Immunity, Vol. 22, 271–274, March, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.03.002

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.

Markus Feuerer, Christophe Benoist, and Diane Mathis
Section on Immunology and Immunogenetics
Joslin Diabetes Center
Department of Medicine
Brigham and Women's Hospital
Harvard Medical School
One Joslin Place
Boston, Massachusetts 02215

Selected Reading

Apostolou, I., and von Boehmer, H. (2004). J. Exp. Med. *199*, 1401–1408.

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Nat. Immunol. *4*, 330–336.

Fontenot, J.D., Rasmussen, J.P., Williams, L.M., Dooley, J.L., Farr, A.G., and Rudensky, A.Y. (2005). Immunity *22*, this issue, 329–341.

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Science *299*, 1057–1061.

Khattri, R., Cox, T., Yasayko, S.A., and Ramsdell, F. (2003). Nat. Immunol. *4*, 337–342.

Liang, S., Alard, P., Zhao, Y., Parnell, S., Clark, S.L., and Kosiewicz, M.M. (2005). J. Exp. Med. *201*, 127–137.

Ohashi, P.S. (2003). Curr. Opin. Immunol. *15*, 668–676.

Sakaguchi, S. (2004). Annu. Rev. Immunol. *22*, 531–562.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). J. Immunol. *155*, 1151–1164.

Shevach, E.M. (2002). Nat. Rev. Immunol. *2*, 389–400.

Stock, P., Akbari, O., Berry, G., Freeman, G.J., DeKruyff, R.H., and Umetsu, D.T. (2004). Nat. Immunol. *5*, 1149–1156.

Van Parijs, L., and Abbas, A.K. (1998). Science *280*, 243–248.