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# SYNERGISM AND COMPLEMENTARITY BETWEEN HUMAN CD1 AND MHC-RESTRICTED T CELLS, TWO LYMPHOID SUBSETS DIRECTED AGAINST DISTINCT ANTIGENIC WORLDS

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## TABLE OF CONTENTS



## 1. ABSTRACT

The antigenic repertoire of T cells has long been considered as exclusively composed of proteins and peptides. This view has been recently challenged by the discovery of CD1 molecules, a set of weakly polymorphic MHC class I-related receptors able to present lipid antigens to T cells. An updated picture of the biology of human CD1-restricted T cells is provided here, which highlights the unique features of these lymphoid effectors and the way they synergize with other innate and adaptive immune players to ensure protective immunity against tumors and pathogens.

## 2. INTRODUCTION

During the first 10 years following their identification (1), CD1 molecules were merely considered as useful markers of intrathymic T cell subsets. The first isolation of CD1-restricted T lymphocytes by S. Porcelli, M. Brenner and colleagues in 1989 (2), and the demonstration soon after that some CD1-restricted clones recognized bacterial antigens of glycolipid nature (3) unveiled unique biological features of the CD1 system, which have been largely confirmed since then by numerous studies, and have been extensively reviewed in several recent papers (see e.g. refs 4-7). In the present review, we will summarize recent findings that have provided important insights into (i) the way CD1 molecules can scan lipids from various subcellular compartements and present them to T cells, (ii) the diversity, specificity and effector functions of CD1-restricted T cells and (iii) the beneficial or deleterious roles played by CD1-restricted T cell responses in a variety of physiopathological contexts. While this overview will primarily focus on human cells, the observations will be put into perspective with studies performed in other species, particularly when addressing the structural and functional features of CD1d-restricted T cells, a lymphoid subset that display an unprecedented level of conservation between several mammalian species.

## 3. HUMAN CD1 GENES AND STRUCTURE

The human CD1 gene cluster is located outside the MHC complex (on chromosome 1, region q23.1) and comprises 5 functional genes designated *CD1A* to *CD1E* (see for reviews 4,7). These weakly or non-polymorphic genes code for five isoforms of which four (CD1a,b,c and d) are found on the cell surface and involved in immune cell activation. CD1 a,b and c isoforms (also referred to as group 1 CD1) (4) are present in primates, and orthologs of some of these isoforms have been identified in several

**Table 1.** Specificity, TCR phenotype and cytokine profiles of human CD1-restricted T cells

| Physiopathological context | Antigen | | CD1 molecule | TCR | Cytokines profile |
|---|---|---|---|---|---|
| - | Sphingolipids | α-GalactosylCeramide | CD1d | $\alpha\beta^{inv}$ | Th1/Th2 |
| | | | CD1d | αβ | Th1/Th2 |
| | | OCH | CD1d | | Th2 |
| **Mycobacterial infections** | Lipopeptides | Didehydroxymycobactins | CD1a | αβ | Undefined |
| | Mycolates | Free mycolic acid | CD1b | αβ | Th1 |
| | | Glucose monomycolates | CD1b | αβ | Th1 |
| | Diacylglycerols | Phosphatidylinositol | CD1b | αβ | Th1 |
| | | Phosphatidylinositol mannosides | CD1b | αβ | Th1 |
| | | | CD1d | $\alpha\beta^{inv}$ | Th1 |
| | | Lipoarabinomannan | CD1b | αβ | Th1 |
| | Sulfoglycolipids | Diacylated sulfoglycolipid | CD1c | αβ | Th1 |
| | Phosphoisoprenoids | Mannosyl-b1-phosphodolichols | CD1c | αβ | Th1 |
| **Self recognition (autoimmunity, tumors,…)** | Glycosphingolipids | GM1 | CD1b | αβ | Th1 |
| | | Sulfatide | CD1a,b,c | αβ | Th1/Th2 |
| | Diverse | Undefined | CD1a | αβ | Th1 and/or Th2 |
| | | Undefined | CD1b | αβ | Th1 and/or Th2 |
| | | Undefined | CD1c | γδ | Th1 |
| | | Undefined | CD1d | $\alpha\beta^{inv}$ | Th1 and/or Th2 |

mammalian species (most notably in cattle and guinea pigs) but are absent in rodents. Orthologs of CD1d (also referred to as group 2 CD1) have been identified in most mammalian species studied so far, including rodents (4,7).

CD1 proteins comprise 3 extracellular Ig-like domains called alpha 1, alpha 2 and alpha 3. The alpha 3 domain, which is highly homologous among CD1 isoforms, shows low but significant homology with MHC class I alpha 3 domain (8). Furthermore like MHC class I, surface CD1 proteins are associated with beta2 microglobulin (9,10). Expression of surface CD1d molecules devoid of beta2m has been documented on some cell types, such as intestinal epithelial cells (11), but the functional significance of these observations remains unclear.

