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NEWS AND COMMENTARY

Regulatory T-cell differentiation

Committed to control: a precocious choice?

Sokol Haxhinasto[1], Christophe Benoist and Diane Mathis

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The prevailing model of regulatory T (T_{reg}) cell lineage commitment in the thymus entails that T_{reg} cells differentiate from $CD4^+CD8^+TCR\alpha\beta^+$ double-positive (DP) precursors after T-cell receptor (TCR) engagement of major histocompatibility complex (MHC):peptide complexes. The discovery that T_{reg} lineage commitment may occur at the earlier $CD4^-CD8^-$ double-negative (DN) stage independently of cognate peptide interactions challenges this model and raises many important questions.

The thymus is the primary lymphoid organ wherein bone-marrow-derived progenitor cells undergo positive and negative selection to shape the repertoire of T lymphocytes that populate the spleen and lymph nodes. In brief, thymocyte selection encompasses a series of well-orchestrated events that ensure progression of immature DN cells into the DP compartment, and culminate in maturation into $CD4^+$ and $CD8^+$ single-positive (SP) cells. Despite the strict rules imposed during selection, self-reactive T cells are still generated, and do appear in the periphery. Recent studies have demonstrated that the thymus is also the major source of regulatory T (T_{reg}) cells,[1] which exert 'dominant' control over self-reactive T cells to prevent autoimmune disease and dampen inflammation in diverse contexts.[2]

To date, the thymic differentiation pathway leading to T_{reg} cells is still an unsolved puzzle for immunologists. The currently popular view, based largely on analogy, is that T_{reg} cells emerge from DP precursors as a consequence of TCR engagement of MHC:peptide ligands of a particular window of affinity/avidity.[3–5] Work by Pennington *et al.*[6] now challenges this view by suggesting that diversion to the T_{reg} lineage happens earlier during thymocyte differentiation and independent of cognate MHC:peptide recognition.

The authors' findings stem from their previous studies that identified an unexpected role for DP cells in *trans*-conditioning DN progenitor cells, strongly influencing their fate.[7] The authors demonstrated that DP cells regulate $\gamma\delta$ T-cell differentiation, identifying RORγt and lymphotoxin β receptor as critical mediators. Extending these observations, Pennington *et al.*[6] reported the enrichment of a population of T_{reg} cells in mice that lack a normal thymus DP population because of a TCR β-chain gene null mutation. Interestingly, this population expressed the 'master' transcriptional regulator Foxp3,[8] which serves to identify naturally occurring T_{reg} cells.

This finding was further extended to other mice with a DP-cell deficiency, specifically mice with a mutation in the gene encoding the pre-Tα (pTα) chain. Absence of pTα blocks thymocyte differentiation at the DN3 and DN4 stages, well before the DP stage, which accordingly drastically reduces the number of DP cells (down to 1% that of wild-type mice). The DP-cell deficiency in $pT\alpha^{-/-}$ mice was also accompanied by an enrichment in $Foxp3^+$ T_{reg} cells. Interestingly, this augmentation was inversely correlated with the size of the DP population: a progressive increase of the DP cells either genetically, through the use of mice that express different levels of pTα, or by construction of appropriate bone-marrow chimeras was mirrored by a progressive decrease and eventual normalization of the representation of $Foxp3^+$ cells.

Although the interpretation of these findings is confounded by the fact that mice with a dearth of DP cells are also lymphopenic, the authors' (perhaps fortuitous) logic led them to hypothesize the existence of a pre-committed sub-population of DN cells that contributes to the T_{reg} pool in a manner independent of the agonist selection that takes place at the DP stage. Using a previously employed clever transgenic system,[7] which tracks putative *trans*-conditioned cells by their upregulation of the *CREM/ICER* gene, and transcriptional profiling, the authors were able to further subdivide the *trans*-conditioned population into two distinct sub-populations: DN2-L (large) and DN2-S (small) cells. The DN2 population from $pT\alpha^{-/-}$ mice more closely resembled the DN2-S sub-population in wild-type animals, hinting at the possibility that DN2-S cells might serve as precursors to T_{reg} cells. Indeed, when the authors tested the differentiation potential of DN2-L and DN2-S cells using fetal thymic organ cultures, the DN2-S cells gave rise to significantly more T_{reg}-like CD4-SP cells, characterized at the transcriptional level.

