IL-17A Induces Signal Transducers and Activators of Transcription–6–Independent Airway Mucous Cell Metaplasia

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Mucous cell metaplasia is a hallmark of asthma, and may be mediated by signal transducers and activators of transcription (STAT)–6 signaling. IL-17A is increased in the bronchoalveolar lavage fluid of patients with severe asthma, and IL-17A also increases mucus production in airway epithelial cells. Asthma therapeutics are being developed that inhibit STAT6 signaling, but the role of IL-17A in inducing mucus production in the absence of STAT6 remains unknown. We hypothesized that IL-17A induces mucous cell metaplasia independent of STAT6, and we tested this hypothesis in two murine models in which increased IL-17A protein expression is evident. In the first model, ovalbumin (OVA)–specific D011.10 Th17 cells were adoptively transferred into wild-type (WT) or STAT6 knockout (KO) mice, and the mice were challenged with OVA or PBS. WT-OVA and STAT6 KO-OVA mice demonstrated increased airway IL-17A and IL-13 protein expression and mucous cell metaplasia, compared with WT-PBS or STAT6 KO-PBS mice. In the second model, WT, STAT1 KO, STAT1/STAT6 double KO (DKO), or STAT1/STAT6/IL-17 receptor A (RA) triple KO (TKO) mice were challenged with respiratory syncytial virus (RSV) or mock viral preparation, and the mucous cells were assessed. STAT1 KO-RSV mice demonstrated increased airway mucous cell metaplasia compared with WT-RSV mice. STAT1 KO-RSV and STAT1/STAT6 DKO-RSV mice also demonstrated increased mucous cell metaplasia, compared with STAT1/STAT6/IL17RA TKO-RSV mice. We also treated primary murine tracheal epithelial cells (mTECs) from WT and STAT6 KO mice. STAT6 KO mTECs showed increased periodic acid–Schiff staining with IL-17A but not with IL-13. Thus, asthma therapies targeting STAT6 may increase IL-17A protein expression, without preventing IL-17A-induced mucus production.

Keywords: asthma; mucous cell metaplasia; IL-17A; STAT6; IL-13

Airway mucus is a hallmark of asthma. Airway epithelial cell remodeling in asthma includes mucous cell metaplasia and mucus hypersecretion, which narrows the airway lumen and limits airflow (1). A major component of mucus consists of mucins, which are large glycoproteins that determine the viscoelasticity of mucus (2). Mucin genes are expressed in many tissues, but

CLINICAL RELEVANCE

Mucous cell metaplasia is a hallmark of asthma, and is mediated by signal transducers and activators of transcription (STAT)–6 signaling and IL-17A. Therapeutics that target STAT6 signaling are currently being developed for use in patients with asthma. The effect of IL-17A on mucous cell metaplasia in the absence of STAT6 remains unknown, and that was the focus of this study. We show, in two *in vivo* models, that IL-17A increases mucous cell metaplasia in the absence of STAT6. Thus, therapeutics targeting STAT6 signaling pathways may not decrease mucus in patients with asthma and increased IL-17A expression.

MUC5AC and *MUC5B* constitute the primary mucin genes expressed in the airway epithelial cells of the lung (3). Previous studies have shown that IL-13, a Th2 cytokine that is increased during allergic airway inflammation, is required for airway mucous cell metaplasia (4–7).

IL-13 is abundant in the sputum of some patients with asthma (8). IL-13 binds to the IL-13 receptor (R), which is comprised of two subunits, IL-4R α and IL-13R α 1. The binding of IL-13 to the IL-13R results in the phosphorylation and activation of the downstream transcription factor signal transducers and activators of transcription (STAT)–6 and the transcription of IL-13–mediated genes. STAT6 and IL-13 are required for maximal airway mucous cell metaplasia in murine models of allergic airway inflammation (4–6, 9). The airway instillation of recombinant IL-13 or the airway epithelial overexpression of IL-13 resulted in increased mucous cell metaplasia and mucus secretion in the airway (6, 10). Further, IL-13–deficient or STAT6-deficient mice exhibited decreased airway mucous cell metaplasia with allergic airway inflammation (4–6, 9).

