HHS Public Access

Author manuscript

Curr Opin Immunol. Author manuscript; available in PMC 2022 December 01.

Published in final edited form as:

Curr Opin Immunol. 2021 December ; 73: 43–49. doi:10.1016/j.coi.2021.09.007.

Functional heterogeneity and adaptation of naive T cells in response to tonic TCR signals

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Abstract

Mature $CD4^+$ and $CD8^+$ T cells constitutively experience weak T cell receptor (TCR) stimulation in response to self-antigens, termed *tonic* (or *basal*) signaling. How tonic TCR signal strength impacts T cell responses to foreign antigens is an active area of investigation. Such studies rely on surrogate markers of tonic signal strength, including CD5, Ly6C, and transgenic reporters of *Nr4a* genes. Recent research indicates that strong tonic TCR signal strength influences basal T cell metabolism, effector differentiation, and TCR signal transduction. T cells that experience the strongest tonic TCR signaling exhibit features of T cell activation and negative regulation. These data suggest a model whereby adaptation to tonic signaling has lasting effects that alter T cell activation and differentiation.

Introduction

T cells are positively selected in the thymus for weak reactivity to self-peptide antigens presented by Major Histocompatibility Complex (self-pMHC). Naive $CD4^+$ and $CD8^+$ T cells continue to experience low-level T cell receptor (TCR) signaling in response to self-pMHC in the periphery, termed *basal* or *tonic* signaling [1,2]. Tonic TCR signaling is sufficient to induce constitutive tyrosine phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the TCR complex and ZAP-70 recruitment to phosphorylated ζ chains, but not the production of IL-2 or clonal expansion [3–5]. A growing body of evidence suggests that tonic TCR signals experienced prior to cognate antigen exposure influence primary and secondary responses of $CD4^+$ T cells. Here, we discuss recent advances in our understanding of how tonic TCR signaling is detected, how naive T cells adapt to varying tonic TCR signal strengths, and the impact on effector responses.

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Conflict of interest statement

Declarations of interest: none.

Markers of tonic signaling

CD5

The surface glycoprotein CD5 is a well-established surrogate marker of tonic signaling [6]. Naive $CD4^+$ and $CD8^+$ T cells expressing the highest levels of CD5 exhibit increased ζ-chain phosphorylation compared to $CD5^{LO}$ cells [7]. The magnitude of CD5 expression can vary between T cells with different TCR specificities, as demonstrated by comparing TCR transgenic populations [7]. However, CD5 expression and TCR specificity are not strictly linked, as two TCR transgenic strains can recognize the same *Listeria monocytogenes* epitope with similar affinity but exhibit different surface levels of CD5 [8,9]. In sum, CD5 has been a useful marker to identify T cells that experience relatively weak or strong tonic signaling.

Reporters of *Nr4a*

The immediate early gene *Nr4a1* (encoding Nur77) is an orphan nuclear receptor in the same family as *Nr4a2* and *Nr4a3* [10]. Antigen-receptor stimulation, but not cytokine stimulation, induces expression of *Nr4a1* in T and B cells [11,12]. Two Nur77-GFP reporter transgenes have been independently generated [11,12]. Like CD5, Nur77-GFP expression is initiated during thymic development and maintained in mature peripheral T cells [11,13]. The level of basal Nur77-GFP in naive T cells is relatively stable, as the majority of sorted cells retain similar Nur77-GFP intensity after adoptive transfer into WT recipients, but not in MHC II-deficient hosts [11,13]. Stimulation with cognate pMHC or TCR crosslinking antibodies leads to rapid upregulation of Nur77-GFP expression [11,14]. While Nur77-GFP expression is sensitive to TCR stimulation induced by self-pMHC interactions, Bending and colleagues recently showed that *Nr4a3* reporter expression is two- to threefold less sensitive to TCR stimulation and is selectively activated by cognate pMHC stimulation [15].

