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Dual TCR T Cells: Identity Crisis or Multitaskers?

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Abstract

Dual TCR T cells are a common and natural product of TCR gene rearrangement and thymocyte development. As much as one third of the T cell population may have the capability to express two different TCR specificities on the cell surface. This discovery provoked a reconsideration of the classic model of thymic selection. Many potential roles for dual TCR T cells have since been hypothesized, including posing an autoimmune hazard, dominating alloreactive T cell responses, inducing allergy, and expanding the TCR repertoire to improve protective immunity. Yet, since the initial wave of publications following the discovery of dual TCR T cells, research in the area has slowed. In this study, we aim to provide a brief but comprehensive history of dual TCR T cell research, re-evaluate past observations in the context of current knowledge of the immune system, and identify key issues for future study.

The identity of a T lymphocyte is largely determined by its TCR. The TCR dictates the specificity and influences T cell fate decisions. This central importance to T cell identity led early immunologists to assume T cells express a single TCR specificity to avoid identity confusion, an extension of Burnet's clonal selection theory of Ab production (1). This assumption shaped our understanding of T cell immunology until 1988 when T cells expressing two in-frame–rearranged TCRβ alleles were cloned from mice (2, 3). Shortly thereafter, T cell clones expressing two different TCR Vα segments were discovered (4, 5). Further investigation indicated that as many as one third of murine T cells express two in-frame–rearranged TCRα transcripts, suggesting that TCRα transcriptional allelic exclusion was virtually absent (6, 7). In 1993, human T cells expressing two different TCR Vα segments on the cell surface were identified, proving the existence of dual TCR–specificity T cells (8). Subsequent studies have estimated that ~10% of αβ T cells express dual surface TCR α-chains (9-11), whereas ~1% express dual surface TCR β-chains (12-16). Despite the abundance of dual TCR T cells and their multiple postulated effects on immunity, modern immunology textbooks provide little consideration of them. In this study, we explore our current understanding of dual TCR T cell biology and examine the consequences of dual TCR expression on immunity.

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Allelic exclusion

TCRβ-chain.

Allelic exclusion is the process by which one allele of a gene is expressed whereas the other is silenced. The discrepancy in prevalence between dual TCRα and dual TCRβ cells can be explained by differences in mechanisms of allelic exclusion. TCR β-chain allelic exclusion is stringent and multifaceted. TCR β-chain rearrangement initiates on one allele during the $CD4^-$ $CD8^-$ double negative stage in an ordered fashion (Dβ-Jβ, then Vβ-DJβ). Several mechanisms combine to prevent simultaneous rearrangement of both alleles, including nuclear localization, chromatin conformation, and accessibility of RAG 1 and 2 (15). Successful rearrangement of an in-frame TCR β-chain results in signaling that halts further rearrangement by inducing RAG protein degradation and initiating the formation of dense chromatin at the TCRβ allele (7, 15, 17, 18). Collectively, these mechanisms severely limit the number of thymocytes expressing two functionally rearranged TCR β-chains.

TCR α-chain.

TCR α-chain rearrangement occurs during the $CD4^+$ $CD8^+$ double positive (DP) stage. In contrast to the sequential rearrangement of the TCRβ locus, TCRα (Vα-Jα) rearrangement occurs on both alleles simultaneously (19, 20). Additionally, the organization of the TCRα locus allows multiple, processive rearrangements of the same allele: proximal Vα and Jα regions pair and distal Vα regions pair to distal Jα regions (21). Therefore, TCRα rearrangement does not have to stop at the formation of an in-frame TCR α-chain, but rather continues until the thymocyte has rearranged a selectable TCR or dies from neglect (20, 22). This lack of transcriptional allelic exclusion is predicted to result in one third of T cells expressing two TCR α-chains, because two thirds of randomly generated reading frames would be abortive (6). Indeed, transcriptional analyses estimate that ~30% of T cells express two functionally rearranged TCR α-chain mRNAs (6, 9, 23-28). The discordance between the fraction of cells expressing two TCR α-chain mRNAs (~30%) and the fraction that express two TCR α-chain proteins on the cell surface (~10%) indicates that posttranslational allelic exclusion mechanisms prevent surface expression of both TCR α-chains on some cells. Two models have been put forth to explain phenotypic allelic exclusion: the competition model and the selective retention model (9, 24, 29-33) (Fig. 1).

