

ASTHMA

Allergic asthma is distinguished by sensitivity of allergen-specific CD4⁺ T cells and airway structural cells to type 2 inflammation

Josalyn L. Cho,^{1,2*} Morris F. Ling,^{1*} David C. Adams,² Lucas Faustino,¹ Sabina A. Islam,¹ Roshi Afshar,¹ Jason W. Griffith,^{1,2} Robert S. Harris,² Aylwin Ng,^{3,4} Giorgia Radicioni,⁵ Amina A. Ford,⁵ Andre K. Han,^{1,2} Ramnik Xavier,^{3,4} William W. Kwok,⁶ Richard Boucher,⁵ James J. Moon,^{1,2} Daniel L. Hamilos,¹ Mehmet Kesimer,⁵ Melissa J. Suter,² Benjamin D. Medoff,^{1,2*} Andrew D. Luster^{1,*†}

2016 © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science.

Despite systemic sensitization, not all allergic individuals develop asthma symptoms upon airborne allergen exposure. Determination of the factors that lead to the asthma phenotype in allergic individuals could guide treatment and identify novel therapeutic targets. We used segmental allergen challenge of allergic asthmatics (AA) and allergic nonasthmatic controls (AC) to determine whether there are differences in the airway immune response or airway structural cells that could drive the development of asthma. Both groups developed prominent allergic airway inflammation in response to allergen. However, asthmatic subjects had markedly higher levels of innate type 2 receptors on allergen-specific CD4⁺ T cells recruited into the airway. There were also increased levels of type 2 cytokines, increased total mucin, and increased mucin MUC5AC in response to allergen in the airways of AA subjects. Furthermore, type 2 cytokine levels correlated with the mucin response in AA but not AC subjects, suggesting differences in the airway epithelial response to inflammation. Finally, AA subjects had increased airway smooth muscle mass at baseline measured in vivo using novel orientation-resolved optical coherence tomography. Our data demonstrate that the development of allergic asthma is dependent on the responsiveness of allergen-specific CD4⁺ T cells to innate type 2 mediators as well as increased sensitivity of airway epithelial cells and smooth muscle to type 2 inflammation.

INTRODUCTION

Asthma affects more than 300 million people worldwide, and most cases are allergic in origin (1, 2). The cardinal features of allergic asthma are eosinophilic airway inflammation, mucus hypersecretion, and airway hyperresponsiveness (AHR) (3). Experimental evidence suggests that allergen-specific T helper 2 (T_H2) cells and their cytokines orchestrate allergic airway inflammation, induce mucus production from airway epithelium, and promote AHR (3–6). Although airway structural cells, including the epithelium and smooth muscle, play an important role in asthma (7, 8), the link between type 2 inflammation and airway structural cell dysfunction is incompletely understood.

Despite systemic sensitization to airborne allergen, not all allergic individuals develop asthma upon allergen exposure. Many of these individuals develop asthma over time (2), suggesting that the pathogenic mechanisms leading to asthma may be incremental and potentially reversible. Thus, identification of differences in the airway response to allergen between allergic asthmatics (AA) and allergic nonasthmatic controls (AC) could provide fundamental insights into asthma pathogenesis and inform therapy. Here, we performed bronchoscopic

segmental allergen challenge (SAC) and characterized changes in airway inflammatory cells, mediators, and T cell subsets, including using class II tetramers, to define allergen-specific CD4⁺ T cells. In addition, we measured mucus and airway smooth muscle (ASM) in vivo using optical coherence tomography (OCT) to determine whether structural changes correlated with type 2 inflammation. Our data suggest that allergic asthma results from both the allergen-specific CD4⁺ T cell response and a greater sensitivity of airway structural cells to type 2 inflammation.

RESULTS

Subject characteristics

We enrolled adults with allergy to cat or dust mite to undergo bronchoscopy with SAC (9–11). AA (*n* = 36) had mild asthma and evidence of AHR as defined by a positive methacholine challenge or bronchodilator responsiveness. AC (*n* = 48) had no history of asthma, no lower respiratory tract symptoms in response to inhaled allergen, and no evidence of AHR. The baseline characteristics of the subjects in the two groups were well matched (table S1). Both AA and AC had normal pulmonary function, but AA had lower percent predicted forced expiratory volume in 1 s (FEV₁) and lower ratio of FEV₁ to forced vital capacity (FVC; FEV₁/FVC). The threshold level of allergen sensitivity was determined by skin prick test titration, and the lowest concentration of extract eliciting a positive skin prick test was used as the allergen concentration for SAC in AA and AC subjects. There were no differences in the number of AA and AC receiving house dust mite (*Dermatophagoides pteronyssinus*) or cat hair extract or in the median dose of allergen administered. For comparison, we also enrolled nonallergic healthy control (HC) subjects (*n* = 5).

¹Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA. ²Division of Pulmonary and Critical Care Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA. ³Center for Computational and Integrative Biology and Gastrointestinal Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA. ⁴Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA 02142, USA. ⁵Marsico Lung Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. ⁶Benaroya Research Institute at Virginia Mason, Seattle, WA 98101, USA.

*These authors contributed equally to this work.

