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Author
Nguyen, Tammie

Publication Date
2013

Tammie Nguyen
ISP Write-Up

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ABSTRACT

Although our understanding of the pathogenesis of asthma remains limited, data now indicate that most patients with asthma have an immune-mediated component. Allergic asthma is thought to involve a response to a specific antigen that triggers a specific adaptive immune response in the lung that requires time. However, new data also implicate the innate immune response, either in concert with or independent of the adaptive response. The broad goal of this project was to explore the role that the cytokine interleukin (IL) 33 and its receptor, ST2, may play in asthma. The first objective was to explore the role of ST2 in a mouse model of asthma in which ovalbumin is used to induce airway hyperresponsiveness and inflammation. We hypothesized that ovalbumin-challenged mice would show increased expression of ST2 compared to unchallenged mice. The second objective was to characterize the stimuli for expression and release of IL-33 from the human airway epithelium using the human airway epithelial cell line BEAS-2B. We hypothesized that IL-33 auto-regulates its expression in airway epithelial cells. By Western blot, we found that expression of the soluble form of ST2 (sST2) appeared higher in lung tissue from ovalbumin-challenged mice compared to lung tissue from control mice. Similarly, by immunohistochemistry, we found higher expression of ST2 in lung tissue of ovalbumin-challenged mice compared to controls. Staining with mouse anti-ST2 antibody also showed ST2 expression in mediastinal lymph node tissue from ovalbumin-challenged mice. By flow cytometry, we found that splenic tissue from ovalbumin-challenged mice had a higher proportion of CD4+ cells that expressed ST2 on the cell surface (CD4+ST2+ cells) compared to control mice. We then studied BEAS-2B cells that had been conditioned with 10 ng/ml of human recombinant IL-33 in growth media or growth media alone for 24 hours at 37°C. However, our results from RT-PCR and ELISA showed that BEAS-2B cells did not express significant IL-33 with or without IL-33 conditioning. This result could be explained by a de-differentiated state of BEAS-2B cells in the culture conditions we used, which did not closely approximate primary bronchial epithelial cells. Overall, our results suggest that ST2 may play a role in asthma, though the interactions of the different isoforms of ST2 are very complex and further work is required to understand exactly how they may affect the pathogenesis of allergic asthma. Our results also suggest that IL-33 may have some role in auto-regulating its own expression in human airway epithelium, though further work in primary airway epithelium or using BEAS-2B cells in different culture conditions to promote differentiation would be needed to explore this further.

BACKGROUND

Asthma is a chronic inflammatory disorder characterized by airway inflammation and hyperresponsiveness to inhaled stimuli. It remains a major health problem that affects as many as 22 million people in the United States alone and 300 million people worldwide, and its incidence has increased significantly over the last 20 to 40 years in developed countries. Allergic asthma is the most common form of asthma; its prevalence is highest during childhood or young adulthood. Ongoing research is attempting to characterize fully the complex immune regulatory pathways responsible for allergic asthma. Recently, Suppressor of Tumorigenicity 2 (ST2) and interleukin (IL)-33, a receptor-ligand pair, have been implicated in mediating the inflammatory responses involved in airway hyperreactivity. However, the complex interactions between ST2 and IL-33 have yet to be fully described.

