The versatility of the αβ T-cell antigen receptor

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Abstract: The T-cell antigen receptor is a heterodimeric αβ protein (TCR) expressed on the surface of T-lymphocytes, with each chain of the TCR comprising three complementarity-determining regions (CDRs) that collectively form the antigen-binding site. Unlike antibodies, which are closely related proteins that recognize intact protein antigens, TCRs classically bind, via their CDR loops, to peptides (p) that are presented by molecules of the major histocompatibility complex (MHC). This TCR-pMHC interaction is crucially important in cell-mediated immunity, with the specificity in the cellular immune response being attributable to MHC polymorphism, an extensive TCR repertoire and a variable peptide cargo. The ensuing structural and biophysical studies within the TCR-pMHC axis have been highly informative in understanding the fundamental events that underpin protective immunity and dysfunctional T-cell responses that occur during autoimmunity. In addition, TCRs can recognize the CD1 family, a family of MHC-related molecules that instead of presenting peptides are ideally suited to bind lipid-based antigens. Structural studies within the CD1-lipid antigen system are beginning to inform us how lipid antigens are specifically presented by CD1, and how such CD1-lipid antigen complexes are recognized by the TCR. Moreover, it has recently been shown that certain TCRs can bind to vitamin B based metabolites that are bound to an MHC-like molecule termed MR1. Thus, TCRs can recognize peptides, lipids, and small molecule metabolites, and here we review the basic principles underpinning this versatile and fascinating receptor recognition system that is vital to a host’s survival.

Keywords: immune system; structures; T-cell receptor; antigen presenting molecules

Abbreviations: α-GalCer, α-galactosylceramide; β2m, β2 microglobulin Ab; Antibody; Ag, Antigen; AGLs, altered glycolipid ligands; C, constant gene segment; CDR, complementarity determining region; CD1, cluster of differentiation 1; D, diversity gene segment; HLA, human leukocyte antigen; iGb3, isoglobotrihexosylceramide; J, joining gene segment; MHC, major histocompatibility complex; MR1, MHC-related protein 1; NKT, natural killer T-cell; PFR, peptide flanking regions; TCR, T-cell receptor; V, variable gene segment.

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Introduction

T-lymphocytes (T-cells), a key arm of the cellular immune system, determine the specificity of the immune response via their αβ T-cell antigen receptor (TCRs). The αβ TCR is highly variable, being comprised of a number of gene segments. Namely, TCRs are diversified via the random re-association of numerous variable (V) and joining (J) genes at the TCR α-locus and V, diversity (D), and J genes at the TCR β-locus of immature T-cells. Further diversity within the TCR is generated via non-nucleotide encoded changes at the V(D)J junctional boundaries. As such, the repertoire of unique TCRs following a process termed thymic selection is comprised of $10^7$–$10^8$ individual receptors.1 This extensive diversity resides mostly in the complementarity determining regions (CDR), three each from the Va and Vβ domains of the TCR, with the CDR3 loops showing the greatest diversity. Collectively, the six CDR loops represent the antigen-binding site of the TCR. The structure of the αβ TCR, which was first solved in 1996, revealed that it resembled the structure of a Fab fragment from an antibody (Ab).2,3 However, unlike Abs, which recognize intact antigens, TCRs recognize processed fragments of antigens that are displayed on the host’s cell surface by antigen-presenting molecules. Moreover, unlike the high affinity (nM) of the Ab–Ag interaction, TCRs generally interact with their ligands with low affinity (μM range).4 The notable exception is the pre-T-cell antigen receptor, an immature form of the TCR, which interacts with itself to enable T-cell development.5,6 Accordingly, a key question in TCR biology arises: how is specificity within the TCR axis maintained against the backdrop of a low affinity interaction? After all, TCR recognition results in an activation signal being transmitted to the T-cell that activates the T-cell, thereby ultimately leading to, for example, the killing of an infected host cell. However, whilst the T-cell, via its TCR recognition characteristics, plays a key role in protective immunity, it can also play a key role in immune dysfunction. For example, aberrant TCR reactivity is linked to T-cell mediated transplant rejection, autoimmunity, and drug-linked hypersensitivities.7–9 Further, whilst it is commonly considered that TCRs recognize peptide-based antigens, it is now becoming clear that TCRs can also bind lipid antigens and small molecule metabolites when presented by specific Ag-presenting molecules, the recognition of which is also crucial for protective immunity and associated with T-cell mediated disease10 (Fig. 1). Accordingly, to understand the role of TCR interactions in biology, it is of enormous value to understand the basic principles underscoring this interaction. This review provides a structural portrait of TCR recognition of peptide, lipid, and vitamin B metabolites.