IL-17A, a cytokine secreted by CD4⁺ Th17 cells, $\gamma\delta$ T cells, natural killer T cells, and innate lymphoid cells, also increased mucous cell metaplasia in murine models, and increased mucin gene expression human airway epithelial cells. IL-17A is increased in the bronchoalveolar lavage (BAL) fluid of patients with moderate to severe asthma (11, 12). In mice, respiratory syncytial virus (RSV) infection during ongoing allergic airway inflammation increased mucous cell metaplasia and mucin protein expression when IL-17A, but not IL-13, was significantly increased in whole-lung homogenates (13). In addition, Chen and colleagues reported that recombinant IL-17A, but not IL-4 and IL-13, up-regulated the mucin gene expression of *MUC5AC* and *MUC5B* in primary human tracheobronchial epithelial cells (3).

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IL-17A secretion from CD4⁺ T cells is negatively regulated by STAT6 (14). IL-13 and STAT6 signaling are currently being targeted for asthma therapy (15–17), and it remains unknown whether down-regulating STAT6 signaling may modulate IL-17A-driven airway mucous cell metaplasia in patients with asthma. Therefore, understanding whether IL-17A-mediated mucous cell hyperplasia occurs in a STAT6-independent model is important for drug development. We hypothesized that IL-17A induces mucous cell metaplasia independently of STAT6. To test our hypothesis, we used two murine models that promote lung IL-17A protein expression, and examined STAT6-independent mucous cell metaplasia. In our first model, we used a previously established design in which ovalbumin (OVA)-specific D011.10 Th17 cells were adoptively transferred into wild-type (WT) or STAT6 knockout (KO) mice, followed by OVA intranasal challenge (18). Using this model, McKinley and colleagues reported increased airway inflammation, airway reactivity, and mucus expression with OVA challenge (18). Further, the Th17-mediated increases in airway inflammation and airway reactivity were resistant to steroid treatment (18). This model allowed us to assess the role of IL-17A directly in STAT6-independent mucous cell metaplasia.

In the second murine model, we challenged WT, STAT1 KO, STAT1/STAT6 double KO (DKO), or STAT1/STAT6/IL-17RA triple KO (TKO) mice with either RSV A2 or uninfected cell culture supernatant (mock viral preparation). We have previously shown that STAT1 KO-RSV mice demonstrated significantly greater lung IL-17A and IL-13 protein expression compared with WT-RSV mice (13, 19), and that STAT1/STAT6 DKO-RSV mice had significantly increased IL-17A protein detected in their lungs compared with STAT1 KO-RSV mice (19). The use of STAT1/STAT6 DKO and STAT1/STAT6/IL-17RA TKO mice allowed us to determine the contributions of RSV-induced IL-17A protein expression in airway mucous cell metaplasia in the absence of STAT6 signaling.

To test our hypothesis further, we examined the direct role of IL-17A in the mucous cell metaplasia of primary murine tracheal epithelial cells (mTECs) cultured from WT and STAT6 KO mice. These experiments in different models defined an IL-17A-dependent, STAT6-independent mechanism for mucous cell metaplasia in airway epithelial cells. Our results suggest that therapeutics targeting STAT6 may not reduce or eliminate airway mucus secretion in asthma patients with increased IL-17A.

MATERIALS AND METHODS

Mice

Female BALB/c mice were purchased from Charles River Laboratories (Wilmington, MA) (20). D011.10 and STAT6 KO mice were purchased from Jackson Laboratories (Bar Harbor, ME). IL-17RA KO and STAT1 KO mice were generated as previously described (20, 21). STAT1/STAT6 DKO and STAT1/STAT6/IL-17RA TKO mice were generated by mating and subsequent genotyping. Investigators adhered to the revised 1996 Guide for the Care and Use of Laboratory Animals (National Research Council).

