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Differential T-Cell Receptor Signaling: A Novel Paradigm for Thymic Selection and Lineage Commitment

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Abstract

The canonical affinity-threshold model of thymic T-cell selection posits that thymocyte fate is determined by TCR-pMHC integrated affinity. However, this model fails to explain high thymocyte deletion rates, diverse TCR affinities in mature repertoires, and precise CD4/CD8 lineage commitment mechanisms. We propose a novel Differential TCR Signaling Model wherein the functional TCR signal is a relative comparison: TCRβ signal strength relative to a dynamic TCRα-derived self-reference threshold (TCR = TCRβ/TCRα). This requires functional partitioning of pMHC recognition: TCRα (via VJ recombination) engages the N-terminal peptide half (Seg1) to establish a tunable self-reference, while TCRβ (via V(D)J recombination) targets the C-terminal half (Seg2) harboring antigenic information. Consequently, thymic selection expands to four distinct checkpoints aimed at achieving Equivalence of Self-Recognition (TCR$\alpha \approx$ TCRβ) on self-ligands, ensuring near-zero differential signaling and stringent central tolerance. TCRα rearrangement becomes an active α-negative selection process, reducing TCRα affinity to balance fixed TCRβ signals. The kinetics of achieving equivalence deterministically dictate lineage fate: strong initial TCRβ signals promote rapid balancing and CD4 commitment, while weaker signals require prolonged tuning and often coreceptor reversal (CD4 → CD8) for MHC I balance, leading to CD8 commitment. This model redefines T-cell development as active, quantitative self-recognition calibration, providing a unified explanation for thymic attrition, repertoire diversity, and lineage choice, and establishing a quantifiable 3:1 self-to-antigen recognition confidence ratio fundamental to immune discrimination.

Subject Areas

Immunology

Keywords

Differential TCR Signaling, Differential Affinity Threshold, Repertoire Diversity, Segregated Peptide Recognition, Four Checkpoints in Thymic Selection, Equivalence of Self-Recognition

1. Introduction

The adaptive immune system, with its exquisite specificity and memory, stands as a cornerstone of vertebrate host defense. Central to this system are T lymphocytes, which orchestrate cellular immunity by recognizing pathogen-derived peptides presented on major histocompatibility complex (MHC) molecules [1]. The ability of T cells to distinguish between self and non-self is paramount, preventing autoimmune destruction while ensuring potent responses against foreign threats. This crucial discriminative capacity is primarily established during thymic development, a tightly regulated process known as T-cell selection [2].

For decades, the prevailing dogma in immunology has centered on the integrated affinity model to explain thymic selection [3]. This model posits that the ultimate fate of a developing thymocyte—whether it is positively selected to survive, negatively selected for deletion, or dies by neglect—is dictated by the overall strength of interaction (affinity) between its T-cell receptor (TCR) and self-peptide-MHC (pMHC) complexes encountered in the thymus. A "Goldilocks zone" of intermediate affinity is theorized to promote positive selection, ensuring the TCR can bind self-MHC, while excessively strong binding triggers negative selection, eliminating potentially autoreactive clones [4].

However, the integrated affinity model faces significant challenges in fully explaining several fundamental observations in T-cell biology. The remarkably high rate of thymocyte attrition, where over 90% of developing T cells fail to successfully navigate selection, seems paradoxical if selection merely requires a minimal functional interaction with self-MHC [5]. Furthermore, the immense diversity of TCR affinities observed in the peripheral T-cell repertoire of healthy individuals appears inconsistent with rigid, universally applied affinity thresholds for selection [6]. Such thresholds would inevitably constrain repertoire diversity, compromising the immune system's capacity to respond to a vast array of pathogens [7]. These discrepancies suggest that T-cell selection is not a simple binary decision based on a single affinity value, but rather a more nuanced, individualized, and active calibration process.

Evidence suggests that TCR signaling is not merely a time-dependent summation [8]. Several studies indicate that TCR signals are spatially and temporally partitioned rather than constituting a single holistic event [9]. Additionally, TCR signaling appears to involve qualitative internal computation rather than simple summation [10]. The distinct behavior of CDR3 regions further suggests differential contributions from TCRα and TCRβ chains [11]. Crucially, recent struc-

tural analyses have provided definitive physical evidence for this differential capacity. Brazin *et al.* [12] demonstrated that the TCRα transmembrane (TM) domain functions as an independent mechanosensor, possessing a unique bipartite helix structure that regulates the signaling threshold. Their findings show that specific residues in the TCRα chain (Arg251/Lys256) independently control the recruitment and dissociation of CD3 subunits, proving that the TCRα axis is structurally competent to set a variable reference threshold against which antigenicity is measured.

Thus, this manuscript proposes a radical departure from the traditional paradigm: the Differential T-Cell Receptor Signaling Model. We hypothesize that a T-cell does not measure the absolute or integrated affinity of its TCR. Instead, it computes a differential signal by actively comparing the intrinsic signal strength generated by its TCRβ chain relative to a dynamic, self-established threshold derived from its TCRα chain. Under this framework, the TCR signal is not a sum, but a comparison: TCR = TCRβ/TCRα (TCRβ signal with respect to TCRα signal). This conceptual shift reframes T-cell recognition from a qualitative binding event to a quantitative, internal computation, where activation is triggered only when the TCRβ signal quantitatively exceeds the TCRα self-reference.

Central to this differential signaling model is a proposed strict molecular partitioning of peptide recognition by the TCR chains. We posit that the TCRα chain, generated through VJ recombination, is preferentially involved in recognizing the N-terminal half of the presented peptide (termed seg1), which in a self-pMHC context primarily comprises self-sequences and thus contributes to establishing a dynamic self-reference [13]. Conversely, the TCRβ chain, characterized by its unique V(D)J recombination and the presence of the Diversity (D) gene segment, is herein specialized for recognizing the C-terminal half (termed seg2), which is more likely to harbor antigenic, non-self elements and serves as the primary detector for antigenicity [14]. This proposed peptide segmentation and differential engagement by TCRα and TCRβ is derived from a comprehensive analysis of existing VDJ recombination research and extensive structural and functional data on TCR-pMHC interactions. In particular, numerous studies employing peptide mutagenesis, alanine scanning, and structural analyses have delineated the distinct roles and contact preferences of the individual TCR CDR loops, providing the collective physical basis for this functional segregation [15].

This structural and functional segregation necessitates four distinct checkpoints in thymic selection. These checkpoints systematically guide the thymocyte through a process of achieving equivalence of self-recognition (TCR$\alpha \approx$ TCRβ) on self-ligands, ensuring that the differential signal is precisely zero in the absence of foreign antigen. This process is not passive; TCRα rearrangement is redefined as an active α-negative selection, wherein TCRα affinity is progressively reduced to meet the fixed TCRβ signal.

The mechanism underlying CD4/CD8 lineage commitment has been a subject of intense debate, largely revolving around the kinetic signaling and strength-of-

signal models [16]. Here, we propose that the efficiency and kinetic trajectory of achieving Equivalence of Self-Recognition (TCR$\alpha \approx$ TCRβ) deterministically dictate CD4/CD8 lineage commitment. Strong initial TCRβ signals (corresponding to MHC II-restricted recognition) facilitate a rapid balancing act, which commits the thymocyte to the CD4+ helper lineage, yielding T cells with inherently higher self-recognition capacity [17]. In contrast, weaker TCRβ signals (corresponding to MHC I-restricted recognition) necessitate a more prolonged and intricate balancing act. This process is kinetically defined by a sequential transcriptional program that frequently involves initial transient CD8 termination followed by a co-receptor reversal (CD4 → CD8) to achieve equivalence in the MHC I context [18], thereby generating highly specific CD8+ cytotoxic T cells.

