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Codification of bidentate pMHC interaction with TCR and co-receptor

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Abstract

A 1983 *Immunology Today* rostrum hypothesized that each T cell has two recognition units: a TCR complex which binds antigen associated with a polymorphic region of an MHC molecule (pMHC) and a CD4 or CD8 molecule which bind to a conserved region of that same MHC gene product (class II or I, respectively). Structural biology has since precisely revealed those bidentate pMHC interactions. TCR $\alpha\beta$ ligates the membrane-distal antigen binding MHC platform whereas CD8 clamps a membrane-proximal MHCI $\alpha 3$ domain loop and CD4 docks to a hydrophobic crevice between MHCII $\alpha 2$ and $\beta 2$ domains. We review how MHC class-restricted binding impacts signaling and lineage commitment, discussing TCR force-driven conformational transitions that may optimally expose the co-receptor docking site on MHC.

Keywords

TCR; antigen recognition; co-receptor; mechanobiology

Introduction

The immune system of vertebrates is comprised of innate and adaptive elements with the former serving as a frontline of defense using pattern recognition receptors and the latter responsible for mediating antigen-specific humoral and cellular immunity. Adaptive T cell and B cell immunity is notable for affording efficient protection through precise immune targeting of effector mechanisms as well as cellular memory that achieves rapid and

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effective recall upon re-exposure to the same pathogens. This review focuses on a unique historical perspective as to the molecular basis of T cell receptor (TCR) recognition and conjoint interaction involving the co-receptors CD4 and CD8. An hypothesis published in 1983 in *Immunology Today*, the precursor of *Trends in Immunology*, was based on biochemical and functional data and is now revisited and placed in the context of structural studies defining the bidentate pMHC interactions at atomic level resolution.

We begin with a discussion of the conundrum of the 1 versus 2 receptor model of TCR recognition under intense investigation in the 1970's followed by its ultimate elucidation based on TCR and co-receptor biology and structure. We then turned to how strict MHC class co-receptor binding preference impacts T lineage commitment and how physical force during cell movement and at the immunological synapse relates to TCR function. Finally, we address how mechanobiology might modulate co-receptor binding site access on MHC, alluding to a vital set of future areas of study.

1 versus 2 receptor models of T cell recognition

In the 1970's, it was known that T lymphocytes recognize antigen in the context of membrane-bound products of the major histocompatibility complex (MHC) with exquisite specificity [1-3]. Two major theories were proposed to explain this dual specificity [4]. One, the single-receptor model or interaction-antigen model, suggested that self-MHC molecules interact with antigen "X" to form a neoantigen which is presented as one entity to a single T cell receptor. The second model, a two-receptor model, assumed that one surface component of the T cell sees antigen "X" and another views the cellular MHC restricting element on the antigen-presenting cell. Independent experimental approaches lined up in favor of the single-receptor model [5, 6]. Notwithstanding, biochemical data to identify the receptors responsible for this dual recognition phenomenon were unavailable. As we shall see, the actual cognate recognition machinery manifests elements of both models.

In 1979 we identified CD4, a molecule expressed on two-thirds of peripheral mature T lymphocytes with helper activity [7-9], defining a subset reciprocal to CD8 cytotoxic T cells [10]. However, the major population of thymocytes, which we termed double positive (DP), co-expressed CD4 and CD8 unlike mature single positive (SP) thymocytes and peripheral T cells [11]. In addition, we observed a small subset of CD4-CD8- (double negative, DN) thymocytes (Box 1). The notion that thymocyte development progressed from DN to DP and then to SP (CD4+CD8- or CD4-CD8+) derived from that study. Acceptance of this idea had to wait several years for immunologists to create and characterize the anti-rodent CD4 mAb equivalents [12-14].