Ly6C

Ly6C is a GPI-linked receptor with unknown function [16] that is upregulated on a subpopulation of naive $CD4^+$ T cells shortly after thymic egress [17]. In contrast to CD5 and Nur77-GFP, Ly6C expression *inversely* correlates with reactivity to self-pMHC, as demonstrated by decreased ζ-chain phosphorylation in $Ly6C^+$ naive $CD4^+$ T cells (Figure 1) [17]. Within the regulatory T cell (Treg) population, Ly6C expression also marks a subset of $Foxp3^+$ cells that experience weaker tonic signaling and exhibit decreased suppressive activity [18,19]. Mechanistically, the downregulation of Ly6C expression is dependent on TCR-induced Ca^{2+} signaling [20].

Combination of markers

Our laboratory investigated whether a combination of markers could improve the dynamic range of tonic signaling that can be detected [13]. The combination of Nur77-GFP plus Ly6C exhibited a broader dynamic range compared to GFP plus CD5 or Ly6C plus CD5. In this scheme, Nur77-GFP^{LO} $Ly6C^+$ cells experience the weakest tonic signaling, and Nur77-GFP^{HI} $Ly6C^-$ cells experience the strongest tonic signals, as shown by ζ-chain phosphorylation [13]. While Nur77-GFP^{HI} $Ly6C^-$ cells express high levels of CD5, high

CD5 expression alone does not solely mark the Nur77-GFP^{HI} Ly6C^{-} subset. These data raise the possibility that the range of tonic signal strength extends further than previously thought. Future studies with new markers or combinations of markers may improve the "resolution" to detect tonic signal strength.

Role of tonic signaling in CD4^{+} T cells

Our recent studies revealed that weak tonic signal strength, experienced by naive CD4^{+} T cells with a Nur77-GFP^{LO} Ly6C^{+} phenotype, consistently correlated with the most robust activation, as reflected by IL-2 secretion, cell division, and ERK phosphorylation [13]. Nur77-GFP^{MED} Ly6C^{+} and Nur77-GFP^{MED} Ly6C^{-} cells, which experience moderate tonic signal strength, mounted IL-2 responses comparable to Nur77-GFP^{LO} Ly6C^{+} cells early (4hr post-stimulation), but the IL-2 responses of GFP^{LO} Ly6C^{+} cells were consistently higher at later time points. These findings are compatible with the concept that strong tonic signaling correlates with short-lived acute responses. However, Nur77-GFP^{HI} Ly6C^{-} cells, which experience extensive tonic signaling, consistently exhibited decreased responsiveness to stimulation. This result is congruent with a "tunable" model where lymphocytes adapt to the amount of tonic signaling they experience [21]. Consequently, cells that experience strong tonic TCR signaling shift their activation threshold and effectively become de-sensitized to subsequent TCR stimulation (Figure 2).

In experimental systems where CD5 was used to mark basal TCR signaling strength, CD5^{HI} cells exhibit greater ERK phosphorylation and IL-2 production in response to acute stimulation [9]. However, at the late stages of the primary response, higher percentages of CD5^{HI} TCR transgenic cells undergo apoptosis than CD5^{LO} cells [8]. A model based on these studies suggests that strong tonic signaling correlates with a robust acute response that is not sustained due to increased cell death [22]. Hence, one potential consequence of naive CD4^{+} T cell heterogeneity is that different clones may engage in primary responses to foreign antigens with different kinetics. Finally, while some of the correlations identified using CD5 and Nur77-GFP as correlate markers of tonic TCR signaling strength overlap, differences remain. Further research is needed to clarify how tonic TCR signaling impacts CD4^{+} T cells at various stages of primary responses.

The molecular pathways activated by tonic TCR signaling remain incompletely understood [1]. A major challenge in studying the basal TCR signaling machinery has been the lack of an in vitro model. However, studies have highlighted how tonic signal strength can impact CD4^{+} T cells. For instance, in a mouse model with impaired NF-κβ signaling, naive T cells express lower levels of the IL-7 receptor α-subunit and exhibit reduced cell survival compared to WT cells [23], suggesting that the downstream effects of basal TCR signaling may affect cell survival. More recent studies add to the complexity and suggest that tonic TCR signaling could have both positive and negative effects on T cell effector function and influence T cell differentiation.