D011.10 Th17 Cell Polarization and Activation

D011.10 CD4⁺ T cells were purified from the spleens of mice as previously described (22). T cells were activated using OVA peptide 323–339 (5 µg/ml; Sigma Chemical Co., St. Louis, MO) and anti-CD28 (1 µg/ml; BD Biosciences, San Jose, CA). T cells were differentiated into Th17 cells, as previously described (22).

Adoptive Transfer of D011.10 Cells into WT Recipient Mice

WT BALB/c and STAT6 KO recipient mice were intranasally challenged with OVA protein (50 µg/mouse in 100 µl; Sigma Chemical Co.) or PBS

on Day –1. On Day 0, 1×10^6 Th17 D011.10 cells were adoptively transferred into WT or STAT6 KO recipient mice. Mice were challenged with 50 µg/mouse OVA protein (WT-OVA or STAT6 KO-OVA) or PBS intranasally (WT-PBS or STAT6 KO-PBS) on Days 1, 2, and 3 after cell transfer. BAL fluid and lungs were collected on Day 4.

Analysis of Inflammatory Cell Infiltration into BAL Fluid

Inflammatory cells were counted in the BAL fluid, as previously described (19). Counts were based on 200 cells, using standard morphologic criteria to classify cells.

Cytokine Measurements

Cytokines were measured in BAL fluid by ELISA (R&D Systems, Minneapolis, MN).

Histopathology of Lungs

Lungs were fixed and sections were stained with periodic acid–Schiff (PAS). Slides were scored by a pathologist blinded to the experimental groups. All airways in the section were scored (15–40 airways) according to a five-point scale (0, no PAS-positive cells; 1, < 5% PAS-positive cells; 2, 5–10% PAS-positive cells; 3, 10–25% PAS-positive cells; and 4, > 25% PAS-positive cells).

RSV Challenge

The A2 strain of RSV was provided by R. Chanock (National Institutes of Health, Bethesda, MD). Viral stocks were generated as previously described (23). Mice were challenged intranasally with 100 µl of 1×10^7 plaque-forming units/ml of RSV A2 or uninfected cell-culture supernatant (mock viral preparation). Lungs were harvested 6 days after the challenge.

Culture of Murine Tracheal Epithelial Cells

Murine tracheas were harvested from WT BALB/c and STAT6 KO mice, and cultured as previously described (24). After 14 days, fully differentiated mTECs were treated with PBS, IL-13 (10 ng/ml), or IL-17 (10 ng/ml) in the basolateral compartment for 3 days.

PAS Staining of mTECs

mTECs were fixed with 4% paraformaldehyde for 20 minutes at room temperature, embedded in paraffin, and PAS-stained. Images were captured with a $\times 60$ objective. One hundred cells were counted on each slide, and the percentage of PAS-positive cells was determined from three independent experiments.

Statistical Analyses

Data are presented as means $\pm$ SEMs, and were analyzed using ANOVA, followed by the Tukey *post hoc* test or a two-tailed *t* test using GraphPad Prism, version 4 (GraphPad Software, La Jolla, CA). Values were significant when $P < 0.05$.

RESULTS

Th17 Cell-Dependent Airway Mucus Production *In Vivo* Is Independent of STAT6

STAT6 mediates airway mucous cell metaplasia in animal models of allergic airway inflammation (9). IL-17A is negatively regulated by STAT6 (14), and IL-17A induces mucin gene expression in human airway epithelial cells (3). However, whether IL-17A induces mucous cell metaplasia in the absence of STAT6 remains unknown. We hypothesized that mucous cell metaplasia is increased in the presence of IL-17A in STAT6 KO mice. Twenty-four hours after the final OVA challenge, lungs were harvested and stained for PAS to determine airway mucous cell metaplasia (Figure 1A). WT-OVA mice and STAT6 KO-OVA mice exhibited a significant increase in the number of