By exploiting T cell cloning techniques [15], alloreactive CTL were derived from both human CD4 and CD8 subsets. CD4 T cells recognized MHC class II products whereas CD8 T cells recognized MHC class I products. These cells could be blocked by appropriate anti-MHC II/I or anti-CD4 or anti-CD8 antibodies [16, 17]. Anti-CD3 mAb inhibited antigen-specific human T cell proliferation to both soluble antigens and alloantigens as well as generation of cytotoxic T lymphocytes (CTL) [18]. The fact that biochemical analysis (see for example [19, 20]) failed to identify differences in peptide maps or electrophoretic

mobility of these molecules on human T cell clones of differing specificities argued that they were invariant structures, incapable of conferring antigen and/or allelic-restricted MHC specificities *per se*. Because each cloned T lymphocyte recognizes antigen in a precise fashion, one could not account for unique specificity on the basis of monomorphic portions of CD3, CD4 or CD8 [21]. Therefore, we reasoned that there had to exist discriminative surface structures on individual clones, referred to as clonotypes or idiotypes. mAbs to such idiotypic structures (T idiotypic = Ti) were produced by immunizing mice with CTL clones, screening resultant antibodies on the immunizing CTL and then selecting those which lacked reactivity with additional clones of different antigen specificities from the same donor [21-23]. Such antibodies inhibited cell-mediated killing and antigen-specific proliferation of the individual immunizing clone without affecting the function of other autologous clones. Like anti-CD3 mAbs, the anti-clonotypic antibodies enhanced IL-2 responsiveness and induced shedding and/or internalization of the Ti structure with CD3 from the cell surface. Data showed that the Ti clonotype was closely associated with CD3 in the membrane of human T cells. Immunoprecipitation and competitive binding analysis revealed that the anti-clonotypes defined a disulfide-linked heterodimer with α and β subunits of approximately 49KD and 43KD, respectively. The heterodimeric clonotype was not physically associated with CD4 or CD8 but instead was in non-covalent association with the invariant CD3 molecules as evidenced by our analyses [19, 21-23].

From the collective data, we proposed a model of T cell cognate recognition ([24] and Fig. 1) in which the antigen-binding structure was comprised of a clonally unique $\alpha\beta$ heterodimeric Ti moiety in complex with CD3. The associative recognition element was either CD4 or CD8 depending on the subset derivation of the individual T lymphocyte. In this model, CD4 and CD8 accessory (“co-receptor”) glycoproteins bind to constant regions of class II or class I MHC, respectively, which are separate from the CD3-linked clonotypic $\alpha\beta$ heterodimeric Ti moiety. The TCR complex, on the other hand, was defined as a CD3-associated Ti $\alpha\beta$ heterodimer working in concert with CD4 and CD8 to mediate MHC-restricted antigen recognition. This view implied that there was a bidentate interaction of the same pMHC with TCR and co-receptor. This proposal has been codified in structural studies over the last 30 years described below. Discovery of the protein tyrosine kinase *lck* and its association with CD4 and CD8 α cytoplasmic tails as well as elucidation of the TCR complex components involving mechanotransduction function since then offer new insights into TCR and co-receptor biology. This review incorporates recent features of signaling and mechanobiology relevant to $\alpha\beta$ T cell development and activation in the model.

Co-receptor biology, a prelude

While CD8 $\alpha\alpha$ homodimers and CD8 $\alpha\beta$ heterodimers are found on the surface of T lymphocytes, CD8 $\alpha\beta$ is the dominant isoform expressed on CTL [25]. Detailed aspects of co-receptor genes and molecular functions are reviewed elsewhere (see [26] and references therein). However, the major function of the co-receptor in T cell-mediated adaptive responses is to deliver the tyrosine kinase *lck* into the area of TCR-pMHC interaction so that exposed immunoreceptor tyrosine-based activation motifs (ITAMs) on CD3 tails can be phosphorylated on tyrosine residues. In turn, this allows for Zap-70 recruitment and the remainder of downstream signaling apparatus to assemble [27]. A zinc clasp tethers *lck* to