ST2

The ST2 receptor (also called T1, DER-4, Fit-1, and IL-1R4) is a member of the IL-1 receptor subfamily. ST2 exists as both a soluble form (sST2) and a transmembrane form (ST2L) (1). ST2 is expressed as these alternative forms from the same gene via the use of alternative promoters (2). Tissue specific expression in adult mice of sST2 has been shown in fibroblasts and the bone marrow and ST2L is expressed in bone marrow, spleen, lymph nodes and lung (2). Additional studies have demonstrated that ST2L is expressed in Th2 lymphocytes, but not in Th1's and this expression is independent of cytokine release (3). Studies from this same group demonstrated that ST2L plays a central role in the development of the Th2 immune response in the lung and that blockade of ST2L significantly attenuated Th2-mediated inflammation, but had no effect on Th1-mediated inflammation (4). Another group's targeted deletion of ST2 resulted in an attenuation of the Th2 response (5). Inhibition of ST2 function *in vivo* with monoclonal antibodies has been associated with attenuating Th2-mediated, but not Th1-mediated, immune responses (6). By contrast, other investigators have found that targeted deletion of
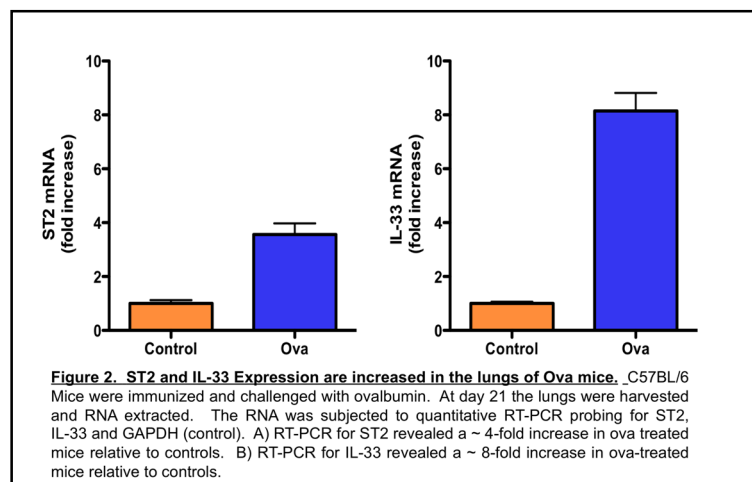
the ST2 gene had no effect on Th2 responses to parasites or allergens (7). The complex interplay between sST2 and ST2L is still not fully understood. The prevailing thought is that sST2 is a decoy receptor that binds ligand and thus attenuates the functional consequence of the ligand. A study of asthmatic patients has demonstrated elevated levels of sST2 in these patients when compared to controls and that these levels are further elevated during exacerbations and correlate with measures of severity (8). In a mouse ovalbumin model of asthma, antigen challenge results in increased expression of sST2 in the lung and a rise in serum sST2 (9). Furthermore, allergic inflammation is reduced by exogenous treatment with sST2, suggesting that sST2 is indeed serving as a decoy receptor for a cytokine that induces allergic inflammation (10).

Interleukin-33
In 2005, Schmitz et al characterized IL-33, a new member of the IL-1 family (11). It is most closely related to IL-18 in this family of cytokines, and like other members is produced in its 266 amino acid "pro-form" of ~30 kDa that is then cleaved to a mature form of 18 kDa. IL-33 is a ligand for ST2 and binding is associated with activation of NF-kB and MAP kinases. IL-33 is identical to a previously described nuclear factor termed NF-HEV (nuclear factor of high endothelial venules), demonstrated to have a DNA-binding domain (12), though little is known about the intra-nuclear function of IL-33 except that in some cells, IL-33 localizes to the nucleus in the resting state and binds to the H2A-H2B histone complex, functioning as a transcriptional repressor (13, 14). The stimulus for migration out of the nucleus is unknown.

The intracellular processing mechanism of release of IL-33 from cells remains controversial. To date there has been very little understanding of the mechanism of release of IL-33 (15). IL-33 lacks a specific signal peptide to enable it to be processed for secretion via the ER-Golgi pathway (16). Initially, the 30 kDa form of IL-33 was thought to be cleaved by caspase 1 to the 18 kDa form, similarly to the processing of IL-1β and IL-18 (17). However, on closer examination, IL-33 appears to be a poor substrate for caspase 1, lacking a caspase 1 cleavage site, and is likely cleaved by caspase 3 (18) and 7 (19) during apoptosis resulting in inactive cleavage products (19, 20). Because these proteins are associated with apoptotic cell death, the conclusion has been that IL-33 is inactivated during apoptosis and may in turn be released intact during cell necrosis (21, 22). However, we know extremely little about the stimuli for release of IL-33. Some have suggested that IL-33 is an endogenous danger signal or alarmin because of the assumption that it is released by cell necrosis (23, 24). Other investigators have found that biologically active IL-33 is released during necrosis (25). Preliminary data from Dr. Timothy Bigby's lab have not shown the 18 kDa form of IL-33 to be present in vivo in mice challenged with ovalbumin, but rather the full-length 30 kDa form is present in large quantities in the allergically inflamed lungs of these mice. This implicates the 30 kDa form as the active molecule.

Studies thus far have demonstrated that IL-33 is most highly expressed in fibroblasts and lung, particularly in airway smooth muscle, and airway epithelial cells (11). Dr. Bigby's preliminary studies in mice now demonstrate significant amounts of IL-33 in the airway epithelium in control animals that significantly increases in both the ovalbumin (see figure at right) and IL-33 models with significant expression in inflammatory cells and alveolar type II cells, and alveolar macrophages.

