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Review

The signaling symphony: T cell receptor tunes cytokine-mediated T cell differentiation

Weishan Huang[1] *and Avery August*[1]

Department of Microbiology and Immunology, Cornell University, Ithaca, New York, USA

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ABSTRACT

T cell development, differentiation, and maintenance are orchestrated by 2 key signaling axes: the antigen-specific TCR and cytokine-mediated signals. The TCR signals the recognition of self- and foreign antigens to control T cell homeostasis for immune tolerance and immunity, which is regulated by a variety of cytokines to determine T cell subset homeostasis and differentiation. TCR signaling can synergize with or antagonize cytokine-mediated signaling to fine tune T cell fate; however, the latter is less investigated. Murine models with attenuated TCR signaling strength have revealed that TCR signaling can function as regulatory feedback machinery for T cell homeostasis and differentiation in differential cytokine milieus, such as IL-2-mediated T_{reg} development; IL-7-mediated, naïve $CD8^+$ T cell homeostasis; and IL-4-induced innate memory $CD8^+$ T cell development. In this review, we discuss the symphonic cross-talk between TCR and cytokine-mediated responses that differentially control T cell behavior, with a focus on the negative tuning by TCR activation on the cytokine effects.
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Introduction

T cell lineage differentiation relies on 2 major signals: 1, via the antigen-specific TCR and the other, via 1 or more cytokine receptors. The TCR is used by conventional T cells to recognize peptide antigens presented by MHC class I or II, expressed by APCs. This occurs over different developmental stages, from antigen-driven selection in the thymus and homeostasis post-thymic emigration to the response of naïve T cells to specific antigen in the periphery during an immune response to generate effector and memory T cells and the response of the latter cells to antigen re-exposure [1, 2]. Along with TCR signaling during T cell development, homeostasis, activation, and reactivation, cytokine signaling is critical for T cell proliferation, subset specialization, memory generation, maintenance, and recall [3–5]. The common γc cytokines, including IL-2, -4, -7, -9, -15, and -21, use IL-2Rγ as part of the receptor complex and signal through JAK/STAT pathways, acting in concert with TCR signals to drive normal T cell homeostasis, as well as immune responses [6]. The effects of these cytokines on regulating the differentiation of specific T cell subsets have been well investigated; however, whether and how TCR signals modulate these cytokine effects are less understood. Here, we summarize recent findings that suggest a critical regulatory role of the TCR and its proximal signalosome in cytokine-mediated T cell development or "TCR tuning."

TCR SIGNALING AS NEGATIVE TUNER IN T CELL DEVELOPMENT AND HOMEOSTASIS

Activation of the TCR by peptide/MHC complexes triggers a downstream signaling cascade that can contribute to a variety of outcomes dependent on the stage of the T cell's life [1, 7]. Upon TCR triggering, Src family kinase Lck is activated, leading to phosphorylation of ITAMs in the TCR/CD3 complex, an event that leads to the recruitment and activation of ZAP70, which phosphorylates further adaptor proteins LAT and SLP-76 [8–12] (see also review; ref. [13]). PI3K is also activated by Lck, catalyzing the generation of phosphatidylinositol (3,4,5)-trisphosphate lipids that interact with and recruit ITK onto the plasma membrane [14]. ITK can then interact with adaptor proteins LAT and SLP-76, which is critical for efficient activation of TCR signaling [15, 16]. Y145 in SLP-76 is involved in signaling downstream of ITK, and T cells expressing the Y145F mutant of SLP-76 exhibit similar developmental and functional defects to those lacking ITK [17, 18]. This ITK/SLP-76 clustering is part of a multiprotein complex that is able to regulate the actin cytoskeleton and other downstream signals (for review, see refs. [7, 19–21]). This multiprotein complex further leads to phosphorylation of PLC-γ by ITK [22, 23]. PLC-γ catalyzes the generation of second messengers, which trigger calcium release [24–27] and the subsequent activation and nuclear translocation of NFAT [13] and activation of PKCθ → Akt → NF-κB [28–30] and RAS → MAPK [13] pathways. These pathways can control multiple events during the T cell's life, including development and differentiation.

