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Indoctrinating T cells to attack pathogens through homeschooling

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Adaptive immunity is predicated on the ability of the T cell repertoire to have pre-existing specificity for the universe of potential pathogens. Recent findings suggest that T cell receptor (TCR)–self-major histocompatibility protein (pMHC) interactions limit autoimmune responses while enhancing T cell response to foreign antigens. We review these findings here, placing them in context of the current understanding of how TCR–self-pMHC interactions regulate T cell activation thresholds, and suggest that TCR–self-pMHC interactions increase the efficiency of the T cell repertoire by giving a competitive advantage to peptide cross-reactive T cells. We propose that self-reactivity and peptide cross-reactivity are controlled by particular CDR3 sequence motifs, which would allow thymic selection to contribute to solving the feat of broad pathogen specificity by exporting T cells that are pre-screened by positive and negative selection for the ability to be 'moderately' peptide cross-reactive.

T cell selectivity and ligand discrimination

To initiate an adaptive immune response, T cells scan antigen-presenting cells (APC) within the secondary lymphoid compartments for pathogen-derived peptides displayed on host pMHC. If a T cell–APC encounter results in intracellular signals that exceed a threshold, naïve T cells are triggered to undergo activation, clonal expansion, and acquire effector cell functions that help orchestrate pathogen clearance [1]. Three antigen-recognition properties are sentinel to the ability of the T cell repertoire to create sterilizing immunity. First, individual T cell clonotypes need to be responsive to a limited set of peptides displayed by host MHC. Second, to provide broad immunological coverage to the plethora of yet-to-be-seen pathogens, the collective T cell repertoire is required to have immense specificity for any and all of the unknown pathogens that might invade the host. Third, the T cell repertoire has to accomplish these feats of specificity and broad pathogen coverage using only several million T cell clonotypes [2–4].

Selective pressures that arise during T cell development contribute to solving these feats of antigen specificity using a 'goldilocks' solution. TCR V(D)J somatic gene rearrangement creates sequence diversity at positions of the TCR CDR3 loops that directly engage pMHC, which gives rise to T cell clonotypes that have their own unique peptide specificity requirements [5]. T cell selection then seeds the mature repertoire with a few million unique T cell clonotypes that express only those TCRs that have a 'moderate' range of peptide cross-reactivity; TCRs that require the engagement of multiple side chains of the peptide to create a strong enough binding reaction to induce T cell activation [6,7]. The structural features that allow post-selection TCRs to be self-tolerant and engage only a limited set of peptides include the pairing of particular TCR V gene combinations with specific CDR3 sequences, as well as through the creation of TCRα to TCRβ interchain interactions that stabilize CDR3 loop conformations [8–10]. Whether there are generalizable structural or sequence feature of CDR3 loops that predispose TCRs to be self-reactive or control the rate of peptide cross-reactivity is poorly understood. The multiplicative effect of millions of T cell clonotypes specific for unique sets of similar (and sometimes dissimilar) peptides, allows the T cell repertoire to have broad pathogen specificity while still limiting dysregulated autoimmune responses [4,11–13].

The actual 'rate' of peptide cross-reactivity and, thus, the likelihood that a given T cell clonotype will enter into an immune response, is a product of the ligand-binding properties of the expressed TCR, the density in which the antigen is expressed, as well as T cell signaling thresholds. The signaling threshold for T cell activation is based on the quality and quantity of TCR interactions with pMHC displayed on APC, and is tunable based on TCR–self-pMHC interactions that occur during development and homeostasis. The ability to select the expressed TCRs and tune the threshold of T cell signaling ensures that a minimum but limited number of T cell clonotypes will respond to any given infection, providing T cell immunity while minimizing detrimental immune and autoimmune responses [14]. A number of recent findings have begun to shed light on how T cell signaling thresholds are set.

