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$\gamma\delta$ T cell surveillance via CD1 molecules

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Abstract

$\gamma\delta$ T cells are a prominent epithelial-resident lymphocyte population, possessing multi-functional capacities in the repair of host tissue, pathogen clearance, and tumor surveillance. Although three decades have now passed since their discovery, the nature of $\gamma\delta$ TCR-mediated ligand recognition remains poorly defined. Recent studies have provided structural insight into this recognition, demonstrating that $\gamma\delta$ T cells survey both CD1 and the presented lipid, and in some cases are exquisitely lipid specific. We review these findings here, examining the molecular basis for and the functional relevance of this interaction. We discuss potential implications on the notion that non-classical MHC molecules may function as important restricting elements of $\gamma\delta$ TCR specificity, and on our understanding of $\gamma\delta$ T cell activation and function.

Keywords

$\gamma\delta$ T cells; CD1d; lipids; crystallography; restriction; T cell receptor

Introduction

$\gamma\delta$ T cells are the most enigmatic among the three cellular branches comprising the adaptive immune system. Like B cells and $\alpha\beta$ T cells, $\gamma\delta$ T cells express a somatically rearranged cell-surface receptor, although much less is known about how antigen recognition by this T cell receptor (TCR) relates to $\gamma\delta$ T cell function. A recent resurgence in the study of $\gamma\delta$ T cells, spurred by the growing appreciation for immune responses in the mucosal and epithelial tissues where $\gamma\delta$ T cells are most numerous, has led to the illumination of several novel functions [1, 2]. These functions include potent IL-17 secretion capacity, antimicrobial peptide production within the intestinal mucosa, and memory recall responses within tissues, functions also shared by innate lymphoid cells (ILCs) and tissue-resident memory T cells (T_{RM}), which among the most hotly investigated cell types in current immunology.

Characterization of $\gamma\delta$ T cell responses to specific antigens has been challenging, in part because the rules learned from extensive work on $\alpha\beta$ TCR recognition have not applied. In fact, the nature of $\gamma\delta$ T cell antigens themselves and a general paradigm for the logic of $\gamma\delta$

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TCR recognition remain poorly defined. Given how profoundly the deciphering of MHC restriction affected our understanding of classical $\alpha\beta$ T cell biology, the recent molecular insights into $\gamma\delta$ T cell recognition of CD1d provide fundamental progress towards decoding the general principles of $\gamma\delta$ TCR recognition. We review these findings here, in the context of the features characteristic of $\gamma\delta$ TCR recognition that present unique challenges, and discuss implications to our understanding of $\gamma\delta$ T cell development and function in both health and disease.

The elusive ligand repertoire of $\gamma\delta$ T cells

A confounding feature in the effort to understand, in a generalizable way, antigen recognition by $\gamma\delta$ T cells is the remarkably poor overlap between characterized ligands of the mouse and human systems. What has emerged is a list of seemingly unrelated molecules, many of which were identified for single $\gamma\delta$ T cell clones (a selection of these is featured in Figure 1) [2]. The heterogeneity of these ligands reflects the heterogeneity of $\gamma\delta$ T cell populations, and this is further complicated by the lack of a clear functional categorization of $\gamma\delta$ T cell subsets akin to that recognized for $\alpha\beta$ T cells (Th1, Th2, Th17, etc...). Given the diverse chemical nature of ligands described thus far, it has been frequently suggested that the $\gamma\delta$ TCR repertoire has not evolved to recognize a particular ligand in the context of an antigen presenting molecule, but rather serves to recognize an unbiased spectrum of antigens, in an adaptive manner similar to antibodies. However, in addition to small molecules with no clear requirement for MHC presentation and structurally diverse proteins of self and microbial origin, the list of ligands also includes MHC-like molecules. Some of these MHC-like proteins do not present antigen, such as the murine non-classical MHC molecule T22, while others such as the CD1 family present lipid antigens (background on CD1 molecules discussed in Box 1). Particularly within the human system, a bifurcation of ligand type coincides with TCR V δ gene segment usage, as discussed below, indicating that the $\gamma\delta$ TCR V genes may have evolved to sample particular antigenic niches for the protection and preservation of the host.