Peptide-mediated immunity

The conventional T-cell response is mediated by TCR recognition of short peptide fragments bound in the binding cleft of MHC class I or class II molecules.2,11,12 In general, MHC-I presents peptides derived from endogenous proteins that are recognized by cytotoxic T-cells whereas MHC-II presents exogenously-derived peptides to T helper cells. The classical human MHC-I genes are split-up into three classes (A, B, and C) that encode a three domain α chain that binds to the nonpolymorphic β2 microglobulin (β2m) protein [Fig. 2(A)]. The human MHC-II genes (DR, DQ, and DP) comprise a polymorphic α- and β-chain, which pair to form the MHC-II molecule [Fig. 2(B)].13 Although the subunit compositions of the two MHC classes are distinct, they are structurally very similar [Fig. 2(A–D)].16,17 The peptide binding cleft, in both cases, is comprised of two anti-parallel α-helices that form a channel in which the peptide can bind in an extended conformation, and eight antiparallel β-sheets. The α-helices and β-sheets provide specific peptide binding pockets in the base of the cleft that are highly variable between different MHC alleles.15,18 Peptides are selected according to their ability to bind to these MHC allele-specific pockets within the floor and sides of the peptide-binding cleft.

Despite these similarities in overall conformation, MHC-I and II present peptides in a distinct manner that is governed by the composition of the MHC peptide-binding cleft. The closed conformation of the MHC-I α1α2 binding cleft [Fig. 2(C)] restricts the length of peptides that can bind (most commonly 9 or 10mers, but longer peptides have been observed19), MHC-I-restricted peptides bind to specific pockets (A–F) in the MHC cleft, although the most important interactions are usually between the primary peptide anchor residues located at, or near, the N- and C-termini of the peptide, and the MHC-I B and F pockets, respectively. These pockets are lined with polymorphic residues that define the size and chemical characteristics of each pocket, and therefore the specific peptide repertoire that can be accommodated by different MHC-I alleles. For example, the HLA-B44 alleles are ideally suited to accommodate glutamic acid and aromatic residues at the B and F-pockets, respectively.20,21 Depending on the length of the peptide, this binding mode squeezes the central peptide residues up so that they extend out the cleft [Fig. 2(E)], exposing peptide side chains for direct interaction with the TCR.22 In contrast, the MHC-II α1β1 binding cleft comprises an opened conformation [Fig. 2(D)] that allows the binding of N- and C-terminally extended peptides. MHC-II restricted peptides typically contain the central binding motif of nine “core” amino acids that bind to the MHC-II cleft via an extensive hydrogen bond network between the MHC-II cleft and the peptide.
Peptide side chains also form contacts with allelic specific pockets of the MHC-II binding groove. Allelic differences within these pockets, usually P1, P4, P6, and P9, govern the specific peptide binding motif and register that can be accommodated by different MHC-II alleles. For example, HLA-DQ8, an allele implicated in celiac disease, exhibits a preference for glutamates at the P1 and P9 pockets. Amino acids that are outside of the "core" region can extend out of either end of the open binding groove like a "hot dog in a bun," forming so called "peptide flanking regions" (PFRs) at both the N- and C-termini (Fig. 2(F)). We have recently shown that modifications to these PFRs can modulate TCR binding affinity and T-cell activation. Further complexity to the MHC-II antigen presentation is added by the presentation of nested sets of peptides in complex within MHC-II at the cell surface. Nested sets share the same constant core sequence of 9 amino acids that associate with binding groove of the MHC-II molecule. However, the PFRs that extend out of the groove can be variable in length and sequence, and are determined by proteolytic processes during the exogenous antigen processing pathway that are not well defined. The effect on antigen recognition, and the mechanisms that control diversity of these sets are still being determined. However, there is evidence...
