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T cell receptor bias for MHC: co-evolution or co-receptors?

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Abstract In contrast to antibodies, which recognize antigens in native form, αβ T cell receptors (TCRs) only recognize antigens as peptide fragments bound to MHC molecules, a feature known as MHC restriction. The mechanism by which MHC restriction is imposed on the TCR repertoire is an unsolved problem that has generated considerable debate. Two principal models have been advanced to explain TCR bias for MHC. According to the germline model, MHC restriction is intrinsic to TCR structure because TCR and MHC molecules have co-evolved to conserve germline-encoded TCR sequences with the ability to bind MHC, while eliminating TCR sequences lacking MHC reactivity. According to the selection model, MHC restriction is not intrinsic to TCR structure, but is imposed by the CD4 and CD8 co-receptors that promote signaling by delivering the Src tyrosine kinase Lck to TCR–MHC complexes through co-receptor binding to MHC during positive selection. Here, we review the evidence for and against each model and conclude that both contribute to determining TCR specificity, although their relative contributions remain to be defined. Thus, TCR bias for MHC reflects not only germline-encoded TCR–MHC interactions but also the requirement to form a ternary complex with the CD4 or CD8 co-receptor that is geometrically competent to deliver a maturation signal to double-positive thymocytes during T cell selection.

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Introduction

Antibodies and T cell receptors (TCRs) are assembled in B and T cell progenitors through the recombination of V, D, and J segments to randomly generate vast repertoires of antigen receptors with highly diverse recognition specificities [1]. Antibodies can bind a virtually unlimited number of biological and non-biological ligands, including proteins, carbohydrates, nucleic acids, and synthetic organic and non-organic compounds. In addition, antibodies recognize antigens in native form. In marked contrast, αβ TCRs recognize only peptide fragments bound to MHC molecules on the surface of antigen-presenting cells in the form of peptide–MHC (pMHC) complexes. This feature of αβ TCRs is known as MHC restriction. The peptides recognized by TCRs are generated by proteolytic degradation of self or foreign antigens within cells expressing MHC class I or class II molecules (antigen processing). MHC restriction enables αβ T cells to identify cells containing intracellular pathogens, foreign proteins, or genetic mutations, and is therefore critical for T lymphocyte function. However, the mechanism by which MHC restriction is imposed on a randomly generated TCR repertoire is a major unsolved problem that has generated much recent controversy.

Two competing hypotheses have been proposed to account for the focus of αβ TCRs on MHC. According to the germline hypothesis, MHC restriction is intrinsic to TCR structure because evolution has conserved germline-encoded TCR sequences with the capacity to bind MHC and has eliminated sequences unable to recognize MHC [2–8]. In this view, specific amino acids in the germline-encoded complementarity-determining region 1 (CDR1) and CDR2 loops of Vα and Vβ have been conserved during evolution because they impose MHC reactivity on TCRs through specific contacts with the MHC α-helices that form the peptide-binding site. Both TCR and MHC molecules are believed to have evolved ~500 million years ago in cartilaginous fish [9], thereby potentially allowing for co-evolution of TCR and MHC to achieve a good fit for each another.

According to the selection hypothesis, by contrast, MHC restriction is not intrinsic to TCR structure but is imposed on the randomly generated TCR repertoire during positive selection in the thymus [5, 7, 10–16]. In this view, preselection CD4$^+$CD8$^+$ double-positive thymocytes express αβ TCRs with the ability to recognize a diversity of ligands, both MHC and non-MHC, in the manner of antibody molecules. However, only TCRs with specificity for MHC are able to signal CD4$^+$CD8$^+$ thymocytes to undergo maturation because of the need for co-receptor function during T cell selection. Thus, MHC restriction is imposed by the CD4 and CD8 co-receptors that promote signaling by MHC-specific TCRs through recruitment of the Src tyrosine kinase Lck to the TCR–pMHC complex following co-receptor binding to MHC. Thymocytes expressing αβ TCRs that do not recognize MHC cannot access Lck and are not positively selected.

In this review, we examine the evidence, both for and against, the germline and selection models that have been proposed to explain the exclusive expression of MHC-restricted αβ TCRs on mature T cells. We address whether these models are mutually exclusive, or whether they can be incorporated into a single view of MHC restriction that features key elements of both models.

Germline model for MHC restriction: pros and cons

TCRs bind pMHC via their six CDR loops, three from the Vα domain and three from Vβ. CDR1 and CDR2 are encoded within the TCR Vα and Vβ gene segments, while CDR3 is formed by DNA recombination involving juxtaposition of Vα and Jα segments for the α chain genes, and of Vβ, D and Jβ segments for the β chain genes. As a result, the somatically generated Vα and Vβ CDR3 loops account for most of the variability of TCR binding sites, whereas variability contributed by CDR1 and CDR2 is restricted to

that already existing in the germline. X-ray crystallographic studies of numerous (>30) TCR–pMHC complexes have demonstrated remarkable similarities in the overall topology of TCR binding, irrespective of MHC class I or class II restriction (Fig. 1a) [4, 17]. Typically, the TCR is positioned diagonally over the center of the composite surface created by the peptide and the MHC α-helices that flank the peptide-binding groove, with the Vα domain situated over the N-terminal half of the peptide, and the Vβ domain over the C-terminal half, although the exact angle and pitch of TCR engagement vary (Fig. 1b) [17]. In general, the loops formed by the germline-encoded Vα and Vβ regions (CDR1α and CDR2α; CDR1β and CDR2β) are positioned over the α-helices of the α2/β1 and α1 domains, respectively, of the MHC class I or class II molecule. By contrast, the somatically generated CDR3α and CDR3β loops usually lie over and contact the MHC-bound peptide.