The two major populations of human $\gamma\delta$ T cells are classified according to their V δ gene segment usage as V δ 2 and V δ 1, the latter also termed “non-V δ 2”, and sometimes grouped with the less prevalent V δ 3 population [3]. V δ 2 cells predominate in human blood where they mostly pair with the V γ 9 chain; this population is stimulated [EA1]by small, phosphorylated metabolites, arising from either altered tumor metabolism or intracellular infection [4, 5], through a mechanism that is just beginning to be unraveled [6]. V δ 1 T cells, however, have been shown to recognize several different molecules of the non-classical MHC family, either with or without loaded antigen [2]. A unifying feature of these V δ 1 ligands is their self-derived origin and stress-regulated expression, suggesting that V δ 1 T cells may be intrinsically autoreactive. The CD1 family member CD1d (see Box 1) has been identified as a V δ 1 ligand by several independent groups, and was also shown to stimulate V δ 3 cells, highlighting its general importance as a “non-V δ 2” $\gamma\delta$ T cell ligand [7-10]. CD1d is also the only $\gamma\delta$ TCR ligand identified in both the human and murine systems, further indicating that some $\gamma\delta$ TCRs may have evolved to sample a specific lipid antigen pool [11].

Structural studies can immediately illuminate several outstanding questions of $\gamma\delta$ TCR recognition. Can $\gamma\delta$ TCRs discriminate antigens in the context of a presenting molecule such as CD1d? Is there evidence of a germline-based “bias” of the TCR for CD1d, and does this suggest the potential for a general “restriction” of $\gamma\delta$ TCRs? Unlike classical $\alpha\beta$ T cells, which are “restricted” by classical MHC molecules through conserved interactions with the MHC helices, it has been difficult to uncover whether $\gamma\delta$ T cell subsets commonly share an affinity for an antigen-presenting molecule. Recent studies have addressed these questions through work on V δ 1 $\gamma\delta$ TCR-CD1d complexes, revealing mechanisms of human $\gamma\delta$ T cell ligand recognition [12, 13].

Discovery of CD1d recognition by $\gamma\delta$ TCRs

To isolate specific CD1d-reactive T cells, CD1d-lipid tetramers were used in two different studies to stain healthy human peripheral blood cells [7, 13]. The lipid ligands loaded into these tetramers differed between the two studies; Bai et al. examined recognition of the lipid sulfatide [7], which is a self-lipid abundant in particular tissues such as the brain, kidney, and gastrointestinal tract [14]. In their study, a majority of CD1d-sulfatide tetramer-staining cells in the blood bore the V δ 1 TCR, averaging at over 80% [7]. Two CD1d-sulfatide reactive TCRs (DP10.7 and AB18.1) identified in this study were the basis for further biochemical and structural studies by Luoma et al. [12]. In contrast, Uldrich et al. identified $\gamma\delta$ T cells specific for CD1d loaded with α -galactosylceramide (α -GalCer), a microbial glycolipid and archetypical agonist of NKT cells [15]. Although $\alpha\beta$ TCR⁺ NKT cells comprised the majority of CD1d- α -GalCer specific cells, V δ 1 TCR⁺ cells were identified in all individuals examined. Single cell sorting led to the identification of the 9C2 clone that was used for biochemical and structural studies.

Below we will first discuss these structures individually, and then identify their commonalities and, importantly, their differences. In this discussion, amino acids will be numbered according to the IMTG-delineated beginning of the V gene segments, allowing for an easier comparison of the two structures.

Key aspects of CD1d recognition by the V δ 1 TCR

The structure of the V γ 4V δ 1 DP10.7 TCR in complex with CD1d-sulfatide is globally very different from that of previously described TCR-ligand structures, although in fact many of the underlying recognition principles bear resemblance to the classical $\alpha\beta$ TCR-MHC-peptide complex [16, 17] (Figure 2C). The DP 10.7 TCR contacts CD1d entirely through the CDR loops of the δ chain, resulting in an overall tilted docking orientation (Figure 2A). This unusual docking mode is reminiscent of the murine $\gamma\delta$ TCR-T22 complex. T22 is also a non-classical MHC molecule, but is distinct in that it does not present antigen and is found exclusively in mice. In this recognition mode, the G8 TCR engages T22 almost entirely through the CDR3 δ loop, resulting in an even more pronounced docking angle [18]. However, unlike the G8 TCR, the DP10.7 TCR utilizes all three loops of the δ chain to contact CD1d-sulfatide. The CDR1 δ loop dominates the interaction footprint with CD1d, bolstered by CDR2 δ loop-mediated ionic interactions. The long CDR3 δ loop, comprised of two D δ gene segments, contributes a sizable interface with the CD1d α 1 helix via Van der

Waals (VdW) interactions. Strikingly, solely non-templated, junctionally encoded residues of the CDR3 δ loop form polar contacts with the sulfatide antigen head group. This bias toward contacts of germline encoded V δ 1 CDR loops with the CD1d surface and junctionally encoded residues of the CDR3 δ loop towards the presented (and potentially variable) lipid ligand is similar to strategies employed by classical $\alpha\beta$ TCR-MHC interactions whereby germline-based recognition of an antigen-presenting molecule appears modulated by the recombined CDR3 loops to mediate fine antigen specificity [19-21].