to suggest that the structural differences in MHC-I and MHC-II antigen presentation have role in governing TCR binding affinity and T-cell immunity. In humans, MHCs are encoded by the human leukocyte antigen (HLA) locus on chromosome 6. This locus is a highly polymorphic region of the human genome, reflecting the continuing need for protective immunity against the virtually unlimited diversity of rapidly-evolving pathogens that might be encountered. The HLA locus spans over 5 mega bases and over 200 genes\(^3\) with more than 7000 HLA allelic sequences identified to date.\(^4\) Normally, individuals express 6 different classical MHC-I and 6 MHC-II molecules that can differ by a single amino acid from each other or by more than 30 amino acids. These polymorphisms, which are mostly found in the MHC binding cleft, dictate the diversity of the peptides presented by each MHC molecule. Importantly, the repertoire of self-pMHCs expressed in the thymus during T-cell selection is also determined in this way. Thus, the selection of naive T-cells that are released into peripheral organs will be different according to an individual's MHC fingerprint. Consequently, MHC polymorphism can change the pMHC structure in a way that can markedly impact on T-cell immunity during pathogen responses, autoreactivity, and alloreactivity.\(^5\) For example, it is accepted that MHC-I is the greatest genetic determinant on disease progression during HIV infection. The expression of certain pMHC-I alleles is strongly linked to protection (such as HLA-B57 or HLA-B27) or progression (such as HLA-B35).\(^6,7\) The mechanism that explains this dichotomy is still unresolved, but it is likely to be linked to the ability of T-cells to recognize different peptide repertoires presented by different MHC alleles. Altered disease susceptibility can be conferred by very subtle differences in the MHC. For example, the B7 supertype HLA-I alleles: B*42:01, B*81:01, B*39:10, and B*67:01, can all present the TPQDLNTML (TL9) epitope from HIV Gag.\(^8\) Interestingly, these alleles confer a distinct level of protection against HIV, even though they differ by only a few residues. Thus, even an identical peptide presented by MHCs that differ at only a few residues can have a marked influence on disease progression in HIV. This observation suggests that small polymorphisms in different MHC alleles can affect peptide presentation to T-cells and alter the robustness of an individual's response. A number of studies have shown how such small changes can impact on TCR recognition.\(^9,10\) Moreover, our understanding of the impact of TCR polymorphism on pMHC recognition is limited.\(^11\) HLA is also the main genetic determinant of autoimmune disease.\(^12\) Presumably, the self peptide repertoire that can be accommodated by individual MHCs determines the T-cell responses that drive these conditions. We have recently demonstrated how the anti-HIV drug abacavir can alter the presentation of self-peptides by HLA-B57 to drive the autoimmune condition: abacavir hypersensitivity syndrome.\(^7,13\) These observations further demonstrate how the polymorphic biochemical nature of MHC can have a central role in determining T-cell immunity.

