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The evolving portrait of $\gamma\delta$ T cell receptor recognition determinants

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Abstract

In $\alpha\beta$ T cells, immunosurveillance is enabled by the $\alpha\beta$ T cell receptor (TCR), which co-recognises peptide, lipid or small molecule antigens presented by MHC and MHC-I like antigen-presenting molecules, respectively. Although $\alpha\beta$ TCRs vary in their antigen recognition modes, in general they co-recognise the presented antigen and the antigen presenting molecule and do so in an invariable ‘end-to-end’ manner. Quite distinctly, $\gamma\delta$ T cells – by way of their $\gamma\delta$ TCR – can recognise ligands that extend beyond the confines of MHC and MHC-I like restrictions. From structural studies, it is now becoming apparent that $\gamma\delta$ TCR recognition modes can break the co-recognition paradigm and deviate markedly from the ‘end-to-end’ docking mechanisms of $\alpha\beta$ TCR counterparts. This minireview highlights the emerging portrait of how $\gamma\delta$ TCRs can recognise diverse epitopes of their antigens in a manner reminiscent to how antibodies recognise antigens.

Keywords

$\gamma\delta$ T cells; $\gamma\delta$ T cell receptor; MR1; CD1; MHC-I like

Introduction

The adaptive immune system is often simplified to a bipartite system comprising of B cells and $\alpha\beta$ T cells. As a part of the humoral arm of adaptive immunity, B cells mediate the recognition of intact pathogen or damage-derived antigens *via* the production of soluble antibodies. In contrast, as drivers of the cell-mediated arm of adaptive immunity, $\alpha\beta$ T cells typically recognise short peptides presented by MHC class I and II molecules through an $\alpha\beta$ T cell receptor (TCR) complex. In addition, unconventional $\alpha\beta$ T cell populations can recognise lipid or other small molecule antigens presented on MHC I-like

molecules, namely CD1 and MR1, respectively (1, 2). Thus, $\gamma\delta$ T cells can recognise a diverse repertoire of antigens when presented by a suitable antigen-presenting MHC or MHC I-like molecule and do so with high specificity to elicit protective immune responses. Many of these B cell and $\alpha\beta$ T cell interactions with antigens have been comprehensively characterised, including structural biology studies that revealed the molecular, and even atomic, details of antigen recognition and thus, cellular activation.

The adaptive immune system in fact includes a third lymphocyte lineage, yielding a tri-cellular arsenal of B cells, $\alpha\beta$, and $\gamma\delta$ T cells that has persisted over 450 million years of jawed vertebrate evolution (3). It has become increasingly evident that $\gamma\delta$ T cells have a central role in immunology with expanding evidence for homeostatic pathogen, cancer, and tissue repair (3). Indeed, an expanding literature implicates peripheral blood $\gamma\delta$ T cell populations in pathogen-driven clonal expansion and potent protection in tuberculosis, malaria and cancers (4–10). Furthermore, $\gamma\delta$ T cells are implicated in cancer-induced stress surveillance (11) although it is currently contested whether the tumour infiltration capacity of $\gamma\delta$ T cells correlate with anti or protumour outcomes in various malignancies and solid tumours (10, 12–14). Such studies have contributed to a burgeoning field of study investigating $\gamma\delta$ T cells as novel cellular immunotherapies. This immunotherapeutic development proceeds despite a lack of clarity around $\gamma\delta$ T cell activation mechanisms, and the role of the $\gamma\delta$ TCR in directing $\gamma\delta$ T cell biology.

While the $\gamma\delta$ TCR can recognise antigens presented on MHC and MHC I-like molecules, the $\gamma\delta$ TCR is not restricted to MHC presentation like its $\alpha\beta$ TCR counterpart. Indeed, the repertoire of known $\gamma\delta$ TCR ligands is incredibly diverse and ever expanding as the literature characterising this enigmatic complex broadens. Several isolated $\gamma\delta$ T cell populations have been characterised from human peripheral blood mononuclear cells (PBMCs), with reactivity to unconventional T cell antigens beyond the confines of the MHC architecture. These antigens exhibit diverse protein architectures such as phycoerythrin (15), Ephrin type-A receptor 2 (16), Annexin A2 (17), histidyl tRNA synthetase (18), ATP synthase, and apolipoprotein A-1 (apoA-I) (19). Other peripheral $\gamma\delta$ T cell populations exhibit reactivity to stress-induced phosphoantigens presented by butyrophilin (BTN) proteins (4, 20–23). $\gamma\delta$ TCRs have also demonstrated the ability to recognise self-ligands in barrier tissues such as the mucosal lining of the gut as well as the peripheral blood as means of stress surveillance and maintenance of homeostasis. In the blood, stress inducible markers such as endothelial protein C receptor (EPCR) (24) or lipids presented by CD1 molecules were found to be antigens for select $\gamma\delta$ T cell populations (25–28).

Tissue-homing $\gamma\delta$ T cells locate to epithelial tissues (skin, lungs, and gut) where they mediate homeostasis, immune surveillance and protection (29–33). Intriguingly, tissue-homing $\gamma\delta$ T cells express similar TCRs suggesting tissue-specific functions. Enteric $\gamma\delta$ T cells are an intra-epithelial lymphocyte subset known to bind BTN-like (BTNL) proteins, with roles in homeostasis and enteropathies including celiac disease, Ulcerative colitis, Crohn's disease and colorectal cancer (34–43).

Thus, to better inform therapeutic approaches, a comprehensive understanding of structural paradigms underpinning $\gamma\delta$ TCR interactions with cognate ligands is needed. Presently

there have only been seven examples of $\gamma\delta$ TCR-ligand complex structures to date, six of which are human (25, 27, 28, 44–47). Here we review recent progress in this nascent field and make comparisons to $\alpha\beta$ T cell receptors.

