

Commentary

The secret of FOXP3 downregulation in the inflammation condition

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Forkhead Box P3+ (FOXP3+) Regulatory T cells (Tregs) are essential mediators of central tolerance [1]. Their role in immune homeostasis is fundamental in restricting excessive inflammatory responses, and as such, the genetic loss of FOXP3 and Treg function presents itself as chronic autoimmunity, known as Immuno-dysregulation Polyendocrinopathy Enteropathy X-linked syndrome [2]. These cells can be classified into thymus-derived, natural Tregs and those that can be induced in the periphery [3-6].

T helper 17 cells (Th17) are regarded as the polar opposite T cell lineage to Tregs, as they share the common differentiation factor TGF- β for induced Treg cells but Th17 cells are pro-inflammatory [7, 8]. However, Th17 polarization requires the presence of IL-6 [7] and the restriction of FOXP3 expression. In this issue, a study conducted by Li's group has provided evidence that in the presence of the Th17 polarization factors TGF- β and IL-6, FOXP3 can be downregulated at the posttranslational level through degradation [9]. Thus, during Th17 differentiation, FOXP3 expression may be controlled at both the gene and protein levels in order to augment Th17 skewing.

The authors constructed a FOXP3-expressing T cell line (under an ubiquitin promoter) to distinguish the effects of IL-6 and TGF- β signals on FOXP3 protein expression rather than its transcriptional silencing. The authors found that only IL-6 plus TGF- β treatment, but neither IL-6 nor TGF- β alone could specifically promote the

downregulation of FOXP3 protein, independent of its transcriptional regulation. This is not strange that TGF- β alone will not affect FOXP3 expression since it induces and promotes FOXP3 expression [10]. Unlike nTregs isolated from animal where IL-6 treatment alone did convert FOXP3+ cells to become Th17 cells and induce FOXP3 downregulation [11, 12], FOXP3-expressing T cell line did not result in similar FOXP3 downregulation although authors did not study Th17 conversion in the current study. It is likely that nTregs express endogenous TGF- β whereas FOXP3-expressing T cell line did not.

Additionally, this study also demonstrated that IL-6 plus TGF- β co-treatment also negatively regulated the gene transcription of FOXP3. This suggests that Th17 skewing signals can negatively regulate FOXP3 expression at both the transcriptional and posttranslational levels. Moreover, the authors found that only the co-treatment of IL-6 plus TGF- β promoted the cell surface expression of the IL-6 receptor alpha subunit, and the activation of its downstream signal pathway as measured by phosphorylated STAT3. However, neither TGF- β nor IL-6 alone could mediate this effect. The significance of this finding is unclear since T cells including Treg cells express higher levels of IL-6 receptors. Indeed, downregulation of IL-6R expression on Tregs corrects to their stability [13]. Lastly, the authors showed that the downregulation of FOXP3 protein by IL-6 plus TGF- β co-treatment could be prevented by the well-known proteasome inhibitor MG-132, which

suggests that FOXP3 protein is targeted to the ubiquitin-proteasome-dependent pathway in this process.

This study addresses an important and timely issue, namely the mechanisms that control Treg stability and the expression of FOXP3 during inflammation. It is likely that this mechanism of action increases the downregulation of FOXP3 during Th17 differentiation. Some studies have suggested that FOXP3 expression is lost to allow for the conversion of Tregs into Teff cells [11, 12, 14, 15]. However, whether this mechanism is involved in the loss of suppressive Treg function during inflammation, by inducing the decrease of FOXP3 protein expression, remains to be further resolved.

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References

- [1] Fontenot JD, Gavin MA and Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; 4: 330-336.
- [2] Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF and Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001; 27: 20-21.
- [3] Sakaguchi S, Sakaguchi N, Asano M, Itoh M and Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; 155: 1151-1164.
- [4] Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S and Horwitz DA. Generation ex vivo of TGF-beta-producing regulatory T cells from CD4+CD25- precursors. *J Immunol* 2002; 169: 4183-4189.
- [5] Lan Q, Fan H, Quesniaux V, Ryffel B, Liu Z and Guo Zheng S. Induced Foxp3+ regulatory T cells: a potential new weapon to treat autoimmune and inflammatory diseases? *J Mol Cell Biol* 2012; 4: 22-28.
- [6] Tran DQ. TGF-beta: the sword, the wand, and the shield of FOXP3(+) regulatory T cells. *J Mol Cell Biol* 2012; 4: 29-37.
- [7] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL and Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; 441: 235-238.
- [8] Zhou X, Wang J, Shi W, Brand DD, Liu Z, Fan H and Zheng SG. Isolation of purified and live Foxp3+ regulatory T cells using FACS sorting on scatter plot. *J Mol Cell Biol* 2010; 2: 164-169.
- [9] Gao Z, Gao Y, Li Z, Chen Z, Lu D, Tsun A, Li B. Synergy between IL-6 and TGF-beta signaling can promote FOXP3 degradation. *Int J Clin Exp Pathol* 2012; 5: 626-633.
- [10] Zheng SG, Wang J, Wang P, Gray JD and Horwitz DA. IL-2 is essential for TGF-beta to convert naive CD4+CD25- cells to CD25+Foxp3+ regulatory T cells and for expansion of these cells. *J Immunol* 2007; 178: 2018-2027.
- [11] Xu L, Kitani A, Fuss I and Strober W. Cutting edge: regulatory T cells induce CD4+CD25-Foxp3- T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. *J Immunol* 2007; 178: 6725-6729.
- [12] Zheng SG, Wang J and Horwitz DA. Cutting edge: Foxp3+CD4+CD25+ regulatory T cells induced by IL-2 and TGF-beta are resistant to Th17 conversion by IL-6. *J Immunol* 2008; 180: 7112-7116.
- [13] Zhou X, Kong N, Wang J, Fan H, Zou H, Horwitz D, Brand D, Liu Z and Zheng SG. Cutting edge: all-trans retinoic acid sustains the stability and function of natural regulatory T cells in an inflammatory milieu. *J Immunol* 2010; 185: 2675-2679.
- [14] Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martinez-Llordella M, Ashby M, Nakayama M, Rosenthal W and Bluestone JA. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat Immunol* 2009; 10: 1000-1007.
- [15] Lu L, Wang J, Zhang F, Chai Y, Brand D, Wang X, Horwitz DA, Shi W and Zheng SG. Role of SMAD and non-SMAD signals in the development of Th17 and regulatory T cells. *J Immunol* 2010; 184: 4295-4306.