Crystallographic analyses of human CD1a, CD1b and murine CD1d isoforms have revealed a quaternary structure very similar to that of MHC class I molecules, with an antigen-binding superdomain composed of two ? helices lying over a platform formed by a set of beta-strands (12-14). The antigen binding groove is significantly deeper and larger and much more hydrophobic than that of MHC class I, thus explaining the trend of CD1 molecules to bind lipid antigens. While the binding groove of CD1d (12) and CD1a (14) comprises two large hydrophobic pockets called A' and F' communicating with a single opening at its center, that of CD1b is made of a network of hydrophobic tunnels called A', C', F' and T', that can accomodate lengthy acyl chains (13,15). These channels communicate with two openings, one located on top of the CD1b groove where the hydrophilic part of glycolipid Ag can protrude, and another one located under the alpha2 helix, which allows protrusion of very long alkyl chains. Analyses of glycolipid / CD1 complexes showed in all instances insertion of the alkyl chains within the hydrophobic pockets and channels, and positionning of hydrophilic components on top of the CD1 groove, where they can interact with T cell receptors. Resolution of the 3D structure of CD1a in complex with sulfatide suggests that the narrow A' pocket, which accomodates the lipidic part of the molecule, acts as a « molecular ruler » to select alkyl chains of restricted length (14). In this regard, sharing between all group 1 CD1 isoforms of this A' pocket is consistent with the ability of all these isoforms to bind sulfatide. Crystal structure of CD1b in complex with short alkyl chain glycolipids (with less than 30 – 40 methylene subunits) shows occupancy of only the A' and C' channels of CD1b (13). As both channels should be readily accessible under neutral conditions, this should allow loading of short tail glycolipids in non-endosomal compartment, as suggested by functional studies (see § 2 and 3). By contrast glycolipids with longer acyl chains occupy all four channels (15), whose accessibility might depend on CD1b conformational changes that are achieved only in an endosomal low pH environment. This would explain the drastic inhibition of T cell responses against of several CD1b-restricted microbial lipids by inhibitors of endosomal acidification (see below).

## 4. CD1-SPECIFIC T CELL RESPONSES

### 4.1. Antigen specificity of CD1-restricted T cells

Since the initial characterization of several T cell clones directed against CD1b and CD1c more than 20 years ago, T cell responses restricted by all CD1 isoforms have been documented in a variety of physiopathological contexts (Table 1). While a growing number of CD1-restricted foreign lipids (mainly of microbial origin) have been characterized, CD1-restricted antigens recognized by self-reactive T cell clones remain in most instances unknown.

#### 4.1.1. CD1-restricted foreign antigens

The vast majority of T cell clones recognizing CD1-restricted microbial antigens have been derived from either mycobacteria-infected patients or BCG-vaccinated healthy donors (Table 1).

- Recognition of didehydroxymycobactins (DDM) by a CD1a-restricted T cell clone has been recently reported (16). Consistent with the intracellular trafficking of CD1a, presentation of these lipopeptides requires internalization of CD1a but does not require loading in low pH compartments, as indicated by efficient CD1a-restricted Ag presentation in the presence of concanamycin B. DDM and related siderophores are involved in iron scavenging: these genes, whose expression is upregulated in low-iron conditions, contribute to mycobacteria survival within macrophages (17). In this regard, CD1a-restricted presentation of DDM may allow early warning of the immune system and rapid elimination of growing intracellular mycobacteria.

- Mycobacterial glycolipids presented by CD1b fall into at least 3 categories : mycolates (free mycolic acid (3) and glucose monomycolate (18)), diacylglycerols (PIM and LAM) (19) and sulfoglycolipids (such as diacylated sulfoglycolipids) (20). Most of these Ag carry long and or complex alkyl chains, and endosomal acidification is needed for their presentation by CD1b (6,19,21,22).

- A large fraction of CD1c-restricted mycobacteria-specific T cells react to mannosyl phosphoisoprenoid Ag (23). The fact that tail-deleted CD1c (which can no longer be targeted to endosomal compartments) can still present these glycolipids to reactive T cell clones indicates that loading of these Ag, unlike that of most CD1b- and CD1d-restricted Ag, does not require an acidic environment (24,25). CD1c may also present diacylated sulfoglycolipids (Ac2-SGL) as suggested by the effect of blocking anti-CD1c antibodies on Ac2-SGL-specific T cell responses elicited in PBL from tuberculosis patients (20).

- The existence of environmental lipid Ag presented by CD1d remains controversial. Schofield and colleagues (26) have described several protozoan glypiated molecules able to activate in a CD1d-restricted fashion murine NKT cells, a highly conserved CD1d-restricted subset in mammals that carry invariant TCR alpha chains with « canonical » junctions (see § 2.2). However these results have not been confirmed in subsequent studies using either murine NKT cells (27,28) or human ones (our own unpublished results). Some mycobacterial diacylglycerols (such as PIM2) can elicit *in vivo* trapping of NKT cells within granuloma but this phenomenon is CD1d-independent. More recently, we obtained strong evidence for the existence of mycobacterial ligands, assigned to PIM4 based on mass spectrometry analyses, able to activate in a CD1d-restricted manner murine and human NKT cells bearing invariant TCR alpha chains. In particular CD1d-restricted recognition of this compound is directly supported by the binding of CD1 tetramers loaded with PIM4 to NKT cell clones (Fischer *et al. unpublished*). Consistent with a role for PIM4-reactive T cells in protective immunity against M. tuberculosis infection, PIM4 triggers IFNgamma production and lysis of CD1d-transfectants by NKT cells but does not trigger Th2 cytokine production (Fischer *et al. unpublished*).

