Duality in the Th17-Treg developmental decision

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F1000 Biology Reports 2009, **1**:5 (doi: 10.3410/B1-5)

The electronic version of this article is the complete one and can be found at: <http://F1000.com/Reports/Biology/content/1/5>

Abstract

Each of the effector CD4 T-cell lineages - Th1, Th2, and the more recently identified Th17 - arises from pluripotent naïve precursors whose developmental fate is largely controlled by cytokines that act in concert with antigenic signals. Remarkably, development of the Th17 lineage has been linked to that of regulatory T cells, which obviate or downregulate Th17 responses to preserve immune homeostasis, through a shared requirement for the cytokine transforming growth factor-beta. Several new studies offer insights into the mechanism whereby the precursors of these subsets are directed into distinct lineages.

Introduction and context

Transforming growth factor-beta (TGF- β) has long been a poster child for pleiotropism in the cytokine world. The diversity of cell types and regulatory processes over which TGF- β holds influence reflects its longevity on the phylogenetic stage and the evolutionary economy gained by molecules that can be adapted for many cellular processes. A common and often confounding feature of the pleiotropy of TGF- β is its paradoxical ability to act in the same pathway to opposite effects. This dualistic nature of TGF- β has recently been extended to developmental decisions that control alternative pathways of CD4 T-cell differentiation. The discovery that there is a shared requirement for TGF- β in the development of anti-inflammatory regulatory T cells (Tregs) and pro-inflammatory Th17 cells has provided an elegant solution for linking the potentially pathogenic Th17 pathway with a potent counter-regulatory pathway that can control it [1–4]. But this has also raised important questions concerning how TGF- β might act at apparently crossed purposes to do this. Several recent studies have built on earlier observations to begin to provide a mechanistic basis by which these two lineages, although linked by a common requirement for TGF- β , go their separate ways [5–9].

Differentiation of Th17 cells from antigen-naïve CD4 T-cell precursors, like the differentiation of Th1 and Th2 cells, is induced by cytokines emanating from innate immune cells that have recognized specific pathogens. In the case of Th17, interleukin (IL)-6 produced by dendritic cells (and perhaps other cells in the local environment) acts in concert with active TGF- β to direct Th17 differentiation. Th17 cells are largely defined by their eponymous cytokines, IL-17A and IL-17F, which are pro-inflammatory by virtue of their direct and indirect effects on neutrophil recruitment. In the absence of IL-6 (and thus pro-inflammatory cues from innate immune cells), TGF- β promotes the differentiation of Tregs. Prior to the discovery of Th17, Tregs had become defined by a master transcription factor, Foxp3, which is both necessary and sufficient to program Treg development and maintenance. Following its discovery [10,11], the Th17 lineage was soon linked to its own master transcription factor, a T-cell isoform of the retinoic acid-related orphan receptor γ , ROR γ t [12]. Interestingly, however, naïve T cells stimulated with TGF- β alone were found to upregulate both Foxp3 and ROR γ t, although they failed to express an appreciable level of IL-17 and progressively extinguished ROR γ t as they differentiated into Tregs [8,13]. Similarly, early Th17 differentiation

induced by TGF- β and IL-6 was accompanied by transient co-expression of ROR γ t and Foxp3, with Foxp3 extinguished as Th17 development progressed [6,8]. These findings raised the possibility that Foxp3 and ROR γ t might engage in an antagonistic competition, the outcome of which could determine whether a cell differentiated as a Treg or a Th17 cell.

Major recent advances

In fact, recent reports have indicated that this is indeed the case, although not before introducing another player into the mix. ROR γ t is one member of a three-member family of nuclear receptors that also includes ROR α and ROR β . Dong and colleagues [9] recently reported that ROR α , like ROR γ t, is expressed by Th17 cells, with similar kinetics and similar functional effects. In the original report linking ROR γ t to Th17, Littman and colleagues [12] observed that mice deficient in ROR γ t were largely Th17-deficient yet retained a small population of Th17 cells. It now appears that ROR α has at least some redundant functions with ROR γ t and can partially compensate for the absence of ROR γ t to drive Th17 development, although there appear to be differences in the gene targets of these factors and these differences will require further study [9].

The link between ROR α and Th17 had been suggested by an earlier report from Ziegler and colleagues [5], who found that human Jurkat T cells transfected with ROR α expressed IL-17A and IL-22. Perhaps more importantly, Ziegler and colleagues established that Foxp3 could inhibit the transcriptional activity of ROR α through physical interaction of the two factors. In yeast two-hybrid screens of a human Treg library that used Foxp3 as bait, ROR α was identified as an interacting partner and Foxp3 was shown to inhibit ROR α activity through direct interaction. A splice variant of Foxp3 that lacks exon 2 was found to lack this repressive activity, and a motif in exon 2 (LQALL, which is similar to the LxxLL motif of other ROR co-activators and repressors) was mapped that bound the carboxy-terminal AF2 domain of ROR α and was essential for its repression [5]. Thus, a basis for direct suppression of an ROR factor by Foxp3 was established, and the domains mediating this interaction were defined.

