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The self-obsession of T cells: how TCR signaling thresholds affect fate 'decisions' and effector function

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Self-reactivity was once seen as a potential characteristic of T cells that was eliminated by clonal selection to protect the host from autoimmune pathology. It is now understood that the T cell repertoire is in fact broadly self-reactive, even self-centered. The strength with which a T cell reacts to self ligands and the environmental context in which this reaction occurs influence almost every aspect of T cell biology, from development to differentiation to effector function. Here we highlight recent advances and discoveries that relate to T cell self-reactivity, with a particular emphasis on T cell antigen receptor (TCR) signaling thresholds.

The physical elimination of self-reactive lymphocyte clones (clonal deletion) is a central tenet of the clonal selection theory, and has been a major focus of immunologists since the introduction of the concept by F. Macfarlane Burnet and Peter Medawar in the 1950s.

Indeed, many self-reactive clones are eliminated daily in the thymus, and numerous studies of mice with transgenic expression of a T cell antigen receptor (TCR) crossed with mice expressing that antigen suggest that clonal deletion is a particularly efficient process[1]. Given this, it would be natural to assume that clonal deletion has a central role in immunological tolerance and that the remaining T cells in healthy animals are not self-reactive. The former assumption is not readily apparent from the experimental literature, however, and the latter is clearly not true. Here we first discuss how pervasive clonal deletion is, how important it is for overall immunological tolerance and how T cells interpret low- and high-affinity interactions to result in the fate of life or death, respectively. Next, we discuss how strong TCR signals can also induce the differentiation of unique lineages, including regulatory T (T_{reg} cells), invariant natural killer T (*i*NKT cells) and intraepithelial lymphocytes (IELs), in the thymus. Finally, we discuss how the 'weak' self-interactions of T cells, which are selected for by positive selection, yield a repertoire of naive T cells with substantial heterogeneity in their ability to respond to foreign antigens.

Clonal deletion in the thymus

For T cells, clonal deletion occurs in the thymus and is most efficient for clones that have high affinity for self antigens presented by professional antigen-presenting cells (APCs), such as dendritic cells[1]. The fact that deleted clones have a higher affinity for self peptides presented on major histocompatibility complex (MHC) molecules than do positively selected clones has been extensively confirmed in both monoclonal and polyclonal experimental models, although it has been unclear precisely what proportion of clones that achieve this high signaling threshold are deleted. It had been widely assumed that the number of clones that interact with any given peptide-MHC complex with high affinity (and are deleted) would be smaller than the number of clones that could interact with low affinity (and are positively selected), because the complementarity-determining region 3 of the TCR is produced by random assortment and nontemplated nucleotide addition. However, several groups have addressed this question with new approaches, and their data suggest that many more clones undergo clonal deletion than positive selection in the thymus. Two groups used an approach that focused on mice deficient in the proapoptotic molecule Bim, which have impaired clonal deletion. These groups used novel transgenically expressed markers (Nur77-GFP)[2] or endogenous markers (Helios)[3] to quantify the strongly signaled cells generated in mice lacking the proapoptotic molecule Bim. They report that 55% (ref. 3) to 57% (ref. 2) of all signaled thymocytes at the $CD4^+CD8^+$ double-positive (DP) stage in the cortex are deleted, and that another roughly 50% of the positively selected $CD4^+$ or $CD8^+$ single-positive (SP) thymocytes were subsequently deleted in the medulla. Thus, more than three-quarters of the cells that respond to self peptide–MHC in the thymus are deleted. These studies are remarkably concordant with those generated by a completely different approach, in which a synchronous cohort of thymocytes developing in normal mice was analyzed, and mathematical modeling of death and differentiation was used to explain the numbers of thymocytes at each stage. Those data suggest 75% of cells that start selection fail to complete it[4]. These data favor the notion that the TCR repertoire has a germline-encoded bias toward recognition of MHC molecules[5], rather than a bias that is strictly rendered by thymic selection processes[6]. It is worth emphasizing that although the T cell repertoire

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is overtly MHC reactive on the whole, thymic selection processes further skew it toward recognition of the specific MHC alleles present in the organism.

As many self-reactive clones are eliminated each day in the thymus, and clonal deletion is considered to be a particularly efficient process from the study of animals with transgenic TCR expression, it might be assumed that clonal deletion has an essential role in immunological tolerance. However, studies have attempted to evaluate clonal deletion from the perspective of a given self antigen, and these reports, which used peptide-MHC tetramers to determine how many self antigen–reactive clones are present in animals that do or do not express the self antigen, suggest that deletion may not be particularly efficient. One such study found only a threefold fewer male-specific cells in male mice than in female mice through the use of tetramers of male antigen and H-2D^b in mice with transgenic expression of the TCRβ chain[7], and similar results were obtained for a tissue-specific antigen in a different transgenic model of TCRβ-chain expression[8]. Although it might be argued that mice with transgenic expression of TCRβ are not a physiologic model because of their elevated frequency of reactive T cells, this type of decrease was also seen in studies using enrichment techniques to quantify MHC class II–restricted self-reactive T cells mice with a normal polyclonal T cell repertoire[9,10]. One consideration is that all of these studies propose a lower TCR avidity of the remaining T cells, consistent with a lower tetramer-staining intensity. Two of the studies included TCR repertoire analysis and found that certain receptor specificities are indeed eliminated from the tetramer-binding pool[7,10]. Thus, from the perspective of a given high-affinity receptor, clonal deletion may be as highly efficient in the polyclonal pool as it is in monoclonal models of transgenic TCR expression. On the other hand, from the perspective of the number of T cells that can react to a particular self peptide, clonal deletion is incomplete. Although tetramers are useful for comparative studies of defined self-reactive T cell populations in mice with specific mutations[11] or in autoimmune-prone strains[12], and transgenic models are useful for exploring deletion mechanisms in animal models, the true measure of clonal deletion efficiency cannot be addressed using either approach alone. In the future, technology will probably allow a comprehensive evaluation of clonal deletion where self-specific T cells are defined at the level of the sequenced TCR repertoire.