#### 4.1.2. CD1-restricted mammalian glycolipids and self-reactive CD1-restricted T cells

Self-reactive T cells restricted by all CD1 isoforms have been isolated (Table 1) (2,29,30). As suggested by a recent study (31), these cells may represent a very significant fraction of the peripheral lymphoid pool, as 14/500 randomly isolated PBL clones turned out to be CD1a, b or c autoreactive. In most instance, the recognized self antigens have not been characterized, though some mammalian sphingolipids (such as sulfatide and GM1 ganglioside) have been found to stimulate T cell responses in the context of either CD1a, CD1b or CD1c (32,33). T cell clones recognizing phosphatidylethalonamine and other common phospholipids have been isolated in the mouse (34) but the generality of these responses, which have not been documented in humans, remains unclear.

#### 4.1.3. CD1d-restricted recognition of alpha -Galactosyl-ceramide

As detailed by Taniguchi and colleagues in a companion review (35), a significant fraction of murine and human CD1d-restricted T cells recognize a marine sponge-derived glycolipid (alpha -galactosyl ceramide, alpha GalCer) in complex with CD1d (30,36-38). The fact that betaGalCer is not recognized indicates that antigenicity of alpha GalCer critically depends on the alpha -anomeric linkage of its sugar, which is never found among mammalian ceramides. Therefore although alpha GalCer has turned out to be an extremely useful reagent to study the activation of CD1d-restricted cells *in vitro* and *in vivo* (35), the relevance of its recognition to mammalian immunity remains largely unclear.

### 4.2. TCR repertoire of CD1-reactive T cells

T cells restricted by group 1 CD1 isoforms express either alpha/beta or gamma/delta TCR (2). The limited TCR repertoire analyses performed so far suggest expression of highly diverse TCR by CD1a, b and c-resticted T cell clones with no evidence of biased V region usage or recurrent junctional motifs (39). However owing to the small number of clones studied to date and their highly heterogeneous reactivity patterns, such conclusions need to be further substantiated by more extensive repertoire studies performed on a much larger panel of group 1 CD1-reactive T cells.

A large fraction of CD1d-restricted T cells carry TCR with highly conserved features that are uniquely conserved within mammals (40,41). Their TCR $\alpha$ chains are almost invariant, as they comprise the same set of rearranged VJ elements (Valpha 14Jalpha 18 in the mouse, Valpha 24Jalpha18 in humans) with a canonical junctional sequence generally devoid of N diversity and generated through a «primordial » rearrangement event (42). These cells, (also referred to as Valpha14$^{inv}$ or Valpha24$^{inv}$ NKT

cells, owing to frequent expression of NK receptors by this subset, see § 5) use a limited set of Vbeta elements (Vbeta2, Vbeta7 and Vbeta8 in the mouse, Vβ11 in humans) but their TCRbeta junctional sequences are quite diverse (42). How heterogeneous is the antigenic repertoire of CD1d-restricted cells bearing invariant TCRalpha chains is still an open question. While all Valpha14$^{inv}$ and Valpha24$^{inv}$ NKT cells recognize CD1d/alphaGalCer complexes, they differ by their ability to be readily activated by CD1d+ antigen presenting cells (30). Moreover some - but not all - of them react to mammalian sphingolipids (such as GD3 (43) or to microbial diacylglycerols (such as PIM4) (unpublished), suggesting a heterogeneous glycolipid repertoire. However these distinct reactivity patterns could also reflect differences in TCR affinities toward a similar set of CD1d/Ag complexes. Irrespective of this issue, these results strongly suggest that the TCRbeta chain influence the CD1d reactivity of mouse Valpha14$^{inv}$ and human Valpha24$^{inv}$ T cells.

CD1d-restricted cells expressing TCRalpha chain distinct from the canonical murine Valpha14 $^{inv}$ or human Valpha24 $^{inv}$ chains have also been described in these two species (38,43-45). Cerundolo and colleagues recently identified in humans a subset of alphaGalCer/CD1d-reactive T cells which carried TCR with diverse Valpha and Vbeta region usage, though a biased Vbeta11 region usage was noticed, suggesting a dominant contribution of this Vbeta region to recognition of CD1d loaded with this particular glycolipid. However clones bearing non-canonical TCR alphachain showed a much lower affinity for alphaGalCer/CD1d than Valpha24$^{inv}$ clones (38). The majority of human bone-marrow derived CD1d-restricted T cells have been found to express non canonical TCR and to recognize better CD1d+ lymphoid cells than CD1d+ Hela cells, unlike Valpha24$^{inv}$ NKT clones (45). Altogether these results suggest recognition of distinct sets of yet unidentified antigenic glycolipids by clones expressing or not canonical TCR.

