innate immune signaling: one links CARD9 to intracellular receptors and MAPK activation; the other links CARD9 to surface receptors and NF-κB activation. It is not yet apparent whether those differences stem from the use of different cell types (macrophages versus dendritic cells) or whether CARD9 is used differently downstream of different receptors. Nevertheless, the data demonstrate that CARD9 is a convergence point for innate immune signaling in response to intracellular bacteria, viruses and fungi.

COMPETING INTERESTS STATEMENT
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T_{reg} cells: guardians for life

Jonathan A Hill, Christophe Benoist & Diane Mathis

Regulatory T cells expressing the transcription factor Foxp3 are known to control autoreactivity during and subsequent to the development of the peripheral immune system. New evidence emphasizes the fact that those cells are constant and powerful guardians against the state of 'horror autotoxicus'.

The immune system has devised many checks and balances to circumvent autoimmune disease. Those mechanisms have been broadly categorized as influencing central or peripheral tolerance, each having a nonredundant function in maintaining antigen-receptor diversity while providing safeguards to effectively curtail self-reactivity. Exceptions to the smooth operation of those well defined modes of tolerance induction have helped to identify critical molecules or cell types, some of which have helped shift the balance of popular opinion as to the importance of central versus peripheral tolerance. The identification of one such molecule, the transcription factor Foxp3, and the realization of its function as a lineage-specific marker of the $CD25^+$ regulatory T (T_{reg}) cell, has produced such a shift. In this issue of *Nature Immunology*, Rudensky and colleagues have evaluated the outcome of acutely ablating Foxp3-expressing cells and thereby demonstrating how important they are[1]. They show that $Foxp3^+$ T_{reg} cells not only control autoimmunity during the time of neonatal lymphopenia but also restrain autoimmunity throughout life.

T_{reg} cells (descendents somehow of the 'suppressor T cells' of the 1970s and 1980s) have occasioned a renaissance of interest since studies in mice showed a requirement for a subset of T lymphocytes expressing the surface marker CD25 (also known as the interleukin 2 receptor-α chain) to control certain autoimmune manifestations[2]. Delineation of that $CD4^+CD25^+$ population provided a new 'handle' on specifically isolating and manipulating T_{reg} cell activity and eventually helped to identify Foxp3 as a factor that controls their differentiation and function[3]. Certain mutations in the human gene encoding Foxp3 result in the clinical entity known as 'IPEX' (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, and a defect in the analogous mouse *Foxp3* gene is found in the scurfy mouse line that has a similar phenotype.

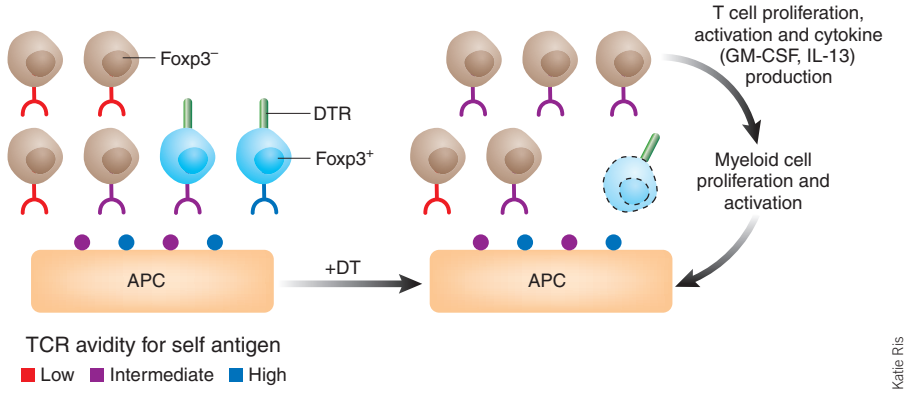

Figure 1 T_{reg} cells control lympho-myeloid cell proliferation in adults. In the normal adult $CD4^+$ T cell repertoire, self-reactive $Foxp3^-$ $CD4^+$ T cells are 'harnessed' by $Foxp3^+$ T_{reg} cells. It is now apparent that ablation of T_{reg} cells through transgenic expression of the DTR in $Foxp3^+$ T cells (and subsequent treatment with diphtheria toxin (+ DT)) causes self-reactive $Foxp3^-$ $CD4^+$ T cell to become activated and to secrete cytokines. That in turn leads to myeloproliferation, thus establishing a vicious cycle of activation that results in autoimmune disease. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-13, interleukin 13; APC, antigen-presenting cell.