Irrespective of the precise degree of deletion efficiency, it is somewhat surprising that there are no autoimmune diseases in which impaired clonal deletion has been shown to be a pathogenic mechanism. It has been assumed that deficiency in the transcriptional regulator Aire in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy reflects autoimmunity due to a failure to clonally delete self-reactive T cells[13]. However, new evidence suggests that Aire is also crucial for the development of tissue-specific T_{reg} cells[14], so this inherited autoimmune syndrome may represent a more complex loss of tolerance. It is remarkable that Bim-deficient mice, which have much larger numbers of self-reactive clones[2,3], do not have widespread autoimmunity, even in a sensitized screen using hematopoietic reconstitution of *Rag1*$^{-/-}$ lymphopenic mice[15]. This is partly because clonal deletion is not needed to purge the repertoire of clones reactive to ubiquitous antigens[16], for reasons related to the nature of differences in TCR signaling in the thymus. In DP thymocytes, high-affinity ligands cause a strong signal of short duration that results in rapid induction of apoptosis[17,18]. In contrast, low-affinity ligands have a 'preferential' ability to result in sustained signals, which are required for positive selection[18,19]. Thus, strong stimuli at the DP stage, even when they fail to induce apoptosis, do not support positive selection and inclusion of those clones in the repertoire[16]. Once cells undergo positive selection and encounter different self antigens in the medulla, they are again susceptible to deletion. However, in this case, if apoptosis is prevented, autoreactive clones will accumulate[20], so any autoimmune pathology that would result from a failure of clonal deletion, as in Bim-deficient mice, is predicted to focus on tissue-specific antigens. The fact that such mice do not develop autoimmunity suggests that high frequencies of self-reactive T cells can be controlled by other tolerance mechanisms. Interestingly, double deficiency in Bim and another proapoptotic protein, Puma, results in spontaneous multiorgan autoimmunity[15]. Thus, a very extensive (or perhaps complete) impairment of clonal deletion is presumably needed to break down the protective effects of tolerance mechanisms such as clonal anergy and extrinsic regulation by T_{reg} cells.

Specialized mechanisms in the cortex

T cell progenitors undergo rearrangement of *Tcrb* and *Tcra* in the thymus. As soon as an intact TCRαβ heterodimer is expressed on the cell surface, the clone is exposed to potential ligands in its environment. The specific environment where this first recognition event occurs in the thymus is the thymic cortex, which is a highly specialized environment established by the activities of the transcription factor Foxn1. This factor is evolutionarily conserved in species that have a thymus[21], which speaks to the importance of the 'first-exposure' environment in establishing a repertoire of T cells that function properly in the animal. It has long been known that cortical, or CD4$^+$CD8$^+$ DP, thymocytes are very sensitive to TCR ligands and can respond to low-affinity ligands more readily than do SP thymocytes[22] (**Fig. 1**). DP thymocytes have a substantially different gene-expression profile from that of SP thymocytes, and at least some of these genes encode molecules presumed to account for DP thymocytes' enhanced sensitivity. Several genes have high expression in DP thymocytes but lower or absent expression in mature T cells, and their products function to support positive selection and/or tuning. These include *Themis*[23], *Tespa1* (ref. 24) and *Scn4b*[25], which encode proteins involved in TCR-proximal signaling events. *Scn4b* encodes the regulatory subunit of a voltage-gated sodium channel (VGSC). Both Ca^{2+} influx and positive selection are inhibited when VGSC activity is blocked in thymocytes[25]. Furthermore, ectopic VGSC expression in mature T cells allows them to respond to low-affinity ligands; thus, VGSCs seem to be crucial for the enhanced sensitivity of thymocytes (**Fig. 1**). Both positive and negative selection elicit Ca^{2+} signaling, although the patterns elicited are distinct, as has been demonstrated directly in thymic tissue by two-photon microscopy[26]. Interestingly, store-operated Ca^{2+} entry, which is crucial for mature T cell responses, is dispensable for positive selection of conventional T cells[27]. Instead, it seems to function in DP thymocytes to promote 'agonist selection' of T cells (*i*NKT cells, T_{reg} cells and IELs)[27]. Mechanistically distinct Ca^{2+} responses are thus at play when thymocytes perceive weak and strong TCR signals. Positive selection causes a gradual increase in the basal concentration of intracellular Ca^{2+} (ref. 28). This effect may also contribute to the 'tuning' of thymocytes, as data from other biological contexts show that elevated basal Ca^{2+} correlates with diminished receptor-elicited Ca^{2+} responses[29].

The precise signaling functions of Tespa1 and Themis remain to be fully elucidated, although Tespa1 deficiency also influences Ca^{2+} signaling[24]. An important study has shown that Themis deficiency allows low-affinity ligands to elicit negative selection–like signaling characteristics[30]. Thus, Themis acts to attenuate or modify weak signals through the TCR to facilitate positive selection. Likewise, Schnurri-2, a zinc-finger protein that may act downstream of Themis, inhibits