Figure 2. ST2 and IL-33 Expression are increased in the lungs of Ova mice. C57BL/6 Mice were immunized and challenged with ovalbumin. At day 21 the lungs were harvested and RNA extracted. The RNA was subjected to quantitative RT-PCR probing for ST2, IL-33 and GAPDH (control). A) RT-PCR for ST2 revealed a ~ 4-fold increase in ova treated mice relative to controls. B) RT-PCR for IL-33 revealed a ~ 8-fold increase in ova-treated mice relative to controls.

IL-33 and ST2 in asthma
In summary, evidence that IL-33 and ST2 are involved in asthma comes from many different types of studies. ST2 receptors are expressed on Th2 cells and IL-33 can induce the release of IL-5 and IL-13 (26). Likewise, IL-33 can differentiate naïve T cells into Th2 cells (27). IL-33 has also been shown to recruit and activate Th2 cells (28). ST2 is expressed and IL-33 has important effects on cell function for

eosinophils, basophils (29), macrophages (30), and dendritic cells. Numerous studies that disrupt, block, or accentuate the interaction of IL-33 and ST2 point to a central role in asthma (5, 7, 31-33). A recent study by Chang et al has demonstrated that influenza induced airway hyperresponsiveness in mice was mediated by IL-33 released by macrophages acting on non-T, non-B cells producing IL-13 (34). Genetic studies have also provided substantial evidence that IL-33 and its receptor, ST2, are important in asthma. In particular, a genome wide association study that demonstrated that the eosinophilia present in many asthmatics correlated with single nucleotide polymorphisms (SNPs) in the ST2 and IL-33 genes (35).

OBJECTIVES

The goal of this project was to understand better the role of IL-33 and its receptor, ST2, in the pathogenesis of asthma. The first objective was to explore the role of ST2 in a mouse model of asthma in which ovalbumin was used to induce airway hyperresponsiveness and inflammation. We hypothesized that ovalbumin-challenged mice would show increased expression of ST2 compared to unchallenged mice. The second objective was to characterize the stimuli for expression and release of IL-33 from the human airway epithelium using the human airway epithelial cell line BEAS-2B. We hypothesized that IL-33 auto-regulates its expression in airway epithelial cells.

METHODS

Tissue harvest

C57BL/6 mice were treated in a 21 day model of immunization with ovalbumin and alum on days 0 and 7, and challenged by intratracheal instillation of ovalbumin on days 17 through 20 (OVA mice). Lung, spleen, and lymph node tissues were harvested from ovalbumin-challenged mice and control mice.

BEAS-2B cell culture

Adherent BEAS-2B cells were grown in plastic T25 and T75 flasks and 6-well plates coated with 0.01 mg/ml fibronectin and 0.03 mg/ml collagen type I. Cells were cultured in 1:1 DMEM:Ham's F12 media containing 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were grown to confluence in T25 flasks, then transferred to T75 flasks. Once the cells were confluent in T75 flasks, they were transferred to 6-well plates at a density of ~300,000-400,000 cells per well. Experiments were done when cells reached 60-70% confluence in the wells. To study possible autoregulation of IL-33 expression by IL-33, BEAS-2B cultures were incubated with 10 ng/ml of recombinant human IL-33 in growth media, or in growth media alone, for 24 hours at 37°C.

Western blot

Western blot was performed using mouse EL-4 cells (a Th2-like mouse lymphoma cell line) and lung tissue isolated from ovalbumin-challenged mice and control mice. For ST2 staining, the membrane was incubated with rabbit anti-mouse ST2 primary antibody overnight at 4°C, followed by HRP-conjugated goat anti-rabbit secondary antibody for 1 hour at room temperature. For β-actin staining (loading control), the membrane was incubated with rabbit anti-mouse β-actin primary antibody overnight at 4°C, followed by HRP-conjugated goat anti-rabbit secondary antibody for 1 hour at room temperature.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded lung tissue from ovalbumin-challenged and control mice. Tissue was deparaffinized & rehydrated in an EtOH gradient, antigen retrieval was performed using a high-pressure cooker, and blocked using H_2O_2 and Avidin/Biotin blocking kit. For ST2 staining, tissue was incubated with rabbit anti-mouse ST2 primary antibody overnight at 4°C, followed by biotin-conjugated goat anti-rabbit secondary antibody for 1 hour at room temperature. Isotype controls were performed using rabbit IgG antibody. Staining was visualized using a Vectastain ABC kit and Vector NovaRED kit. Tissue was counterstained with hematoxylin, acid rinse solution, and bluing solution. Tissue was dehydrated using a reverse EtOH gradient then mounted on a slide.

