if the mutant protein is indeed expressed, it might have acquired a new, proinflammatory "gain of function" that is distinct from the activity of the wild-type version of Nod2. Finally, these studies bring ideas for possible treatments for Crohn's disease: targeting hnRNP A1 directly to influence its ability to drive *IL10* transcription might bypass the block erected by mutant Nod2.

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The Foxo and the hound: chasing the *in vivo* regulation of T cell populations during infection

Elia D Tait & Christopher A Hunter

T cell expansion and contraction during the immune response to pathogens are regulated by a wide variety of cell-intrinsic and cell-extrinsic factors. A new study identifies a role for CTLA-4 signaling and activation of the Foxo3 transcription factor in modulating T cell populations.

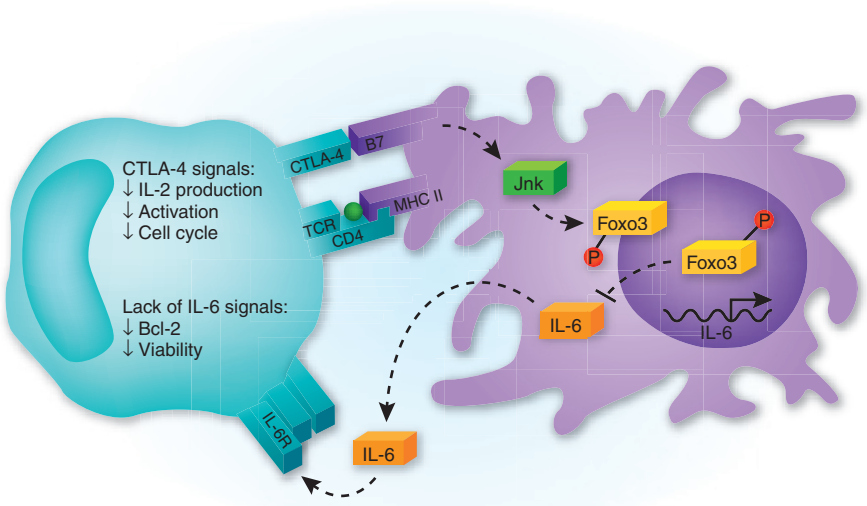
In this issue of *Nature Immunology*, Dejean *et al.* provide new insight into the role of the forkhead box (Fox) transcription factor Foxo3 in limiting the magnitude of T cell responses[1]. The Fox family of transcription factors participate in a multitude of biological processes, including the cell cycle, metabolism and the stress response. The activity of proteins in the Foxo subfamily is predominantly regulated by phosphorylation status and subcellular localization, but these transcription factors are also subject to a number of post-translational modifications, including acetylation and ubiquitination. Growth factors mobilize numerous kinases, including Akt, that phosphorylate Foxo proteins, leading to their export from the nucleus and inactivation. In contrast, stress stimuli mobilize a different set of kinases, notably Jun N-terminal kinase (Jnk), that promote nuclear import of Foxo and the initiation of Foxo-dependent gene regulation programs[2]. These transcription factors can modulate gene expression by binding promoters at Foxo-binding sites to recruit transcriptional machinery, by competing for promoter binding sites with other factors, and by dynamically regulating interactions between cofactors and various elements of the transcriptional machinery[3].

In the past decade, immunologists have come to appreciate the importance of the Fox family in coordinating immune responses, particularly in the context of Foxp3 expression by T regulatory (T_{reg}) cells. The Foxo subfamily members (in mammals, Foxo1, Foxo3, Foxo4 and Foxo6) have also been shown to influence T cell function. T cell receptor (TCR) and co-receptor ligation modulate the activity of Foxo proteins that upregulate prosurvival programs in the presence of growth factors and initiate apoptotic pathways in the absence of mitogen or cytokine[2]. In 2004, Pandiyan *et al.* demonstrated that signaling through the inhibitory receptor cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) protected T helper type 2 (T_H2) cells from apoptosis via Foxo3 inactivation and subsequent upregulation of the survival protein Bcl-2 (ref. 4). Consistent with a proapoptotic, regulatory role for Foxo3, Lin *et al.* reported that Foxo3a antagonizes signaling by the transcription factor NF-κB and that mice carrying a mutated *Foxo3a* allele underwent spontaneous T cell hyperproliferation and inflammation[5]. In 2007, it was shown that Foxo3a participates in the maintenance of memory $CD4^+$ T cells by coordinating signals from cytokine receptors and the TCR[6]. Thus, Foxo proteins appear to have an intrinsic role in T cell homeostasis and in limiting T cell activity.

Dejean *et al.* examined how Foxo3 influences T cell population expansion and contraction during infection by lymphocytic choriomeningitis virus (LCMV). In this model, the absence of Foxo3 led to an exaggerated antigen-specific T cell accumulation, with largely normal contraction[1]. Whereas a previous study had suggested that Foxo3 was "largely dispensable"[5] in modulating T cell apoptosis and was involved exclusively in spontaneous T cell population expansion, Dejean *et al.* showed that antigen-specific T cells in Foxo3-deficient mice had enhanced expression of the prosurvival factor Bcl-2 and decreased binding to the apoptosis marker annexin-5. Notably, the expansion of T cell populations observed in the Foxo3-deficient mice was not T cell intrinsic and was instead dependent on increased production of the cytokine interleukin-6 (IL-6) by Foxo3-deficient dendritic cells (DC)[1]. IL-6 has long been regarded as proinflammatory, and it also has a strong prosurvival effect on activated T cells[7]. Blockade of IL-6R α-chain restored the magnitude of the LCMV-driven T cell response in the Foxo3-deficient environment to a level comparable to that observed in wild-type mice. Finally, Dejean *et al.* showed that CTLA-4 ligation induced nuclear localization and activation of Foxo3 and inhibited the production of IL-6 in DCs *in vitro*[1]. These findings provide a previously unsuspected mechanism by which CTLA-4–expressing T cells can limit their own survival; by initiating Foxo3 activation and nuclear import in DCs, T cells modulate DC cytokine production and thereby influence their own viability (**Fig. 1**).