On the basis of these results, the authors proposed a model whose major tenet is that T_{reg} lineage commitment in the thymus occurs at the DN2 stage, independent of cognate peptide interactions, dependent, instead, on DP cell *trans*-conditioning of DN2 cells. They argue for the existence of two distinct DN2 populations, the *trans*-conditioned DN2-L subset, which gives rise to conventional DP cells and ultimately SP cells, and the unconditioned DN2-S cells, a subset of which eventually differentiates into T_{reg} cells (Figure 1).

However, there is an important caveat to this set of conclusions. Bosco *et al.*[9] had previously observed the paucity of DN thymocytes and enrichment of $Foxp3^+CD4$-SP thymocytes in pTα-deficient mice. In a very careful and detailed series of experiments, they demonstrated that many of the latter represented recirculation of peripheral $Foxp3^+CD4^+$ cells into the lymphopenic $pT\alpha^{-/-}$ thymus. The key experiment

S Haxhinasto, C Benoist and D Mathis are at the Section on Immunology and Immunogenetics, Joslin Diabetes Center, Department of Medicine, Brigham and Women's Hospital; Harvard Medical School, One Joslin Place, Boston, MA 02215, USA.
E-mail: cbdm@joslin.harvard.edu
[1]Sokol Haxhinasto is a Damon Runyon Fellow supported by the Damon Runyon, Cancer Research Foundation.

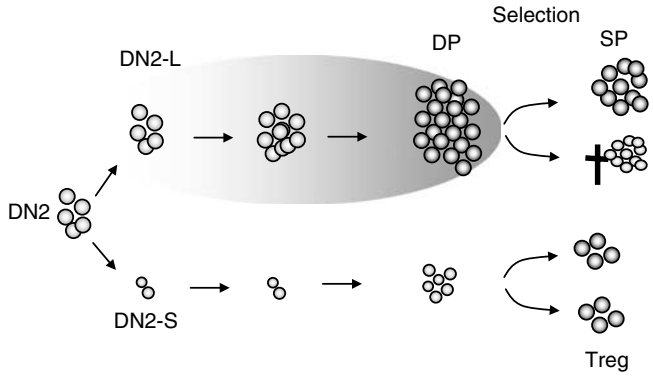

Figure 1 DP *trans*-conditioning dictates T_{reg} lineage commitment. DP cells condition DN2-L cells and regulate their differentiation into conventional SP cells. DN2-S cells are refractory to *trans*-conditioning by DP cells and serve as precursors to T_{reg} cells.

involved 'parabiotic' mice constructed by anastomosing the vasculature of pTα-deficient and wild-type animals: T_{reg} cells from the wild-type parabiont could be found in the $pT\alpha^{-/-}$ thymus, but not vice versa.

Regardless of this issue, the identification of progenitor DN2 cells with distinct differentiation potentials provides further insight into the heterogeneity of thymic progenitors, and raises additional questions about the mechanisms underlying thymocyte differentiation to ensure an apt immune repertoire. Do the DN2-S cells from pTα-mutant mice give rise to T_{reg} cells in an fetal thymic organ culture (FTOC)? What are the similarities and differences in the global transcriptional profiles between T_{reg} cells generated strictly by DN2-S or the few generated by DN2-L cells? Is there a mutual 'conditioning' between these two precursor populations for optimal generation of the T_{reg} compartment? What makes the DN2-S cells less sensitive to *trans*-conditioning by DP cells and, what are the molecular signals involved in this process?

The work by Pennington *et al.*[6] predicts that early in the life of a normal mouse, when the first wave of thymocytes is undergoing differentiation, so that the DP population is less represented than later in life, the thymus and periphery would be enriched in T_{reg} cells. Is this true and, if so, what is the significance of this early T_{reg}-cell appearance? In addition, the DP-cell deficiency in $pT\alpha^{-/-}$ and $TCR\beta^{-/-}$ mice is analogous to lymphopenia observed in chronic infections or even bone-marrow transplantations, and therefore it would be interesting to see how the T_{reg} population is affected in those settings.

The study of Pennington *et al.*[6] conditions our thinking in new ways to describe the intricacies of thymocyte differentiation, and provide a framework for further experimentation.

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