Structures of $\alpha\beta$ TCR complexed to conventional and unconventional ligands

Thanks to a concerted global effort over recent decades, a multitude of $\alpha\beta$ TCR structures have been determined in complex with peptides bound to MHC allomorphs, reviewed in (48). This catalogue of $\alpha\beta$ TCR-pMHC structures has invariably shown a conserved end-to-end docking mode whereupon $\alpha\beta$ TCRs sit atop the MHC antigen presenting platform. This end-to-end docking mode allows for the hypervariable complementarity determining region (CDR) loops of the $\alpha\beta$ TCR to co-contact the presented peptide and the MHC molecule, termed co-recognition (49). Within this conserved ‘end-to-end’ TCR-pMHC binding, the $\alpha\beta$ TCR has, with notable exceptions (50–52), demonstrated a canonical docking topology that typically transcends differences in affinity, MHC molecules, and presented antigens. Namely, $\alpha\beta$ TCR recognition of MHC I adopts a well-established ‘diagonal’ docking modality in which V α contacts the α 2 helix of MHC I and the V β is positioned over the α 1 helix (48, 53). Here, the CDR1 and CDR2 loops contact the α helices, leaving the hypervariable CDR3 loop to predominantly recognise the presented peptide. $\alpha\beta$ TCR recognition of pMHC II is similar to that of MHC I, where overall docking topology remains largely consistent across both MHC classes whereby the V α contacts the β chain α helix and the V β contacts the α chain α helix (48).

The $\alpha\beta$ TCR can also recognise unconventional MHC I-like ligands such as MR1 that present small molecule metabolites, and those of the CD1 family that present lipids (1). These $\alpha\beta$ TCR-ligand-MHC-I like ternary complexes can markedly differ from TCR-pMHC complexes. For example, in the case of MR1, small metabolites such as those generated from vitamin B metabolism are encased in its antigen binding cleft (2). Due to the size of these metabolites, they are often less accessible to the TCR during recognition. Further, characterisation of Natural Killer T cell TCRs revealed a parallel docking mode over the distal end of the antigen presentation cleft of CD1d (54). Autoreactive TCRs from CD1-reactive T cells can bind CD1a and CD1c without co-contacting the lipid, thereby breaking the co-recognition paradigm (55, 56). Despite variability in orientation and docking angle, the $\alpha\beta$ TCR invariably sits atop these MHC I-like molecules in a highly conserved ‘end-to-end’ manner.

First insights into the $\gamma\delta$ TCR

The first insights into how the $\gamma\delta$ TCR endows this cell lineage with unique antigen recognition principles was garnered from early work characterising unbound receptor structures. The first such study determined the structure of a single variable (V) δ domain of the human ES204 (V γ 1V δ 3) TCR, an HLA-A2 reactive clone isolated from peripheral blood (57, 58). This was followed by a structure of the human G115 (V γ 9V δ 2) TCR isolated from peripheral blood with reactivity to phosphoantigen stimulation (20, 59). These structures revealed that the $\gamma\delta$ TCR variable domains formed a typical IgV fold comprised

of two β -sheets of antiparallel β -strands that expose the CDR loops for antigen engagement, analogous to $\alpha\beta$ TCRs and immunoglobulins (Ig). The ES204 TCR structure provided the first structural evidence for the antibody-like capacity of the $\gamma\delta$ TCR, as analysis of the V δ 3 structure revealed close structural similarity to the V_H domain of human IgM, despite sequence similarity to the V α domain (57). Here, the close structural alignments between antibodies and the ES204 V δ domain were theorised to stem from fundamental similarities in the framework region (57). Namely, the ES204 V δ 3 structure revealed that the C' β -strand that follows the CDR2 loop is three-residues longer in V δ domains than in the V α domain, and more comparable to those observed in the V_H or V_L domains of antibodies. The V δ 2 domain of the G115 TCR exhibited a shorter C' β -strand due a shortened CDR2 δ loop, yet the carbon backbone of this framework region remained similar to an antibody.(59). This shortening of the CDR2 δ loop in compensation for shorter β -strands is observed in the V_L domain of antibodies (57).

In both the ES204 TCR and G115 TCR, the CDR loops display a unique surface with vestiges of both $\alpha\beta$ TCRs and antibodies. Namely, the CDR1 δ loop positioning is nearly identically to the V α domain in both the ES204 TCR and the G115 TCR. Conversely, the CDR2 δ loop most closely resembles antibody CDR2 loops as a consequence of the shared pairing of the C' β -strand prior to the CDR2 δ loop and the C' β -strand subsequent to the CDR2 loop, however similarity to antibodies manifests differently between the ES204 TCR and the G115 TCR (57, 59). Both CDR2 δ loops are positioned at an angle congruent with antibodies and in the ES204 TCR, the CDR2 δ loop is more aligned with the V_H domain than that of the V α domain (57). Meanwhile, in the G115 TCR, the CDR2 δ is positioned more similarly to the V_L domain as they share a shortened CDR2 loop (59). The two $\gamma\delta$ TCR structures differ markedly when comparing their CDR3 δ loop structure. In the ES204 TCR, the 10 amino acid (aa) long CDR3 δ loop is positioned more in line with the V α domain whilst the 15 aa long CDR3 δ loop in the G115 TCR is similar to the V_H domain, suggesting that CDR3 δ loop may exist on a spectrum in degree of similarity to either the V α or V_H domains depending on loop length (57, 59). Broad analysis of an array of $\gamma\delta$ TCRs shows that the range of CDR3 δ loop length (8–21 aa) is more similar to long CDR3 loop lengths of the V_H domains (3–25 aa) of antibodies rather than $\alpha\beta$ TCRs (6–12 aa) (60). This large range leads to greater protrusions when the CDR3 δ loop is longer, and this is more reminiscent of antibodies instead of the relatively flatter surface of the $\alpha\beta$ TCR. Together these structures showed for the first time that the $\gamma\delta$ TCR displays elements of both $\alpha\beta$ TCR and antibody structures, leading to many questions and speculation about how these receptors would bind antigens, a question that remained hotly contested until further structural studies emerged.

Peculiarities in $\gamma\delta$ TCR interactions

Despite a diverse breadth of potential cognate antigens, current $\gamma\delta$ TCR-ligand complexes have focused upon recognition of MHC I-like molecules via unprecedented mechanisms. Superposition of the $\gamma\delta$ TCR-ligand complex structures solved to date immediately reveals the extreme diversity of docking interactions with MHC I-like molecules (Figure 1A). Below, we will summarise the key aspects of each individual complex and identify unifying principles underpinning these interactions.