### 4.3. Interaction between TCR and CD1/Ag complexes

Contribution of TCR to recognition of several glycolipid Ag in the context of CD1 has been formally documented through TCR mutagenesis and transfection approaches (e.g. ref 39). More recently surface plasmon resonance analyses of the interactions between soluble TCR from murine Valpha14$^{inv}$ clones and CD1d/alphaGalCer demonstrated high affinity binding (in the $10^{-6}$ – $10^{-7}$ M range), with fast association rates and very slow dissociation rates when compared to « conventional » TCR/MHC/peptide interactions (46,47). These high affinity interactions might be a general feature of non conventional T cell subsets, as similar high affinity / high avidity interactions were noted between a murine gamma/delta TCR and an MHC class I b protein antigen (48). Although resolution of the 3D structure of a TCR in complex with CD1 has not been achieved yet, primary contacts between the TCR and the hydrophilic part of glycolipid antigens are strongly suggested by the dramatic effects on TCR recognition of even small changes in the sugar moeties of CD1-restricted glycolipids (18,36).

### 4.4. CD4 / CD8 phenotype of CD1-restricted T cells and contribution to TCR/CD1 interactions

Contribution of CD4 and CD8 coreceptors to TCR/CD1 interaction remains debated, and may depend on the CD1 isoform recognized and the species studied.

Group I CD1-specific T cells have been initially identified within peripheral T cells devoid of CD4 and CD8 coreceptors (hereafter referred to as double negative, DN) (2). However subsequent studies have shown that a large fraction of CD1a, b and c-restricted clones are actually CD4+ and CD8+ (20,49,50). For instance out of ten recently isolated CD1-restricted T cell clones directed against mycobacterial Ag, 8 were CD8+ and 2 were CD4+ (20). Accordingly substantial CD1-restricted responses have been detected within both CD4+ and CD8+ PBL derived from PPD+ donors and mycobacteria-infected patients (51,52). It is still unclear whether or not coreceptor expression on T cells directed against group 1 CD1 molecules actually reflect co-engagement of TCR and coreceptors in the recognition process, as is the case for conventional MHC-restricted T cells. While blocking anti-CD8 mAb do not readily inhibit antigenic activation of CD1a- and CD1c-restricted T cell clones, partial inhibition is achieved if these Ab are used in conjonction with suboptimal concentrations of anti-CD2 Ab (49). This is consistent with a direct engagement of CD8 coreceptors by these CD1 isotypes but a non-specific effect of anti-CD8 antibodies cannot be ruled out. Surface plasmon resonance studies have so far failed to demonstrate specific interactions between CD8 and group 1 CD1 molecules (53). However interpretation of these negative results has been hampered by the very low affinity of such interactions, which are expected to be in the 100 microM range based on analysis of CD8/MHC class I binding (54).

With respect to CD1d-restricted NKT cells, both human and murine cells bearing respectively canonical Valpha24 or Valpha14 TCR, are either CD4+ or DN (42,53). As these cells never express heterodimeric CD8 molecules in the mouse, this suggests that CD8 increases the avidity of Valpha14$^{inv}$ TCR for self CD1d/Ag complexes, thus resulting in intrathymic negative selection of the CD8alpha/beta Valpha14$^{inv}$ subset (42,53). Accordingly forced expression of CD8 results in early deletion of T cells bearing Valpha14$^{inv}$ TCR (53). While human NKT cells frequently express intermediate levels of CD8alpha/alpha homodimers (55), they are generally devoid of heterodimeric CD8alpha/beta coreceptors, of presumably higher affinity than CD8alpha/alpha for classical MHC class I (54). Therefore this is consistent with the negative selection of the CD8alpha/beta Valpha24$^{inv}$ subset. However peripheral NKT cells bearing heterodimeric CD8alpha/beta have been described in some cases (56), suggesting either escape from intrathymic deletion or weaker contribution of CD8 to CD1d/TCR interaction in humans than in the mouse. Specific interaction between CD8 and human CD1d has been recently supported by a recent analysis of alphaGalCer/CD1-reactive clones bearing non-canonical TCR (38). Unlike Valpha24$^{inv}$ clones, these cells are either

CD8alpha/beta or CD4+ but are never DN. Moreover their activation by alphaGalCer-loaded CD1d+ cells is CD8-dependent, as suggested by blocking experiments using anti-CD8 antibodies (38).

The existence of CD1-restricted clones bearing CD4 naturally raises the possibility that this coreceptor may interact with CD1 molecules as well. Consistent with this hypothesis, we recently documented inhibition of CD1d-mediated activation of Valpha24$^{inv}$ T cell clones by either CD4-specific mAb or soluble molecules interfering with CD4 interactions (e.g. HIVgp120) (unpublished). Along this line, the dissociation rates of interactions of soluble CD1d/alphaGalCer complexes on CD4 Valpha24Vbeta11 clones were found to be much slower than on DN clones expressing the very same TCR (unpublished). Specific binding of CD4 to CD1d is also consistent with structural data, showing conservation between CD1d and MHC II of most residues interacting with CD4 (12). CD4/CD1d interactions may have important functional consequences, as they could result in potentiation of some effector functions and explain the functional dichotomy observed between human CD4 vs DN NKT cells in humans (see § 5). On a philogenetic standpoint, the unique ability of some CD1 isoforms (such as human CD1d) to bind both CD4 and CD8 coreceptors would be consistent with the view that CD1 represent ancestors of both MHC class I and class II molecules.

