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Nature and nurture in Foxp3$^+$ regulatory T cell development, stability, and function

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ABSTRACT

Foxp3$^+$ regulatory T lymphocytes (Treg) are critical homeostatic regulators of immune and inflammatory responses. Their absence leads to fulminant multiorgan autoimmunity. This review explores recent studies that have altered our emerging view of the development, stability, and plasticity of these cells. Treg appear not to be a single entity, but a family of immunomodulatory cell types with shared capabilities. On a first level, Treg may alternatively form in response to developmental cues in the thymus as a distinct lineage of CD4$^+$ T cells or adaptively, in response to environmental cues received by mature conventional CD4$^+$ T lymphocytes. These 2 populations bear distinct specificity, stability, and genetic profiles and are differentially used in immune responses. Secondarily, in a manner analogous to the generation of T helper (Th)-1, Th2, and other T cell subsets, Treg may further specialize, adapting to the needs of their immunologic surroundings. Treg therefore comprise developmentally distinct, functionally overlapping cell populations that are uniquely designed to preserve immunologic homeostasis. They combine an impressive degree of both stability and adaptability.

1. The T regulatory lineage

Lineage: descent in a line from a common progenitor. (*Merriam Webster*)

Tracing one's lineage is important to many who seek to understand their origins and character. The extent to which our complex traits indeed mirror those of our ancestors is uncertain. However, within us, cellular lineages do exist that possess stable, generationally transmitted traits. These traits are the product of self-perpetuating genetic programs that commit a cell and its descendants to a particular fate.

Although not scientifically defined, several common features characterize cellular lineages: (1) induction by developmental cues of a phenotypic profile in a common progenitor; (2) maintenance of the profile through the altered expression of 1 or more genetic regulators; (3) stabilization of the profile, typically through cell-intrinsic feedback loops or epigenetic modifications; and (4) in the absence of terminal differentiation, robust transmission of the profile to progeny after cellular division. Conventional CD4$^+$ and CD8$^+$ T cells are examples of cellular lineages. Their thymic progenitors are imprinted with characteristic transmissible gene expression patterns that maintain a broad range of lineally preserved qualities. Overlaid on these lineage assignments are a variety of additional qualities. Thus, T cells can mature into T-helper (Th)-1, Th2, Tc1, Tc2, and a host of other subsets that manifest stereotyped effector responses when stimulated by antigen (Ag) [1]. Unlike differentiation into CD4 or CD8 T cells, which occurs developmentally, subset differentiation results from a T cell's integration of local environmental cues to optimize the cell's response to a particular immunologic challenge.

CD4$^+$ Foxp3$^+$ regulatory T lymphocytes (Treg) are a subclass of CD4$^+$ T cell receptor (TCR) $\alpha\beta^+$ T cells that are essential to preserve immune homeostasis [2,3]. Absence of Treg or the Foxp3 transcription factor they express leads to the rapid development of fulminant multiorgan autoimmunity. Unlike other CD4$^+$ T cell subsets that form extrathymically from conventional CD4$^+$ T cells (Tconv), Treg can develop as a separate population in the thymus. Consequentially, Treg are often referred to as a distinct lineage. Yet the extent to which these cells are an independent lineage or a metastable maturation state that is interconvertible with Tconv has been a topic of debate [4,5]. Indeed, Treg exhibit both a high degree of stability with preserved phenotypic and functional properties and an acute sensitivity and adaptability to environmental inputs. Further, Treg differentiation is not restricted to the thymus, where natural Treg (nTreg) are generated. Environmental Ags and extrathymic signals can upregulate Foxp3 in Tconv, converting them into induced Treg (iTreg) [6,7]. Circulating Treg therefore include 2 populations, 1 thymically derived that appears to meet the criteria for a cellular lineage and a second that forms adaptively and seems not to. In this review we explore the development, stability, and plasticity of these different forms of Treg.

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2. TCR specificity and Foxp3 expression in thymic Treg differentiation

TCR α- and β-chain gene rearrangement and expression in early thymic precursors prompts a series of TCR recognition-dependent events leading to differentiation into $CD4^+$ or $CD8^+$ T cell subclasses. Expression of Th-inducing POZ/Kreuppel factor is critical for $CD4^+$ T cell differentiation, and its absence is associated with the "helper-deficient" mutant phenotype. Runx3, in turn, is required for $CD8^+$ T cell differentiation [8,9]. The actions of these factors are further overlaid on a complex landscape of alterations, including in Notch, Gli1, GATA3, Ikaros, and Tox. Ultimately, these changes lead to robust lineage assignments.

The sina qua non for Treg is the sustained expression of a single transcription factor, Foxp3 [10,11]. Foxp3 can bind the promoters and influence the expression of hundreds of genes [12,13]. Importantly, Foxp3, in a complex with other transcription factors, particularly CBFβ and Runx1, can bind its own promoter and support its own expression [14]. Such positive feedback is important in ensuring continued gene expression and lineage persistence. However, Foxp3 expression is not in itself sufficient for Foxp3 transcription. Endogenous Foxp3 is not upregulated in Tconv transduced with a Foxp3 transgene [15], indicating that Foxp3 alone cannot induce its own expression. An evolutionarily conserved element in the Foxp3 promoter, termed the Treg-specific demethylated region, is heavily methylated in Tconv but demethylated in Treg, potentially limiting the activity of transduced Foxp3 in this regard [16,17]. Indeed, inhibitors of DNA methylation help stabilize Treg Foxp3 expression, whereas germline deletion of the TSDR results in loss of Foxp3 [14,18]. Additional transcription factors not directly induced by Foxp3 are also important for Foxp3 expression [14,19]. Therefore, the presence of Foxp3 defines Treg, but Treg generation is not a simple matter of Foxp3 upregulation.