Setting TCR signaling thresholds

The consensus model of T cell ligand discrimination is based on the concept of kinetic proofreading [15,16]. That is, the commencement of TCR signaling is not instantaneous following TCR engagement with pMHC, and requires the TCR to be bound for a period of time to allow the initiation of productive signaling (Box 1). The first step in this process requires TCR engagement of pMHC to

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Box 1. T cell activation: affinity, dwell time, and force

A number of models have been proposed to explain the potency of pMHC ligands to induce T cell activation. T cells can only measure three parameters of the TCR–pMHC binding event: the number of TCRs engaged with a pMHC complex at any one time, the length of time in which each receptor is bound, and the amount of force applied to the TCR–pMHC binding event. The number of TCR simultaneously engaged with a pMHC is dependent upon the equilibrium affinity (K_D) of the TCR–pMHC interaction and the concentration of pMHC being presented. Evidence supporting K_D-based receptor occupancy models of TCR signaling comes from studies showing a correlation between K_D and ligand potency [75,76], and from the fact that ligands can induce qualitatively distinct biological outcomes depending upon their concentration [77]. Kinetic proofreading models hypothesize that TCR must be engaged long enough to complete a series of signaling events, including co-receptor recruitment and TCR phosphorylation [15]. Increases in the dwell time of the TCR–pMHC engagement raise the probability that any single TCR–pMHC engagement will surpass the threshold amount of time required to initiate T cell activation and undergo clonal expansion [16,78]. More recently, the force that pMHC can apply to the TCR has been correlated with T cell activation [79]. Stronger TCR–pMHC binding events can stay bound for longer periods of time, allowing an accumulation of force to be applied to the TCR [80,81]. The application of force may induce TCR constant domains to undergo allosteric conformational changes, allowing interactions with the extracellular portion of CD3 components to initiate the release of CD3 and TCRζ cytoplasmic domains from the plasma membrane [82–85]. Given that affinity, dwell-time, and force are not independent parameters, how the interplay of these variables allows for distinct biological outcomes, including positive selection, T cell homeostasis, and T cell antagonism, as well all clonal expansion and T cell exhaustion, remains a work in progress.

result in the phosphorylation of TCR associated ITAM-containing TCRζ and CD3 chains by the Src family kinase, Lck [17]. How TCR signals are transmitted across the cell membrane is an area of intense research (Box 2).

Within the cytoplasm of T cells, the ability of the TCR complex to transduce activating signals stems from competing phosphorylation and dephosphorylation reactions. The T cell co-receptors, CD4 and CD8, are critical to this process because they enhance TCR signaling by delivering the Src family kinase Lck to pMHC-bound TCRs, and for CD8, through stabilizing the TCR–pMHC interaction [17–19]. The phosphorylation status of Lck itself determines whether the kinase has enzymatic function. Lck has two major tyrosine phosphorylation regulatory sites. Tyr394, which when phosphorylated by *trans*-autophosphorylation or by Fyn, the other Src kinase, stabilize the active conformation. By contrast, the phosphorylation of Lck Tyr505 in the absence of phosphorylated Tyr394, promotes the inactive, auto-inhibited conformation of Lck. In unperturbed T cells and thymocytes, a basal level of active Lck is maintained. When the active form of Lck is recruited to the TCR complex, it initially phosphorylates the two tyrosines within the CD3 and TCRζ-chain ITAMs. Doubly phosphorylated TCRζ-chain ITAMs recruit ZAP70, a kinase that is subsequently activated by a second round of Lck-mediated phosphorylation, which then relays the signal downstream by phosphorylating LAT and SLP76 (thoroughly reviewed in [17,20]).

The signal activation cascade that occurs when the TCR engages strongly activating ligands results in the increase in intracellular Ca^{2+}, activation of the Carma1/Bcl10/Malt1 (CBM) and Ras-ERK pathways. Consequently NFAT and NF-κB translocate to the nucleus and AP-1 becomes phosphorylated to induce gene transcription. Competitive to the activating signal are phosphatases, such as CD45, SHP-1, and PTPN22, which dephosphorylate Lck at Tyr394, ZAP-70, Lat, SLP-76, and Vav1, as well as kinases such as CSK, which phosphorylates Lck at Tyr505 [17,21]. Weak TCR–pMHC binding events are thought to trigger a negative feedback loop leading to rapid recruitment of the phosphatase SHP-1, followed by receptor desensitization, through the inactivation of Lck kinase and through a still being defined THEMIS:GRB2:SHP1 complex [21–24].

