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Regulatory T Cells GATA Have It

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The factors that control regulatory T (Treg) cell homeostasis and function are still being defined. In this issue of *Immunity*, Wang et al. (2011) demonstrate that the Th2 cell-associated transcription factor GATA-3 helps control Foxp3 expression in Treg cells and is required for their proper functional activity in vivo.

$CD4^+$ helper T cells adopt one of several functional fates defined by their production of distinct proinflammatory cytokines or their regulatory capacity. This functional specialization is thought to be due to their differential expression of "master" transcription factors that turn on unique programs of gene expression controlling T cell function and migration (Reiner, 2007). Work over the last 15 years has clearly established that a specialized population of MHC class II-restricted $CD4^+$ regulatory T (Treg) cells expressing the transcription factor Foxp3 play a critical role in preventing autoimmunity and limiting immune-mediated inflammation. Additionally, Treg cell-mediated immune modulation can block tumor eradication and suppress T cell responses during infection with bacterial, viral, and parasitic pathogens. As such, clinical manipulation of Treg cell activity is a promising new therapeutic approach that may prove useful in treating autoimmunity, blocking graft rejection, promoting tumor clearance, and eradicating persistent infections.

Because of their therapeutic potential, the development, homeostasis, and function of Treg cells have been extensively studied. As a result of this intense scrutiny, a host of molecules required for Treg cell function have now been identified. Foxp3 itself is generally considered the "master" transcription factor that directs Treg cell differentiation, and deficiency in Foxp3 leads to a complete absence of functional Treg cells associated with rapid development of multiorgan autoimmunity. More recently, several additional transcription factors have been found to help control Treg cell function, homeostasis, and migration in different immunological contexts (Rudensky, 2011). These include the Ikaros family transcription factor Eos, which binds directly to Foxp3 and helps mediate Foxp3-dependent gene silencing; NF-AT, which can form a cooperative binding complex with Foxp3; Runx1,which forms a complex with Foxp3 and CBF-β and is essential for Treg cell function; STAT5, which is activated upon IL-2 receptor signaling in Treg cells and helps induce and stabilize Foxp3 expression; IRF4, loss of which in Treg cells results in dysregulated Th2 cell responses and hyper-IgG production; STAT3, activation of which by IL-10 is essential for Treg cell-mediated suppression of Th17 cells; and T-bet, which helps control Treg cell migration and homeostasis during Th1 cell responses. In this issue of *Immunity*, Wang et al. (2011) add GATA-3 to this list, demonstrating that this transcription factor contributes to Treg cell homeostasis by stabilizing expression of Foxp3.

GATA-3 is a broadly expressed transcription factor that is essential for the normal development of numerous tissues, including the central nervous system, mammary epithelium, kidneys, and skin. It is therefore not surprising that loss of GATA-3 is embryonic lethal due to defects in development of multiple organ systems. Within the hematopoietic system, GATA-3 is required for T cell development and $CD4^+$ helper T cell specialization (Hosoya et al., 2010). GATA-3 is expressed by early T cell progenitor cells in the thymus, and in the absence of GATA-3 T cell differentiation is blocked at the double negative 1 (DN1) stage of development. In mature T cells, GATA-3 is best known for its ability to regulate expression of the Th2 cell-associated cytokines IL-4, -5 and -13, and as such GATA-3 is widely considered to be the "master transcription factor" of Th2 cell development. However, GATA-3 now joins T-bet and RORγt as transcription factors important in the functional specialization of $CD4^+$ effector T cells, that are also expressed by and function within Treg cells.

The importance of GATA-3 for appropriate Treg cell function was demonstrated by conditional deletion of this gene specifically in $Foxp3^+$ Treg cells. This resulted in dysregulated T cell activation, overproduction of multiple effector cytokines such as IFN-γ, IL-4, and IL-17, and development of multiorgan autoimmunity by ~16 weeks of age. Thus, unlike T-bet and RORγt, whose expression in Treg cells is associated with Th1 or Th17 cell-polarizing conditions, respectively (Campbell and Koch, 2011), GATA-3 appears to more generally impact Treg cell function and is required for regulation of Th1, Th2, and Th17 cell responses. However, although the ability of GATA-3-deficient Treg cells to inhibit effector T cell proliferation was impaired, the relatively late onset of autoimmune disease in the $Gata3^{fl/fl}$-Foxp3-Cre mice compared with completely Treg cell-deficient mice indicates that Treg cells are still at least partially functional even in the absence of GATA-3. In this respect, the phenotype of these mice more closely resembles that of either IL-2- or CD25-deficient animals, in which Treg cells develop normally but are functionally and homeostatically impaired (Fontenot et al., 2005). In fact, like CD25-deficient Treg cells, Treg cells lacking GATA-3 were outcompeted by wild-type cells in mixed bone marrow chimeras and showed other proliferative and homeostatic defects. Thus, the combination of decreased function and altered homeostasis of GATA-3-deficient Treg