Abbreviations: $^{-/-}$ = deficient, γc = cytokine receptor common γ chain, Akt = protein kinase B, CICD = cytokine-induced cell death, CIS1 = cytokine-induced Src homology 2 protein 1, Dok-1 = downstream of tyrosine kinase 1, Eomes = eomesodermin, Foxp3 = forkhead box p3, GFI-1 = growth factor independence 1, HIF-1α = hypoxia-inducible factor 1-α, IMP = innate memory phenotype, *i*NKT = invariant NK T cell, ITK = IL-2-inducible T cell kinase,
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1. Correspondence: Dept. of Microbiology & Immunology, College of Veterinary Medicine, VMC 5121, Cornell University, Ithaca, NY, USA. E-mails: weishan.huang@cornell.edu (W.H.); averyaugust@cornell.edu (A.A.); Twitter: http://www.twitter.com/CornellPathogen

Although TCR signaling is necessary and a positive regulator in the differentiation of $CD4^+$ naïve progenitors to Th1, Th2, and Th17 cells [31–33], it has been shown to have a more complex role in the differentiation of $CD4^+$ naïve progenitors to T_{regs} [34–37]. In $CD8^+$ T cells, TCR signals can contribute to regulatory feedback circuits that optimize $CD8^+$ T cell homeostatic maintenance regulated by the cytokine IL-7 [38]. In addition, attenuated TCR signaling functionally enhances IL-4-induced development of IMP $CD8^+$ T cells from $CD8^+$ naïve thymic progenitors [39]. The latter findings reveal the versatility of TCR signaling in modulating cytokine-mediated T cell responses beyond previously held ideas about its role in this process. Therefore, intracellular signals triggered by the TCR may contribute to the tuning of cytokine-mediated signals. In the next sections, we discuss recent data indicating that TCR-triggered pathways negatively regulate or "tune" the response of T cells to cytokine-mediated signals, thus regulating T cell homeostasis and differentiation.

TCR SIGNALING NEGATIVELY TUNES IL-2-MEDIATED T_{REG} DIFFERENTIATION

$CD4^+$ T_{regs} are important immune regulators that promote self-tolerance in prevention of autoimmunity [40, 41] and act to restrain inflammatory responses to pathogens [42, 43]. tT_{regs} develop from $CD4^+$ SP T cells in the thymus when they receive high signals via the TCR (upon encounter with high levels or high affinity antigen/MHC complexes) and the costimulatory receptor CD28. These developing tT_{regs} express the transcription factor Foxp3 and can further survive by up-regulating the IL-2R, stabilizing the T_{reg} phenotype [44–48]. Likewise, naïve $CD4^+$ T cells in the periphery can be skewed toward the T_{reg} fate when their TCR is triggered in the presence of the IL-2 and TGF-β, leading to the generation of iT_{regs} [49–53]. TCR signals that trigger the development of T_{regs} have been under intense study, and the strength of the TCR signal has been suggested to be a crucial parameter in the development of T_{regs} (e.g., tT_{regs} [54–57]). TCR signals are critical for the induction of Foxp3, as well as Foxp3-independent effects that lead to the development of T_{regs} (e.g., iT_{regs} [58, 59]). The balance between IL-2 and TGF-β is critical for iT_{reg} abundance and population size [60], and IL-2 signaling through STAT5 is indispensible for the survival of Foxp3-expressing cells during tT_{reg} generation and homeostasis [45, 46]. The availability of IL-2 signaling can adjust the sensitivity of T_{reg} to TCR signals during homeostatic proliferation, whereas TCR signals have been shown to be dispensable in the presence of elevated IL-2 [61]. Under pathogenic conditions, iT_{regs} have been shown to be insensitive to activation-induced cell death but are very sensitive to IL-2 deprivation-induced death; TCR religation triggers an ERK and PI3K/mTOR-mediated loss of Foxp3 expression, resulting in the activation of an effector program in these cells, whereas the presence of TGF-β can attenuate the loss of Foxp3 [62]. TGF-β signaling activates the transcription factors Foxo1 and Foxo3a, which promote Foxp3 expression in iT_{regs} [50, 53, 63]. This transcriptional activation of Foxp3 can be repressed by activation of the PI3K/Akt/mTOR pathway downstream of TCR [37] (**Fig. 1**). Intriguingly, Foxp3 negatively regulates TCR signaling circuits by directly suppressing components of the TCR proximal signalosome, including ZAP70 and ITK, as well as IL-2 [64], which may be a critical route for maintenance of tT_{regs}. This cross-talk among TCR, IL-2, and TGF-β signaling pathways thus enables the TCR to act as a tuner of T_{reg} differentiation (Fig. 1).