Box 2. Transmitting TCR signals across the cell membrane

Several nonmutually exclusive models have been proposed to explain how TCR engagement allows signals to be transmitted across the cell membrane, eventually resulting in the phosphorylation of the CD3ε and TCRζ cytoplasmic domains. Under nonactivating conditions, the CD3ε and TCRζ cytoplasmic domains of the T cell receptor complex are thought to bind to the inner leaflet of the plasma membrane, resulting in the insertion of the aromatic tyrosine in the ITAMs to be buried within the plasma membrane [86,87]. This sequestration of the ITAM, mediated by electrostatic interactions between acidic phospholipids and clusters of basic residues within the CD3ε and TCRζ cytoplasmic domains, may limit spurious phosphorylation of the TCR complex in the absence of pMHC binding [88]. When TCR engage agonist pMHC ligands, a mechanical shear force is applied to the TCR, due to the dynamic movement of these proteins bound to cell membranes. The force exerted on the TCR is thought to induce allosteric conformational changes within the constant domains, including changes to the A-B loop within the TCRα chain, as well as conformational changes within the F-G loop of the TCRβ chain, allowing the TCR constant domains to change their interactions with CD3εγ heterodimer [79–85]. Within the cytosol, mechanical forces on the TCR, TCR multimerization, and/or changes in the local environment may induce CD3ε and ζ cytoplasmic domains to be to be released from the plasma membrane, allowing the associated ITAM to be accessible for phosphorylation [86,89]. Changes in the charge property of lipids have also been ascribed to increases in the Ca^{2+} concentration [90]; however, the exact mechanisms involved are still incomplete because it has been noted that other divalent cations are able to induce this release of ITAMs, and that the magnesium concentrations in the cell far exceed the concentrations of free calcium [17]. Contrary to the idea that ITAMs need to first be released from the plasma membrane, it has also been suggested that the phosphorylation of TCRζ by Lck induces the dissociation of the TCRζ chain from the plasma membrane, which then facilitates TCR–CD3 clustering required for full T cell activation [88]. These and other models and pathways not discussed here, indicate that although many details of TCR signaling are known, the question of 'How does T cell receptor signaling begin?' is still not fully understood [17,18].

Creating an 'autoimmunity buffer'

Mature T cells have tremendous selectivity; T cell activating signals can be generated from as few as one to ten pMHC ligands within the T cell–APC contact area [25,26]. These findings raise a specificity conundrum. If thymocytes can, and indeed are required by positive selection, to functionally engage as few one to ten self-pMHC complexes, why does the mature T cell repertoire not chronically induce fulminant autoimmunity?

Part of the answer to this riddle was observed 25 years ago by Yagi and Janeway, who showed that developing thymocytes are 30–100 times more sensitive to antigen

than mature T cells [27]. These early experiments using superantigens were followed up using TCR transgenic mice and sets of altered peptide ligands (APLs) to show that mature T cells become desensitized to both ligand potency and ligand density [28–30]. Allowing thymocytes to undergo positive selection on very weak ligands as well as somewhat stronger ligands ensures that the subsequent T cell repertoire has a range of self-pMHC reactivity profiles. The ability of thymocytes to incorporate different strengths of self-pMHC signals appears to be critical for T cells to differentiate into distinct T cell lineages. Selection of conventional naïve T cells requires the least amount of signaling derived from self-pMHC to undergo positive selection and be maintained through homeostasis. By contrast, anti-inflammatory regulatory T cells, unconventional innate-like T cells, including NKT cells and CD8αα IELs are thought to require stronger signals derived be selected on stronger 'agonist' self-pMHC complexes [31].