The V γ 5V δ 1 9C2 TCR/CD1d- α GalCer complex structure revealed a docking mode even more globally similar to various $\alpha\beta$ TCR-ligand structures [13, 22-24]. This TCR utilizes both γ and δ chains for ligand recognition, conferring a much less titled docking mode (Figure 2B). Similar to that noted in the DP10.7/CD1d structure, the CDR1 δ loop makes a substantial contribution to the interface with CD1d. The CDR2 δ loop makes effectively no contact, save a single VdW interaction. The CDR3 δ loop makes many contacts with the CD1d helices, but in this case, none with the α GalCer antigen head group. Though the γ chain is involved in recognition, very few germline residues of the CDR1 γ and CDR2 γ loops participate in the interaction. Rather, the CDR3 γ plays a central role in contributing both to CD1d recognition and in contacting the α GalCer head group.

Conserved features of V δ 1+ TCR recognition of CD1d

There are many important similarities between these two structures. Both TCRs utilize the germline CDR1 δ loop as an anchor to the CD1d molecule itself. In fact, the same residue, Trp30, is responsible for a majority of the footprint in both structures via contact with the CD1d α 2 helix. The CDR3 δ loops of both TCRs form a large VdW surface with the α 1 helix of CD1d, and CDR3 junctional residues, of either the γ or δ chain, dictate antigen specificity by contacting the lipid head groups. Strikingly, the V γ germline-encoded residues are minimally involved in recognition in both complexes; in fact, no V γ residues contribute to CD1d recognition in the case of the DP10.7-CD1d/sulfatide complex structure and instead all TCR contacts with CD1d-sulfatide are mediated by the V δ 1 domain. The ancillary use of V γ -encoded residues in the contact interface may explain the diversity of V γ gene segment usage observed among the repertoire of characterized CD1d-restricted V δ 1 TCRs in both studies. Perhaps the main role of the V γ domain is to partner and stabilize the V δ 1 domain and, in some cases, provide additional contacts dependent on the lipid antigen presented. Additionally, both TCRs were also solved in the unliganded form, and comparisons with their respective bound TCRs revealed minimal CDR loop conformational change. This suggests these TCRs do not adapt extensively to the CD1d-lipid surface, reminiscent of interactions documented for other innate-like TCRs, such as iNKT and MAIT for which structures of unliganded and liganded TCRs are highly similar [24, 25]. Overall, both of these structures suggest that V δ 1 TCRs broadly survey CD1d molecules with a heavy bias towards V δ 1-encoded residues, yet discriminate different lipid antigens through the highly variable CDR3 loops.

Divergent features of V δ 1+ TCR recognition of CD1d

Surprisingly, there are many notable differences between these V δ 1+ $\gamma\delta$ TCR-CD1d complexes, setting this system apart from other “innate-like” and “semi-invariant” T cell

populations such as iNKT and MAIT cells where the TCR footprint is highly conserved regardless of the presented antigen or variability in V β chain usage [26-28] (see Box 2 for discussion of antigen recognition by other innate T cells). We will highlight several key differences in both CD1d and lipid antigen contacts.

The first obvious distinction between these two complexes is the overall docking mode of the TCRs. The extreme tilt of the DP10.7 limits the potential for γ chain contacts, whereas the 9C2 TCR is docked more evenly (Figure 2A,B). The shift in docking footprints also affects contacts mediated by the CDR2 δ loop, which is positioned more directly over the CD1d α 2 helix in the DP10.7 TCR structure but rotated away in the 9C2 TCR structure. Moreover, the docking angles upon the CD1d surface are rotated when compared to each other, resulting in a different footprint and, importantly, non-overlapping CD1d contact residues (Figure 2A, 2B, 3). This difference also illustrates the utility of the shared CDR1 δ loop residues, which employ two distinct sets of CD1d interaction partners in the two complexes, and may suggest that the V δ 1 TCR can adapt to diverse CD1d-presented lipid antigens via alteration of germline contacts

Overall the most obvious difference in lipid antigen contact between the two structures is which TCR chain is involved. As discussed previously, both TCRs utilize variability encoded in the CDR3 loops to contact the antigen head group, but the DP10.7 TCR binds sulfatide via the CDR3 δ loop, whereas the 9C2 TCR binds α GalCer through the CDR3 γ loop. A less obvious difference, yet one that has implications for lipid antigen recognition by other $\gamma\delta$ TCRs, is the origin of the CDR3 loop residue contacts. Though two germline D δ 2 residues of the DP10.7 TCR juxtapose the galactose moiety of sulfatide, all polar contacts are contributed by non-templated CDR3 δ residues, which specifically bind the sulfate group. The requirement of these non-templated residues for recognition was demonstrated by mutagenesis of both the CDR3 δ and probing of the sulfatide antigen itself, highlighting their role in discriminating the chemical identity of the lipid antigen. However, in the 9C2 TCR structure, polar contacts with the α GalCer head group are also contributed by the V γ 5 germline encoded Arg101. In the context of this docking mode, Arg101 may confer specific recognition of α -linked sugar moieties as found on glycosphingolipids of microbial origin. [29]

What is the basis of the differences between these two structures?