Although the differences between MHC-I and MHC-II antigen presentation generate different challenges for TCR binding at the atomic level, all of the structural data currently available demonstrate that TCRs bind both pMHC-I and II with a fixed polarity: TCR\( \alpha \) chain sitting over the N-terminus of the peptide and the TCR\( \beta \) chain sitting over the C-terminus and make similar interactions with the bound peptide and MHC surface.\(^14\) However, although the general TCR-pMHC binding mode is...
similar, the flexibility and structural and energetic versatility of this interaction (Fig. 3) has made it difficult, if not controversial, to identify a fixed set of structural rules that explain the general binding mode.4,61–66 For example, during antigen binding, TCRs can “tilt” over a ~45° range (relative to the pMHC cleft) (Fig. 3(A)), they can bind centrally, or towards the N- and C-terminus of the peptide (Fig. 3(B)), and they can “swivel” over the pMHC surface at a wide range of angles (32°–80°) (Fig. 3(C)).9,20,67 Moreover, to compound matters, TCRs can exhibit marked flexibility upon binding, the peptide can also undergo TCR-induced conformational changes and bulged peptides can act as “obstacles” for TCR ligation.53,54,68–71 This enigmatic binding mode represents a major hurdle for predicting the effect of altering TCR-pMHC binding for therapeutics and medicine. Part of the reason for this degree of versatility is probably due to the extremely polymorphic nature of the MHC platform. Our understanding of pMHC recognition by the TCR is further complicated by the fact that individual receptors can recognize huge numbers of peptides within the same MHC groove.72,73 This inherent receptor crossreactivity, or poly-specificity, is essential for recognition of the vast milieu of different antigens that is crucial for full immune coverage.74 The ability of individual TCRs to cross-react with multiple peptides is inherent in their very selection as all T-cells in the periphery must bear a TCR that weakly recognized a self-pMHC in order to obtain a positive selection signal in the thymus. Several lines of evidence show that the human TCR repertoire is also strongly coupled to MHC expression. For example, we have shown that most individuals that express HLA-B8 mount a common T-cell response (LC13) to the Epstein-Barr virus antigen, FLRGRAYGL.50 However, individuals that also express HLA-B44 mount a distinct T-cell response through different TCRs.58,72 The deletion of the LC13 T-cell response in HLA-B8/HLA-B44 individuals was found to be due to alloreactivity (T-cell responses against nonautologous cells). LC13 T-cells recognized a self-peptide bound to HLA-B44,47,76 so in these individuals, LC13-like T-cells were presumably deleted in the thymus. These results have strong implications for organ transplantation as they demonstrate that individuals with certain MHC types may mount strong T-cell responses against other MHC types. These observations demonstrate the importance of tailored tissue transplantation to avoid the likelihood to allosresponses that could lead to organ rejection.77 Thus, MHC polymorphisms can shape the T-cell repertoire expressed by an individual.

Precisely how the balance between self-tolerance and T-cell cross-reactivity is achieved is not understood. The polymorphic nature of the MHC and the variability of the TCR raise several interesting biochemical questions. The first is: how can the TCR bind to a composite ligand, in which it makes contacts with the self-MHC surface, whilst retaining specificity for nonself peptides presented in the MHC binding cleft? The second is: how does the TCR maintain a generally fixed binding mode to the MHC surface whilst retaining a large degree of variability during interactions with different ligands? These apparently contrary observations seem to be specific to the TCR-pMHC interaction as TCR binding to nonpolymorphic ligands, such as CD1d (discussed later) obey comparatively strict conformational and biochemical rules. Although there are not yet enough published TCR-pMHC structures to fully answer these questions, there are a number of examples that demonstrate that the system works under a high level of peptide specificity. Indeed, our own recent observations show that TCR-peptide specificity is maintained even in the presence of affinity-enhancing TCR-MHC interactions.78 We further demonstrated that single amino acid substitutions in the MHC-bound peptide, that had a minimal structural impact, could substantially reduce TCR binding indirectly by altering solvent interactions during binding.79 The biochemical rules that
enable the TCR to exhibit this level of sensitivity, whilst retaining a high level of cross-reactivity are still to be determined.

Although the TCR-pMHC interaction has been, by far, the best studied TCR recognition system, the highly polymorphic nature of the MHC antigen binding platform, and the high level of diversity within the pMHC specific TCR repertoire, has made it difficult to determine the biochemical rules that govern the interaction. However, the TCR can also interact with other less polymorphic molecules, such as the CD1 and MR1 family, using a more restricted set of TCRs. The rest of this review will cover some of our recent structural and biochemical discoveries in these systems, which provide more general clues about the molecular rules that govern T-cell immunity.

**Lipid-mediated immunity**

Many T-cells have been identified that respond to nonpeptide antigens presented by “MHC-like” molecules from the CD1 protein family. CD1 proteins are ideally suited to present lipid-based antigens to T-cells due to the hydrophobic nature of their antigen binding pockets, which are vastly deeper than the peptide anchoring grooves of MHC molecules. There are five isoforms of CD1 molecules in humans, CD1a-e, although CD1e is not involved in Ag-presentation. Each CD1 isoform has a distinct architecture and volume within the Ag-binding cleft that permits it to present a differing array of self- or foreign lipids for T-cell surveillance. Presently, whilst there are binary structures of each CD1 isoform, our structural insight into lipid-mediated recognition is restricted to the advances that have been made in CD1d-lipid mediated immunity.