Flow cytometry

Flow cytometry was performed on splenic tissue collected from ovalbumin-challenged and control mice. CD4+ T cells were isolated using a magnetic column, followed by fixation only or fixation and permeabilization. Cells were blocked with an Fc-receptor blocker and an Avidin/Biotin blocking kit. Cells were washed and incubated with the following antibodies in the dark for 1 hour at room temperature: 1)

isotype control (unconjugated rabbit IgG); 2) CD4 (APC); 3) ST2 (FITC); 4) CD4 and ST2. Data was acquired using a digital flow cytometer (BD FACS Canto II) and analyzed using Graph Pad Prism Software.

ELISA
BEAS-2B cells were conditioned with 10 ng/ml of human recombinant IL-33 in growth media, or in growth media alone, for 24 hours at 37°C. ELISA was performed on supernatant and cell lysates collected after 24 hours of conditioning using an R&D human IL-33 duoset kit. A 96-well plate was coated overnight with goat anti-human IL-33 capture antibody. The plate was then washed and blocked with reagent diluent at room temperature for 1 hour. 100 μl of each sample or standard was added per well and incubated at room temperature for 2 hours. The wells were then washed, and biotinylated goat anti-human IL-33 detection antibody was added and incubated at room temperature for 2 hours. Streptavidin-HRP and substrate solution were used for visualization. Optical densities were determined using a spectrophotometer at a wavelength of 450 nm with a wavelength correction of 540 nm. A standard curve was plotted using Microsoft Excel and an equation for the line of best fit was determined. IL-33 concentrations were determined based on the optical densities measured and the standard curve equation.

RNA extraction and cDNA formation
RNA was extracted from conditioned and unconditioned BEAS-2B cells using Trizol and chloroform. Samples were vortexed thoroughly and centrifuged at 14000 RPM at 4°C for 15 minutes. The RNA layer was carefully collected from each sample after centrifuging and isopropanol was added. Samples were again centrifuged at 14000 RPM at 4°C for 5 minutes. Supernatant was removed and the remaining pellet was washed with 75% ethanol. RNA was dissolved in RNase/DNase-free water. Samples were warmed at 55°C for 5 minutes to dissolve the RNA. RNA optical density, purity, and concentration were determined using a nanodrop at a wavelength of 260 nm. cDNA was then made using the extracted RNA, 10nM dNTPs, primer, and RNase/DNase-free water. To each sample, 10x RT buffer, 25mM $MgCl_2$, 0.1M DTT, RNase out, and Superscript II RT were also added. PCR was performed at 42°C for 50 minutes, then 70°C for 15 minutes. RNase H was added to each cDNA sample. cDNA was stored at -20°C for real-time quantitative PCR.

Real-time quantitative PCR
Real-time quantitative PCR (RT-PCR) with SYBR green detection was performed using cDNA made from extracted mRNA as above to quantify the expression of IL-33 and GAPDH (housekeeping gene) in conditioned and unconditioned BEAS-2B cells. Cross threshold (C_T) values were recorded. Using these C_T values, the fold change was calculated as follows:

$$\text{Fold Change} = 2^{-\Delta(\Delta CT)}$$
$$\text{where } \Delta C_T = C_{T,\,target} - C_{T,\,GAPDH}$$
$$\text{and } \Delta(\Delta C_T) = \Delta C_{T,\,stimulated} - \Delta C_{T,\,control}$$

RESULTS

Verification of sST2 and ST2L expression in mouse EL-4 cells
Our first objective was to explore the role of ST2 in a mouse model of asthma in which ovalbumin is used to induce airway hyperresponsiveness and inflammation. Using mouse EL-4 cells, sST2 and ST2L were found to be expressed as approximately 37 kDa and 60 kDa proteins, respectively (Figure 1). These molecular weights corresponded with the expected molecular weights of sST2 and ST2L based on their primary sequence. Of note, the predominant band was the lower molecular weight, corresponding to the sST2 protein.

