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MHC Restriction: Where Are We Now?

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Discovery of MHC Restriction

"Interaction between immune lymphocytes and cells expressing antigens expressed by LCM virus is, however, apparently confined to a histocompatible system perhaps because it is only in this situation that the necessary intimacy of contact is achieved…… An alternative possibility that must be considered in LCM is that the process of virus maturation through the cell membrane causes changes in self-components, which are recognized only within the syngeneic or semiallogeneic system….. The cytotoxic T cell may thus be recognizing altered self'' (99)

So records the critical interpretation of experiments performed by Rolf M. Zinkernagel and Peter C. Doherty, which documented the ability of T cells from lymphocytic choriomeningitis virus (LCMV)-infected mice to kill LCMV-infected target cells *in vitro*, only if the T cells and the targets shared at least one H-2 antigen. Of course, we now understand the extraordinary insight of this interpretation. In 1996, the same year that Zinkernagel and Doherty were awarded the Nobel Prize for their discovery of major histocompatibility complex (MHC) restriction, the first structures of T cell receptor-peptide+MHC (TCR-pMHCI) complexes were reported, for mouse (26) and human (23), enabling the direct visualization of this interaction. Indeed, these initial structures and those following show simultaneous TCR binding of self-MHC and either self- or non-self-peptide for the express purpose of detection of altered self. In the joint recognition of self and non-self, this receptor-ligand interaction is unprecedented in biology, highlighting the perceptiveness of Doherty and Zinkernagel's interpretation. Of course, at the time Zinkernagel and Doherty's experiments were performed, both murine transplantation antigens and the human lymphocyte antigen (HLA) system had been discovered, and their influence on transplantation outcomes as well as differential susceptibility to infectious diseases had been noted [reviewed in Zinkernagel and Doherty (100)]. However, the purpose and mode of action of major histocompatibility antigens were unknown. It was speculated that the extreme polymorphism of the MHC served to protect the body from invasion by cells (e.g., tumor cells) from another individual (11), to facilitate cell-cell recognition for differentiation and morphogenesis of multicellular organisms (8), or to drive elimination of self-reactive antibody-producing cells (43), among other theories. In an attempt to address the question of whether the lytic activity of antiviral cytotoxic T lymphocytes (CTLs), which were responsible for fatal choriomeningitis, could be correlated with H-2 haplotype, Doherty and Zinkernagel tailored the ^{51}Cr release assay to allow for analysis of cytolysis by relatively few LCMV-specific CTLs. In doing so, they made the striking finding that unveiled the mechanism of MHC restriction, namely that CTL effectors could kill virally infected targets only if the two cells were H-2 compatible.

Defining Characteristics of the TCR-pMHC Interaction

Over 40 years have passed since these original observations and our understanding of MHC restriction of TCR recognition has progressed significantly. Extreme diversity is inherent in all components of the interaction; generation of the TCR by a somatic recombination process allows a theoretical diversity of $\sim 10^{15-18}$ different TCRs, of which $\sim 2.5 \times 10^7$ can be found within any individual (17); the MHC genes are the most polymorphic genes of the human genome, and the peptide cargo that can be bound by MHC is virtually limitless. As a consequence, TCR recognition of pMHC is unparalleled in the diversity of the interacting surfaces. Our understanding of the nature of TCR-pMHC interactions can be almost completely attributed to structural studies—over 50 ternary structures of unique TCRs interacting with cognate pMHCI or pMHCII have been solved—which have provided direct, high-resolution visualization of these interactions, enabling conserved patterns of TCR-pMHC binding to be identified (reviewed in La Gruta *et al*., Rossjohn *et al*. (50,70).

Notably, the extreme diversity in the interacting ligands is reflected in considerable variation in the manner of the

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interaction; namely in the relative contribution to binding of the various molecules, in the angle at which TCR docks over pMHC (pMHCI: average $\sim 63°$; pMHCII: average $\sim 76°$), and in the extent of contact between the TCR and pMHC (buried surface area ranges from 1,240 to 2,400 A^2) (70). Thus, the interaction is able to simultaneously maintain specificity, while accommodating the variable nature of the ligands. The diversity inherent in the TCR-pMHC interaction is perhaps best exemplified by the observation that within both MHCI and MHCII-restricted TCR recognition, the same TCR V gene elements can interact with multiple pMHC complexes, with no conservation in the interactions between TCR and MHC in the different contexts (70).