†Corresponding author. Email: aluster@mgh.harvard.edu

Airway inflammation after SAC

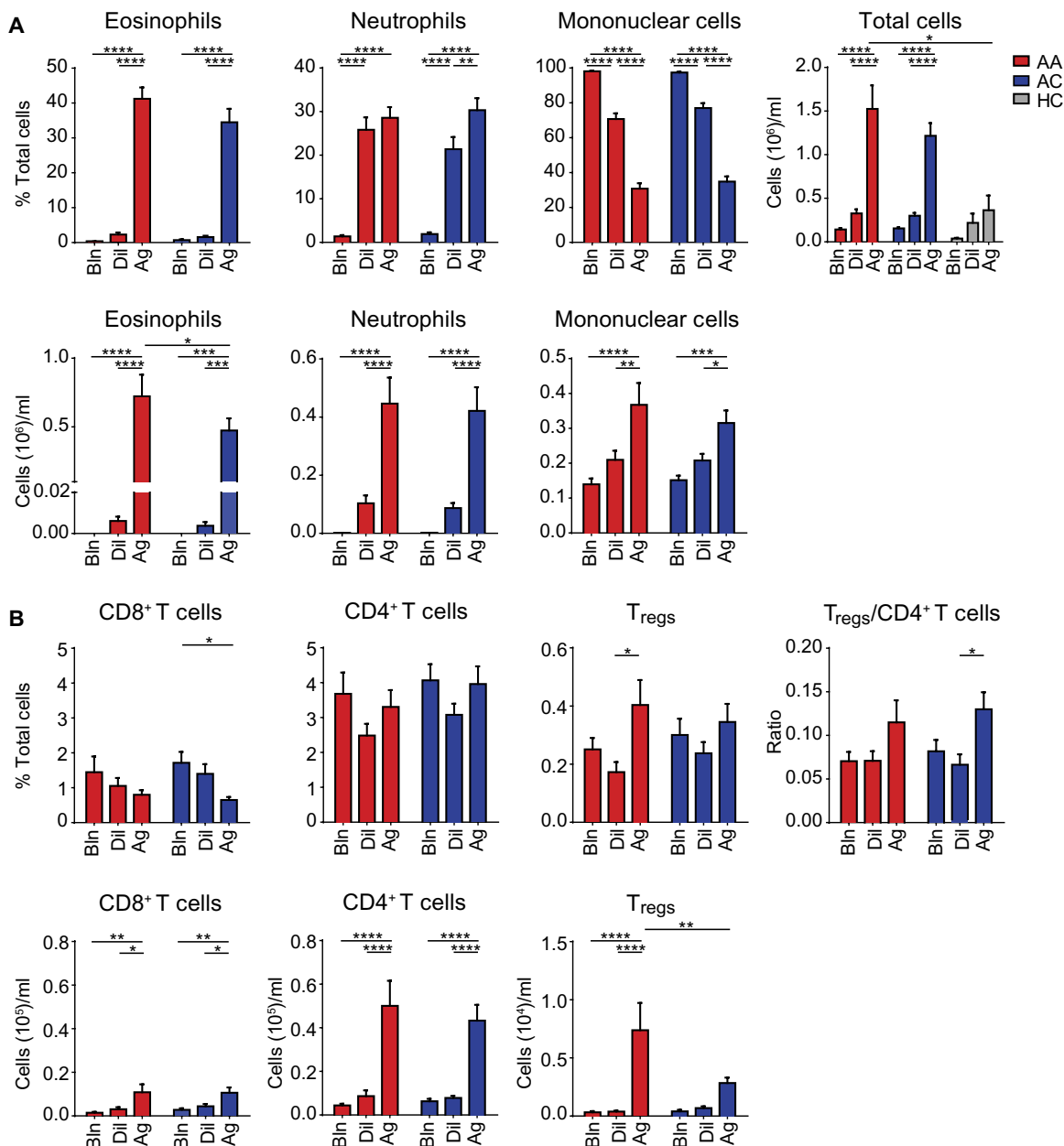
Bronchoscopic SAC was performed by obtaining a baseline bronchoalveolar lavage (BAL) followed by administration of diluent and allergen in two separate lung subsegments. Twenty-four hours later, BAL was performed in the diluent- and allergen-challenged airways. Cell differential counts in BAL from baseline and 24 hours after SAC revealed eosinophilic inflammation in only the allergen-challenged segment in both AA and AC (Fig. 1A). BAL cell counts and differentials did not differ at baseline or after diluent exposure between AA and AC. In response to allergen, the number of total cells, mononuclear cells, and eosinophils per milliliter of BAL fluid increased in both AA and AC. Although there was no significant difference in the percentage of eosinophils from the allergen-challenged segment between groups, AA had a higher eosinophil count (0.72×10^6 cells/ml AA versus 0.48×10^6 cells/ml AC;

$P < 0.05$). In contrast, HC did not exhibit a significant increase in total cells in either the diluent- or allergen-challenged segment (Fig. 1A).

T cell responses to allergen

The numbers and phenotype of proinflammatory allergen-specific T cells and anti-inflammatory regulatory T cells (T_{regs}) may determine whether allergen exposure results in asthma (9, 12). There were no differences in the number of $CD8^+$ T cells, $CD4^+$ T cells, or T_{regs} at baseline between groups (Fig. 1B). Although the percentage of $CD4^+$ T cells in the BAL did not change, the total number of $CD4^+$ T cells increased similarly in both AA and AC after allergen challenge. The number of T_{regs} in the BAL fluid after allergen challenge increased in AA and was higher compared to AC (0.74×10^4 cells/ml AA versus 0.28×10^4 cells/ml AC; $P < 0.01$). There was a small but nonsignificant increase in the number

Fig. 1. Airway cellular response after SAC. (A) Percentages and numbers of eosinophils, neutrophils, and mononuclear cells in BAL fluid at baseline and 24 hours after diluent or allergen exposure in AA (red) and AC (blue) subjects ($n = 36$ AA, $n = 48$ AC). Total cell counts are also shown [HCs shown in gray ($n = 4$)]. **(B)** Percentage and number of $CD8^+$ T cells and $CD4^+$ T cells in the BAL ($n = 31$ AA, $n = 44$ AC). Percentage and number of T_{regs} in the BAL and ratio of T_{regs} to total $CD4^+$ T cells ($n = 22$ AA, $n = 23$ AC). Bln, baseline; Dil, diluent; Ag, allergen. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by two-way analysis of variance (ANOVA).



of T_{regs} in AC after allergen challenge. There were no significant differences in the percentage of T_{regs} or in the ratio of T_{regs} to total $CD4^+$ T cells between AA and AC before or after allergen challenge (Fig. 1B).

T_{reg} phenotype in response to allergen

Although we observed higher numbers of T_{regs} in the airways of AA compared to AC, we reasoned that T_{regs} in AC may be more suppressive. T_{regs} can be subdivided into subpopulations that correlate with their suppressive capacity based on CD45RA and Foxp3 expression, with $CD45RA^{\text{low}}\text{Foxp3}^{\text{hi}}$ T_{regs} representing the most suppressive T_{regs} (Fig. 2A) (13). We therefore assessed the total number and distribution of T_{reg} subpopulations in the BAL before and after allergen challenge in a subgroup of AA ($n = 7$) and AC ($n = 10$). The number of suppressive T_{regs} ($CD45RA^{\text{low}}\text{Foxp3}^{\text{hi}}$, group II) and nonsuppressive T_{regs} ($CD45RA^{\text{high}}\text{Foxp3}^{\text{int}}$, group III) increased in response to allergen in both groups but only achieved statistical significance in AC. There were no significant differences between groups in either the percentage or number of T_{reg} subpopulations (Fig. 2B) or in the ratio of suppressive T_{regs} (groups

I and II) to T effector and memory cells (Te+m; group IV) (Fig. 2C). $CD4^+\text{CD}25^{\text{high}}$ T_{regs} were sorted from the allergen-challenged BAL, and genome-wide transcriptome analysis was performed (fig. S1). There was a trend toward higher levels of interleukin-9 (IL-9) ($P = 0.05$), CTLA4, and IKZF2 (HELIOS) expression in T_{regs} sorted from AA after allergen challenge (Fig. 2D) that did not reach statistical significance after correction for multiple comparisons.