The desensitization of mature T cells creates an 'autoimmunity buffer' that may be critical to avoid autoimmunity. For thymocytes to undergo positive selection and be exported into the mature T cell repertoire, the TCR–self-pMHC binding event has to propagate TCR signal transduction. The TCR–pMHC potency or dwell time threshold that separates positive selection from negative selection is quite narrow [19,32]. Without T cell signaling desensitization, the narrow window between positive and negative selection could pose great risk for the development of autoimmunity. This would be particularly true for autoreactive T cells that express self-reactive TCRs that are near the threshold for negative selection [33], target antigens that are expressed at much higher levels in the peripheral tissues as compared to the thymus (such as myelin), or target self-antigens that have temporal expression patterns [34]. However, based on *in vitro* assays, T cell signaling desensitization ensures that a self-pMHC ligand would need to be presented minimally at a 30–100-fold greater density in a peripheral tissue, as compared to the thymus, for a T cell to avoid negative selection and cause autoimmunity [27–30].

Difference in TCR signaling thresholds and pathways between thymocytes and mature T cells arise from both developmentally programmed changes and through dynamic tuning of T cell signal thresholds. Developmentally programmed changes include the differential expression of miRNAs, short noncoding RNAs that alter gene expression by targeting specific mRNA molecules for degradation or translational repression. The expression of miR-181a, for example, correlates with changes in ligand sensitivity because it is highly expressed in pre-selection thymocytes and is downregulated following TCR signaling and differentiation into mature T cells [35]. miR-181a amplifies TCR signaling by repressing multiple negative regulators in the TCR signaling pathway, including nonreceptor-type tyrosine phosphatase SHP-2, PTPN22, and the ERK-specific phosphatases, dual specificity phosphatases (DUSP5 and DUSP6). Repression of miR-181a targets is required for proper thymocyte positive and negative selection *in vitro*, due to altered TCR-signaling thresholds, while the inhibition of miR-181a results in the development of mature T cells that are overtly self-reactive [36]. Analysis and interpretation of deficiency of the entire miR-181 family is somewhat complicated: the miR-181 family is composed of six mature miRNAs that are encoded in three independent paralog precursor transcripts on three separate chromosomes, and is a critical regulator of cellular metabolism [37].

Developmentally programmed changes in TCR signaling also arise from the differential expression of T cell signaling molecules. This has been documented for the signal transduction cascade leading to NF-κB activation: while thymocytes mature by and large normally in animals deficient in PKCθ, Carma1, Bcl10, or Malt1, peripheral T cells carrying these deficiencies are unable to respond to strong TCR stimuli, indicating that positive and negative thymocyte selection minimally involve the CBM complex that is necessary for peripheral responsiveness [38–41]. Conversely, the adaptor molecules, thymocyte-expressed positive selection-associated-1 (Tespa1), and thymocyte-expressed molecule involved in selection (Themis) and a voltage-gated Na^+ channel (VGSC) are highly expressed in double negative and immature TCR^{lo} DP thymocytes, and their expression levels are reduced or absent in mature single positive thymocytes and in mature T cells [42–47]. The expression of both Tespa1 and VGSC provides mechanisms that allow weak positive selection signals to induce sustained Ca^{2+} signals that are required for CD4+ T cell development [47,48]. Themis, however, is thought to be a negative regulator of TCR signaling, based on the observation that Themis-deficient thymocytes respond to positively selecting self-pMHC in a fashion similar to that observed in wild type thymocytes interacting with higher-affinity ligands and from observations that the expression of Themis desensitizes TCR-signaling in mature T cells [24,42]. Thus, the developmental changes in TCR signaling thresholds are a product of differentially expressed positive and negative regulators of signaling, the balance of which is required to allow T cells to be less sensitive to self-pMHC ligands presented in the periphery, as well as to allow T cells to develop into different lineages.