Despite the flexibility of the interaction, key structural characteristics have emerged, which are highly conserved across virtually all interactions. The TCR makes contact with both the peptide cargo and the MHC (co-recognition), the TCR binds over the top of the peptide, the peptide is contacted by at least one of the highly variable TCR CDR3 loops, and the TCR binds pMHC with a highly conserved polarity. That is, the TCR α chain sits over the top of the MHCI $\alpha 2$ helix or MHCII β chain, while the TCR β chain is positioned over the MHCI $\alpha 1$ helix or MHCII α chain (70,72). Recently, examples of deviations from these tenets of TCR recognition have been reported (discussed below) and have the potential to advance our understanding of the key requirements for TCR recognition of pMHC.

Models to Explain TCR Recognition of pMHC

Given the inherent random rearrangement process, and the consequent diversity of TCRs, one overarching question that is yet to be definitively answered is, what drives MHC restriction? Here we discuss our current understanding of the TCR's obsession with MHC by examining evidence from the perspective of the two current models: the germline encoded and selection theories of MHC restriction.

Germline-encoded hypothesis

The germline encoded model of TCR-MHC bias stems from an idea first proposed by Jerne (43), suggesting that the TCR and MHC genes have, over millions of years of co-evolution, developed an intrinsic bias for co-recognition. It suggests that the intrinsic bias of TCRs for MHC molecules is driven by evolutionarily conserved amino acid motifs encoded by both the TCR and MHC molecules. Recent reports of complete TCR-pMHC structures solved by X-ray crystallography have identified multiple conserved pairwise interactions between the TCR and the MHC molecule [termed ''interaction codons'' (25)] leading to the hypothesis that TCRs are genetically biased toward MHC recognition. The germline-encoded theory of MHC restriction relies on evolutionarily conserved pair-wise interactions between the TCR and pMHC, and suggests that any deviation from ''interaction codons'' would result in unfavorable or low- affinity interactions. The germline-encoded model is supported by data that show up to 30% of the preselection repertoire is reactive to MHC molecules (61,62,83,98). However, it does not support unconventional modes of antigen recognition, namely TCRs that recognize MHC-independent antigens (4,35,58,68,81,90) or reversed polarity recognition of TCRs on MHCI and MHCII (6,32).

Selection hypothesis

Some immunologists argue that germline-encoded bias of TCRs for MHC molecules is minimal and that MHC restriction is driven ultimately by constraints imposed on the TCR during positive selection and the nature of TCR signaling (67,92). TCRs themselves do not possess intrinsic signaling capacity, and instead, TCR signaling relies on the delivery of Lck to the CD3 complex when associated with the cytoplasmic tails of CD4 or CD8 co-receptors (87). Thus, the CD4 and CD8 co-receptors act to focus the TCR onto the MHC molecule. This requirement for co-receptor facilitation of signaling prevents development of T cells expressing TCRs that are not specific for MHC, ensuring MHC restriction. Early support for this model was found when mice lacking expression of co-receptors developed a diverse repertoire of $\alpha\beta$ T cells that could react to conformational epitopes independent of MHC presentation (90), suggesting that co-receptors are critical for MHC restriction. This model is also supported by the fact that that at least 70% of the preselection repertoire has no intrinsic capacity to recognize MHC (61,62,83,98) and also supports the many reports of unconventional modes of antigen recognition, namely two distinct examples of reversed polarity docking over MHCII and MHCI (6,32), atypical binding modes such as recognition of super-bulged peptides (88) and TCR recognition of MHC-independent ligands (4,35,58,68,81,90).