The intensity of TCR signaling has been suggested be an important factor in regulating T_{reg} development, but its definitive role is unclear. Whereas it is reported that development of tT_{regs} require high TCR signals [57, 65], it has also been suggested that TCR signals may need to be attenuated early after activation for optimal iT_{reg} development [59]. Other data also suggest that low antigen dosage or impaired TCR signaling favors tT_{reg} and iT_{reg} differentiation [34–37, 60, 66]. Although TCR activation is required to initiate T_{reg} differentiation, high TCR signaling triggered by high antigen dose or high concentration of anti-CD3ε antibody induces

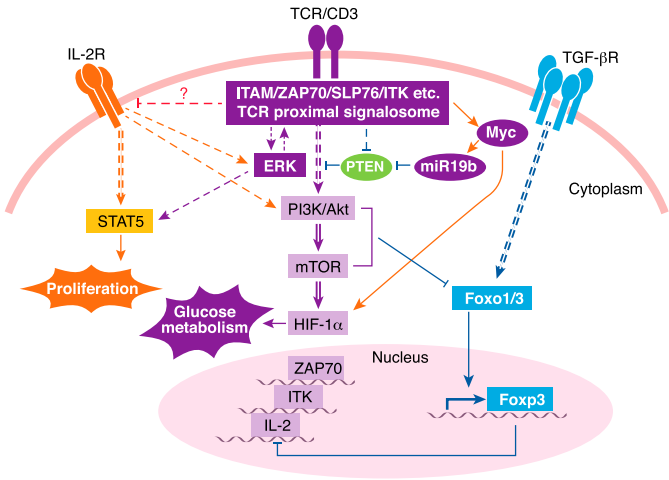

Figure 1. TCR tuning of IL-2-mediated T_{reg} differentiation. Under T_{reg} differentiation conditions, TGF-β activates transcriptional factors Foxo1/3a to enforce Foxp3 expression, whereas IL-2 activates STAT5, PI3K/Akt/mTOR, and ERK pathways to regulate cell proliferation and metabolism. TCR engagement activates the proximal signalosome involving ITAM/ZAP70/SLP-76/ITK to activate further ERK and PI3K/Akt/mTOR signaling, triggering PTEN turnover and Myc/miR19b-mediated targeting of PTEN to release PI3K/Akt/mTOR signaling from PTEN suppression. Active PI3K/Akt/mTOR is essential for glucose metabolism and can suppress Foxo-mediated Foxp3 expression. Foxp3, in turn, directly suppresses expression of IL-2, ITK, and ZAP70, further regulating PI3K/Akt/mTOR-mediated suppression of Foxp3 expression. Of note, the TCR proximal signalosome can negatively tune IL-2/STAT5 signaling strength, although the details are currently unclear.

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iT_{reg} = induced Foxp3-expressing conventional regulatory T cell, LAT = linker for activation of T cells, miR19b = microRNA 19b, mTOR = mammalian target of rapamycin, OTI = transgenic TCR recognizing OVA peptides 257–264 presented by MHCI, PIAS = protein inhibitor of activated STAT, PKC = protein kinase C, PLC = phospholipase C, PLZF = promyelocytic leukemia zinc finger, PTEN = phosphatase and tensin homolog, PTP = protein tyrosine phosphatase, sγc = soluble extracellular domain of cytokine receptor common γ chain, SHIP-1 = Src homology 2-containing inositol phosphatase-1, SHP-1 = Src homology 2-containing phosphatase-1, SLP-76 = Src homology 2 domain-containing leukocyte protein of 76 kDa, SOCS = suppressor of cytokine signaling, SP = single-positive, T_{reg} = Foxp3-expressing conventional regulatory T cell, tT_{reg} = thymic-derived Foxp3-expressing conventional regulatory T cell, WT = wild-type, Y145 = tyrosine 145

strong activation of Akt/mTOR signaling that favors an effector $CD4^+$ T cell fate and diminished iT_{reg} development [35, 36] (Fig. 1). Interestingly, however, high affinity antigen given at a low dose or in disrupted periods increases the abundance of iT_{regs}, suggesting complex regulation of T_{reg} development by antigen potency, concentration, and duration of TCR signals [35].

Genetically modified mice that have impaired TCR signaling also exhibit altered T_{reg} development, with enhanced tT_{reg} abundance in vivo and iT_{reg} differentiation in vitro. Mice carrying mutated TCR ζ chains that disrupt all 6 ITAMs and thus, have attenuated TCR activity show increased frequency of T_{regs} [67]. Likewise, mice that carry the SLP-76 Y145F mutation, which affects its interaction with ITK [66], or those that lack ITK [34, 68] exhibit increased frequency of tT_{reg} in the thymus and/or periphery. Furthermore, naïve $CD4^+$ T cell precursors inversely respond to incremental TCR signals under iT_{reg}-differentiating conditions in vitro, with a reduced proportion of iT_{reg} in the culture, and those that have reduced TCR signals as a result of the absence of ITK are unresponsive to the gradient of TCR signaling [34]. These findings support the view that $CD4^+$ T cell differentiation into T_{regs} occurs at a higher frequency in the face of attenuated TCR signaling. Most interestingly, the reduction in TCR signals in the absence of ITK is accompanied by an enhanced responsiveness of the IL-2/STAT5 signaling pathway [34] and enhanced expansion in response to IL-2 in vivo [68], suggestive of a regulatory role for TCR signals in regulating T_{reg} differentiation by tuning the signals they receive from IL-2.