Tonic signal strength influences effector functions and cell fate decisions

Th1-polarized CD5^{HI} cells express lower levels of Tbet and produce less IFNγ relative to CD5^{LO} cells upon stimulation [24]. Similarly, strong tonic signaling correlates with

impaired T follicular helper cell differentiation [25]. In contrast, naive $CD4^+$ T cells that experience increased tonic signaling, such as $CD5^{HI}$, $Ly6C^–$, or $Nur77\text{-}GFP^{HI}$ $Ly6C^–$ populations have a higher propensity for Foxp3 expression under induced Treg differentiation conditions [13,17,26]. This functional heterogeneity may reflect mechanisms to attenuate highly self-reactive cells or divert them from an inflammatory effector state. However, in a lymphopenic environment, strong tonic signal strength correlates with increased autoreactive potential, as $Ly6C^–$ naive $CD4^+$ cells induce more severe disease in an adoptive transfer model of colitis compared to $Ly6C^+$ cells [17]. A correlation between basal TCR signaling and immunopathology can also be observed in mice that harbor mutations in the TCR signaling pathway [27–29]. The ZAP-70 mutation in SKG mice renders ZAP-70 hyporesponsive and thus allows positive thymic selection of T cell clones that otherwise would have undergone negative selection, resulting in an arthritis-like disease [30]. In the SKG mouse model of rheumatoid arthritis, $Nur77\text{-}GFP^{HI}$ naive $CD4^+$ cells have increased arthritogenic potential compared to $Nur77\text{-}GFP^{LO}$ cells [27]. Likewise, T cells expressing a point mutation in LAT Tyrosine 136 experience weaker tonic signaling but paradoxically induce a Th2 lymphoproliferative disorder [28]. More specifically, weaker tonic signaling reduces the constitutive nuclear export of histone deacetylase 7, a transcriptional repressor of *Nr4a1* and *Irf4* [31]. Furthermore, a point-mutation in Rasgrp1 increases tonic mTORC1 signaling, which skews $CD4^+$ T cells toward Th2 differentiation and instigates immunopathology in mice [29]. Together, these studies underscore that (i) TCR signaling can influence T helper effector function and cell fate decisions and (ii) strong tonic TCR signals correlate with increased autoimmune pathology if tolerance is compromised.

Potential mechanisms of negative regulation

An elegant study by Trefzer et al. investigated the effects of chronic antigen stimulation on $CD4^+$ T cells in the absence of infection by utilizing a TCR transgenic mouse model in which cognate antigen expression is inducible [32]. In contrast to acute cognate antigen exposure, chronic exposure impaired cytokine production and induced gene expression signatures that bear similarities with gene expression patterns in anergic and exhausted T cells. Although constitutive cognate antigen stimulation differs from the constitutive low-level TCR stimulation T cells experience from self-pMHC interactions, T cells may similarly adapt to strong self-pMHC signals.

CD5 expression positively correlates with higher expression of IκB (a negative regulator of NFκB) [33], suggesting that self-reactive naive T cells potentially counterbalance an increased capacity of tonic signaling by expressing negative regulators of TCR signaling. A negative regulator of strong tonic signaling may also be CD5 itself. CD5 deficiency results in hyperresponsive TCR signaling in thymocytes, and mature $CD5^{HI}$ T cells exhibit a decreased TCR-induced calcium flux, consistent with CD5 as an inhibitor of TCR signaling [34–36]. Recent analyses of the CD5 interactome by mass spectrometry have highlighted several potential binding partners in mouse $CD4^+$ T cells. One analysis identified negative regulators such as the E3 ubiquitin ligase Cbl-b and the phosphatase Ubash3a in the CD5 signalosome [37]. An independent analysis identified a required role for Tyrosine 429 of CD5 in the recruitment of c-Cbl, Cin85, and CrkL, which assemble molecular complexes

that included both negative regulators (phosphatases SHIP-1 and Ubash3a) and positive regulators (PI3K) [38]. Moreover, CD5 also has a reported pro-survival role in mature T cells [39]. Hence, CD5 may have both positive and negative regulatory roles in TCR signaling, although further research is necessary to define the underlying mechanisms.