Murine $\gamma\delta$ TCR – T22

The first $\gamma\delta$ TCR complex structure solved was that of murine G8 (V γ 4V δ 10) TCR recognising murine non-classical MHC-I like molecule, T22 (Figure 1B) (44). Approximately 0.4% of murine splenic $\gamma\delta$ T cells react to T22 and its close relative T10 (61). As T22 is unable to present antigens due to a partially collapsed antigen presenting platform, the G8 TCR bound the T22 molecule itself at ~ 67 nM, a remarkably strong affinity for a TCR that is more typical for antibodies (62). The G8 TCR docked onto T22 in a manner that deviated from the canonical docking mode observed in $\alpha\beta$ TCR-pMHC complexes. Namely, the $\gamma\delta$ TCR binds at an almost a sideways angle, distal from what would be the antigen binding cleft of T22. The interface is dominated by the δ -chain which contributes 89% of buried surface area (BSA) with a minor contribution of 11% from the CDR3 γ loop (Table 1). This contrasts with $\alpha\beta$ TCR-pMHC complexes which typically display a more balanced contribution from the two chains and the six CDR loops encoded within, reviewed in (48). For the G8 TCR, the 12 aa long CDR3 δ loop contributes $\sim 70\%$ of the BSA as it protrudes into the cavity created by T22's $\alpha 1$ helix and the underlying β -sheet (Figure 2A) (44). Interacting residues or contacts were spread across germline-encoded (conserved across generations) and non-germline residues that resulted from recombination. Specifically, the germline-encoded hydrophobic tryptophan (Trp) residue Trp93 was shown to be a driver of reactivity, with alanine mutagenesis of this residue implied that absence of this residue and other hydrophobic residues had significant impact on TCR binding (62). Intriguingly, the hydrophobic residues driving this reactivity were shared across all T22 reactive $\gamma\delta$ TCRs (63), including the murine KN6 (V γ 4V δ 5) TCR which had a non-germline encoded Trp within the CDR3 δ loop that was implicated *via* alanine mutagenesis as a driver of T22 binding (62). This mode of recognition was shown to mimic a single-chain shark antibody which utilised a homologous CDR3 loop to bind a similar cavity within lysozyme (62). The G8 TCR-T22 complex provided a prefatory glimpse into the unique rules of $\gamma\delta$ TCR-ligand binding, suggesting a prominent role for apical CDR3 δ Tryptophan's that were common across the T22-reactive $\gamma\delta$ T cells isolated.

$\gamma\delta$ TCR-pMHC-I

Despite sometimes being overlooked as bona fide ligands, multiple examples of MHC I and MHC II reactive $\gamma\delta$ TCRs have been identified, including several human $\gamma\delta$ TCR clones reactive to various MHC molecules such as HLA-B*07 (64), HLA-A*24 (65) and HLA-A*02 (antigen for ES204 TCR) (58). Currently, there is one structure of a $\gamma\delta$ TCR interacting with a classical MHC I molecule, namely the 5F3 (V γ 8V δ 1) TCR binding HLA-A*02 presenting melanoma associated antigen, MART-1 peptide (Figure 1C) (45). 5F3 TCR was isolated from human hematopoietic stem and progenitor cells and bound HLA-A*02-MART1 at relatively strong affinity for physiological TCRs at ~ 2.9 μ M (45, 66). The overall docking topology of this interaction was highly reminiscent of canonical $\alpha\beta$ TCR-pMHC-I interactions as the V γ and V δ co-recognises HLA-A*02 and MART-1 peptide in an 'end-to-end' manner. However, there are some distinctions. As the δ and α chain are homologs, and the V δ 1 domain sits atop the $\alpha 1$ helix and the V γ 8 domain atop the $\alpha 2$ helix, this is considered a 180° flip of what is typical with $\alpha\beta$ TCRs (45, 48). Here, there is also a δ -chain bias as it is responsible for $\sim 59\%$ of the interface (Table 1). All loops, with the exception of CDR2 δ loop are involved in binding HLA-A*02-MART1 to varying degrees. Germline

CDR1 δ loop residues Trp29 and Trp30, in particular, contribute predominantly by forming extensive van der Waals interactions with the α 1 helix on the antigen binding platform of HLA-A*02 (Figure 2B). However, only the CDR3 δ and CDR1 γ loops are directly in contact with the MART-1 peptide with non-germline encoded Trp98 of the CDR3 δ loop being the major contributor (Figure 2A), forming more van der Waals interactions cross the peptide with Ala3, Gly4, and Ile7 (45). Conversely, the CDR2 γ loop is responsible for most of the contacts to HLA-A*02 with positively charged Arg56 underpinning a network of hydrogen bonds, van der Waals interactions, and a salt bridge with Glu154 on the α 1 helix of the MHC I molecule. The 5F3 TCR-HLA-A*02-MART1 structure revealed a recurrence of both a δ -chain bias and Trp residues acting as major contacts, the latter may have implications for $\gamma\delta$ TCR recognition broadly, as the Trptophans were germline and thus conserved across all V δ 1⁺ TCRs.

$\gamma\delta$ TCR-CD1-lipid

Several populations of human $\gamma\delta$ T cell populations isolated from peripheral blood and the intestinal epithelium have exhibited reactivity towards several members of the CD1 family (25–28, 67–70). To date there are three $\gamma\delta$ TCR-CD1 structures, two with CD1d presenting different lipid antigens (25, 27) and one with CD1a (28).

The two CD1d structures include the DP10 (V γ 4V δ 1) and 9C2 (V γ 5V δ 1) TCRs, in complex with CD1d presenting sulfatide or α -Galactosylceramide (α -GalCer) (Figure 1D & 1E) (25, 27). Both DP10 and 9C2 TCRs were isolated from peripheral blood whereby, 0.004–0.01% of CD3⁺ PBMCs and 0.05–3.5% of $\gamma\delta$ TCR⁺ PBMCs were reactive to CD1d-sulfatide and CD1d- α -GalCer respectively (25, 27, 71). The DP10 and 9C2 TCRs bound CD1d-sulfatide and CD1d- α -GalCer with comparable affinities ($\sim$ 5.6 μ M & $\sim$ 16.0 μ M, respectively), which demonstrated moderate affinity interactions that fell within the range expected of physiological TCRs (66). Of the two, only the DP10 TCR displayed lipid-specific reactivity (sulfatide), whilst the 9C2 TCR was broadly reactive to endogenous lipids. The DP10 and 9C2 $\gamma\delta$ TCRs bound atop the antigen presentation cleft of the CD1d molecule although DP10 was positioned centrally above the presented sulfatide lipid, whereas 9C2 was positioned slightly closer to the distal end of the antigen presentation platform (25, 27). Interestingly, both $\gamma\delta$ TCR-CD1d-lipid complexes were dominated by the δ -chain, the DP10 δ -chain exclusively mediated CD1d-sulfatide recognition whereas the 9C2 δ -chain comprised 75% of the CD1d- α -GalCer interface. More specifically, the DP10-CD1d-sulfatide interface involved germline encoded CDR1 δ and CDR3 δ loop residues as the main mediators of the interaction with the CD1d molecule, contributing 32% and 57% to BSA, respectively (Table 1). Here, the germline encoded Trp29, Trp30 of the CDR1 δ loop, and Trp99 of the CDR3 δ loop (Figure 2C) made key contributions to the recognition of CD1d. These three aromatic residues formed extensive covalent bonds and van der Waals interactions with the α 1 and α 2 helices of the CD1d antigen presentation platform. However, only the CDR3 δ loop contacted the presented sulfatide as junctionally encoded aromatic Phe101 formed a hydrogen bond with the sulfate moiety of the sulfatide. With the 9C2 TCR, the CDR1 δ loop contributed a majority 40% to CD1d binding, whereby Trp29 and Trp30 formed mainly van der Waals interactions with the α 2 helix of CD1d (Figure 2D). The 9C2 CDR3 δ loop also contributed 28% to BSA as almost the entirety of the

