1 Role of TNFSF11 and Group 2 Innate Lymphoid Cells in Type 2 Inflammation in Chronic Rhinosinusitis with Nasal Polyps

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RATIONALE: Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by type 2 inflammation with accumulation of group 2 innate lymphoid cells (ILC2s). Although a member of TNF superfamily (TNFSF), TNFSF15, is known to induce production of type 2 cytokines in ILC2s, the role of other TNFSFs in ILC2s and the importance of TNFSFs in ILC2-mediated inflammation in CRSwNP have not been elucidated.

METHODS: We investigated the presence of TNFSFs in nasal polyps (NPs) by microarray, real-time RT-PCR and Lumines, and the expression of TNFSF receptors (TNFRSFs) in human ILC2s by RNA-sequencing and flow cytometry. Purified human ILC2s were stimulated with recombinant TNFSFs and thymic stromal lymphopoietin (TSLP) and the production of type 2 cytokines from ILC2s was evaluated by Luminex.

RESULTS: Although TNFSF15 was not elevated, mRNAs for TNFSF6, TNFSF11, TNFSF14, and TNFSF18 were significantly elevated in NPs compared to normal uncinate tissue and mRNAs for their receptors, TNFRSF6, TNFRSF11A, TNFRSF14, and TNFRSF18 were detected in human ILC2s. Among identified TNFSFs, only recombinant TNFSF11 and agonistic anti-TNFSF11A antibody were able to induce the production of IL-13 in blood ILC2s. We confirmed that TNFSF11 protein was significantly elevated in NP tissue and TNFRSF11A protein was detected on ILC2s from blood and NPs. The presence of mRNA (n=60) and protein (n=96) for TNFSF11 significantly correlated with IL-13 in sinonasal tissue. Interestingly, TSLP enhanced the TNFSF11-mediated production of IL-13 in blood ILC2s (5.0-fold; p<0.01, n=7).

CONCLUSIONS: TNFSF11 together with TSLP may play an important role in the ILC2-mediated type 2 inflammation seen in patients with CRSwNP.

2 Identification of MEK2 and CBX7 as Top Steroid Resistant Genes in Airway ILC2s and Lymphocytes from Asthma

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RATIONALE: We reported that BAL ILC2s and lymphocytes (abbreviated as lymphoid cells) from asthmatic patients were steroid resistant in a TSLP-dependent manner. The mechanism of this steroid resistance is poorly understood.

METHODS: The induction of steroid resistant genes was studied by real-time PCR and flow cytometry. Interaction of MEK2 and CBX7 was studied by immunofluorescence staining and co-immunoprecipitation. Lymphoid cells were isolated from blood and BAL obtained from asthmatic patients, and from human lungs obtained from transplant-rejected donors.

RESULTS: Using a genome-wide gene knockdown approach an earlier study identified about 50 steroid resistant genes in leukemic cells. We selected top 17 steroid resistant genes from this screen and examined their Dexamethasone (Dex) sensitivity in TSLP and IL33-stimulated lymphoid cells. Three of these 17 genes—CBX7 (a polycomb group repressor/co-activator), MEK2 and TLR2 were resistant to Dex. Unexpectedly, their induction by TSLP but not by IL33 was further augmented by Dex. TSLP induced MEK2, which translocated to the nucleus and physically interacted with CBX7. MEK2 and CBX7 cross-regulated each other’s expression indicating a positive feedback regulation. BAL lymphoid cells from refractory asthmatic patients showed increased expression of MEK2 and CBX7 as compared to disease controls. BAL lymphoid cell type 2 cytokine (IL5 and IL13) production in refractory asthma was resistant to Dex, which was reversed by Trametinib and MS37452, inhibitors of MEK2 and CBX7, respectively.

CONCLUSIONS: We identified MEK2 and CBX7 as top steroid resistant genes in lymphoid cells from refractory asthma. MEK2 and CBX7 inhibitors are likely to benefit steroid-resistant asthma.

3 Group 2 innate lymphoid cells display ILC3-like functional plasticity in asthmatics and non-human primates

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RATIONALE: The underlying mechanisms that drive neutrophilic asthma are poorly understood. Recently group 2 innate lymphoid cells (ILC2s) have been described as an important cellular player in severe asthma. Our laboratory has shown that following ozone exposure in mice, ILC2s acquire an ILC3-like phenotype and produce IL-17A and IL-22. We hypothesized that ILC2s display ILC3-like functionality in asthma and produce IL-17A and IL-22 to promote neutrophilia.

METHODS: To study cytokine production in ILC2s, we recruited severe asthma patients from the UC Davis Asthma Network Clinic and acquired non-human primates (NHP, Rhesus macaque). Human and NHP subjects underwent lung function measurements. Human subjects were segregated into severe asthma and control groups, while NHP were segregated into AHR+ and AHR- groups. ILC2 and ILC3 numbers were assessed via flow cytometry of PBMCs. Cytokine production was studied after restimulation and intracellular staining (severe asthma patients) or qPCR (non-human primates) of ILC2s isolated via FACS.