The competition model posits that the TCR α-chains compete for pairing with TCR β- or CD3 chains, with the more favorable pairing dominating the cell surface (30, 32, 34) (Fig. 1A). A transgenic T cell hybridoma line expressing a single TCR β-chain and two different TCR α-chains demonstrated preferential TCRβ pairing and surface expression of one TCR α-chain over the other (34). Similar competition between transgenic and endogenous TCR subchains was reported in TCR transgenic mouse models (32). There is also evidence that interaction between the Vα and Vβ CDR3s can influence pairing; specifically, the HY-specific TCR C6 β-chain pairs efficiently with the C6 α-chain but poorly to other TCR α-chains because of steric incompatibility at CDR3 (35). However, multiple interactions between TCRα, TCRβ, and the CD3 subunits also occur in the conserved TCRα C region (36-38); thus, whereas cases of CDR3 steric incompatibility may exist, most TCR α- and β-chains may pair adequately. Furthermore, artificially increasing

TCRβ or CD3ζ expression in an attempt to overcome the effect of competitive binding did not eliminate TCRα phenotypic allelic exclusion, indicating that alternative mechanisms of TCRα surface-expression exclusion exist (9).

The selective retention model posits that in thymocytes with two rearranged TCR α-chains one TCR α-chain transduces signals that promote its persistence on the cell surface, whereas the other TCR α-chain does not. This model is based on observations that TCR signaling protects the TCR from surface downmodulation during auditioning for positive selection (9, 30, 33, 39) (Fig. 1B). However, the mechanism by which this occurs is not entirely understood. Based on available evidence, we propose a model whereby weak TCR–pMHC engagement initiates conformational changes to the TCR complex that prevent internalization.

TCRs on DP thymocytes are constitutively internalized and degraded. ZAP70 and Src-like adaptor protein (SLAP) serve as adaptor proteins that bring the ubiquitin ligase c-Cbl in proximity to CD3ζ, targeting the TCR complex to the lysosome for degradation (9, 40-44). This process ensures the low level of surface TCR expression characteristic of auditioning thymocytes. During auditioning for positive selection, TCR signaling protects the TCR from internalization, but only for that receptor, not a second receptor that is not positively selected. Thus, in the selective retention model, phenotypic allelic exclusion would be established via preferential internalization of the excluded TCR. Indeed, a dual transgenic TCR mouse model demonstrated that proteasomal inhibition did not improve surface expression of the excluded TCR in DP thymocytes; instead, the excluded TCRs were found in internalized vesicles (39).

How might TCR signal strength alter constitutive internalization during positive selection? Following TCR engagement, conformational changes release the CD3 ζ-chains from the lipid bilayer exposing their ITAMs (45). CD3 subchain ITAMs are then phosphorylated in an ordered fashion directly related to the strength of TCR–pMHC interaction: lower-affinity interactions induce low-level CD3ζ ITAM phosphorylation, whereas stronger ones induce robust ITAM phosphorylation on CD3ζ and the other CD3 subchains (46, 47). The popular model predicts that TCR–pMHC engagement brings CD4/8–associated Lck into proximity of the CD3ζ ITAMs to phosphorylate two tyrosine residues that serve as a docking site for inactive ZAP70 (48). Constitutive docking of inactive ZAP70 on CD3ζ has been observed in developing thymocytes, indicating low tonic TCR signaling induced by self-peptide–MHC is common (47, 49, 50). When Boyd et al. (33) analyzed prepositive selection thymocytes expressing transgenic Vα2-containing and endogenous Vα11-containing chains, they found that cross-linking with anti-Vα2 Ab resulted in the retention of Vα2 on the cell surface and the corresponding loss of Vα11. Conversely, cross-linking the transgenic Vβ8.1 chain or CD3ε resulted in downregulation of both Vα2 and Vα11 (33). These findings suggest that TCRα and its unique associated CD3 chains (CD3ζ and δ) protect the TCR complex from internalization (33). Taken together with the ITAM phosphorylation order, it seems likely that CD3ζ ITAM phosphorylation (and perhaps the docking of inactive ZAP70) resulting from weak TCR stimulation could prevent TCR internalization, whereas both unengaged TCRs and high-affinity TCRs would be internalized and degraded (Fig. 2). We posit that in thymocytes with two functionally rearranged TCR α-chains, the net result of these

differences in TCR signaling is selective retention of low-affinity TCRs on the cell surface and phenotypic allelic exclusion of both unengaged and high-affinity TCRs. This model is consistent with the capacity for dual TCRα expression to promote positive selection of cells with otherwise unselectable (unengaged) TCRs and also to allow cells with high-affinity TCRs to escape clonal deletion as discussed later.

Whether the TCRα phenotypic allelic exclusion established in thymocytes is maintained in mature T cells is not known, but the similar proportion of dual-surface TCR T cells among postselection thymocytes and peripheral T cells implies some level of preservation. The mechanisms that maintain TCRα allelic exclusion in mature T cells have not been described, but it is logical to surmise the mechanism that establishes TCRα allelic exclusion also maintains it, with the notable exceptions that mature T cells exhibit low SLAP expression and express Cbl-b rather than its homologue c-Cbl (43, 51). Consequently, in mature T cells, unengaged/internalized TCRs are frequently recycled to the cell surface rather than constitutively degraded. Therefore, phenotypic allelic exclusion could be maintained if the selected TCR continues to receive low-level stimulation, thus preventing its internalization. Indeed, mature T cells frequently encounter self-peptide–MHC capable of minimum TCR stimulation (52). In contrast, high-affinity TCR–pMHC interactions in mature T cells cause widespread phosphorylation of the CD3 chains. Once internalized, these highly phosphorylated TCRs are directed to lysosomes and degraded, rather than recycled, leading to TCR downmodulation; this would also alter surface TCR composition in dual TCR T cells (41, 53, 54) (Fig. 2). Altogether, we propose that both unengaged and strongly engaged TCRs are frequently internalized. In mature T cells, unengaged TCRs persist in endosomes, which can be recycled back to the cell surface, whereas strongly engaged TCRs are degraded in lysosomes. Conversely, low-affinity TCR interactions prevent internalization, thereby maintaining TCR surface expression and phenotypic allelic exclusion.