Effect of ovalbumin challenge on ST2 expression in mouse lung tissue
Expression of ST2 in lung tissue from ovalbumin-challenged C57BL/6 mice was compared to lung tissue from control mice using Western blot. Qualitatively, sST2 expression appeared higher in lung tissue from ovalbumin-challenged mice compared to lung tissue from control mice (Figure 2). We also studied expression of ST2 in mouse lung tissue using immunohistochemistry. Staining with mouse anti-ST2 antibody showed higher expression of ST2 in lung tissue of ovalbumin-challenged mice compared to controls (Figure 3). Staining with mouse anti-ST2 antibody also showed ST2 expression in mediastinal lymph node tissue from ovalbumin-challenged mice (Figure 3). Lymph nodes from unchallenged control mice were too small at the time of tissue harvest to be isolated for comparison.

Effect of ovalbumin challenge on surface expression of ST2 in mouse splenic tissue
ST2 expression was also studied in CD4+ cells isolated from mouse splenic tissue using flow cytometry. We found that splenic tissue from ovalbumin-challenged mice had a higher proportion of CD4+ cells that expressed ST2 on the cell surface (CD4+ST2+ cells) compared to control mice (Figure 4). However, when these cells were permeabilized, there was no difference is the percent of CD4+ cells that expressed ST2 when comparing ovalbumin-challenged to control mice.

**Effect of IL-33 conditioning on IL-33 expression in human airway epithelial cells**
Next, we aimed to characterize the stimuli for expression and release of IL-33 from the human airway epithelial cell line BEAS-2B. Firstly, we wanted to determine if IL-33 expression could be auto-induced by conditioning with IL-33. BEAS-2B cells were grown to confluence and treated with or without IL-33 as detailed in the methods section. Real-time quantitative PCR (RT-PCR) was used to quantify IL-33 expression in conditioned and unconditioned cells. We found that there was a 1.87-fold increase in IL-33 expression in IL-33-conditioned BEAS-2B cells compared to unconditioned cells (Table 1). ELISA was also used to characterize IL-33 expression. Supernatant and cell lysate from IL-33-conditioned and unconditioned BEAS-2B cells were collected. We found a mean IL-33 concentration of 164.5 pg/ml, SE 14.92, in supernatant collected from IL-33-conditioned BEAS-2B cells (Figure 5). However, this measured amount of IL-33 likely represented residual amounts of recombinant IL-33 that had been initially added to the media as part of the treatment condition. No IL-33 was detected in supernatant from unconditioned BEAS-2B cells. When comparing IL-33 expression in cell lysates, there was no difference detected between cell lysate collected from IL-33-conditioned versus unconditioned cells (Figure 5).

Figure 1: sST2 and ST2L expression in mouse EL-4 cells
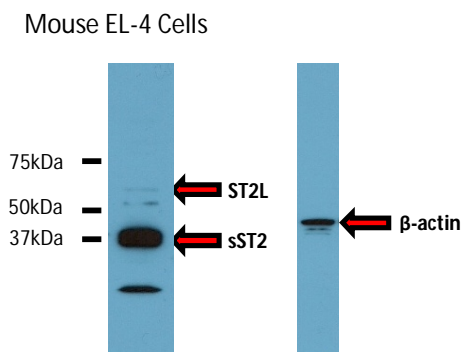
Mouse EL-4 Cells

sST2 was expressed in mouse EL-4 cells as a 37kDa protein, and ST2L was expressed as a 60kDa protein. These molecular weights correspond with the expected molecular weights of sST2 and ST2L based on their primary sequence. The predominant band corresponded with the lower molecular weight protein, sST2.

Figure 2: sST2 expression in lung tissue of ovalbumin-challenged and control mice