Dynamic tuning of TCR sensitivity is initially set during T cell selection and continues to occur during mature T cell homeostasis. During development, the expression level of negative regulators of TCR signaling can be adjusted following thymocyte interactions with endogenous self-pMHC to fine-tune the TCR signaling sensitivity of mature T cells. For example, T cells that more strongly recognize self-pMHC increase the expression of CD5, a cell surface molecule that can negatively regulate TCR signals through association with the phosphatase SHP-1, whereas T cells that have weak interactions with self-pMHC express lower levels of CD5 [12,14,31,49–51]. Dynamic tuning of TCR sensitivity continues to occur postselection. During homeostasis, T cell interactions with self-pMHC can regulate the expression of negative regulatory molecules, influence cytokine responsiveness and limit autoimmune responses [14,52–54]. However, it is clear that T cell interactions with self-pMHC do not solely result in limiting T cell reactivity. Continuous TCR–self-pMHC interactions are required for proper lymphocyte homeostasis, and to maintain the effector functions. These processes ensure that peripheral T cells remain capable of recognizing peptides displayed by host-MHC molecules [55]. Moreover, recent findings

suggest that the quality (signal strength) of TCR–self-pMHC interactions are important in determining the response of a T cell to foreign antigens.

TCR–self-pMHC interactions prepare T cells for effector responses

Once exported from the thymus, the naïve T cell repertoire continually interacts with self-pMHC ligands displayed on APC. At the repertoire level, these TCR–self-pMHC interactions are required for naïve T cells to undergo homeostatic proliferation, regulate cytokine responsiveness, and endow T cells with a heightened sensitivity towards foreign antigens through inducing partial phosphorylation of the TCRζ chain [31,55–58]. TCR–self-pMHC interactions are also required for $CD4^+$ $FoxP3^+$ T cells to properly function, through the regulation of mTOR signaling and the expression of, among others, IRF4 and CTLA-4 [59,60].

It has been evident since the earliest experiments that individual naïve T cell clonotypes likely interpret self-pMHC interactions differently. By transferring polyclonal T cell populations into lymphopenic hosts, it was observed that some naïve T cells failed to undergo homeostatic proliferation. Similar studies using monoclonal and polyclonal T cell populations demonstrated that the capacity of self-pMHC ligands to regulate T cell homeostasis is clonotype specific and that the peripheral expression of ligands that induce T cell positive selection can provide T cell homeostatic signals [55,61,62]. Likewise, competition among $CD4^+$ T cells in unmanipulated hosts is probably caused by the limited availability of particular self-pMHC complexes [63]. However, deciphering this heterogeneity in naïve T cell reactivity to self-pMHC is intrinsically challenging; the interaction of self-pMHC complexes with the clone-specific TCR is of very low affinity and only a few self-peptides have been identified that induce positive selection of T cells or affect on peripheral T cell function.

Whether TCR–self-pMHC signal strength, and not simply the presence or absence of a positively selecting ligand, regulates naïve T cell survival and function has been addressed by overexpressing positively selecting ligands and through the use of TCR signaling reporters and surrogates. Allen and colleagues have recently provided direct evidence using gp250, a self-peptide that induces the positive selection of AND TCR transgenic T cells, that the expression level of a positively selecting ligand influences the frequency at which T cells with a particular specificity undergo positive selection, as well as the frequency in which these T cells are present in the mature T cell repertoire [64,65]. Experiments using reporters of TCR signaling further support the model that the quality of the TCR signals generated by self-pMHC regulates T cell function. Of particular value is the expression of CD5, which may function as a rheostat to weaken or strengthen TCR signaling [12,31,50,51,66]. Paradoxically, increasing levels of CD5, which one might expect to reduce TCR signals, positively correlate with the degree of basal phosphorylation of TCRζ and, following TCR stimulation, the rapid induction of Erk phosphorylation and production of IL-2 [67,68]. Consistent with these findings, $CD5^{hi}$ $CD4^+$ and $CD8^+$ T cells express higher levels of GFP than do $CD5^{lo}$ naïve T cells in TCR signaling reporter Nurr77-gfp mice. Naïve $CD5^{hi}$ T cells also show increased expression of multiple genes involved in T cell activations, including Eomes, T-bet, Helios, and Id3 [69]. These functional and transcriptional changes have led several groups to test the hypothesis that responses to foreign antigens may be influenced by the quality of the interactions of a T cell clonotype with self-pMHC complexes.