Effect of dual TCRα expression on T cell signaling

As discussed above, phenotypic allelic exclusion in T cells with two functionally recombined TCRs either arises through competition for the pairing chains or through selective retention of the positively selected TCR α-chain on the cell surface. For T cells in which selective retention determines phenotypic allelic exclusion, the excluded TCRα reaches the cell surface but is preferentially internalized. It, therefore, follows that both TCR α-chains are likely expressed on the cell surface, albeit with one of them typically below the limit of detection. Likewise, depending on their relative affinities for the pairing TCR β-chain, occasional surface expression of the typically excluded TCR α-chain may also occur in the competitive exclusion model. Because engaging a small number of TCRs is sufficient for activation (55, 56), one might question whether TCRα phenotypic allelic exclusion results in functional monospecificity or if signaling through the secondary excluded TCRα could activate the cell. In a TCR transgenic model, dual TCRα T cells with disproportionate TCRα expression levels were stimulated with the cognate Ag of either the minor TCR or the dominant TCR, and proliferation was measured (39). Stimulation of the dominant TCR drove robust proliferation, whereas stimulation of the minor TCR resulted in minimal proliferation over background (39). These data seem to indicate that functional monospecificity can exist in dual TCRα T cells if one

of the TCRs is expressed on the cell surface at a level insufficient to activate the cell. However, in this transgenic system, the minor TCR α-chain had fewer transcripts (39). In contrast, normal dual TCRα T cells might be expected to have similar amounts of each TCRα transcript because TCRα allelic exclusion primarily occurs posttranslationally (4, 29). Therefore, the observed monospecificity could have been due to peculiarities of the transgenic system (39). Conversely, other models have demonstrated that TCRs incapable of promoting positive selection when solely expressed could survive selection and respond to Ag in the periphery when a second selectable TCR was coexpressed (57, 58). The unselected TCR specificity was rarely detected by flow cytometric analysis in naive mice but became common following immunization. This outcome could result from robust clonal expansion of a very small number of dual TCR T cells with equivalent initial surface expression of both TCR α-chains. However, the same outcome could result from signaling-induced changes in TCR surface composition such that the unselected TCR α-chain becomes detectable on the cell surface (57) (Fig. 2B). Further investigation is required to understand the plasticity of TCR α-chain surface composition in dual TCRα T cells.

In contrast to cells with disproportionate TCRα surface expression, cells commonly considered to be dual TCRα T cells have detectable and similar levels of both surface TCRs. These cells have repeatedly been shown to respond to both epitopes equivalently to T cells solely expressing either TCR specificity [i.e., they are functionally dual Ag specific (59-66)]. However, the outcome of the response was dictated by the original stimulation. That is, if the initial TCR stimulation resulted in a memory or regulatory T (T_{reg}) cell response, then that response was maintained following subsequent stimulation of the second TCR (63-66).

Can engagement of one TCR affect the function of the second TCR? This question is relevant to understanding how dual TCR expression affects tolerance to both self- and foreign antigens. Different model systems have led to disparate conclusions regarding the degree to which one TCR influences the other in dual TCR T cells (56, 59-62, 67, 68). In one TCR transgenic model, inhibition of one TCR with a TCR-specific antagonist resulted in some level of inhibition of the second TCR not directly bound by the antagonist, although the effect on the second TCR was 10- to 20-fold less pronounced (59). Conversely, another study concluded that the two TCRs can function independently, without cross-regulation. In this in vivo system, dual TCRα T cells tolerized through a TCR recognizing a self-tumor Ag were fully capable of proliferating in response to stimulation of the secondary, foreign Ag-specific TCR (62). Furthermore, the proliferation induced by activating the secondary TCR broke tolerance of the self-specific TCR (62). In a TCR transgenic model expressing both OT-I and P14 TCRs, stimulation of P14 led to downregulation of P14 surface expression and a slight increase in OT-I expression; in contrast, stimulation of OT-I downregulated both TCRs, although the effect on P14 was less pronounced (56). These various effects between model systems have been attributed to differences between CD4 and CD8 T cell biology, the assays used to measure TCR signaling inhibition, the surface proportion of the two TCRs, affinity for peptide–MHC, and the method of tolerance induction, underscoring the complex and context-dependent nature of T cell inhibition (59, 62, 69, 70). Furthermore, since the publication of these studies, numerous T cell–inhibitory pathways have been elucidated (CTLA-4, programmed cell death protein 1 [PD-1], lymphocyte-activation gene 3 [LAG-3], and T cell Ig and mucin domain protein 2 [TIM-2]). As we learn more about these and other

T cell–inhibitory pathways, it will be important to evaluate how each pathway influences TCR signaling and tolerance in dual TCR T cells.