Initially, no consistent use of particular TCR residues to contact particular MHC residues was evident in these TCR–pMHC structures, as might be expected if TCR and MHC molecules had co-evolved. However, subsequent analyses of complexes involving common V segments and MHC alleles revealed the conservation of specific TCR–MHC interactions [3, 4, 7, 18]. For example, crystal structures of six mouse TCRs expressing Vβ8.2 bound to I-A^b, I-A^k, or I-A^u MHC class II molecules showed a close convergence of CDR1β and CDR2β contacts with the α1 helix of these I-A alleles (Fig. 1c) [3, 18]. This pairwise interaction motif comprises TCR residues CDR1β Asn31, CDR2β Tyr48, and CDR2β Glu54 contacting I-A residues Gln61α, Gln57α, and Lys39α, respectively. However, it is important to note that these germline-encoded interactions are not atomically identical (Fig. 1c), indicating a degree of flexibility ('wobble') on the order of several angstroms to accommodate differences in the Vα partner or the peptide. These and related findings demonstrating conserved interactions between germline-encoded CDR1 and CDR2 loops and particular MHC alleles support the hypothesis that the canonical diagonal docking orientation of TCR on MHC observed in TCR–pMHC complexes is the result, at least in part, of co-evolution of TCR and MHC molecules [2–8].

At the same time, it is evident from the structural database that the 'rules' governing germline-encoded interactions are not straightforward, even when a particular V region recognizes the same MHC allele. For example, structures of a single TCR in complex with different peptides bound to H-2K^b showed that the detailed interactions made by CDR1 and CDR2 with the MHC helices differed considerably, due to repositioning of the TCR [19]. The alternative TCR–MHC contacts were attributed to selection by the peptide of the germline-encoded interactions that were optimal for that particular peptide ('peptide editing') [20]. Similarly, different peptides were found to substantially

Fig. 1 Canonical docking orientation of TCR on pMHC. **a** Structure of a representative TCR–pMHC complex, viewed down the MHC peptide-binding groove. The complex involves TCR 2C bound to the MHC class I molecule H-2K^b and peptide dEV8 (PDB accession code 2CKB). **b** Footprint of the Vα and Vβ CDR loops of TCR 2C on pMHC, illustrating the canonical diagonal docking topology. **c** Close superposition of the contacts of Vβ CDR1 and CDR2 with the α1 helix of I-A MHC class II in six different TCR–pMHC complexes (1U3H, 3C61, 2Z31, 2PXY, 1D9K and 3C60). All six TCRs express Vβ8.2 but different Vαs. *Inset* germline-encoded interactions of TCR residues CDR1β Asn31, CDR2β Tyr48, and CDR2β Glu54 with I-A residues Gln61α, Gln57α, and Lys39α
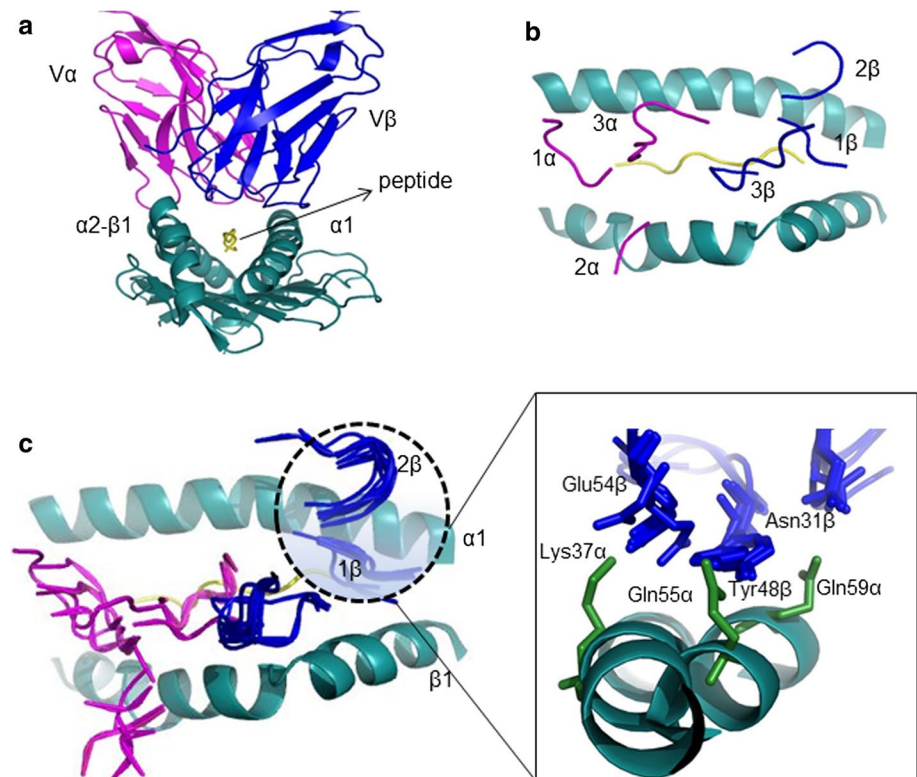
alter the docking modes of TCR 42F3 on H-2L^d [5] and of TCR DMF4 on HLA-A2 [21]. In addition to peptide editing, CDR3 can modulate interactions between CDR1 and CDR2 and MHC ('CDR3 editing') [3, 6, 22]. For example, the structures of two TCRs that use the same Vα to recognize the same peptide bound to HLA-DR1 showed that CDR1 adopts markedly different conformations in the two TCRs, resulting in a different set of contacts with MHC (Fig. 2a, b) [6]. The distinct CDR1α conformations result from differences in the length and sequence of CDR3α that are transmitted to CDR1α (Fig. 2c, d).