CD1d-lipid complexes bind to TCRs that are expressed by the innate-like Natural Killer T-cells (NKT cells), which are categorized into two classes depending on their gene usage and responsiveness to certain antigens. For example human Type I NKT cells respond to the potent lipid, α-galactosylceramide (α-GalCer), and typically utilize a “semi-invariant” T-cell receptor (iNKT TCR), possessing a restricted TCR α-chain (TRA10-TRAJ18) paired with a TRBV25-1-encoded β-chain. The mouse Type I NKT TCRs express homologous genes to that of the human system, although show greater variation in TCR β-chain usage. Moreover, both the mouse and human Type I NKT TCRs show considerable diversity within the hypervariable CDR3β loop. Type II NKT cells do not express such a restricted T-cell repertoire, are not activated by α-GalCer, and are considered to bind an array of antigens distinct from Type I NKT cells.

Structures of human and mouse CD1d with a number of self and foreign lipid antigens revealed how such molecules are contained within two deep hydrophobic clefts, termed the A’ and F’-pockets, of the protein. For example, the acyl and sphingosine chains of α-GalCer reside within the A’-pocket and F’-pocket, respectively, while the polar α-linked galactosyl headgroup protrudes out from the cleft, and is thus the only region of the antigen that is directly accessible to the NKT TCR.

Type I NKT TCRs can recognize α-linked glycolipids and β-linked glycolipids, microbial lipids, and self-lipids, with the functional outcome of such an engagement being influenced by the antigen itself, as well as the nature of the responding NKT TCR repertoire. The lipid molecules vary in the structure and size of the polar head group, length, and saturation of hydrocarbon tails, linkage between sugar and sphingosine chain including the nature of the atom the linkage is carried on and the distance of the linkage from the base of the sugar moiety. Prior to our structural analyses, central questions in the field for Type I NKT TCR recognition were: (i) How does the NKT TCR bind a CD1d-Ag? (ii) How does variation in the NKT TCR repertoire impact on recognition? (iii) How does NKT TCR recognition dictate functional outcome? (iv) How can the same NKT TCR bind distinct Ags bound to CD1d?

Type I NKT TCRs dock in a parallel mode over the antigen entry portal directly over the F’ pocket of CD1d. This docking mode is conserved for human and mouse Type I NKT TCR-CD1d-Ag recognition. Typically, the total buried surface area (BSA) of the human Type I NKT TCR upon binding with CD1d-α-GalCer is ~900 Å², significantly below that of typical TCR-pMHC-I interactions (~1200–2500 Å²). The TCR α-chain contributed ~2.5-fold more contacts than the TCR β-chain. Here, the CDR1α loop contacted CD1d, while the CDR3α contacted CD1d and the antigen head group. The interactions involving the TCR β-chain were restricted to interactions with CD1d that were mediated via the CDRβ3 loop. Studies on the mouse Type I NKT TCRs, possessing the Vβ8.2, Vβ7, and Vβ2 chains, have been very informative in assessing the impact of NKT TCR repertoire on CD1d-Ag recognition. While the overall parallel docking mode is conserved between the Type I NKT TCRs, differences at the respective interfaces translate to differing affinities and functional outcomes towards CD1d-Ag complexes. These TCR β-chain mediated differences were also observed to subtly impact on some of the TCR α-chain mediated contacts. Importantly, the variations in the CDR3β loop of the NKT TCR were shown to impact on CD1d auto-reactivity in an Ag-independent manner—thereby this loop can fine-tune the functional response to any given ligand, which is important in the context of responding to ligands that typically display weak reactivity.
In regards to Type I NKT TCR recognition of different CD1d-restricted Ags, the field has been focused on understanding (i) the fine specificity of the NKT TCR and (ii) binding to Ags exhibiting diverse chemistries. α-GalCer is a phase I therapeutic, a potent agonist, and has served as a benchmark for development and discovery of altered glycolipid ligands (AGLs) that can be used to modulate NKT cell activity owing to the ligand's ability to stimulate both pro- and anti-inflammatory responses. Thus, AGLs have been synthesized that vary in their lipid composition and/or polar head group that modulate potency, or skew the ensuing cytokine response to be either pro or anti-inflammatory. Modifications in the acyl chain, whilst not impacting on NKT TCR recognition, can nevertheless impact on lipid loading of CD1d, while modifications in the sphingosine chain can indirectly impact on NKT TCR recognition. The cytokine-skewing properties of different AGLs seem more attributable to the pharmaco-kinetic properties of the ligand itself as opposed to any NKT TCR recognition affinity-based considerations. Alterations of the glycolipid polar head group can have a marked effect on NKT TCR recognition, with fine specificity differences residing between human and mouse Type I NKT TCRs. In essence, these structures provide a platform for rationally designing Type I NKT TCR therapeutics.