These findings illustrate the importance of the interactions that take place between DCs and T cells in regulating the expansion and contraction of T cell populations during infection. In addition, this work identifies a new role for Foxo3 in modulating this process. Understanding how T cell responses are regulated and maintained during infection has obvious implications for vaccine development. To this end, multiple DC–T cell interactions that result in the activation of these two

Elia D. Tait and Christopher A. Hunter are in the Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.
e-mail: chunter@vet.upenn.edu



Kim Caesar

Figure 1 T cells can limit their own accumulation by multiple mechanisms mediated by CTLA-4 signaling and the Foxo3 transcription factor. During antigen presentation, B7 molecules on DCs engage CTLA-4 expressed on activated T cells or T_{reg} cells. The resulting signals promote direct control of T cell activation and expansion through downregulation of IL-2 production, decreased T cell activation and decreased cell cycle progression. Also, the B7–CTLA-4 interaction initiates signaling in the DCs that mobilizes nuclear import and activation of Foxo3, which indirectly controls T cell population expansion by limiting DC production of the prosurvival cytokine IL-6. T cells with limited access to IL-6 are more susceptible to apoptosis and have lower expression of Bcl-2. MHC II, major histocompatibility class II.

populations have been characterized. The CD40-CD40L interaction is one example of a mechanism through which T cells influence their own activation and accumulation by optimizing DC responses. In contrast, the interactions between T cells and DCs that govern the contraction and control of T cell numbers are less well defined[8]. DCs may imprint T cells during activation with apoptotic programs, and T cell population expansion is certainly limited by T cell–mediated DC killing[9]. Also, interactions of CTLA-4 and programmed death-1 with their respective ligands intriusically modulate T cell responses and are important in controlling autoimmunity[8]. Specifically, mice lacking CTLA-4 develop spontaneous lymphoproliferative disease (similar to that of the Foxo3-deficient mice described previously[5]), indicating that this molecule is vital in maintaining T cell homeostasis[10]. Overall, however, the signals that orchestrate the fine balance between too much and too little T cell population expansion during an immune response are still unclear.

The findings of Dejean *et al.* suggest a scenario in which activated T cells that express CTLA-4 (or T_{reg} cells that constitutively express CTLA-4; ref. 11) can induce nuclear localization of Foxo3, and this active Foxo3 then limits the production of IL-6 by DCs and thus controls T cell population expansion (**Fig. 1**)[1]. Certain aspects of this model need to be tested further, however. Some previous studies have shown that antibody-mediated blockade of CTLA-4 can lead to enhanced T cell responses during infection[12] and cancer[13], and the model outlined in **Figure 1** suggests that antagonizing CTLA-4 *in vivo* during LCMV infection should lead to an increase in the expansion of T cell populations[1]. However, in agreement with other studies[14], Dejean *et al.* found that this was not the case: LCMV-driven T cell responses were unaffected by CTLA-4 blockade *in vivo*[1]. These negative data serve as a reminder that blocking CTLA-4 has different effects during different types of immune responses[1,4,8,12–14]. Thus, the effect of CTLA-4 signaling on the expansion and contraction of the T cell population overall might vary depending on context. The apparent contradiction between the model described in **Figure 1** and the negative *in vivo* data of Dejean *et al.*[1] also indicates that there may be ligands other than CTLA-4 that lead to Foxo3 activation *in vivo*. The model proposed by Dejean *et al.* prompts other questions regarding the biology and function of Foxo3 in response to CTLA-4 signaling. The lack of Foxo3-binding sites in the *Il6* promoter[1] implies that Foxo3 may regulate *Il6* transcription through indirect means or by upregulating a broader anti-inflammatory gene expression program that includes downregulation of IL-6 production. Overall, the principles established by Dejean *et al.* are important in advancing our understanding of the role of DC–T cell interactions in controlling T cell populations, and they highlight some of the contradictions and future directions in this field.

In total, the current literature suggests that CTLA-4 and Foxo3 have important roles in the control of spontaneous and antigen-induced T cell population expansion. In this issue, Dejean *et al.* make an important contribution by providing evidence linking these pathways[1]. In the context of treating human disease, particularly autoimmune disease, controlling the immune response using therapies that mimic natural cell-intrinsic immunoregulation is an appealing strategy. CTLA-4–Ig, a fusion between CTLA-4 and IgG that mimics CTLA-4, was initially thought to act as an antagonist of B7-CD28–mediated signaling that would downregulate inappropriate T cell population expansion in the context of autoimmunity; we now appreciate that it has additional effects, including inducing the immunosuppressive indoleamine-2,3-dioxygenase pathway[8]. This fusion protein is now used for the management of rheumatoid arthritis, but the true biological mechanism behind its clinical success is not entirely clear. Whether Foxo3 signaling is also influenced during treatment with CTLA-4–Ig remains to be tested. Although CTLA-4 signaling and the *in vivo* biology of Foxo3 are still not fully understood, the work of Dejean *et al.*[1] prompts exciting new questions about the basic biology and clinical relevance of these factors.

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