## 5. INTRACELLULAR TRAFFICKING AND MODALITIES OF ANTIGEN LOADING ON HUMAN CD1 MOLECULES

Intracellular trafficking of CD1 molecules greatly differs from one isoform to another : this heterogeneity results in lipid loading in distinct subcellular compartments by each CD1 isoform, and allows efficient scanning of the whole cellular lipid content by the CD1 system (see for recent reviews 6,7).

In brief, soon after their translocation into the ER, CD1 chains associate with beta2m and are stabilized upon loading with endogenous short tail lipids, such as phosphatidylinositol (PI). Then they exit to the cell surface where CD1 isoforms may interact with adaptor protein complexes (AP), that govern their subsequent delivery to particular subcellular compartments. CD1a, which is devoid of tyrosine-based late endosome sorting motifs, remains on the cell surface, in clathrin-coated pits and early sorting endosomes (57), where PI can be exchanged with short tail lipid antigens of either endogenous or exogenous origins (see § 2.1). Binding of AP2 to YXXZ endosome sorting motifs carried by all other CD1 isoforms allows their delivery to late endosome. While CD1c remains in the endosome (24,25), CD1b and CD1d can be further translocated to lysosomes / MIIC through a process dependent on AP3 in the former case (58), or possibly on a functional dileucine motif in the latter case (6). In this regard unlike their murine orthologs, human CD1d does not associate with AP3 (6).

The endosomal / lysosomal compartments may provide optimal conditions for CD1-loading of glycolipids with long and/or complex acyl chains, for at least 3 reasons. Firstly as mentionned above, the loading efficiency of long tail lipids is greatly increased under acidic conditions (6,19,21-22). In this respect compartmentalization of lipid loading according to lipid length has been directly documented through a comparative analysis of CD1b loading of glucose monomycolate with acyl chains of different lengths (59). Secondly several endosomal proteases may contribute not only to intracellular trafficking of CD1d, but also to optimal processing of either Ag or endosomal enzymes involved in Ag delivery. These assumptions are supported by the reduced NKT cell development in mice and altered processing of digalactosylceramide associated with defects in cathepsin S and L (60,61). Thirdly several recent studies have highlighted the key role played by saposins, a family of endosomal lipid-transfer proteins, in lipid loading into endosomal CD1 isoforms. SAP-C, but not other saposins, seems to play a key role in lipid loading into CD1b (62), whereas SAP-A or GM2 activator proteins may contribute to CD1d lipid loading, depending on the lipid structure (63,64). Prosaposin deficiency seems to differentially affect development/activation of human vs murine NKT cells : while it abrogates development of murine CD1d-restricted cells bearing Valpha14$^{inv}$ TCR (64), it has limited effect on the *in vitro* activation of human CD1d-selfreactive clones bearing Valpha24$^{inv}$ TCR (63). These differences may reflect either distinct readouts used in these studies (*in vitro* vs *in vivo* activation may require distinct T cell activation thresholds), distinct modalities of intracellular trafficking of human vs murine CD1d and/or recognition of distinct endogenous glycolipids by selfreactive CD1d-restricted T cell clones in each species. Consistent with either of the latter two possibilities, tail-deleted CD1d mutants are still able to activate human, but not murine, CD1d-restricted clones bearing canonical TCRalpha chains (29).

## 6. TISSUE DISTRIBUTION OF HUMAN CD1 MOLECULES

Group I CD1 isoforms are found on cortical thymocytes (which express CD1a,b and c), on subsets of B cells and on several professional antigen-presenting cells including dendritic cells (DC) (4,7). CD1c is expressed by B cells from germinal center and mantle zone of lymph node and marginal zone from the spleen (7). DC subsets differ by their expression patterns of CD1 isoforms, Langherans cells being CD1a+ and CD1c+ but devoid of CD1b, whereas dermal and interdigitating DC strongly express CD1b (65,66). Upregulation of group I CD1 molecules in response to some soluble factors (such as GM-CSF) may explain their expression on infiltrating APC (macrophages, DC, astrocytes…) in various physiopathological situations associated with chronic inflammation (such as autoimmunity and cancer) (7). This local upregulation of CD1 isoforms could result in triggering of CD1-restricted T cells, as suggested by the isolation of several sphingolipid-specific T cells derived from multiple sclerosis patients (32). However the physiopathological significance of these cells, which are also detected within the peripheral repertoire of healthy donors, remains to be established (32,33). While trafficking

of CD1 isoforms changes upon DC maturation, CD1 surface expression is already high on immature DC, unlike that of class II, and is not dramatically modified upon maturation (67). Accordingly glycolipid antigens can be at least as efficiently presented by immature than mature DC (68).