Among the available TCR–pMHC structures, there are number of cases of TCRs that bind MHC using amino acids that are not consistent with the co-evolution hypothesis. For example, several autoimmune TCRs were found to engage their self-pMHC ligands with altered binding topologies that produce unusual TCR–MHC contacts [23]. Perhaps the most extreme case involves the complex between a human TCR (Ob.1A12) and a self-peptide from myelin basic protein (MBP) bound to HLA-DR2b (Fig. 3a) [24]. In contrast to other TCRs, which typically contact the MHC α-helices primarily through the germline-encoded CDR1 and CDR2 loops [17], the majority of contacts to MHC made by Ob.1A12 are mediated by the somatically generated CDR3 loops. In another complex, between human TCR Hy.1B11 and MBP bound to HLA-DQ1, a highly tilted binding mode precludes direct interactions between Vα and the MHC β1 α-helix, such that only a single germline-encoded CDR of Hy.1B11, CDR2β, engages HLA-DQ1 (Fig. 3b) [25]. Importantly, unusual docking topologies are not restricted to autoimmune TCRs, which have escaped negative selection. For example, in complexes involving viral peptides that bulge out of the MHC class I binding groove, the TCR was found to focus on the peptide bulge and make only limited contacts with MHC (Fig. 3c) or, alternatively, to largely bypass the bulged peptide and make extensive contacts with the extreme N-terminal end of the MHC molecule (Fig. 3d) [26]. It should be emphasized, however, that none of these atypical TCR–pMHC complexes violate the basic docking polarity observed in canonical complexes, in which Vα is positioned over the C-terminus of the peptide and Vβ over the N-terminus.

Intriguingly, an antibody (25-D1.16) raised against a specific pMHC class I complex (pOV8–H-2K^b) has been shown to bind pMHC in an orientation closely resembling the canonical diagonal mode used by TCRs, with V_L over the N-terminus of the peptide and V_H over the C-terminus [27]. Since the CD8 co-receptor was not involved in selecting this antibody, one interpretation of this result is that TCR has an intrinsic propensity for MHC, and that MHC, not the co-receptor, determines the selection of TCRs that bind with canonical orientation [27]. It should be noted, however, that antibodies have no evolutionary relationship with MHC, and that another antibody (Hyb3) was found to

Fig. 2 Editing of germline-encoded TCR–pMHC interactions by CDR3. **a** Interactions between CDR1α of TCR G4 and the HLA-DR1 β1 α-helix in the G4–mutTPI–DR1 complex (4E41). The side chains of contacting residues are shown. **b** Interactions between CDR1α of TCR E8 and the HLA-DR1 β1 α-helix in the E8–mutTPI–DR1 complex (2IAM). TCRs G4 and E8 use the same Vα region (AV13.1) but have different CDR3α sequences. CDR1α adopts different conformations in the two TCRs, resulting in different contacts with HLA-DR1. **c** Effect of CDR3α on the conformation of CDR1α of TCR G4. Hydrogen bonds are indicated by *red dotted lines*. **d** Effect of CDR3α on the conformation of CDR1α of TCR E8
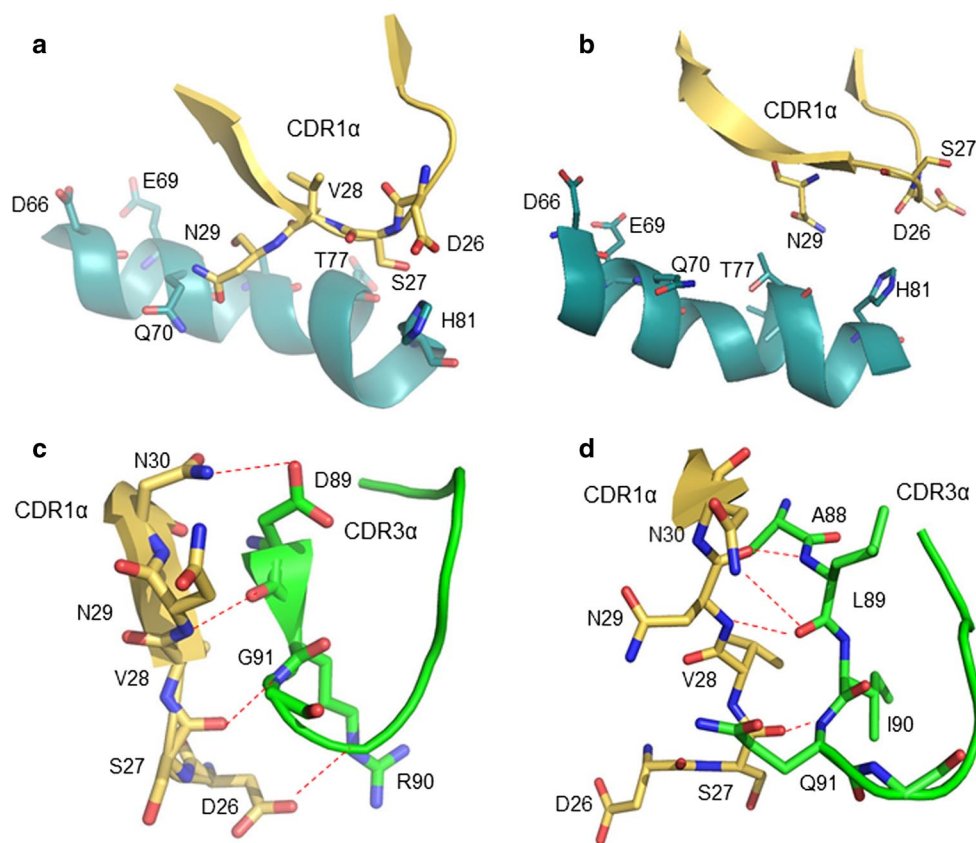
assume a completely reversed orientation over its pMHC class I ligand (MAGE-A1–HLA-A1), with V_L over the C-terminus of the peptide and V_H over the N-terminus [28].