the cytoplasmic tail of both CD4 and CD8 α , facilitating co-receptor delivery of this essential kinase to the TCR-engaged pMHC ([28] and references therein). This co-receptor coupling of lck and bidentate pMHC binding skews $\alpha\beta$ TCR repertoire selection toward a pMHC ligand specification. In contrast, mice lacking co-receptors and MHC show no $\alpha\beta$ TCR preference for pMHC ligands, instead possessing antibody-like, MHC-independent receptor specificities [29]. Careful analysis of the TCR-pMHC-CD8 trimolecular interaction revealed a two-stage process, the first involving TCR-specific pMHC binding, thereafter triggering a kinase-dependent second stage delayed by approximately one second that initiates CD8 binding to the TCR-engaged pMHC [30].

The affinity of CD4 for pMHCII is extremely weak (200 μ M or higher) [31] with that of CD8 $\alpha\beta$ for pMHCI several fold stronger [32, 33]. By contrast, ~ 1 μ M affinities of TCR-pMHC interactions in solution are not uncommon [34]. The half-life of TCR-pMHC is ~ 1000 times longer than that of CD4-pMHC; hence, little pMHC binding is contributed by the co-receptor ectodomain. Likely, CD8 $\alpha\beta$ offers more pMHC interaction energy than CD4. Given that not all co-receptor molecules are linked to lck (*infra vide*) and only one-third or so of lck molecules are catalytically active in T cells with that amount not upregulated upon TCR and co-receptor engagement [35], a dynamic process of a rapid entry and exit of co-receptors into the TCR-pMHC site is critical. If a co-receptor were loaded with inactive lck and bound tightly to pMHC, it could function as a dominant negative inhibitor of TCR activation. In contrast, a fast on and off rate of co-receptor-pMHC interaction increases the probability of bringing an active kinase in contact with an accessible CD3 tail ITAM.

Structural biology of co-receptors

Structural immunology advanced knowledge at the molecular level as to how TCR co-receptors function [21]. The first breakthrough was the structure of CD4 N-terminal two-domain fragment [36, 37]. Later, detailed results about co-receptor engagement of MHC molecules emerged from subsequent structures of CD8 in complex with pMHCI [38-41] and CD4 in complex with pMHCII [42, 43]. These findings along with structures of TCR in complex with MHC (reviewed in [26]) have confirmed and extended in exquisite detail the functional observations.

While CD4 and CD8 belong to the same immunoglobulin superfamily (IgSF), they are remarkably distinct in overall architecture. CD4 consists of four abutting Ig-like domains, a short stalk, a TM and a short cytoplasmic tail. By contrast, the CD8 receptor is a disulfide-linked dimeric transmembrane molecule existing in two isoforms, either as an $\alpha\beta$ heterodimer or as an $\alpha\alpha$ homodimer. Each CD8 subunit consists of a single Ig-like domain at the N-terminus followed by a lengthy stalk, rich in prolines and O-linked glycosylated adducts, a TM and a short cytoplasmic tail (reviewed in [26]). As reviewed here, the strategies employed by CD4 and CD8 to bind pMHCII and pMHCI, respectively, are strikingly different.

Three CD8 $\alpha\alpha$ /pMHCI complex structures have been solved. These include human CD8 $\alpha\alpha$ in complex with HLA-A2 [38], mouse CD8 $\alpha\alpha$ in complex with H-2K^b [39] and mouse

CD8 $\alpha\alpha$ in complex with non-classical MHC Ib molecule, TL [40]. These complex structures are very similar to the more recent structure of CD8 $\alpha\beta$ in complex with H-2D^d [41]. The common feature is that the N-terminal Ig-like domains of the two CD8 subunits form a structural unit similar to the V-module of an Fab molecule, with the CD8 dimeric headpiece binding to the $\alpha 3$ domain of the pMHC I molecule in a fashion analogous to an antibody binding to antigen. Six CDR-like loops of the CD8 dimer clamp onto the CD loop of pMHC $\alpha 3$ domain, contributing to CD8/MHCI recognition (Fig. 2A) [39]. This CD loop of MHC I is rigidified by a network of internal hydrogen bonds formed by conserved residues in the loop. Such a pre-configuration is energetically favorable for binding, enabling a conserved residue Gln226 in the loop to poke into a crevice formed by the two CD8 subunits to mediate a key recognition role in all CD8/MHCI complex structures (Fig. 2A).