Self-pMHC reactivity biases increase the efficiency of T cell responses

By co-transferring polyclonal $CD5^{hi}$ and $CD5^{lo}$ T cells into recipient mice, the groups of Germain and Jameson have recently demonstrated that polyclonal $CD5^{hi}$ CD4 and $CD5^{hi}$ CD8 T cells, respectively, outcompete $CD5^{lo}$ T cells in primary responses to multiple pathogen challenges. For CD8 T cells, the immunodominance of $CD5^{hi}$ T cells was maintained at the memory phase and during recall responses. The immunodominance of $CD5^{hi}$ T cells appears not to be due to a greater intrinsic ability of these T cells to undergo TCR-induced proliferation, because both $CD5^{lo}$ and $CD5^{hi}$ T cells proliferate similarly to *in vitro* activation using α-CD3 and α-CD28 [67,69]. Although there is a strong consensus regarding the increased basal TCR signaling and improved functional characteristics, clonal analyses of the $CD5^{hi}$ versus $CD5^{lo}$ T cell subsets suggests the mechanisms that underlie differences in the T cell response during pathogen challenge are diverse. Using pMHC tetramer staining as a measure of TCR-pMHC binding strength, Mandl *et al*. argue that $CD5^{hi}$ T cells express TCRs that are intrinsically of higher affinity for both self-peptides and foreign peptides, and that this higher affinity for foreign peptides gives these T cells a competitive advantage during clonal expansion [67]. Alternative to this TCR-intrinsic affinity model, Fulton *et al.* suggest a T cell-intrinsic model in which increasing strength of TCR–self-pMHC interactions more efficiently poise naïve T cells to proliferate and integrate pro-inflammatory signals following pathogen challenge [69]. These findings are somewhat of a paradox to the idea that strong T cell interactions with self-pMHC dampen T cell reactivity and limit autoimmunity [52]. It is possible, however, that the different experimental approaches elucidated different aspects of peripheral T cell interactions with self-pMHC; stronger subthreshold interactions with self-pMHC enhance T cell responses until the threshold is met and receptor desensitization, anergy, and deletion occur.

The complexities of immune responses and the importance of maintaining T cell diversity suggest that the response of individual T cell clonotypes to pathogen challenge diverge from the general features of the polyclonal repertoire. Indeed, study of two CD4 T cells specific for an identical epitope from *Listeria monocytogenes* ($LLO_{190–205}$), in which one is $CD5^{hi}$ and the other is $CD5^{lo}$, demonstrated that the $CD5^{lo}$ clonotype undergoes greater clonal expansion during a primary immune response. This occurred despite the two TCR having near-identical affinities for the IA^b-LLO peptide complex and the $CD5^{hi}$ clonotype having increased basal levels of phosphorylated TCRζ and ERK [68,70]. Immune response dynamics and functional heterogeneity likely reconcile these differences. Consistent with all of the models of CD5 expression, the

Box 3. Ensuring immunological diversity

Effective immune responses occur when polyclonal T cells target the invading pathogen. However, during immune responses, there is a competitive advantage for T cells with a strong reactivity for the pathogen over T cells with a weak reactivity for the pathogen. Thus, T cell competition based on antigen reactivity could result in the entire immune response being dominated by progeny of just a few T cell clones. Although a focused T cell response may initially be successful in attacking cells harboring the invader, pathogens often have the ability to escape narrow oligoclonal T cell response through clonal exhaustion or through deleterious mutations within the T cell epitope [91–95]. To limit these effects, several additional layers of T cell competition ensure clonal diversity of the overall naïve T cell repertoire, as well as during immune responses [96]. During homeostasis, the mature T cell repertoire is subject to intraclonal competition, likely for access to self-pMHC ligands presented by APC and cytokines that provide survival signals [53,55]. This form of competition ensures that there are relatively few numbers of any individual clonotype, allowing the space for a large number of unique T cell clonotypes to exist [4]. During immune responses, T cell intrinsic and extrinsic mechanisms limit oligoclonality and ensure individual clones do not overly dominate the T cell response during the priming phase. These include co-inhibitory molecules, such as CTLA4, selective apoptosis of T cell clonotypes, and T cell competition [21,68,97,98]. Intrinsic T cell signaling, T cell competition for self-ligands, and cytokines further impacts the transition from activated to memory T cell formation [99].