The work of Gomez-Rodriguez et al. [34] recently reveals a potential mechanism for this TCR tuning of IL-2-mediated T_{reg} differentiation. TCR activation results in down-regulation of the phosphatase PTEN in naïve $CD4^+$ T cells and T_{regs} [69]. PTEN turnover alters the T cell response to IL-2, with resultant enhanced PI3K/Akt pathway activation, in addition to STAT5 phosphorylation [69] (Fig. 1). In the face of impaired TCR signal in $Itk^{-/-}$ T cells, PTEN degradation is attenuated, coupled with inefficient activation of the Akt/mTOR pathway and hyperactive responsiveness to IL-2 [34]. In support of the proposal that PTEN degradation is impaired, $Itk^{-/-}$ T cells exhibit impaired Myc and miR19b up-regulation, which normally represses PTEN expression (Fig. 1). The weakened mTOR and Myc pathways in $Itk^{-/-}$ cells are also likely to be the leading reasons for a decreased expression of the transcription factor HIF-1α and accompanying reduction in glucose metabolism, thus affecting energy production and proliferation in these cells [34]. Thus, TCR signals mediate regulation of PTEN, which is regulated by signals coming from ITK. PTEN then regulates IL-2 distal signaling and impacts the T_{reg} differentiation. However, it is yet unclear whether the TCR proximal signalosome acts directly on IL-2 proximal signaling pathways to modulate signaling sensitivity.

"CORECEPTOR TUNING": TCR SIGNALS ACT IN A NEGATIVE-FEEDBACK LOOP TO FINE TUNE IL-7-MEDIATED, NAÏVE $CD8^+$ T CELL HOMEOSTASIS

T cell homeostasis in the periphery is critical for maintenance of immunocompetence, and the survival and homeostasis of naïve T cells require IL-7 signaling [2, 70–74]. The level of IL-7R expression is tightly controlled to optimize IL-7 consumption in support of T cell homeostasis [75]. The Singer group [76] has shown that naïve $CD8^+$ T cell homeostasis is regulated by a negative-feedback loop, in which the IL-7R is transcriptionally repressed via signals induced by γc cytokines, including IL-7 itself. These naïve $CD8^+$ T cells require a GFI-1-dependent pathway to dampen IL-7R expression in response to IL-7 or other γc cytokines [76]. In addition, naïve $CD8^+$ T cells are subjected to modulation by a second regulatory feedback circuit: CD8 coreceptor-assisted, TCR-mediated, negative tuning of IL-7/IL-7R signaling [77].

In response to γc cytokines, including IL-2, IL-4, IL-7, and IL-15, $CD8^+$ T cells up-regulate CD8 expression, which does not occur in response to non-γc cytokines, such as IL-6 and TNF-α [38, 78]. However, the engagement of TCR signals, with assistance of the CD8 coreceptor, during IL-7 stimulation can down-regulate IL-7 signaling. This reduced IL-7/STAT5 signaling activity, in turn, down-regulates CD8 expression, which reduces TCR/CD8 signaling and alleviates the TCR-mediated suppression of IL-7R expression and signals [38]. The CD8-mediated TCR signaling that suppresses IL-7R expression is the driving force for the oscillation of IL-7 and TCR signaling and is termed coreceptor tuning [77] (**Fig. 2**).

When released from this coreceptor tuning constraint, IL-7 can trigger signals necessary for $CD8^+$ T cell proliferation under normal homeostasis. However, prolonged IL-7 signaling paradoxically induces CICD [77]. When the IL-7R is constitutively expressed on $CD8^+$ T cells, the intrinsic oscillation driven by TCR/CD8-mediated, negative feedback or coreceptor tuning is disrupted, and IL-7-driven $CD8^+$ T cell proliferation is elevated, accompanied by significant secretion of cytotoxic cytokine IFN-γ, which leads to CICD through auto- and paracrine effects (Fig. 2) [77]. This negative-feedback loop, IL-7R → CD8 → TCR ⊣ IL-7R (Fig. 2), thus forms a circuit that acts as a cell-intrinsic rheostat