Prior to structural elucidation, there were disparate views as to where CD8 β would localize [38, 39]. However, since the stalk region of CD8 α chain is significantly longer (about 7-10 residues) than the corresponding region of CD8 β chain, this CD8 β chain was predicted to be positioned closer to the T cell membrane [39], a notion confirmed by the CD8 $\alpha\beta$ complex structure (Fig. 2B) [41]. Furthermore, in the CD8 $\alpha\beta$ complex structure the CD8 β subunit at this “top” position exclusively contacts the MHC $\alpha 3$ domain. By comparison in all known CD8 $\alpha\alpha$ complex structures, the CD8 α subunit at this “top” position has more contacts with $\alpha 2$ and/or $\beta 2$ domains of MHC I. However, CD8 $\alpha\beta$ and CD8 $\alpha\alpha$ affinities for pMHC are similar (30-60 μ M) [33]. Within T cells, the CD8 β chain brings CD8 $\alpha\beta$ into the lipid raft so that the CD8 α -associated lck can be in close proximity to raft-associated TCR activation complexes [44]. Conversely, the stronger CD8 $\alpha\alpha$ binding to the non-classical TL molecule may function to sequester and redirect CD8 $\alpha\alpha$ away from the TCR, thereby modifying lck-dependent signaling [40].

Fig. 3 demonstrates the binding mode of the N-terminal two-domains of CD4 to the pMHC II molecule murine I-A^k and a 13-residue peptide from hen egg conalbumin (CA) (Fig. 3A) [42]. Unanticipated was the manner in which the CD4 N-terminal domain wedges between the two membrane-proximal $\alpha 2$ and $\beta 2$ domains of MHC II. The protruding C’ strand of the CD4 domain 1 (D1) N-terminal corner makes a mini-antiparallel β structure with the D strand of MHC II molecule (Fig. 3A). This docking mode brings the key MHC II-binding residue, Phe43 of CD4 into a conserved hydrophobic pocket of MHC II. Based on this structure and that of the same CA/I-A^k pMHC in complex with the D10 TCR, we proposed a ternary TCR/pMHC II/CD4-D1-D4 model revealing a V-shape in the absence of direct contacts at the vertices between TCR and CD4 within the complex [42]. This model was confirmed by an experimentally determined ternary complex structure TCR/DR4/CD4 and offering a direct snapshot of bidentate interaction (Fig. 3B) [43]. Collectively, the structural pictures nicely explain our earlier observation on the two-receptor requirement for T cell function (Fig. 1).

Co-receptor tuning and glycobiology

Aside from their binding specificities, CD8 and CD4 differ with respect to their ectodomain flexibility and tunable biology [45, 46]. The combined presence of O-glycans and prolines in the stalk CD8 subunits indicates that the stalks most likely adopt extended and stiffened