Impact of dual TCRα expression on thymic selection

Positive selection.

During positive selection a dual TCRα thymocyte has three possible outcomes (Fig. 3A1-3). 1) Both TCRs are retained on the cell surface (Fig. 3A1). Because only 10–15% of all TCRs are capable of positive selection, the chance that a given cell would produce two selectable TCRs is calculated to be 1–2.25%; this scenario might only account for some of the dual surface TCRα-expressing T cells (71, 72). 2) One TCR is retrained on the cell surface, whereas the other is excluded (Fig. 3A2). The cumulative avidity would determine the cell's fate. In those that survive, phenotypic allelic exclusion may not be absolute, and these cells would have the potential to express both TCRs on their surface in response to cognate Ag. This would explain observations from TCR transgenic mice in which foreign-reactive (57) or autoreactive (58) TCRs are not selected unless a second, selectable TCR is coexpressed. These first two mechanisms underlie the ability of dual TCR expression to expand the TCR repertoire. 3) The final possible outcome is that neither TCR is preferentially retained. If neither TCRα allele rearranges a TCR capable of signaling, the cell dies by neglect (Fig. 3A3).

Negative selection.

Many have hypothesized that dual TCR expression can decrease negative selection efficiency (8, 26, 62, 67, 73-75). If a dual TCR thymocyte retains equivalent surface expression of both TCR specificities (only one of which has high affinity for self-peptide–MHC), the overall avidity could be diminished, allowing the cell to avoid clonal deletion (Fig. 3B1). However, phenotypic allelic exclusion likely prevents equivalent surface expression of both TCR specificities in most of these cells. The biased surface expression would be expected to limit the influence the second TCR has on overall avidity, resulting in appropriate deletion of the majority of strongly self-reactive dual TCRα thymocytes (Fig. 3B2). The effect of dual TCR expression on negative selection and its downstream impacts remain an area of debate that will likely continue until better tools to detect dual TCR T cells are created.

Agonist selection.

Recently we demonstrated that dual TCR expression might also limit agonist selection of thymic T_{reg} cells (76). Thymocytes that survive positive and negative selection in the cortex migrate to the medulla as single positive (SP) thymocytes where they encounter a new assemblage of self-peptide–MHC. Here, strongly self-reactive $CD4^+$ T cells may be clonally deleted or undergo agonist selection to become T_{reg} cells (77). In NOD mice lacking dual TCRα expression ($TCR\alpha^{+/-}$), we observed an increased ratio of T_{reg} cells to SP cells in the thymus and less apoptosis among the signaled CD4SP population, indicating increased T_{reg} commitment and decreased clonal deletion (76). This suggests that dual TCRα-expressing T_{reg} cells should be rare, yet human thymic and peripheral T cells expressing both Vα12 and Vα2 TCRs were three times as frequent in the $CD25^+$ T_{reg} cell–enriched population versus

the $CD25^{-}$ population, leading to the interpretation that dual TCRα expression may be common in T_{reg} cells (78). However, both Vα12 and Vα2 were individually enriched in the $CD25^{+}$ thymic and peripheral populations, which could imply that these Vα regions favor T_{reg} commitment; this effect would be compounded in cells expressing both V α-chains, resulting in an even higher rate of T_{reg} commitment (78).

Although the exact mechanisms that determine thymic T_{reg} cell commitment are not fully elucidated, transient strong interactions with self-peptide–MHC are required (77, 79-83). It is reasonable that surface expression of a secondary TCR could reduce overall avidity, thereby limiting TCR signal strength and T_{reg} cell lineage commitment (Fig. 3C1). Although many dual TCRα T cells would be expected to have downregulated the unselected TCR and display phenotypic allelic exclusion at the SP stage (24, 29) (Fig. 3C2), it is worth noting that thymocytes encounter previously unseen self-peptide:MHC combinations in the medulla. Therefore, it is likely that medullary thymocytes perceive different TCR signal strength than they experienced in the cortex. This change in self-peptide–MHC exposure theoretically could alter TCR surface composition (Fig. 3C3). Presently, how TCR signaling affects surface TCR expression during agonist selection of T_{reg} cells is unknown. Whether dual TCR expression impacts conversion and homeostasis of peripheral T_{reg} cells has not been studied. Elegant studies of T_{reg} cell lineage commitment in the context of single TCR expression are helping to inform new hypotheses regarding how dual TCR expression might impact agonist selection (82, 84).