As described above, there are several major distinctions between the two $\gamma\delta$ TCR docking modes in these structures. Both papers reporting these structures highlight that CD1d may be a potential germline “restriction” element for V δ 1 TCRs, and thus the question of whether these docking modes are conserved in other V δ 1 TCR-CD1d interactions emerges as centrally important. As CD1d is a non-polymorphic molecule, the variability of the TCRs is the obvious basis for these two docking modes. The main distinctions between these two TCRs are highlighted below, with commentary on how these differences may affect the mode of CD1d recognition.

V γ gene segment usage

Perhaps the most fundamental difference between the two TCRs is their different V γ gene segment usage. The DP10.7 TCR utilizes V γ 4, whereas 9C2 utilizes V γ 5, both of which are members of the same human V γ gene family. However, the γ chain has no involvement in DP10.7 TCR recognition of CD1d-sulfatide, and there are only three V γ encoded residues that are involved in 9C2 recognition of CD1d- α GalCer. Two of these three residues are conserved in the V γ 4 segment, and comparison of the two unliganded TCRs show that these residues are in the same position with similar side-chain orientation. The only V γ -encoded residue involved in 9C2 interactions and not shared with the DP10.7 TCR is Arg101, which is only involved in a single VdW interaction with the CD1d molecule but is important for recognition of the α GalCer antigen. Thus, the distinct modes of CD1d engagement observed in these structures does not appear to be dictated by the different V γ chain usage of the TCRs and instead may be due to differences encoded in the diverse CDR3 δ and γ loops.

CDR3 loop length and diversity

Aside from the different V γ gene usage, the two crystallized TCRs differ in their CDR3 loop sequences and have very different CDR3 loop lengths. A unique structural feature of $\gamma\delta$ TCRs is the long CDR3 δ loop, which can utilize multiple D δ gene segments owing to specialized recombination signal sequences [30, 31]. This feature makes the CDR3 δ loop on average the longest of all loops among recombined TCR and immunoglobulin receptors [1, 32]. The median human CDR3 δ loop length is 14 amino acids, which is also the length of the DP10.7 TCR CDR3 δ loop [32]. The 9C2 CDR3 δ loop is shorter than average at 11 amino acids. Superimposition of the DP10.7 TCR structure, or the AB18.1 TCR structure determined in the unliganded form, onto the liganded 9C2 TCR shows that these CDR3 δ loops, without considerable conformational changes, would clash with the CD1d helices and the lipid antigen (Figure 4). So TCRs endowed with average to long CDR3 δ loops may be forced to adopt an alternate footprint with a tilted docking mode to avoid a steric clash, in the process limiting the ability of the γ chain to contact CD1d.

On average, CDR3 γ loops are somewhat shorter and more constrained in length than the CDR3 δ loop, averaging at seven residues in humans [1, 32]. In this case there is a very notable difference between the two TCRs used in these structures. The DP10.7 TCR has a CDR3 γ of average length, totaling eight amino acids. However, the 9C2 TCR has an exceptionally long CDR3 γ at thirteen amino acids, outside of the range reported in an analysis of human CDR3 γ lengths [32]. This long CDR3 γ is used by the 9C2 TCR to recognize both CD1d and the lipid antigen, contributing to the shifted contacts of the δ chain.

Clearly, additional structures of V δ 1 TCR-ligand complexes will clarify whether features of the TCR or antigen confer use of these (or additional) docking modes. The fact that these TCRs are quite different in sequence outside the conserved CDR1 and CDR2 loops of the V δ 1 domain yet still recognize CD1d highlights how important diversity is in determining the V δ 1 TCR footprint, setting this T cell population distinctly apart from the conserved iNKT and MAIT cell recognition modes (Box 2).

Towards a more global understanding of $\gamma\delta$ T cell activation

The role of TCR stimulation in $\gamma\delta$ T cell activation

These structural studies provide much needed data for beginning to reconcile $\gamma\delta$ T cell ligand engagement with the unique functional roles of $\gamma\delta$ T cells that straddle the boundaries of innate and adaptive immunity. $\gamma\delta$ T cells are commonly described as innate-like lymphocytes, owing to their ability to rapidly respond to markers of cellular stress [33]. The initiation of this activity has not been well understood due to the lack of well-defined, TCR-specific ligands. Inferring the identity of $\gamma\delta$ TCR ligands has been difficult due to the variability of $\gamma\delta$ T cell populations between species and even between tissue types. Indeed, some tissue-specific populations express completely invariant receptors, such as the murine dendritic epidermal T cell (DETC) population that survey the skin, or semi-invariant receptors, as found on V γ 9V δ 2 cells in human blood, while others have diversity in both their chain pairing and have high CDR3 loop diversity (such as with the V δ 1⁺ cells that recognize CD1d in the blood and the gut). Indeed, it has been shown that for some $\gamma\delta$ T cell populations this innate-like activity derives from TCR-independent signaling through cytokines rather than from a TCR-specific response to a stimulatory antigen [34, 35]. But for those populations where TCR ligands have been defined, what is the role of antigen ligation in T cell activation?

