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How smart can it be: transcriptional regulation of T helper cells by SMAR1

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Scaffold/matrix attachment region binding protein 1 (SMAR1) shares structural homology with SATB1, Cux, and Bright.[1] SMAR1 was originally identified as a factor that is highly expressed in the double-positive (DP) thymocytes and binds to the MARβ sequence located 400 bp upstream of the T cell receptor β (TCRβ) enhancer.[1] Chattopadhyay *et al.*[1] have elucidated the potential mechanisms of action of SMAR1 to function as a transcription factor in regulating T helper (Th) cell differentiation.

Th cells are important components in the adaptive immune system. Th1 and Th2 cells, described in the original Th cell paradigm, are joined by other newly defined Th cell subsets.[2] Among them, Th17 cells make the signature cytokines interleukin (IL)-17, IL-17F, and IL-22. These cells are involved in clearance of extracellular pathogens, particularly at mucosal surfaces, where IL-17 induces recruitment and differentiation of neutrophils and IL-22 is required for production of antimicrobial peptides. Th17 cells, along with Th1 cells, are often responsible for autoimmune diseases and may also contribute to tumor progression owing to the role of their cytokines in inflammation and tissue repair. Another subset of $CD4^+$ T cells, the regulatory T cells (Tregs), suppress effector T-cell responses and prevent their potentially pathogenic effects in disease settings.[3]

The Th cell differentiation program is largely controlled by cytokines produced in response to microbial products by innate immune cells. Specific cytokine signaling pathways along with TCR induce and activate a series of transcriptional regulators that interact with each other and/or their target genes in complex networks. For each Th cell differentiation program, certain transcription factors have been identified as key regulators. These transcription factors include T-bet for Th1 cells and GATA3 for Th2 cells.[2] Foxp3 was identified as a lineage-specific regulator and marker for Tregs and controls the expression of multiple genes that mediate Treg cell functions.[4] Retinoic acid–related orphan receptor γt (RORγt) has an essential role for Th17 cell differentiation. In the presence of proinflammatory cytokines (i.e., IL-6, IL-21, or IL-23) and low concentrations of transforming growth factor-β (TGF-β), RORγt expression is upregulated, whereas Foxp3 expression and function are inhibited in TCR-activated $CD4^+$ T cells, thus tipping the balance in favor of the Th17 cell fate. Under such cytokine conditions, RORγt-induced IL-23R expression on T cells confers responsiveness to IL-23, which further promotes Th17 cell differentiation and its *in vivo* pathogenicity. In contrast, in the absence of proinflammatory cytokines, high concentrations of TGF-β will favor Foxp3 expression and result in Treg cell differentiation.[5] RORγt cooperates with a series of other essential transcription factors in guiding the differentiation of Th17 cells.[6,7] The interaction between RORγt and other factors can be either antagonistic or cooperative. For an example, the interaction between RORγt and Foxp3 inhibits RORγt activity in the induction of IL-17 and IL-23R expression. On the other hand, synergistic induction of Th17-related cytokine transcription is achieved by RORγt, signal transducer and activator of transcription factor 3 (STAT3), and Ahr. Together, these data suggest that multiple transcription factors modulate Th17 and Treg cell differentiation. The regulation of this reciprocal fate decision between Th17 cells and Tregs has been intensively studied.

Mirlekar *et al.*[8] and Chemmannur *et al.*[9] have reported the role of SMAR1, a transcription factor, in regulating adaptive immune responses in mucosal tissues of the gut and lung using Lck-Cre-driven T-cell-specific SMAR1 knockout mice ($SMAR1^{\Delta T}$). Using an acute dextran sodium sulfate (DSS)-induced colitis model, Mirlekar *et al.*[8] reported exacerbated disease as revealed by gut histopathology and weight loss in $SMAR1^{\Delta T}$ mice. Interestingly, the gut Tregs appeared normal under the steady state and at the early stage of DSS treatment but were reduced when the disease progressed, suggesting that lack of SMAR1 in T cells may lead to compromised Treg compartment in an inflammatory setting. Accordingly, upregulation of gut

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homing molecules in CD4$^+$ T cells and enhanced proliferation and production of inflammatory cytokines IL-17, tumor necrosis factor α and interferon-γ by effector T cells were observed in SMAR1$^{\Delta T}$ mice during the DSS-induced colitis, presumably due to downregulation of Foxp3$^+$ Tregs. Consistent with the role of IL-17 in neutrophil recruitment, more myeloid cells, including neutrophils and dendritic cells, were found in the gut of SMAR1$^{\Delta T}$ mice during the gut inflammation. IL-6, a key cytokine secreted by antigen-presenting cells such as dendritic cells and important for Th17 cell differentiation, was found upregulated in the colon of SMAR1$^{\Delta T}$ mice, thus may further enhance Th17 responses in a positive feedback loop.