Analysis of the Preselection Repertoire

Positive selection in the thymus, and therefore continued T cell development, depends on the TCR-pMHC interaction delivering a survival signal to T cells. As a consequence, the postselection TCR repertoire is altered to reflect ''successful'' TCRs. For this reason, immunologists have relied on analyses of $\alpha\beta$ TCRs expressed by preselection thymocytes to understand intrinsic MHC reactivity without selection bias. Studies have used mice that lack the expression of MHCI and MHCII molecules such that thymocytes are blocked at the $CD4^+CD8^+TCR\alpha\beta^+$ stage (62,86,98) or $TCR\alpha^{-/-}$ mice (47), which are unable to express TCR on their cell surface. Both of these T cell populations have intact $TCR\alpha\beta$ gene rearrangements that allow the sampling of ''untested'' preselection TCRs for the presence or absence of inherent MHC reactivity by germline-encoded recognition motifs. The ability to test the MHC reactivity of the preselection repertoire using MHC-deficient models has revealed that a substantial proportion (15–30%) of TCRs has the capacity to be activated by pMHC molecules (61,62,83,98), a relatively high frequency if we consider that there are 10^{15-20} theoretical TCR rearrangements possible, but arguably a low frequency if intrinsic affinity for MHC molecules is an evolutionarily encoded feature of TCRs. Similarly, the use of $TCR\alpha^{-/-}$ mice to study MHC reactivity of unselected $TCR\alpha$ sequences was consistent with these findings and fascinatingly, pairing of unselected $TCR\alpha$ sequences with $TCR\beta$ sequences containing a modified germline region derived from frog, shark, or trout retained a high frequency of pMHC-reactive cells (47), suggestive of a highly evolutionarily conserved TCR bias for MHC reactivity.

Another approach used to resolve this biological question was the generation of mice lacking MHCI, MHCII, CD8, and CD4 (Quad-KO mice) or expressing a mutant variant of Lck that cannot associate with co-receptors (90,91), both of

which circumvented the requirement for MHC recognition during T cell development. Surprisingly, in this context, when Lck was not constrained to a co-receptor, αβ T cells were positively selected, expressing TCRs that recognized non-MHC-associated epitopes in a manner akin to antibody recognition of foreign antigens. Specifically, Quad-KO mice were found to positively select αβ T cells specific for multiple conformational epitopes of CD155, an abundant molecule in the thymus, without any detectable reactivity toward MHC molecules. This study was the first evidence that MHC restriction was imposed at least, in part, by selection events and driven by co-receptor binding to MHC molecules. One thing to note is that CD155-reactive TCRs made up around 40% of the TCR repertoire in QuadKO mice. While this was taken as supporting evidence for the selection model, the fact that most of the repertoire was reactive to a single molecule was also interpreted as evidence for the importance of germline-encoded recognition, given the apparently limited capacity of TCRs to recognize non-MHC-restricted epitopes.

More recently, the MHC-independent TCR repertoires from Quad-KO mice were compared with MHC-restricted TCR repertoires from intact mice of different MHC haplotypes (B6 and B10.BR mice). Interestingly, while ~33% of TCR sequences were shared between individual B6 and B10.BR mice, individual MHC-independent TCR repertoires derived from Quad-KO mice shared only 3% of TCR sequences, with the lack in sequence overlap between mice resembling the extent of overlap usually found in antibody repertoires (57). These data indicate that thymic selection samples from a very large repertoire of preselection TCRs and drives selection of a much smaller pool of MHC-restricted TCRs.

Ultimately, studies of the preselection repertoire have yielded results that provide evidence and support for both germline-encoded and selection models of MHC restriction. While it appears that germline-encoded amino acids are not strictly required for the development of αβ TCRs restricted to MHC molecules, evolutionary pressures appear to have predisposed a level of complementarity between the TCR and the MHC.

A Structural Perspective on TCR-pMHC Recognition

Conserved residues in the TCR-MHC interaction

The very first TCR-pMHC complexes reported showed that the germline-encoded CDR1 and CDR2 loops made contact with the MHC molecule, whereas the most variable CDR3 loop typically lay centrally over the peptide. These early structural studies revealed three key residues on MHCI (65, 69, and 155; termed the ''restriction triad'') that were contacted by nearly all TCRs identified at that point in time, suggesting that these key positions predetermined, and were the minimum requirements for, MHC restriction of TCRs. However, mutation of any or all of these three residues did not abolish TCR recognition of MHC (12), suggesting that reproducible contacts to the ''restriction triad'' were dispensable and perhaps represented incidental recognition motifs due to the fairly reproducible nature of TCR-pMHC docking.