Implications in disease

Autoimmunity.

Many have speculated that dual TCRα expression might allow self-reactive thymocytes to escape clonal deletion because of reduced surface expression of the self-reactive TCR, effectively decreasing avidity and allowing the self-reactive TCRs to stow away (8, 26, 62, 67, 73-75, 85). Several TCR transgenic mouse models have demonstrated this effect (73, 75, 85). However, most of these models have premature TCR expression, an unnaturally high number of dual TCRα T cells, and a heavily skewed TCR repertoire. TCR transgenic systems thus describe a “can-happen” scenario by which dual TCRα expression might allow a self-reactive thymocyte to avoid clonal deletion. In contrast, a meaningful impact of dual TCRα expression on negative selection in the context of a normal (nontransgenic) TCR repertoire has not been described (26, 86, 87). Therefore, in the normal state, phenotypic allelic exclusion appears competent to maintain high-fidelity clonal deletion of the majority of potentially hazardous self-reactive dual TCRα thymocytes (85). The seemingly contradictory observations regarding the impact of dual TCRα expression on agonist selection of T_{reg} cells further confounds our understanding of how dual TCRα T cells contribute to autoimmunity and emphasizes the need for more direct evidence demonstrating how dual TCRα expression affects T_{reg} cell lineage commitment (76, 78).

Allergy.

Two studies have demonstrated that pathogen recognition by one TCR can result in cross-activation of the other allergen-specific TCR, triggering immune responses to an otherwise

innocuous Ag (66, 88). As with the autoimmune hazard theory, these conclusions derive from TCR transgenic systems; how common this phenomenon is and whether it contributes to allergic disease in organisms with normal immune systems remains unknown.

Alloreactivity.

The alloreactive T cell population has been shown to contain a very high proportion of dual TCRα T cells (89-92). Furthermore, alloreactive responses were measurably decreased in mouse models lacking dual TCRα T cells (TCRα$^{+/-}$) (72-75). More information on dual TCR expression in alloreactivity and graft-versus-host disease can be found in recent reviews (93, 94).

Evolutionary pressure

The question of whether dual TCR expression is subject to evolutionary pressures is complex. Dual TCR expression has several hypothesized negative risks, including alloreactivity, allergy, and autoimmunity. Outside of pregnancy, alloreactivity would not be expected to exert any natural evolutionary selective pressure. Severe forms of allergy and autoimmunity that impair reproductive fitness have been selected against over evolutionary time. Yet virtually all humans have dual TCRα T cells, suggesting that dual TCRα expression is either not strongly selected against or that the risk of disease predilection is balanced by the benefit of immune protection.

Dual TCR expression has been hypothesized to expand the repertoire to include TCR specificities that would otherwise not survive selection. This expansion may provide evolutionary benefit by improving protective immunity (57, 95). If true, dual TCR expression would only offer an evolutionary benefit if both TCRs can signal from the cell surface. However, many T cells have two rearranged TCR α-chains but because of phenotypic allelic exclusion are presumed to be monospecific, negating the proposed evolutionary benefit. It is the opinion of the authors that the dynamic nature of TCR surface expression makes it likely that dual TCR T cells are functionally dual specific. In support of this view, it has been suggested that TCRs recycled from the cell surface can serve as an intracellular store of functional TCR that can be rapidly directed to the immune synapse after ligand engagement of surface TCRs (96). In T cells with two rearranged TCR α-chains, this intracellular store contains surface-excluded TCR α-chains that could be mobilized, overcoming phenotypic allelic exclusion and allowing the cell to be activated in response to this second specificity (Fig. 2B). Thus, the fraction of T cells with two different surface TCR specificities is likely larger than one in ten, perhaps as high as one in three (6).

An alternative view is that dual TCR expression exists as a byproduct of the processive nature of TCR α-chain rearrangement. To optimize the chances that a positively selected TCR is generated, both TCRα alleles undergo multiple processive rearrangements simultaneously (21). This greatly increases the size of the TCR repertoire screened during positive selection. Because both processive rearrangement and dual TCR expression require continued availability of the rearrangement machinery, it is plausible that the evolutionary benefit is caused solely by the repertoire expansion provided by processive rearrangement. In this scenario, dual TCRα expression occurs as a byproduct but does not provide any

evolutionary benefit per se. In contrast, it is theoretically possible to achieve the similar-sized repertoire of TCR α-chains if processive rearrangement were to occur sequentially rather than simultaneously, if a mechanism were to exist that specifically prevented dual TCRα expression while allowing processive rearrangement. Such a mechanism would limit dual TCRα expression like dual TCRβ expression but might reduce the efficiency of TCRα rearrangement relative to simultaneous recombination of both alleles by increasing the average time required to form a selectable TCR. Indeed, thymocytes with one functional TCRα allele have decreased selection efficiency relative to thymocytes with two functional alleles and are outcompeted in bone marrow chimera models as a result (87, 90). However, animals with only one TCRα allele are still capable of forming a seemingly normal immune system free of any obvious deleterious phenotype, indicating that simultaneous TCRα rearrangement improves thymocyte development efficiency but is not necessary for normal immune function (87, 90). Viewed this way, an evolutionary benefit of simultaneous rearrangement of the TCRα alleles is that it improves thymocyte selection efficiency (i.e., fewer thymocytes are wasted, resulting in net energy conservation for the organism).