Characterization of allergen-specific $CD4^+$ T cells

Although the number of $CD4^+$ T cells was not different between AA and AC at baseline or after SAC, we hypothesized that there could be differences in allergen-specific $CD4^+$ T cells. We used dust mite (Der p 1) and cat (Fel d 1) peptide-specific major histocompatibility complex (MHC) class II tetramers to identify allergen-specific $CD4^+$ T cells (Fig. 3A, fig. S2, and table S2). There were very few detectable allergen-specific $CD4^+$ T cells in the airways at baseline and after diluent exposure (Fig. 3B). However, allergen-specific $CD4^+$ T cells markedly increased in the airways of both AA and AC after

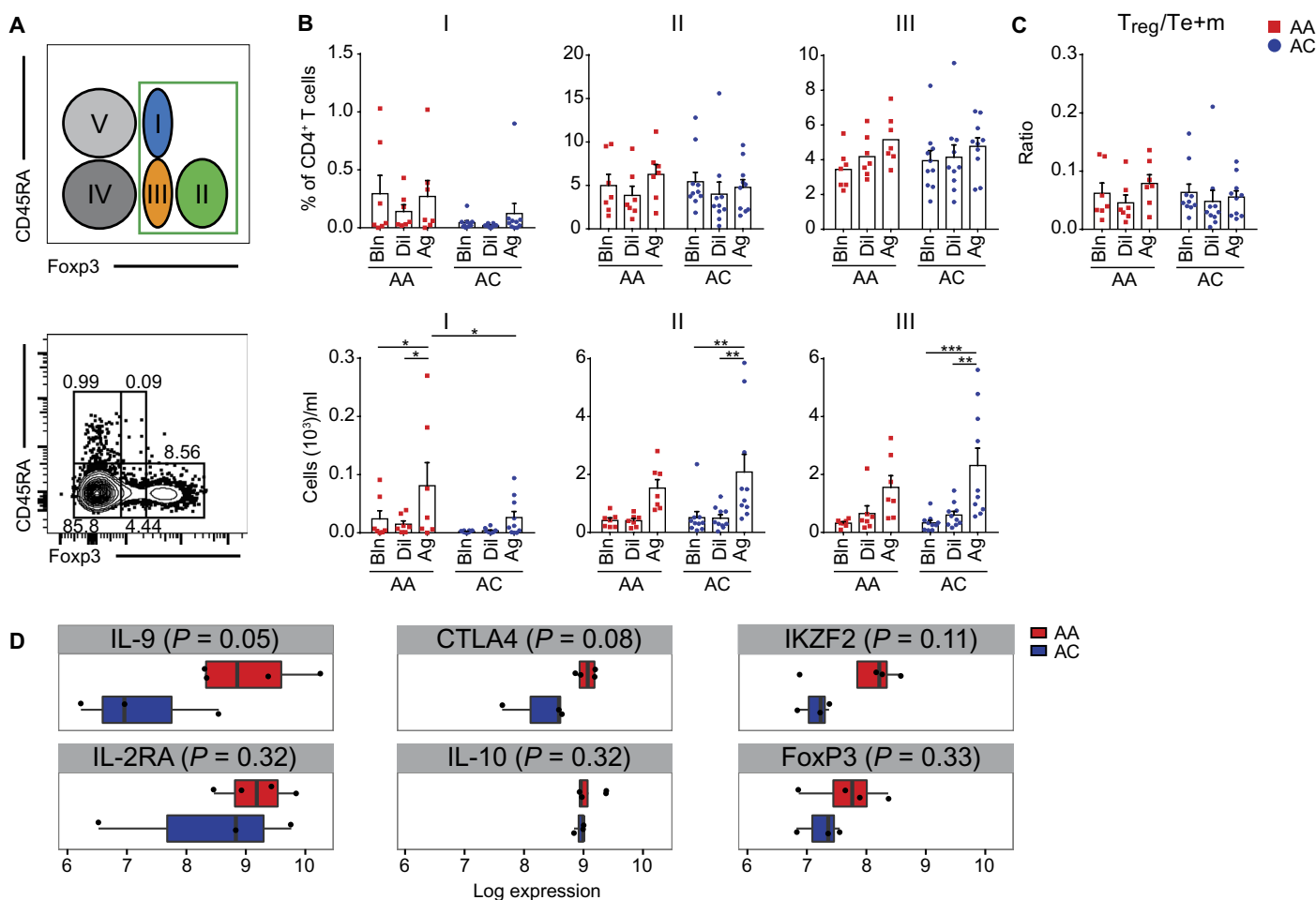


Fig. 2. Phenotyping of T_{regs} . (A) Functional T_{reg} subpopulations (green box) and a representative flow plot. I, naïve T_{regs} ; II, suppressive T_{regs} ; III, nonsuppressive T_{regs} ; IV, effector and memory $CD4^+$ T cells (Te+m); V, naïve $CD4^+$ T cells. (B) Percentages and numbers of T_{reg} subpopulations in AA (red squares) and AC (blue circles) subjects ($n = 7$ AA, $n = 10$ AC). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by two-way ANOVA. (C) Ratio of suppressive T_{regs} (I and II) to Te+m cells (IV) ($n = 7$ AA, $n = 10$ AC). (D) Expression levels of six genes relevant for T_{reg} function from a genome-wide transcriptome microarray analysis performed on sorted cells obtained after SAC from AA (red bars) and AC (blue bars) subjects. Data are displayed as medians (bar), interquartile range (box; 25th to 75th percentiles), and minimum and maximum values (whiskers). Nominal P values were determined using linear models for microarray.

allergen challenge (Fig. 3B). Surprisingly, there were no significant differences between AA and AC in the percentage or number of allergen-specific CD4⁺ T cells in the BAL under any condition examined. However, allergen-specific CD4⁺ T cells from AA had higher expression of the type 2-associated innate immune receptors IL-33R (ST2) (33.6% AA versus 15.28% AC; $P < 0.05$) and CRTH2 [prostaglandin D₂ (PGD₂) receptor] (26.8% AA versus 3.24% AC; $P < 0.01$) after allergen challenge (Fig. 3C). Moreover, the expression of IL-33R, CRTH2, and IL-25R was enriched on allergen-specific CD4⁺ T cells compared to bulk CD4⁺ T cells after allergen challenge only in AA ($P < 0.01$, $P < 0.0001$, and $P < 0.01$, respectively) (Fig. 3D). No significant differences in the expression of the type 2 chemokine receptor CCR4 were observed in either allergen-specific CD4⁺ T cells compared to bulk CD4⁺ T cells or between AA and AC.

Cytokine profile after allergen challenge

Inflammatory cytokines play an integral role in the pathogenesis of asthma (3, 14, 15). We therefore measured BAL levels of 44 cytokines and chemokines, many of which have been implicated in asthma pathogenesis (3, 14, 15). At baseline, AA had slightly higher absolute levels of IL-21, IL-33, CXCL1, and CCL21 (fig. S3). In response to allergen, absolute levels of the type 2 cytokines IL-4, IL-5, IL-9, IL-13, CCL11, and CCL26, as well as the neutrophil-associated cytokines IL-1 α , IL-1 β , CXCL1, and CXCL8, were increased in AA compared to AC (Fig. 4A and fig. S3). Interferon- γ , IL-12p40, and IL-17A were increased after allergen challenge in both AA and AC and were not different between groups (fig. S3). Given the importance of cytokines in both initiating and propagating allergic airway inflammation (3, 14, 15), we examined the correlation of cytokine levels to cellular inflammation. Levels of IL-4, IL-5, IL-9, IL-13, and CCL26 correlated to the number of eosinophils in the allergen-challenged segment in both groups (Fig. 4B) and with IL-33R expression on allergen-specific CD4⁺ T cells in both groups (Fig. 4C).