The authors hypothesized that SMAR1 might have a role in regulating the reciprocal relationship between Th17 and Treg cells during their *in vitro* differentiation. Interestingly, SMAR1 is expressed at a higher level in Tregs (both natural Tregs and TGF-β-induced Tregs (iTregs)), compared with that in naive T cells. Consistent with the *in vivo* findings showing the reduction of Tregs during inflammation, the Foxp3 expression was markedly reduced in SMAR1-deficient CD4$^+$ T cell stimulated by TGF-β *in vitro*, suggesting that SMAR1 is also important for iTreg cell differentiation. In contrast, more Th17 cells were differentiated in the presence of IL-6 and TGF-β, consistent with the model of reciprocal fate decision between Treg and Th17 cells. Intriguingly, although SMAR1-deficient CD4$^+$ T cells had compromised Foxp3 expression and enhanced IL-17 expression during *in vitro* differentiation, these phenotypes were largely rescued by co-culturing the mutant cells with wild-type T cells. These data suggest that the phenotypes of SMAR1 deficiency could be due to cell-extrinsic mechanisms that remain to be determined.

The authors further went on to show the increased expression of transcription factors STAT3 and RORγt in SMAR1-deficient CD4$^+$ T cells stimulated with TGF-β or TGF-β and IL-6, consistent with the role of these factors in the inhibition of Foxp3 expression and function and promotion of Th17 cell differentiation. Mechanistically, SMAR1 was found to bind to the promoter of STAT3 in Tregs induced *in vitro* by TGF-β, and deletion of a SMAR1-binding site enhanced the STAT3 promoter activity in the luciferase reporter assay, suggesting that SMAR1 may directly inhibit STAT3 transcription in Tregs (**Figure 1**).

Besides its role in the regulation of Treg cell differentiation, SMAR1 also modulates the function of Tregs. IL-10, an effector cytokine produced by Tregs, was reduced in SMAR1-deficient CD4$^+$ T cells stimulated with TGF-β. Accordingly, Tregs purified from SMAR1$^{\Delta T}$ mice had impaired suppressive function both *in vitro* to inhibit effector T-cell proliferation and *in vivo* to suppress gut inflammation in a CD45RB^{hi}-transfer model of colitis.

Chemmannur *et al.*[9] reported another role of SMAR1 in Th2 cell differentiation and allergic airway disease. Using the same animal model (i.e., SMAR1$^{\Delta T}$ mice), the authors noticed that SMAR1$^{\Delta T}$ mice were resistant to ovalbumin-induced allergic airway inflammation. Compared with those from wild-type control mice, naive CD4$^+$ T cells isolated from SMAR1$^{\Delta T}$ mice were differentiated more readily to Th1 and Th17 cells. However, *in vitro* Th2 differentiation was impaired in the absence of SMAR1. Reverse transcriptase–PCR revealed lower expression of SMAR1 in Th1 and Th17 cells, compared with that in naive CD4$^+$ T cells. In contrast, more SMAR1 mRNA was detected in Th2 cells, consistent with a positive role of SMAR1 for Th2 cells. It remains to be determined whether the expression of SMAR1 in Th2 cells and Tregs, both of which showed higher expression of SMAR1, is similar or different. Despite the unknown mechanism by which SMAR1 expression was downregulated in Th1 and Th17 cells, the authors showed that SMAR1 promoter contains GATA3-binding sites that were bound by GATA3 in Th2 cells, suggesting that the key Th2 transcription factor GATA3 may regulate SMAR1 transcription. Indeed, in luciferase reporter assay, GATA3 can activate the transcription of a SMAR1 promoter construct that contained GATA3-binding sites. Furthermore, the authors showed that SMAR1 can directly regulate IL-17 and T-bet transcription by binding to their promoters in Th2 cells, presumably inhibiting the transcription. The correlation between the enhanced binding of SMAR1 and the recruitment of histone deacetylase 1 with reduced active histone mark (e.g., H3K9Ac) at the MAR regions of the IL-17 and T-bet loci supports a model of repression of these genes by SMAR1 in Th2 cells. Careful examination of these molecular events in SMAR1-deficient CD4$^+$ T cells will provide direct evidence regarding whether SMAR1 is responsible for these epigenetic changes, thus leading to the altered gene expression. Although Treg

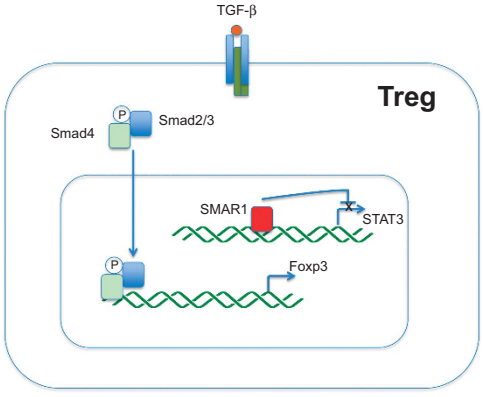

Figure 1 Transcriptional regulation of regulatory T cells (Tregs) by Scaffold/matrix attachment region binding protein 1 (SMAR1). Transforming growth factor-β (TGF-β)-induced Smad signaling promotes Foxp3 expression in CD4$^+$ T cells. SMAR1 promotes Foxp3 expression by binding to signal transducer and activator of transcription factor 3 (STAT3) locus to suppress the transcription of STAT3, an inhibitor of Foxp3 in Tregs.