Lung Tissue from OVA and Control (CTL) Mice
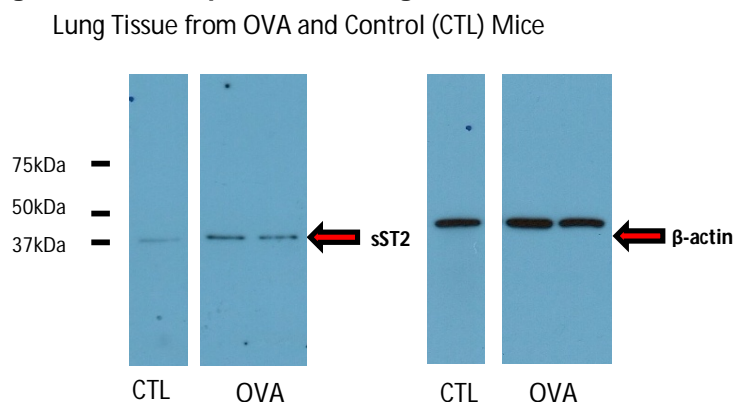
Lung tissue was harvested from C57BL/6 mice immunized and challenged with ovalbumin and control unchallenged mice. Western blot was performed using rabbit anti-mouse ST2 primary antibody followed by HRP-conjugated goat anti-rabbit secondary antibody, with β-actin as a loading control. Qualitatively, sST2 expression appeared higher in lung tissue from ovalbumin-challenged mice compared to lung tissue from control mice.

Figure 3: ST2 is induced in immunized and challenged mouse lungs
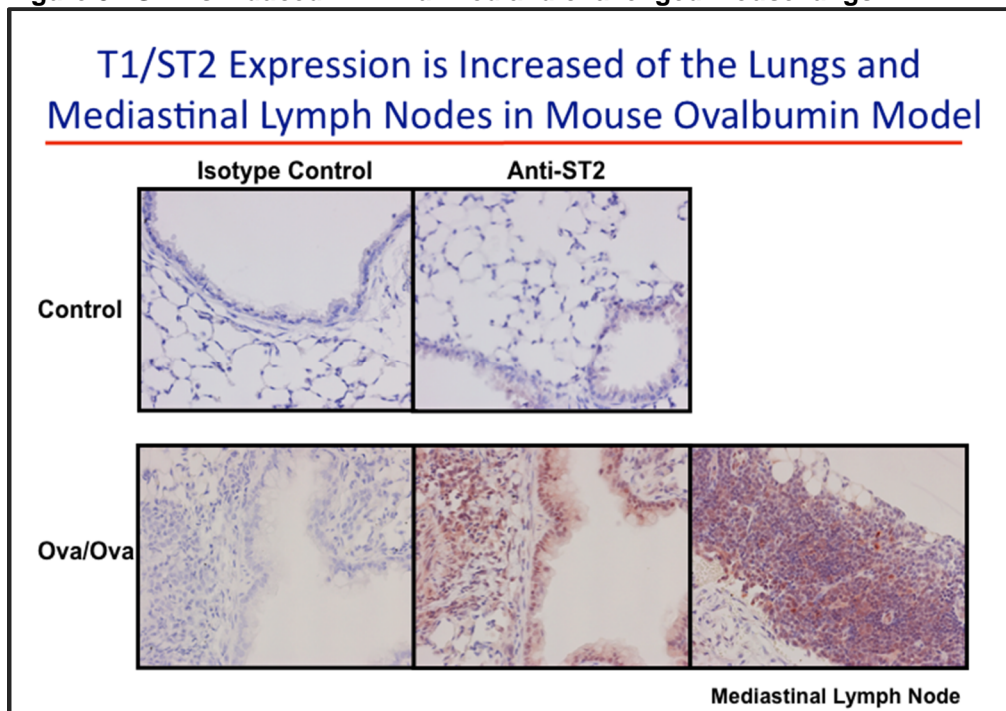
Immunohistochemistry was performed on lung and lymph node tissue from ovalbumin-challenged mice and unchallenged control mice using rabbit anti-mouse ST2 primary antibody followed by biotin-conjugated goat anti-rabbit secondary antibody.   There was higher expression of ST2 in lung tissue of ovalbumin-challenged mice compared to controls. There was also ST2 expression in mediastinal lymph node tissue from ovalbumin-challenged mice. Lymph nodes from unchallenged control mice were too small at the time of tissue harvest to be isolated for comparison.