In striking contrast to the TCRα loci, multiple mechanisms limit simultaneous recombination of the TCRβ loci, consistent with strong selective pressure against dual TCRβ expression. Why TCRβ recombination has evolved to limit simultaneous recombination is not known but could relate to the need to maintain appropriate γδ T cell numbers. The TCR β-, TCR γ-, and TCR δ-chains all recombine during the double negative stage of thymocyte development and vie for the fate of the cell. If a functional γδ TCR forms before a TCR β-chain, then the cell will develop into a γδ T cell and vice versa. Simultaneous TCRβ recombination would favor αβ T cell lineage commitment and reduce γδ T cell numbers (87); this effect could negatively impact γδ T cell–mediated barrier immunity (97, 98). If so, simultaneous TCRβ recombination would be expected to be selected against. Several other selective pressures might also participate in driving the dichotomy between TCRα and TCRβ allelic exclusion.

Conclusions

The initial discovery of dual TCR T cells was unexpected (2, 3). That as much as 10% of the peripheral T cell population expresses two detectable surface TCR specificities was even more striking. Given that one third of T cells have two productively rearranged TCR α-chains and the apparent plastic nature of TCR surface composition, we speculate that any T cell expressing two rearranged TCR α-chains in the cytosol has the potential to be functionally dual specific.

Whereas posttranslational allelic exclusion describes how surface TCRα composition is established during positive selection, little is known about how dual TCRα surface expression is maintained. Because TCRs constitutively recycle, phenotypic allelic exclusion must be actively maintained in the periphery. The simplest explanation is that the mechanisms that establish phenotypic allelic exclusion also maintain it. We propose that the selected TCR continues to phosphorylate CD3ζ through frequent low-level interaction with self-peptide–MHC in the periphery, consistent with findings of tonic CD3ζ phosphorylation

(48-50). Constitutive TCR recycling would then preferentially endocytose the unselected TCR, effectively maintaining phenotypic allelic exclusion.

Surface TCR expression is central to the hypothesized impact of dual TCR expression on cell fate, signaling, and immunity. Because T cell identity depends on TCR specificity and signal strength, it is natural to question whether expression of a second TCR specificity would confuse fate decisions and cell responses. Does dual TCR expression create an identity crisis within the T cell, resulting in inappropriate responses to stimuli (i.e., allergy or autoimmunity), or is it an efficient means to build multitasking T cells able to recognize multiple Ags, thereby improving protective immunity? The major limitation to answering this and other questions regarding dual TCR T cell biology in the context of normal immunity is the inability to detect all dual TCR T cells, so it remains unclear how often these scenarios occur, how they are regulated, and how they impact immune function. To advance this field, new tools must be generated to improve our ability to detect dual TCR expression in organisms with normal T cell repertoires.

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Abbreviations used in this article:

DP	double positive
SLAP	Src-like adaptor protein
SP	single positive
T_{reg}	regulatory T

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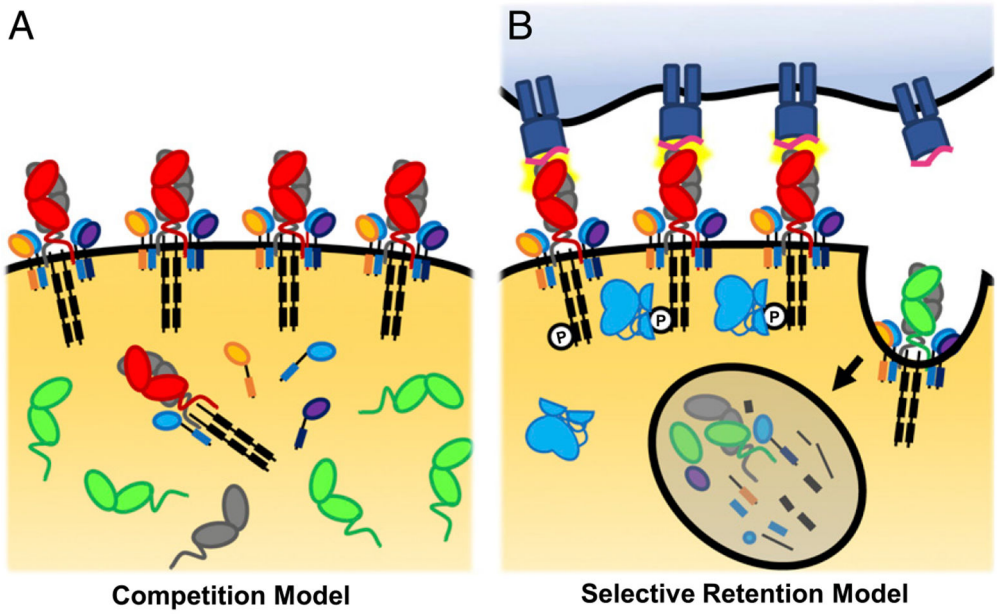

FIGURE 1.