$CD5^{hi}$ T cells produced greater IL-2 responses following antigenic and nonspecific stimulation, arguing there are intrinsic differences in the responsiveness of the two T cell lines to antigen receptor stimulation. Although this might portend the $CD5^{hi}$ T cells to undergo greater clonal expansion, the $CD5^{hi}$ T cells in fact showed a greater disposition to undergo apoptosis, potentially through IL-2-mediated activation-induced cell death. Nevertheless, some of the $CD5^{hi}$ T cells were maintained and indeed dominated the immune response during a secondary challenge. Thus, during polyclonal T cell response to pathogens, the immune system has multiple mechanisms in place to limit clonal dominance and maintain immunological diversity (Box 3).

Concluding remarks

How might self-pMHC reactivity benefit the efficiency of the T cell repertoire? An effective adaptive immune system is predicated on creating a T cell repertoire that is capable of providing immunological coverage to the universe of pathogens, an impossible feat if each TCR were overly specific for a particular antigen. By requiring thymocytes to functionally engage self-pMHC complexes, the very nature of positive selection itself biases the mature T cell repertoire to be peptide cross-reactive. This occurs because thymocytes expressing increasingly peptide cross-reactive TCRs will have a higher likelihood of recognizing one of the 10^3–10^5 self-peptides presented on host MHC and, thus, receive a positive selecting signal [71–74].

Thymic and peripheral T cell recognition of self-pMHC complexes, leading to the modulation of CD5 levels and basal levels of phosphorylated TCRζ and ERK, is likely to follow the same general rules that govern mature T cells engaging foreign antigens. Increases in CD5 levels will undoubtedly be associated with increases in the TCR–self-pMHC binding strength and the density in which the self-pMHC is expressed. Increases in CD5 levels could also occur from T cells being able to functionally engage multiple distinct self-peptide presented on MHC molecules (Figure 1).

T cell efficiency gains through increasing peptide cross-reactivity, however, have to be balanced to minimize overt self-reactivity within the pro-inflammatory T cell repertoire. Restraining the development and clonal dominance of $CD5^{hi}$ T cells in the mature T cell repertoire is thymic selection. T cell developmental processes instruct developing thymocytes to undergo positive or negative selection based on the affinity or dwell time of TCR–self-pMHC interactions. Reflective of this, thymocytes that express TCR with increasingly stronger binding strength for a particular self-pMHC ligand have a higher likelihood of being eliminated in the thymus by negative selection. Thus, $CD5^{hi}$ T cells may be skewed towards recognizing high-density, weak affinity/short dwell time self-pMHC ligands or have the ability to weakly recognize multiple unique self-pMHC ligands. Given that there are relatively few high-density self-pMHC available to induce homeostatic signals, one consequence of peripheral TCR–self-pMHC interactions may be to increase the efficiency of the T cell repertoire by allowing T cells with increased