Collectively, this work presents a unified, mechanistic framework that integrates the molecular genetics of TCR rearrangement, the structural basis of pMHC recognition, and the dynamics of thymic selection into a coherent model of differential signaling. This model not only resolves long-standing paradoxes in T-cell development but also profoundly redefines our understanding of self-tolerance as an active, quantified, and continuously calibrated process. By partitioning recognition based on the TCR α and β chains, the model formally establishes a structural and functional 3:1 self-to-antigen recognition confidence ratio (four MHC-dominant loops to two peptide-dominant loops) crucial for accurate immune discrimination [19].

2. Redefining T-Cell Receptor Engagement and Signaling

2.1. Critique of the Traditional Integrated Affinity Model

The established immunological framework, often referred to as the Affinity-Threshold Model, relies on the concept that T-cell fate—whether a cell is positively selected, negatively selected, or deleted by neglect—is determined by the overall integrated affinity (signal quantity) of the TCR$\alpha\beta$ heterodimer for the peptide-MHC (pMHC) complex [20]. This perspective maintains that successful maturation depends on the TCR-pMHC interaction falling within predefined affinity windows defined by generalized selection thresholds. However, this traditional integrated affinity model struggles to provide a conclusive mechanistic explanation for several stringent and biologically significant observations during thymocyte development.

A primary challenge to the integrated model is the massive deletion of developing thymocytes, where more than 90% fail the positive selection process [21], despite the fact that all TCR chains inherently carry components for self-recognition. If selection were merely a test of minimal interaction capacity with self-pMHC, this high rate of deletion suggests a fundamental inefficiency in receptor architecture or necessitates a more complex selection process than simple quantitative thresholds [22]. Furthermore, the reliance on a few unique affinity thresholds for both positive and negative selections is fundamentally unrealistic given the highly variable and overlapping affinity ranges observed in mature T cells circulating in

healthy systems [23]. If a single overall affinity threshold were to govern selection, it would necessarily lead to a T-cell repertoire severely restricted in diversity, contradicting the essential requirement for vast diversity in adaptive immunity.

The functional constraint imposed by repertoire diversity indicates that the selection process must establish an internal, personalized threshold for each unique thymocyte. Such an individualized reference point cannot be derived solely from the overall, externally measured affinity of the TCR$\alpha\beta$ heterodimer. This suggests that the T cell must possess an internal mechanism for calibrating its reactivity based on self-reference signals established during maturation, which we propose is the Equivalence of Self-Recognition.

2.2. The Central Hypothesis: Differential T-Cell Receptor Signaling, TCR = TCRβ/TCRα

The core novelty proposed herein is the Differential T-Cell Receptor (TCR) Signaling Mechanism, which fundamentally redefines how the TCR interprets peptide-MHC (pMHC) engagement. Instead of measuring total integrated affinity (signal quantity), the T-cell measures the relative signal strength between its two constituent chains: the TCRα axis and the TCRβ axis (**Figure 1(A)**).

Under this model, the functional TCR signal is not the summation of interactions, but is defined by the TCRβ signal strength with respect to the TCRα reference threshold:

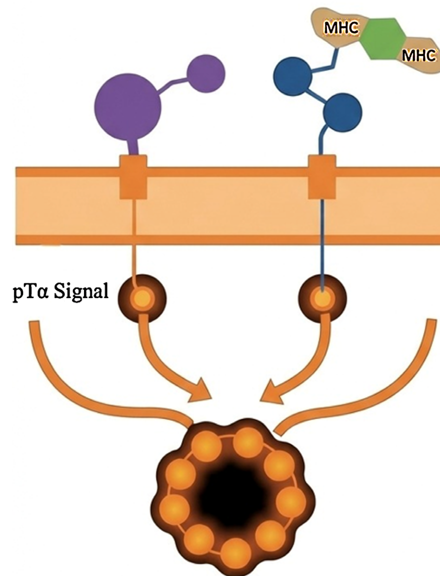
$$\text{TCR signal} = \frac{\text{TCR}\beta}{\text{TCR}\alpha}\text{; Or TCR} = \text{TCR}\alpha\beta = \frac{\text{TCR}\beta}{\text{TCR}\alpha} \approx \text{TCR}\beta|_{\text{TCR}\alpha} \approx \text{TCR}\beta - \text{TCR}\alpha$$

In this framework, the TCRα interaction provides a variable, self-tuned threshold that modulates the downstream signal generated by TCRβ [24]. T-cells only initiate a biological action when a functional difference is perceived between these two axes.

Signal transmission is critical to maintaining this differential. The CD3 subunits associated with the TCRα and TCRβ transmembrane (TM) domains are postulated to be continuously signaling-competent, generating unliganded basal signals due to intrinsic cellular and molecular dynamics [25]. These basal signals serve as the self-reference points. Consequently, a T-cell only transitions from a quiescent state when a differential signal—a functional difference, rather than absolute magnitude—is detected (**Figure 1(B)**).

The term "unliganded basal signals" describes low-level, constitutive signaling activity that persists in the absence of overt ligand engagement [26]. Such tonic signaling has been documented across multiple immune receptors and reflects the intrinsic biochemical dynamics of receptor complexes rather than a discrete activation event [27]. From a metabolic perspective, reliance on continuous signaling differences is feasible, as cells are known to integrate signaling inputs over time rather than respond solely to absolute engagement thresholds [28].

Importantly, the model proposed here does not assume that cell fate decisions are triggered by a single deterministic signal, but rather that relative differences



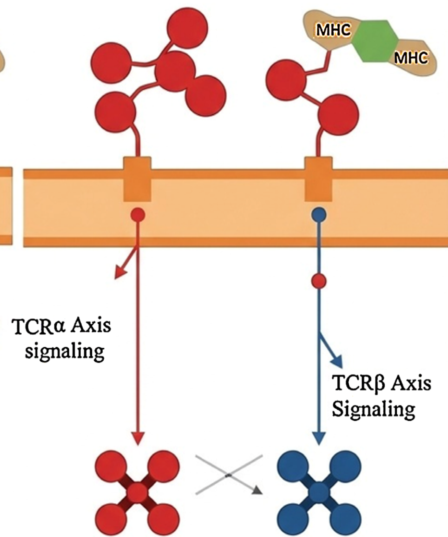


Figure Labels: This schematic differentiates between the signaling characteristics of the pre-TCR and mature TCR$\alpha\beta$ complexes. Figure A focuses on the pre-TCR, comprising the single-domain pTα associated with TCRβ. It emphasizes that pTα, through its signaling-competent transmembrane domain, generates an intrinsic signal even without MHC ligand binding, establishing a crucial initial TCRα threshold. Figure B transitions to the mature TCR$\alpha\beta$ complex, illustrating its interaction with MHC. Crucially, it depicts both the TCRα and TCRβ chains, each with two distinct extracellular domains, initiating separate, yet coordinated, signaling pathways via their respective transmembrane and intracellular domains, collectively termed TCR$\alpha\beta$ signaling.

Figure 1. Distinct signaling modalities in early T-cell receptor development.

in basal signaling intensity may bias developmental trajectories [29]. Similar principles have been described in tonic TCR signaling and basal cytokine receptor activity, where graded signals contribute to survival, tuning, and lineage bias [30]. In this context, unliganded basal signals are treated as a theoretical framework rather than an experimentally established deterministic mechanism.