Competition model versus selective retention model. (**A**) The competition model of phenotypic allelic exclusion proposes that the two rearranged TCR α-chains (red and green) compete for either the pairing TCR β-chain (gray) or CD3 chains (blue, orange, purple, and black). The TCR α-chain with the highest binding affinity for TCRβ and/or the CD3 chains will preferentially form complete TCRs and gain access to the cell surface, whereas the lower-affinity binding TCR α-chain would be excluded from the cell surface. (**B**) The selective retention model is based on the observation that TCRs that recognize self-peptide–MHC are retained on the cell surface because of signaling-mediated protection from the TCR internalization machinery. TCRαβ pairs that do not recognize self-peptide–MHC are endocytosed and degraded and are, therefore, excluded from the cell surface.

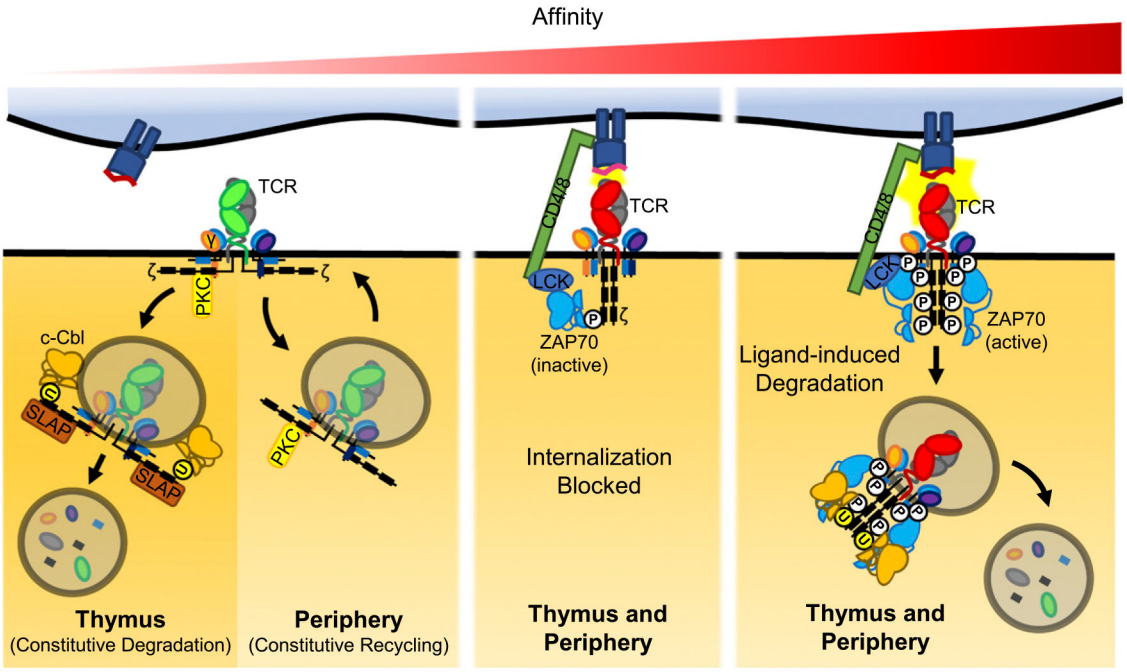

FIGURE 2.

TCR ligand affinity dictates surface expression. TCR are constitutively internalized in developing thymocytes and mature T cells. In DP thymocytes, internalized TCR are constitutively degraded. ZAP70 or SLAP serve as adaptors to mediate the ubiquitination of the CD3 ζ-chain by c-Cbl, which directs the cell to the lysosome for degradation. In contrast, internalized TCR are routinely recycled to the cell surface via the CD3γ–PKC pathway in mature T cells. During positive selection, TCRs engaged with self-peptide–MHC are retained on the cell surface. In dual TCR T cells, this method could result in preferential surface expression of one TCR, whereas the other is constitutively degraded, thus establishing phenotypic allelic exclusion. We predict that similar low-level self-peptide–MHC recognition in the periphery maintains phenotypic allelic exclusion. Conformational changes to CD3 ζ-chain following TCR engagement free the CD3ζ ITAMs from the lipid bilayer to be phosphorylated and serve as docking sites for inactive ZAP70. We suspect this interferes with the constitutive recycling pathway machinery to maintain surface expression of the engaged TCR. Higher-affinity TCR interactions result in Lck-mediated activation of ZAP70, which leads to the spread of CD3 chain ITAM phosphorylation. Strongly activated TCR is then internalized and degraded through a distinct mechanism. Selective downmodulation of TCR following high-affinity interactions also have the potential to alter TCR composition on dual TCR T cells, indicating dual TCR surface composition is a likely dynamic.