We also compared the increase in cytokines after allergen challenge relative to baseline and diluent levels. The increase in IL-1 β , IL-4, IL-5, IL-9, IL-13, IL-10, CCL11, and CCL21 in the allergen-challenged segment relative to baseline was higher in AA versus AC ($P < 0.05$) (fig. S4A). Only the increase in IL-4, IL-9, IL-13, and IL-10 in the allergen-challenged segment relative to diluent was greater in AA ($P < 0.05$) (fig. S4B).

Characterization of the mucin response to allergen

Increased production of mucus in the airway is a phenotypic hallmark of asthma (3). Mucins are the gel-forming components of airway mucus (16), and therefore, we used refractometry (17) to measure total mucin in BAL fluid from AA, AC, and HC before and after SAC. Baseline levels of mucin were similar in all three groups. Both AA and AC had a significant increase in mucin after allergen challenge (7.1 μ g/ml baseline versus 52.5 μ g/ml after allergen for AA, $P < 0.0001$, and 7.0 μ g/ml baseline versus 27.4 μ g/ml after allergen for AC, $P < 0.01$; Fig. 5A). However, AA had significantly higher levels of mucin after allergen challenge compared to both AC and HC (52.5 μ g/ml AA versus 27.4 μ g/ml AC versus 6.5 μ g/ml HC; $P < 0.01$ and $P < 0.001$, respectively). Airway mucus composition has been shown to be altered in asthmatics with increased levels of the gel-forming glycoprotein mucin MUC5AC (16, 18, 19). We therefore measured MUC5AC at baseline and after allergen challenge with a mass spectrometry (MS)-based proteomics platform. There were no differences between groups in MUC5AC levels at baseline. However, MUC5AC levels increased in AA BAL after allergen challenge and were significantly higher compared to AC {normalized spectral intensity [SI(N)] 5.6×10^9 versus 1.1×10^8 , $P < 0.05$ } (Fig. 5B). We also performed *in vivo* OCT to examine whether there are differences in mucus quality between AA and AC. The average OCT signal intensity (*p*OCT) of mucus within the airway lumen was determined as a surrogate of particulate density (Fig. 5C) (20–22).

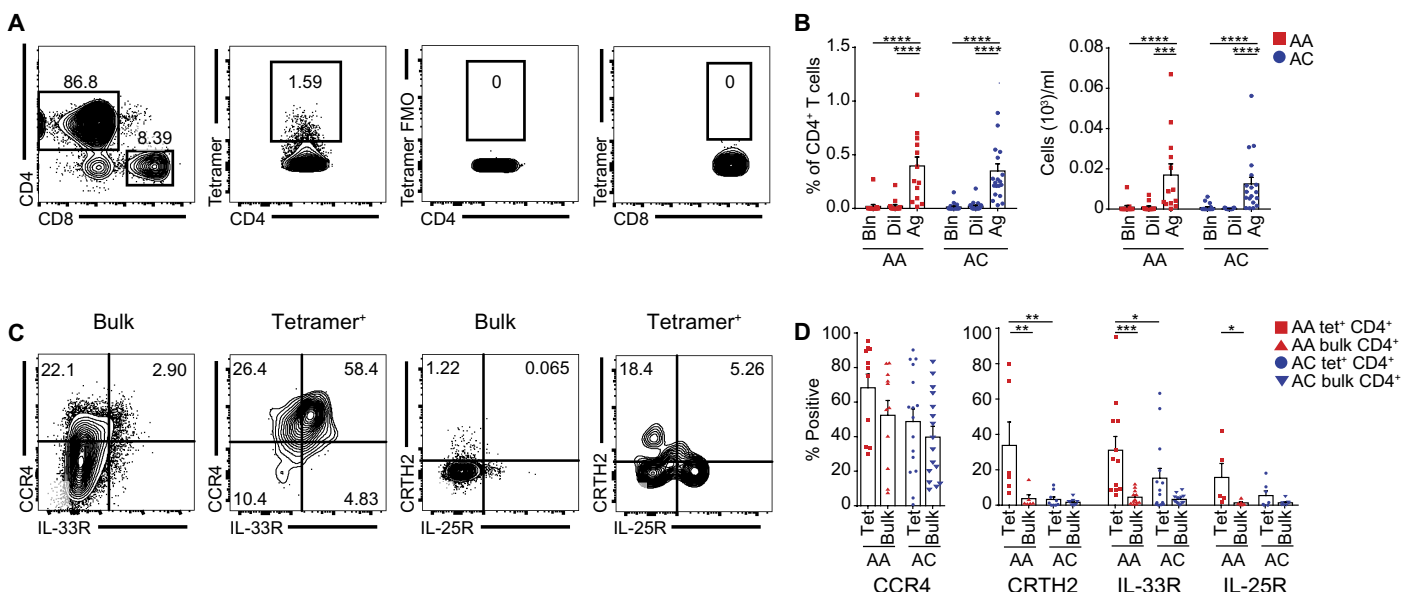


Fig. 3. Phenotyping of allergen-specific CD4⁺ T cells. (A) Representative gating for tetramer⁺CD4⁺ T cells. FMO, fluorescence minus one. (B) Percentage and number of tetramer⁺CD4⁺ T cells in the BAL for AA (red squares) and AC (blue circles) subjects ($n = 13$ AA, $n = 19$ AC). (C) Representative gating for TH2 markers on bulk and tetramer⁺CD4⁺ T cells from an AA subject. (D) Percentage of tetramer⁺CD4⁺ (AA, red squares; AC, blue circles) or bulk CD4⁺ (AA, red triangles; AC, blue triangles) T cells expressing the TH2 markers CCR4 ($n = 11$ AA, $n = 16$ AC), CRTH2 ($n = 6$ AA, $n = 9$ AC), IL-33R ($n = 12$ AA, $n = 14$ AC), and IL-25R ($n = 5$ AA, $n = 7$ AC). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by two-way ANOVA.

Mucus *p*OCT intensity correlated to total mucin (Fig. 5D), and at baseline, AA, AC, and HC had similar *p*OCT intensity (Fig. 5E). However, *p*OCT intensity increased after allergen challenge compared to baseline and diluent only in AA and was greater than *p*OCT intensity in AC and HC (81.6 AA versus 68.1 AC versus 61.2 HC; $P < 0.05$ for both) (Fig. 5E). Furthermore, *p*OCT intensity correlated with the levels of IL-4 ($R^2 = 0.3802$; $P < 0.05$) and IL-13 ($R^2 = 0.4656$; $P < 0.05$) only in AA (Fig. 5F).