As discussed above, crystal structures of several mouse TCRs expressing Vβ8.2 bound to I-A MHC class II allotypes highlighted a conserved interaction between TCR residue CDR2β Tyr48 and I-A residue Gln57α (Fig. 1c) [3, 18]. To test whether thymic selection of the TCR repertoire is controlled by germline-encoded TCR–MHC contacts, retrogenic mice were constructed that expressed a single, rearranged Vβ8.2 chain in which CDR2β Tyr48 was mutated to Ala [4]. In agreement with a crucial role for this residue in thymic selection, the CDR2β Tyr48Ala mutation resulted in a striking reduction in positive selection of both $CD4^+$ and $CD8^+$ T cells, even though any possible TCR α chain was available to pair with the mutated β chain. Interestingly, CDR2β Tyr48 is evolutionarily conserved in Vβ sequences from sharks and frogs, which are only distantly related to mouse and human. Moreover, chimeric TCRs containing xenogenic Vβs could recognize antigens presented by mouse I-A, suggesting that structural features of the TCR which mediate MHC specificity were selected early in evolution and maintained between species that last shared a common ancestor >400 million years ago [29].

Importantly, all αβ TCRs that have been structurally characterized to date derive from T cells which have

undergone thymic selection to be MHC-specific. No structural information is available for TCR–ligand complexes involving αβ TCRs that have not been prescreened in the thymus for MHC reactivity. To unambiguously answer the question of whether evolutionary pressure or positive selection is the more important factor in imposing MHC reactivity on the TCR, it will be necessary to determine the frequency of MHC-reactive versus MHC-nonreactive T cells in the thymus before these cells have undergone positive selection. Several studies of the preselection T cell repertoire have found a significant genetic bias toward MHC reactivity [30, 31], in agreement with the germline model for MHC restriction. If, however, co-receptors impair signaling by MHC-independent TCRs, as indicated by studies of mice lacking CD4 and CD8 (see below) [11–13, 16], the preselection repertoire may be less biased toward MHC recognition than is generally believed [11].

Recently, Holland et al. [15] employed an elegant retrogenic mutagenesis approach to determine whether the bias of αβ T cells for MHC ligands is intrinsic to the TCR, as proposed by the germline model for MHC restriction. The germline-encoded CDR1β and CDR2β loops of a mouse TCR (C6) specific for the MHC class I molecule H-2K^k were extensively diversified by directing V(D)J recombination to these CDRs through insertion of recombination signal sequences into the center of CDR1β or CDR2β.
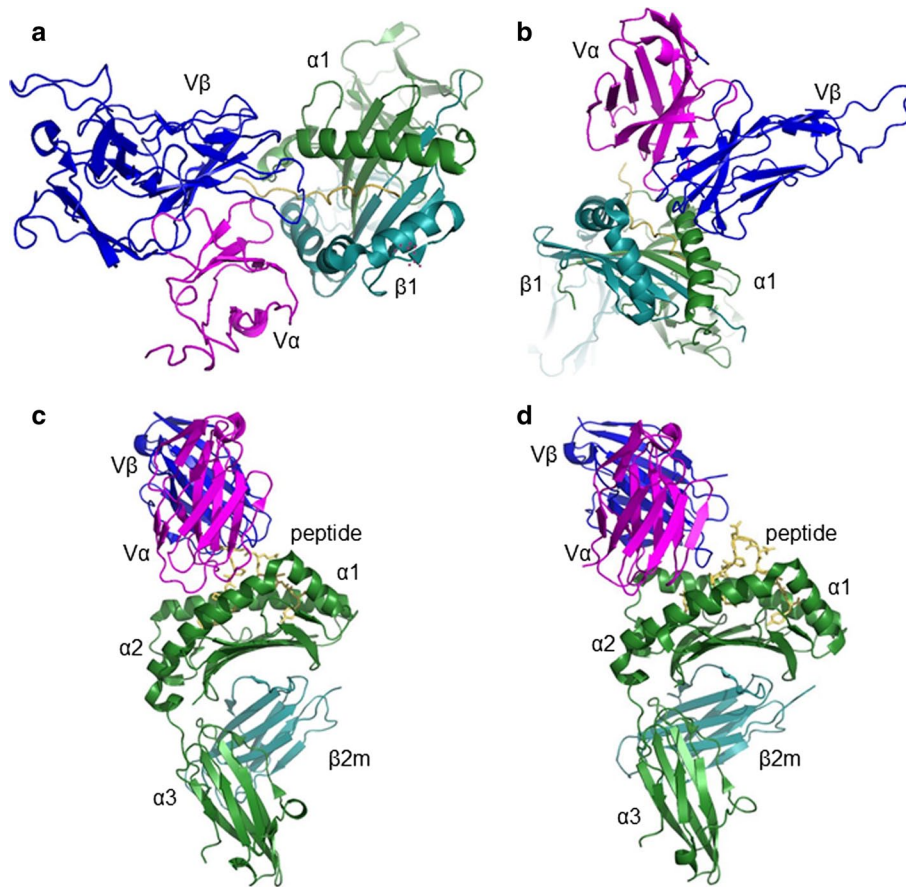
Fig. 3 TCR–pMHC complexes with atypical docking topologies. **a** Top view of the complex between TCR Ob.1A12 and MBP bound to HLA-DR2a (1FYT). The footprint of Ob.1A12 on MPB–DR2a is shifted towards the N-terminus of the bound peptide compared to the canonical docking mode (Fig. 1b), and most contacts to MHC are mediated by the CDR3 loops. **b** Side view of the complex between TCR Hy.1B11 and MBP bound to HLA-DQ (3PL6). The highly tilted binding mode of TCR Hy.1B11 prevents the Vα domain from con- tacting MHC. **c** Side view of the complex between TCR CA5 and a bulged viral peptide bound to HLA-B35 (4JRX). The TCR straddles the central region of the bound peptide but makes limited contacts with MHC. **d** Structure of the complex between TCR SB47 and a bulged viral peptide bound to HLA-B35 (4JRY). The TCR largely cir- cumvents the bulged peptide by establishing an extensive footprint on the extreme N-terminal end of the MHC molecule

