**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.