The differential model intrinsically links the perception of tolerance (self-recognition) to a specific signal inequality, and activation (antigen recognition) to the reversal of that inequality. Tolerance is maintained when the TCRα self-reference signal is minimally greater than the TCRβ signal (TCRα > TCRβ on self-pMHC), establishing a non-activating equilibrium. Activation occurs only upon a productive antigen encounter, which reverses the inequality (TCRβ > TCRα). This mechanism transforms tolerance from a qualitative issue (bind/don't bind) into a quantitative comparison problem, allowing for the precise calibration of self-tolerance. The system permits mild autoreactivity in the circulation only due to the non-zero

margin ($\mathbf{\partial}$) established during the *Equivalence of Self-Recognition*, providing crucial protection against autoimmunity while maintaining necessary T-cell responsiveness (**Figure 1(B)**).

Finally, the T-cell requires not just the generation of a differential signal, but the persistence of that signal. Without persistence, random momentary dynamics or transient binding events could falsely trigger *T-cell activation*, irrespective of true pMHC engagement. This requirement for persistent differential signaling inherently connects this novel quantitative model to the well-established concepts of kinetic proofreading and the stability/duration of productive TCR-pMHC interactions [31].

3. Mechanistic Foundations

The following sections outline the proposed theoretical mechanisms of the Differential TCR Signaling Model. It is important to distinguish between established immunological principles (supported by the cited literature) and the novel hypothetical postulates introduced here. Where the model extends beyond current experimental consensus, this is explicitly noted, and the proposed mechanisms should be interpreted as testable hypotheses rather than confirmed biological realities [32].

We postulate that the invariant pTα chain establishes the initial ceiling for the self-recognition axis. This proposed role of pTα as a 'maximum self-reference' is consistent with observations of its ligand-dependent but non-specific signaling [33], but remains a theoretical construct to be validated.

3.1. Mechanistic Foundations of the Differential Signaling Model: Initial Threshold Setting by Pre-TCR (pTα)

The differential signaling paradigm is initiated during the pre-TCR stage (the $CD4^+CD8^+$ double-positive stage), where the pre-TCR complex (pTα/TCRβ) is expressed. The invariant pTα chain is structurally distinct from TCRα, and its extracellular domain is known to be non-polymorphic, suggesting it plays a role in generating a critical, constant signal [34].

Mechanistically, we propose that the pTα chain establishes the ceiling for the self-recognition axis. It is postulated to hold the equivalent of the maximum possible TCRα affinity (pTα = TCRα~max~) (**Figure 3** & **Figure 4**). By setting the α-axis threshold at its theoretical maximum, the system ensures that the nascent, rearranged TCRβ self-recognition signal is always evaluated below this fixed ceiling (pTα > TCRβ) (**Figure 2(A)**, **Figure 1**). This design prevents catastrophic deletion due to the initial, potentially high, TCRβ self-reactivity and ensures that the subsequent, highly variable TCRα rearrangement process begins from the highest achievable self-reference point. This provides the maximum calibration range necessary to fine-tune the subsequent TCRα against a fixed TCRβ [35].

pTα/TCRβ signal is known to be ligand-dependent (relying on TCRβ interaction with self-MHC molecules), but it is not ligand-specific [36]. This initial

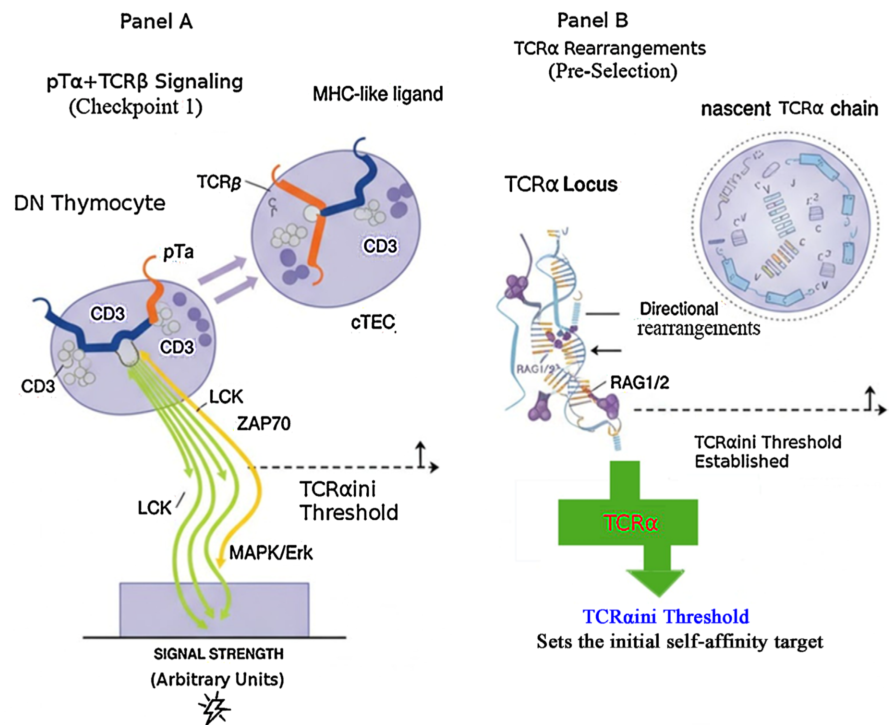


Figure Labels: Panel A illustrates Checkpoint 1, β Positive Selection (PSL0), where a double-negative (DN) thymocyte engages an MHC-like ligand on a cortical thymic epithelial cell (cTEC). Signaling through the pre-TCR (TCRβ with pTα) confirms minimal TCRβ self-recognition and initiates an internal computation of initial TCRα affinity, setting the TCRαini threshold. This signal activates downstream pathways (LCK, ZAP70, MAPK/Erk). Panel B depicts the subsequent TCRα rearrangements (Pre-Selection), where the TCRαini threshold is established, serving as the initial self-affinity target for nascent TCRα chains.

Figure 2. pTα + TCRβ signaling establishes the initial TCRα rearrangement threshold, TCRαini

TCRβ-MHC interaction, dominated by the germline-encoded CDR1β and CDR2β loops, intrinsically biases the thymocyte toward CD4 lineage development (MHC II preference) [37]. The non-zero signal generated by TCRβ during this phase must satisfy the β-selection criteria to progress to the next stage.

This essential ligand interaction is critical for setting the groundwork for the TCRα rearrangement phase. The pTα/TCRβ-MHC interaction is required to estimate an initial, personalized TCRα value (TCRα~ini~) for the subsequent rearrangement. If pre-TCR signaling were entirely ligand-independent, the extensive proliferation of double-positive thymocytes would lead to massive redundancy. Instead, the ligand-dependent nature of the pre-TCR signal, which reflects the inherent self-MHC recognition capacity of the TCRβ chain, provides the necessary personalized TCRα~ini~, thus optimizing the efficiency of subsequent thymic selection (Figure 2(B)).

Signal Transmission and Functional Segregation:

The physical architecture of the TCR-CD3 complex provides the necessary infrastructure to generate and compare differential signals. The signaling machinery

Figure Labels: α negative selection (Checkpoint 2): active reduction of TCRα self-affinity. This checkpoint occurs at the double-positive (DP) stage. Panel A illustrates that the thymocyte, now expressing both TCRα and TCRβ, encounters a self-pMHC ligand. The goal is to actively reduce the TCRα chain's self-affinity via iterative VJ recombination (Panel B), driven by a differential signal where the TCRα affinity must be lowered to approach the TCRβ signal, achieving TCR$\alpha \leq$TCRβ (equivalence) on self-ligands. Thymocytes that achieve this equivalence proceed, while those that fail due to persistently high TCRα self-affinity undergo apoptosis.