Other approaches to understanding the germline-encoded bias of TCRs have identified germline-encoded motifs in $V\beta8.2^+$ TCRs (βY46, βY48, and βE54) make reproducible contacts with different pMHC molecules (60). Artificially modifying these conserved residues substantially diminished, but did not abolish, naive T cell development (75), indicative of an important but nonessential role in TCR-MHC recognition. Further evidence of the plasticity and flexibility of TCR-MHC recognition was demonstrated in an elegant study by Holland *et al.* (38), in which extensive modifications were made to the CDR1 and CDR2 loops of a Vα8.3 and Vβ11 TCR by redirecting Rag recombination to the germline-encoded residues. This approach generated substantially altered CDR1 and CDR2 loops in the TCR genes, yet allowed development of a high proportion of $CD4^+$ and $CD8^+$ T cells (38). Moreover, the importance of evolutionarily conserved residues in the MHCII molecule was tested by introducing amino acid substitutions into either the α (A64Q) or β chain (R70A and T77A) of MHCII in mice. These mutations resulted in minor shifts in the TRAV and TRBV usage of positively selected T cells, but surprisingly did not have any effect on the frequency or diversity of thymocytes that were positively selected (80).

A key feature that has emerged from numerous TCR-pMHC structures is the highly reproducible docking orientation of the TCR over the pMHC, with the TCRα chain sitting over the MHCI α2 helix or MHCII β chain, and the TCRβ chain sitting over the MHCI α1 helix or MHCII β chain (Fig. 1a). The reproducible docking polarity has been interpreted as clear evidence of germline-encoded specificity between the TCR and MHC molecule. This was in agreement with the observation from multiple TCR-pMHC structures that have identified reproducible and conserved ''interaction codons'' in the CDR1 and CDR2 regions of αβTCRs. Indeed, studies have demonstrated conserved pairwise interaction between CDR1β and CDR2β of Vβ8.2 TCR in complex with pMHCII complexes (16,69,74,75,84,94). These conserved pairwise interactions between the TCR and pMHC were considered key drivers of MHC restriction and suggested that the docking polarity was ''hardwired.''

These data were ostensibly supported by studies that used a library of pMHC molecules to determine the relative contribution of the TCR CDR3-peptide interface in driving TCR-pMHC docking polarity. In these studies, several alternative peptides were found to be recognized by a single TCR, all of which were recognized by the TCR in a conventional docking topology (9). Similar studies have been performed by modifying the CDR3α sequence of the TCR or by identifying TCRs cross-reactive to different pMHC. Modification of CDR3α sequence affected cross-reactivity to different ligands and its binding affinity, but did not affect TCR-pMHC docking orientation (45). Likewise, a single TCR has been shown to cross-react to two distinct pMHC, yet this TCR maintains the conventional docking orientation (13). These findings were taken as evidence that germline CDR1 and CDR2 loops, rather than the largely non-germline-encoded CDR3, played dominant roles in driving MHC restriction (24). These conclusions are muddied somewhat by the observation that the major conserved Y48, Y46, and E54 residues also made key contacts with MHC-independent conformational epitopes, including in the TCRs specific for CD155 found in Quad-KO mice (86), raising the argument that these residues have been evolutionarily conserved to drive enhanced reactivity of the TCR to ligands more globally.

Organization of the CD3-TCR-pMHC complex

The weight of evidence indicates that evolutionarily conserved complementarity between the TCR and the MHC