loop (Asp98 to Lys106) formed extensive van der Waals interactions with both α helices of CD1d. In contrast to the DP10 TCR, the γ -chain of 9C2 TCR is also involved in recognition of the CD1d-lipid complex. Although the 9C2 γ only minorly contributed to BSA (25%) (Table 1), Arg103 and Tyr111 of the CDR3 γ loop were the sole mediators of α -GalCer recognition, forming extensive hydrogen bonds and van der Waals interactions with the galactose moiety.

The most recently published $\gamma\delta$ TCR-CD1-lipid structure is that of CO3 (V γ 4V δ 1) TCR in complex with CD1a presenting sulfatide (Figure 1F) (28). Here, ~0.070% of CD3⁺ $\gamma\delta$ TCR⁺ PBMCs isolated from the peripheral blood were broadly CD1a reactive (28). The CO3 TCR binds the CD1a-sulfatide complex at ~22 μ M, consistent with other $\gamma\delta$ TCR-antigen interactions and $\alpha\beta$ TCR-CD1a interactions (55). The CO3 TCR was also found to be lipid permissive as the loading of different lipopeptides and endogenously loaded lipids had little effect on binding (28). This CO3 $\gamma\delta$ TCR-CD1a-lipid structure was a highly novel interaction whereby the $\gamma\delta$ TCR co-contacted CD1a and β_2 microglobulin (β_2 m) at the extreme end of the antigen binding cleft in a fashion described as ‘sideways’ as opposed to the canonical ‘end-to-end’. Notably, there is no co-recognition of the presented sulfatide as the CO3 TCR only contacted CD1a but nevertheless a CD1a-bound lipopeptide can impact CO3 TCR recognition *via* indirect means, namely by altering the conformation of the CD1a cleft. Here, the δ -chain once again dominated the majority of the interaction, contributing 65% to BSA (Table 1). The CDR1 δ and CDR3 δ loops were primarily responsible for the interactions with both the CD1a molecule and the β_2 M, contributing 20% and 40% to BSA respectively. Once more, the germline encoded Trp31 formed extensive van der Waals interactions with the β_2 M while the non-germline encoded Trp99 formed a mixture of hydrogen bonds, van der Waals, and *pi*-stacking interactions with the CD1a heavy chain (Figure 2E). As with previously examined gdTCR complexes, there is a persistence of tryptophan residues on the δ -chain in mediating interactions with the MHC-I like molecule. The γ -chain only contributed ~35% of the BSA, where framework residues (Arg48, Tyr51, Leu61, Glu62) proximal to the CDR2 γ loop primarily formed van der Waals interactions with His21 of the CD1a heavy chain. Together these CD1- $\gamma\delta$ TCR structures demonstrate a continued involvement of germline Tryptophan’s in the recognition of MHC-I like molecules, despite unprecedented variances in antigens or docking mechanisms.

$\gamma\delta$ TCR-MR1

Following on from the interest in $\gamma\delta$ T cell-CD1 interactions, novel interactions with human MR1 were observed (46). Here, <0.1–5% of $\gamma\delta$ T cells in the peripheral blood were broadly specific for MR1 and from these $\gamma\delta$ T cell clones, G7 (V γ 9V δ 1) and G83.C4 (V γ 5V δ 3) were isolated (46). The co-complex structure of G7 $\gamma\delta$ TCR-MR1 presenting 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), a pyrimidine formed as a metabolite of the riboflavin pathway (72), was solved first (Figure 1G) (46). G7 TCR bound MR1–5-OP-RU at moderate affinity of ~ 4.7 μ M akin to $\alpha\beta$ MAIT TCR recognition of MR1–5-OP-RU (~1.65 μ M) (2). Like the CO3 TCR, G7 TCR overturned any preconceived notions of a canonical binding modes adopted by $\gamma\delta$ TCRs as it binds to the underside of the MR1 molecule within the α 3 domain without co-contacting the presented 5-OP-RU (46). Again, there is a prevailing dominance of the δ chain in ligand interaction as the

V δ 1 contributed a majority 84% to BSA (Table 1). Here, CDR1 δ and CDR3 δ loops were the primary mediators of contacting the α 3 domain, contributing 22% and 42% to BSA respectively. This is due to the predominance of aromatic residues in the CDR1 δ and CDR3 δ loops at the interface, a recurring motif in MHC I-like molecule recognition. Again, key Tryptophan's (Trp29, Trp30, and Trp106) formed key van der Waals interactions with the α 3 domain of MR1 (Figure 2F). Similarly to the CO3 TCR, the framework regions of the γ -chain made crucial contacts to the MR1 molecule and were responsible for almost the entire γ -chain contributions to the BSA. Here, Arg59, Lys60, Glu61, and Ser62 on the γ chain formed a mixture of van der Waals and hydrogen interactions with Glu219 of the MR1 α 3 domain.