Figure 3. Initial TCR$\alpha\beta$ signaling (TCR = TCRβ/TCRβ = TCR$\beta_{TCR\alpha}$).

associated with the α and β chains is segregated, ensuring the independent assessment required for the differential mechanism [38]. The TCRβ axis signal is known to rely on the TCRβ transmembrane (TM) domain associating with the $CD3\gamma\varepsilon$ and ζζ heterodimers. Conversely, the TCRα axis is specifically postulated to be carried by the TCRα TM domain associating with $CD3\delta\epsilon$ and ζζ heterodimers [39].

Mechanistically, the structural architecture required for this differential signaling has now been confirmed. Brazin *et al.* [12] identified a specific, force-sensitive interaction between the TCRα connecting peptide (CP) and the CxxC motif of the CD3δ subunit, establishing a dedicated TCRα-CD3δ signaling axis. Furthermore, they demonstrated that the TCRα TM domain exists in a dynamic equilibrium between an "L-state" (inactive) and an "E-state" (extended/active). Mutations that alter this TCRα equilibrium (e.g., R251L) drastically shift the sensitivity of the T-cell, effectively lowering the self-reference threshold and causing hypersensitive

"early activation" even with weak ligands. This confirms our hypothesis that the TCRα chain physically encodes a variable threshold rather than simply acting as a passive partner.

Furthermore, the TM domains of TCRα and TCRβ are defined as signaling competent, capable of generating unliganded basal signals continuously [40]. This continuous, low-level signaling is necessary to maintain a constant self-reference point (TCRα), allowing T-cells to sense antigens even in the absence of stable ligand engagement (*i.e.*, by instantly reacting to the subtle disruption of the basal equilibrium).

A critical consequence of the differential model is the shift in the functional relevance of TCR affinity. The primary purpose of thymic training is the rigorous establishment of the necessary balance (TCR$\alpha \approx$ TCRβ)—the Equivalence of Self-Recognition. This implies that the overall affinity (K~D~) of the TCR-pMHC interaction, as measured externally (e.g., by surface plasmon resonance), is a secondary, *qualitative* parameter [41]. The decisive factor in T-cell fate is the quantitative comparison of the two internal signals, determined by the differential engagement of the TCRβ chain (harboring the antigenic CDR3β) against the established TCRα self-reference (**Figure 3(B)**). This represents a crucial divergence from the affinity-centric humoral immune response.

3.2. Molecular Partitioning of Peptide Recognition: Seg1 and Seg2

The validity of differential signaling is intrinsically linked to the ability of the TCRα and TCRβ chains to structurally and functionally partition the pMHC epitope into distinct, non-overlapping self-sensing and non-self-sensing components.

3.2.1. Structural and Genetic Basis of Segregation

Peptide recognition by antigen receptors is often described in terms of molecular partitioning, whereby conserved structural features contribute disproportionately to self-recognition, while variable elements preferentially mediate antigen discrimination. While this partitioning provides a useful conceptual framework, existing crystallographic and biophysical data indicate that such separation is not absolute [42]. Structural variations in peptide-receptor interfaces, including differences in docking geometry, contact residues, and conformational flexibility, demonstrate that self and non-self recognition can partially overlap at the molecular level [43].

Accordingly, the model presented here does not assume strict molecular partitioning in an invariant structural sense, but rather proposes a bias in recognition roles that emerges statistically across receptor repertoires [44]. In this view, molecular partitioning reflects a probabilistic tendency shaped by structural constraints and selection pressures, rather than a rigid deterministic rule.

Structural partitioning of the pMHC ligand is mandatory for the differential mechanism to succeed. We propose that the TCRα chain preferentially interacts with the N-terminal half of the peptide (Seg1), while the TCRβ chain targets the

C-terminal half (Seg2) [45]. Functionally, this segregation means that the TCRα axis strictly recognizes self-sequences, and critically, only Seg2 can harbor sufficient non-self elements to be antigenic (**Figure 4**).

This functional specialization is profoundly underpinned by the genetics of TCR recombination [46]. The TCRα chain is generated via V(J) recombination, analogous to immunoglobulin light chains (IgL), resulting in CDR3α loops with restricted junctional diversity. This restricted diversity predisposes the TCRα axis to recognize the relatively constant, self-like profile of Seg1 and the MHC helices, thereby establishing the self-reference threshold. In stark contrast, the TCRβ chain uses V(D)J recombination, incorporating the unique Diversity (D) gene segments (analogous to immunoglobulin heavy chains). The presence of D genes exclusively in TCRβ directly enables the high junctional diversity (up to 10^{15} combinations) necessary for non-self or antigen recognition (Supplementary **Figure 5**) [47].

Consequently, the complementarity-determining regions (CDRs) are specialized. The highly variable CDR3β, driven by V(D)J recombination, is dedicated to antigen recognition. Conversely, the less diverse CDR3α primarily reinforces self-sequence recognition.

The structural implications of this segregation impose a critical constraint on antigenicity. For a peptide to successfully activate a T-cell, it must possess two characteristics: first, it must maintain a Seg1 sequence that is sufficiently self-like to ensure stable TCRα docking and generation of the self-reference signal (TCRα/Seg1 > 0); second, it must possess non-self (diverse) sequences in Seg2 to drive activation (TCRβ > TCRα). If the Seg1 sequence were strongly antigenic, the TCRα interaction would be disrupted, either preventing stable docking or failing to generate the necessary self-reference, which would ultimately result in T-cell deletion or anergy (**Figure 4**). This establishes a powerful structural barrier against widespread autoimmunity.

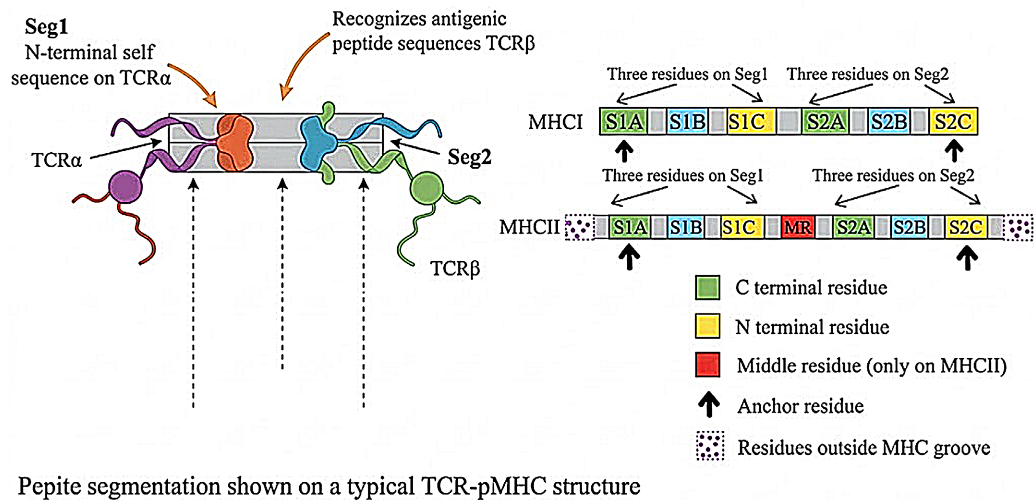
3.2.2. Variability and Diversity: Defining Antigenicity

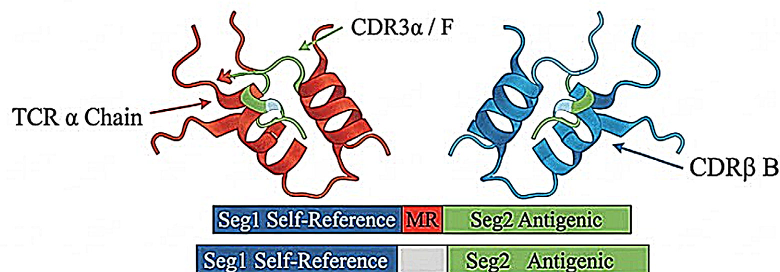
The capacity of the immune system to distinguish self from non-self sequences relies on detecting specific features within the pMHC structure, defined here as variability and diversity. Self-sequences are characterized by smooth variability, reflecting features common to the host proteome. In contrast, pathogen or non-self sequences introduce discontinuity.