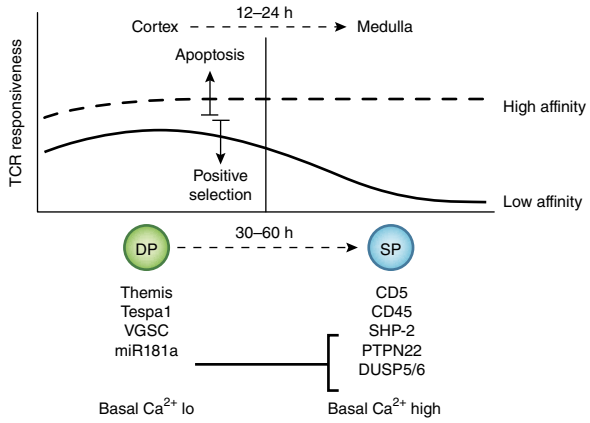

Figure 1 TCR sensitivity changes as cells mature from cortical (DP) to medullary (SP) thymocytes. DP thymocytes reside in the cortex and respond efficiently to both low- and high-affinity TCR ligands. High-affinity ligands can trigger apoptosis (clonal deletion), whereas low-affinity ligands are more likely to induce survival and differentiation (positive selection). DP thymocytes migrate to the medulla 12–24 hours after positive selection. At the same time, they begin differentiating to the SP stage, a process that is not complete for another 1–2 d. In the medulla, SP thymocytes remain responsive to high-affinity ligands, but their response to low-affinity ligands decreases, a process sometimes referred to as 'tuning'. DP thymocytes have a unique gene-expression profile that serves two purposes. First, it allows them to interpret graded TCR signals in a 'digital' fashion, to induce life or death in the cortex. Themis is hypothesized to be crucial for this feature. Second, it allows them to respond more efficiently to low-affinity ligands than do SP thymocytes. Evidence suggests that Tespa1, VGSC and miR-181a all contribute to this property. miR-181a represses expression of several phosphatases (SHP-2, PTPN22, DUSP5 and DUSP6) that are known to negatively regulate TCR signaling. Expression of CD45 and CD5 is upregulated in SP thymocytes independently of miR181a. These expression changes, together with an increase in the basal concentration of intracelluar Ca^{2+}, are thought to contribute to the developmental tuning of the TCR response.

apoptosis in response to positive-selection signals in DP thymocytes[31]. These unique signals transduced by the TCR during positive selection must be sustained for a surprisingly long time. Blocking the TCR signal 24–48 hours after initiation with antibodies to MHC[19], inhibitors of the kinase Erk[19] or inhibitors of the tyrosine kinase Zap70 (ref. 18), or by induced loss of Zap70 (ref. 32), all affect positive selection. Intriguingly, cortex-to-medulla migration occurs about 12–24 hours after initiation of positive selection[28], (**Fig. 1**), which suggests that at least some of this sustained signaling may occur in the medulla. Nonetheless, there is little evidence that medullary epithelial cells[33] or any MHC ligands in the medulla[34] are needed for positive selection, so this issue needs further investigation.

It is important to consider how multiple factors that act at different levels of the signaling pathway are regulated in concert to tune TCR responsiveness. The microRNA miR-181a has high expression in DP thymocytes but not in SP thymocytes and has been shown to repress expression of several phosphatases, including SHP-2, PTPN22, DUSP5 and DUSP6, to alter TCR responsiveness[35,36]. Its ectopic expression in mature T cells enhances their sensitivity[35], and its loss in DP thymocytes impairs clonal deletion[37] and agonist selection of *i*NKT cells[36]. In contrast, the expression of negative regulatory proteins, such as CD45 and CD5, increases during T cell development[38] (**Fig. 1**). CD5 is an interesting membrane protein that can negatively regulate signaling, yet its expression seems to be directly proportional to the TCR signal strength initially perceived[39]. Although the precise mechanisms of how these changes over time act together to influence the signal network remain to be fully elucidated, it is clear that specific mechanisms have evolved to allow cortical thymocytes to 'preferentially' respond to low-affinity TCR ligands, and in such a way as to not undergo apoptosis.

It is not only cortical thymocytes that are specialized for selection; the cortical APCs are as well (**Fig. 2**). Cortical thymic epithelial cells (cTECs) display a repertoire of peptides distinct from that of other APCs. The cTEC-unique peptides, which have been called 'private peptides', are apparently crucial for selection of the repertoire, as deletion of cTEC-unique genes such as *Psmb11* (which encodes the β5t subunit of the proteasome), *Ctsl* (which encodes cathepsin L) or *Prss16* (which encodes a thymus-specific serine protease) results in reduced $CD8^+$ or $CD4^+$ T cell numbers[40]. In the case of β5t, this unique private peptide diversity is suggested to be important for positive selection rather than reducing the effect of negative selection (either of which could result in lower T cell numbers)[41]. Interestingly, naive $CD8^+$ T cells that are $CD5^{lo}$ and have lower reactivity for self are selectively affected by β5t deficiency, which suggests that private ligands on cTECs enhance selection of T cells with low affinity for self[41]. How having a distinct peptide repertoire expressed by cTECs favors positive selection has been discussed elsewhere[40] and will not be reviewed in detail here. Suffice it to say that the naive T cell repertoire in normal animals contains clones with a range of reactivity to self ($CD5^{lo}$ to $CD5^{hi}$). In the steady state, these clones do not respond to their selecting self ligands, either because the specific peptides are not presented by peripheral APCs and/or because the TCR signaling threshold is adjusted by 'tuning'. It is important to emphasize that positive selection does not absolutely require expression of private peptides—indeed, cells selected on 'public' peptides (those shared between cTECs and other cell types) have the opportunity to reencounter those ligands in the periphery, which leads to enhanced basal TCR signaling (**Fig. 3a**). Studies have shown that a relatively abundant self peptide–MHC complex is able to positively select $CD4^+$ T cells of defined foreign antigen specificity[42], in support of this concept. What affect a range of self-reactivity in the naive repertoire has on peripheral T cell function is discussed in greater detail below.