The engineered TCR β genes were transduced into TCR β-deficient hematopoietic stem cells, which differentiate into T cells, and transferred to recipient mice. Rearrange- ment of the target germline CDRs in transduced immature thymocytes generated highly diverse CDR1β and CDR2β sequences, including the original germline sequences [15]. Because the mutated TCRs must engage self-pMHC ligands to permit the development of mature T cells (posi- tive selection), this system constitutes a potent screen for identifying features of germline CDRs that are required for binding MHC. The composition of CDR1β and CDR2β in mature CD4$^+$ and CD8$^+$ T cells present in the periphery was then determined. Remarkably, these germline CDRs were found to tolerate a high degree of structural diver- sity, with multiple amino acid substitutions and variable length, yet still productively engage pMHC. Moreover, the original germline CDR1β and CDR2β sequences were not

highly represented among the >150 variant CDRs that were able to direct positive selection. The apparent dispensabil- ity of germline CDR sequences for αβ T cell development was confirmed by their successful replacement with arti- ficial loops lacking all germline amino acids and by ger- mline CDR sequences from αδ TCRs, which are not MHC- restricted [15].

The functional capacity of peripheral T cells lacking germline CDRs was assessed by examining the ability of these T cells to recognize allogeneic MHC molecules, since reactivity to non-self MHC is a hallmark feature of T cells. Mature T cells expressing TCRs with non-germline CDRs were capable of orchestrating a competent immune response to MHC-mismatched skin transplants, resulting in graft rejection [15]. In addition, these T cells mediated T cell-dependent Ig isotype switching in B cells, showing that TCRs with non-germline CDRs can recognize pMHC

complexes presented by B cells. However, further assessment of functional capacity will require the isolation and detailed characterization of antigen-specific clones bearing mutant TCRs with non-germline CDRs.

The surprising finding that T cell selection and peripheral T cell activation are not dependent on germline CDR structure suggests that the TCR is not predetermined, or hardwired, to engage MHC ligands, as proposed by the germline model for MHC restriction [2–8]. This versatility is more compatible with an antibody-like, or opportunistic, strategy for ligand engagement. An opportunistic strategy is also suggested by an energetic study of the interaction of TCR A6 with its pMHC class I ligand, Tax–HLA-A2 [32]. Surprisingly, the strongest interaction was between CDR3α and the HLA-A2 α1 helix, demonstrating that somatically generated TCR loops can significantly influence MHC restriction.

Selection model for MHC restriction: pros and cons

The selection (or co-receptor) hypothesis for MHC restriction maintains that MHC bias is imposed on the TCR by the CD4 and CD8 co-receptors during thymic selection [10–14]. The principal role of the CD4 and CD8 co-receptors is to recruit the Src tyrosine kinase Lck to the TCR–pMHC complex following co-receptor binding to MHC, resulting in assembly of a TCR–pMHC–CD4 or TCR–pMHC–CD8 ternary complex [33–36]. Recruitment of Lck occurs via its association with the cytoplasmic tail of CD4 or CD8. The accompanying increase in the local concentration of Lck promotes phosphorylation of immunoreceptor tyrosine activation motifs (ITAMs) in the cytoplasmic tails of CD3 subunits (CD3ε, -γ, -δ, and -ζ) associated with the TCR in the TCR–CD3 complex, leading to the recruitment and activation of Zap-70. Activated Zap-70 phosphorylates LAT and SLP-76, which function as scaffolds to recruit other signaling molecules to the downstream T cell signaling apparatus that regulates T cell activation, proliferation, and differentiation.