Diversity is considered a specialized state of variability, characterized by an abrupt rise or fall in the signal or a state of extremely high or low variability compared to the neighboring segment. The genetic components of the TCR repertoire directly reflect this specialization:

1) V and J genes are primarily germline-encoded and evolved to capture and recognize the smooth, self-like features of host pMHC.

2) D genes, exclusive to the TCRβ chain, are described as featureless relative to self-sequences, allowing the TCRβ axis to exclusively detect the abrupt discontinuity (diversity) characteristic of non-self sequences in Seg2.





This panel illustrates the proposed functional segregation of peptide recognition by the TCR chains, a cornerstone of the differential TCR signaling model. The peptide presented by MHC is conceptually divided into two segments: Seg1 (N-terminal half), primarily recognized by the TCRα chain. In the self-pMHC context, Seg1 is enriched in self-like sequences. TCRα, generated via VJ recombination, engages Seg1 through its complementarity-determining regions (CDRs), with a highlighted role for CDR3α. This interaction establishes a dynamic self-reference threshold (TCRα signal). Seg2 (C-terminal half): Primarily recognized by the TCRβ chain. Seg2 is proposed to harbor antigenic, non-self elements. TCRβ, generated via V(D)J recombination and possessing greater junctional diversity due to D-gene segments, engages Seg2 through its CDRs, with a specialized role for the highly diverse CDR3β. This interaction provides the antigenic signal (TCRβ signal). Structural Depiction: The schematic shows a peptide bound within the MHC groove, demarcated into Seg1 and Seg2. TCRα (blue) is shown engaging Seg1 via its CDR loops (with three representative contact residues: S1A, S1B, S2A). TCRβ (green) is shown engaging Seg2 via its CDR loops (with three representative contact residues: S2A, S2B, S2C). An important distinction is indicated: For MHC II, a middle residue (MR) is depicted between Seg1 and Seg2, a feature noted as absent in MHC I. This highlights potential structural differences in peptide presentation between MHC classes that may influence TCR engagement and coreceptor function. Functional Implication: This molecular partitioning enables the core signaling computation of the model: TCRsignal = TCRβ / TCRα. Activation occurs only when the antigenic signal from TCRβ (via Seg2) quantitatively exceeds the self-reference signal from TCRα (via Seg1). This segregation ensures that T-cell reactivity is fundamentally a differential measurement, refining self/non-self discrimination.

Figure 4. Molecular partitioning of peptide recognition into Seg1 and Seg2.

This differential sensing explains the quantitative requirement for the TCRα chain diversity. Although TCRα only recognizes self, it requires a vast number of combinations (>10^4 in mice) [48]. This is necessary because self-variability encompasses the immense and constantly changing repertoire of proteins expressed in an individual. The TCRα repertoire must be diverse enough to accurately sample this wide range, establishing a robust and accurate self-reference point for

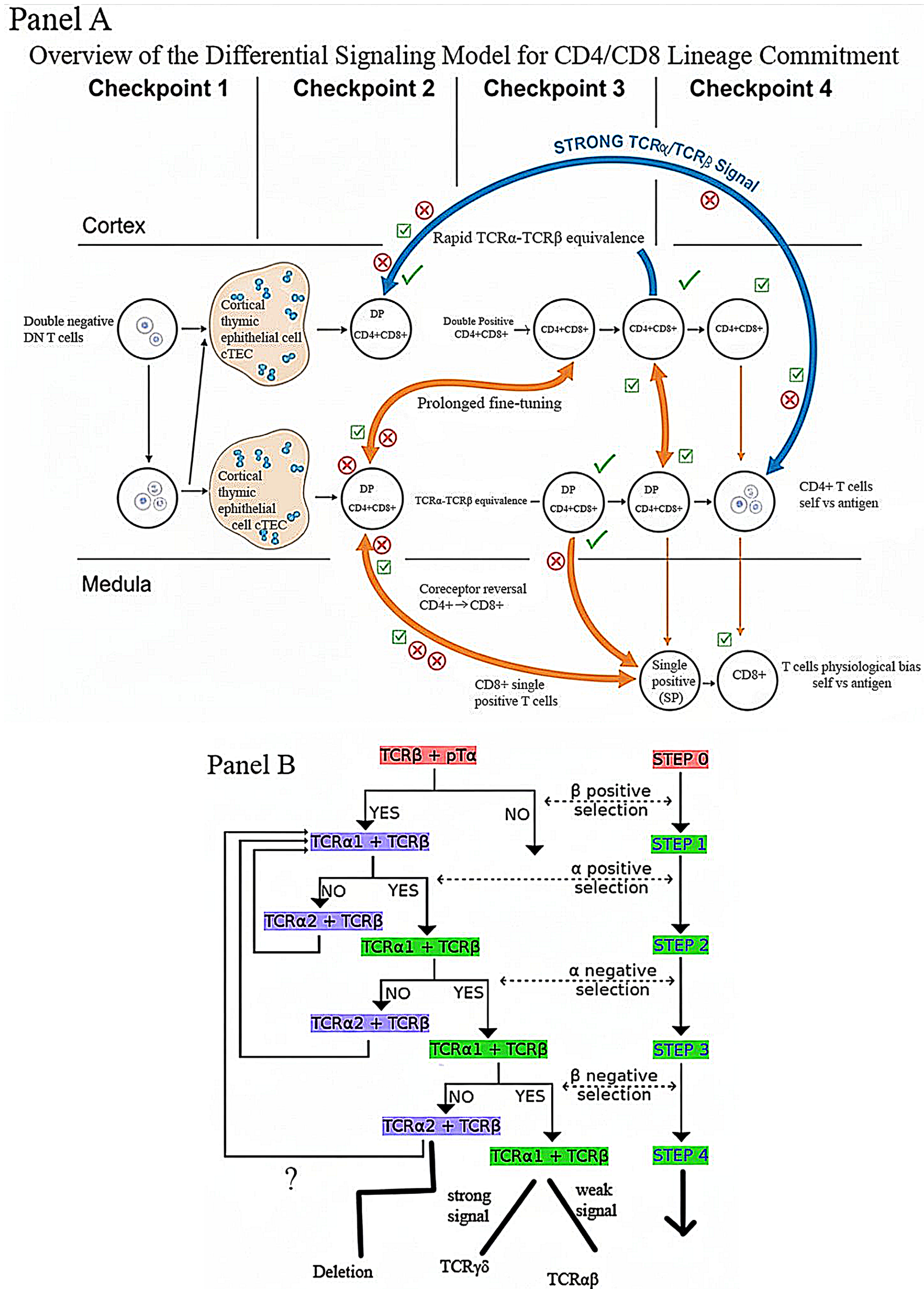


Figure Labels: Thymic selection. Overview of the differential TCR signaling model for thymic selection and CD4/CD8 lineage commitment. This schematic illustrates the four checkpoints of thymic T-cell development and the deterministic nature of CD4/CD8 lineage choice within the proposed Differential TCR Signaling Model. Initial TCRβ signal strength (weak vs. strong) on self-pMHC dictates the developmental pathway. Stronger signals (blue pathway) lead to more rapid TCRα/TCRβ equivalence, promoting CD4 lineage commitment. Weaker signals (orange pathway) necessitate a prolonged fine-tuning process, often involving coreceptor reversal ($CD4^+$ to $CD8^+$), to achieve equivalence and commit to the CD8 lineage. Checkpoints 1 - 4 delineate the stages of TCRα/TCRβ balancing for stringent central tolerance. This process ultimately generates T-cells with a physiological bias towards identifying self versus antigen.

