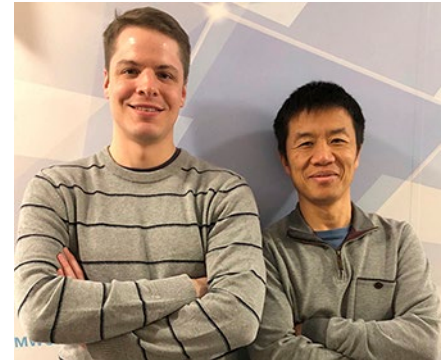## INSIGHTS

TRIMming TGF-β signals in Th17 cells

Aaron S. Rapaport and Wenjun Ouyang

The precise downstream mediators of TGF-β signaling in Th17 and T reg cells remain unclear. In this issue of *JEM*, Tanaka et al. report that Trim33 transduces TGF-β signals in Th17 cells to generate an optimal proinflammatory cytokine profile.

Upon activation, naive CD4^{+} T cells can differentiate into one of several effector or regulatory lineages, including Th1, Th2, Th17, induced T reg (iT reg), and other subsets, which possess unique effector functions that regulate various host immune responses. For example, Th17 cells preferentially produce IL-17, IL-22, and IL-21, which are essential for host defense against extracellular pathogens, but also contribute to the pathogenesis of many autoimmune diseases such as psoriasis and multiple sclerosis (Weaver et al., 2006). By contrast, iT reg cells secrete immunosuppressive cytokines TGF-β and IL-10, which in general repress inflammation and induce immune tolerance. The lineage determination of a CD4^{+} cell is mainly directed by the different cytokines present during primary stimulation. Interestingly, TGF-β is not only a potent inducer of T reg cells but also is indispensable for the development of the highly inflammatory Th17 cells (Weaver et al., 2006). Low concentrations of TGF-β in combination with the proinflammatory cytokines IL-1β, IL-6, and IL-23 strongly induce Th17 differentiation, while TGF-β in combination with IL-2 drives iT reg cell differentiation.

Binding of TGF-β to the TGF-β receptor complex results in phosphorylation of receptor-Smad (R-Smad) dimers composed primarily of Smad2 and Smad3 (Travis and Sheppard, 2014). In the canonical TGF-β signal transduction model, R-Smad then associates with Smad4 to form heterotrimeric complexes that translocate to the nucleus and mediate transcription of immunoregulatory genes. However, the contributions of this canonical pathway in both Th17 and iT reg differentiation are complicated. Smad4 in T cells is required for proper iT reg differentiation, but is dispensable for Th17 differ-

entiation (Kim et al., 2006; Yang et al., 2008). Similarly, Smad3 was found to enhance iT reg differentiation, while inhibiting differentiation of Th17 cells (Martinez et al., 2009). Controversial results have been reported for the role of Smad2. Although earlier studies showed a reduction but not abolishment of the development of both Th17 and iT reg cells (Malhotra et al., 2010), another study indicated that TGF-β-dependent in vitro Th17 differentiation is largely normal (Lu et al., 2010). Nevertheless, the double ablation of Smad2 and Smad3 in T cells almost completely abolished the TGF-β–dependent Th17 induction. Interestingly, the induction of RORγt is normal under the same conditions (Takimoto et al., 2010). Instead, the double knockout T cells produce significantly higher amounts of IL-2 that partially repress Th17 development. Together, these data support a partially and redundantly necessary role for both Smad2 and Smad3 in Th17 differentiation. Thus, there is speculation that Smad4-independent signaling contributes to Th17 differentiation downstream of TGF-β. And in this issue of *JEM*, Tanaka et al. report that Trim33 directs TGF-β signaling in Th17 cells, enhancing the proinflammatory functions of this helper T cell subset. Trim33 has been reported to bind to Smad2/3 and direct noncanonical TGF-β signaling. However, its function in T cells is unknown. Trim33 protein contains several motifs that suggest disparate activities. The N-terminal region is characterized by a tripartite motif (TRIM) composed of a RING finger, two B-box domains, and a coiled-coil. TRIM-containing proteins are known to engage in E3 ubiquitin ligase activity. A middle linker region has been shown to bind Smad2 (He et al., 2006). Two C-terminal motifs, a PHD finger and Bromo domain, are commonly found

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in chromatin remodeling proteins and may interact with certain histone modifications.