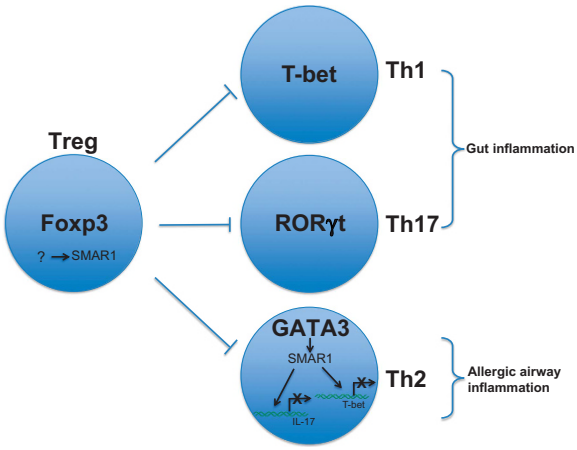

Figure 2 Scaffold/matrix attachment region binding protein 1 (SMAR1) regulates T helper (Th) responses in disease. SMAR1 expression in regulatory T cells (Tregs) is important for suppressing pathogenic Th1 and Th17 responses in colitis models. Genetic ablation of SMAR1 in T cells leads to impaired Th2 cell differentiation *in vitro*, potentially rendering resistance to ovalbumin-induced allergic airway inflammation in SMAR1$^{\Delta T}$ mice. IL, interleukin; RORγt, retinoic acid–related orphan receptor γt.

compartment is compromised in the gut during inflammation, it is unknown whether Treg compartment in the airway is impaired in SMAR1$^{\Delta T}$ mice upon ovalbumin-induced inflammation. It is possible that reduced Tregs and/or impaired Treg function in the lung of SMAR1$^{\Delta T}$ mice during allergic inflammation may lead to aberrant upregulation of Th1 and Th17 responses, which in turn suppress Th2 responses in the ovalbumin model of allergic airway inflammation *in vivo*.

SMAR1 is important for regulating the crosstalk between Tregs and other effector Th cells (e.g., Th1, Th2, and Th17 cells) and perturbation of SMAR1 *in vivo* leads to altered immune responses in various diseases of mucosal tissues (e.g., the models of colitis and allergic airway inflammation) (**Figure 2**). However, the role of SMAR1 in Tregs especially *in vivo* requires rigorous examination. Factor(s) that are responsible for induction of SMAR1 in Tregs remain to be identified. It is also unclear whether lack of key positive factors or mechanism of active suppression of SMAR1 expression is responsible for the lower expression of SMAR1 in Th1 and Th17 cells. As Lck-Cre-mediated gene deletion occurs in all T cells during early development, Foxp3-Cre-mediated deletion of SMAR1 may provide specific insights into the cell-intrinsic role of SMAR1 in Tregs. It would be interesting to determine whether SMAR1 is involved in the plasticity of Tregs and whether loss of SMAR1 will promote the re-differentiation of Tregs to other Th cell lineages (i.e., the plasticity of Tregs).[10] Aberrant expression of STAT3 correlates with the down-regulation of Foxp3 in SMAR1-deficient CD4$^+$ T cells. It would be interesting to determine whether genetic ablation of STAT3 expression or pharmacological inhibition of STAT3 activity can rescue the Treg phenotypes of SMAR1$^{\Delta T}$ mice. Given the initial discovery of binding of SMAR1 to the TCRβ locus,[1] whether the TCR signaling is perturbed in SMAR1-deficient CD4$^+$ T cells and thus contributes to some of the phenotypes of SMAR1$^{\Delta T}$ mice need to be determined in the future. The similarity of SMAR1 to other chromatin regulators (e.g., SATB1) prompts an interesting hypothesis that SMAR1 regulates Treg and other Th cell differentiation epigenetically, for example, by organizing the higher order of structure of chromatin to impact gene expression. Better understanding of the role of SMAR1 in regulating Th cell differentiation may benefit from future global transcriptome and cistrome analyses of SMAR1 in T cells.

From a translational perspective, it would be interesting to identify potential polymorphisms in SMAR1 gene in humans and their association with altered susceptibility to inflammatory bowel disease or allergic inflammation. Any therapeutic attempt to target SMAR1 (e.g., pharmacological antagonists of SMAR1) as a potential treatment in human allergic inflammation (or in other Th2 hypersensitivities) has to be made by caution, given the impairment of Treg compartment by SMAR1 deficiency in other disease settings (e.g., mouse models of inflammatory bowel disease).

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CONFLICT OF INTEREST

The author declared no conflict of interest.

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