Figure 5. Four checkpoints in thymic selection.

Seg1 against which TCRβ measures the antigenicity of Seg2.

The concept of individualized variability profiles also provides a mechanistic explanation for phenomena like alloreactivity (graft rejection). Since the smoothness of variability is maintained precisely at the individual level (encoded by personal MHC alleles), the introduction of foreign MHC molecules (even from a close family member) presents a fundamental discontinuity or imbalance in the established self-variability profile [49]. The host's T-cells recognize the non-host pMHC as a disruption of their internal self-reference, failing to tolerize against donor pMHCs, which results in rejection.

3.3. The Four Checkpoint Model of Thymic Selection

The differential signaling framework necessitates a complete re-evaluation of thymic selection, expanding the traditional two selection stages (positive and negative) into four distinct, sequentially executed checkpoints that operate along the separable α and β axes to achieve Self-Recognition Equivalence (TCR$\alpha \approx$ TCRβ) (**Table 1**, **Figure 5**).

Table 1. The four checkpoints of thymic selection.

Checkpoint	Selection Axis	Checkpoint name in accordance with differential signaling	Checkpoint name with traditional existing models	Mechanistic Goal
1	TCRα (with pTα)	β Positive Selection (PSL0)	Pre-TCR checkpoint	Confirm minimal TCRβ self-recognition and estimate TCRαini
2	TCRα	α Negative Selection	Positive Selection	Establish Equivalence (TCR$\alpha \approx$ TCRβ) by actively reducing TCRα self-affinity via directional rearrangements
3	TCRβ (with TCRα)	β Negative Selection	Negative Selection	Delete autoreactive cells where the TCRβ signal overtly exceeds the TCRα threshold (TCR$\beta \gg$ TCRα)
4	TCR$\alpha\beta$ (CD8 SP)	α Positive Selection (PSLI)	Post-reversal checkpoint	Confirm minimal self-recognition on MHC I after coreceptor reversal (CD4 → CD8)

This table presents a comprehensive overview of the four redefined checkpoints within the differential TCR signaling model for thymic T-cell development. It systematically breaks down each checkpoint by its specific selection axis (e.g., TCRβ with pTα, TCRα), a unique checkpoint name, and its corresponding traditional nomenclature. The table's primary focus is on the "Mechanistic Goal" of each stage, providing clarity on how developing thymocytes achieve stringent central tolerance and make definitive CD4/CD8 lineage choices through successive TCRα/TCRβ balancing on self-ligands.

3.3.1. Checkpoint 1. *Β*-Positive Selection (PSL~0~) and TCR*α*~ini~ Estimation

β positive selection is the initial stage, corresponding to pre-TCR signaling, where the pTα/TCRβ complex interacts with self-MHC ligands (PSL~0~) [50]. This step serves the primary purpose of ensuring that the newly rearranged TCRβ chain possesses the requisite minimal self-recognition capacity necessary for development. Given that TCRα lacks a variable domain, the interaction during this stage is highly likely mediated only by the germline-encoded CDR1β and CDR2β with

the MHC molecule, with minimal or no direct interaction by CDR3β with Seg2 [51].

A notable finding related to this stage is that the diversity of PSL~0~ ligands is not essential for double-positive (DP) proliferation, unlike the subsequent selection stage. This difference confirms that β positive selection focuses narrowly on assessing the inherent, MHC-restricted self-recognition capacity of the TCRβ chain itself—a structural assessment—rather than its affinity across a wide array of self-peptides. This checkpoint is structural and quantitative, setting the initial signal.

This checkpoint concludes by estimating the initial TCRα value (TCRα~ini~) necessary for the next stage. The value of TCRα~ini~ is determined by the strength of the TCRβ interaction, predominantly CDR1/2β-MHC. This estimation is vital to avoid redundant rearrangement cycles, ensuring the subsequent, costly, and time-consuming TCRα gene rearrangement begins from a tailored starting point based on the fixed TCRβ chain's inherent self-affinity (**Table 1** & **Figure 5**).

3.3.2. Checkpoint 2. α-Negative Selection: TCRα Rearrangement as Affinity Reduction

VJ recombination of the TCRα chain is inherently stochastic and generates a broad spectrum of receptor variants with differing affinities [52]. In isolation, such random variation would be expected to produce nondirectional fluctuations in signaling strength [53]. However, directionality emerges when these stochastic outcomes are evaluated against pre-established signaling constraints imposed by earlier developmental checkpoints [54].

In the framework proposed here, the term "α-negative selection" does not imply a guided modification of receptor structure, but rather a selective filtering process. TCRα variants that increase signaling above a tolerated range are preferentially eliminated, while variants that reduce signaling toward a reference window are more likely to persist [55]. As a result, although recombination itself is random, the population-level outcome reflects a systematic bias toward reduced affinity. This directional effect arises from selection pressures acting on random variation, rather than from intrinsic directionality within the recombination process itself [56].

The second checkpoint is defined as α-negative selection, executed through the process conventionally known as TCRα rearrangement. This is an exhaustive search for a suitable TCRα chain that can pair with the fixed TCRβ. The selection mechanism during this phase is dictated by a systematic reduction of the TCRα self-reactivity.

It is hypothesized that TCRα rearrangement proceeds with decreasing affinity (starting from pT$\alpha \approx$ TCRα~max~). This directional search dramatically increases the efficiency of the tuning process compared to a random search, allowing the process to terminate within the restricted DP lifespan (approximately two weeks). The objective of α-negative selection is to limit TCRα reactivity such that Equivalence (TCR$\alpha \approx$ TCRβ) is achieved. The massive deletion of DP thymocytes (the >90%

failure rate) is thus attributed not to a lack of minimal self-recognition capacity, but to the failure to achieve this critical TCRα/TCRβ balance within the restricted developmental timeframe. This process is fundamentally a test of functional balance, not merely structural stability, as confirmed by the mandatory requirement for specific ligands (PSL~II~) during rearrangement (**Table 1** & **Figure 5**) [57].

3.3.3. Checkpoint 3. β-Negative Selection: Definition and Requirements of Equivalence; The Balance of Self-Recognition

Equivalence represents the balanced state of self-recognition that must be achieved before a T-cell is released into the periphery. It is the condition under which the differential signal generated by the TCR$\alpha\beta$ heterodimer approaches zero on self-ligands (**Figure 4**).