Foxp3 is also not required for its own transcription. A subset of T cells in mice with a deleted Foxp3 coding sequence still actively transcribe from the Foxp3 promoter [20]. Presumably these cells, which exhibit some properties of Treg but are not suppressive, would have differentiated into Treg if the Foxp3 coding sequence had been present. These "would be" Tregs do exhibit decreased retention of Foxp3 promoter transcription compared with wild-type Treg when transferred into lymphopenic SCID mice, indicating a role for Foxp3 in persistent gene expression.

For most Treg, Foxp3 is first expressed in a subset of CD4 single positive (SP) cells within the thymic medulla [21]. This SP stage of differentiation is the last maturation stage prior to egress of the newly formed mature T cell. A Treg differentiation program may be induced in these SP cells, leading to rapid Foxp3 upregulation, or initiate in an earlier precursor with a delay in Foxp3 expression. Some data indicate a multistage commitment process that may include priming steps necessary for Foxp3 commitment at early stages of thymocyte development [22]. Supporting a model of earlier Treg commitment, CD28 is required for the expansion of $Foxp3^-$ Treg precursors in the thymus [23]. Likewise, Treg develop in transgenic mice in which the class II major histocompatibility complex is limited to the thymic cortical epithelium, which interacts primarily with earlier $CD4^+CD8^+$ precursor cells [24–26].

Much as for $CD4^+$ and $CD8^+$ T cells, Treg development is TCR specificity dependent. T cells from mice transgenic for any of several rearranged Tconv-derived TCR, when bred on a Rag-deficient background so that they cannot rearrange and express endogenous TCR, fail to develop Treg [27–30]. Therefore, expression of a Tconv-derived TCR prevents Treg differentiation. On a Rag-sufficient background, allelic nonexclusion of the TCR α-chain allows the formation of dual receptor T cells that coexpress transgenic TCR $\alpha\beta$ as well as endogenous TCR α paired with transgenic TCR β. This second TCR complex can promote Treg differentiation in a fraction of transgenic T cells. Depending on the efficiency of allelic exclusion and the specific transgene, different numbers of Treg are observed. As additional evidence indicating a developmental role for TCR specificity, several groups have demonstrated that Treg and $CD4^+$ Tconv possess distinct TCR repertoires [31–33]. Treg and Tconv TCR sequences exhibit only limited overlap in either the thymus or the lymphoid tissue.

The different repertoires of Treg and Tconv endow these cells with distinct functional properties, with a particularly enriched self-specificity among Treg. T cells transduced with Treg TCR exhibit increased activation and proliferation after transfer into syngeneic mice when compared with TCR from Tconv [31]. Spontaneously activated T cells present in Foxp3-deficient mice exhibit repertoire overlap with Treg from Foxp3-sufficient mice, implying that these self-reactive cells would have differentiated into Treg had Foxp3 been available [34,35]. A mouse expressing a Treg TCR transgene exhibited high levels of thymic deletion, indicating specificity for self Ag [36]. Finally, provision of cognate Ag can promote the thymic development of Treg in TCR transgenic (Tg) mice, indicating that high-affinity ligand can prompt Treg differentiation [37]. These findings have led to the hypothesis that a subset of developing thymocytes with an affinity for self Ag intermediate between that required for positive and negative selection develop into Treg [38–40].

Even in the absence of inflammation or disease, lymphoid tissue draining different locations possesses Treg with distinct repertoires. Interestingly, these Treg display enriched specificity for regional tissue-restricted Ags [41,42], implying that specificity governs localization. In the setting of autoimmune diseases, responding Treg are also enriched for tissue-specific reactivity [32,43,44]. Although the extent of the antiself bias among Treg compared with Tconv has been disputed [45], Treg clearly form a predominantly distinct group of cells when compared with other $CD4^+$ T cells. Indeed, public Treg-associated TCR sequences can be identified [32,43], indicating a conserved skewing in Treg recognition across individuals.

Importantly, possession of a particular TCR specificity is necessary but not sufficient for Treg differentiation. Whereas the enforced transgenic expression of monoclonal Tconv-derived TCR induces conventional $Foxp3^-$ T cell differentiation in the thymus, only a minority of T cells from thymocytes expressing transgenic Treg-derived TCR develop into Treg [36]. An explanation for this came with the production of chimeric mice incorporating variable numbers of hematopoietic or thymic progenitor cells with fixed Treg-derived TCR admixed with unmanipulated progenitor cells [32,46,47]. When only very small numbers of precursors express a monoclonal Treg TCR, the extent of Treg differentiation is proportional to precursor numbers. However, Treg production rapidly saturates as larger numbers of Treg-TCR Tg precursors are added to the wild-type cells. The additional transgenic T cells differentiate into Tconv that express the Treg-derived TCR. Therefore, Treg but not Tconv development is quantitatively limited for an individual TCR. Implicitly, niches permissive for Foxp3 induction in thymocytes with a particular specificity are limited, and the default pathway for thymocytes that exceed this niche capacity is formation of Tconv. Specific types of Ag-presenting cell types may form these niches. For example, thymic stromal lymphopoietin produced by Hassal's corpuscles within the thymus has been demonstrated to convert migratory dendritic cells (DCs) into cells capable of upregulating Foxp3 in thymocytes [48].

In summary, Treg possess features of other cellular lineages. Differentiation occurs at a specific developmental checkpoint. Inductive signals, particularly the quality of the TCR's signal to thymic self Ag but also other constitutively available signals, such as through CD28 and CD40, guide development. This leads to the