The reason this question is important, particularly in terms of V δ 1⁺ T cell recognition of CD1d-lipid, is that these cells appear to be stimulated by endogenous lipid antigens [7, 36], a hallmark of auto-reactivity. Indeed, some V δ 1 TCRs examined appeared to be somewhat lipid-independent, promiscuously recognizing a range of lipid antigens presented by CD1d [12, 13]. How can we reconcile this auto-reactivity with disease surveillance? Is tonic engagement of self-antigens the norm, resulting in constitutive, low-grade signaling as seen with murine DETCs [37]? If so, what signals then initiate effector functions relevant to disease? It is plausible that expression of CD1d may be up- or down-regulated in specific tissues, and therefore may be acting itself as a stress-modulated ligand, either for proinflammatory or regulatory effector functions. In this way, the level of expression of CD1d, rather than the specific antigen presented, could be of greater functional importance to activation. For example, CD1d levels are reduced on intestinal epithelial cells (IECs) in patients with inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease [38], providing a potential mechanism for modulation of T cell activity in these diseases.

An alternative role for this system may be to monitor the microbial environment through presentation of microbial lipids, or in the context of disease, through detection of altered-self lipids presented by CD1d. For example, the cellular composition of lipids is altered during tumorigenesis, and certain lipids, including sulfatide, are upregulated in some epithelial cancers [14, 39-41]. Uldrich et al. demonstrated reactivity to the microbial lipid α -GalCer, and even though the affinity and reactivity to this lipid was much less than that observed in the case of iNKT cells (16 μ M compared to 0.5 μ M, and evidenced by lower tetramer staining intensity to V δ 1 cells than iNKT cells), it sets an important precedent that microbial lipids may be stimulatory. Directly relevant to sulfatide reactivity, some pathogens, such as *Trypanosoma cruzi* [42] and *Mycobacterium tuberculosis* [43], can produce sulfatide-like

compounds, and it is also likely that other bacterial species and stressed cells make antigens that can be presented by CD1 molecules to these $\gamma\delta$ T cells ([44] and reviewed in [45]).

Returning to the scenario where TCRs engage self-antigens in an auto-reactive fashion, we propose that additional, TCR-independent signals can be used in a “Signal-2”-like mechanism to confer a disease- or stress-specific response in these cells (Figure 5). This Signal-2 could be through cytokines or through NK activating receptors such as NKG2D. In humans, NKG2D is a high-affinity receptor for stress-induced ligands like MHC class I-related molecule A (MICA) and UL16-binding proteins (ULBP1 and 2) [46]. Previous work on NKG2D expressed on $V\delta 1^+$ $\gamma\delta$ T cells in colorectal cancer infiltrates demonstrated the importance of both NKG2D and TCR ligation in activation of these cells [47, 48], with MICA proposed as the ligand for both receptors. However, the affinity of these TCRs for MICA was exceeding low ($\sim 1\text{mM}$) [49]. Our discovery that CD1d-presented sulfatide is a highly specific ligand for one of these TCRs, $\delta 1\text{A/B-3}$, supports a Signal-1/Signal-2 mechanism for activation, whereby the TCR receives a specific signal through recognition of CD1d-lipid which is then modified or enhanced by a Signal-2 through engagement of the activating receptor NKG2D by stress-induced MICA. Now that $\gamma\delta$ TCR ligands are becoming better defined, we propose that the mode of activation for some of these $\gamma\delta$ T cell populations (such as those $V\delta 1^+$ T cells that respond to CD1d) may be a complex amalgamation of direct signals through the TCR and co-stimulatory signals through cytokines or non-TCR activating receptors.

Lastly, that some human $\gamma\delta$ T cells can recognize self-ligands presented by CD1d has implications for the thymic selection of these cells. In mice, T22 reactive- $\gamma\delta$ T cells including IELs can develop independently of $\beta 2$ -microglobulin ($\beta 2\text{m}$), suggesting they do not require positive selection, although they still recognize T22 [50]. Could human $V\delta 1$ cells develop in a similar way, or does CD1d act as a developmental restricting element in selection of these CD1d-specific $\gamma\delta$ T cells in humans? If so, this opens up important questions as to the selecting lipid ligand and other signals required for productive selection.