To study the functions of Trim33 in T cells, Tanaka et al. (2018) conditionally ablate Trim33 in mouse T cells. Trim33 conditional KO (cKO) mice exhibit reduced severity of experimental autoimmune encephalomyelitis, a model of multiple sclerosis that is highly dependent on Th17 cells. CD4^{+} T cells isolated from disease tissue produce less IL-17. In vitro, *Trim33*^{−/−} CD4 T cells are less able to differentiate into Th17 cells under TGF-β–dependent conditions. Notably, Foxp3 induction and iT reg differentiation are unchanged in Trim33 cKO cells. Remarkably, Tanaka et al. (2018) also observed increased production of IL-10 in Th17 cells. The regulation of IL-17 and IL-10 in these cells is at the transcriptional level without broader impacts on other Th17 programs. Indeed, chromatin immunoprecipitation sequencing analysis of Trim33 in Th17 cells shows Trim33 binding at both the *Il17a* and *Il10* genomic loci, with the binding peaks exhibiting significant overlap with Rorγt binding sites. The authors also show that both Rorγt and Smad2 coimmunoprecipitate with Trim33 in Th17 cells, and that Smad2 is essential for Trim33 binding at the

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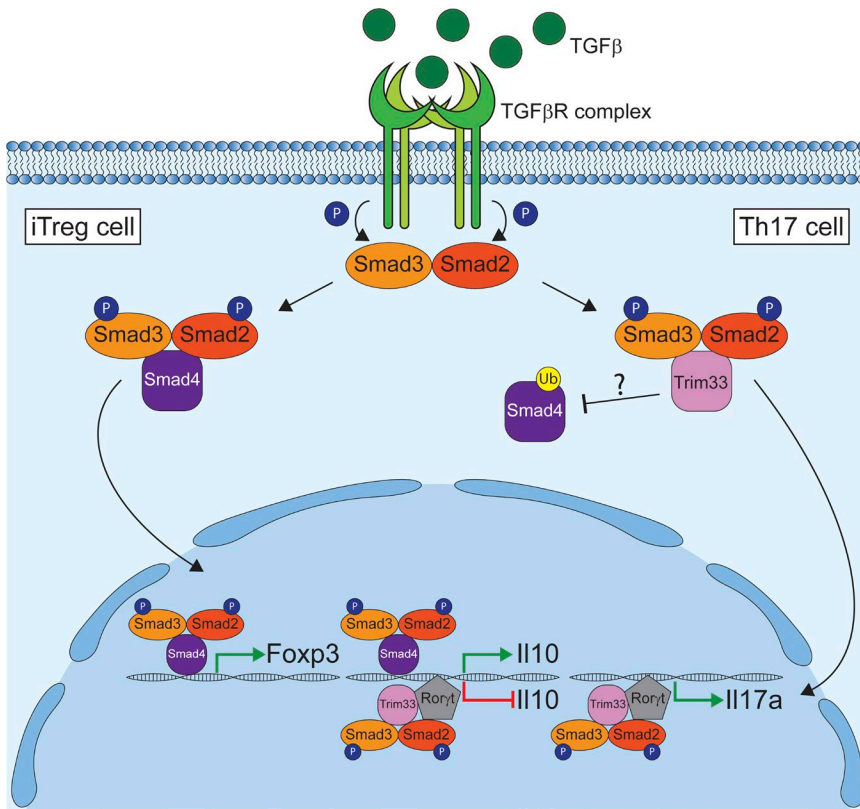
TGF-β signal transduction in iT reg versus Th17 cells. Upon binding of TGF-β to its receptor, the R-Smad components Smad2 and Smad3 are phosphorylated. In iT reg cells, Smad2/3 then associate with Smad4, forming a heterotrimeric complex that mediates canonical TGF-β signaling. Smad2/3/4 translocate to the nucleus and enhance transcription of a regulatory gene signature, notably *Foxp3* and *Il10*. Here, Tanaka et al. (2018) describe a related but contrasting signaling pathway in Th17 cells. Phosphorylated Smad2/3 associates with Trim33, forming a signaling complex that mediates noncanonical TGF-β signaling. Trim33 may also cause the degradation of Smad4 via ubiquitination. Upon translocation to the nucleus, Smad2/3/Trim33 cooperate with RORγt to repress *Il10* and amplify *Il17a* transcription, thereby optimizing Th17 cytokine responses.