Strong self-reactivity drives distinct T cell fates

Although is it abundantly evident that strong TCR signals can drive clonal deletion, evidence increasingly suggests that they can also drive the survival and differentiation of other T cell populations, including T_{reg} cells expressing the transcription factor Foxp3, *i*NKT cells and IELs. As for T_{reg} cells, support for the concept of agonist selection has been extensively reviewed elsewhere[43,44] but includes three seminal observations. First, coexpression of an agonist ligand in mice with transgenic TCR expression promotes T_{reg} cell development[45], at least in some models. Second, $Foxp3^+$ T_{reg} cells have a repertoire that overlaps that of autoreactive T cells derived from Foxp3-deficient mice[46]. Third, T_{reg} cells show signs of stronger and/or more recent activation in both the thymus and the periphery of TCR signal–reporter mice[47]. Such data have led to the frequent discussion of a model in which T_{reg} cell selection is supported optimally by TCR affinity for self peptide–MHC class II complexes that are intermediate between positive selection and clonal deletion. One study examined a panel of six TCRs with varying degrees of reactivity for a model antigen and then studied the thymic development of cells with transgenic and/or retrogenic expression of those TCRs in mice that express the model antigen as a self antigen[48]. There was a direct correlation between the degree of antigen reactivity and T_{reg} cell development; negative selection was apparent only with the most self-reactive TCRs. These findings would support the model stated above. However, the range

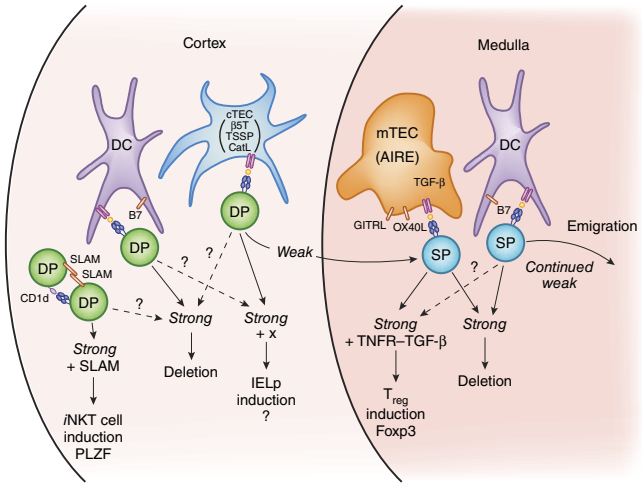

Figure 2 The anatomic context of TCR signaling is crucial for thymocyte fate. In the cortex, TCR signal strength is central in the fate outcome of DP thymocytes, but extrinsic factors provided by APCs in the cortex are also key. Weak interactions between the nascent TCR and self peptide–MHC complexes on cTECs are crucial for positive selection. The peptide-MHC repertoire expressed by cTECs is unique, owing to the function of several gene products involved in endogenous or endosomal proteolysis, such as β5t, thymus-specific serine protease and cathepsin L. Although this unique repertoire is essential for efficient positive selection, its involvement is not fully understood. Strong interactions between the newly expressed TCR and self peptide–MHC complexes in the cortex classically induces apoptosis (clonal deletion), and costimulation by B7 family members enhances this outcome. Strong TCR signals can also induce cells to become IEL precursors (IELp), although it remains unclear how frequently this occurs (relative to deletion), if it requires unique extrinsic factors, and whether IELps express a transcription factor that acts as a 'master regulator' of this fate. For *i*NKT cells, other DP thymocytes expressing CD1d are a crucial APC. The semi-invariant *i*NKT TCR must recognize a high affinity lipid ligand–CD1d complex, but SAP-dependent signals from homotypic interactions between members of the SLAM family on DP thymocytes is also required. These integrated signals result in the upregulation of PLZF. As for the medulla, the cells positively selected by weak TCR signals in the cortex will migrate to the medulla and, by downregulating expression of the inappropriate coreceptor, will form the medullary SP thymocyte pool. SP thymocytes interact with distinct APCs, including medullary TECs (mTECs), which express the nuclear factor Aire. As Aire promotes the expression of tissue-specific antigens, mTECs also display a unique peptide-MHC repertoire. Strong interactions between the TCR and new peptide-MHC complexes displayed by mTECs or dendritic cells (DCs) classically induce apoptosis (clonal deletion), and both express members of the CD28 ligand B7 family as a source of costimulation. Strong TCR signals can also induce CD4$^+$ SP cells to become T_{reg} cells through induction of Foxp3. T_{reg} cell induction requires signals from members of the TNFR family, including OX40 and GITR, whose ligands are expressed on mTECs. The induction of T_{reg} cells also requires TGF-β, which is abundantly produced by mTECs. As for IELp cells in the cortex, it remains unclear how frequently the induction of T_{reg} cells occurs (relative to deletion). SP thymocytes that continue to weakly recognize self peptide–MHC ligands ultimately emigrate from the thymus and form the naive T cell pool in peripheral lymphoid organs.

of reactivities over which T_{reg} cell development was observed was surprisingly broad, which would provide an explanation for the observed overlap of the TCR repertoires of T_{reg} cells and non-T_{reg} cells and would suggest either that the TCR signaling thresholds among positive selection, clonal deletion and T_{reg} cell development are not rigid or that the natural variation in signaling capacity between cells with identical TCRs is large. Theoretical modeling posits an integration of different signal thresholds together with a loss of TCR sensitivity over time that allows the divergent fate outcomes of positive selection and T_{reg} cell induction, an interesting idea that will need further experimental testing[49]. In the future, full understanding of the role of the antigen receptor will require comprehensive elucidation of the overlap of the TCR repertoires of conventional CD4$^+$ T cells, T_{reg} cells and cells that bear MHC class II–restricted TCRs and undergo deletion.