Figure 1. Th17 cells induced airway mucus expression independent of signal transducers and activators of transcription (STAT)-6. DO11.10 Th17 CD4⁺ T cells were adoptively transferred into wild-type (WT) or STAT6 knockout (KO) recipient mice. Recipient mice were intranasally treated with PBS or ovalbumin (OVA) daily for 3 consecutive days. Lungs were harvested 24 hours after the final challenge. (A) Representative sections of periodic acid-Schiff (PAS)-stained sections. Black arrowheads denote PAS-positive cells. (B) Quantification of PAS staining scores as a percentage of airways with a score of 1–4, as determined by a pathologist blinded to groups. * $P < 0.05$, compared with PBS-stimulated mice for respective group ($n = 7$ –8 for each group), according to ANOVA.

cells with PAS staining compared with WT-PBS or STAT6 KO-PBS mice, respectively (Figure 1B). No significant difference was evident in airways scoring 1–4 for PAS staining among WT-OVA and STAT6 KO-OVA mice. These data suggest that the Th17 cell expression of IL-17A induces airway mucous cell metaplasia independent of STAT6.

IL-17A and IL-13 Are Significantly Increased in the BAL Fluid of STAT6 KO-OVA Mice

Th17 cells comprise a plastic T-cell lineage and are not irreversibly differentiated, and may secrete cytokines typically secreted by other T-cell subsets (25). Therefore, before the adoptive transfer, we determined whether DO11.10 Th17 cells maintained their Th17 phenotype. Cell-culture supernatants were harvested from DO11.10 Th17 differentiated cells 4 days after T-cell activation and differentiation. IL-17A and IL-13 protein expression in cell-culture supernatants was analyzed by ELISA. DO11.10 Th17 cells expressed $2,901 \pm 78.5$ pg/ml of IL-17A, but IL-13 protein was not detectable in DO11.10 Th17 cells (data not shown).

For our *in vivo* model, DO11.10 Th17 cells were adoptively transferred into WT and STAT6 KO mice, and the BAL fluid was collected 24 hours after the final OVA challenge. Both WT-OVA and STAT6 KO-OVA mice had increased IL-17A

protein detected in their BAL fluid, compared with recipient WT-PBS and STAT6 KO-PBS mice (Figure 2A). IL-17A protein expression was significantly increased in the BAL fluid of STAT6 KO-OVA mice compared with WT-OVA mice. IL-13 protein expression in BAL fluid was also significantly increased in WT-OVA and STAT6 KO-OVA mice, compared with WT-PBS and STAT6 KO-PBS mice (Figure 2B). IL-13 protein expression was significantly higher in STAT6 KO-OVA mice compared with WT-OVA mice.

Th17 cells also produce cytokines other than IL-17A, including IL-17A, IL-17F, IL-6, IL-21, and IL-22 (26). We analyzed the cytokines present in the BAL fluid, and found that IL-17A was increased in STAT6 KO-OVA mice compared with STAT6 KO-PBS mice. IL-17F, IL-21, and IL-22 were not significantly increased in the BAL fluid of STAT6 KO-OVA mice, compared with STAT6 KO-PBS mice or WT-OVA mice (data not shown). These results suggest that STAT6 signaling negatively regulates the expression of both IL-17A and IL-13 in the airways.

STAT6 KO-OVA Mice Have Increased Airway Neutrophils Compared with WT-OVA Mice

Increased IL-17A results in increased neutrophil infiltration into the airway (27). Therefore, to ensure that the adoptively transferred DO11.10 Th17 cells maintained their Th17 phenotype, we examined the infiltration of inflammatory cells into the airway. Twenty-four hours after the final intranasal challenge, WT OVA and STAT6 KO-OVA mice both had significantly increased neutrophils in their BAL fluid, compared with WT-PBS and STAT6 KO-PBS mice (Figure 3A). STAT6 KO-OVA mice also had significantly increased neutrophils in their BAL fluid, compared with WT-OVA mice. These results suggest that STAT6 signaling negatively regulates IL-17A-mediated neutrophil infiltration into the airway. Lymphocytes were also significantly increased in WT-OVA mice compared with WT-PBS mice, as well as in STAT6 KO-OVA mice compared with STAT6-PBS mice (Figure 3B). However, no difference in lymphocyte numbers in BAL fluid was found between WT-OVA and STAT6 KO-OVA mice. Minimal numbers of eosinophils were evident in the BAL fluid of WT-OVA mice ($1.1 \times 10^4 \pm 0.4 \times 10^4$ cells/ml), but no eosinophils were detected in the BAL fluid of STAT6 KO-OVA mice (data not shown). Taken together, these data suggest that adoptively transferred DO11.10 Th17 cells recruit neutrophils and lymphocytes to the lung, and that STAT6 negatively regulates neutrophil recruitment.