conformations, similar to those observed for leukosialin and mucins. That said, there remains some CD8 stalk flexibility in counter-distinction to the rigidity of the four concatamerized CD4 Ig-like domains, accommodating the variability of TCR docking onto pMHC I. In addition, developmentally regulated glycosylation of the CD8 $\alpha\beta$ stalk modulates pMHC binding [45]. Immature DP thymocytes bind MHC I more avidly than mature CD8 SP thymocytes. This differential binding is governed by developmentally programmed O-glycan modification of several CD8 β stalk threonine residues proximal to the CD8 β headpiece and controlled by ST3Gal-I sialyltransferase [45, 46] in the medulla where SP thymocytes reside. ST3Gal-I induction and attendant core 1 sialic acid addition to CD8 β on mature thymocytes decreases CD8 $\alpha\beta$ -MHC I avidity by altering CD8 $\alpha\beta$ domain-domain association and/or orientation. Hence, glycans on the CD8 β stalk appear to modulate the ability of the distal binding surface of the dimeric CD8 globular head domains to clamp MHC I. The DP stage facilitates efficient elimination of autoreactive TCR specificities by negative selection through this enhanced co-receptor function working in tandem with highly specific TCR-pMHC triggered apoptosis [47]. Once a thymocyte has differentiated to the CD8 SP stage, however, CD8 $\alpha\beta$ O-glycan sialylation reduces the strength of the CD8 $\alpha\beta$ co-receptor interaction with pMHC, mandating a greater requirement for TCR-pMHC interaction to achieve a subsequent activation threshold in mature T lineage cells [45]. While no such modulation of O-glycans is known to occur on the CD4 molecule, N-glycan branching of TCR and CD4 and CD8 co-receptors alters ligand affinity boundaries for positive selection [48].

Strict MHC class co-receptor binding preference in T lineage commitment

The differentiation of DP thymocytes into CD4 or CD8 SP thymocytes is an example of bipotential cell fate determination (reviewed in [49]). A stochastic selection model that postulates that co-receptor gene expression occurs randomly has been discredited [50]. Instructional models postulating that TCR signals during positive selection specifically direct termination of irrelevant co-receptors have been controversial [51-53]. A more recent variant of the instructional model is a duration-of-signal model whereby TCR signaling of long duration terminates CD8 gene expression while TCR signaling of short duration terminates CD4 gene expression. Recent data have shown that positive selection in DP thymocytes *per se* invariably decreases surface CD8, thereby selectively disrupting MHC class I-restricted signaling [54]. The kinetic signaling model proposed by Singer et al. incorporates aspects of this signaling duration model in conjunction with more recent common cytokine receptor γ -chain (γ_C) signaling biology, viewing positive selection and lineage choice as sequential events [49, 55]. Positive selection induces all DP thymocytes, regardless of TCR specificity, to transiently terminate CD8 gene expression resulting in CD4⁺CD8^{low} thymocytes [56]. Persistent TCR engagement blocks IL-7 signaling and fosters CD4⁺ SP development with loss of CD8. In contrast, disruption of TCR engagement permits IL-7 signaling and results in “co-receptor reversal” with CD8 SP development. IL-7 signaling induces RUNX3 transcription that initiates CD8 expression and also activates the cytotoxic gene transcriptome.

Our structural survey of almost 300 MHC class I and class II molecules reveals that CD4 exclusively binds to MHC class II while CD8 exclusively binds to MHC class I molecules

[57]. In contrast, the interacting domains of TCR and MHC molecules manifest no intrinsic structural differences distinguishing the two classes of MHC interactions; nor are TCR complex components distinct [26]. Thus, TCR-pMHC interactions are unlikely to differentially regulate CD4 or CD8 co-receptor expression *per se*. Rather, the preference of the TCR for a specific pMHC dictates which co-receptor subsequently enters into the preformed TCR-pMHC complex. During positive selection, DP thymocytes first become intermediate cells ($CD4^+CD8^{low}$). If during positive selection, one MHCII-restricted TCR on the $CD4^+CD8^{low}$ cell surface encounters an MHCII molecule that presents a self-peptide on the stromal cell, the “snug” fit of CD4 into the MHCII molecule’s $\alpha 2/\beta 2$ domains on the cell surface brings Lck into close proximity with exposed CD3 cytoplasmic tail ITAMs in the TCR complex. If successful ITAM phosphorylation and downstream activation persists, transcriptional factors like GATA3 and Th-POK are induced, promoting $CD4^+$ T cell differentiation by preventing *Cd4* gene silencing [49]. This successful signaling eventually terminates CD8 expression, and the thymocyte differentiates into $CD4^+$ SP cell. If during positive selection an MHCI-restricted TCR on one $CD4^+CD8^{low}$ thymocyte surface encounters an MHCI that presents a self-peptide, then CD4 fails to contact the MHCI molecule. Consequently, TCR signaling is disrupted with loss of GATA3 and Th-POK transcription. Instead, IL-7R signaling is initiated to up-regulate RUNX3, thereby silencing *Cd4* and activating *Cd8* expression. CD8 co-receptor function is operative. As RUNX3 is also linked to the cytotoxic program, this dichotomy normally ensures that CD8 SP development is coupled to the CTL program.