Regulation of CD1d expression greatly differs from that of other CD1 isoforms. Unlike group 1 CD1 molecules, CD1d is expressed at low levels on most professional APC (monocytes, macrophages, DC) and is not upregulated upon DC maturation (69). It is expressed at higher levels on a large fraction of B cells, within subsets that do not necessarily overlap with those expressing CD1 b or c isoforms (7). However like group 1 CD1, CD1d is expressed by cortical thymocytes, activated T cells (though at low levels) but not by resting T cells (70). CD1d is also expressed on several non-hemopoietic tissues, most notably on intestinal epithelial cells (11). Interestingly both heat shock proteins and inflammatory factors have been involved in the regulation of CD1 expression on the latter cell type (71).

## 7. PHENOTYPE AND EFFECTOR FUNCTIONS OF HUMAN CD1-RESTRICTED T CELLS

T cells directed against mycobacterial antigens presented by group 1 CD1 isoforms typically display a proinflammatory « Th1 » profile, and produce large amounts of TNFalpha and IFNgamma (49,50). Furthermore these cells efficiently kill in a perforin-dependent manner mycobacteria infected macrophages and inhibit bacterial replication through release of microbicidal compounds (such as granulysin) (49,72). CD1a, b and c self reactive clones are similarly polarized towards Th1 cells (31). By contrast clones directed against mammalian sphingolipids restricted by group 1 CD1 are either Th1 or Th2 (32,33).

Murine and human CD1d-restricted T cells bearing canonical TCR frequently express surface receptors found on NK cells (such as CD161, a member of the NKRP1 C-type lectin family), and for these reasons have been classically referred to as « NKT » cells (see for reviews 42,53). However such a nomenclature brings confusion as on the one hand not all murine Valpha14$^{inv}$ or human Valpha24$^{inv}$ express NKR, and on the other hand NKR (including NKRP1 members) are frequently found on conventional MHC-restricted T cells with a memory phenotype (42,73). Besides CD161, CD1d-specific cells with canonical TCR typically display several hallmarks of memory cells, as they are CD122+, CD3dim, CD45R0+, CD45RA-, CD62L-, CCR7- and frequentyl express CD94 (73,74). Like several other non-conventional T lymphocytes (such as gamma/delta T cells), acquisition of these phenotypic features parallels actual numerical expansion of this subset, which occurs early in life presumably upon recurrent exposure to yet unidentified environmental or self antigens. For these reasons, CD1d-restricted NKT cells have been sometimes referred to as « natural memory cells » (42).

While there is no clearcut functional dichotomy between DN and CD4+ Valpha14$^{inv}$ subsets in the mouse (53), functional polarization of Valpha24$^{inv}$ T cells clearly correlates with their coreceptor phenotype (73,74). While DN cells produce primarily Th1 cytokines (such as IFNgamma and TNFalpha), are NKG2D+ and Perforin-hi upon IL2/IL12 exposure, CD4+ Valpha24$^{inv}$ cells produce both Th1 and Th2 cytokines, upregulate CD95L and perforin upon PMA / Calcium ionophore (but not IL2 or IL12) exposure (73,74). Like group 1 CD1-restricted T cells, human Valpha24$^{inv}$ T cells inhibit the growth of intracellular *M. tuberculosis*, presumably through production of granulysin (75). Like Valpha24$^{inv}$ clones, alphaGalCer/CD1d-specific clones with non-canonical TCR produce either IL4, IFNgamma, or IL13 (38). However it not known whether or not their functional polarization correlates with a particular coreceptor phenotype. Finally unlike Valpha24$^{inv}$ NKT cells, bone marrow-derived CD1d-restricted T cells are frequently CD8+, mainly Th2-biased, and suppress MLR (45).

## 8. CROSSTALK BETWEEN CD1-RESTRICTED T CELLS AND OTHER INNATE / ADAPTIVE IMMUNE EFFECTORS

### 8.1. Dendritic cells

Incubation of immature DC (iDC), which express all CD1 isoforms, with self reactive clones directed against group 1 or group 2 CD1 leads to rapid upregulation of CD83, CD86 and surface MHCII, and an increased ability of DC to trigger proliferation of alloreactive T cells (31,76). Further DC maturation and polarization is controlled by both the strength of TCR/CD1 interactions and the secreted / membrane costimuli provided by the reactive T cell clone. In the presence of a weak TCR stimulus, CD1-restricted cells may promote generation of tolerogenic (IL10-producing) DC in response to limiting amounts of bacterial stimuli whereas a strong TCR stimulus will promote IL12-producing DC and subsequent Th1 responses (31). Functional polarization of DC is also critically dependent on the strength of the microbial stimuli. At high concentrations (in the microg range), LPS directly triggers a burst of IL12 by DC, which results in rapid DC « exhaustion » in the absence of T cell costimuli (77). However in the presence of CD1d-restricted invariant T cells, LPS-induced IL12 potentiates IFNgamma production by CD1d-restricted clones in response to low affinity interactions established between their TCR and self CD1d/Ag complexes. This leads then to sustained DC polarization and subsequent induction of Th1 responses (78). Potentiation by IL12 of IFNgamma production in response to low affinity TCR interactions has been similarly documented for conventional T cell responses (see for a review ref.79). It may represent an important mechanism allowing priming of Th1 responses, particularly in microbial immunity where IL12 production by DC can be promoted following interactions between microbial patterns and Toll like receptors.