Concluding remarks

These two structures of $\gamma\delta$ TCRs in complex with CD1d provide the first molecular insight into models of $\gamma\delta$ T cell recognition in humans. While both TCRs utilize the $V\delta 1$ gene segment and bind to CD1d with contacts associating with both the presenting molecule and respective antigen, there were surprising differences between the two complexes. The CD1d contacts and general angle of binding were different between the two structures, and notably, only one of them had any γ chain involvement. These differences stimulate several exciting questions about the overarching nature of $\gamma\delta$ T cell antigen recognition. What specific features govern the ability of $V\delta 1^+$ cells to bind to CD1d and what differences are found in TCRs that bind unloaded CD1d compared with CD1d presenting sulfatide, α -GalCer or other antigens? What is the general role of the γ chain in binding to CD1d? If more structures are solved will we be able to identify a canonical binding mode or will each TCR have its own unique docking characteristics? What, if anything, can these structures tell us about the binding of other types of $\gamma\delta$ T cells? Perhaps most interestingly, could $V\delta 1^+$ cells, in general, represent a subset of $\gamma\delta$ T cells that recognize antigen in a traditional, TCR-like

manner, while other $\gamma\delta$ subsets detect antigen directly? Finally, the first described ligand for human $\gamma\delta$ T cells was a Group 1 CD1 molecule, CD1c [51]. How do $\gamma\delta$ T cells “see” CD1c, what role does this perform in vivo, and do the other Group 1 CD1s also present antigen to these cells? Whatever the characteristics these binding “rules” eventually display, a better understanding of these and other biochemically validated $\gamma\delta$ T cell ligands will aid in the understanding of the general role of TCR signaling in human $\gamma\delta$ T cell-mediated diseases.

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Box 1**CD1 molecules: lipid-presenting members of the MHC family**

The protein fold characteristic of classical MHC molecules exhibits remarkable utility, as members of the CD1 family of MHC-like molecules have evolved to utilize this overall structure to bind and present lipid antigens to T cells [52]. The CD1 family in humans comprises five members, CD1a-e, and divided into three groups: group 1 (CD1a-c), group 2 (CD1d) and group 3 (CD1e). Interestingly, CD1d is the only member conserved between mice and humans, which combined with its identification as a restricting element for invariant natural killer (iNKT) cells [53], has led to extensive study of its structure and function.

The extreme polymorphism of MHC molecules, which facilitates their presentation of a tremendous diversity of peptide antigens, contrasts with the non-polymorphic nature of CD1 molecules. Given that the extensive variability self-, microbial-, and environmental-derived lipids lies within the polar head group, the hydrophobic binding pockets of CD1 molecules are suited to bind and present broad lipid repertoires via non-specific interactions with hydrophobic lipid tails. In fact, the different members of the CD1 family have structurally unique binding pockets, which together can accommodate lipid tails from 20 to 80 carbons in total size [54]. CD1a and CD1d have two hydrophobic pockets, which are well suited for binding 8-26 carbon chains of self- and microbial-derived glycolipids [55-58]. CD1c and CD1b have a larger and more complex pocket architecture, imparting their ability to present very long and branched lipid chains characteristic of mycobacterial species [59, 60]. Several recent reviews have detailed the unique structures, biological sources, and functions of CD1-presented lipid antigens [61-63]. CD1 molecules, most notably CD1d, can be expressed on epithelial cells of non-hematopoietic origin, facilitating its role as an antigen-presenting molecule for tissue-resident rapidly-responding T cells [64]. Together, the family of CD1 molecules can comprehensively subject an extensive repertoire of lipids to T cell immune surveillance.

The most well-studied CD1-specific T cell population are iNKT cells of the $\alpha\beta$ T cell lineage, which explosively produce IFN- γ and IL-4 upon stimulation with CD1d-presented agonist lipids. The lipid α -galactosylceramide (α -GalCer, also called KRN7000), originally isolated from commensal bacteria of a marine sponge through a screen for anti-cancer compounds, potently stimulates iNKT cells and therefore has been seminal in the deciphering of iNKT cell biology [15, 65]. Intriguingly, α -GalCer and other sphingolipids are also produced by commensal bacteria of the human intestine [66]. The characterization of T cells specific for human group 1 CD1 molecules has been met with slower progress, though recently, several non-invariant $\alpha\beta$ T cell populations specific for CD1a, b, and c have been described [67-73]. CD1c has also been described as a $\gamma\delta$ T cell ligand, though specific stimulatory lipid antigens have yet to be identified [51, 74].