Figure 4: Splenic tissue from ovalbumin-challenged mice demonstrate a higher proportion of CD4+ cells that expressed ST2 on the cell surface (CD4+ST2+ cells)
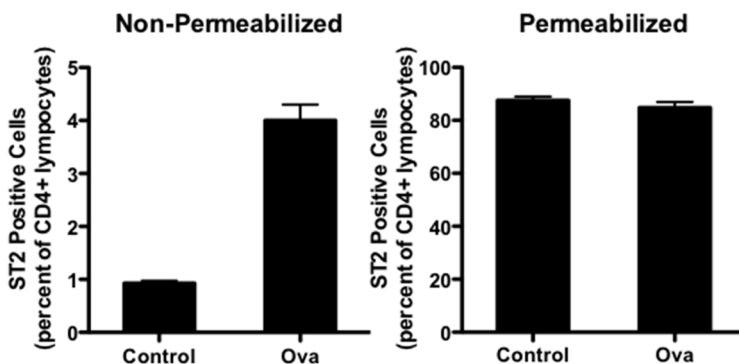
Splenic tissue was harvested from ovalbumin-challenged and control mice. CD4+ T cells were isolated using a magnetic column, followed by fixation only or fixation and permeabilization. Flow cytometry was done using the following antibodies:1) isotype control (unconjugated rabbit IgG); 2) anti-CD4 (APC); 3) anti-ST2 (FITC); 4) anti-CD4 and anti-ST2. Splenic tissue from ovalbumin-challenged mice had a higher proportion of CD4+ cells that expressed ST2 on the cell surface (CD4+ST2+ cells) compared to control mice. However, when these cells were permeabilized, there was no difference is the percent of CD4+ cells that expressed ST2 when comparing ovalbumin-challenged to control mice.

Table 1: Real time quantitative PCR to measure IL-33 expression in BEAS-2B cells

	Condition	IL-33 Ct	GAPDH Ct	ΔCt	Δ(ΔCt)	Fold change =2^-[Δ(ΔCt)]
Sample #1	Control - growth media only	39.04	18.97			
Sample #2	Control - growth media only	40	19.86			
Sample #3	Control - growth media only	38.06	21.28			
	Average	39.03	20.04	18.99	0	1
Sample #4	Growth media + IL-33 24 hours	39.98	21.47			
Sample #5	Growth media + IL-33 24 hours	39.88	22.61			
Sample #6	Growth media + IL-33 24 hours	39.90	21.41			
	Average	39.92	21.83	18.09	-0.9022	1.87

Real-time quantitative PCR was performed using cDNA made from extracted mRNA of BEAS-2B cells conditioned with 10 ng/ml IL-33 in growth media or growth media alone for 24 hours. GAPDH was used as a housekeeping gene. Cross threshold (Ct) values were recorded. Fold change = $2^{-\Delta(\Delta Ct)}$, where $\Delta Ct = Ct_{IL-3} - Ct_{GAPDH}$ and $\Delta(\Delta Ct) = \Delta Ct_{stimulated} - \Delta Ct_{control}$. There was a 1.87-fold increase in IL-33 expression in BEAS-2B cells that had been conditioned with IL-33 compared to control BEAS-2B cells.

Figure 5: IL-33 concentrations measured by ELISA performed on supernatant and cell lysate collected from IL-33-conditioned and unconditioned BEAS-2B cells
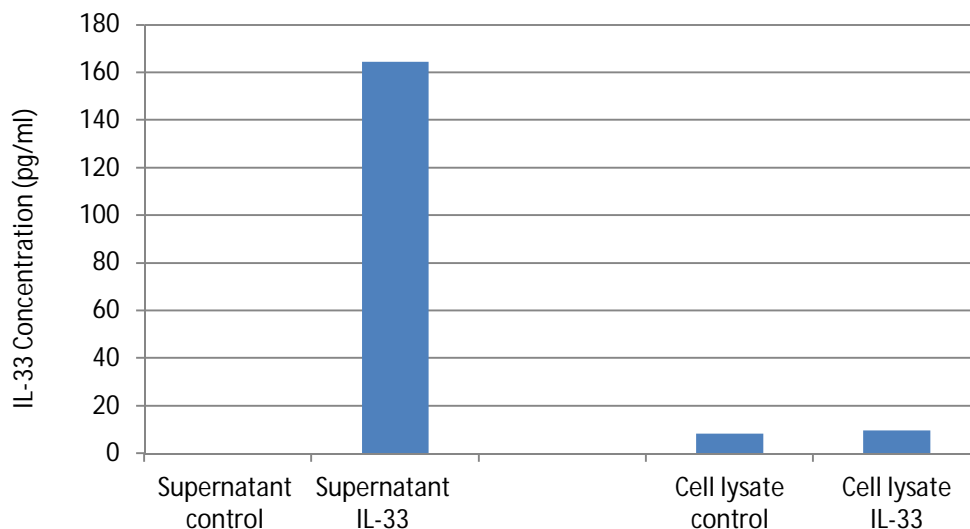
BEAS2B cells were conditioned with 10 ng/ml of human recombinant IL-33 in growth media or in growth media alone for 24 hours. ELISA was performed on supernatant and cell lysates collected after 24 hours of conditioning using a 96-well plate coated with goat anti-human IL-33 capture antibody, followed by biotinylated goat anti-human IL-33 detection antibody, and streptavidin-HRP and substrate solution for visualization (R&D duoset). The mean concentration of IL-33 in supernatant from conditioned BEAS-2B cells was 164.5 pg/ml, SE 14.92. No IL-33 was detected in supernatant from unconditioned BEAS-2B cells. There was no difference in IL-33 concentration in cell lysate collected from conditioned versus unconditioned BEAS-2B cells.