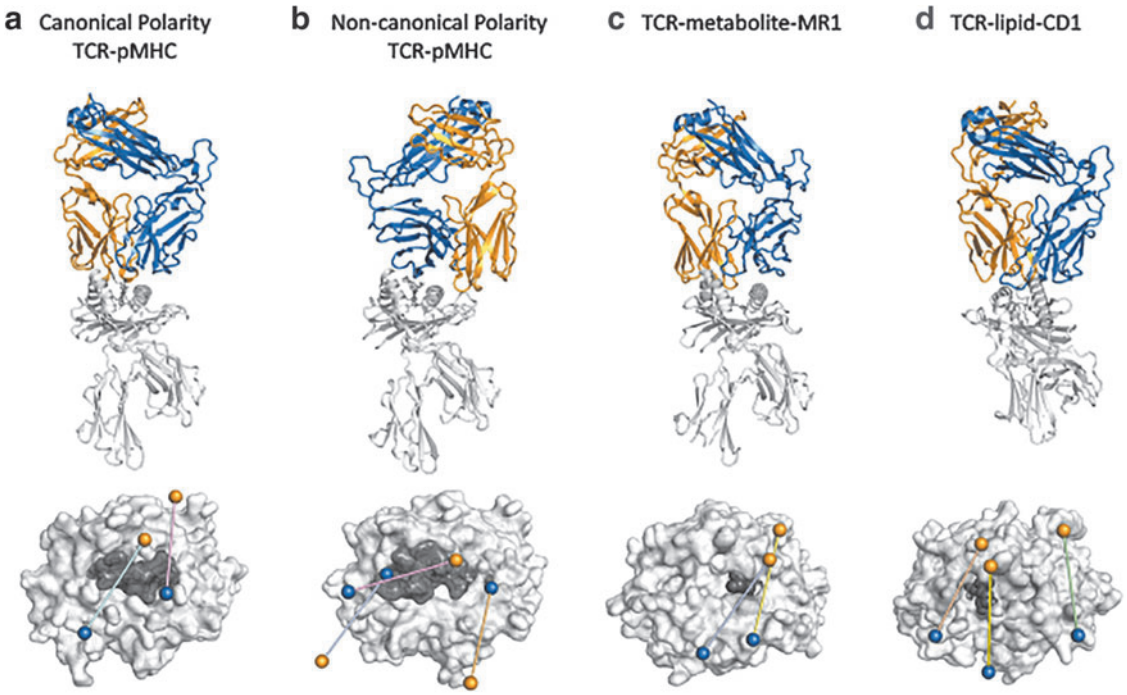

FIG. 1. Comparison of docking orientations for TCR-MHC and MHC-like interactions. *Above:* Ribbon structures of selected TCR-MHC and TCR-MHC-like interactions. *Bottom:* Top view of the TCR docking footprint on top of the MHC or MHC-like molecule. *Orange* and *blue spheres* represent the centers of mass for TCRα and TCRβ variable domains, respectively. The MHC molecule is colored *light gray* and the antigen is colored in *dark gray*. **(a)** Canonical docking topology of the LC13-HLA-B8^{EBV} complex (*above*) and the footprints of the LC13 TCR (*cyan*; PDB: 1MI5) and the ''superbulged'' peptide-centric TCR SB27 over HLA-B*3508^{EBV} (*pink*; PDB: 2AK4) (*bottom*). **(b)** Noncanonical topology of the reversed NP1-B17-H-2Db^{NP366} complex (*above*) and the footprints of the NP1-B17 TCR (*blue*; PDB: 5SWZ), the reversed FS18 TCR over HLA-DR4^{proinsulin} (*orange*; PDB: 4Y19), and the nonsignaling 42F3 TCR over H-2:Ld^{p3A1} (*pink*; PDB: 3TJH) (*bottom*). **(c)** Unconventional MAIT M33.64-MR1$^{5\text{-}OP\text{-}RU}$ complex (*above*) and the footprints of the MAIT M33.64 TCR (*yellow*; PDB: 5D5M) and MAV36 TCR over MR1$^{5\text{-}OP\text{-}RU}$ (*blue* PDB: 5D7L) (*bottom*). **(d)** Unconventional 3C8-CD1c^{monoacylglycerol} complex (*above*), and the footprints of the 3C8 TCR (wheat; PDB: C09), the BK6 TCR over CD1a^{LPC} (*green*, PDB: 4X6C), and the PG90 TCR over CD1b^{PG} (*blue*; PDB: 5WKI) (*bottom*). EBV, Epstein-Barr Virus; HLA, human lymphocyte antigen; MAIT, mucosal associated invariant T; PDB, Protein Data Bank; TCR-MHC, T cell receptor-major histocompatibility complex.

predisposes, but does not mandate, MHC recognition by TCRs. The ultimate driver of MHC restriction by TCRs, as proposed by the selection model, is the requirement for the TCR-ligand interaction to result in a signal that is achieved by pMHC recognition by the simultaneous binding of MHC by the CD4 and CD8 coreceptors, which co-localizes Lck to the intracellular ITAMs of CD3. That is, the main driver for MHC restriction is TCR signaling, rather than TCR binding. The question that remains is how the requirement for this signal mandates the precise nature of the TCR-MHC interaction.