### 8.2. NK, T and B cells

Early interaction between CD1-restricted cells and DC results in dramatic upregulation of IFNgamma and IL2 production by the former and IL12 by the latter, which in combination allow broad activation of lymphoid cells.

This phenomenon has been well documented *in vivo* in mice treated with αGalCer, in which activation of CD1d/alphaGalCer-reactive T cells results in rapid upregulation of activation markers on NK, T and B cells, potentiation of NK cytolytic activity and adaptive Th1 responses (see for reviews 42,53). In most instance, these effects were shown to be dependent on CD40/CD40L interactions between CD1d-restricted cells and DC and were inhibited by blocking anti-IL12 Ab. Such an adjuvant effect on proinflammatory responses mediated through activation of CD1d-restricted T cells has not been formally documented *in vivo* in humans, but is strongly suggested by several *in vitro* studies (see above). Human Valpha24$^{inv}$ were recently found to be as efficient as conventional CD4+ T cell in promoting proliferation of naive and memory B cell and inducing Ig production *in vitro*, CD4 NKT cells being more potent than DN ones (80). B cell help is CD1d-dependent but does not require exogenous Ag. Altogether this suggests a direct role for NKT in regulating B cell proliferation and functional maturation (80). It is still unclear how a generalized B cell activation can be avoided here : is Valpha24$^{inv}$ T cell activation controlled through transient upregulation of a restricted set of endogenous glycolipids or does this phenomenon remains restricted to specific sites (eg inflammatory) to which both Vα24$^{inv}$ T and B cells would migrate ?

## 9. ANTIMICROBIAL CD1-RESTRICTED T CELL RESPONSES

Direct contribution of group 1 CD1-restricted T cells to antimycobacterial immunity is suggested by their ability to recognize a variety of mycobacterial lipids (see § 2), to produce proinflammatory factors, to kill mycobacteria-infected macrophage and to release microbicidal compounds (see § 5). A role for these cells in immunity against a wider array of bacterial infections is supported by the existence of CD1-restricted antigens derived from non-mycobacterial microorganisms, such as Haemophilus influenza (81), and by the ability of self-reactive CD1a,b and c-specific T cells to potentiate conventional peptide-specific antibacterial Th1 responses through priming of IL12-producing DC (78). These assumptions, drawn from in vitro studies, are also supported by ex vivo analyses of group 1 CD1 expression and CD1-restricted responses in infected patients, and by in vivo studies performed in animal species expressing group 1 CD1 isoforms. In particular BCG-immunized subjects display a significant pool of CD8+ CD1-restricted T cells recognizing BCG-infected DC (52). Responses against live BCG-infected DC are mainly inhibited by anti-CD1b, to a lesser extent by anti-CD1a and CD1c, but not by anti-MHCI or II Ab (52). CD1c-restricted responses to mycobacterial phosphoisoprenoids are observed in M. tuberculosis-infected patients but not in healthy donors (23). Asymptomatic M. tuberculosis infected patients show stronger CD1-restricted responses than non infected donors, and the reduced responses observed in patients with active tuberculosis are restored upon chemotherapy (51). Finally guinea pigs vaccinated with mycobacterial lipids, which can be presented by CD1b or CD1c in this species (82), show reduced *M. tuberculosis* burden and reduced pathology after aerosol challenge (83).

A role for CD1d-restricted T cells in immunity against a wide array of infections is suggested by numerous *in vivo* studies performed in rodents, showing increased susceptibility of CD1d-deficient mice to bacterial (e.g. *P. aeruginosa*, *M. tuberculosis*, *B. burgdoferi*…), parasitic (e.g. *L. donovani*, *T. gondii*, *P. berghei*) and viral (e.g. herpes simplex, respiratory syncitial or encephalomyocarditis virus) infections (see for reviews 7,35). While in most models CD1d-deficiency was associated with impaired control of microbial infection, it turned out be beneficial to the host in few cases, either due to improved control of bacterial replication or to reduced immunopathology (7). The limited *in vitro* and *ex vivo* data obtained on human CD1d-restricted cells similarly suggest a prominent beneficial contribution of this subset to protective immunity against infections. In particular *in vitro* results indicate that like group 1 CD1-restricted cells, human CD1d-specific T cells may contribute to anti-infectious immunity both directly, e.g. through killing of mycobacteria-infected macrophages and inhibition of bacterial replication (75), and/or indirectly through priming of Th1 responses (see § 6)). Further altered CD1d-restricted responses observed in HIV- or HCV-infected patients (84,85) could partly explain patient impaired ability to control viral infection.

## 10. HUMAN CD1-SPECIFIC T CELLS AND ANTITUMOR IMMUNITY

Expression of some group 1 CD1 isoforms by leukaemic cells (86) and *in vitro* killing of CD1-expressing tumor cell lines by some self-reactive CD1-restricted CTL (2) suggest an implication of these lymphocytes in antitumor immunity. Accordingly a recent expression profiling of chronic B lymphocytic leukaemias indicates strong downregulation of CD1b and CD1c (when compared to normal B cell counterparts), which could reflect a tumor escape from CD1-restricted T cell immune responses (87). As already mentionned, self reactive CD1a,b,c-specific clones may also indirectly contribute to antitumor immunity through potentiation of NK cytolytic activity and potentiation of antitumor Th1 immunity, owing to their ability to promote maturation of IL12-producing DC (see § 6).