Tammie Nguyen
ISP Write-Up

DISCUSSION

Asthma remains a major health problem that affects as many as 22 million people in the United States alone and 300 million people worldwide, and its incidence has increased significantly over the last 20 to 40 years in developed countries. Recently, ST2 and IL-33, a receptor-ligand pair, have been implicated in mediating the inflammatory responses involved in airway hyperreactivity. However, the complex interactions between ST2 and IL-33 have yet to be fully described.

Our first objective was to explore the role of ST2 in a mouse model of asthma in which ovalbumin was used to induce airway hyperresponsiveness and inflammation. Our findings suggest that ST2 is strongly induced by immunization and challenge with ovalbumin. Based on the expected molecular weights of the ST2 proteins, it would appear that sST2 was the more predominant form that was increased in lung tissue of ovalbumin-challenged mice compared to control mice as found on Western blots. Because sST2 may play an anti-inflammatory role as a decoy receptor, the increased expression of sST2 in ovalbumin-challenged mice may serve to attenuate allergic inflammation. We also found that lung tissue and lymph node tissue from ovalbumin-challenged mice demonstrated higher levels of ST2 as seen by immunohistochemistry. However, the antibodies that were available to us for use in immunohistochemistry experiments could not distinguish with certainty between the different isoforms of ST2, so we could not say definitively which isoform of ST2 was increased in these tissues.

Conversely, our flow cytometry results suggest that the increased expression of ST2 in CD4+ cells from mouse splenic tissue seen after ovalbumin challenge was localized to the cell surface, whereas intracellular ST2 levels in CD4+ cells were similar between challenged and unchallenged mice. Since ST2L is the transmembrane form of ST2, our results would suggest that ST2L, rather than sST2, was the predominant form that was increased in mouse secondary lymphoid tissue following ovalbumin challenge. It would be informative to immunoblot these cells for ST2, which could help to determine if the surface and internalized protein were predominantly the ST2L versus sST2 isoforms. The interactions of the different isoforms of ST2 appear to be very complex and further work is required to understand clearly the significance of these proteins in the pathogenesis of allergic asthma.

Our next objective was to determine if IL-33 may autoregulate its own expression in human airway epithelium. We used RT-PCR and ELISA to determine if conditioning BEAS-2B human epithelial cells with IL-33 would affect the level of expression of IL-33. Our results showed that BEAS-2B cells did not express significant IL-33 with or without IL-33 conditioning. It is possible that BEAS-2B cells did not express significant IL-33 because of a lack of differentiation of the epithelial cell line compared to primary epithelium. Primary epithelium may require specific growth factors or other cell-cell signals that mediate differentiation and possible downstream effects of IL-33 exposure, and we were not able to achieve this state of differentiation or response to IL-33 with the BEAS-2B cell line. The next step would be to determine if IL-33 may autoregulate its own expression in primary mouse or human airway epithelium rather than in an airway epithelial cell line. It would also be beneficial to find different culture conditions for BEAS-2B cells that would have them more closely approximate normal human bronchial epithelial cells.

One limitation of this study was not being able to clearly distinguish between sST2 versus ST2L in immunohistochemistry experiments. Also, for RT-PCR it would be beneficial for future experiments to design primers that specifically identify ST2L versus sST2, which would allow for probing the epithelial cells in a more sensitive manner. It would have been informative to study IL-33 and ST2 expression in the same cells or tissues to understand better how they may be interacting. Future studies should also explore possible dose- or time-dependent relationships between IL-33 conditioning of airway epithelial cells and subsequent effects on IL-33 expression. Future studies should also aim to clarify the interactions of ST2L versus sST2 with IL-33 and how these interactions may correlate with the clinical presentation of asthma.

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