Tolerized and anergic T cells express high levels of Nur77 [40–42], and Nur77-deficiency impairs the induction of tolerance and exhaustion [40,41]. Furthermore, Nur77 deficient $CD4^+$ T cells exhibit enhanced basal and maximal respiration and glycolytic capacity [43] in addition to enhanced IL-2 secretion upon stimulation [44], consistent with a role for Nur77 as a negative regulator of T cell activation. Moreover, extensive tonic signaling results in elevated levels of CBL-b and GRAIL, E3 ubiquitin ligases that negatively regulate TCR signal transduction and are associated with T cell anergy [13,45,46].

Increasing tonic signal strength attenuates metabolism

Ectopic expression of Scn5a, the pore-forming subunit of a voltage-gated sodium channel, enhances tonic TCR signal strength as reflected by elevated CD5 expression [47,48]. Increasing tonic signal strength by ectopic expression of Scn5a resulted in impaired cell expansion during a primary response to *L. monocytogenes* infection [48]. Furthermore, Scn5a-expressing cells have a decreased basal and maximal respiration rate and glycolytic rate [49]. These results suggest that strong tonic signaling limits the basal metabolism of naive T cells, perhaps to limit the autoimmune potential of self-reactive T cells.

Role of tonic signaling in $CD8^+$ T cells

High CD5 expression positively correlates with increased persistence of antigen-specific $CD8^+$ T cells during a primary response [50], suggesting a positive correlation between tonic signal strength and the magnitude of naive $CD8^+$ T cell responses to foreign/agonist pMHC. Jameson's group compared $CD8^+$ cells specific to the self-antigen tyrosinase-related protein 2 (Trp2) harvested from WT and Trp2-deficient mice [51]. Although the Trp2-specific T cells were phenotypically and transcriptionally similar, Trp2-specific T cells from Trp2-deficient mice induced greater pathology in an adoptive transfer model of vitiligo. Moreover, a positive correlation between the expression of CD5 and the protein tyrosine phosphatase non-receptor type 2 (PTPN2), a negative regulator of TCR-proximal signal transduction, was detected in naive $CD8^+$ T cells [52]. These findings are consistent with the concept that negative feedback from strong self-pMHC interactions reduces the pathogenic potential of the most self-reactive naive $CD8^+$ T cells.

Strong tonic signaling is associated with virtual memory cells

Strong tonic signaling in $CD8^+$ T cells positively correlates with the conversion of naive cells into antigen-inexperienced $CD44^{HI}$ memory phenotype cells [53], so-called virtual memory (VM) cells. Mouse models that enhance tonic signaling, such as Dock2 mutant mice and mice expressing a chimeric CD8 that couples with Lck at superphysiological stoichiometry, illustrate this correlation [54,55]. Furthermore, TCR sequencing of VM cells revealed enrichment of distinct clonotypes that, upon re-expression, possessed higher self-reactivity compared to TCRs isolated from the naive repertoire [56].

Tonic signaling in human T cells

Transcriptional analysis of human $CD5^{LO}$ vs. $CD5^{HI}$ naive $CD4^+$ T cells revealed upregulation of genes associated with TCR signaling in $CD5^{HI}$ cells [57], consistent with a previous study that demonstrated increased CD5 staining intensity on antigen-experienced $CD4^+$ T cells compared to naive cells [7]. The transcriptional profile of naive human $CD8^+$ $CXCR3^+$ cells was more similar to naive murine $CD5^{HI}$ than $CD5^{LO}$ $CD8^+$ T cells [58]. Consistent with this finding, CXCR3 expression in the murine naive $CD8^+$ population is limited to the $CD5^{HI}$ compartment [50]. Further studies are needed to build on our understanding of the functional implications of tonic signaling in naive human T cells. There appears to be some similarity in the functional capacities of human and mouse $CD5^{LO}$ and $CD5^{HI}$ $CD4^+$ cells. Re-stimulation of activated human naive $CD4^+$ T cells revealed differences in cytokine production; $CD5^{LO}$ cells produced higher levels of IFNγ under Th1 conditions [57], consistent with previous results in mice [24].