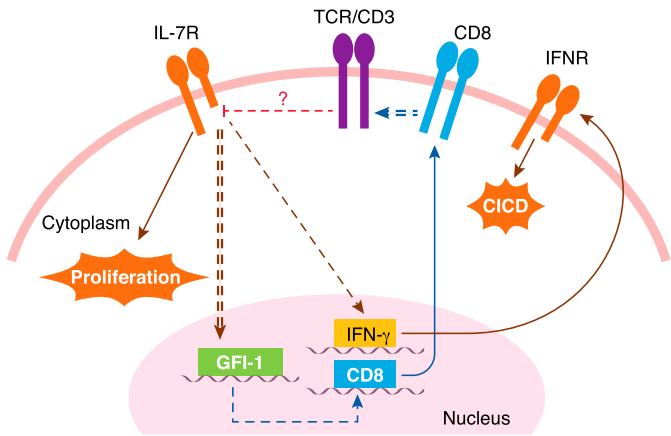

Figure 2. TCR/CD8 coreceptor tuning of IL-7-mediated $CD8^+$ T cell homeostasis. IL-7/IL-7R signaling is critical for naïve $CD8^+$ T cell homeostasis. IL-7R induces high levels of IFN-γ that induce CICD through auto- and paracrine mechanisms, which counteract the homeostatic proliferation. To prevent CICD, IL-7R activation induces GFI-1-dependent CD8 expression, which potentiates TCR-mediated negative tuning of IL-7R expression and thus, IFN-γ-induced CICD. The IL-7R → CD8 → TCR ⊣ IL-7R negative-feedback loop drives cell-intrinsic IL-7R and TCR oscillatory signaling.

for tuning naïve CD8$^+$ T cell homeostasis through TCR/CD8 and IL-7-mediated signaling oscillations.

TCR SIGNALING NEGATIVELY TUNES IL-4-INDUCED IMP CD8$^+$ T CELL DEVELOPMENT

Innate memory T cells were discovered recently during the characterization of T cell phenotypes in *Itk*$^{-/-}$ mice, in which CD8 SP thymocytes were first found to be increased significantly [79–81]. These cells were later shown to express memory T cell markers CD44 and CD122 and the transcription factor Eomes and are endowed with rapid effector cytokine production capacity upon stimulation [82–86]. Although these phenotypes are typical characteristics of memory T cells derived from conventional T cell activation in the periphery, *Itk*$^{-/-}$ CD8 SP thymocytes gain them in the thymus during development, independently of peripheral stimulation, and have thus been termed memory-like or IMP T cells. The development of IMP T cells shares the early stages of conventional T cell differentiation and likely diverges from the double-positive stage. However, whereas IMP T cell development is dependent on hematopoietic cell–MHC expression, it can be independent of the thymic MHC and even the entire thymus, regardless of the presence of ITK [84, 86, 87].

Cells with similar phenotypes to the *Itk*$^{-/-}$ IMP CD8$^+$ T cells have been observed in mice expressing the SLP-76 Y145F mutant (the mutant that disrupts ITK/SLP-76 coupling as described in earlier section) and others [18, 88, 89]. Ablation of the IL-4R blocks the elevation of the IMP CD8$^+$ T cells in *Itk*$^{-/-}$ mice, supporting a critical role for IL-4 in development of IMP CD8$^+$ T cells in the absence of ITK [39, 88]. In WT mice, *i*NKT cells are able to produce IL-4 in a PLZF-dependent manner and thus, were originally proposed to be the source of IL-4 for the development of IMP T cells [88]. However, *Itk*$^{-/-}$ *i*NKT cells are severely impaired in number as well as in production of IL-4 [90–92], and so, the proposed candidates for the source of IL-4 have been suggested to be a subset of NKT-like γδ T cells [93, 94] and/or a CD4$^+$ PLZF^{hi} population of thymocytes [88] that are both capable of IL-4 production and are expanded in the absence of ITK. It is shown recently that *i*NKT and γδ T cells are dispensable for development of IMP CD8$^+$ T cells in the absence of ITK [39]; thus, it is likely that *Itk*$^{-/-}$ CD4$^+$ PLZF^{hi} thymocytes produce sufficient IL-4 to drive development of *Itk*$^{-/-}$ IMP CD8$^+$ T cells [88, 95]. Furthermore, *Itk*$^{-/-}$ CD8$^+$ T cells exhibited better responsiveness to IL-4 than WT cells [39]. Intriguingly, similar to the case with T$_{reg}$ differentiation, the reduced TCR signaling manifest in the absence of ITK results in enhanced, IL-4-induced IMP development, suggesting that TCR signaling functions during development of IMP CD8$^+$ T cells to tune IL-4 signals negatively [39]. Indeed, provision of exogenous IL-4 to OTI-*Rag*$^{-/-}$ mice in vivo results in the up-regulation of the Eomes protein and conversion of a significant population of naïve CD8$^+$ T cells to the IMP, which was enhanced in the absence of ITK. When cultured with IL-4 in vitro, naïve OTI-*Rag*$^{-/-}$ CD8 SP thymocytes preferentially develop an IMP-like phenotype [39] (**Fig. 3**), and the frequency of these IL-4-induced, IMP-like CD8$^+$ T cells is inversely correlated to the amount of TCR signals provided [39]. Of further interest is the finding that naïve, peripheral CD8$^+$ T cells lacking ITK express elevated Eomes mRNA but lower Eomes protein, and provision of exogenous IL-4 induced significantly higher expression of Eomes protein in *Itk*$^{-/-}$ cells compared with WT cells, likely, in part, through translation of the premade Eomes mRNA [39]. These data suggest that *Itk*$^{-/-}$ CD8$^+$ T cells receive weak TCR signals during development and may be primed to respond to IL-4 signals to become IMP cells.