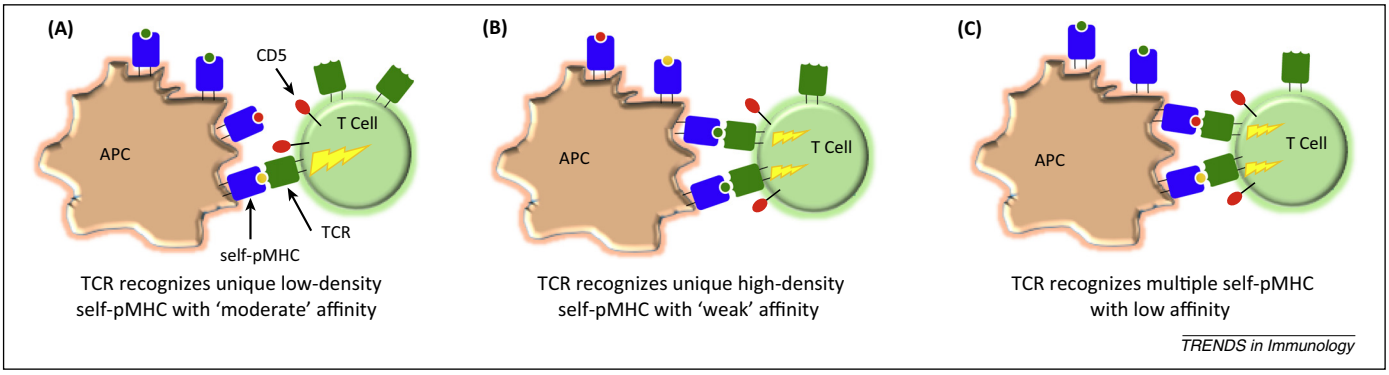

Figure 1. Does homeostasis increase the efficiency of the mature T cell repertoire through promoting T cell cross-reactivity? T cell interactions with self-major histocompatibility complex proteins (pMHC) ligands can promote responsiveness of T cell towards foreign antigens through inducing the partial phosphorylation of TCRζ, allowing for the rapid induction of Erk phosphorylation and production of IL-2 following T cell receptor (TCR) recognition of pMHC. The nature of the self-pMHC ligands that drive this process are not well defined. Three possibilities exist: T cells may generate homeostatic signals from **(A)** 'moderate' affinity interactions with self-pMHC that are expressed at a low density; **(B)** 'weak' affinity interactions with self-pMHC that are expressed at a high density; or **(C)** 'weak' affinity interactions with multiple self-pMHC. Given that thymic development selects against T cells that have stronger interactions with self-pMHC [depicted in (A)], and there are relatively few high-density self-MHC ligands presented in the periphery [depicted in (B)], cross-reactive T cells that can interact weakly with multiple self-pMHC [depicted in (C)] would have a competitive advantage over T cells that are more peptide specific. Abbreviation: APC, antigen-presenting cell.

peptide cross-reactivity to outcompete more peptide-specific T cells. These peripheral selection processes may also underpin the limiting of T cell diversity in aged individuals following thymic involution.

How might TCRs be created that have increased frequencies of self-reactivity and peptide cross-reactivity? TCR expressed on mature T cells are selected in part on their ability to be peptide cross-reactive. To create TCRs that have a beneficial range of peptide cross-reactivity, thymic selection equips mature T cells with TCR that carry structural features that allow TCRs to have 'moderate rates' of peptide cross-reactivity [6]. Somatic gene recombination creates TCR with variations in pMHC specificity and rates of peptide cross-reactivity by pairing different TCR Vα and Vβ gene segments with rearranged CDR3 V(D)J sequences, with the majority of TCR diversity and, thus, control of ligand specificity arising from CDR3 sequences. Given that CDR3 sequences are the hot spot of TCR diversity [5], we predict that the control of peptide cross-reactivity will derived from these sequences. In particular, we hypothesize that thymic selection equips mature T cells with TCR that carry particular types of amino acid at the tips of CDR3 loops that allow TCRs to have 'moderate rates' of peptide cross-reactivity. Unique CDR3 sequences may also create loops with greater or lessor flexibility, regulating the number of peptide features CDR3 residues can engage. Future experiments will be needed to determine if CDR3 self-reactivity and peptide cross-reactivity sequence motifs can be identified, and how these recognition properties regulate the development of inflammatory and anti-inflammatory T cells, as well as immune and autoimmune T cell responses.

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