To understand how TCR-pMHC docking topology can influence TCR signaling, we must look at what is currently known about the structural assembly of the TCR-CD3 complex. Early mutational studies to understand the assembly of the TCR-CD3 complex have suggested that the CD3εδ and εγ heterodimers congregate on one side of the TCR (49). Later, the first ternary complex of the TCR-pMHC-CD4 was solved by the Mariuzza group (95); it was then suggested that the CD3 heterodimers are positioned inside the TCR-pMHC CD4 arch, thereby facilitating intracellular localization of Lck to the CD3 ITAMS (48,95). All of these early data are in agreement with the most recent structural assembly of the TCR-CD3 complex, first proposed from studies using solution NMR (36) and recently confirmed by the first cryo-electron microscopy structure of the complete TCR-CD3 complex (19), which similarly indicates a ''sided'' orientation of the CD3 complex in relationship to the TCR. Based on these observations, it has been proposed that the highly reproducible TCR-pMHC docking orientation may reflect the structural positioning that is most conducive to signaling either by facilitating Lck proximity to the CD3 ITAMS (48,67) or by affecting TCR clustering by dimer exclusion or partial occupancy (1).

Unorthodox TCR Recognition of pMHC

Much of what we know about TCR-pMHC complexes have come from TCRs that are well represented in immune or expanded repertoires, and therefore may actually represent a biased view of the fittest TCRs that have the capacity to induce strong TCR signaling. In this study, we look at unconventional TCR recognition of pMHC- or MHC-like ligands for further insight into how generalizable these observations are in similar, but distinct models of recognition. In 2005, the first unorthodox modes of TCR-pMHC

recognition were identified for two TCRs specific for myelin basic protein-derived peptides presented by HLA-DR2, where, instead of sitting centrally over the peptide, the TCR lay over the N-terminal end of the peptide (34,52). In both of these cases, it was hypothesized that this unusual mode of docking negatively impacted on the affinity of the interaction, allowing these autoreactive T cells to escape negative selection (48,67).

More recently, there have been two discoveries of TCRs that recognize the pMHC complex in a completely reversed orientation (Fig. 1b). Before these structures, no reversed orientation TCRs had been observed, although their presence had been suggested (82). First, two reverse orientation human HLA-DR4-restricted TCRs were derived from Tregs induced by *in vitro* stimulation with tolerogenic proinsulin-pulsed DCs (6). More recently, two TCRs, bound in a reversed orientation to mouse H-2D^b loaded with an immunodominant influenza A virus (IAV)-derived nucleoprotein peptide, were isolated from the preimmune repertoire (32). The identification of these reversed polarity TCRs is significant as they are the first examples of TCR-pMHC complexes to deviate from conventional docking polarity, and the mouse TCRs also represent the first TCR-pMHC complexes solved from the preimmune repertoire. The analysis of preimmune TCRs has allowed us to take an unbiased look at TCR-pMHC interactions, independent of their ability to support robust T cell activation. The two reversed MHCI-restricted TCRs interacted primarily through germline-encoded TCRβ framework regions and resulted in a TCR-pMHCI interaction of moderate affinity, suggesting that energetically favorable interactions can be achieved in the absence of conserved germline-encoded interactions (25). Reversed polarity TCR-pMHCI complexes from the preimmune repertoire was found to drive poor signaling and immune expansion after viral challenge, despite having moderate affinity for pMHC, providing further evidence for structural constraints imposed on the TCR-pMHC complex for effective signaling, which were independent of binding strength (32). It remains to be determined how a reversed TCR-pMHC docking topology might negatively impact signaling, and by extension, how canonical docking facilitates signaling. Another example of unusual TCR-pMHC recognition is the CD8 TCR recognition of an unusually long 13 amino acid Epstein-Barr Virus (EBV) peptide that bulges out of the MHC peptide binding groove. Structural analysis of this TCR-pMHC complex revealed a highly peptide centric mode of antigen recognition that made minimal contacts with MHCI. Interestingly, although the TCR made minimal contacts with the MHC, it retained the canonical docking orientation over the MHCI and was capable of transducing a TCR signal and killing target cells (Fig. 1a) (88). In addition, Adams *et al.* demonstrated that different peptides presented by the same MHCI molecule can alter the TCR-pMHC docking topology. Peptides that induced a canonical TCR-pMHC ''fit'' were capable of signaling, whereas one peptide, which resulted in a significantly altered TCR-pMHC docking orientation, reduced the capacity for the TCR to induce a signal, independent of binding affinity (Fig. 1b) (1). One explanation for the lack of signaling was that the unusual TCR-pMHC docking angle exceeds tolerances allowed for productive arrangement of the TCR-CD3-CD8 complex.