The precise condition for equivalence of self-recognition is defined as TCR$\alpha \approx$ TCRβ on PSLs, where the T-cell signal, defined differentially, approaches zero. This must satisfy the condition $\delta > TCR\alpha - TCR\beta \approx 0$, where δ represents a non-zero margin of acceptable difference. For this equivalence to hold on self-peptides, the CDR3β-Seg2 interactions must be rigorously minimized, approaching zero [5]. If CDR3β demonstrated strong affinity for Seg2 on self-peptides, the T-cell would auto-activate (TCRβ > TCRα) and be eliminated during this subsequent β-negative selection checkpoint (**Table 1** & **Figure 5**).

A complex mechanism involving the potency of PSLs is required to guide TCRα rearrangement toward this stable zero-signal state. PSL potency is defined as the inverse of Seg2 diversity. As the rearrangement proceeds and TCRα affinity systematically decreases, the TCRβ signal must also be kept low to maintain the inequality TCRα > TCRβ. This requires that the thymus present increasingly potent PSLs (*i.e.*, PSLs with minimal Seg2 diversity) in the later stages of rearrangement. This necessary increase in PSL potency dictates a strict spatial and sequential distribution of PSL types (PSL~II~) within the thymic cortex [16].

3.3.4. Checkpoint 4. α-Positive Selection: Fine-Tuning Equivalence: Biasing and Coreceptor Reversal

Due to the genetic constraints imposed by the limited number of Vα and Jα genes, perfect Equivalence (TCRα = TCRβ) cannot always be reached solely through gene rearrangement. Two ancillary mechanisms are utilized to fine-tune the differential signal to within the narrow margin (δ): Biasing (Post-Translational Modification) and Coreceptor Reversal (**Figure 4**) [18].

Biasing, potentially achieved via post-translational modifications (PTMs) such as TCR glycosylation patterns, can restrict conformational flexibility, thereby adjusting the affinity up or down slightly. This is used to "nudge" TCRα or TCRβ values toward equivalence when the differential signal is low but non-zero (**Figure 6**, **Figure 3**, & **Figure 4**).

When the differential signal is very low, but equivalence cannot be met even with Biasing, and further TCRα rearrangement is deemed impossible or inefficient, Coreceptor Reversal (CD4 → CD8) is initiated. Coreceptor reversal is a dra-

matic attempt to solve the Equivalence problem by changing the pMHC context. The T-cell switches its primary restriction from MHC II (encountered during TCRα rearrangement) to MHC I [58].

This rescue mechanism exploits the structural difference between MHC I and MHC II polymorphism patterns. The shift tends to lower TCRβ affinity (as its CDR1/2β loops move from the highly polymorphic MHC IIβ helix to the less polymorphic MHC I regions) and simultaneously increases TCRα affinity (as its CDR1/2α loops move to the highly polymorphic MHC α1 helix). This combined effect forces the differential ratio back toward the necessary balance (TCR$\alpha \approx$ TCRβ), allowing equivalence to be achieved in the CD8 lineage when it failed in the CD4 lineage (**Table 1**, **Figure 5**, & **Figure 4**).

Because the MHC context changes completely upon reversal, CD8 Single Positive (SP) thymocytes must undergo a final test of self-recognition capacity on MHC I ligands, termed α Positive Selection (PSL~I~). This is necessary to confirm minimal fitness in the new restriction environment. CD4 SP cells do not require a separate final positive selection test because they were continuously assessed for self-recognition against MHC II throughout the extended α-negative selection phase (TCRα rearrangement).

3.4. Differential Signaling Dictates CD4/CD8 Lineage Commitment

3.4.1. Differential Signaling Dictates CD4/CD8 Lineage Commitment

The choice between CD4 and CD8 lineages is not a stochastic process but a deterministic outcome based on the efficiency and strength of the self-recognition balancing act, specifically quantified by the initial TCRβ signal strength and the difficulty encountered during the TCRα affinity tuning process [59].

During early thymocyte development, signaling initiated by the TCRβ chain precedes the formation of a complete $\alpha\beta$ TCR and therefore cannot establish a definitive affinity threshold on its own [60]. Instead, early TCRβ-dependent signaling is proposed to impose a preliminary constraint on the permissible signaling range within which subsequent TCRα-mediated interactions are evaluated [61].

In this framework, TCRβ signal strength does not act as a deterministic predictor of CD4 or CD8 lineage commitment, but rather biases the developmental landscape by shaping the sensitivity of downstream signaling pathways [62]. Once the TCRα chain is expressed and a complete receptor is formed, $\alpha\beta$ TCR signaling is interpreted relative to this preconditioned signaling environment, allowing lineage commitment to emerge through integration of sequential signals rather than through a single predictive event [63].

3.4.2. Signal Strength Determines Lineage Pathway

The strength of the initial TCRβ signal, derived predominantly from CDR1/2β-MHC II interactions, defines the commitment path. All thymocytes start with a default bias toward CD4 due to the initial interaction with MHC II [29].

CD4 Lineage Commitment: A strong initial TCRβ signal (implying high inherent self-affinity of the β chain) simplifies the Equivalence problem. A strong signal

requires TCRα to only execute a small, rapid affinity reduction to achieve balance against the TCRα~max~ starting point. This results in fewer TCRα rearrangement iterations, a shorter developmental time period (~30 hours), and high, sustained signals that definitively commit the cell to the CD4 lineage. CD4 T-cells, therefore, arise from precursors possessing inherently strong self-recognition capacity [17].

CD8 Lineage Commitment: Conversely, a weak TCRβ signal (mild CDR1/2β-MHC interaction) makes equivalence difficult to reach. Since weak signals require extremely fine adjustments over numerous, prolonged TCRα iterations, the process extends the developmental timeline significantly (~4 days), increasing the likelihood that the thymocyte will fail to achieve equivalence on the MHC II platform and undergo coreceptor reversal (CD4 → CD8). CD8 T-cells are, by necessity of this mechanism, the product of a more prolonged and precisely tuned self-recognition balancing act than CD4 T-cells [64].

3.4.3. Functional Consequences and Confidence Bias

The differential tuning mechanism establishes critical functional biases. The analysis predicts that CD4 T-cells possess a far stronger basal self-recognition capacity than CD8 T-cells, which explains why regulatory T-cells (T~regs~), which require robust confidence in self-tolerance, predominantly derive from the CD4 lineage [25]. In contrast, CD8 T-cells require exceptionally high specificity and sensitivity to prevent bystander damage; their protracted development ensures they are more precisely tuned in their self-recognition equivalence.

This mechanism dictates the quantitative bias of immune discrimination. The four-checkpoint process entails three distinct stages dedicated to establishing and confirming self-recognition (CDR1/2β selection, TCRα affinity reduction, and CDR3α self-minimization) compared to only one primary component dedicated to antigen detection (CDR3β). This establishes a 3:1 self-recognition confidence bias (Self: Antigen). However, because TCRβ's ability to detect antigen is measured against the rigorously tuned TCRα self-reference, the antigen recognition status is determined with triple accuracy, ensuring high confidence when the T-cell decides that non-self is present (TCRβ > TCRα).

The proposed 3:1 self-to-antigen recognition confidence ratio arises from the cumulative contributions of three dominant self-referential components: i) tonic signaling via pre-TCR/pTα-MHC interactions [65], ii) sustained germline-encoded CDR1/2α/β-MHC contacts that establish a stable docking platform [66], and iii) co-receptor-mediated signal amplification (CD4-MHC II or CD8-MHC I) [11]. These collectively create a robust 'self-background' against which a single dominant antigen-specific signal—delivered primarily through the highly diverse CDR3β-peptide interaction [4]—is compared. This structural and functional asymmetry ensures that antigen detection is validated against a multi-layered self-reference, increasing the fidelity of immune discrimination.