Medullary specialization for T_{reg} cell induction

Transgenic and retrogenic models using TCRs cloned from T_{reg} cells have confirmed the crucial role of antigen receptor signaling[50], yet they also make clear that a particular TCR specificity alone is

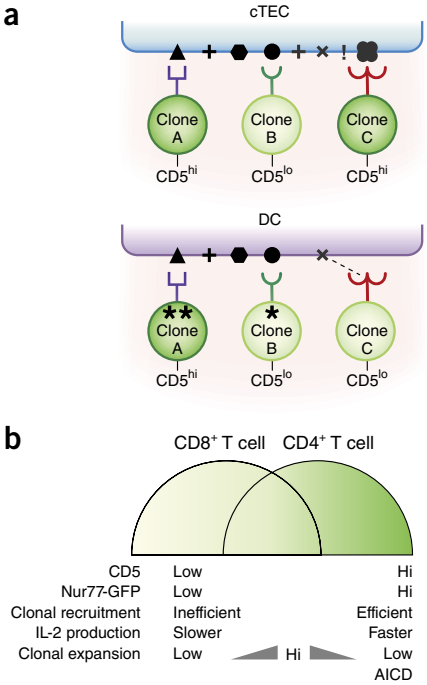

Figure 3 Self-reactivity establishes the activation potential of naive T cells. (**a**) Naive T cells, as a population, display variable abundance of CD5, and this heterogeneity reflects the strength with which the TCR of a particular clone recognizes self peptide–MHC. This has been confirmed with Nur77^{GFP} mice, in which CD5 expression correlates with GFP expression. An additional means by which CD5 heterogeneity may arise is by the recognition of public self peptide–MHC versus private self peptide–MHC. We consider self peptide–MHC complexes displayed by both positive-selecting APCs in the thymic cortex (cTECs) and dendritic cells (DCs) in the periphery as 'public'. 'Private' self peptide–MHC complexes are those displayed only by cTECs, owing to their unique expression of proteolysis factors. A clone selected on 'private' peptide–MHC complexes will fail to continue to recognize the same peptide-MHC complexes in the periphery, which results in lower CD5 expression. CD5^{hi} clones have more basal phosphorylation of TCRζ than do CD5^{lo} clones and have altered gene-expression patterns compared with those of CD5^{lo} clones, factors that contribute to their activation potential. (**b**) The abundance of CD5 on naive T cells correlates with their ability to respond to foreign antigens during an immune response. CD5^{lo} cells are recruited less efficiently into immune responses, produce IL-2 more slowly and undergo less clonal expansion. Interestingly, naive CD4$^+$ T cells express more CD5 overall (and GFP in Nur77^{GFP} mice) than do naive CD8$^+$ T cells and are more effective at rapidly producing IL-2. Because CD4$^+$ T cells can undergo activation-induced cell death (AICD) when overstimulated, the CD4$^+$ clones with the very highest CD5 expression will not be as well represented in memory response.

not sufficient to induce T_{reg} cell differentiation, as the generation of T_{reg} cells is detectable only at low precursor frequencies and is never observed to be complete (with 100% of the cells being Foxp3$^+$). Thus, there is a limiting 'niche' for T_{reg} cell development at the level of competition for TCR ligands or for other factors. In addition to TCR signaling, T_{reg} cell differentiation requires multiple other factors, including the coreceptor CD28, interleukin 2 (IL-2), the morphogen TGF-β and signaling via the lipid kinase PI(3)K[44], as well as costimulation by members of the TNF receptor (TNFR) superfamily[51]. The availability of one or more of these factors may account for the observation that T_{reg} cell development is dependent on medullary epithelial cells[33] (**Fig. 2**). An intriguing idea that has been proposed is that thymocyte apoptosis may enhance the generation of T_{reg} cells through the production of TGF-β by phagocytic cells, particularly in the medulla[52]. Likewise, certain TNFR ligands, such as OX40L and GITRL, are also expressed on medullary APCs[51]. Furthermore, it may be an intrinsic property of medullary thymocytes to respond to such cues, as semimature SP thymocytes that reside in the medulla have high susceptibility to *Foxp3* induction[53]. This may reflect the requirement for TNFR signaling, as the expression of members of the TNFR superfamily, such as GITR, is upregulated only in medullary thymocytes through a process dependent on the kinase TAK1 during positive selection[51]. In this context, it is interesting that tumor-infiltrating self antigen–specific T_{reg} cells are dependent on Aire, expressed in medullary epithelial cells[14]. Although Aire-deficient mice are not profoundly lacking in T_{reg} cells, the extent to which the T_{reg} cell repertoire is affected by Aire deficiency, and whether this contributes to the autoimmune pathogenesis in mice and humans, is now of great interest.

Signal thresholds in *i*NKT cell development

*i*NKT cells have a unique semi-invariant α-chain variable region 14–α-chain joining region 18 (V_α14-J_α18) TCR that recognizes lipid molecules presented by CD1d. These cells develop in the thymus as do other αβ T cells, but they have many characteristics distinct from those of conventional T cells, including their dependence on homotypic interactions between members of the signaling lymphocytic-activation molecule (SLAM) family and the use of other DP thymocytes as APCs, which makes the cortex a unique environment for their development (**Fig. 2**). *i*NKT cells have a previously activated phenotype, as do T_{reg} cells, which suggests that they perceive strong TCR signals during development. Indeed, analyses of transcription factor Egr-2– reporter mice[54] and nuclear hormone receptor Nur77–reporter mice[47] are consistent with this idea. A crucial role for strong TCR signaling comes from observations that *i*NKT cell development requires a full complement and immunoreceptor tyrosine-based activation motifs[55] and is abrogated in mice lacking miR-181a-1 and miR-181b-1 (ref. 36). A published study has more fully explored the extent to which TCR affinity for agonist self ligands controls *i*NKT cell differentiation[56] through the use of mice with transgenic expression of a TCRβ chain that increases affinity for self lipid–CD1d complexes when paired with canonical V_α14-J_α18 rearrangements. *i*NKT cells that express the high-affinity canonical V_α14-J_α18 rearrangements do not mature in these transgenic mice, which proves that *i*NKT cells are subject to clonal deletion. A small proportion of cells escape deletion in these mice and utilize a noncanonical V_α14-J_α18 rearrangement with a 40-fold lower affinity for self ligands. However, those TCRs do not lead to the efficient induction of the *i*NKT cell lineage–specific transcription factor PLZF and thus display aberrant proliferation, trafficking and effector function[56]. This study thus suggests that proper *i*NKT cell differentiation requires a specific window of TCR signal perception together with other anatomically restricted lineage-induction factors, similar to the differentiation of T_{reg} cells (**Fig. 2**).

