Human IL–10 Producing T Cells Specific for *Mycobacterium tuberculosis*.

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IL–10 producing Mtb–specific $CD4^+$ T cells can be detected in pulmonary TB patients with persistent anergy. Aim of the study was to define the spectrum of ex vivo frequencies of IL–10 producing Mtb–specific $CD4^+$ and $CD8^+$ T cells in adults. Peripheral blood mononuclear cells were collected from uninfected adults and subjects with latent tuberculosis infection or active tuberculosis. Monocyte–derived dendritic cells (DC) were infected overnight with Mtb (MOI=50:1) and incubated with different concentrations of positively selected autologous $CD4^+$ and $CD8^+$ T cells in an IL–10 ELISPOT assay. In all subjects we detected additional IL–10 producing cells with the addition of T cells to Mtb–infected DC, compared to Mtb–infected DC alone. We next focused on $CD8^+$ T cells and asked if they represent the additional IL–10 producing cells. Autologous DC were left uninfected or infected with Mtb (MOI=20:1). After overnight incubation, positively selected $CD8^+$ T cells were added and incubated overnight. Then, $CD8^+$ T cells were positively selected from these cultures using magnetic beads, and RNA was isolated and subjected to RT–PCR. Relative quantitation of IL–10 RNA showed that $CD8^+$ T cells were induced to produce IL–10 in response to Mtb–infected DC, suggesting that T cells are a source of the augmented IL–10 production previously seen in co–cultures of Mtb–infected DC with T cells. We next sought to isolate IL–10 producing Mtb–specific $CD8^+$ T cells using a limiting dilution T cell cloning approach. T cells from wells exhibiting growth were analyzed by ELISPOT for their production of IFN–γ and/or IL–10 in response to Mtb–infected autologous DC. In all donors, IFN–γ producing $CD8^+$ T cells were most frequently isolated. Most donors also had IL–10 producing $CD8^+$ T cells, the majority of which also produced IFN–γ. Finally, we confirmed that IL–10 has the potential to inhibit IFN–γ $CD4^+$ T cell responses to Mtb antigens.

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