Mechanobiology of the TCR

Models for the TCR complex have been presented elsewhere ([58, 59] and references therein). The $TCR\alpha\beta$ heterodimer with a vertical dimension of $80\text{\AA}$ projecting from the cell membrane assumes a central position, flanked on either side by the shorter ($40\text{\AA}$) CD3 heterodimers, CD3 $\epsilon\delta$ on the “left” $TCR\alpha$ side and CD3 $\epsilon\gamma$ on the “right” $TCR\beta$ side. CD3 $\zeta\zeta$ lacks a folded ectodomain but positions near **TCR α** . The width of each of the CD3 $\epsilon\delta$ and CD3 $\epsilon\gamma$ components are comparable in size to that of the $TCR\alpha\beta$ heterodimer, impeding lateral **bending** movement of the $TCR\alpha\beta$ heterodimer upon pMHC binding. The 5-10 amino acid rigid CD3 connecting peptide (CP) segments [58, 60] contrast sharply with the long (19-26aa) and flexible TCR α and β CP linking their respective constant domains to the transmembrane (TM) segments, suggesting that the $\alpha\beta$ TCR heterodimer may bend and extend relative to the squat and inflexible CD3 heterodimers under force.

The simple notion that the TCR might be a mechanosensor has been confirmed by several independent studies. Kim et al. provided the first direct evidence of the influence of mechanical force in TCR activation [58]. Using an optically trapped bead coated with pMHC or anti-CD3 monoclonal antibody for engaging the TCR, T cells were mechanically triggered by applying an oscillating tangential force to the cell surface while monitoring their activation via intracellular calcium flux. Importantly, piconewton (pN) force application with cognate pMHC but not irrelevant pMHC triggered activation. Additional mechanosensor evidence was provided by other studies [61-63]. Prediction that a nonlinear mechanical response such as catch bond formation might facilitate TCR-based recognition [58] was elegantly confirmed by Zhu and coworkers using a **biomembrane force probe**

(BFP) [64]. Note that while Kim et al. [58] applied tangential force via optical tweezers and Liu et al. [64] applied normal force using BFP to stimulate T cell activation, the application of the latter uses pMHC arrayed on RBCs, making it difficult to exclude tangential load.

Physical forces are generated through T lymphocyte movement during immune surveillance as well as by cytoskeletal rearrangements at the immunological synapse following cessation of cell migration ([65] and references therein). Nevertheless, the mechanistic explanation for how TCRs distinguish between foreign and self-peptides bound to a given MHC molecule has been unclear. Using optical tweezers and DNA tether spacer technology which permit pN force application and nm scale precision, Lang et al. [65] determined how bioforces relate to self versus non-self discrimination. Single molecule analyses involving isolated $\alpha\beta$ heterodimers as well as complete TCR complexes on T lymphocytes reveal that the FG loop in the β subunit constant domain allosterically controls both the variable domain module's catch bond lifetime and peptide discrimination via force-driven conformational transition. In contrast to slip bonds that release under physical load, catch bonds become stronger so that TCR-pMHC single bond lifetimes extend. For a representative CD8-derived $\alpha\beta$ TCR heterodimer like N15 that binds a rabies family vesicular stomatitis peptide, the bond lifetime goes from 0.3 seconds at zero force to >3 seconds at 15 pN. Such forces are readily achievable in biological systems. The extended bond lifetime under force fosters greater TCR subunit conformational alterations, membrane lipid perturbation, facilitation of the exposure of sequestered CD3 cytoplasmic tail ITAMs that allow full signaling to generate "digital" activation responses.