tion to enhance invariant natural killer T cell development (Doisne et al., 2009).

Even within Th17 cells, the full extent of Trim33 function may still be incompletely understood. For example, c-Maf was identified as another transcription factor downstream of TGF-β in both T reg and Th17 cells (Pot et al., 2009; Rutz et al., 2011). c-Maf functions in these cells to promote IL-10 production while inhibiting IL-22 production. Given that Trim33 is shown by Tanaka et al. (2018) to bind to the *Il10* locus and associate with RORγt, it is possible that Trim33 and c-Maf can form a regulatory circuit. Finally, additional work is needed to dissect various domain functions of Trim33 in Th17 cells. Trim33 is reported to function in multiple ways, including ubiquitinating target proteins through its N-terminal TRIM motif, binding to transcriptional cofactors via its middle linker region, and altering transcription through the chromatin-binding activity of its C-terminal region. Here, the authors report that Trim33 enhances the Th17 response through direct interactions to transcription factors and subsequent epigenetic remodeling. How Trim33 protein motifs, either individually or cooperatively, mediate these functions remains undetermined.

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Il17a and *Il10* loci. Mechanistically, Trim33 can cooperate with Rorγt and Smad2 to regulate the transcription of *Il17a* and *Il10*. In addition, the authors provide evidence that Trim33 promotes permissive histone modifications at the *Il17a* locus and repressive histone modifications at the *Il10* locus. While the Smad4 heterotrimeric complex promotes *Il10* transcription downstream of TGF-β, the Trim33 complex antagonizes that function at the same locus. Finally, *Trim33*^{−/−} Th17 cells express higher Smad4 protein levels. The mechanism of Smad4 antagonism by Trim33 in T cells remains unclear, although it may be partially due to Smad4 ubiquitination via the Trim33 RING domain.

The branching of the TGF-β signal transduction pathway is being increasingly appreciated as a driver of cell fate and function in the immune system. The data presented here by Tanaka et al. (2018) place Trim33 as a central mediator of TGF-β signaling in Th17 cells, partially through antagonism of

canonical Smad4-dependent signal transduction. A recent study describes how Smad4 establishes the gene signature of natural killer cells by restricting noncanonical TGF-β signaling (Cortez et al., 2017). The same study also suggests that noncanonical TGF-β signaling directs the differentiation of related type 1 innate lymphoid cells (ILC1), opening the possibility of Trim33 contributing to ILC1 establishment. The contributions of Smad4/Trim33-independent TGF-β signaling in lymphocyte differentiation and function also remain incomplete. For example, while Smad2/3 double-deficient T cells phenocopy the lethal inflammation seen in TGFβRII cKO mice, Smad4 or Trim33 deficiency in T cells does not recapitulate this (Li et al., 2006; Takimoto et al., 2010). This suggests unforeseen synergistic effects of Smad4/Trim33 signaling, or that other mediators of Smad2/3 signaling optimize T cell tolerance and function. Relatedly, one study has indicated that Smad4/Trim33-dependent and -independent branches all func-