A role for the ITK-containing signalosome in the IL-4-induced generation of IMP CD8$^+$ T cells is supported by findings from Carty and colleagues [96, 97], who have reported in conference abstracts that IL-4 induced enhanced STAT6 and Akt activation in SLP-76 Y145F innate-like CD8 SP thymocytes compared with conventional CD8 SP thymocytes. This negative tuning of IL-4 signals by the TCR may be facilitated or modulated by the reciprocal interaction between downstream STAT6 and ERK [98] (Fig. 3). PI3K activity has been shown to be important for IL-4-induced expression of IFN-γ and Eomes in CD8$^+$ T cells in vitro [99], and it is possible that as seen for T$_{reg}$ differentiation, TCR/ITK regulation of PTEN may influence the level of IL-4 signaling. Given the fact that peripheral, naïve *Itk*$^{-/-}$ CD8$^+$ T cells carry higher levels of preformed Eomes mRNA without efficient translation until IL-4 is provided [39], it is likely that enhanced Akt activity downstream of IL-4 is coupled with mTOR activity to regulate protein synthesis [100] (Fig. 3). Overall, these results reveal a suppressive function of the TCR proximal signalosome on STAT6 and Akt signaling, tuning IL-4-mediated IMP CD8$^+$ T cell differentiation, in part, via regulation of expression of Eomes. Under conditions of reduced TCR signaling, there may be enhanced IL-4-induced signaling, contributing to enhanced IMP CD8$^+$ T cell development (Fig. 3).

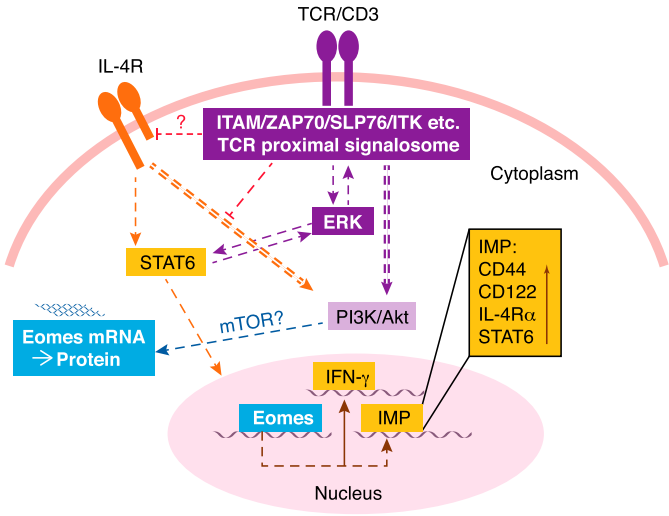

Figure 3. TCR tuning of IL-4-induced IMP CD8$^+$ T cell development. IL-4 drives STAT6-dependent Eomes expression in naïve CD8 SP thymic progenitors, leading to development of the IMP. IL-4 activates PI3K/Akt pathways and drives Eomes translation, likely involving mTOR-mediated translational machinery. TCR signals also activate PI3K/Akt but suppress IL-4R signaling.

Similarly to IL-7-induced up-regulation of CD8, discussed in coreceptor tuning, IL-4/STAT6 stimulation of $CD8^+$ T cells induces a significant increase in CD8 expression [38]. Thus, it is likely that coreceptor tuning may also be involved in IL-4-mediated IMP $CD8^+$ T cell differentiation and homeostasis, as IMP $CD8^+$ T cells accumulate under conditions of high levels of IL-4 and attenuated TCR signal strength. One interesting difference is the cytokine-modulated expression of the cytokine receptor: IL-7 signaling leads to IL-7R down-regulation, whereas IL-4 stimulation induces IL-4R expression [38], which may further complicate the outcome of TCR tuning on cytokine signaling. Nevertheless, IL-4/TCR and IL-7/TCR interplay shares similarities in cytokine-induced coreceptor expression and TCR tuning of a cytokine-mediated cell response, suggestive of a general mechanism used by the TCR to tune cytokine-induced T cell differentiation.

