257 IL-13 Decreases IL-17a Production In Mouse Th17 Cells Through An IL-10 Dependent Mechanism

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RATIONALE: IL-13 is an important cytokine in allergic airway inflammation and is a potential therapeutic target for asthma. We have shown that IL-13 inhibits IL-17A production by mouse CD4+ polarized Th17 cells. However, the mechanism by which this occurs remains unknown. In this study we hypothesized that IL-13 inhibited IL-17A production in an IL-21 dependent manner.

METHODS: Splenic CD4+ T cells from BALB/c mice were activated and polarized to Th17 cells with IL-13 (0-10ng/ml). Recombinant IL-21 and anti-IL-10 were added in select experiments. Supernatants were harvested 4 days after polarization and analyzed for cytokine production by ELISA.

RESULTS: As expected IL-17A was decreased in Th17 cells polarized with IL-13 (10ng/ml). IL-21 was also significantly decreased in Th17 cells with IL-13 as early as 3 days after polarization. IL-13 had no effect on IL-17A levels when IL-21 was added at the time of Th17 polarization. IL-10 was significantly increased in Th17 cells in the presence of IL-13 (10ng/ml) compared to Th17 cells. When Th17 cells were polarized in the presence of IL-13, anti-IL-10 prevented the IL-13-mediated decrease in Th17 cytokine production. $p<0.05$, $n=5$-8 for all experiments conducted.

CONCLUSIONS: The ability of IL-13 to attenuate IL-17A is lost in presence of IL-21 or anti-IL-10. These data suggest that IL-13 increases IL-10 production which then inhibits IL-21 production leading to decreased IL-17A production in Th17 cells. Overall, this study depicts a mechanism as to how IL-13 inhibits IL-17A production to help better understand potential implications of inhibiting IL-13 in patients with Th17 mediated diseases.

258 Novel Actions of MEK1 and the Nuclear Co-receptor SMRT in Regulating T cell Function

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RATIONALE: We reported increased expression of MEK1 in CD4 T cells from asthma. MEK1 regulates gene transcription by activating ERK1/2. We asked if MEK1 exerted a direct effect on gene transcription.

METHODS: CD4 T cells were isolated by negative selection from peripheral blood. MEK1 interaction with SMRT was studied by co-precipitation. MEK1 binding to the c-Fos promoter was studied by ChIP. SMRT knockdown was done by siRNA. Cytokine expression was studied by real-time PCR and ELISA.

RESULTS: MEK1 translocates to the nucleus and binds to the c-Fos promoter. MEK1 interacts with the nuclear co-receptor and gene repressor SMRT/NCOR2. The interaction increases upon T cell activation. MEK positively regulates SMRT expression. T cells from asthmatic patients have a qualitatively altered distribution and expression of SMRT. SMRT knockdown CD4 T cells have reduced IL-2 (5-fold), IL-4, IL-10 (both 2-fold) and IFN-[[Unsupported Character - Symbol Font γ]] (1.4-fold) ($P<0.05$) production in response to anti-CD3/CD28 but not PMA/ionophore stimulation. There was a proportionate reduction in cytokine mRNA. SMRT knockdown T cells are unable to proliferate (CFSE dilution) but survival is unaffected. Mechanistically, SMRT knockdown T cells have impaired signaling through the p38 but not ERK1/2, AKT or NFkB pathways. These cells have reduced JunB but normal levels of c-Fos, c-Jun, Egr2 and Foxp3 expression.

CONCLUSION: We have identified a direct and novel nuclear action of MEK1. MEK1 binds to the promoter of c-Fos. It interacts with and regulates SMRT. SMRT functions as a positive regulator of T cell cytokine production and proliferation. SMRT affects TCR/CD28 proximal signaling pathways.

259 Inducible, Antigen Specific IgE-suppression By Gamma-delta T Cells

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RATIONALE: A regulatory subset of gamma-delta T cells (Vgamma4+ gamma-delta T cells, Vg4 cells) can be induced to be IgE-suppressive by repeatedly challenged with inhaled antigen, we now determine the antigen specificity of these regulatory cells by a criss-cross experiment using different antigens. The targets of these cells in IgE response were also examined

METHODS: Vg4 cells from mice exposed to inhaled ovalbumin (OVA) or hen egg lysozyme (HEL) were transferred to gamma-delta T cell-deficient mice shortly before immunization by the same or mismatched antigen. Serum total and antigen-specific IgE levels were examined after 14 days. In some experiments, CFSE-labeled OT II cells were co-transferred with Vg4 cells into T cell-deficient mice followed by OVA/alum immunization, the proliferation and differentiation of OT II cells were measured 4 days later by surface/intracellular flow cytometry

RESULTS: "Inhaled OVA"-prepared Vg4 cells can only suppress the IgE response elicited by OVA/alum, but not that by HEL/alum, vice versa. OVA/alum-driven differentiation of OT II cells into Th2 cells (indicated by IL-4/IL-13-producing cells) was forestalled by co-transferred Vg4 cells.

CONCLUSIONS: IgE-suppressive Vg4 cells induced by inhaled antigen are antigen-specific, and they exert their function through redirecting the differentiation of antigen-specific alpha beta T cells.

260 Performance of FDA-Approved Serologic Testing for Latex Allergy in an At-Risk Population

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RATIONALE: Due to the lack of a FDA-approved skin testing reagent, diagnosis of latex allergy must rely on patient history and serologic assays. There has not been a recent large-scale study of skin and serologic testing in a low prevalence population to determine the PPV and NPV of serologic testing for latex allergy.

METHODS: Health care workers underwent duplicate skin testing with Clone 600 extract and serologic testing for latex specific IgE measured by Pharmacia ImmunoCap. Sensitivity, specificity, PPV and NPV of the serologic assay were determined using skin prick testing as the gold standard for sensitization to latex.

RESULTS: There were 792 paired skin and serologic tests. 40 skin tests were positive (5%). The sensitivity of the Pharmacia ImmunoCap assay was 35% and the specificity was 98%. The PPV was 48.3% while the NPV was 96.6%. There were 15 false positive serologic tests (1.9% of individuals tested) and 26 false negative tests (3.3%).

CONCLUSIONS: Using an at risk population, with a 5% prevalence of latex allergy, we demonstrate that the performance of the FDA-cleared ImmunoCap serologic test for latex allergy has much lower sensitivity than previously reported. There were high rates of false positive and false negative results in relation to the prevalence of allergy in the population tested. This confirms suspicions that this serologic test should only be used for patients with a history of latex allergy and not used for screening the population with a low prevalence of latex sensitization.