Next, the V δ 3⁺ G83.C4 TCR was determined to bind MR1–5-OP-RU at strong affinity of $\sim$ 0.57 μ M. This affinity is relatively stronger than that of $\alpha\beta$ TCR-MR1 interactions and is also considered a strong affinity for $\alpha\beta$ TCRs overall (2, 66). The co-complex structure revealed that it too, had a docking topology that deviated from the norm of $\alpha\beta$ TCR binding (47). The G83.C4 TCR binds MR1 on the upper side away from the antigen presentation cleft without co-recognition of 5-OP-RU (Figure 1H). Being the first human non-V δ 1⁺ TCR in complex with an antigen solved, underlying patterns observed in V δ 1⁺ complexes were also observed here with key differences. The δ -chain continues to dominate the interaction with MR1, contributing 71% towards BSA as the CDR3 δ loop (37%) is a driver for the unconventional docking topology (Table 1). In contrast to V δ 1⁺ structures, Trp29 and Trp30 were absent from the V δ 3⁺ domain. Instead, key contacts to MR1 were primarily made *via* germline-encoded negatively charged Asp103 and Asp107 on the CDR3 δ loop. Here salt bridges were formed with positively charged Arg61 on the α 1 helix and Arg167 on the α 2 helix respectively. Additional hydrogen bonds and van der Waals interactions were formed between Asp103 and both the α 1 and α 2 helices. Here, non-germline encoded Trp100 on the CDR3 δ loop contributed indirectly to the interaction, forming van der Waals interactions with the polar end of the α 1 helix (Figure 2G) (47). Despite the lesser role aromatics may play in V δ 3⁺ TCR bind to MHC I-like structure, its presence in the interaction is consistent with human V δ 1⁺ and murine $\gamma\delta$ TCR recognition of MHC I-like molecules. Minor contribution from the γ domain is mainly evenly distributed between the CDR2 γ and the CDR3 γ loops (11%) where each loop forms a complex of hydrogen bonds, salt bridges and van der Waals interactions with the α 1 and α 2 helices respectively.

V δ -domain centric binding

A striking observation of the four V δ 1⁺ TCR structures in complex with MHC I-like molecules (DP10, 9C2, G7, and CO3) is their markedly different angles and paratopes of recognition, highlighting an unpredictability of $\gamma\delta$ TCR ligand binding. Despite such variances, there are commonalities across these $\gamma\delta$ TCR-ligand interfaces including the conserved involvement of germline-encoded CDR1 and CDR3 loop residues yet, this does not lead to common docking mechanisms as observed in some $\alpha\beta$ TCR-peptide-MHC interactions (73). Specifically, CDR1 δ loop encoded Tryptophan's are major mediators in all of the V δ 1⁺ TCR complex structures yet do so uniquely within each complex (Figure 2). From the singular V δ 3⁺ TCR-antigen complex solved; hydrophobic residues still played a key role in supporting the interaction with MHC-like molecule. However,

whether this underlying pattern can be observed in other V δ ³⁺ interactions remain to be seen. Furthermore, whether these observations hold true for V δ ²⁺ and V δ ⁵⁺ populations is unknown.

An emergent trend across all these $\gamma\delta$ TCR complexes is the V δ domain dominance at the binding interface. So far, all published complex structures are of $\gamma\delta$ TCRs bound to an MHC or MHC I-like molecules, leaving open an unexplored realm of co-complex structures with other ligands (Table 2). Further support for a prevailing V δ reliance has been demonstrated in $\gamma\delta$ TCR activation assays involving CD1b (70) and CD1c (67) which showed reactive V δ ¹⁺ populations with varying V γ domains. Reactivity towards other ligands, including MICA (74) and non-MHC I-like ligands like phycoerythrin (15), and Ephrin type-a receptor 2 (75) has also been observed in $\gamma\delta$ T cell populations isolated from human peripheral blood. For many of these highly unconventional ligands, reactive populations expressed a common δ -chain, implying a high degree of δ -chain reliance in binding. Phycoerythrin and Ephrin type-a receptor reactive $\gamma\delta$ T cells all expressed a V δ 1 chain (15, 75). It is possible that the capabilities of the CDR3 δ for generating long and hyper-diverse loops may play a role in driving the dominance of the δ -chain in these interactions.

$\gamma\delta$ TCR-B7-like

Potential exceptions to the V δ centric binding paradigms are a population which respond to the B7-like molecules butyrophilin or butyrophilin-like (BTNL) molecules. BTNs are a family of immunoglobulin proteins related to B7 proteins such as PD1, a checkpoint inhibitor for $\alpha\beta$ T cell activation (20). The former utilises a V γ 9 domain and to recognise BTN2A1 (21–23), whereas the latter relies upon a V γ 4 domain to bind BTNL3 in a BTNL3-BTNL8 heterodimer (42, 43). These $\gamma\delta$ T cell populations are some of the most numerous with V γ 9V δ ²⁺ T cells accounting for up to ~20% of circulating peripheral blood $\gamma\delta$ T cells (20, 22).

A number of studies have been made into understanding the underpinnings of $\gamma\delta$ TCR mediated recognition of phosphoantigens (pAgs) *via* butyrophilins (BTN), BTN2A1 and BTN3A1, drivers of the recognition of intracellular pAgs (22, 23, 76, 77). Phosphoantigens are accumulated in cells either due to infection with pAg-producing microbes and parasites or due to dysfunctional metabolic pathways often present in cancers or cells under stress (4, 20, 78, 79). Phosphoantigens bind to the intracellular domain of BTN3A1 (76), and are thought to induce a conformational change that promotes BTN2A1 association (80, 81). It is hypothesised that this conformational change propagates to the extracellular region, leading the exposure of a $\gamma\delta$ TCR binding surface on BTN2A1 (82). Whilst there is an established restriction of BTN2A1 binding to the V γ 9 domain, direct recognition of the extracellular V domain of BTN3A1 by the positively charged CDR2 δ residues of the V δ 2 domain has only recently been demonstrated *via* mutational analyses (83). BTN3A1 was also recently demonstrated to form a dimer with non-pAg sensing isoforms BTN3A2 or BTN3A3, which enable the cell surface trafficking of BTN3A1 and the subsequent recognition of pAgs and BTN3A1 by V γ 9V δ 2 TCRs (23, 84).

Recently, the first glimpses into how a V γ 9V δ 2 TCR interacts with BTN2A1 was afforded by an elegant structural study and resultant complex crystal structure (PDB: 8DFW),

described in detail in (83). This novel structure revealed that the BTN2A1 IgV domain was bound by the side of the V γ 9 domain. The CDR γ loops had little involvement in BTN2A1 binding, with all major contacts stemming from germline-encoded framework regions. This interaction centred upon a positively charged Arginine (Arg20) on the β strand preceding the CDR1 γ loop. This residue is absent from other V γ domains and could explain the specificity of V γ 9⁺ TCRs for BTN2A1. Thus, the V δ domain dominance that underpins $\gamma\delta$ TCR interactions MHC-like molecules is starkly contrasted in the first example of a BTN reactive $\gamma\delta$ TCR structure where the γ chain drove binding.