Characterization of ASM

Increased ASM mass is an important feature of airway remodeling in moderate to severe asthma, and it has been suggested that ASM mass increases in the setting of type 2 inflammation (23). However, whether ASM mass increases in mild asthmatics or allergic nonasthmatics is unknown. We developed a novel technique to measure ASM in vivo using orientation-resolved OCT (OR-OCT) (24). To determine whether differences in ASM mass distinguish AA from AC, we performed OR-OCT at baseline and measured ASM thickness and band width (Fig. 6A). AA subjects had significantly increased ASM thickness (70.3 μ m AA versus 35.4 μ m AC versus 35.9 μ m HC; $P < 0.05$ for both) and ASM band width compared to AC and HC (380.3 μ m AA versus 327.3 μ m AC versus 331.7 μ m HC; $P < 0.01$ and $P < 0.05$, respectively) (Fig. 6B). There were no significant differences in ASM thickness or band width between AC and HC.

DISCUSSION

Here, we identified a number of novel differences in the airway immune response to allergen and in airway structural cells between allergic individuals with and without asthma. AA had slightly higher levels of eosinophils and type 2 cytokines in the airways in response to allergen and a marked increase in expression of the innate type 2 receptors IL-33R and CRTH2 on allergen-specific CD4⁺ T cells compared to AC. In addition, AA had higher levels of mucin and qualitatively different mucus after allergen challenge. Finally, we measured ASM in vivo using OR-OCT and demonstrated that AA had increased ASM mass compared to AC.

Previous studies have documented that allergic nonasthmatics develop prominent type 2 airway inflammation in response to allergens despite a lack of asthma symptoms (25–33). However, these studies were limited by a small number of subjects, different methods for allergen challenge, and circumscribed analyses of the immune response. In our comparatively large cohort, we found a greater type 2 inflammatory response to allergen in AA compared to AC, characterized by an increase in airway eosinophils and type 2 cytokines.

We extensively characterized the T_{reg} response to allergen and found that AA had greater accumulation of T_{reg} in the airways after SAC. We found no differences in the numbers or percentage of suppressive T_{reg}s between groups. Of interest, gene expression analysis suggested that AA T_{reg}s expressed more IL-9 compared to AC T_{reg}s. Although these studies

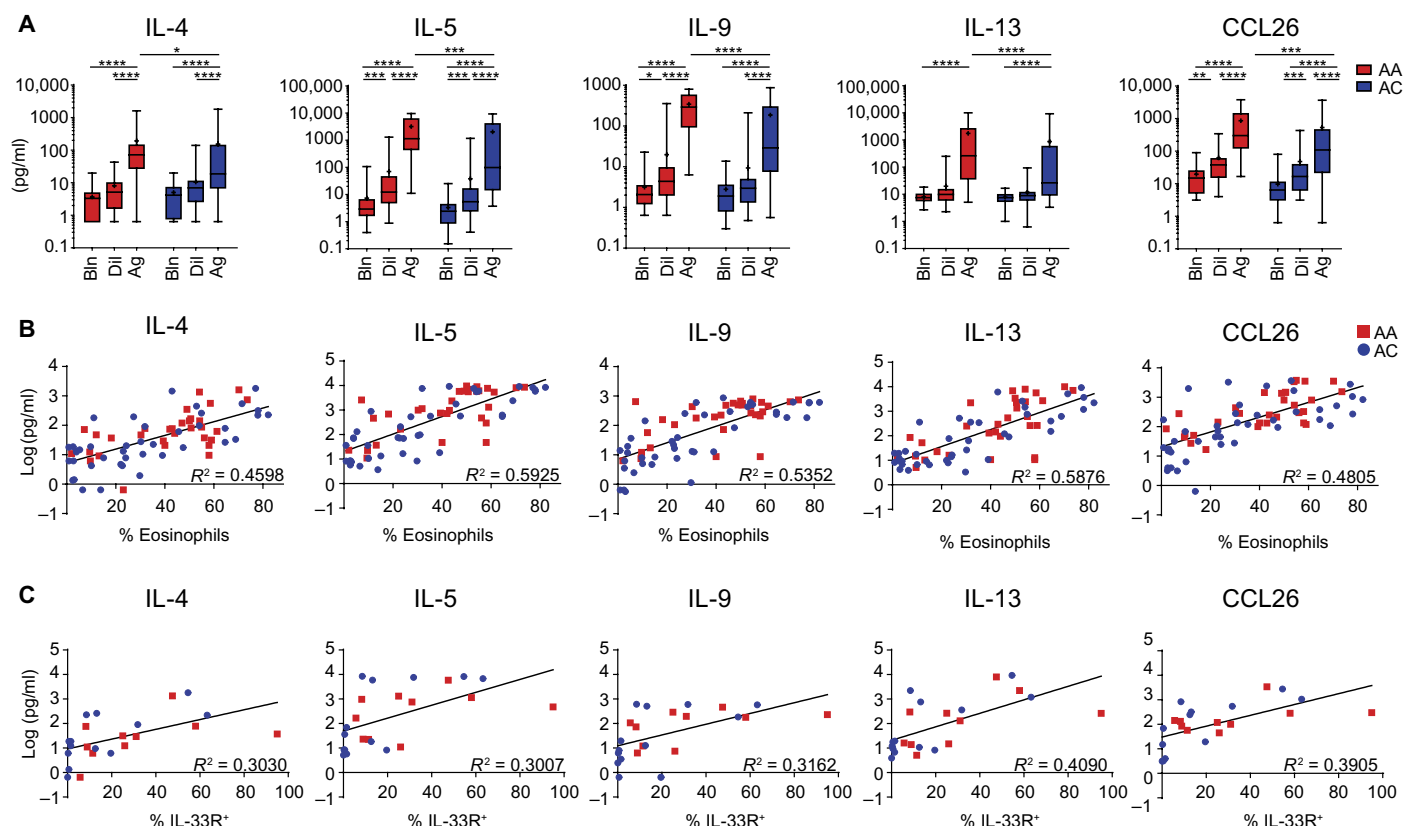


Fig. 4. T_H2 cytokine levels and correlations. (A) Protein levels of IL-4, IL-5, IL-9, IL-13, and CCL26 in BAL fluid ($n = 32$ AA, $n = 41$ AC). Data are presented as median (bar), interquartile range (box; 25th to 75th percentiles), and minimum and maximum values (whiskers); cross denotes the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by two-way ANOVA. (B) Correlation between IL-4, IL-5, IL-9, IL-13, and CCL26 levels in BAL fluid and the percentage of eosinophils in the allergen-challenged segment ($n = 32$ AA, $n = 41$ AC). Pearson's correlation, $P < 0.0001$ for all. Red squares, AA; blue circles, AC. (C) Correlation between IL-4, IL-5, IL-9, IL-13, and CCL26 and percent IL-33R⁺ allergen-specific CD4⁺ T cells in the allergen-challenged segment ($n = 10$ AA, $n = 13$ AC). Pearson's correlation, $P < 0.001$ for IL-13, $P < 0.01$ for all others.