TCR Recognition of Unconventional MHCI-Like Ligands

Unlike classical MHCI and MHCII molecules, MHCI-like molecules such as CD1 and MR1 are monomorphic (63) and present lipid or metabolite antigens, rather than peptides, to TCRs expressed by ''unconventional'' T cells such as mucosal associated invariant T (MAIT) cells, natural killer T cells (NKT), and subsets of $\gamma\delta$ T cells (reviewed in Godfrey *et al.* (30)). The three key tenets of conventional TCR-pMHC binding, namely the TCR binding over peptide, TCR co-recognition of both the MHC and the peptide cargo, and the conserved docking polarity of the TCR, have also been observed for TCR recognition of nonclassical MHC molecules. This includes recognition of HLA-E (37,85) and MR1, which presents metabolites to MAIT cells, and to atypical MR1-restricted T cells (14,21,27,28,65) (Fig. 1c).

However, analysis of TCR recognition of CD1 molecules provides an interesting exception. CD1 molecules are a family of MHCI-like antigen-presenting molecules (CD1a, CD1b, CD1c, and CD1d proteins) that are specialized in lipid antigen presentation. Typically, the hydrophobic chains are sequestered within the CD1 cleft, while the polar headgroups protrude from the cleft and are potentially available for TCR contact. There have been a number of TCR-CD1 ternary complexes solved (70,71), in which the TCR has been observed to co-recognize the CD1 protein and the surface-exposed polar headgroup, including recognition of mycobacterially derived lipids (33) and self-phospholipids (77,78). However, two distinct autoreactive TCRs in complex with CD1c (3C8) (93) and CD1a (BK6) (7) showed that the TCRs exclusively contacted the CD1 molecule and made no contact with the lipid ligand (Fig. 1d), contravening the co-recognition tenet. Human CD1 molecules have relatively enclosed ligand binding pockets, termed the A' and F' pockets, above which sit the closed A' roof and the F' portal, respectively. Small ligands can bind entirely within these pockets, while ligands with sufficiently large headgroups can protrude through the F' portal. Although the BK6 TCR avoided lipid contact by assuming a left-shifted footprint over the CD1a A' roof, the 3C8 TCR made critical contacts with the F' portal, facilitated by the complete burial of the ligand within the pocket. This presents a scenario in which moieties with large headgroups may obstruct TCR-CD1 interactions, while small ''headless'' moieties, such as fatty acids, monoacylglycerols, and squalene, may facilitate the interaction and drive T cell activation (15). Thus, it appears that, unlike in conventional TCR-pMHC recognition, co-recognition may in some instances serve as an impediment to T cell activation.

In both modes of CD1 recognition, the TCR docks over the CD1 with the canonical topology defined by conventional TCR-pMHC interactions, that is, Vα-chain is positioned over the α2-helix and the Vβ-chain resides over the α1-helix. The docking angle is 66° for 3C8 and 110° for BK6 (Fig. 1d) and differs from the TCRs co-recognizing CD1d and polar headgroups ($\sim$90°), but fits within the observations made for MHCI (37° to 90°), MHC II (44° to 115°), and MR1 [82° to 89°; (70)] (Fig. 1). Interestingly, even human $\gamma\delta$ TCRs, which are known to recognize antigens independent of MHC (51), have been shown to recognize CD1d with a conserved polarity and docking angle

(74° to 84°) (70,89). Such conservation in docking polarity in the absence of obvious utilization of conserved germline-encoded motifs provides further evidence that the docking polarity facilitates signaling in a manner unrelated to binding.

However, some extreme docking modalities that appear to support productive signaling have also been observed. For example, type I NKT cells, which use a largely invariant Vα14-Jα18 TCR in mice and Vα24-Jα18 TCR in humans, are known to dock over CD1d-αGalCer in an orientation that is almost parallel to the antigen binding groove (conserved angle of 5° to 17°) (10), although more ''conventional'' angles of TCR-MHC docking have also been shown for type II NKT, which are more diverse in their TCRα and TCRβ gene usage (3,29,66,71).