The reduced frequency and/or impaired *in vitro* response of CD1d-restricted (mainly Valpha24$^{inv}$) T cells derived from patients with advanced cancers suggests a role for CD1d-restricted responses in antitumor immunity (88-91). Human CD1d-restricted cells kill *in vitro* a variety of CD1d+ tumors, such as T cell leukaemias (30), myelomonocytic leukaemias (92) and multiple myelomas (90), and release antitumor cytokines (such as IFNgamma) upon TCR engagement. Therefore they may exert direct antitumor activity against CD1d+ targets. Accordingly the fact that freshly isolated Valpha24$^{inv}$ PBL from progressive myeloma patients, but not from non-progressive ones, show reduced IFNγ production upon *in vitro* stimulation with alphaGalCer, suggest their implication in immune surveillance against this tumor (90). However as the vast majority of tumor cells are CD1d-, it is likely that like their murine counterpart, human CD1d-restricted T cells

primarily contribute to antitumor immunity through indirect mechanisms, e.g. via potentiation of NK and/or conventional Th1 responses, possibly upon recognition of tumor-derived glycolipids crosspresented by DC, as suggested by a recent study (43). In this regard, alphaGalCer-stimulated Valpha24$^{inv}$ T cells have been shown to trigger NK cell cytotoxicity through IL2 and IFNgamma production (93,94).

## 11. ROLE OF CD1-RESTRICTED RESPONSES IN TOLERANCE AND AUTOIMMUNITY

As already mentionned sphingolipid-reactive T cells restricted by group 1 CD1 isoforms have been isolated from multiple sclerosis patients but their pathogenic role remains unclear as cells with similar reactivities have been also found in healthy donors (32,33). Nonetheless the fact that the recognized lipid antigens (sulfatide and gangliosides) are present in large amounts in neural tissues and that Th-dependent humoral responses against sulfatide are associated with several immunopathological processes (such as IDDM and cardiomyopathy associated with chronic Chagas disease) (95,96) suggest a direct role in the pathogenesis of organ-specific autoimmune diseases.

While a wealth of observations document a role, either deleterious or beneficial, of murine CD1d-restricted T cells in a vast array of autoimmune and allergic diseases (7,35), data supporting a similar contribution of human CD1d-restricted responses in these diseases remain scarce. Several studies suggest a decreased frequency of Valpha24$^{inv}$ T cells in patients with diabetes (97,98), systemic sclerosis (99), lupus (100), rheumatoid arthritis and inflammatory bowel disease (101) and multiple sclerosis (102). However a more recent in depth analysis of the frequency, phenotype and function of alphaGalCer/CD1-reactive NKT cells failed to confirm either decreased frequency or functional alterations of this subset in IDDM patients (103). In particular although the frequency of NKT cells was highly variable from one individual to another, their frequency and IL4 production were not modified during the course of IDDM (102). Discrepancy with previous results could be explained by the fact that NKT cells were initially identified by means of Valpha24-specific mAb (a large fraction of which are not stained by alphaGalCer/CD1d tetramers), and that several studies focused on DN subsets and therefore missed the CD4 subset, shown to be the major IL4 producer. Finally implication of Valpha24$^{inv}$ T cells in feto-maternal tolerance has been suggested, on the basis of expression of CD1d on villous and extravillous trophoblasts and an increased frequency of CD4+ Valpha24$^{inv}$ cells within decidual lymphocytes (104).

## 12. CONCLUSIONS AND PERSPECTIVES

The unique ability of CD1-restricted cells (i) to recognize a broad set of lipid antigens derived from either self tissues or environmental pathogens, (ii) to contribute either directly or indirectly to protective immune responses against tumors and infectious agents and (iii) to regulate positively or negatively a variety of immunopathological processes suggest their key and mandatory implication in both the early polarization and possibly termination of innate and adaptive immune responses. Like several other non-conventional subsets (such as gamma/delta T lymphocytes), CD1-restricted cells share several features with innate effectors (high frequency, recognition of conserved stimuli, swift activation kinetics…) which place them at frontier between innate and adaptive immunity. These features also make them attractive candidates for immunotherapeutic approaches, and accordingly clinical trials using synthetic agonists targeting a significant fraction of these lymphocytes, such as αGalCer, have been already initiated (105). However several important issues will need to be addressed before we can foresee broad clinical applications for compounds targeting CD1-reactive lymphocytes. For instance CD1-restricted T cell subsets targeted alphaGalCer can have either a beneficial or deleterious roles in immunity against e.g. tumors or pathogens. These somewhat opposite roles played by CD1-restricted cells could be explained not only by their broad effector functions, but also by their reactivity patterns towards conserved Ag whose upregulation on activated lymphocytes or transformed / infected cells may result in either immune suppression or immune activation. The use of agonists able to selectively activate Th1 or Th2 responses by CD1d-restricted cells may circumvent some of these problems and allow optimal exploitation of the broad biological activities of these lymphocytes.

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