Several issues regarding the *in vivo* operation of $Foxp3^+$ T_{reg} cells remain unresolved. First, *Foxp3* deletion or mutation leads to severe autoimmune lymphoproliferative disease in very young mice. Because such germline mutations in *Foxp3* could alter T cell homeostasis at early stages of development, a clear understanding of how $Foxp3^+$ T cells regulate peripheral immune homeostasis in the normal adult immune system has been lacking. Second, many studies involve the transfer of mature $CD25^-$ T cells into a lymphopenic host, a condition that induces autoimmunity that can be prevented by cotransfer of $CD25^+$ T cells (and other subtypes); however, results generated by such an experimental protocol probably depend on

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the unique 'empty' host environment[4,5]. Third, mice depleted of T_{reg} cells through neonatal thymectomy or antibody-mediated depletion have a milder autoimmune phenotype than mice with germline *Foxp3* mutations. However, *in vivo* depletion of T_{reg} cells using antibody to CD25 in a mature host does not eliminate all Foxp3$^+$ T_{reg} cells, given that a substantial population of Foxp3$^+$ T_{reg} cells are CD25$^-$ (refs. 6,7). Moreover, strategies for depletion using antibodies to CD25 may also eliminate recently activated effector T cells (and B cells). To circumvent those issues, Rudensky and colleagues introduced the human diphtheria toxin receptor (DTR) into the 3′ untranslated region of mouse *Foxp3*, which results in T_{reg} cell–specific expression of the human DTR and the ability to achieve depletion of those cells by the administration of diphtheria toxin.

Using the '*Foxp3*^{DTR}' mice, the authors made many interesting and unique observations. Initial studies validated the DTR model, showing more than 97% depletion of Foxp3$^+$ cells within 48 hours of diphtheria toxin administration. They assessed regeneration of the T_{reg} cell compartment after transient depletion and found it to be robust. Within 4 days, a substantial population of Foxp3$^+$ cells reappeared in the thymus (about 50% of the wild-type population), whereas the peripheral compartment lagged behind (only 6–8%). However, the thymus and all peripheral lymphoid organs examined had a fully re-equilibrated Foxp3$^+$ population by between 10 and 15 days after depletion.

The faster kinetics of repopulation in the central versus peripheral lymphoid tissues suggests that the thymus could be the principal site for T_{reg} cell generation, consistent with findings identifying T cell receptor (TCR) repertoires in different organs[8,9]. However, the possibility of thymus-independent peripheral 'conversion' cannot be excluded in this context; further experiments in thymectomized mice should help clarify the issue.

Because of the rapid regeneration of Foxp3$^+$ cells, the authors used a regimen of constant diphtheria toxin administration to determine if T_{reg} cell ablation in neonates resulted in a 'phenocopy' of the disease that manifests in Foxp3-deficient mice, and indeed it did. The only notable difference was a reduction in gross clinical tail skin lesions in *Foxp3*^{DTR} mice.

Next, the authors depleted 3-month-old adult *Foxp*^{DTR} mice of Foxp3$^+$ T_{reg} cells. *Foxp*^{DTR} mice treated with diphtheria toxin succumbed to disease with an extremely rapid onset, with some mice dying within 10 days and all mice progressing to terminal disease within 3 weeks. Progressive, eventually extensive T cell activation was made manifest by the expression of diagnostic cell-surface markers. Moreover, within 7 days of T_{reg} cell depletion, there was a massive expansion of myeloid populations in the lymph nodes, including dendritic cells, macrophages, neutrophils and natural killer cells, which may have been the result of excessive cytokine production (such as interleukin 13 and granulocyte-macrophage colony-stimulating factor) by activated CD4$^+$ T cells in the T_{reg} cell–ablated environment (**Fig. 1**). Although the T_{reg} cell–mediated control of dendritic cell activity proposed by Rudensky and colleagues is a likely hypothesis and is consistent with some other published data, their study does not demonstrate a direct effect or explore the relevance of expansion of other cell populations (such as natural killer cells) that might also contribute to the autoimmunity manifested after T_{reg} cell depletion.