In addition, if we assume that the canonical docking orientation is mandated by the need for appropriate colocalization of coreceptor-associated Lck, the driver for the canonical docking polarity of TCRs expressed on coreceptor-negative NKT cell, MAIT cell, and γδ T cell raises becomes less clear. One likelihood is that, for NKT cells and MAIT cells, the recognition modes reflect those that are conducive to signaling during positive selection at the $CD4^+$ $CD8^+$ double-positive (DP) stage, when coreceptors are able to contribute to signaling (5,76). The situation is less clear with γδ T cells, which do not go through DP selection, but egress to the periphery after the DN3 stage (64). A greater understanding of unconventional T cell development and preselection TCR repertoires is needed to fully appreciate the drivers of TCR-MHC-like ligand recognition and their similarity to conventional TCR-pMHC recognition.

TCR-pMHC Mechanotransduction

Of course, TCR signaling is not binary and it is well established that the strength of the TCR-pMHCI interaction substantially impacts T cell activation and function (18,20,96,97). One of the key recent shifts in our appreciation of how TCR binding of pMHC drives T cell signaling has emerged from a change in the biophysical measurement of interaction ''strength'' and by accounting for the conditions of force under which physiological recognition of antigen occurs. The gold standard measure of TCR-pMHC interaction strength has long been Surface Plasmon Resonance, which utilizes isolated molecules (at least one of which is in the fluid phase) to determine the intrinsic or ''three-dimensional'' affinity of the TCR for pMHC. These three-dimensional (3D) measurements of off- and on-rates have also been used to yield the total ''dwell time'' of a TCR on a pMHC complex, which, along with affinity, have broadly, but not universally, shown correlations with the extent of T cell activation (2,31,46,59,73,97). More recently, two-dimensional (2D) measurements, which directly measure molecular interactions at cell-cell junctions, were thought to better characterize TCR-pMHC interaction strength in the context of the cellular membrane, and are proposed to better correlate with T cell signaling/activation (41,42,44,55). In addition, because physiological TCR-pMHC interactions occur under conditions of force (22,40,53,56), such 2D measurements are typically performed under conditions of applied mechanical force. Using such measurements, a number of recent studies have observed that productive (signal inducing) TCR-pMHC interactions correlate with the ability to form bonds that strengthen with increased force (catch-bonds), while unsuccessful TCR-pMHC interactions are thought to form slip-bonds, whose strength diminishes or is lost with applied force (41,55,79).

However, a couple of very recent studies have called into question whether catch- versus slip-bond formation is a cause or a consequence of a productive TCR-pMHC interaction (39,54). One study found that the formation of TCR-pMHC-CD8 catch bonds was dependent on the kinase activity of Lck and its ability to localize to CD8 and CD3, with inhibition of Lck kinase activity and mutation of several CD3ζ ITAMS resulting in a reduced ability of TCR and CD8 to form a catch bond with pMHC (39). A more recent study showed, in a cell-free system that precluded the contribution of the cellular response to catch bond formation, that intrinsic catch bonds were not formed by any of the five agonist TCR-pMHC ligand pairs studied, and that off-rates of binding were the best predictor of activation potency (54). Thus, our understanding of how the TCR-pMHC-coreceptor bond formation is initiated and changes over the duration of the encounter, and how that drives (or is driven) by downstream signaling events continues to evolve.

Summary

From the initial observations by Zinkernagel and Doherty over 40 years ago of the need for T cells to recognize ''altered self,'' our understanding of TCR recognition of peptide+MHC has made significant advances. However, alongside a more detailed understanding of the interaction comes additional questions around precisely why T cells must limit themselves to MHC, when a greater range of ligand binding is demonstrably possible, and mechanistically, how MHC restriction achieves the necessary T cell survival and activation signals. The answer may well lie in the study of noncanonical or poorly signaling TCRs to understand the absolute requirements for effective TCR-pMHC recognition, continued advances in structural biology providing resolution of multimolecular complexes, and cryo-EM providing information on the dynamics of molecular localization and organization before and after TCR ligation of pMHC.

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