In a collaborative study, Ziegler's and Littman's groups extended these findings, establishing similar interactions between Foxp3 and ROR γ t in direct analyses of Th17 development in the mouse system [6]. In agreement with the previous study of ROR α [5], Foxp3 was found to directly bind ROR γ t via exon 2 and repress its effects. Moreover, repression of ROR γ t-mediated effects by

Foxp3 was critically dependent on the dose of TGF- β used. High doses of TGF- β repressed ROR γ t function via increased Foxp3; but at lower doses, TGF- β cooperated with signals initiated by IL-6 to overcome Foxp3-mediated repression of ROR γ t. Therefore, true to its pleiotropic nature, TGF- β could drive fundamentally different outcomes on the same cellular target via differential effects of dose and co-signaling context. The repressive effects of Foxp3 were dependent on both exon 2 and the DNA-binding forkhead domain in this study, although the forkhead domain was dispensable in another study [8].

In reciprocal fashion, the repressive effects of Foxp3 could be reversed by IL-6 as well as by IL-21 and IL-23, which like IL-6 are STAT3-activating cytokines important in Th17 differentiation. All of these cytokines both suppress the expression of Foxp3 and attenuate its effects under conditions of enforced expression. Similar findings were independently reported by Ichiyama and colleagues [7]. Thus, Th17-promoting cytokines may act through STAT3-dependent pathways to reverse the Foxp3-mediated repression of ROR γ t and ROR α in developing Th17 cells via both transcriptional and post-transcriptional blockade of Foxp3.

In complementary studies, Yang and colleagues [8] used dual-reporter mice that permit identification of IL-17F- and Foxp3-positive T cells to demonstrate co-expression of IL-17F and Foxp3 in a subset of Th17- or Treg-polarized cells *in vitro* and *in vivo*. Remarkably, it was shown that Tregs could express IL-17 and downregulate Foxp3 if stimulated with IL-6, suggesting that mature Tregs can be subverted to Th17-like cells late in their development. Using mice with targeted deficiencies of STAT3, ROR γ or ROR α , or deficiency of both ROR α and ROR γ , Yang and colleagues [8] further demonstrated that only STAT3-deficient mice failed to downregulate Foxp3 following IL-6 stimulation, whereas the ROR γ and ROR α mutants continued to inhibit Foxp3 despite greatly reduced expression of IL17, indicating that the down-regulation of Foxp3 by STAT3 activation is independent of ROR α or ROR γ t.

Future directions

Collectively, these studies support a model of divergent Treg and Th17 differentiation in which early co-induction of the master transcription factors of both pathways gives way to reciprocal amplification or suppression of either Foxp3 or ROR α and ROR γ t, contingent upon the balance between local levels of active TGF- β or L-6 (Figure 1). Although specific details from these studies are in conflict (for example, the requirement for the

Figure 1. Reciprocal development of Th17 and regulatory T cells (Tregs) effected by the balance of Foxp3 and ROR transcription factors.

Naïve CD4 T cells activated by antigen (Ag) in the presence of transforming growth factor-beta (TGF-β) induce co-expression of the transcription factors Foxp3, RORα, and RORγt in early precursors fated for either Treg or Th17 differentiation. Foxp3 binds to the ROR factors α and γt via a motif in exon 2, repressing their transcriptional activity. Contingent upon the balance of sustained levels of TGF-β or interleukin (IL)-6, Foxp3 expression and repressive activity are enhanced or repressed, respectively, leading to divergence of the Treg and Th17 pathways. In cells directed to the Th17 lineage, upregulation of the inducible component of the IL-23 receptor, IL-23R, pairs with the constitutively expressed, shared IL-12 and IL-23 receptor component, IL-12Rβ1, to confer responsiveness to IL-23, which activates downstream aspects of the Th17 developmental program. Cells directed to the induced Treg lineage express cytokine genes, such as *Ebi3*, that distinguish them from the cytokine genes expressed by mature Th17 cells (for example, *Il17a* and *Il17f*). ROR, retinoic acid-related orphan receptor; RORγt, T-cell isoform of the retinoic acid-related orphan receptor γ.

forkhead domain of Foxp3 in repressing RORα or RORγt), there is remarkable concordance of most of the major findings. Key questions remain, however. What are the unique and redundant functions of RORα and RORγt in the Th17 developmental pathway? Do these orphan receptors require ligands for their function, and if so, what are the ligands? Are these family members co-expressed in the same cells or are they preferentially expressed in emerging subsets of the Th17 lineage (for example, IL-10-expressing or interferon-gamma-expressing Th17 cells)? How is alternative splicing of exon 2 of Foxp3 regulated, and how critical is this to the derepression of RORα or RORγt? What are the cis-regulatory sites targeted by these factors for activating or suppressive effects on Th17- and Treg-associated gene targets? Given the potential autoimmune dangers

inherent in the transition of Tregs to Th17 cells, how often and under what circumstances might this conversion occur physiologically, and what are the regulatory implications of this late plasticity of the Treg pathway? Clearly, insights into the pleiotropic roles for TGF-β in regulating both Th17 and Treg pathways promise additional surprises as these and other questions are addressed in upcoming studies.

Abbreviations

IL, interleukin; ROR, retinoic acid-related orphan receptor; RORγt, T-cell isoform of the retinoic acid-related orphan receptor γ; TGF-β, transforming growth factor-beta; Treg, regulatory T cell.

Competing interests

The authors declare that they have no competing interests.

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