■ BENCH TO BEDSIDE

Turncoat regulatory T cells

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Researchers interested in combating diseases ranging from autoimmunity to cancer have taken an interest in regulatory T cells (T_{reg} cells), a $CD4^+$ T cell subset known for its ability to dampen the immune response. People and mice genetically deficient in a transcription factor that defines the lineage, forkhead box protein-3 (FoxP3), are afflicted with severe, multisystem autoimmune disease. Extensive research has shown that administration of T_{reg} cells can ameliorate disease in mouse models of autoimmunity. On the basis of such data, a few human trials of T_{reg} cells have been initiated.

A recent study by Zhou *et al.*[1] should insert a note of caution about the use of T_{reg} cells in the clinic. The researchers find that the T_{reg} cell lineage in mice is not nearly as stable as has been thought. The expression of FoxP3 can wax and wane, and with it the expression of a host of other molecules that help determine cellular phenotype. Cells that once were suppressive can become pathogenic and potentially contribute to disease—not prevent it.

For a decade and a half, T_{reg} cells have been at the center of immunological investigation, and we now know a great deal about their capacity to keep immune responses to pathogens and host tissues at bay. Two types of T_{reg} cells are now well recognized: natural T_{reg} cells, which arise in the thymus, and inducible T_{reg} cells, which differentiate from naive $CD4^+$ precursors in the periphery. Both subsets express CD4 and the interleukin-2 (IL-2) receptor α chain (CD25). Importantly, FoxP3 expression is necessary and sufficient to impart suppressive function; at least in mice, its expression is a reliable indicator of suppressive activity. T_{reg} cells produce anti-inflammatory cytokines including IL-10, transforming growth factor-β and IL-35, but the molecular bases of suppression *in vivo* remain elusive. The notion that successful experiments in animal models can be translated into humans is predicated on the idea that T_{reg} cells are a homogenous population of cells with a stable phenotype—and there's the rub.

Classically, $CD4^+$ T helper cell subsets have been viewed as terminally differentiated lineages with little flexibility. In line with this concept, T_{reg} cells have many features of a stable lineage, including the expected epigenetic modifications[2]. But Zhou *et al.*[1] look closer, using an elegant lineage-tracing approach that allowed them to differentiate between cells with sustained FoxP3 expression and cells that expressed FoxP3 at any point during their ontogeny. They found that FoxP3 was transiently expressed in a substantial proportion of cells. Moreover, 'ExFoxP3'cells lose more than expression of FoxP3—they also lose expression of CD25 (IL-2 is a major regulator of FoxP3). These ExFoxP3 cells can become pathogenic, secreting interferon-γ (IFN-γ) and IL-17. These cytokines activate other innate immune cells, resulting in the recruitment of inflammatory cells.

How do T_{reg} cells become traitors? Zhou *et al.*[1] suggest that this transformation is dependent on the cells' location and their environment. Apparently, an inflammatory milieu in the context of self-antigen can induce loss of FoxP3 expression and associated surface receptors. Others have noted that T_{reg} cells are not a homogenous population. $FoxP3^+$ cells with low CD25 expression can lose expression of this transcription factor and become effector T cells, whereas cells with high CD25 expression are more resistant to such 'conversion'[3]. In addition, $FoxP3^+$ cells that home to Peyer's patches, specialized lymphoid structures in the gut, can also lose expression of FoxP3 and acquire characteristics of follicular helper T cells[4].

New subsets of helper T cells (T_H cells) continue to be recognized, such as T_H9, T_H17, T_H22 and follicular helper T cells. Along with these discoveries, the perception of the stability of $CD4^+$ T cell subsets continues to evolve, and several mechanisms have emerged to account for their flexibility. First, the expression of 'master regulator' transcription factors is more fluid than initially recognized. Cells can coexpress more than one master regulator, for example, FoxP3 and Rorγt[5] or FoxP3 and T-bet[6], with Rorγt being a driver of IL-17 production and T-bet being a key regulator of IFN-γ. Second, although epigenetic modifications preserve phenotype, loci encoding some master regulators such as Gata-3 and T-bet have both permissive and repressive marks, affording the opportunity for re-expression[7]. Third, miRNAs are key regulators of phenotype. For instance, T cell deficiency of Dicer and Drosha, enzymes that process miRNAs, destabilize the phenotype of T_{reg} cells[8].

What are the implications of the study by Zhou *et al.*[2] and related findings for the prospects of human T_{reg} cell therapy? If FoxP3 expression is not stable and T_{reg} cells are heterogeneous, then administration becomes problematic. The situation in humans is further complicated by the finding that FoxP3 is expressed in activated human T cells and is not a reliable indicator of suppressive function; $FoxP3^+$ cells with suppressor capability exist along with $CD45RA^-FoxP3^+$ cells that secrete IL-17 and IFN-γ[9]; the latter cells, of course, could contribute to inflammation.

These confounding factors raise the specter that we may not really understand what we are enumerating when we quantify 'T_{reg} cells' in humans. Given that FoxP3 expression is not indicative of suppressive activity, what cells should be infused to treat patients? An equally fundamental question is that if mouse T_{reg} cells are more flexible than previously appreciated, why does infusion of T_{reg} cells in mouse models work at all? Clearly, we need to better understand what factors influence FoxP3 expression and maintain high levels of expression. Conversely, what factors terminate expression and how can they be blocked? A robust protocol for *in vitro* expansion is also needed, along with some kind of quality control for stable suppressive activity. The essential challenge, however, is that we still do not really understand why T_{reg} cells are suppressive. This is not a trivial question.

Despite many advances, our knowledge of T_{reg} cell biology is still limited. At the very least, we should proceed with extreme caution in any trials in which administration T_{reg} cells for autoimmunity is considered. Certainly, one would want some assurance that a T_{reg} cell with some degree of stability is being infused and that measuring its stability *in vivo* would be a component of the study. Proposed studies should be limited in scope and accompanied by vigorous efforts at the bench to ascertain what governs the stability and activity of this subset of cells.

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