1 CD1-expressing antigen presenting cells and *M. tuberculosis* lipids followed by detection of polyclonal T cell proliferation or the release of Interferon-γ directly. This approach has been taken to demonstrate the recognition of CD1c-restricted T cells recognizing phosphomycoketides selectively expanded in human *M. tuberculosis* infected patients, providing evidence for an in vivo immune response [61]. Also, CD1b-restricted responses against glycerol monomycolate were detected ex vivo in latent tuberculosis patients and BCG vaccinated individuals, but not in healthy controls or patients with active tuberculosis [36]. Glucose monomycolate was recognized by ex vivo T cells from latent tuberculosis patients, but not by T cells from healthy donors, patients with active tuberculosis, or BCG vaccinated individuals [55]. CD1b-restricted responses against mycobacterial sulphoglycolipid have been demonstrated in both latent and active *M. tuberculosis* infected patients, but not in healthy controls [40]. Finally, responses to mycolic acid were detected in active tuberculosis patients but not in healthy controls. During treatment, the mycolic acid-specific T cell population contracted and became mostly undetectable at 6 months after initiation of treatment, but T cells specific for the mycobacterial proteins ESAT-6 and CFP10 were still detectable [54].

An alternative approach to demonstrate group 1 CD1 T cell responses during mycobacterial infections in human subjects is the use of tetramers made from group 1 CD1 proteins. Two advantages of using tetramers is that CD1-restricted,

antigen specific T cells can be visualized directly ex vivo without stimulation, and antigen specific cells can be physically captured in a live state after flow cytometric sorting. Tetramer studies of human group 1 reactive T cells were first reported last year using a glucose monomycolate-loaded CD1b tetramer. A small CD4+ T cell population was clearly detectable in tuberculosis patients, while no such population could be detected using unloaded CD1b tetramer. Sorting of this population confirmed the CD1b restriction and antigen specificity of these cells in an Interferon-γ ELISPOT assay and provides new avenues of research into TCR diversity and natural effector functions [53].

5 Characteristics of Group1 CD1-Restricted T Cells

The different mammalian group 1 CD1 genes and the chemical diversity of the antigens that can be presented by the group 1 CD1 proteins can only exert an effect on the course of an infectious disease, if there are also group 1 CD1 responsive immune cells. The evidence that T cells respond to antigen presentation by CD1 is very strong, but interactions of CD1 with a receptors other than the TCR were recently demonstrated in reports that showed that Ig-like transcript 4 (ILT4) expressed on monocytes can bind CD1c and CD1d [71, 72]. Binding of molecules other than TCR has also been demonstrated for classical MHC class I molecules and is by no means inconsistent with the activation of T cells as the main function of these molecules.

Very low precursor frequency MHC class I- and II-restricted T cells expand upon primary exposure to their cognate antigen, and this forms the basis of immunological memory. Therefore it was a surprise that invariant NKT cells, a subset of T cells that are restricted by CD1d, exist as a population with a very high precursor frequency with an activated phenotype. Immunization with their cognate antigen does not durably change their precursor frequency and phenotype. It is unknown whether CD1a-, CD1b-, and CD1c-restricted T cells behave like MHC class I- and class II-restricted cells, or rather like NKT cells. Pathogen-specific CD1a-, CD1b-, and CD1c-restricted T cell responses are virtually undetectable in blood in naïve individuals, and they appear upon priming with antigen either by infection in vivo with CD1-presented antigen-producing pathogens or by experimental immunization with CD1-presented antigens. These responses can be detected several months after the initial exposure with antigen or immunization, but it is unknown how long they persist in the absence of antigen. Thus, the question whether group 1 CD1-restricted T cells generate years-long durable memory responses like conventional MHC-restricted T cells has not yet been resolved.

Double-negative (CD4-CD8-) cells were among the first CD1a-, CD1b-, and CD1c-restricted T cells that were described [27]. This observation is likely attributable to the fact that early studies of CD1 specifically deleted CD4+ cells from cultures to reduce alloreactivity rather than the lack of naturally occurring CD4 cells that recognize group 1 CD1 proteins. More recent studies have detected many CD4+ CD1b-restricted clones, and T cells sorted with CD1b tetramers bound to glucose monomycolate are predominantly or exclusively CD4 positive [26, 36, 53, 73, 74].