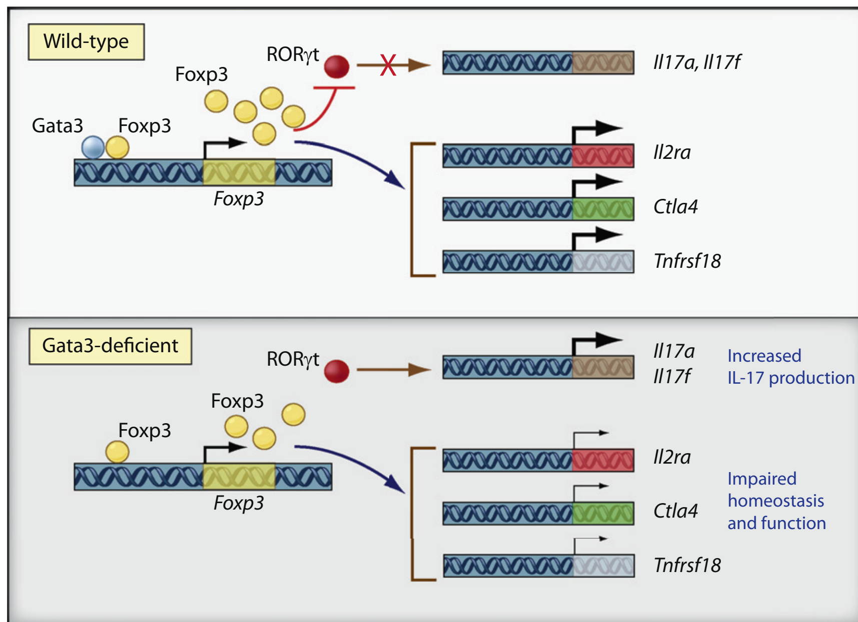

Figure 1. Control of Treg Cell Activity by GATA-3
GATA-3 and Foxp3 act cooperatively to promote Foxp3 expression, which in turn prevents IL-17 production by blocking RORγt function,and establishes the transcriptional signature of Treg cells. Reduced Foxp3 expression in the absence of GATA-3 results in unchecked RORγt activity and production of IL-17 and decreased transcription of genes essential for Treg cell function and homeostasis in vivo.

cells probably underlies their inability to ultimately prevent development of autoimmune disease.

A clue to the mechanism by which GATA-3 controls Treg cell activity comes from the observation that expression of Foxp3 was reduced ~50% in GATA-3-deficient Treg cells at both the mRNA and protein levels, suggesting that GATA-3 helps control Foxp3 expression. Indeed, chromatin immunoprecipitation analysis revealed that GATA-3 binds to a conserved element in the Foxp3 locus, where it can function as a transcriptional enhancer. Attenuated Foxp3 expression has been associated with decreased Treg cell activity and development of autoimmunity in diabetic NOD mice (Grinberg-Bleyer et al., 2010) and in mice rendered Foxp3-insufficient through insertion of luciferase and GFP into the 3′-untranslated region of the Foxp3 gene (Wan and Flavell, 2007). Thus, decreased Foxp3 expression likely accounts for at least some of the functional defects observed in GATA-3-deficient Treg cells (Figure 1). Consistent with this, Treg cells lacking GATA-3 had decreased expression of a number of other genes characteristic of Treg cells and could readily undergo Th17 cell differentiation in response to IL-6 + TGF-β, a process that is normally blocked in Treg cells because of Foxp3's ability to inhibit RORγt function (Zhou et al., 2008). One caveat of these studies is they do not distinguish between how GATA-3 influences Foxp3 expression in the so-called "natural" Treg cells that arise in the thymus versus Treg cells induced in the periphery (iTreg cells) by activation in the presence of TGF-β. These two modes of Foxp3 induction depend on different DNA elements and unique (although overlapping) sets of transcription factors (Rudensky, 2011). In fact, previous studies have shown that GATA-3 can inhibit TGF-β-induced Foxp3 expression, and thereby limit iTreg cell generation (Mantel et al., 2007). Thus, whether GATA-3 acts as an enhancer or repressor at the Foxp3 locus appears to be context dependent and is likely controlled by the available binding/interaction partners and changes in chromatin structure.

The GATA family of transcription factors helps specify cell fate in multiple cell types by controlling expression of a wide range of gene products, including those influencing cell proliferation and death. GATA-3 can also restrict $CD4^+$ T cell differentiation into other functional lineages such as Th1 cells. Thus, in addition to its ability to promote Foxp3 expression, GATA-3 probably influences Treg cell homeostasis and function through regulation of multiple gene targets, and further defining the mechanisms of GATA-3 function in Treg cells is likely to yield new insights into the molecular control of their homeostasis and function. Moreover, the fact that GATA-3, which is normally associated with Th2 cell development, has a critical role in specifying the Treg cell fate adds to a growing body of evidence indicating that the transcriptional control of $CD4^+$ effector T cell specialization is far more complex than previously appreciated. Indeed, the identification and characterization of cell populations expressing various combinations of Foxp3, T-bet, RORγt, and GATA-3 calls for a re-evaluation of the idea that differential expression of "master transcription factors" strictly underlies the functional differentiation of $CD4^+$ T cells. Instead, it appears that these molecules can act in a combinatorial fashion and that differences in the timing, extent, and context of their expression can alter their functions, resulting in $CD4^+$ T cells with a wide range of effector and regulatory phenotypes. The work from Wang et al. (2011) further highlight the complex and sometimes contradictory ways in which GATA-3 can influence the functional specialization of $CD4^+$ T cells. Key issues requiring further investigation include better understanding how GATA-3 expression is controlled in Treg cells, identifying the co-factors required for its function as either an enhancer or repressor at the Foxp3 locus, and determining why expression of GATA-3 in Treg cells does not lead to production of the potentially inflammatory cytokines IL-4, −5 and −13.