4. Discussion and Conclusion

The molecular and cellular analyses presented herein necessitate a fundamental

restructuring of the model governing T-cell development and selection. The traditional concept of integrated affinity is supplanted by the Differential TCR Signaling Paradigm, wherein T-cell fate is dictated by a precise, quantitative comparison between the intrinsic signaling capacities of its two receptor chains, actively setting an individualized self-reference threshold (Figure 6).

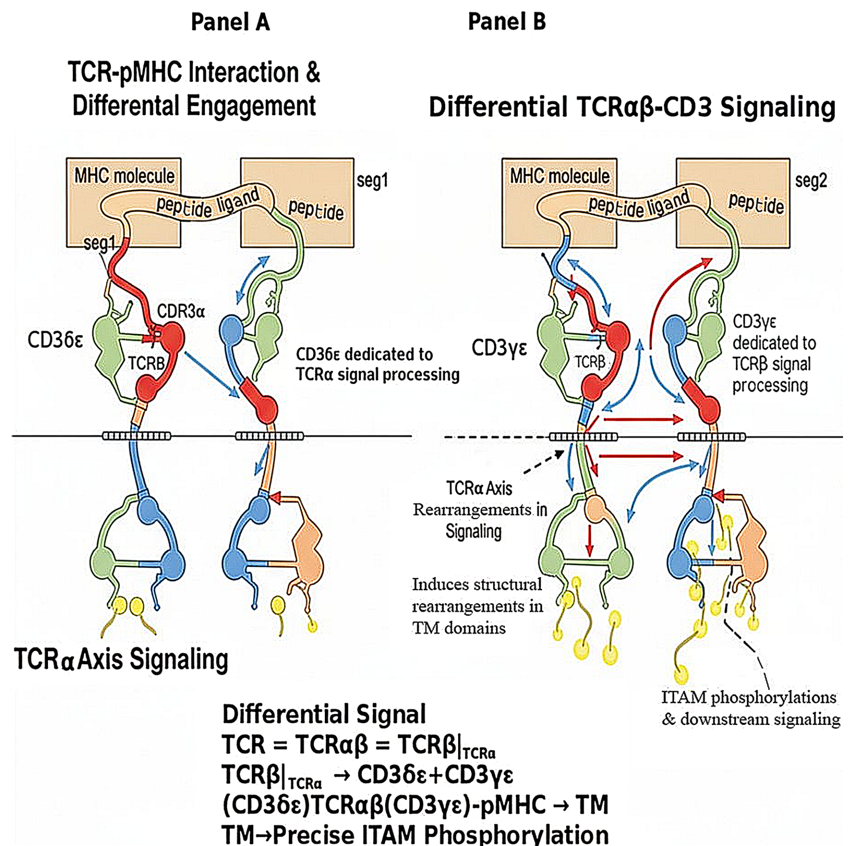


Figure Labels: Deconstructing TCR-CD3 signaling: from peptide contacts to ITAM phosphorylation. This figure elucidates an advanced model of T-cell receptor (TCR)-CD3 complex function, highlighting the precise interplay between its components from peptide engagement to signal transduction. Panel A meticulously illustrates the TCR-pMHC interaction, depicting TCRα and TCRβ (both with two extracellular domains) making specific contacts with the peptide via CDR3α ("seg1") and CDR3β ("seg2"), respectively. Crucially, it visualizes the consistent association of TCRα with CD3$\delta\varepsilon$ and TCRβ with CD3$\gamma\varepsilon$, proposing a functional dedication where CD3$\delta\varepsilon$ exclusively processes TCRα signals, and CD3$\gamma\varepsilon$ handles TCRβ signals, all at the extracellular level. Panel B transitions into the signaling phase, where the "differential signal" (TCRβ|TCRα) is transmitted through these dedicated CD3 pathways. This figure postulates that such unit-specific signal processing initiates sequential structural rearrangements within the transmembrane and intracellular domains of the entire TCR-CD3 complex. This refined understanding is crucial for precisely explaining ITAM phosphorylation kinetics and the subsequent propagation of downstream TCR signaling, offering a more granular view of T-cell activation.

Figure 6. Functional segregation of CD3 subunits is supported by Brazin *et al.* (2018), who mapped the specific interaction between TCRα-CP and CD3δ signaling.

This mechanism is critically enabled by the mandatory functional segregation

of the pMHC epitope: TCRα uses VJ rearrangement to recognize the Seg1 (N-terminal, self-like) domain to establish the variable threshold, while TCRβ uses V(D)J rearrangement to recognize the Seg2 (C-terminal, antigenic) domain to determine activation status.

Tolerance is actively maintained by achieving the state of Equivalence of Self-Recognition (TCR$\alpha \approx$ TCRβ), where the differential signal approaches zero on self-ligands. This equivalence is secured through four distinct selection checkpoints, modifying the traditional two [5]. This includes the definition of TCRα rearrangement as an α-negative selection process characterized by a directional decrease in TCRα affinity and the rigorous sequential tuning of PSL potency [32].

The process of achieving equivalence is also the mechanism for CD4/CD8 lineage commitment. T-cells requiring rapid, strong self-recognition commit to the CD4 lineage, while those requiring prolonged, fine-tuning commit to the highly specific CD8 lineage, often requiring coreceptor reversal to solve the equivalence equation within the MHC I context. Ultimately, this framework demonstrates that the adaptive immune system is primarily dedicated to the precise, quantitative confirmation of self-tolerance, establishing a 3:1 self-to-antigen recognition confidence ratio as the definitive metric of immune discrimination.

The 'Dissociative Model' of activation proposed by Brazin *et al.* [12] aligns perfectly with the Differential Signaling Paradigm. They observed that force-induced straightening of the TCRα-TM domain dictates the release of CD3 subunits to initiate signaling. In our framework, this is the physical manifestation of the comparison TCRβ > TCRα. The TCRα chain's resistance to straightening (controlled by its affinity for self-Seg1) represents the 'Self-Reference' threshold. Only when the antigenic force on TCRβ (Seg2) exceeds this stabilizing TCRα threshold does the conformational switch occur, releasing the CD3 modules for activation. This physically validates the concept that T-cell activation is a differential computation of forces between the two chains.

The Differential TCR Signaling Model presented here reframes thymic selection as an active, quantitative balancing process between TCRα-mediated self-reference and TCRβ-mediated antigen sensing. By introducing the concept of Equivalence of Self-Recognition (TCR$\alpha \approx$ TCRβ) and expanding selection into four distinct checkpoints, this model addresses long-standing paradoxes in thymocyte attrition, repertoire diversity, and lineage commitment [67]. It provides a mechanistic link between the structural segregation of peptide recognition, the genetic design of TCR recombination, and the functional outcome of central tolerance.

While the model is consistent with a growing body of structural and signaling data—most notably the mechanosensory role of the TCRα transmembrane domain [12]—it ultimately represents a theoretical framework that makes specific, testable predictions. Future experiments examining the independent signaling capacities of TCRα and TCRβ [45], the peptide-segment preferences of each chain [48], and the dynamics of affinity adjustment during TCRα rearrangement will be crucial for validation.

If supported, this paradigm would not only refine our understanding of T-cell development but also offer new perspectives on autoimmune disease, alloreactivity [41], and the design of TCR-based immunotherapies.

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Conflicts of Interest

The author declares no conflicts of interest.

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