Some highly self-reactive clones divert to IELs

The intestinal epithelium is interspersed with a heterogenous lymphocyte population. IELs expressing TCRαβ and CD8αα are referred to as 'natural' IELs and are thought to be important for mucosal tolerance and homeostasis[57]. Such natural IELs are generally thought to be derived from thymic precursors and to require TGF-β[58] and IL-15 (ref. 59) for survival. This natural IEL pool has an activated phenotype and contains 'forbidden clones', and experiments with animal models with transgenic TCR expression suggest that IELs are dependent on high-affinity TCR interactions in the thymus[60]. It has been shown that rescuing cells from thymic clonal deletion through deficiency in CD28 or transgenic overexpression of the antiapoptotic protein Bcl-2 leads to increased IEL development[61], which suggests that the precursors of gut IELs are cells destined for clonal deletion in the thymus. It is unclear why cells destined for deletion in the thymus would sometimes traffic to the gut and adopt this fate, nor is it understood how frequently it occurs and whether it is stochastic or regulated by specific mechanisms. Nonetheless, there are many such cells in the gut, and their potential to serve an important role in mucosal tolerance and homeostasis merits further examination.

Peripheral self-reactivity and naive T cell homeostasis

The majority of thymocytes that emerge after thymic selection are 'conventional' naive TCRαβ T cells rather than the agonist-selected populations discussed above. Considerable evidence suggests that these naive T cells maintain a low response to self peptide–MHC ligands and that those interactions are important for sustaining a basal TCR signaling and naive T cell homeostasis. At the same time, accumulating evidence indicates that the strength of the interaction of a T cell with self peptide–MHC complexes is not uniform among naive T cell clones and that even within this 'low-affinity' range, the extent of self-recognition has a substantial effect on the T cell's ability to respond to homeostatic cues and to react to foreign peptide–MHC ligands in an immune response. Hence, as for developing thymocytes, a continuum in intensity of self-awareness has relevance for function and maintenance of the peripheral naive T cell pool. Furthermore, as discussed below, accumulating data indicate that the effect of self peptide–MHC interactions in the periphery is not identical for CD4$^+$ T cells and CD8$^+$ T cells, which suggests an unexpected layer of subset specialization.

It has been known for many years that naive CD8$^+$ or CD4$^+$ T cells exhibit basal TCR signaling (manifest as partial phosphorylation of the TCRζ chain) and that this is lost with deprivation of self peptide–MHC class I or class II molecules, respectively[62–65]. Likewise, naive T cells have basal expression of green fluorescent protein (GFP) in mice with transgenic expression of GFP from the *Nr4a1* (encoding Nur77) locus (Nur77^{GFP} mice), but this expression is rapidly lost when naive CD4$^+$ T cells are deprived of self MHC class II molecules[47]. However, the relevance of these weak TCR signals has been more difficult to discern. Initial studies of T cell homeostasis have suggested an essential role for self peptide–MHC ligands in the survival of naive T cells[66,67], but interpretation of those findings has been complicated by the discovery that recognition of self peptide–MHC complexes is one of the cues that induces naive T cells to proliferate and acquire memory phenotype in situations of lymphopenia (often used in studies of T cell homeostasis)[62,68]. There is general consensus that recognition of self peptide–MHC complexes elicits lymphopenia-induced proliferation (LIP) of both CD4$^+$ T cells and CD8$^+$ T cells and that similar

encounters by the TCR are needed for the survival of naive CD8+ T cells in a lymphocyte-replete environment. Determining whether the survival of CD4+ T cells requires the binding of TCRs to self peptide–MHC class II complexes has proven more controversial[62,68]; however, comprehensive studies have suggested a more complex picture in which competition with CD8+ T cells (but not with other CD4+ T cells) enforces dependence of the survival of naive CD4+ T cells on self peptide–MHC class II (ref. 69), although the molecular mechanisms for this regulation are currently unclear. And whereas the maintenance of memory CD8+ T cells seems to be independent of the recognition of self peptide–MHC (and even of TCR expression)[62,68], studies of a TCR-loss model have shown that a subset of memory-phenotype CD4+ T cells continue to depend on TCR signals for maintenance[70].

Heterogeneity in self-sensitivity

Part of the difficulty in getting a clear impression of how self peptide–MHC complexes affect T cell homeostasis is that not all naive T cells respond to homeostatic cues in the same way. Comparing the responses of naïve T cells from mice that express different transgenic TCRs has revealed radically divergent capacities to proliferate and acquire memory-like properties during LIP[68]. Likewise, there is notable variability in the capacity of naive CD8+ T cells expressing different transgenic TCRs to respond to the cytokines IL-7 and IL-2 (refs. 71,72). Relating this heterogeneity to self peptide–MHC recognition is inherently difficult. For conventional T cells, the affinity of the TCR for self peptide–MHC ligands is predicted to be very low. The peptide-MHC complexes that drive thymic positive selection bind the TCR with low affinity (and similar ligands can promote naive T cell homeostasis), and it is likely that multiple self peptides can engage a given TCR in such interactions, which makes it impractical to identify and characterize all relevant self peptide–MHC complexes in a physiological setting[73,74].