Shifting shape complementarity and buried surface area contributions

Although the BSA contribution of each chain varies across $\gamma\delta$ TCR complexes, we aimed to probe how BSA and shape complementarity (sc) (85) of the entire interface compares to that of $\alpha\beta$ TCRs. Previous analyses of a large repertoire of $\alpha\beta$ TCR-MHC complexes revealed that average total BSA was ~1910 or 2030 Å² between MHC I and MHC II complexes, respectively (48). This value, amongst the $\gamma\delta$ TCR complexes is slightly lower on average, at ~1780 Å. The smallest value seen in DP10 TCR-CD1d presenting sulfatide with a total BSA of ~1310 Å, this is within the range of $\alpha\beta$ MHC I complexes but is smaller than known $\alpha\beta$ TCR-MHC II complexes.

In regard to sc, with 0 being considered not at all fitting and, a value of 1 being considered to be a perfect fit, most of the $\gamma\delta$ TCR-complexes were similarly well fitting. The lowest sc values amongst existing $\alpha\beta$ TCR complexes were 0.41 and 0.48 for MHC I and MHC II respectively. Whereas the lowest sc value for a $\gamma\delta$ TCR-ligand complexes was 0.56 for the G7 gdTCR-MR1 complex. (Table 1). Shape complementarity has also been observed to be a poor correlate for affinity in $\alpha\beta$ -TCR-pMHC complexes (48). Shape complementarity in antibody-antigen complexes (0.64 – 0.68) is generally higher than those of $\alpha\beta$ TCR-pMHC complexes (85). Considering the structural attributes that $\gamma\delta$ TCRs share with both the $\alpha\beta$ TCR and antibodies, sc is yet another aspect worth examination as further $\gamma\delta$ TCR-antigen complexes are solved.

Conclusions

Inroads have been made in recent decades in the effort to unravel the complexity of the $\gamma\delta$ TCR interactions. As the new structural evidence suggests, $\gamma\delta$ TCR complexes can be entirely unpredictable. When viewing all existing $\gamma\delta$ TCR-MHC I complexes superposed to together, it becomes apparent that each complex is distinct in docking topology, akin to antibody-antigen complexes rather than the canonical ‘end-to-end’ binding of conventional $\alpha\beta$ TCR-pMHC complexes. Nevertheless, some recurring patterns are emerging in the $\gamma\delta$ TCR recognition of MHC-like molecules. For example, there is a strong dominance of the δ -chain and the omnipresence of a tryptophan’s in mediating interactions, this is a concept that grows as the portrait of $\gamma\delta$ TCR recognition continues to evolve. These recurring patterns are only observed in $\gamma\delta$ TCR-MHC I-like structures, perhaps suggesting that these motifs are unique to MHC I-like recognition. Notably, the increased presence of hydrophobic CDR loop residues in $\alpha\beta$ TCRs have been linked to reactivity to self-antigens presented *via* MHC molecules(86).

Furthermore, the antigen co-recognition paradigm is absent in multiple existing $\gamma\delta$ TCR-MHC I-like structures. Instead, direct recognition of the MHC-like molecule proceeds and is reminiscent of antibody-like recognition. These observations corroborate noted structural similarities of $\gamma\delta$ TCR variable domains and antibodies which suggested that the conformational flexibility of $\gamma\delta$ TCR CDR3 loops were reminiscent of immunoglobulins (57, 60).

The veracity of these emerging trends as solid rules for $\gamma\delta$ TCR engagement is yet to be established and exemplifies the importance of further structural studies to deconvolute the nature of how $\gamma\delta$ TCRs interact with ligands. In this vein, study of the $\gamma\delta$ TCR with the context of CD3 signalling apparatus could be highly informative as would further insight into the signalling capacity of $\gamma\delta$ TCRs with conflicting observations of hypo or hyper responsive $\gamma\delta$ T cells following antigen stimulation (28, 46, 47). Such structural studies will be revealing for $\gamma\delta$ T cell biology and highly informative for the burgeoning field of $\gamma\delta$ T cell-based immunotherapies.

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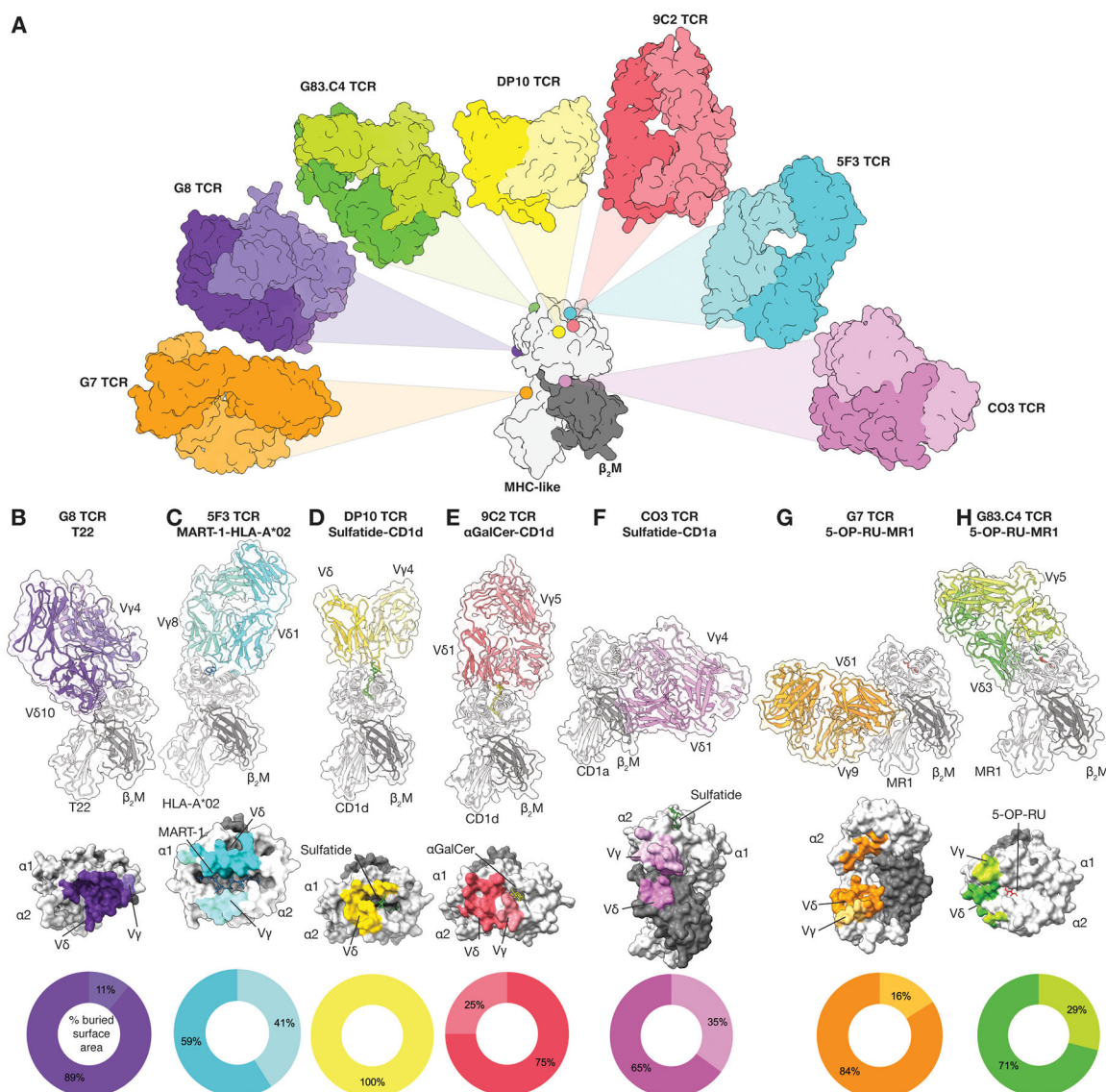