RESULTS: Severe asthma patients and AHR+ NHP had increased numbers of circulating ILC2s compared to healthy controls or AHR- NHP, respectively. ILC2s from severe asthma patients had constitutive, increased expression of IL-13 and IL-17A compared to healthy controls, however upon restimulation IL-13 and IL-17A production was reduced and IL-22 production increased. ILC2s isolated from AHR+ NHP displayed increased IL-4, IL-22, and GATA3 gene expression compared to AHR-NHP.

CONCLUSIONS: We propose that ILC2 displaying ILC3-like functionality are potentially important in neutrophilic asthma, and how IL-17A and IL-22 produced in conjunction with IL-5 and IL-13 might impact inflammation will be important for future studies.
**TSLP and IL-33 Reciprocally Regulate Each Others Lung Protein Expression and Receptor Expression on ILC2 following Aeroallergen Challenge in Mice**

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**RATIONALE:** The epithelial cell-derived danger signal mediators, TSLP and IL-33 are consistently associated with adaptive Th2 immune responses. TSLP and IL-33 synergistically promoted group 2 innate lymphoid cell (ILC2) activation to induce innate allergic inflammation. However, the mechanisms regulating the synergism by which TSLP and IL-33 regulate the synergistic activation of ILC2 are unknown.

**METHODS:** BALB/c background WT mice pre-administered with recombinant TSLP (rTSLP) or vehicle, TSLP receptor (TSLPR) deficient mice, and IL-33 receptor (ST2) deficient mice were challenged intranasally with *Alternaria*-extract or vehicle. BALF and lungs were harvested to detect IL-33 release (1 hr after challenge) and TSLP protein expression (6 hr after challenge) by ELISA. Whole lung cells from WT mice challenged intranasally with rTSLP or rIL-33, and isolated lung ILC2 stimulated with rTSLP or rIL-33 in vitro were used to measure TSLPR and ST2 expression on ILC2 by flow cytometry.

**RESULTS:** rTSLP pre-administration increased *Alternaria*-induced IL-33 release into BALF, and TSLPR deficiency decreased the *Alternaria*-induced IL-33 release. ST2 deficiency decreased *Alternaria*-induced TSLP expression in the lung. Further, rTSLP administration increased ST2 expression, and rIL-33 administration increased TSLP expression on lung ILC2 in vivo and in vitro.

**CONCLUSIONS:** TSLP and IL-33 reciprocally increased each other’s protein expression or release in the lung, and each other’s receptor expression on lung ILC2. This may be one mechanism by which TSLP and IL-33 synergistically activate ILC2 and augment ILC2-mediated allergic airway inflammation.

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**IL33 Receptor Deficiency Leads To Steroid Resistant Asthma Due To TSLP-driven Increase In IL9+ILC2s And Mast Cells**

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**RATIONALE:** IL33 plays an important role in asthma. We examined the role of the IL33 receptor (ST2) in a mouse model of chronic asthma.

**METHODS:** We studied tripe allergen (dust mite, ragweed and Aspergillus)-induced asthma in ST2 knockout (KO) and wild-type control mice. We measured airway hyperresponsiveness (AHR) by flexivent, inflammatory indices by ELISA, histology and real-time PCR, and ILC2s in lung single cell preparations by flow cytometry.

**RESULTS:** The AHR level was elevated in allergen-treated ST2 KO mice and was comparable to that from allergen-treated WT controls. Peribronchial and perivascular inflammation and mucus production were largely similar in both groups. Persistence of asthma in ST2 KO mice was associated with an increase in the level of TSLP, IL9 and IL13 but not IL5 in bronchoalveolar lavage (BAL). ST2 deletion caused a reduction in IL13+ CD4+ T cells, Foxp3+ Tregs and IL5+ ILC2s but unexpectedly, induced an overall increase in ILCs (CD45+lin-CD25+ cells), IL13+ ILC2s, the emergence of a TSLP-R+ IL9+ ILC2 population and an increase in intraepithelial mast cells in the lung. AHR in ST2 KO mice was steroid resistant. An anti-TSLP antibody abrogated AHR, inflammation and mucus production in allergen-treated ST2 KO mice, which was associated with a reduction in IL9+ and IL13+ ILC2s in the lung.

**CONCLUSIONS:** IL33 receptor deficiency paradoxically increases TSLP production, which stimulates the emergence of IL9+ and IL13+ ILC2s and mast cells, and development of steroid-resistant asthma. An anti-TSLP antibody abrogates all features of asthma in this model and is likely to be beneficial in steroid-resistant asthma.