TCR CONDUCTS THE TUBA PLAYERS TO TUNE THE CYTOKINE PITCH

Although it is well accepted that Th2 cell differentiation requires effective TCR triggering, negative tuning of IL-4-mediated signaling by TCR ligation has also been reported in Th2 cells [32]. Strikingly, during the first 12 h following TCR triggering, naïve $CD4^+$ T cells exhibit potent but transient suppression of IL-4-induced tyrosine phosphorylation of IL-4Rα, JAK1/3, STAT6, and insulin receptor substrate 2 [101]. This suppressive effect of TCR triggering on naïve $CD4^+$ T cells also occurs following IL-2-induced STAT5 and IL-6-induced STAT3 activation, suggestive of a general phenomenon of negative tuning by TCR for cytokine-mediated signaling in $CD4^+$ T cells [101]. As full cytokine signaling activity returns ~20 h post-TCR ligation, this transient desensitization of cytokine signaling by TCR ligation may be a mechanism to tune preferentially specific programming to enhance T cell effector function before enrichment of the resultant population by cytokine-mediated T cell expansion and/or differentiation. $CD4^+$ T cells defective in proximal TCR signalosome, such as in the absence of ITK, also have defects in IL-4-mediated Th2 [25, 102, 103] and IL-6/TGF-β-mediated Th17 [33] differentiation, and we speculate that TCR tuning of cytokine responses may play a role in this process as well.

WHO ARE THE TUBA PLAYERS?

Among all of the examples depicted above, there is a missing link between TCR activation and the suppression of cytokine signaling, in which the early engagement of the regulatory machinery may be the tuner during transient and/or long-term suppression. Downstream of γc cytokine/cytokine receptor triggering, the JAK/STAT signaling pathways are the most prominent players [3, 104–106]. There are data that support a regulatory role for TCR signaling in tuning the γc expression/sensitivity and the downstream JAK/STAT6 activation.

TCR stimulation can lead to activation and increased expression of calpain [107], which has been demonstrated to be able to catalyze the proteolysis of γc [108] in a calcium-dependent fashion. The levels of intracellular calcium and calpain activity are inversely correlated with responsiveness of the IL-2/γc/STAT5 pathway [108, 109]. Furthermore, the inhibition of calpain can delay the progression of skin-graft rejection and multiple sclerosis in murine disease models, with delayed development of T cell effectors and/or enhanced T_{reg} function [109, 110], similar to what has been observed in cases of impaired TCR proximal signalosome. Given the critical role of TCR proximal signaling in regulating calcium influx into T cells (see reviews; refs. [7, 21]), calcium-activated calpain may serve as the tuba player conducted by TCR in tuning down the γc expression and thus, the downstream cytokine signaling activity. Alternatively, it has been shown recently that activated T cells produce an alternatively spliced γc mRNA, encoding the sγc, which is secreted and competes with membrane-bound, full-length γc to alter T cell responses to IL-2 and IL-7, shown to lead to impaired survival and enhanced Th17 effector function in T cells [111]. sγc acts to tune down the immune-regulatory effects mediated by IL-2 and IL-7 during TCR activation, which may occur in $CD8^+$ T cells as well, but it is unclear how activated TCR signaling triggers γc mRNA alternative splicing, the latter being pervasive in activated T cells [112]. Ca^{2+}-independent TCR proximal signaling, mediated by PKC and Ras, was shown to be critical for alternative splicing of PTP CD45 during T cell activation [113]. It is likely that T cells have evolved Ca^{2+}-dependent and -independent pathways downstream of TCR to modulate the intensity of γc interaction with cytokines.