While MHC-restricted TCRs are often considered to be flexible the NKT TCR appeared to exhibit less flexibility. Thus, in recognizing distinct antigens, such as microbial-based antigens, or β-linked self-ligands, a priori it was unclear whether the NKT TCR would adopt distinct or similar docking modes upon recognizing such lipids. Regardless of the nature of the antigen, the Type I NKT TCR adopts a common footprint on CD1d, and in this manner the Type I NKT TCR acts like an innate pattern recognition receptor. Surprisingly however, while the Type I NKT TCR was observed to remain rigid upon engagement of these distinct ligands, the ligands themselves were observed to be malleable within the cleft. This was most dramatically observed for the β-linked ligands, and ostensibly the self-Ag, isoglobotrihexosylceramide (iGb3), a 3-carbon based glycolipid. This ligand, which projected out of the cleft in the binary structure, was completely flattened upon NKT TCR engagement, in which the β-linked sugar moiety adopted a similar conformation to that of the α-GalCer. Thus, to recognize distinct ligands, the NKT TCR operates via an induced fit molecular mimicry mechanism and a significant energetic penalty is incurred via such a recognition mode, although the ligand itself was shown to form stabilizing interactions with CD1d itself, thereby partly compensating the need to bulldoze the β-linked ligand.

Figure 4. Docking modes of TCR-pMHC, NKT TCR-CD1d-lipid, and MAIT TCR-MR1-metabolite complexes. (A) TCR-pMHC1 complex, pdb code; 1MI5. (B) A TCR-pMHCII ternary complex, pdb code; 1FYT. (C) A human NKT TCR in complex with CD1d-α-galactosylceramide (αGalCer), pdb code; 2PO6. (D) MAIT TCR in complex with MR1-ribityl derivative, pdb code; 4LCW. The antigen binding face of each antigen presenting molecule (ribbon, middle panel; surface, bottom panel) with respective ligands depicted in stick (A–C) or spheres (D).
While Type II NKT TCRs do not recognize α-linked ligands, some of them can recognize β-linked ligands such as the self-lipids, sulfatide, and lysosulfatide. Recently, two Type II NKT TCR-CD1d-sulfatide/lysosulfatide complexes have revealed that the mode of Type II NKT TCR recognition can be markedly different to Type I NKT TCR recognition. Namely, these Type II NKTs dock orthogonally and over the A-pocket of CD1d with a higher BSA at the interface than for Type I NKT TCRs. Accordingly, Type II NKT TCRs can exhibit a completely different network of contacts with CD1d-antigen molecules in comparison to Type I NKT TCRs, and better resembles the overall docking mode of MHC-restricted TCRs. Moreover, whereas germline encoded residues dominated the interaction between Type I NKT TCRs and CD1d, the nongermline encoded CDR3 loops played a major role in interacting with the CD1d-sulfatide, and the sulfatide head-group was not flattened upon engagement. Accordingly, Type I and Type II NKT TCR recognition can be markedly different, underscoring the different functional roles Type I and Type II NKT cells can play.

While CD1d is mostly considered a ligand for αβ NKT cells, most recently our laboratories have provided the first structural insight into how CD1d is recognized by γδ TCRs. γδ T-cells are distinct from αβ T-cells, and are considered to recognize a wide range of ligands in a manner that can be antigen independent. As such, the nature of the ligands that γδ T cells can respond to remains mostly unclear. Nevertheless, our recent study showed that a Vδ1+ γδ T cells could recognize CD1d-αGalCer in an Ag-dependent manner. The mode of docking was distinct from Type I and Type II NKT TCR-CD1d recognition, in which the CDR1δ loop defined CD1d restriction, while the CDR3γ loop mediated contacts with the α-GalCer ligand. Thus, distinct T-cell lineages can recognize the same Ag-presenting molecule via different recognition mechanisms.

