Title: Repeated IL-4 stimulation impairs STAT1 signaling and confers CD4+ T cell resistance to IL-27-mediated suppression

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Body: Background: Th2 cells play critical roles in the pathogenesis of allergic asthma. Established Th2 cells have been shown to resist reprogramming toward Th1 cells. The inherent Th2 stability poses a significant barrier in treating allergic diseases. We sought to understand the mechanisms by which asthmatic CD4+ T cells develop resistance to IL-27-mediated inhibition. Methods: We isolated and cultured CD4+ T cells from healthy individuals and allergic asthmatics and tested whether IL-27 can inhibit IL-4 production. STAT1 phosphorylation was analyzed by Western blot and flow cytometry. SOCS mRNA expressions were measured by quantitative PCR. The small interfering RNA method was used to knockdown SOCS3 mRNA expression. Main Results: We demonstrate that CD4+ T cells from asthmatic patients resisted human IL-27-mediated suppression of IL-4 production. We observed that repeated exposures to Th2-inducing conditions rendered healthy human CD4+ T cells resistant to IL-27-mediated inhibition. Using an in vitro murine culture system, we further demonstrated that repeated or higher doses of IL-4, but not IL-2, stimulation upregulated SOCS3 mRNA expression and impaired IL-27-induced STAT1 phosphorylation. Py-STAT1 expression was impaired in Th2 memory cells circulating in blood isolated from asthmatics, but not from normal subjects. Knockdown of SOCS3 expression restored IL-27-mediated inhibition. Conclusions: Our findings demonstrate that differentiated Th2 cells can resist IL27-induced reprogramming toward Th1 ells by downregulating STAT1 phosphorylation and upregulation of SOCS3 and may explain why asthmatic CD4+ T cells are resistant to IL-27-mediated inhibition.