The results obtained with adult *Foxp3*^{DTR} mice indicate substantial 'harnessed' self-reactivity in the peripheral CD4$^+$ T cell population. Results from other published studies concur that the TCRs of CD25$^+$ and CD25$^-$ T cells are distinct but do have some overlap[8,9]. Most TCRs on CD25$^+$ cells have been found to be self reactive, whereas the opposite is true of TCRs on the CD25$^-$ cells analyzed. However, certain Foxp3$^-$ T cells with TCR specificities also represented in Foxp3$^+$ T cell populations 'preferentially' expand their populations in lymphopenic hosts, suggesting that this population, when not diverted to the T_{reg} lineage in the thymus, may be restrained in the periphery by Foxp3$^+$ T cells[9]. Once Foxp3$^+$ T cells are removed (as in *Foxp3*^{DTR} mice), such Foxp3$^-$ T cells can expand their populations in the presence of self antigen.

Similarly, the scurfy autoimmune phenotype depends both on T_{reg} cell ablation and on a population of Foxp3$^-$ CD4$^+$ T cells with self-reactive TCRs (which act as effectors). Using mice expressing a foreign antigen–specific (SMARTA) TCR transgene on a recombinase-activating gene–deficient genetic background (which lack Foxp3$^+$ T cells), Rudensky and colleagues generated data consistent with those previous findings, as they did not find dendritic cell proliferation (a 'surrogate marker' of abnormal self-reactive T cell activation) in the mice. Moreover, depletion of CD4$^+$ T cells after T_{reg} cell ablation in *Foxp3*^{DTR} mice prevented the dendritic cell proliferation found in T_{reg} cell–depleted mice replete with CD4$^+$ T cells. Thus, it seems that self-reactive Foxp3$^-$ CD4$^+$ T cells mediate the disease in *Foxp3*^{DTR} mice, although the effector cell types directly responsible for tissue damage have not yet been conclusively pinned down.

Many issues remain to be explored in terms of the control of autoreactivity by T_{reg} cells. Even though Foxp3 deficiency in mice and humans leads to autoimmune phenotypes, there is a restriction on the organs targeted. For example, in scurfy mice, inflammatory cell infiltration is not seen in many organs, such as the central nervous system, joints and small intestine[10]. Is such organ restriction due to the TCR specificity of CD4$^+$ T cells and manifestations of major histocompatibility complex restriction, or are there specific features of the tissues or local vasculature that restrict infiltration? Nor is it clear whether T_{reg} cells act mainly in target organs or in draining lymphoid tissues[11]. Foxp3$^+$ T cells are normally found in the lung, liver and skin; hence, the suppression responsible for curtailing excessive inflammation in tissues may be imposed by resident T_{reg} cells rather than by regulatory cells in the draining lymph nodes. Finally, what is the relationship between T_{reg} cells and other regulatory T lymphocyte populations such as Tr1 or T helper type 3 cells? As shown by Rudensky and colleagues, the last two cell types are incapable of restraining autoimmune inflammation in the periphery: but are they simply less important reinforcements or do they 'patrol' different territories?

In addition, the molecular mechanisms by which T_{reg} cells exert their inhibition *in vivo* are still fuzzy. For example, the precise function of Foxp3 itself in those cells is still not fully understood: is it required transiently to establish a specific transcription profile during lineage commitment, or is it needed throughout the life of a T_{reg} cell to maintain suppressor function, or both? Rudensky and colleagues have certified Foxp3$^+$ T cells as a dominant force in controlling lympho-myeloid cell proliferation and activation in the adult steady-state immune system, and the experimental model presented in their paper will facilitate the exploration of additional aspects of T_{reg} cell biology.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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