Tonic signaling strength and adoptive cell therapy (ACT)

Chimeric antigen receptor (CAR) T cell therapy is an individualized treatment strategy that relies on harvesting a patient's T cells, expanding them in vitro, and transducing them with a synthetic T cell receptor that can recognize and eliminate tumor cells upon reinfusion into the patient [59]. Some degree of tonic signaling mediated by the endogenous TCR seems beneficial for CAR T cell therapy since deletion of the TCR negatively affected CAR T cell persistence in vivo [60]. However, too much basal signaling may be detrimental since tonic signals through the synthetic CAR T cell receptor are associated with T cell exhaustion [61,62]. Furthermore, TCRs that were engineered to have increased affinity for self-MHC resulted in diminished responsiveness upon stimulation [63]. Minguet and colleagues recently demonstrated that mutating a previously unknown Lck binding motif in CD3ε impaired the recruitment of Lck to the TCR complex and attenuated T cell activation [64]. CARs incorporating this mutated binding motif induced enhanced anti-tumor responses, possibly due to reduced CAR tonic signals [64]. Hence, determining the "optimum" amount of tonic signaling for T cells used in immunotherapy may further improve the therapeutic efficacy of ACT.

Concluding remarks

The view of naive T cells as a functionally homogenous group of cells is under revision as increasing evidence reveals further heterogeneity. How the effects of tonic TCR signal strength influence T cell responses in different contexts (i.e., autoimmunity, infection, cancer) remain incompletely understood. Further studies are also needed to identify the molecular mechanisms that regulate adaptations to varying strengths of tonic TCR signaling, including at the signaling, transcriptional, and epigenetic levels.

Acknowledgments

This was supported in part by NIAMS K01 AR06548 (to B.A.) and Winship Cancer Institute #IRG-17-181-06 from the American Cancer Society (to B.A.). We thank Wendy Zinzow-Kramer and Wan-Lin Lo for critical reading of the manuscript.

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Highlights

- Surrogate markers of tonic TCR signaling include CD5, Ly6C, and Nur77-GFP
- Tonic (or basal) TCR signaling in response to self-antigens can vary in intensity
- Naive $CD4^+$ T cells adapt to the strength of tonic TCR signals they experience
- Extensive tonic TCR signals may induce negative feedback