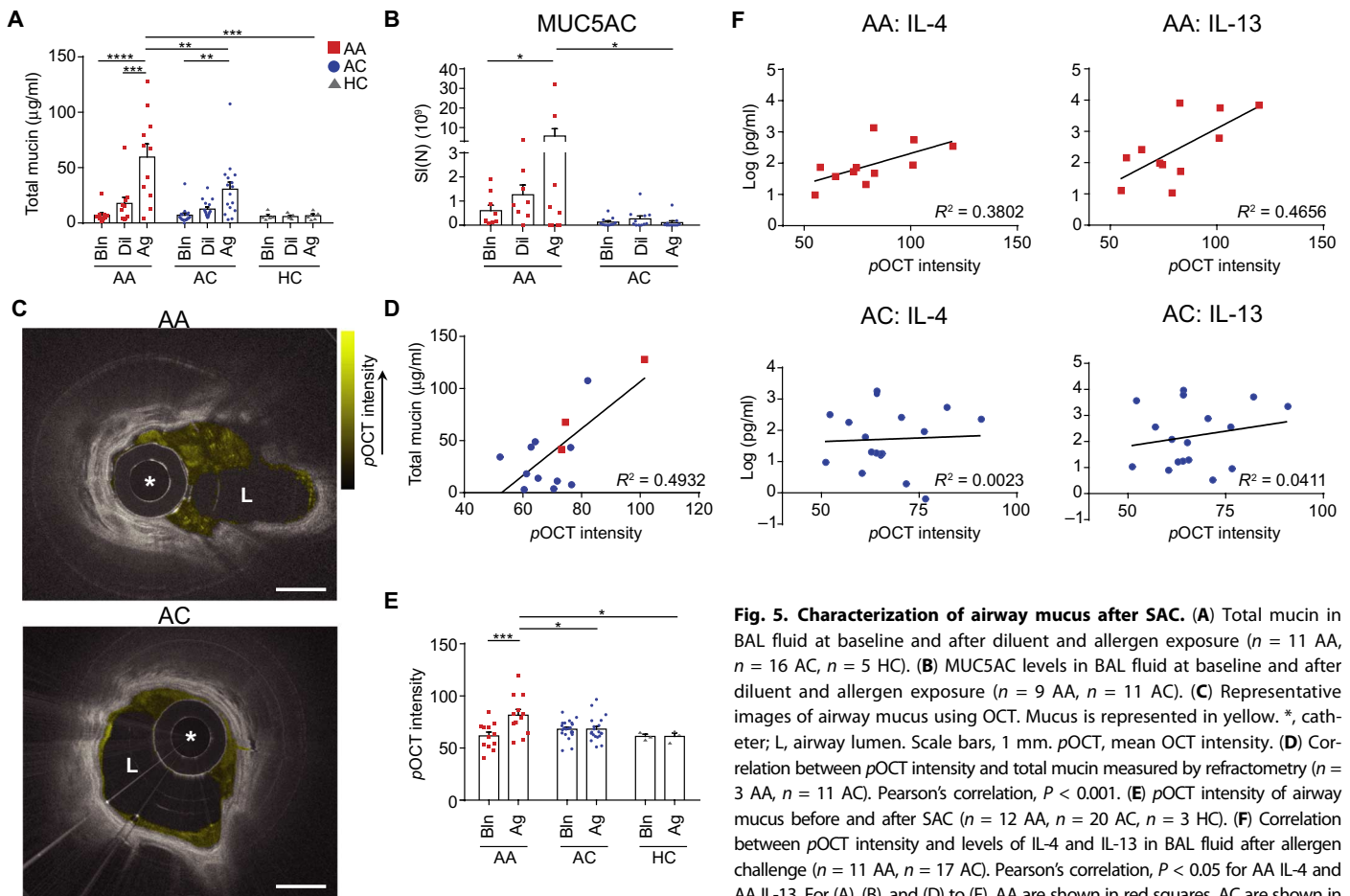


Fig. 5. Characterization of airway mucus after SAC. (A) Total mucin in BAL fluid at baseline and after diluent and allergen exposure ($n = 11$ AA, $n = 16$ AC, $n = 5$ HC). (B) MUC5AC levels in BAL fluid at baseline and after diluent and allergen exposure ($n = 9$ AA, $n = 11$ AC). (C) Representative images of airway mucus using OCT. Mucus is represented in yellow. *, catheter; L, airway lumen. Scale bars, 1 mm. pOCT, mean OCT intensity. (D) Correlation between pOCT intensity and total mucin measured by refractometry ($n = 3$ AA, $n = 11$ AC). Pearson's correlation, $P < 0.001$. (E) pOCT intensity of airway mucus before and after SAC ($n = 12$ AA, $n = 20$ AC, $n = 3$ HC). (F) Correlation between pOCT intensity and levels of IL-4 and IL-13 in BAL fluid after allergen challenge ($n = 11$ AA, $n = 17$ AC). Pearson's correlation, $P < 0.05$ for AA IL-4 and AA IL-13. For (A), (B), and (D) to (F), AA are shown in red squares, AC are shown in blue circles, and HC are shown in gray triangles. For (A), (B), and (E), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, by two-way ANOVA.

may have been underpowered and require follow-up, these data suggest that AA may have more pathogenic "T_H2-type" T_{regs}, which have been described in a mouse model to express T_H2-type cytokines and contribute to allergic disease (34).

One of the novel aspects of our analysis is the use of peptide-MHC class II tetramers to define the allergen-specific CD4⁺ T cell population recruited into the airways in response to allergen (35, 36). Using this technology, we found a marked increase in the numbers of allergen-specific CD4⁺ T cells in the BAL 24 hours after allergen challenge in both groups, suggesting recruitment of these cells into the airway.

More detailed phenotyping of T cells demonstrated enrichment of CRTH2, IL-25R, and IL-33R expression in allergen-specific CD4⁺ T cells compared to bulk CD4⁺ T cells only in AA. Allergen-specific CD4⁺ T cells from AA expressed higher levels of IL-33R and CRTH2 when compared to AC. Because IL-33R and CRTH2 are enriched on T_H2-polarized cells (37), our data suggest that AA subjects accumulate more T_H2-polarized allergen-specific effector cells in the airways after allergen challenge. It is interesting to note that the levels of IL-33 in the airway at baseline were higher in AA compared to AC, suggesting that AA may be primed for more robust type 2 inflammation in response to allergen in the lung. Higher expression of IL-33R and CRTH2 on allergen-specific CD4⁺ T cells may also enhance responsiveness to type 2 signals from innate immune cells, such as PGD₂ from mast cells and IL-33 from

epithelial cells in the lung. In addition, it has recently been shown that IL-33 drives IL-13 expression from T_H2 cells independently of the T cell receptor, and thus, IL-33R⁺ CD4⁺ T cells may be an important source of IL-13 in allergic asthma (38). Furthermore, AA have been shown to have higher levels of PGD₂ in the airway compared to AC in response to allergen challenge (32). Thus, our data suggest that allergen-specific CD4⁺ T cells drive the development of type 2 inflammation in allergic individuals. Furthermore, differences in the sensitivity of allergen-specific CD4⁺ T cells to innate type 2 signals may explain the increase in eosinophils, cytokines, and mucin in the airways of AA compared to AC.