Figure 2. The expression of IL-17A and IL-13 protein is increased in STAT6-OVA bronchoalveolar lavage (BAL) fluid. (A) IL-17A and (B) IL-13 protein expression was determined in the BAL fluid of OVA-challenged or PBS-challenged WT or STAT6 KO recipient mice after the adoptive transfer of DO11.10 Th17 CD4⁺ T cells. * $P < 0.05$, compared with PBS in the respective murine strain, and † $P < 0.05$, greater than WT OVA-stimulated mice ($n = 6$ –7 mice for each group), according to ANOVA.

Figure 3. Increased neutrophil infiltration in STAT6-OVA mice compared with WT-OVA mice. The infiltration of (A) neutrophils and (B) lymphocytes was measured in the BAL fluid of OVA-challenged or PBS-challenged WT or STAT6 KO recipient mice after the adoptive transfer of DO11.10 Th17 CD4⁺ T cells. * $P < 0.05$, greater than PBS-stimulated mice for respective group, and † $P < 0.05$, greater than WT OVA-stimulated mice ($n = 6-7$ for each group), according to ANOVA.

RSV-Induced Mucous Cell Metaplasia in STAT1 KO Mice Is Independent of STAT6

Using the Th17 polarized D011.10 adoptive transfer model, we showed that Th17 cytokines increased mucous cell metaplasia in the absence of STAT6. We confirmed these findings in another *in vivo* model with increased endogenous IL-17A protein expression. Previously we have shown that RSV-challenged STAT1/STAT6 DKO (STAT1/STAT6 KO-RSV) mice have increased RSV-induced lung IL-17A protein expression compared with RSV-challenged WT (WT-RSV) or STAT1 KO (STAT1 KO-RSV) mice (19). This model allowed us the opportunity to examine mucous cell metaplasia when STAT6 was absent. We also generated STAT1/STAT6/IL-17RA TKO mice to determine directly the role of IL-17RA signaling in mucous cell metaplasia. We hypothesized that STAT1/STAT6 DKO-RSV mice have increased mucous cell metaplasia compared with WT-RSV mice, whereas the STAT1/STAT6/IL-17RA TKO-RSV mice would not have increased airway mucous cell metaplasia.

WT, STAT1 KO, STAT1/STAT6 DKO, and STAT1/STAT6/IL-17RA TKO mice were RSV-challenged or mock-challenged intranasally, and lungs were harvested 6 days after challenge and stained with PAS to examine airway mucous cell metaplasia. We chose 6 days because we previously showed that it is the maximal time for lung IL-17A expression and increased airway mucous cell metaplasia in response to RSV challenges in STAT1 KO mice (28). STAT1 KO-RSV and STAT1/STAT6 DKO-RSV mice showed a significant increase in the number of PAS-staining airway epithelial cells, compared with WT-RSV mice (Figure 4). However, STAT1/STAT6/IL-17RA TKO-RSV mice displayed virtually no PAS-positive staining airway epithelial cells. Mock challenge resulted in almost absent PAS-positive staining in airway epithelial cells among all strains of mice. These data suggest that in this model, in the setting of endogenous IL-17A expression, IL-17RA signaling is required for STAT6-independent airway mucous cell metaplasia.

