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Previews

Green T_R Cells

Identification of the transcription factor Foxp3 as a "master regulator" of regulatory T (T_R) cells was a major discovery. A new study by Fontenot et al. (2005), reported in this issue of *Immunity*, provides novel insights into T_R cell biology by tracking their behavior in mice expressing a GFP-Foxp3 fusion-protein reporter.

All higher organisms harbor autoreactive T cells, which somehow survived central tolerance induction (i.e., negative selection during thymocyte differentiation) and have the potential of inducing organ-specific autoimmune disease (Ohashi, 2003). Therefore, in order for self-tolerance to be maintained, several peripheral tolerance mechanisms have evolved, including deletion, anergy, and active control of autoreactive T cells. (Van Parijs and Abbas, 1998).

Since the middle of the 1990s, a subset of T cells expressing the high affinity IL-2 receptor alpha chain (CD25) has emerged as a focus for immunologists interested in immunoregulation (Sakaguchi et al., 1995). $CD4^+CD25^+$ T_R cells are now known to play a central role in the maintenance of immunological homeostasis and self-tolerance in a number of autoimmunity, allergy, and infection models (for review see Sakaguchi [2004] and Shevach [2002]).

Nonetheless, a means of definitively identifying T_R cells has been elusive. CD25 expression is most commonly employed as a marker, and glucocorticoid-induced TNF receptor-related gene (GITR), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), neuropilin-1, or the integrin α_E (CD103) have also been used, but none of these molecules is really restricted to T_R cells. They are also expressed on T effector cell precursors upon activation, with the exception of neuropilin-1 and CD103, although the latter molecule can be induced in the presence of TGF-β. Recently, Foxp3, a member of the well-known and diverse forkhead transcription factor family, was identified as a master switch in T_R cell differentiation and function (Hori et al., 2003; Fontenot et al., 2003; Khattri et al., 2003). Because Foxp3 is not upregulated in recently activated $CD4^+CD25^-$ T cells, it seemed to be an excellent candidate for a specific marker of T_R cells.

By using mice harboring a GFP-Foxp3 fusion-protein reporter knockin allele, Fontenot and colleagues have now explored the role of Foxp3 in the hematopoietic system; in particular, the relationship between Foxp3 expression, cell-surface display of CD25, and T_R activity (Fontenot et al., 2005). In control studies, they first established the functional integrity of Foxp3 in reporter mice by demonstrating that T_R cells isolated from them had normal suppressive activity. Expression of the transcription factor fusion protein in the peripheral immune system was largely restricted to a small population of $TCR\beta^+CD4^+$ T cells (which constituted 97% of the $Foxp3^{gfp+}$ cells). Tiny populations of $CD8^+$ T cells as well as CD4/CD8 double-positive and double-negative T cells that made $Foxp3^{gfp}$ could also be discerned. Based on expression of $Foxp3^{gfp}$ and CD25, $CD4^+$ T cells could be divided into four subpopulations. Perhaps surprisingly to some, Foxp3 expression and T_R cell function were not well correlated with display of CD25. Notably, less than 50% of the $Foxp3^{gfp+}$ lymphocytes isolated from the lungs exhibited high levels of CD25. It seems that $CD25^{high}Foxp3^{gfp+}$ and $CD25^{lo/neg}$ $Foxp3^{gfp+}$ $CD4^+$ T cells represent the pool of regulatory T cells, whereas the $CD25^{high}Foxp3^{gfp-}$ population has an activated/effector phenotype with no regulatory potential (at least under the conditions analyzed in this study).

Compatible with a breakdown in peripheral self-tolerance, mice with a deficiency in Foxp3 show a rapid, fatal lymphoproliferative autoimmune syndrome at 3–4 weeks of age. Given that Foxp3-deficient mice display a much more severe autoimmune phenotype than do mice depleted of $CD25^+$ cells, there has been speculation that Foxp3 might have an additional as-yet-uncharacterized role. However, Fontenot et al. (2005) made several arguments against this notion. First, this transcription factor was not expressed in any non-T cell populations, as was clearly evident from examining expression of the Foxp3-GFP-reporter in lymphocyte-deficient $RAG^{o/o}$ mice. Second, a T cell-specific ablation of Foxp3 was sufficient to induce the full lymphoproliferative autoimmune syndrome observed in standard Foxp3-deficient mice. Third, a lack of Foxp3 did not influence the effector responses by T cells from Foxp3/RAG double-deficient T cell receptor transgenic mice. This finding argues against any cell-intrinsic function for Foxp3 in effector T cells, suggesting an exclusive role in regulatory T cells.

Most striking, and in contrast to previous assumptions (Stock et al., 2004), "adaptive" Foxp3-expressing T_R cells were not induced (as a form of feedback regulation) during the course of an acute immune response. There was no induction of this transcription factor after 7 days of in vitro culture in the presence of antigen and no de novo generation of $Foxp3^{gfp+}$ cells in the course of an acute pathogen-driven immune response. Whether the system used by Fontenot et al. (2005) was sufficient to rule out any extrathymic induction of T_R cells remains in question, particularly in light of recent reports of the conversion of naive T cells into T_R cells in vivo (Liang et al., 2005; Apostolou and von Boehmer, 2004).

In another interesting, though still somewhat preliminary, set of experiments, the important question of T_R cell differentiation in the thymus was addressed. Expression of Foxp3 was largely restricted to CD4 single-positive thymocytes; however, minor populations of CD8 single-positive, double-positive, and double-negative thymocytes were also detectable. Foxp3 expression was strictly dependent on TCR/MHC molecule in-

teraction. Surprisingly, and consistent with the data from the periphery, a fraction of the Foxp3^{gfp+} cells represented CD8$^+$ thymocytes dependent on expression of MHC class I molecules.

Overall, this study has established the validity of Foxp3 as a specific marker for regulatory T cells and has reported a novel mouse line of tremendous potential value in studies on immunoregulation. It has also raised some intriguing questions. Can the newly discovered CD8$^+$Foxp3^{gfp+} T cells exert regulatory function comparable with that of CD4$^+$CD25$^+$ T_R cells? If so, in what context(s) do they emerge as important control elements? Foxp3^{gfp+} T_R cells isolated from diverse sites showed some striking phenotypic differences; for example, peripheral organs were enriched in the CD25$^{lo/neg}$Foxp3^{gfp+} population, which had an activated phenotype and included proliferating cells. Might these cells be the key to self-tolerance within tissues? Unlike CD4$^+$CD25$^+$ T_R cells, other immune cells with regulatory potential, including NKT cells and Tr1 cells, express no or low levels of Foxp3; thus, it is unlikely that this transcription factor and the gene-expression program it specifies is the only means of establishing tolerance dominantly. What is the master regulator of these cell-types and when do they come into play?

The powerful in vivo model introduced by Fontenot et al. (2005) opens the door for new insights into T_R cell biology. There is sure to be an onslaught of studies on antigen-specific systems, as well as adaptations to a diversity of pathological situations, including autoimmunity, chronic infection, transplantation, and tumorigenesis.

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