It is important to note, however, that there was considerable overlap between groups in measures of inflammation. Moreover, cytokine levels correlated with eosinophils and IL-33R expression on allergen-specific CD4⁺ T cells in both AA and AC. This suggests that there is a continuum of allergic inflammation in both groups rather than a critical threshold of allergic inflammation that determines the asthma phenotype. Thus, it seems unlikely that the magnitude of the type 2 response by itself can fully explain the marked phenotypic differences between AA and AC with respect to asthma symptoms and AHR.

Perhaps, the most marked differences we observed were in the response of airway epithelium to allergen and in ASM. Airway epithelial cells alter both the production and composition of mucus in response to IL-4 and IL-13 (16, 18), and mucus hypersecretion is an important

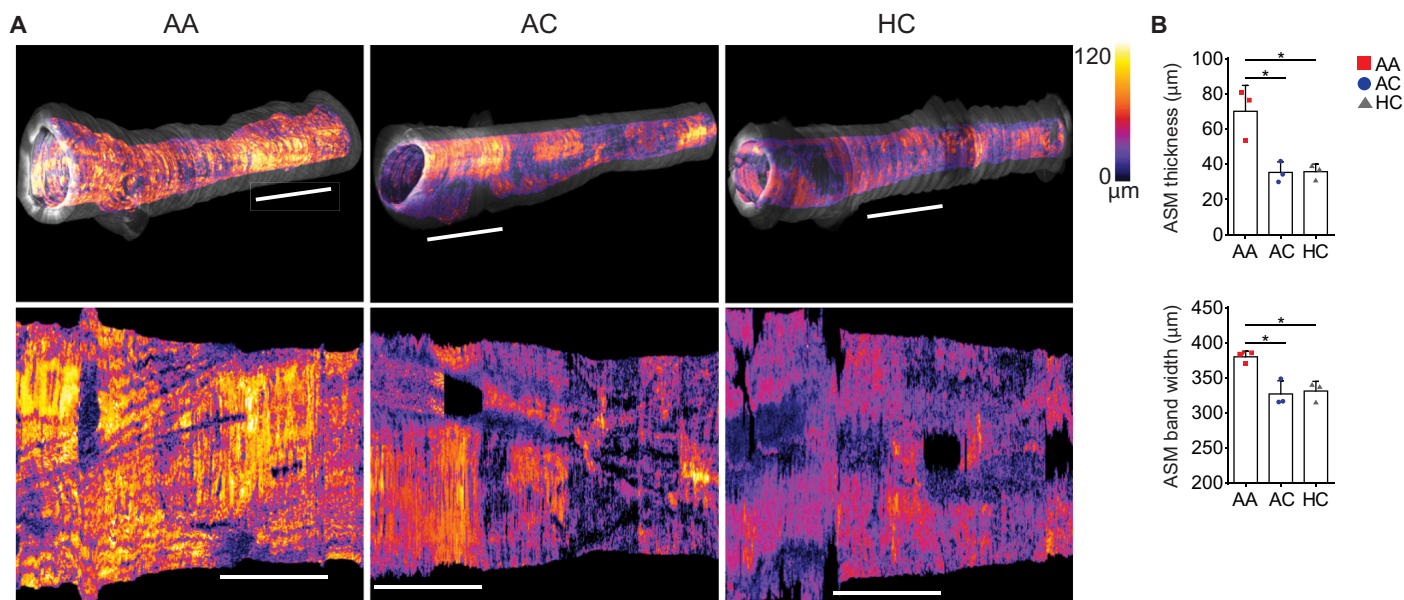


Fig. 6. Characterization of ASM. ASM measurements were obtained using OR-OCT. (A) Top panels are volumetric reconstructions from circularized data, with ASM represented in terms of thickness (color) overlaid on airway structure (grayscale). Bottom panels are the same ASM data unrolled circumferentially and “flattened” for ASM quantification. Scale bars are corresponding 6 mm regions of comparable airway perimeter from each data set, where the ASM distribution was quantified. (B) Quantification of ASM thickness and band width ($n = 3$ AA, $n = 3$ AC, $n = 3$ HC). Red squares, AA; blue circles, AC; gray triangles, HC. * $P < 0.05$ by two-way ANOVA.

determinant of symptoms in asthma (16). However, the mucus response to SAC in AA and AC has not been previously studied. Using multiple modalities, including refractometry, MS, and a novel OCT-based method to measure mucus in vivo, we demonstrated both quantitative and qualitative differences in mucus between AA and AC. Although AC increased mucus production in response to allergen, it was significantly less than AA and not different than HC. Mucus particulate density as determined by OCT (p OCT) was increased after allergen challenge and correlated with IL-4 and IL-13 levels only in AA. Furthermore, only AA had a significant increase in MUC5AC levels in the BAL after allergen challenge. MUC5AC is induced in epithelial cells by type 2 cytokines, and its levels are elevated in human asthma (16, 39–41). MUC5AC has also recently been shown to be required for AHR in mice (42). These results suggest that the airway epithelium in AA is more responsive to type 2 inflammation than in AC and contributes to the development of the asthma phenotype.

It is also thought that ASM contributes to AHR in asthma (5, 23). Previously, it has not been possible to measure ASM in vivo, and studies of ASM mass have relied on histological measurements. We have developed a novel OR-OCT technology (24) that allows for in vivo assessment of ASM. Surprisingly, we found increased ASM thickness and band width in AA compared to AC, although our AA cohort had mild asthma and normal pulmonary function. Differences in ASM measurements were observed despite significant overlap in the levels of type 2 inflammation observed in response to allergen in both groups and enrollment of subjects with only mild asthma. Our data could suggest that individuals with increased ASM are predisposed to the development of asthma in the setting of type 2 inflammation. Alternatively, ASM in AA may have increased sensitivity to type 2 inflammatory or other epithelial cell-derived signals that result in AHR and ASM hyperplasia and/or hypertrophy. Further studies are needed to define the mechanistic links between type 2 inflammation and changes in ASM mass and function in AA subjects.