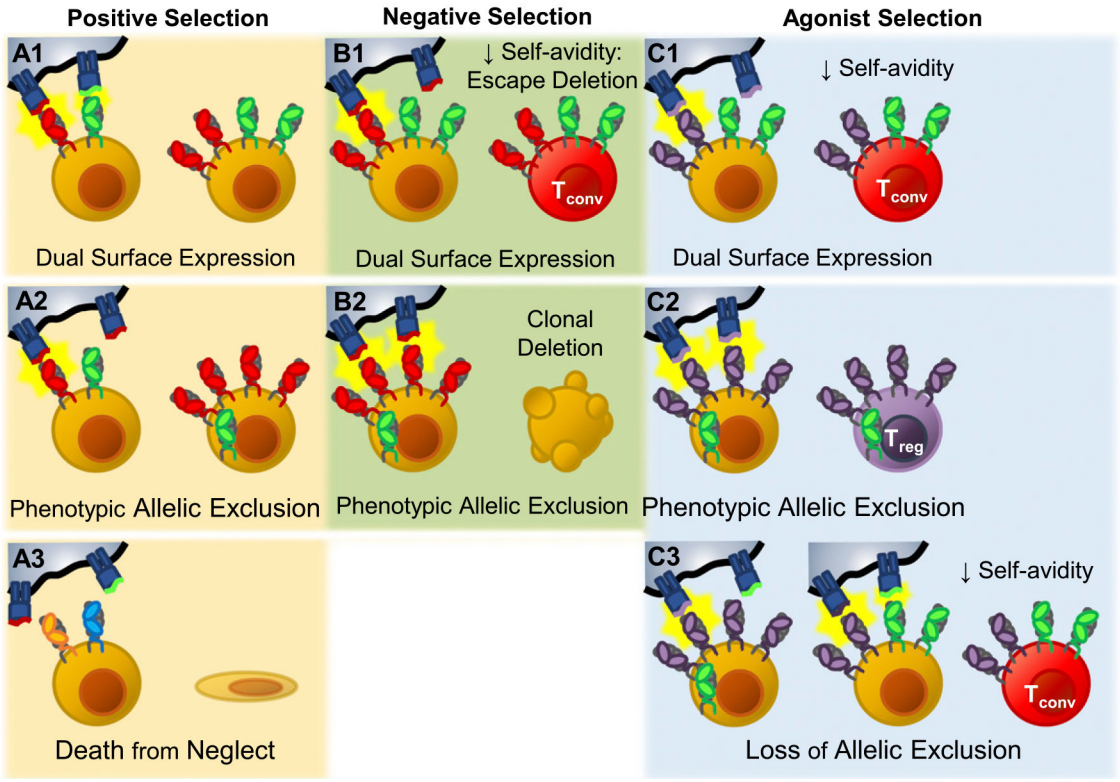

FIGURE 3.

Effects of dual TCR expression on thymic selection. During positive selection, dual TCR–expressing thymocytes have three potential outcomes. (**A1**) Both TCRs are retained on the cell surface (Dual TCR T cell). (**A2**) Only one of the two TCRs is retained. The excluded TCR is preferentially internalized and degraded, leaving the other TCR to populate the cell surface, establishing phenotypic allelic exclusion. Phenotypic allelic exclusion is likely plastic; thus, these cells retain the potential to recognize both specificities. (**A3**) Neither TCR induces positive selection, and the cell dies from neglect. (**B1**) During negative selection, thymocytes that express two surface TCRs may experience decreased overall avidity to self-peptide–MHC relative to sole expression of the self-reactive TCR. In this situation dual TCR expression has the potential to allow the strongly self-reactive TCRs to escape clonal deletion. (**B2**) If phenotypic allelic exclusion has been established and the self-reactive TCR dominates the cell surface, avidity would likely be similar to sole expression of the self-reactive TCR, and the cell would undergo clonal deletion normally. Agonist selection of T_{reg} cells takes place in the medulla where thymocytes are tested against new self-peptide–MHC combinations. (**C1**) If the thymocyte enters the medulla expressing two TCR specificities on its cell surface, it is possible that the resulting decrease in avidity could limit agonist-mediated commitment to the T_{reg} cell lineage. (**C2**) If the phenotypic allelic exclusion established in the cortex is maintained in the medulla, agonist selection would be expected to function normally, and T_{reg} cell–biasing TCRs would drive T_{reg} cell commitment. (**C3**) However, if encountering new self-peptide–MHC combinations alters

phenotypic allelic exclusion, it is possible that self-avidity could be reduced, effectively rerouting T_{reg} cell–biasing TCRs into conventional T cell lineages.