Concluding remarks

While conjoint interaction of co-receptor with pMHC has been verified at an atomic level, important details continue to emerge. Foster resonance energy transfer (FRET) microscopy between TCR complex and CD8 components shows interaction within seconds between CD3 ζ and CD8 $\alpha\beta$ during antigen recognition that is independent of CD8 ectodomain binding to pMHC. Instead, this stage involves CD3 ζ -lck association via lck linkage to the CD8 cytoplasmic tail via its zinc clasp. After several minutes, an interaction between the CD8 ectodomain and MHCI follows [66]. These separate but sequential processes involved in co-receptor function further refine and extend the two stage cooperative TCR-pMHC-CD8 trimeric interaction reported by Jiang et al. [30]. Moreover, Casas et al. suggest that free lck (i.e. coreceptor unlinked) can initiate ITAM phosphorylation to allow additional CD8-bound lck to interact with ITAMs via its SH2 domain, dragging associated co-receptor into the TCR complex.

Very recently, Palmer and colleagues [67] have examined the dwell time ($T_{1/2}$) for MHCI and MHCII ligands on intact thymocytes that threshold for negative selection in pre-selection DP thymocytes as 0.9 and 0.2 seconds, respectively, findings inconsistent with the similar affinity thresholds for pMHCI- vs. pMHCII-specific TCRs **measured by SPR in the absence of co-receptors**. Strikingly, they determined that those co-receptors with associated active lck are only 1.8% and 0.16% of total CD4 and CD8 molecules, respectively, implying that multiple co-receptors must sequentially associate with a preformed TCR-pMHC bimolecular complex in order to engage an active enzyme with the capacity to trigger

downstream thymocyte activation. Mathematical modeling suggested that the probability of lck-bound co-receptor is critical to predict observed differences in antigen dwell-time thresholds used by MHCI- and MHCII-restricted thymocytes that control thymocyte fate. According to this view, sufficiently long TCR-pMHC interaction enables lck-mediated phosphorylation of ZAP-70 with downstream phosphorylation of LAT and SLP76 to initiate negative selection.

These data show that bidentate pMHC interaction with TCR and co-receptor controls digital T lineage activation responses such as negative selection and, by extension, TCR activation *per se*. That CD8 has less associated lck compared to CD4 is given as the explanation for why negative selection for MHCI-restricted TCRs requires a longer dwell-time than MHCII-restricted TCRs. In addition, it is possible that the single TM spanning pMHCI molecule adopts multiple conformations with full access to the MHC $\alpha 3$ site for CD8 docking available only after TCR-pMHCI interaction. pMHCII is preconfigured, by contrast, given its two transmembrane spanning links. Moreover, as described previously [26], differences in presenting platform orientations may exert greater mechanical force during cell movement in the case of TCR-pMHCII ligation. Clearly, the understanding of this bidentate pMHC biology involving TCR and co-receptor molecules will require a melding of immunology, cell signaling and mechanobiology (Box 2).

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Highlights

Functional data implying conjoint TCR and co-receptor binding to pMHC have been structurally established.

Binding modes of CD4 and CD8 to pMHCII and pMHCI, respectively, are distinct.