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Excitable T Cells: $Ca_V1.4$ Channel Contributions and Controversies

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Store-operated CRAC channels encoded by the Orai genes mediate calcium entry in T cells. In this issue of *Immunity*, Omilusik et al. (2011) record $Ca_V1.4$-mediated voltage-gated calcium currents in T cells and address their role for T cell development and function.

T cell activation requires calcium (Ca^{2+}) entry across the plasma membrane (PM). This Ca^{2+} influx is preceded by emptying of internal Ca^{2+} stores, a process referred to as "store-operated" Ca^{2+} entry. The corresponding channels were initially described as Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Its molecular machinery, whereby the endoplasmic reticulum (ER) Ca^{2+} sensor STIM clusters upon ER Ca^{2+} depletion, translocates to plasma membrane (PM) adjacent ER where it directly activates ORAI channels was recently identified (references in Hogan et al., 2010). STIM exists in two isoforms, STIM1 and STIM2, whereas the mammalian ORAI family contains three members, ORAI1-3. T cells from patients with severe combined immunodeficiency were shown to have a loss-of-function mutation in ORAI1 (Feske et al., 2006). In ORAI1-deficient mice, Gwack et al. (2008) reported a strong reduction in Ca^{2+} entry after T cell receptor stimulation or store depletion in differentiated effector T cells, consistent with the data in human patients, and a weaker reduction in naive T cells. Vig et al. (2008), using a different targeting strategy that may result in a hypomorphic allele, reported a significant CRAC reduction in mast cells but only a mild phenotype in T cells. Both groups showed strong expression of the homolog ORAI2 in naive T cells, which may compensate for the loss of ORAI1. Interestingly, development of T and B cells was normal in ORAI1-deficient mice, which points toward the involvement of other Ca^{2+} influx channels during maturation.

In this issue of *Immunity*, Omilusik et al. (2011) show evidence that, in addition to store-operated CRAC channels encoded by the ORAI genes, voltage-gated $Ca_V1.4$ channels, which are activated by depolarization of the membrane potential, are required for proper development and function of naive T cells. T cells thus share characteristics with electrically excitable cells such as neurons and muscle cells. Several reports have described that voltage-gated Ca^{2+} channels and their β subunits are expressed in T cells (references in Omilusik et al., 2011), although evidence for functional importance of Ca_V channels was based on pharmacological tools that are used at high concentrations and may have indirect effects on other ion channels as well. Involvement of Ca_V channels was supported by the finding that their $\beta3$ and $\beta4$ subunits can modulate Ca^{2+} signals and T cell function (Jha et al., 2009). Nevertheless, Ca_V current recordings, a detailed Ca^{2+} signal analysis, or mechanistic explanations were still lacking.

Omilusik et al. (2011) now measure Ca_V currents in T cells. Currents were recorded in naive ($CD44^{low}$) murine $CD4^+$ and $CD8^+$ lymphocytes and were absent in cells from *Cacnalf* $^{-/-}$ mice, which do not express $Ca_V1.4$ on the PM according to surface biotinylation. Currents are small, do not show Ca^{2+}-dependent inactivation (a hallmark of $Ca_V1.4$) in contrast to most voltage-gated Ca^{2+} channels, and result in a current-voltage relationship (IV) that has a peak potential of more than 0 mV (expected in 100 mM Ba^{2+} as charge carrier due to surface potential effects). Omilusik et al. (2011) supplement their electrophysiological recordings with measurements of intracellular Ca^{2+} concentrations by using flow cytometry. To eliminate the disadvantage of single wavelength Ca^{2+} measurements, which is mostly used in flow cytometry if a UV laser is not available for Indo-1 (a ratiometric emission dye), the authors did mix Fluo-4 and Fura-Red and analyzed the median ratio between Fluo-4 and Fura-Red. This is certainly better than single wavelength measurements, even if we do not fully understand how ratios between 100 and 1000 reflect the median ratios of the Fluo-4 and Fura-Red intensities. The quantification of the measurements may also be hampered by relative differences of Fluo-4 and Fura-Red concentrations in individual cells. Nevertheless, Ca^{2+} signals are clearly reduced in naive ($CD44^{low}$) $CD4^+$ or $CD8^+$