According to the selection model, it is the targeted delivery of Lck to TCR by CD4 or CD8 during thymic selection that imposes MHC restriction on the developing αβ TCR repertoire [13]. In immature CD4$^+$CD8$^+$ double-positive thymocytes, all available Lck is bound to the co-receptors, such that TCRs can only access Lck when they bind to the same pMHC ligand as CD4 or CD8 in the TCR–pMHC–CD4 or TCR–pMHC–CD8 ternary complex. In this way, the co-receptors promote signaling by MHC-specific TCRs in the thymus but prevent signaling by TCRs that might engage non-MHC ligands, thereby explaining the MHC bias of TCRs in the periphery. However, in the absence of CD4 and CD8 co-receptors, Lck should be freely available

to all TCRs, so that TCRs that bind non-MHC ligands could potentially deliver maturation signals to immature thymocytes to drive positive selection.

To test this hypothesis, Van Laethem et al. [11] eliminated co-receptor sequestration of Lck by constructing mice deficient in both CD4 and CD8, and that additionally lacked MHC class I and class II (so-called quad-deficient mice). As predicted, genetic deletion of CD4 and CD8 allowed the generation of mature αβ T cells expressing MHC-independent TCRs with antibody-like recognition properties [11, 12]. In particular, two TCRs isolated from quad-deficient mice recognized CD155, the mouse homolog of the human poliovirus receptor, in its native (i.e. unprocessed) form and independently of MHC. These αβ TCRs bound different conformational epitopes on CD155 with affinities of ~200 nM, which is 10- to 100-fold higher than the micromolar affinities of TCRs for pMHC, but comparable to the affinities of primary antibodies prior to affinity maturation. Moreover, in addition to CDR3 residues, recognition of CD155 involved the same germline-encoded CDR2β residues that contact MHC in several crystal structures of TCR–pMHC complexes [3, 18], and that were required for thymic selection of an MHC-restricted retrogenic TCR [26].

The finding that identical CDR2β residues can mediate recognition of both MHC and non-MHC ligands is not particularly surprising, since these solvent-exposed residues are likely to contact any large ligand (e.g., a protein) that occupies the TCR antigen-binding site. It does, however, argue against the concept that evolutionarily conserved CDR residues uniquely promote TCR binding to MHC and are the molecular basis for MHC restriction [2, 7, 8]. Nevertheless, evolution may have optimized germline-encoded CDRs to facilitate MHC engagement across the spectrum of alleles present within the species [15], but without eliminating MHC-independent TCRs from the preselection repertoire [11, 12].

As a further test of the selection model, Van Laethem et al. [16] examined the impact of Lck availability on the mature αβ TCR repertoire. This model predicts that Lck that is not sequestered by CD4 and CD8 (co-receptor-free Lck) may be available to promote signaling by MHC-independent αβ TCRs in the thymus and selection of MHC-independent T cells in the periphery. The N-terminal region of Lck contains two cysteine residues, at positions 20 and 23, that are required for noncovalent association with the cytoplasmic tails of CD4 and CD8 [37]. To determine if co-receptor-free Lck promoted thymic selection of MHC-independent αβ TCRs, a transgene encoding an Lck mutant with alanine substitutions at positions 20 and 23 to abolish binding to CD4 and CD8 was introduced into Lck$^{-/-}$ mice. In contrast to peripheral αβ T cells from mice expressing wild-type Lck that only reacted against MHC-expressing

stimulator cells, peripheral αβ T cells from transgenic mice expressing the Lck mutant reacted against both MHC-expressing and MHC-deficient stimulator cells [16].

This result indicates that MHC-independent αβ TCRs do exist in the thymus, but are normally prevented from signaling positive selection by Lck sequestration. It should be noted, however, that many fewer polyclonal αβ T cells were generated by co-receptor-free than co-receptor-associated Lck [16]. One possible explanation for the inefficiency of co-receptor-free Lck in promoting thymic selection is that co-receptor-free Lck must be passively captured in microclusters of ligand-bound αβ TCRs, whereas CD4 and CD8 deliver co-receptor-associated Lck directly to microclusters of pMHC-bound αβ TCRs. Another explanation is that there are simply many fewer MHC-independent than MHC-restricted TCRs expressed in the preselection repertoire, as argued by the germline model [2, 7, 8]. In this regard, it should also be pointed out that the self-protein CD155 is the only thymic selecting ligand so far identified in quad-deficient or Lck mutant mice [12, 16]. Moreover, ~40 % of T cell hybridomas generated from quad-deficient mice were reactive with CD155. If MHC-independent αβ TCRs were highly represented in the preselection repertoire, as the selection model proposes, one would expect the existence of a wide variety of selecting ligands, well beyond CD155. The identification of such ligands, accompanied by sequence analysis of the clonal diversity of MHC-independent TCRs, would significantly bolster the selection model. In the absence of such data, it can be plausibly argued that TCRs, being antibody-like molecules, will inevitably find some non-MHC ligands to bind in a thymic environment devoid of MHC and co-receptors.