Mechanobiology plays an important role in TCR-pMHC interaction

Mechanical force during TCR-pMHC interaction may optimize exposure of the co-receptor binding site on MHC

Box 1**Schematic of co-receptor expression in thymocytes and peripheral T cells**
[11] **Fig. 3.**

Box 1 legend: The thymus is divided into immature and mature compartments corresponding to cortex and medulla, respectively. In the medulla, maturing thymocytes are observed. There, CD4 and CD8 co-receptor expression matches that found in the peripheral lymphoid compartment, namely being reciprocal and non-overlapping. Cortical thymocytes, on the other hand, arise from DN (CD4-CD8-) lymphoid stem cells seeded via blood at the corticomedullary junction (DN1), then differentiate into DN2 thymocytes where they lose B and NK potential, followed by DN3 thymocytes where TCR β expression and pre-TCRs are expressed, and a DN4 stage transitioning to become DP (CD4+CD8+) cortical thymocytes. The $\alpha\beta$ TCR is first expressed at the DP stage where positive selection leads to differentiation of thymocytes to CD4 or CD8 SP (single positive) thymocytes. As described later in the text, co-expression of CD4 and CD8 at the DP stage allows co-receptor selection to be driven by a given TCR's preference for pMHC ligand specificity. Positive and negative thymic selection events begin at this stage.

Box 2**Outstanding questions**

1. Do force-driven conformational transitions linked to TCR mechanobiology alter accessibility of co-receptor docking sites on pMHC I or pMHC II molecules?
2. Does the combination of non-equilibrium signaling events driven by the mechanosensor mechanism and lck delivery to the TCR-pMHC complex by co-receptors account for kinetic proofreading, the mechanism allowing biological discrimination between two highly-related peptide ligands?
3. What is the role of free lck in “priming” TCR signaling?
4. In mature T cells, how could other molecules at the immunological synapse influence the mechanobiology of the TCR, the co-receptor or both?
5. Will further details analysis of trimolecular TCR-pMHC-coreceptor complexes facilitate development of strategies (chemical, recombinant proteins or other biologics) to manipulate T cell activation *in vivo*?
6. Among DN thymocytes, does the absence of co-receptors afford MHC-independent as well as MHC-dependent specificities among pre-TCRs, assuming they possess ligand binding capacity?

Fig. 1. Model of the human T cell cognate recognition apparatus circa 1983

Each T lymphocyte displays two recognition units on its surface; the Ti_n -T3 complex and the associative recognition structure, CD8 or CD4. The CD8 and CD4 glycoproteins bind to non-polymorphic regions of class I (▶) and class II (◆) MHC gene products, respectively. In contrast, the Ti_n -T3 complex recognizes specific antigen in the context of a polymorphic MHC gene product (◐). Note that the precise domains of class I and class II which the T cells recognize are unknown and assigned above only for purposes of illustration. At that time, the various CD3 components were not yet defined and hence, only a single CD3 subunit was pictured. This figure was reproduced with permission from Ref. 24.

Fig. 2. Structures of CD8αα and CD8αβ in complex with an MHC I molecule

A) Mouse CD8αα homodimer in complex with H-2K^b (PDB code: 1BQH). The CD8αα dimer clamps onto the CD loop of MHC I α3 domain as the major binding region. The conserved CD loop Q226 is shown. The CD8αα dimer reaches MHC I from the T cell that would be positioned above in this view. B) Structure of mouse CD8αβ heterodimer in complex with H-2D^d (PDB code: 3DMM). The CD8β subunit is at the predicted “upper position”, closer to the T cell membrane than CD8α “below”.

Fig. 3. Structures of CD4 in complex with pMHCII molecule

A) Structure of human CD4 N-terminal two-domain molecule in complex with murine I-A^k (PDB code: 1JL4). Note how the top of CD4 N-terminal domain 1 wedges between $\alpha 2$ and $\beta 2$ domains of the pMHCII molecule. B) A ternary complex structure of the entire human CD4 ectodomain and TCR MS2-3C8 bound to the same pMHCII molecule, HLA-DR4 (PDB code: 3TOE). The complex assumes a V-shaped configuration just as the binary complex shown in A).