**Vitamin B metabolite mediated immunity**

Similar to γδ T-cells, there are some αβ T-cell populations with undefined Ag specificities. A prime example concerned Mucosal-associated invariant T-cells (MAIT cells). This innate-like T-cell population can be up to 10% of the entire T-cell population and is found in all mammals. In humans, they are mostly focused in the mucosa and also express an invariant TCR α-chain (TRAV1–2, TRAJ33) (hence their name), paired with a limited range of TCR β-chains. MAIT cells, which play a key role in protective immunity, are known to be restricted to the MHC-I like molecule, termed MR1, but it was unknown what Ags bound to MR1 to stimulate MAIT cells. Surprisingly, MR1 did not present lipids or peptides—as widely speculated.

A combination of mass spectrometry and structural biology showed that MR1 was ideally suited to bind metabolites of vitamin B (e.g., folic acid and riboflavin derivatives). While the folic acid derivative was shown not to activate MAIT cells, the riboflavin derivatives did. Thus, MAIT cells can be activated by small molecule metabolites, thereby diversifying the nature of Ags that T-cells can recognize. Why recognize precursors of vitamin B? Humans do not synthesize vitamin B, yet many bacteria and yeast do. Hence, MAIT cell reactivity towards vitamin B precursors represents a previously unappreciated mechanism for self and non-self-discrimination. The structural basis of MAIT TCR recognition of MR1 presenting defined vitamin B precursors was also recently revealed (Fig. 4). Namely, human MAIT TCR-MR1-Ag complexes and a xenoreactive MAIT TCR-MR1 complex (with no defined ligands or heterogeneous mixtures—the interpretation of which is severely compounded by low resolution structures) shared some similarities to TCR-pMHC docking. However, the high-resolution human MAIT TCR-MR1-Ag complexes showed that the vitamin B precursors are mostly shielded within the MR1 cleft for direct TCR docking. Nevertheless, upon engagement, the human MAIT TCR prizes open the MR1 cleft to expose the occluded ligand. While the folic acid derivative did not contact the MAIT TCR directly, the riboflavin precursors did—and it is these additional contacts with the MAIT TCR that relates to their ability to stimulate MAIT cells.

**Concluding Remarks**

The αβ TCR is a versatile receptor that can recognize multiple different ligands, as well as different classes of ligands. It is a recognition system that can show remarkably specificity as well as cross-reactivity. Such patterns of reactivity are presently impossible to predict and any considerations of modeling such precarious ternary complexes are, at best, misinformed. The TCR receptor recognition axis represents a system whereby small shifts (<1 Å) in regions of the helices of the MHC, or CD1, or the Ag itself, can have a profound impact on immune outcome. Indeed, one amino acid difference between HLA molecules, which can subtly alter the substructure of the MHC, or the nature of the peptide bound, can lead to T-cell mediated transplant rejection. Hence, high-resolution structural studies are well suited to understand the impact of such changes on TCR recognition. Moreover, while we have a growing database of unique TCR-pMHC structures, the concept of TCR-MHC hardwiring, and any overarching “rules” pertaining to this recognition event, remains highly controversial, with generalizations being proffered based on limited independent examples. Hence, further TCR based studies are required to lift the fog relating to this
defining paradigm in T-cell immunity. Further, as the overall folds of the TCR, MHC, and MHC-like molecules are conserved, one could be forgiven for assuming that the fundamental correlates under-scoring the MHC-restricted response, for example, would be mirrored by lipid and metabolite mediated immunity. This is certainly not the case, and many more structural and biophysical studies are required before we get a firm understanding of the basic principles under-scoring TCR recognition. While the field of Ab–Ag recognition was considered to be “done” many years ago, key discoveries in this field are regularly being made. It would be prudent to anticipate a similar situation in the field of TCR recognition.

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