Role of CD4 and CD8 in MHC restriction: a structural perspective

As discussed above, the selection model argues for a critical role for the CD4 and CD8 co-receptors in imposing MHC restriction on the developing αβ TCR repertoire [13]. The molecular basis for this role may be understood in terms of the crystal structure of a complete TCR–pMHC–CD4 ternary complex involving a human autoimmune TCR (MS2-3C8), a self-peptide from MBP bound to HLA-DR4, and CD4 (Fig. 4a) [14]. The TCR–pMHC–CD4 complex resembles a pointed arch whose apex is formed by the α2 and β2 domains of the MHC class II molecule and the D1 domain of CD4. The TCR and CD4 molecules each make an angle of ~65° with the T cell surface, with no direct contacts between TCR and CD4.
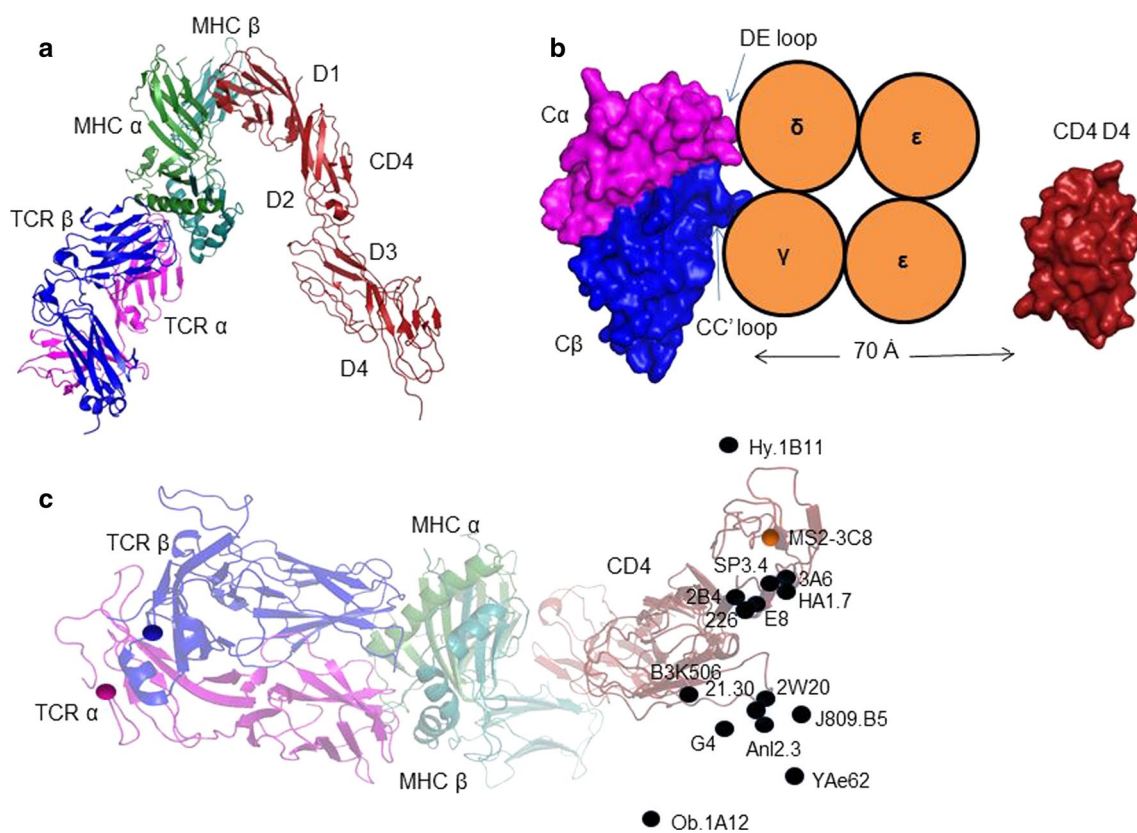
The wide separation (~70 Å) between the membrane-proximal TCR Cα/Cβ module and CD4 D4 domain provides ample room for the placement of TCR-associated CD3εγ, εδ, and ζζ subunits (Fig. 4b), which transmit activation signals to the T cell [38]. Although no crystal structure is available for the TCR–CD3 complex, mutational studies have identified docking sites for the ectodomains of CD3εδ and CD3εγ, which interact with the TCR through adjacent Cα DE and Cβ CC′ loops, respectively [39–41]. Based on these results, CD3εγ and CD3εδ would be situated inside the TCR–pMHC–CD4 arch, wedged between the TCR and T cell membrane (Fig. 4b). The ectodomain of CD3ζ, which is only nine amino acids long, has not been implicated in interactions with the TCR ectodomain, and so is not shown in Fig. 4b. However, mutational analysis of the transmembrane regions of TCR and CD3 subunits has established that CD3ζζ is associated with TCRα in the T cell membrane [42].

By fixing anchor points for TCR and CD4 on the T cell membrane, the arch-shaped TCR–pMHC–CD4 complex imposes constraints on the orientation of CD3 relative to Lck associated with CD4 on the cytoplasmic side of the membrane [14]. Figure 4c shows the position of the C-terminus of CD4 observed in the complex with TCR MS2-3C8 and HLA-DR4, as well as the predicted position of the C-terminus of CD4 in hypothetical ternary complexes constructed using 15 other TCR–pMHC class II structures, both human and mouse. Except for the human autoimmune TCR Ob.1A12 [24], the C-termini of CD4 in these modeled complexes are grouped in a loose cluster that includes the C-terminus of CD4 in the MS2-3C8–MBP–DR4–CD4 complex. Differences in the position of the CD4 membrane anchor point result from variations in the diagonal docking topology of the TCR–pMHC complexes, which places CD3εγ and CD3εδ inside the TCR–pMHC–CD4 arch, opposite CD4 (Fig. 4b). If the TCR–pMHC docking polarity were reversed (i.e. Vα over the C-terminus of the peptide and Vβ over the N-terminus), CD3εγ and CD3εδ would be positioned outside, rather than inside, the TCR–pMHC–CD4 arch. The much greater distance between CD4-bound Lck and CD3 ITAMs would likely impede ITAM phosphorylation by Lck, thereby preventing positive selection of T cells bearing TCRs with the reversed polarity, or their activation in the periphery. In this view, the diagonal docking topology of TCR–pMHC complexes reflects the requirement to form a ternary complex with the CD4 or CD8 co-receptor that is geometrically able to deliver a maturation signal to CD4$^+$CD8$^+$ thymocytes during T cell selection [10, 14].