Figure 1. Docking topology of $\gamma\delta$ TCRs upon MHC-like molecules.

A. Expanded alignment of $\gamma\delta$ TCR complex structures upon an MHC I-like molecule. All molecules are represented as a surface with the γ -chain of each TCR in a lighter shade and the δ -chain in a darker shade of the orange (G7 TCR), purple (G8 TCR), green (G83.C4 TCR), yellow (DP10 TCR), red (9C2 TCR), blue (5F3 TCR), and pink (CO3 TCR). Dots and projections symbolise the approximate centre of the interface. **B-H)** Contribution of $V\gamma$ and $V\delta$ towards BSA at the interface of the TCR and MHC-like molecule. Complexes are initially displayed in cartoon form within a silhouette of the surface of the complex. The BSA of the MHC-like molecule corresponding to each chain of the $\gamma\delta$ TCR is highlighted in their respective colours with the $V\gamma$ chain being depicted in the lighter shade. **B.** Murine G8 TCR in complex with T22. PDB: 1YPZ. **C.** 5F3 TCR in complex with HLA-A*02 presenting MART-1 peptide. PDB: 6D7G. **D.** DP10 TCR in complex with CD1d presenting sulfatide. PDB: 4MNG. **E.** 9C2 TCR in complex with CD1d presenting α GalCer. PDB: 4LHU. **F.** CO3 TCR in complex with CD1a presenting sulfatide. PDB: 7RYN. **G.** G7 TCR

in complex with MR1 presenting 5-OP-RU. PDB: 6MWR. **H.** G83.C4 in complex with MR1 presenting 5-OP-RU. PDB: 7LLI. Molecular graphics and analyses were performed with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, USA.

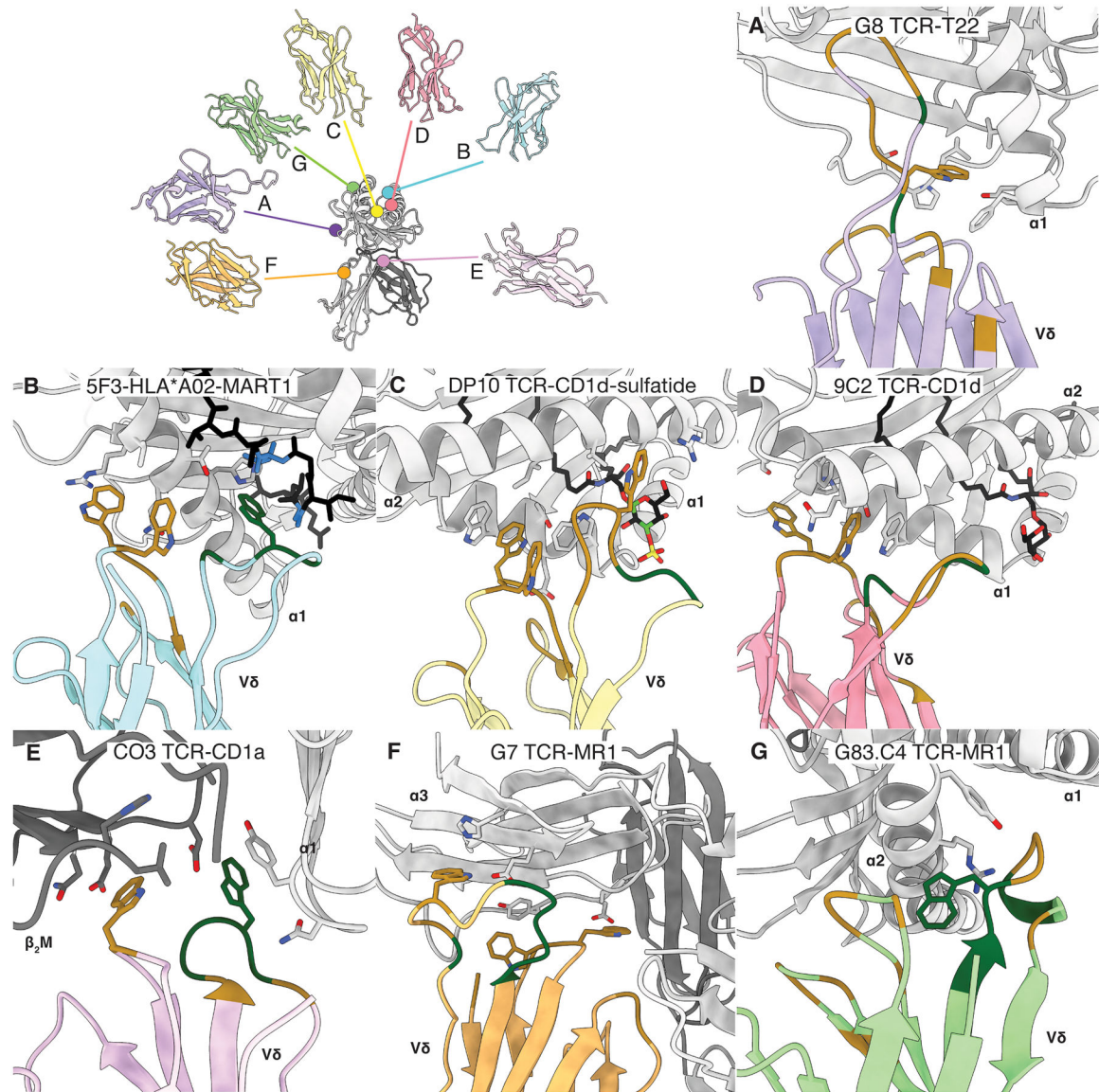


Figure 2: Key tryptophan residues in the germline and non-germline encoded regions of $\gamma\delta$ TCR paratopes.