Together, the CD1 family of antigen-presenting proteins has evolved for the specialized presentation of lipid antigens to T cells, and current research initiatives include the

further functional and biochemical characterization of group 1 T cell recognition, and the recognition of both group 1 and 2 CD1 molecules by $\gamma\delta$ T cells.

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Box 2**Antigen recognition by other innate-like T cells**

CD1d molecules presenting lipid antigens, specifically, α -GalCer, were originally described as antigens for invariant NKT (iNKT) cells [15, 28] rather than V δ 1 T cells. These cells are considered semi-invariant due to their limited gene usage; human iNKT TCRs nearly exclusively use TRAV10 (V α 24) and TRAJ18 (Ja18) along with TRBV25 (V β 11) [28]. Structural studies of iNKT binding to CD1d revealed an unusual binding mode, with the TCR tilted and parallel to the antigen binding pocket of CD1d [25]. Interestingly, both human and mouse iNKT:CD1d interactions display a similar docking angle [75], suggesting that this binding mode is also invariant.

Recently a second type of invariant $\alpha\beta$ T cell has also been identified [27, 76], called mucosal-associated invariant T (MAIT) cells, due to their prevalence in intestinal tissues. The TCR α domains of human MAIT cells preferentially contain TRAV1-2 joined to TRAJ33 [27, 76], but a minor proportion of cells also use TRAJ12 and TRAJ20 [77]. Although both iNKT cells and MAIT cells possess mostly invariant TCR α -chains, MAIT cells are more variable due to the usage of multiple TCR β segments (TRBV20, TRBV6, and TRBV2) plus higher diversity CDR3 β rearrangements [78]. These diverse β sequences may play a role in modulating MR1 recognition [77] or in antigen specific encounters [79-82].

Unlike iNKT cells and V δ 1 T cells, which bind to CD1d, MAIT cells are restricted by MR1 [83], which like CD1d, is a non-polymorphic, MHC-like molecule. Whereas CD1d is capable of presenting both self and non-self lipid ligands (Box 1), to date, the only stimulatory MR1 ligands that have been characterized derive from the riboflavin (vitamin B2)-biosynthesis pathway [81, 84], which is only present in certain microbial species. Although mammals cannot make riboflavin, synthesis can occur in commensals as well as pathogenic bacteria, so although not true “self” molecules, these small metabolites are presumably present at high levels even in the normal intestinal mucosa.

Like classical MHC class I molecules and CD1d, MR1 is also β 2m associated. Structural analyses of the MR1:MAIT TCR interaction [24, 79-81], have shown that MR1 has a mostly closed groove, which only allows TCR access to a portion of the metabolite containing a ribityl group. This minimal access to antigen explains why while another vitamin B metabolite, the folic acid (vitamin B9) metabolite 6-formyl pterin (6-FP), which lacks the ribityl group, is non-stimulatory despite binding MR1 [24, 80, 84]. Multiple ligand conformations have been noted in MR1, but MAIT TCRs recognizing two different antigens display very conserved binding modes, and in both cases, the TRAJ33-encoded residue Y95 α was responsible for contact with the ribityl group of the antigen [79, 80].

Similarly, the CDR3 α loop is important for iNKT binding to CD1d: α -GalCer [28, 85]. However, despite CDR3 α dominance for antigen recognition in both cases, the general binding angles in iNKT and MAIT cell interactions are quite different. MAIT TCRs bind perpendicularly to MR1 in a manner more like classical $\alpha\beta$ TCR:MHC docking [16, 17, 27], whereas iNKT cells adopt a more parallel, tilted conformation during CD1d

recognition (Figure 2D) [28]. So, while the binding mode of iNKT and MAIT TCRs are distinct from each other, within each group there is little variability in overall structure, unlike the two V δ 1 structures described here.

Highlights

1. V δ 1+ $\gamma\delta$ T cells engage CD1 molecules using a diverse structural footprint.
2. Recognition of CD1d-lipid is dominated by the V δ 1 domain.
3. The CDR3 loops make important contacts in lipid-antigen discrimination.
4. Auto-reactivity of V δ 1+ $\gamma\delta$ T cells may be modulated by co-stimulatory signals.

Figure 1. Ligands recognized by $\gamma\delta$ TCRs

A selection of reported $\gamma\delta$ TCR ligands are shown, grouped by species-specificity and by structural class. MHC-like ligands include the non-classical molecules T10/T22 [86, 87], Endothelial protein C receptor (EPCR) [88], MHC class I-related molecule A (MICA) [47], and CD1 family members CD1c and CD1d [8, 11, 36, 51, 74], as well as the classical class II MHC molecule I-E^k [89]. MHC-unrelated ligands include HSV glycoprotein I [90], phosphoantigens [4, 5, 91], F1-ATPase [92], histidyl tRNA synthetase [93], and phytoerythrin [94]. A comprehensive list can be found in recent reviews [1, 2].