IL-17A-Mediated Mucus Production in mTECs Is Independent of STAT6

In vivo we have demonstrated in two murine models that IL-17A mediates mucous cell metaplasia in a STAT6-independent fashion, but we wanted to determine the direct effect of IL-17A on airway epithelial cell mucous metaplasia in the absence of STAT6. We hypothesized that IL-17A increases mucus production in fully differentiated, primary mTECs. To test this, we isolated mTECs from WT and STAT6 KO mice, and cultured them at an air-liquid interface to become differentiated epithelial cells. Primary

mTECs from WT and STAT6 KO mice were stimulated with PBS, IL-13, or IL-17A from the basolateral compartment for 3 days. Cells were examined by PAS staining, and scored by the percentage of PAS-positive cells. IL-13 increased PAS staining in WT mTECs compared with PBS-treated WT mTECs (Figures 5A and 5B). IL-17A, and not IL-13, increased cells with PAS staining in STAT6 KO mTECs compared with PBS-treated mTECs. These results suggest that IL-17A induces mucus production in differentiated airway epithelial cells in the absence of STAT6.

DISCUSSION

Asthma is a heterogeneous disease with many different phenotypes, leading to variable responses to therapeutics. IL-13 and STAT6, a downstream transcription factor, are deemed central mediators of airway mucous cell metaplasia in allergic asthma (4, 5, 7, 9). IL-17A also increases epithelial-cell mucous cell metaplasia, and IL-17A was detected in the BAL fluid and sputum of patients with severe or uncontrolled, corticosteroid-resistant asthma (11, 12). In this study, we examined IL-17A mucous cell metaplasia in the absence of STAT6. Understanding this mechanism is important, because therapeutics targeting the STAT6 pathway are being developed for the treatment of asthma (16, 17, 29–33). These patients demonstrate increased levels of mucus production, but with variable expressions of IL-13 or periostin, an IL-13 marker protein (34), calling into question the absolute IL-13 dependence of airway mucus production in asthma.

We examined the effects of Th17 cytokines on airway mucus in two different *in vivo* models. In the first model, we adoptively transferred OVA-specific Th17 cells into WT or STAT6 KO recipient mice, and examined mucous cell metaplasia after OVA allergen challenge. WT-OVA and STAT6 KO-OVA mice exhibited increased PAS staining, compared with WT-PBS or STAT6 KO-PBS mice, respectively. In the second model, we challenged WT, STAT1 KO, STAT1/STAT6 DKO, and STAT1/STAT6/IL-17RA TKO mice with RSV. We previously showed that RSV challenge significantly increased IL-17A production in STAT1/STAT6 DKO mice, compared with WT and STAT1 KO mice (19). Therefore, we extended these findings in the present study by examining the role of STAT6 signaling in airway mucous cell metaplasia. We found that STAT1 KO-RSV mice had increased airway mucous epithelial cells, compared with STAT1/STAT6 DKO-RSV mice. We also found that STAT1/STAT6 DKO-RSV mice had significantly increased airway mucous cells compared with either WT-RSV mice or STAT1/STAT6/IL-17RA TKO-RSV mice. These data indicate that IL-17RA signaling is required for STAT6-independent mucous cell metaplasia in the lungs of mice.

Determining the extent of IL-17A-mediated, STAT6-independent mucus production is important for developing therapeutics that target airway mucus production. In murine models, IL-13 readily increases mucus production in the lungs (7, 35). However, in primary human tracheobronchial epithelial cells, IL-17A, and not IL-13, increased mucin gene expression (3). In our *in vitro* model, we used primary differentiated mTECs from WT or STAT6 KO mice to analyze the number of PAS-positive cells after IL-13 or IL-17A stimulation. IL-17A induced mucus production in STAT6 KO mTECs. IL-13 induced mucous cell metaplasia in the WT mTECs, but IL-13 did not induce mucous cell metaplasia in the STAT6 KO mTECs. These models confirm that IL-17A increases airway epithelial cell mucus production in a STAT6-independent manner.