Cartoon representation of the interfacial tryptophan residues from the TCR δ -chain, with germline and non-germline residues coloured brown and dark green, respectively for the following: **A.** G8 TCR in complex with murine T22. PDB: 1YPZ. **B.** 5F3 TCR in complex with HLA-A*02 presenting MART-1 peptide. PDB: 6D7G. **C.** DP10 TCR in complex with CD1d presenting sulfatide. PDB: 4MNG. **D.** 9C2 TCR in complex with CD1d presenting α GalCer. PDB: 4LHU. **E.** CO3 TCR in complex with CD1a presenting sulfatide. PDB: 7RYN. **F.** G7 TCR in complex with MR1 presenting 5-OP-RU. PDB: 6MWR. **G.** G83.C4 in complex with MR1 presenting 5-OP-RU. PDB: 7LLI. Contact residues on the MHC I-like molecule are also displayed. Molecular graphics and analyses were performed with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, USA.

Table 1:Buried surface areas of all known $\gamma\delta$ TCR-ligand complex structures

Complex	K _D	BSA (Å ²)	BSA (%)	BSA (%)	sc
G8 TCR-T22 (V γ 4V δ 10)	67 nM	T22: 930			
		TCR: 940	γ : 11	CDR1 γ : 0	0.66
				CDR2 γ : 0	
				CDR3 γ : 11	
			δ : 89	CDR1 δ : 12	
				CDR2 δ : 2	
DP10 TCR-CD1d-sulfatide (V γ 4V δ 1)	5.6 μ M	CD1d: 590			
		TCR: 720	γ : 0		0.82
			δ : 100	CDR1 δ : 32	
				CDR2 δ : 1	
				CDR3 δ : 57	
9C2 TCR-CD1d- α GalCer (V γ 5V δ 1)	16 μ M	CD1d: 930			
		TCR: 900	γ : 25	CDR1 γ : 4	0.75
				CDR2 γ : 0	
				CDR3 γ : 18	
			δ : 75	CDR1 δ : 40	
				CDR2 δ : 0	
G7 TCR-MR1-5-OP-RU (V γ 9V δ 1)	4.7 μ M	MR1: 1030			
		TCR: 1090	γ : 16	CDR1 γ : 0	0.56
				CDR2 γ : 0	
				CDR3 γ : 0.1	
			δ : 84	CDR1 δ : 22	
				CDR2 δ : 0	
5F3 TCR-HLA-A*02-MART1 (V γ 8V δ 1)	2.9 μ M	MHC: 990			
		TCR: 1000	γ : 41	CDR1 γ : 7	0.71
				CDR2 γ : 22	
				CDR3 γ : 10	
			δ : 59	CDR1 δ : 22	
				CDR2 δ : 0	
G83.C4 TCR-MR1-5-OP-RU (V γ 5V δ 3)	0.57 μ M	MR1: 1000			
		TCR: 970	γ : 29	CDR1 γ : 3	0.62
				CDR2 γ : 11	
				CDR3 γ : 11	
			δ : 71	CDR1 δ : 17	
				CDR2 δ : 19	

Complex	K _D	BSA (Å ²)	BSA (%)	BSA (%)	sc
CO3 TCR-CD1a-sulfatide (V γ 4V δ 1)	22 μ M	CD1a: 550 β ₂ M: 210 TCR: 730	γ : 35 δ : 65	CDR3 δ : 37	0.65
				CDR1 γ : 1	
				CDR2 γ : 6	
				CDR3 γ : 5	
				CDR1 δ : 20	
				CDR2 δ : 0	
				CDR3 δ : 44	

Table 2:

All presently known human $\gamma\delta$ TCR ligands.

Known Ligands	$\gamma\delta$ TCR	Known Presentation	Ref.	Structure
MHC/MHC-like				
HLA:peptide	V δ 1 ⁺	Peripheral blood	(45, 87)	5F3 TCR-HLA-A*02-MART1
MICA	V δ 1 ⁺	Peripheral blood	(74)	N/A
CD1a	V δ 1 ⁺	Peripheral blood	(28)	CO3 TCR-CD1a-sulfatide
CD1b	V δ 1 ⁺	Peripheral blood	(70)	N/A
CD1d	V δ 1 ⁺ /V δ 3 ⁺	Peripheral blood	(25, 27)	9C2 TCR-CD1d- α GalCer DP10 TCR-CD1d-sulfatide
CD1c	V δ 1 ⁺	Peripheral blood	(67)	N/A
MR1	V δ 1 ⁺ /V δ 3 ⁺	Peripheral blood	(46, 47)	G7 TCR-MR1-5-OP-RU G83.C4 TCR-MR1-5-OP-RU
Butyrophilins				
BTN2A1/BTN3A1	V γ 9V δ 2 ⁺	Peripheral blood	(4, 20–23).	G115 TCR-BTN2A1
BTNL3/BTNL8	V γ 4 ⁺	Enteric organs	(42)	N/A
Other				
Phytoerythrin (PE)	V δ 1 ⁺	Unknown	(15)	N/A
EphA2	V γ 9V δ 1 ⁺	Peripheral blood	(75)	N/A
hMSH2	V δ 2 ⁺	Peripheral blood	(88)	N/A
histidyl tRNA synthetase	V γ 3V δ 2 ⁺	Peripheral blood	(18)	N/A
Annexin A2	V δ 3 ⁺	Unknown	(11)	N/A
EPCR	V γ 4V δ 5 ⁺	Peripheral blood	(24)	N/A