Figure 2. Overall structures of TCR-ligand complexes. Top panels

(A) Side-view of the DP10.7 TCR-CD1d-sulfatide complex (Protein Data Bank accession code 4MNG). The crystallized TCR construct was a single-chain version; thus shown are the variable domains only. The TCR docks at a tilted angle such that only the δ chain (violet) makes contact with CD1d-sulfatide. (B) Side-view of the 9C2 TCR-CD1d- α GalCer complex (Protein Data Bank accession code 4LHU). The full-length TCR structure was determined, but the variable domains only are shown here for comparison purposes with (A). The 9C2 TCR is docked such that both the γ (blue) and δ (purple) chains contact the CD1d- α GalCer surface. In both A and B, CD1d is shown in light grey and the lipid ligands are shown in yellow. (C) Structure of a classical $\alpha\beta$ TCR-peptide-MHC (2C TCR-H-2 K^b-dEV8 peptide) complex (Protein Data Bank accession code 2CKB). Shown are the variable domains only. The TCR is docked centrally and diagonally above the MHC footprint. Both the TCR α chain (blue) and β chain (green) contribute to MHC (light blue) and peptide contact (orange). (D) Structure of an iNKT TCR-CD1d- α GalCer complex (Protein Data Bank accession code 2PO6). Shown are the variable domains only. The NKT15 clone shown here prominently uses the α chain (blue) to engage the α GalCer ligand (yellow) and the CD1d surface (grey). The β (green) chain is docked at the extreme end of the CD1d and makes less extensive CD1d contacts. Bottom panels, shown are cartoon representations of TCR docking upon CD1d/MHC surfaces, corresponding to TCR shown in upper panel. View is looking down upon the MHC/CD1d surface; each TCR chain is depicted as a circle. Cartoons of lipid ligands or peptides also depicted. Acyl chains shown as black lines, lipid head groups shown as yellow/orange circles, peptide as orange line. TCR chain colors

are the same as upper panels. Dotted line indicates TCR chain does not make contact with CD1d-lipid.

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Figure 3. Two TCRs, two footprints

(A) Different footprints of DP10.7 and 9C2 TCR upon CD1d. CD1d surface shown in light grey, lipid (sulfatide) shown in yellow. DP10.7 TCR δ chain (purple) and γ chain (light pink) CDR loops, and 9C2 TCR δ chain (green) and γ chain (light green) CDR loops from complex structures are shown upon CD1d surface. Loops that are not involved in the interaction are shown as transparent (CDR1,2,3 γ of DP10.7 TCR, CDR2 γ of 9C2 TCR). (B) CD1d surface residues contacted by the DP10.7 (left) and 9C2 (right) TCRs. TCR loops that are involved in recognition are shown above the CD1d surface (white), and participating CD1d residues are colored according to which TCR chain makes contact (CD1d residues contacted by the TCR δ chain shown in pink, contacted by γ chain shown in blue, contacted by both chains shown in green). The pattern of CD1d contact residues clearly differs between the two TCRs: the DP10.7 TCR is docked more centrally above the lipid portal, whereas the 9C2 TCR is docked closer to the extreme A' end of CD1d.

Figure 4. CDR3 loop length may govern TCR docking mode

(A). Shown are the CDR3 δ loops of three CD1d-specific V δ 1 TCRs, the DP10.7 (purple), AB18.1 (green) and δ 1A/B-2 (cyan) aligned to the CD1d- α GalCer bound 9C2 TCR (gold). The surface of CD1d is shown in transparent light grey. Docking of the AB18.1 and DP10.7 TCRs based on V domain alignment with the 9C2 TCR results in steric clashes of their CDR3 δ loops with CD1d, as can be seen by the submerging of these loops under the CD1d surface. Additional TCR loops have been removed for increased clarity.

Figure 5. Cartoon representation of the Signal 1/Signal 2 model

In this model, CD1d (shown in cyan) recognition can provide either a potent signal derived from alter-self lipids or lipids from microbial origin sufficient to stimulate a Vδ1 T cell (yellow). This we call “Signal 1”. In some cases, this CD1d-lipid specific signal is of low to moderate intensity, deriving from engagement of a CD1d-self lipid complex to produce a “self-reactive” signal that requires additional enhancement from a “Signal 2”, here shown as the activating receptor NKG2D (orange) engaging the stress-induced MICA (red) on the target cell. From the structures derived thus far, we hypothesize that the Vδ1 domain (pink) will dominate in this interaction, in some cases contributing all contacts in the interface with CD1d-lipid. In cases where the CD38 loop is of a different length or amino acid composition, or if the lipid has a large or structural complex head group, an alternate TCR footprint will be used in engagement, resulting in contacts derived from the Vγ domain (green).