In our first *in vivo* model, we adoptively transferred D011.10 Th17 cells into WT or STAT6 KO mice, and challenged the mice with OVA. Th17 cells comprise a plastic T-cell lineage, and are known to switch cytokine-expression phenotypes depending on

Figure 4. Respiratory syncytial virus (RSV)-induced mucus production is STAT6-independent in STAT1 KO mice. Mice were intranasally challenged with RSV A2, and lungs were harvested, fixed, paraffin-embedded, and stained with PAS to determine mucus production. (A) Representative sections of PAS-stained sections. Black arrowheads denote PAS-positive cells. (B) Quantification of PAS staining scores as a percentage of airways with a score of 1–4, as determined by a pathologist blinded to groups. * $P < 0.05$, compared with WT-RSV mice ($n = 7$ –10 for each group), according to ANOVA. DKO, double knockout; IL-17R, IL-17 receptor; TKO, triple knockout.

the cytokine milieu in the local environment (25). Therefore, in this study, we also wanted to determine whether Th17 cells were changing their cytokine-expression profile. We analyzed IL-13 protein expression in the BAL fluid of mice after OVA challenge. D011.10 Th17 cells did not produce detectable IL-13 at the time of T-cell adoptive transfer. However, when lungs were harvested from STAT6 KO-OVA mice or WT-OVA recipient mice, IL-13 protein expression was significantly increased compared with respective STAT6 KO-PBS and WT-PBS control mice. Increased IL-13 likely contributed to the observed increased mucous cell metaplasia in WT-OVA mice. STAT6 KO-OVA mice also showed significantly increased IL-13 protein expression in BAL fluid, compared with WT-OVA mice. We previously reported that the RSV infection of STAT1/STAT6 KO mice significantly increased IL-13 protein expression, compared to WT mice (19), demonstrating that STAT6 negatively regulates IL-13 protein expression in an *in vivo* model of airway inflammation.

Our results also showed that STAT6 signaling negatively regulated airway IL-17A expression in STAT6 OVA mice. IL-4 and IL-13 both signal through STAT6, and we previously demonstrated in murine and human differentiated Th17 cells that IL-13 inhibited IL-17A protein expression (22, 36). Further, we also reported that lung IL-17A protein expression and airway neutrophils were increased in STAT1/STAT6 DKO-RSV mice compared with WT-RSV, STAT1 KO-RSV, and STAT6 KO-RSV mice (19). IL-17A is known to cause increased neutrophil infiltration into the lung (37), and therefore our observed increase in neutrophil infiltration is likely attributable to increased IL-17A protein expression in the BAL fluid of STAT6 KO-OVA mice.

Therapeutics are currently being developed to target IL-13 or STAT6. For example, the therapeutic YM-341619 suppressed STAT-6 dependent gene expression. YM-341619 administered orally to rats during OVA-induced allergic inflammation decreased eosinophil recruitment to the lungs, and decreased airway reactivity (17). Antibodies that target IL-13, IL-4, or the IL-4R α chain of the IL-13R decreased the activation of STAT6, and are currently undergoing human clinical trials to reduce asthma

symptoms and mucus production (16, 29–33). Based on our findings, STAT6-targeted and IL-13-targeted therapeutics may not eliminate the symptoms associated with mucous cell metaplasia and mucus production in patients with asthma and increased IL-17A protein expression. Inhibiting IL-13 or STAT6 in patients may increase IL-17A protein expression and the neutrophil-driven symptoms of asthma, and may not reduce mucus production. Therefore, the careful immunologic phenotyping of individual

Figure 5. IL-17A evokes mucus production in differentiated tracheal epithelial cells from STAT6 KO mice. Primary murine tracheal epithelial cells (mTECs) were cultured at an air–liquid interface for 2 weeks. Cells were then stimulated with PBS, IL-13, or IL-17A basolaterally for 3 days. Transwell membranes containing cells were harvested and embedded in paraffin. (A) PAS-stained cross-sectional cuts of mTECs. (B) Percentage of PAS-positive cells in mTECs from three independent experiments. * $P < 0.05$, compared with PBS-treated mTECs for specific murine strain ($n = 4$ –6 for each group), according to ANOVA.

patients with asthma should precede the use of IL-13 and STAT6 therapeutics, to ensure therapeutic efficacy and to avoid unintended side effects.

Author disclosures are available with the text of this article at www.atsjournals.org.

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