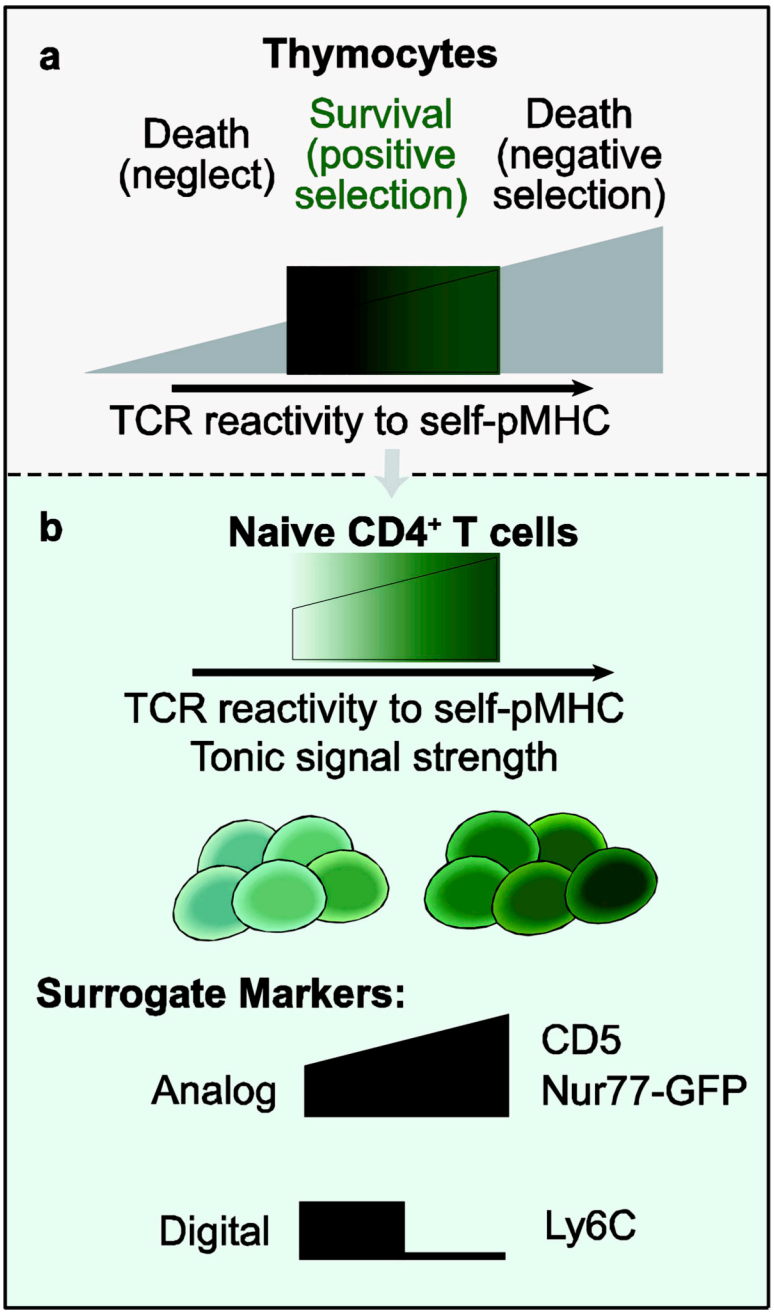

Figure 1.

TCR reactivity to self-pMHC during development and in the periphery. **a)** There is a broad range of self-reactivity in the immature $CD4^+$ $CD8^+$ double-positive population. Positively selected thymocytes (in green) exhibit self-reactivity that is neither too weak nor too strong. **b)** Self-reactivity persists in the periphery, and naive $CD4^+$ T cells experience varying strengths of tonic TCR signaling. Surrogate markers of tonic signal strength in mice include CD5, Ly6C, and the Nur77-GFP transgene.

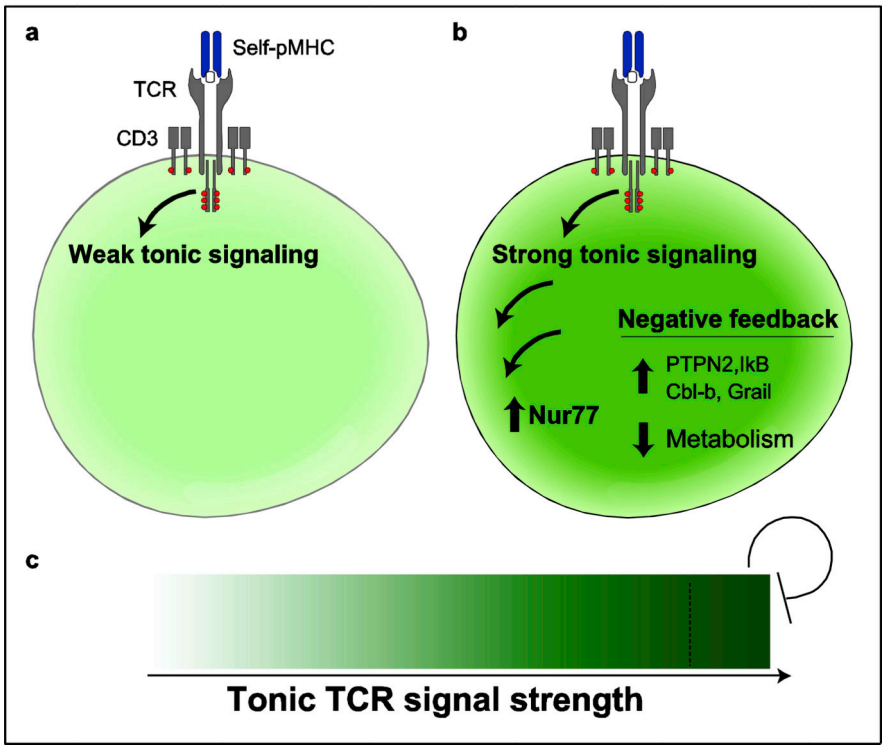

Figure 2.

Adaptation to tonic signals through negative feedback. **a)** Individual naive T cells that exhibit relatively weak reactivity to self-pMHC induce weak tonic TCR signals. **b)** $CD4^+$ T cells that exhibit strong reactivity to self-pMHC induce more extensive tonic TCR signaling, which results in higher expression of Nur77, and correlates with higher expression of negative regulators of TCR signaling and decreased basal metabolism. **c)** Naive $CD4^+$ T cells that experience the most extensive tonic signal strength have attenuated responsiveness.