

***In Vitro* Generation of IL-10 Producing Regulatory CD4⁺ T cells is induced by Immunosuppressive Drugs and Inhibited by Th1 and Th2-Inducing Cytokines.** Anne O'Garra, Daniel J. Cua, André Boonstra, David F. Richards, Chad Crain, Huub F. Savelkoul, René de Waal-Malefyt, Robert L. Coffman, Catherine M. Hawrylowicz and Franck J. Barrat. Anne O'Garra & Franck Barrat were previously at DNAX Research Institute, Palo Alto, California 94304-1104, USA: Anne O'Garra is now at: The National Institute for Medical Research, Mill Hill, London NW7 1AA, UK: Franck Barrat at: Dynavax Technologies Corp., Berkeley, CA 94710, USA.

We show that a combination of the immunosuppressive drugs, Vitamin D3 and Dexamethasone, induced human and mouse naïve CD4⁺ T cells to differentiate *in vitro* into regulatory T cells. In contrast to the previously described *in vitro* derived CD4⁺T cells, these cells produced only IL-10, but no IL-5 and IFN- γ , and furthermore retained strong proliferative capacity. The development of these IL-10-producing cells was enhanced by neutralization of the Th1 and Th2 inducing cytokines IL-4, IL-12 and IFN- γ . These immunosuppressive drugs also induced the development of IL-10 producing T cells in the absence of APC, IL-10 acting as a positive autocrine factor for these T cells. Furthermore, NF- κ B and AP-1 activities were inhibited in the IL-10 producing cells described here as well as key transcription factors involved in Th1 and Th2 subset differentiation. The regulatory function of these *in vitro* generated IL-10 producing T cells was demonstrated by their ability to prevent central nervous system inflammation, when targeted to the site of inflammation, and this function was shown to be IL-10-dependent. Generating homogeneous populations of IL-10 producing T cells *in vitro* will thus facilitate the use of regulatory T cells in immunotherapy.

Barrat.F.J, Cua, D.J., Boonstra, A., Richards, D.F., Crain, C., Savelkoul, H.F., de Waal-Malefyt, R., Coffman, R.L., Hawrylowicz, C.M., and O'Garra, A. *In Vitro* Generation of IL-10 Producing Regulatory CD4⁺ T cells is induced by Immunosuppressive Drugs and Inhibited by Th1 and Th2-Inducing Cytokines. Submitted.

Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra, A. 2001. 1 α ,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol.* 167(9):4974-80.

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra, A. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol.* 19:683-765.

O'Garra., A., and Arai, N. . 2000. The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends Cell Biol.* 10(12):542-50. Review.

Castro AG, Neighbors M, Hurst SD, Zonin F, Silva RA, Murphy E, Liu YJ, O'Garra A. 2000. Anti-interleukin 10 receptor monoclonal antibody is an adjuvant for T helper cell type 1 responses to soluble antigen only in the presence of lipopolysaccharide. *J Exp Med.* 192(10):1529-34