Fortunately, studies of mice have revealed valuable phenotypic markers that correlate with the intensity of the recognition of self by the TCR. Of these, the marker CD5 has proven the most reliable (**Fig. 3a**). Analyses of CD4+ or CD8+ naïve T cells from both normal mice and mice with transgenic TCRs expressed have revealed that increasing expression of CD5 correlates positively with the degree of basal phosphorylation of TCRζ[64,65,75], the capacity to rapidly produce IL-2 and induce phosphorylation of Erk after T cell stimulation[64] and the capacity of T cells to undergo LIP[72,76,77]. We have observed that CD5^{hi} naive CD4+ and CD8+ T cells also show higher GFP expression than CD5^{lo} naïve T cells in Nur77^{GFP} mice, and that CD5^{hi} and CD5^{lo} naive CD8+ T cells have different gene-expression profiles (K.A.H., S.C.J. and R. Fulton, unpublished data); this suggests that the CD5^{hi} pool has improved readiness for activation and functional differentiation. CD5 itself is a cell-surface molecule that can negatively regulate TCR signals through association with the phosphatase SHP-1 and has been proposed to function as a rheostat in dampening TCR signaling during thymic development[39]. Indeed, the abundance of CD5 surface expression is set during thymic development and is typically maintained in the periphery[64,78], although deprivation of the interaction of T cells with self peptide–MHC in the periphery can cause a decrease in CD5 expression[79]. Because T cells with the highest expression of CD5 seem to show the strongest reactivity to self peptide–MHC, the proposed rheostat function of CD5 seems to be insufficient to normalize TCR reactivity for all clones, which makes this marker valuable for the isolation of cells with different degrees of active self-sensitivity. Studies of CD5-deficient T cells from mice with transgenic TCR expression support the concept that this molecule restrains TCR signaling, yet loss of CD5 does not effectively normalize the differences in the responses of clones normally selected into CD5^{hi} or CD5^{lo} pools[64,65]. Hence, although CD5 expression levels have proven to be an effective and useful marker to subset naïve T cells, the functional relevance of CD5 itself is controversial.

Intriguingly, differences emerge in comparisons of naive CD4+ and CD8+ T cells (**Fig. 3b**). Expression of GFP in Nur77^{GFP} reporter mice is higher in naive CD4+ T cells than in naive CD8+ T cells[47], and there is a notably greater range in the abundance of phosphorylated TCRζ between resting CD5^{lo} and CD5^{hi} populations within the CD4+ T cell pool than in the CD8+ T cell pool[65]. Further, although pharmacologically induced IL-2 production increases with CD5 expression on both CD4+ and CD8+ T cells, the overall IL-2 expression is notably higher in CD4+ T cells[64]. On the other hand, CD5^{hi} naive CD8+ T cells are induced to proliferate when exposed to sustained stimulation with a high dose of IL-2 (in the absence of foreign peptide–MHC ligands), but this response is not observed for naive CD4+ T cells (regardless of their CD5 expression). This cytokine-induced proliferation correlates with the 'preferential' localization of CD122 (the β-chain of the receptor for IL-2) to lipid rafts in CD5^{hi} CD8+ T cells[72]; hence, the failure of naive CD4+ T cells to engage in this response may reflect the lower expression of CD122 on CD4+ T cell subsets than on their CD8+ T cell counterparts. We have observed that CD5^{hi} naive CD8+ T cells show elevated expression of factors associated with early T cell activation (including T-bet, Eomes, CXCR3 and Xcl-1), as well as patterns of gene expression that indicate improved preparation for responsiveness and cell division (K.A.H., S.C.J. and R. Fulton, unpublished data), yet such changes have not been reported for naive CD4+ T cell subsets. Thus, there may be additional features of the CD5^{hi} and CD5^{lo} populations among naive CD4+ T cells that are distinct from those of CD8+ T cell populations.

Although basal TCR signaling and the associated surface abundance of CD5 are considered to reflect the intensity of self-recognition by naive T cells, this does not necessarily mean they reflect the affinity and/or avidity of the interaction of the TCR with self peptide–MHC. Instead, such signals might reflect the tissue distribution of suitable self peptide–MHC ligands (**Fig. 3a**). The frequency with which a given T cell would encounter a relevant self peptide–MHC complex might dictate its steady-state basal TCR signaling properties. A potential reflection of this is the finding that selection of CD8+ clones from mice with some transgenic TCRs strongly depends on β5t expression, whereas for others it is independent of β5t. Intriguingly, this generally correlates with the CD5 expression status of the T cells, with CD5^{hi} cells being less dependent on β5t[80]. Consistent with that finding, the populations of CD8+ T cells selected in mice lacking β5t show enrichment for CD5^{hi} cells. We are tempted to conclude that cells selected on self-MHC molecules containing β5t-independent 'public' peptides may encounter the very same peptide-MHC complex on other cells in the periphery (imparting a basal TCR signal), whereas this would be less likely for cells selected on ligands containing private β5t-dependent peptides. It is currently unclear whether the same pattern applies to CD4+ T cells selected on private or public peptides presented by MHC class II molecules. This concept has been discussed elsewhere[40] and will not be further explored here.

Self-sensitivity regulates reactivity to foreign antigens

Although the degree of sensitivity to self peptide–MHC complexes affects the basal intensity of TCR signaling, gene-expression patterns and the proliferative response to lymphopenia, those differences, perhaps surprisingly, do not seem to affect steady-state survival of naive T cells. Hence, studies of CD5^{hi} and CD5^{lo} polyclonal CD4+ and CD8+ T cells

have reported similar maintenance (and preservation of their $CD5^{hi}$ or $CD5^{lo}$ status) after adoptive transfer into a lymphocyte-replete environment[64,65] (K.A.H., S.C.J. and R. Fulton, unpublished data). These data suggest that a minimum TCR signal suffices for T cell survival, whereas greater self-awareness provides naive $CD8^+$ T cells with greater sensitivity to homeostatic opportunities.