JAK/STAT activation is regulated by multiple inhibitory mechanisms [114], among which are some components known to be induced by TCR signaling. These include proximal as well as distal downstream modulators, such as PTP (including CD45, SHP-1, and SHIP-1), SOCS (CIS1 and SOCS1–7), and PIAS that can suppress cytokine responses [114–117]. CD45, expressed on the surface of T cells, can regulate an essential axis of the negative feedback downstream of TCR by recruiting an adaptor protein (Dok-1), suppressing IL-2-induced signaling [118]. TCR ligation induces the assembly of signaling complexes that include Dok-1/2, SHIP-1, and growth factor receptor-bound protein 2, which negatively tune LAT/ZAP70 phosphorylation and IL-2 production [119]. IL-4-induced STAT6 activation exhibits transient hypersensitivity in *Dok-1*$^{-/-}$ splenocytes [120]. Of interest, given the findings with TCR tuning of IL-4 to induce $CD8^+$ IMP cells, is the finding that overexpression of Dok-1 suppresses STAT6 activation and GATA3 expression in $CD4^+$ T cells [121]. Another PTP, SHP-1, can be recruited to lipid rafts in a TCR signaling-mediated manner [122], and its expression is gradually enhanced in $CD8^+$ T cells exhibiting increased TCR affinity over time [123], suggesting that the TCR may be able to orchestrate cytokine signaling through activation and/or expression of SHP-1. In support of the idea that SOCS can tune T cell development and homeostasis, *SOCS1*$^{-/-}$ $CD8^+$ T cells exhibit IL-7/IL-15-dependent hyperproliferation in lymphopenic hosts [124]. Furthermore, SOCS3, a well-known, cytokine-induced regulatory gene, can be up-regulated by TCR triggering as well [125]. TCR triggering also induces the expression of CIS1, which can attenuate IL-2-induced STAT5 activation [126]. Although a modest change in STAT1-related cytokine signaling has been observed in *Pias4*$^{-/-}$ mice, no overt difference in lymphocytes has been determined [127, 128]. However, given

the high homology of the 5 PIAS family members [129], this may be a result of compensation as a result of functional redundancy. Thus, the role of PIAS in T cells and their functional behavior downstream of TCR are of considerable interest but remain to be elucidated.

Whereas it is unclear whether calpain, PTP/Dok, SOCS, and/or PIAS are downstream participants in the TCR-mediated negative tuning described above, TCR triggering can indeed activate and/or induce expression of some members of these groups, making them promising candidates to bridge the TCR in tuning γc cytokine expression and signaling during T cell differentiation and homeostasis (**Fig. 4**).

CONCLUDING REMARKS

The examples of TCR signals tuning γc cytokine (IL-2/IL-4/IL-7) signaling, discovered so far, suggest that the TCR is not just a receptor for activation of T cells but is also a rheostat that can tune cytokine responses to control diverse, effective outcomes. Depending on the situation, TCR tuning of cytokine effects may create a window of time that allows T cell effector programming before the cells go on with cytokine-driven population expansion. This may be an essential mechanism for T cell memory formation to potentiate antigenic specificity. In the cytokine milieu, cytokine-driven T cell homeostasis and differentiation are thus modulated by TCR signals through the cell-extrinsic antigenic stimulation or cell-intrinsic alteration in TCR signaling strength. We suggest that this property of the TCR may be exploited to optimize the development of specific T cell lineages and/or memory responses.

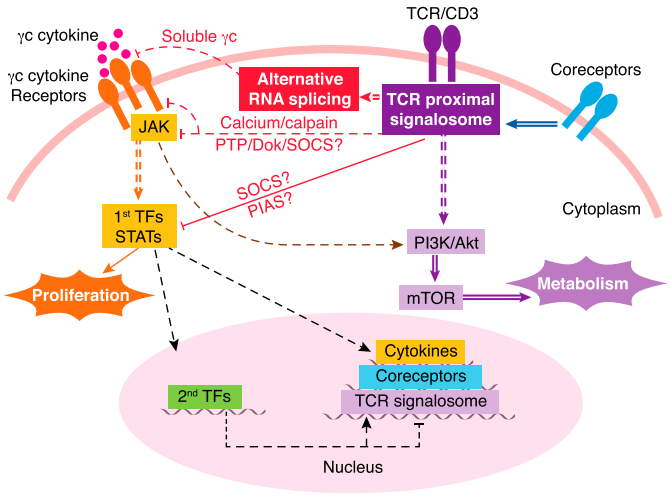

Figure 4. Generalized model for TCR tuning of cytokine-mediated T cell differentiation and homeostasis. γc cytokines (IL-2/IL-4/IL-7) activate the receptor complex and the downstream JAK/STAT and PI3K/Akt signaling pathways. Active STAT can enhance cell proliferation and directly or indirectly modulate expression of effector components, such as cytokines, coreceptor, or TCR signalosome components (e.g., Eomes → IFN-γ, GFI-1 → CD8, Foxp3 ⊣ IL-2/ITK). TCR triggering (with assistance from the coreceptor) suppresses cytokine receptor signaling, likely through modulating receptor complex expression or receptor/JAK/STAT signaling cascade via alternative splicing of RNA, calcium → calpain, PTPs, Dok, SOCS, and/or PIAS. TF, transcription factor.

AUTHORSHIP

W.H. and A.A. wrote the manuscript.

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DISCLOSURES

The authors declare no competing financial interests.

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