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INVITED COMMENTARY

All regulators great and small: when Treg need small RNAs to fulfill their commitment

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$CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) are a small subset of $CD4^+$ T cells that are crucial in the maintenance of immunological self-tolerance as well as for the active regulation of pathogenic effector T cells in inflammatory responses and after allogeneic transplantation. Mouse and human Treg are characterized by the constitutive high and sustained co-expression of the interleukin (IL)-2 receptor α-chain (CD25), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and of the transcription factor forkhead box p3 (Foxp3). Besides being used as phenotypic markers to identify Treg, these molecules are critical for their development, homeostasis, and suppressive function. In particular, Foxp3 is a master regulator of Treg function as demonstrated in clinical and experimental conditions in which the gene was mutated, deleted or overexpressed [1,2].

Naturally occurring Treg are selected in the thymus (tTreg), but a subset of Treg can also be induced *in vitro* or in the periphery *in vivo* (pTreg) from conventional uncommitted $CD4^+$ T cells following antigenic stimulation in the presence of specific immunomodulatory molecules. However, as compared to tTreg, pTreg might represent a less stable lineage, in particular in inflammatory or lymphopenic conditions [3]. Indeed, while a peripheral microenvironment enriched in TGF-β and IL-2 favors pTreg differentiation, the presence of inflammatory cytokines (in particular IL-6) skews naïve $CD4^+$ T cells toward IL-17-producing T-helper (Th17) cells [4,5] and may even destabilize previously differentiated Treg. Moreover, Treg selected from adult peripheral blood were shown to be heterogeneous, containing a subset of apparently differentiated Treg that can downregulate Foxp3 expression and produce effector cytokines in the absence of TGF-β or in a lymphopenic environment [6].

As Foxp3 constitutes a lineage specification factor for Treg development and suppressive activity, it is critical to understand how its expression is regulated to ensure Treg stability [7]. The specific transcriptional program of Treg is initiated by antigenic stimulation via the T-cell receptor together with CD28 downstream signaling that induce a set of nuclear factors, including Foxp3, that assemble to repress

or activate a particular set of target genes. Foxp3 in turn binds to accessible demethylated regulatory elements of the *Foxp3* locus including the conserved noncoding sequence 2 (CNS2; also called Treg-specific demethylated region, TSDR), thus perpetuating its own expression via a positive auto-feedback loop [8,9]. However, while Foxp3 alone appears to be sufficient to maintain stable lineage commitment in steady-state conditions, other factors may be required to stabilize its expression in disease settings and in the presence of pro-inflammatory cytokines [10].

Besides DNA methylation and histone modifications, epigenetic changes involving small RNAs have been described, allowing another level of regulation in gene expression. MicroRNAs (miRNAs) are highly conserved endogenously produced small single-stranded noncoding RNAs, which bind to target mRNAs and mediate post-transcriptional repression or mRNA degradation [11]. miRNAs are specifically expressed in certain cell subsets and contribute to the regulation of gene expression in physiologic processes such as cell differentiation, maturation, and proliferation. They were also shown to be involved in the homeostasis and differentiation of innate and adaptive immune cells, with disruption in their expression resulting in immunodeficiency or autoimmunity. Studies on mice deficient for the endoribonuclease Dicer, an important enzyme for the production of mature miRNAs, have revealed the key role of miRNAs in T- and B-cell development, effector functions, and survival. Importantly, selective deletion of Dicer in tTreg was shown to lead to the loss of Foxp3 expression and uncontrolled autoimmunity [12,13], highlighting the role of miRNAs in controlling important transcription factors involved in T-cell differentiation and lineage commitment. In this regard, *Foxp3* as well as other important genes involved in immune regulation were shown to contain predicted miRNA-binding sites in the 3′untranslated regions (UTR) of their mRNA.

Over the past years, individual miRNAs (e.g. miR-155, miR-146a, miR-17~92 cluster) have been associated with key immune regulatory pathways, including T-cell differentiation and function. Compared to conventional T cells, a number of miRNAs (e.g. miR-10a, miR-155, miR-146a) were shown either to be enriched in Treg or to affect their function. Interestingly, *in vivo* deletion experiments have shown that while individual miRNAs were dispensable for Treg-suppressive function in steady-state conditions, these miRNAs were important for Treg homeostasis during an immune response [14]. In this issue of *Transplant International*, Xie *et al.* [15] demonstrate a critical role for miR-26a in promoting Treg expansion and prolonging MHC-mismatched allograft survival in a mouse skin transplantation model. This is the first report of the role of miR-26a in the setting of transplantation as this miRNA was previously mainly described as a regulator in carcinogenesis. Of particular interest, the data presented in this study suggest the role of miR-26a in regulating the Treg/Th17 balance in the periphery by inhibiting IL-6 and promoting IL-10 expression. Whether miR-26a only affected cytokine production and the microenvironment in which naïve alloreactive $CD4^+$ T cells differentiated, or directly controlled the expression of Foxp3 and the stability of already differentiated functional Treg, remains, however, to be further investigated. Overall, this study illustrates that while the differentiation of T-cell subsets, including Treg, is dependent on specific transcription factors in physiologic states, miRNAs may provide another level of epigenetic regulation to fine-tune the differentiation program in inflammatory and pathologic conditions.

In recent years, specific miRNA signatures have been associated with a variety of human diseases including cancer, autoimmune diseases, and graft outcome after transplantation. As miRNAs are highly stable in biological fluids, cells, and tissues, current research is mainly validating their use as biomarkers for clinical diagnostic and therapeutic monitoring. In kidney transplantation, distinct miRNA expression profiles were associated with operational tolerance and acute cellular and antibody-mediated rejection [16]. The study by Xie *et al.* alludes to possible miRNA-based therapeutic strategies to promote stable pTreg differentiation *in vivo* and transplantation tolerance. Nonetheless, considering the diversity and context-dependent suppressive mechanisms of Treg, overexpressing or inhibiting a single miRNA may not be sufficient to control complex immune pathways in response to an allograft.

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