However, multiple studies have suggested the response to foreign antigen is influenced by the sensitivity of naive T cells to self peptide–MHC complexes, although the exact nature and basis of that effect remains controversial. It has been reported that the abundance of CD5 on naive $CD4^+$ T cells not only corresponds with the intensity of basal TCR signaling but can also be used to predict the capacity of these cells to bind specific foreign peptide–MHC ligands (as revealed by staining with peptide-MHC tetramers)[65]. That finding led to the intriguing hypothesis that the interaction with self peptide–MHC during thymic positive selection not only provides a signal for T cell maturation but also produces T cells that are better able to bind foreign peptide–MHC. However, other studies have reached divergent conclusions. Two strains of mice have been generated with T cells that have transgenic expression of TCRs that bind the same foreign peptide–MHC ligand with very similar affinity yet were selected into $CD5^{hi}$ or $CD5^{lo}$ populations (with corresponding differences in basal TCR signaling)[64,81]. Likewise, studies of normal polyclonal $CD8^+$ T cells and $CD8^+$ T cells from mice with transgenic TCR expression did not indicate a consistent difference in the binding of foreign peptide–MHC tetramers to $CD5^{hi}$ populations versus $CD5^{lo}$ populations (K.A.H., S.C.J. and R. Fulton, unpublished data).

There is better consensus for the concept that the intensity of the basal encounter with self peptide–MHC affects reactivity to foreign antigen. Two aspects of this concept have been explored: the contribution of the recognition of self peptide–MHC during encounter with a foreign antigen, and the effects of self peptide–MHC on intrinsic properties of naive T cells before encounter with a foreign antigen. Davis and colleagues first proposed that nonstimulatory self peptide–MHC complexes may serve as 'coagonists' to assist the T cell response to foreign peptide–MHC ligands[82]. However, it remains unclear whether coagonists contribute to all T cell responses, and even where a coagonist role has been observed, there has been considerable controversy over whether coagonist encounters were peptide specific, with divergent conclusions drawn for $CD4^+$ T cells and $CD8^+$ T cells[82,83]. A published report has nicely resolved this discrepancy by showing that there are two modes of coagonist contribution, in which the importance of the affinity of the TCR for the coagonist peptide-MHC depends on the strength of the binding of the coreceptor to the foreign peptide–MHC target[84], because the coreceptor CD8 is thought to have a higher affinity for MHC class I molecules (albeit not all allelic forms) than that of the CD4–MHC class II interaction, the specificity of a TCR for coagonist ligands may be a much more stringent limitation for $CD4^+$ T cells than for $CD8^+$ T cells[84].

Three studies have investigated the foreign antigen–specific responses of $CD5^{hi}$ naive $CD4^+$ T cells versus those of $CD5^{lo}$ naive $CD4^+$ T cells[64,65] and of naive $CD8^+$ T cells (K.A.H., S.C.J. and R. Fulton, unpublished data) through the use of polyclonal models and models with transgenic TCR expression. These reports include studies of the properties of $CD5^{hi}$ or $CD5^{lo}$ naive T cells before antigen encounter and in situations in which the possibility of a coagonist role could be excluded to determine whether and how sensitivity to self peptide–MHC (reflected as CD5 abundance) alters the intrinsic capacity of naive T cells to respond to foreign ligands. As discussed above, $CD5^{hi}$ populations among both $CD4^+$ and $CD8^+$ naive T cell subsets show evidence of the increased basal TCR signaling and improved functional characteristics, and this has been found to correlate with greater expansion in response to foreign antigen[65] (K.A.H., S.C.J. and R. Fulton, unpublished data). Whereas Mandl *et al.*[65] correlated this response pattern with changes in the binding of foreign peptide–MHC by the TCR on $CD5^{hi}$ naive $CD4^+$ T cells, We found that the $CD5^{hi}$ naive $CD8^+$ T cells show more efficient clonal recruitment into the immune response and superior augmentation of their response by inflammatory cues (K.A.H., S.C.J. and R. Fulton, unpublished data). In apparent contrast to those findings, studies of two mouse strains with T cells that have transgenic expression of TCRs that recognize the same foreign peptide–MHC ligand but are positively selected into $CD5^{hi}$ and $CD5^{lo}$ populations, respectively, have shown a different pattern, whereby the $CD5^{lo}$ clones show greater expansion than the $CD5^{hi}$ group during the primary immune response[64,81]. A possible resolution of these discrepancies reflects the possibility that the $CD5^{hi}$ population among naive $CD4^+$ T cells maintains a higher basal activation state than that of $CD5^{hi}$ naive $CD8^+$ T cells (**Fig. 3b**) and thus may be more susceptible to activation-induced cell-death pathways after strong stimulation. Indeed, Allen and colleagues[64] suggest that the poor expansion of their $CD5^{hi}$ TCR transgenic clone is not due to ineffective early activation but is instead due to IL-2-driven apoptosis. In this context, we note that, relative to that on polyclonal cells, the CD5 expression on this '$CD5^{hi}$' clone was greater than on the clones analyzed by Mandl *et al.*[64,65], which potentially suggests an even greater degree of self peptide–MHC reactivity. Hence, superior intrinsic sensitivity in the $CD5^{hi}$ naive T cell pool may (at least in the $CD4^+$ T cell subset) come at the cost of greater vulnerability to elimination mechanisms. It is also worth noting that in secondary immune responses, Persaud *et al.*[64] have reported that the $CD5^{hi}$ clones expand to a greater extent than do the $CD5^{lo}$ clones. Whether this arises from avoidance of IL-2–induced cell-death pathways during the recall response or some other mechanism is unclear.

These reports are consistent in that the $CD5^{hi}$ populations of naive $CD4^+$ or $CD8^+$ T cells have enhanced intrinsic reactivity to foreign peptide–MHC stimulation, but the underlying mechanism and immediate consequences for clonal recruitment and expansion are harder to predict and may depend on where cells lie on the scale of self-reactivity.

Conclusions

T cells continually experience a broad range of self-reactivity that begins when they first express a surface receptor in the thymus and continues throughout their lifespan and residency in peripheral lymphoid organs or tissues. The developmental stage of the T cell and the affinity and costimulatory or cytokine context of these interactions affect what kind of response is ultimately made and maintained. Thus, self-interactions are crucial to the sophisticated and highly controlled adaptive immune response.

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