The early in vitro studies on reactivity of CD1a-, CD1b-, and CD1c-restricted T cell clones and also polyclonal T cell responses have emphasized assays that determine cell proliferation, cytotoxicity, or Interferon-γ release, which are anti-mycobacterial effector mechanisms. Indeed, most known mycobacterial lipid-specific T cells can efficiently kill infected cells, which is a phenotype that suggests that these cells might contribute to immunity against mycobacteria [27, 46, 73, 75]. Interestingly, the release of granulysin, a mycobactericidal peptide, in cytolytic granules been described and has been shown to be able to kill *M. tuberculosis* bacteria [76]. However, T cell clones may show functional drift during in vitro culture. Therefore, candidate effector functions previously identified in clones must now be tested using new methods to survey fresh polyclonal T cells.

A conceptual problem with a primary cytotoxic and mycobactericidal role is that CD1a, CD1b, and CD1c are not expressed on macrophages, which form the bulk of the infected cells during tuberculosis. Also, arguing against a mainly cytotoxic and mycobactericidal function in vivo are the low percentages of circulating CD1b-restricted cells that have been detected compared to the percentages of cytotoxic MHC class I-restricted T cells during acute viral infection [53, 54]. The relatively low absolute precursor frequency in the blood raises the question as to whether these cells might migrate from the blood toward infected sites. CD1-restricted T cells might have helper functions by which the effect of a small number of antigen-specific T cells might be amplified through other effector cells, a concept that is well established for NKT cells.

It must be noted that our view of the T cell response is determined by the choice of the T cell assays. Because release of Interferon-γ is thought to be beneficial to the host during mycobacterial infection, and sensitive reagents for its detection have been widely available, it may have been the preferred readout for T cell function for convenience sake rather than the dominant natural effector function of cells in vivo. Whether CD1a-, CD1b-, and CD1c-restricted T cells perform other tasks in vivo, including regulatory, Th1, Th2, Th17-like helper-like functions, or have completely novel functions, remains to be established. These questions could be addressed with more unbiased and descriptive techniques like gene expression profiling. The recently described technology of detecting CD1-restricted T cells using antigen-loaded tetramers should help in the elucidation of the function of these cells because the Th1 biasing effects of in vitro culture can be avoided and diverse effector functions might be detected.

Another piece of information about a T cell subset lies in the TCR itself. The available TCR sequences of T cells that recognize CD1a-, CD1b-, and CD1c-presented antigens show diverse variable region usage, incorporate non-template encoded amino acids, and otherwise do not contain any features that stand out that distinguish these TCRs from the TCRs of conventional peptide specific MHC class I- or II-restricted T cells. Due to the limited number of available TCR sequences from group 1 CD1-restricted T cell clones with known antigen specificity, little is known about whether subpopulations with invariant characteristics exist. With the advance of tetramer technology and discovery of novel group 1 CD1-presented antigens, more TCR sequences will become available and invariant group 1

CD1-restricted T cells may be identified. However, the possibilities of diverse and invariant group 1 CD1-restricted are not mutually exclusive. In fact, CD1d presents lipid antigens to invariant NKT cells, but also to T cells that do not fit the criteria of invariant T cells [77–79].

6 Concluding Remarks

The CD1 family of antigen presenting molecules is a varied group of molecules that is unevenly spread among mammalian species. The functions of the CD1a, CD1b, and CD1c isoforms might be as different from each other as from CD1d. Although all the CD1 isoforms can present mycobacterial lipids to T cell clones, data suggest that for CD1a self-antigen reactive T cells are very common. In contrast, CD1b has most convincingly and repeatedly shown to present mycobacterial antigens in physiologically relevant settings. It is the right time now to to focus on animal models and study of fresh human T cells in the ex vivo setting to expand the knowledge of group 1 CD1-restricted T cells, bringing it to a level that equals our extensive knowledge of classical MHC- and CD1d-restricted T cells, and prepare the road for application of lipids in vaccines against tuberculosis.

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