A role for CD4 and CD8 in constraining the docking topology of TCR–pMHC complexes is also suggested by the demonstration that TCR signaling can be modulated by peptides that are recognized in divergent TCR–pMHC binding orientations [5]. In this study, yeast-displayed pMHC libraries were used to discover large selections of peptides reactive with a single TCR (42F3) that recognizes

Fig. 4 Orientation of TCR and CD4 in TCR–pMHC–CD4 complexes. **a** Crystal structure of a TCR–pMHC–CD4 complex (MS2-3C8–MBP–DR4–CD4) oriented as if the TCR and CD4 molecules are attached to the T cell at the bottom and the MHC class II molecules is attached to an opposing APC at the top (3T0E) [14]. **b** Top view of the MS2-3C8–MBP–DR4–CD4 complex, as if looking down on the T cell. The membrane-proximal TCR Cα/Cβ domains and the CD4 D4 domain are depicted in surface representation. Other domains and pMHC are omitted for clarity. The proposed arrangement of the ectodomains of CD3εγ and CD3εδ [36] is shown in relation to docking sites identified by mutational analyses [34–36]. The Ig-like ectodomains of CD3εγ and CD3εδ are drawn as *orange ovals*. In this arrangement, only CD3γ and CD3δ contact the TCR. CD3ε projects away from the TCR, towards CD4. **c** Bottom view of the MS2-3C8–MBP–DR4–CD4 complex, as if looking up from inside the T cell. On the *left side*, the C-termini of the extracellular portions of the α and β chains of TCR MS2-3C8, as defined in the crystal structure [14], are indicated by *pink* and *blue spheres*, respectively. On the *right side*,

the C-terminus of the extracellular portion of CD4 in the complex with MS2-3C8 and HLA-DR4 is marked by an *orange sphere* labeled MS2-3C8. The *right side* also shows the predicted position of the C-terminus of CD4 in 15 hypothetical ternary complexes constructed using other TCR–pMHC class II structures [human: HA1.7 (1JH8), Ob.1A12 (1YMM), 3A6 (1ZGL), E8 (2IAM), Hy.1B11 (3PL6), G4 (4E41); SP3.4 (4GG6); Ani2.3 (4H1L); mouse: B3K506 (3C5Z), 2W20 (3C6L), YAe62 (3C60), 21.30 (3MBE); J806.B5 (3RDT); 2B4 (3QIB); 226 (3QIU)]. In each case, the C-terminus of CD4 is marked by a *black sphere* labeled with the name of the corresponding TCR. The TCR–pMHC–CD4 complexes were modeled by superposing each TCR–pMHC class II structure onto the MS2-3C8–MBP–DR4–CD4 complex through the Cα/Cβ domains of the TCRs. With the exception of TCR Ob.1A12, the C-termini of CD4 in the modeled TCR–pMHC–CD4 complexes form a cluster that includes the C-terminus of CD4 in the MS2-3C8–MBP–DR4–CD4 complex. Variability in the position of the CD4 membrane anchor point is due to differences in the docking orientation of the TCR–pMHC complexes

an endogenous peptide presented by the MHC class I molecule H-2L^d. Surprisingly, one of these peptides failed to induce T cell activation in functional assays, despite an affinity for 42F3 typical for TCR–pMHC interactions. Structural analysis of the non-signaling TCR–pMHC complex revealed a docking mode that differed substantially from those of signaling-competent complexes, which all shared a similar canonical diagonal topology. By contrast, the TCR orientation in the non-signaling complex is nearly parallel to the null peptide, suggesting that this docking mode may have exceeded the geometric constraints

imposed by the CD8 co-receptor for productive signaling to occur, as described above for CD4 (Fig. 4c).

Conclusions

In this review, we have sought to present a balanced view of the evidence, both for and against, the germline and selection models for MHC restriction of the αβ T cell repertoire. We conclude that these models are not mutually exclusive, and that both play a role in determining TCR

specificity, although current evidence seems to favor the selection model as making the greater contribution. This conclusion is broadly consistent with the unifying hypothesis recently proposed by Garcia [7] to reconcile differing views of TCR germline bias for MHC, according to which co-receptor binding and germline predisposition are tightly coupled. Thus, while the preselection TCR repertoire is not hardwired to bind only MHC, evolutionary pressures have likely biased germline TCR sequences toward MHC recognition. In particular, germline-encoded interactions between TCR and MHC probably evolved from TCR–pMHC docking topologies that are geometrically compatible with formation of signaling-competent TCR–pMHC–CD4 or TCR–pMHC–CD8 complexes during positive selection. In addition, Holland et al. [15] have shown that MHC restriction can be imposed on TCRs with multiple amino acid substitutions at positions that may have been subjected to co-evolutionary pressures.

Ideally, it would be desirable to comprehensively evaluate the ligand specificity of the preselection αβ TCR repertoire, in order to determine the frequency of MHC-reactive versus non-reactive T cells before these cells have undergone thymic selection. A further question is whether the TCRs expressed by MHC-reactive preselection T cells all engage pMHC in the canonical diagonal manner of postselection T cells, or whether highly divergent binding modes are observed that are incompatible with generating signals to induce thymocyte maturation. Such studies should become feasible with on-going technical advances in the analysis of preselection $CD4^+CD8^+$ thymocyte repertoires.

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