There are several important limitations to our study that should be considered. SAC provides an intense and isolated allergen challenge that does not fully recapitulate aeroallergen exposure to the entire lung, and BAL only samples the airspaces, so cell types and mediators within lung tissue may not be well represented in our analyses. Furthermore, we only studied subjects 24 hours after challenge using just one dose of allergen based on the individual subject’s cutaneous “threshold” reaction. Thus, it is possible that we missed important differences between these groups in the initiation or resolution of inflammation that would be evident with different doses or at different time points. Finally, we only enrolled subjects with mild asthma. Previous studies have demonstrated that the response to SAC is not different between mild and moderate asthmatics (43), but the response in severe asthmatics may be different (44, 45).

Our results suggest that type 2 airway inflammation is necessary but not sufficient for the allergic asthma phenotype and that increased sensitivity of airway structural cells to type 2 inflammation is also required. Whether structural cells in asthmatics are intrinsically more sensitive to type 2 inflammation or develop altered responses in the setting of chronic inflammation remains to be determined. Future investigations into mechanisms that determine responsiveness of airway structural cells to allergen and type 2 inflammation may reveal important new therapeutic targets for asthma that can alter the course of this chronic disease.

MATERIALS AND METHODS

Study design

The primary aim of this study was to compare the immune and airway response to SAC in aeroallergen allergic subjects with asthma (AA) and without asthma (AC). A group of HCs was also included. Participants were recruited via advertisement in the Massachusetts General Hospital outpatient clinics and around the Boston metropolitan area. Interested volunteers were screened for eligibility with a full medical

history, baseline spirometry and methacholine challenge, and allergen skin testing. To determine eligibility for tetramer studies, human leukocyte antigen subtyping was performed on peripheral blood samples by the American Red Cross reference laboratories. Detailed inclusion and exclusion criteria as well as the study protocol are provided in the Supplementary Materials. Subjects gave their written informed voluntary consent before testing and sample collection. Eligible subjects underwent SAC with OCT imaging and collection of BAL fluid before and after allergen challenge for analysis. The study was approved by the Partners HealthCare Institutional Review Board.

Allergen skin testing

Standardized allergen extract for cat hair and *D. pteronyssinus* were purchased from Greer Laboratories. AA and AC had a positive skin prick test to either cat hair or dust mite extract, whereas HC had negative skin tests to allergens. The threshold level of allergen sensitivity was determined by skin prick test titration using serial threefold dilutions of extract (46). The lowest concentration of extract eliciting a positive skin prick test (3-mm wheal diameter) was used as the allergen concentration for SAC in AA and AC subjects; for HC, cat hair extract (500 allergy units/ml) was administered.

Segmental allergen challenge

SAC was performed as previously described (9–11). Briefly, a prechallenge BAL was obtained from the lingula using 4 × 30-milliliter (ml) aliquots of normal saline. Diluent (2 ml) was then administered to the anterior segment of the right upper lobe followed by administration of allergen (2 ml) to the lateral segment of the right middle lobe. Twenty-four hours later, BAL samples were obtained from the diluent- and allergen-challenged lung segments.

Cytospins

Cell differential counts for BAL were determined by enumerating mononuclear cells, neutrophils, and eosinophils on cytocentrifuge preparations (9, 10).

Flow cytometry

BAL cells were stained with fluorescent-conjugated antibodies against CD3, CD4, CD8, CD25, CD45RA, CCR4, CRTH2, IL-33R, and IL-25R (BioLegend). Some cells were stained for Foxp3 (eBioscience). Phycoerythrin (PE)-conjugated MHC class II tetramers containing either house dust mite (Der p 1) or cat hair (Fel d 1) peptides (table S2) were prepared in our laboratory as previously described (35, 47, 48). Baseline and diluent samples underwent enrichment for tetramer-positive cells with anti-PE microbeads and magnetic column selection (Miltenyi Biotec) as previously described (35, 47, 48). Samples were run on an LSRFortessa flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star).

Cytokine and chemokine analysis

Cytokines and chemokines were analyzed by Milliplex MAP kit (EMD Millipore) using 30-fold concentrated BAL supernatant. BAL was concentrated in Amicon Ultra-15 centrifugal filter units (EMD Millipore) and used in the kit according to the manufacturer's instructions. Cytokines and chemokines were measured on a Luminex 200 and analyzed using xPONENT 3.1 software (Luminex Corp.).

Transcriptome analysis

BAL cells from the allergen-challenged segment were enriched for CD4⁺ T cells by negative selection using magnetic beads (Stemcell Technolo-

gies). Cells were stained with CD4 and CD25, and CD4⁺CD25^{high} T_{regs} were sorted to >97% purity with FACSARIA II (BD Biosciences). RNA was purified with the RNeasy Plus Mini Kit (Qiagen). Global gene expression was profiled using a HumanHT-12 v4 Expression BeadChip according to the manufacturer's instructions (Illumina). Image analysis, bead-level processing, and quantile normalization of array data were performed using the Illumina LIMS platform, BeadStudio. A gene-wise linear model was fitted to the data and was performed in R programming language (www.r-project.org/), using functions from Linear Models for Microarray Data (49). For each probe, a moderated *t* statistic (with SEs moderated across genes) was computed using a Bayesian model. For each transcript, log-transformed ratios were computed for the allergen segment relative to the baseline or diluent segment.

Mucin measurement

Total mucin concentration was measured in BAL samples using differential refractometry (T-rEX, Wyatt Technology) coupled with size exclusion chromatography as described previously (17).

MUC5AC assessment

BAL samples were prepared for analysis as previously described (50). Briefly, 250-μl BAL samples were denatured, reduced, and alkylated. Samples were then buffer-exchanged into a digestion buffer (pH 8.0) (50 mM ammonium bicarbonate) using a HiTrap Desalting column (GE Healthcare). Samples were digested with trypsin at 37°C overnight. The final digest was vacuum-dried to remove bicarbonate salts. The digest peptides were resolubilized in 20 μl of 0.1% formic acid water or were stored –30°C until liquid chromatography–MS/MS (LC-MS/MS) was run. The LC-MS/MS was performed with a Dionex UltiMate 3000 RSLCnano system coupled to a Hybrid Quadrupole-Orbitrap mass spectrometer with a Nanospray source (Q Exactive, Thermo Fisher Scientific). Peptides were analyzed by a data-dependent top 10 method dynamically choosing the most abundant precursor ions from the survey scan (300 to 1650 Th) for higher-energy collisional dissociation fragmentation. Proteins identified from the BAL were quantified using a label-free method termed the normalized spectral intensity [SI(N)]. SI(N) is defined as the cumulative fragment ion intensities for all spectra counted for a protein (51).

OCT mucus assessment

Mucus was automatically detected using custom image segmentation algorithms. The suite of algorithms used a two-step identification process. In the first step, the sheath and tissue lumen were identified. The second step looked for mucus in the region between sheath and tissue lumen using a combination of thresholding and intelligent exclusion of image artifact. All the data were checked for accuracy, and frames in which the mucus content was ambiguous were excluded. Mucus particulate content was assumed to be proportional to the scattering intensity of the mucus (20), and the average quantity of particulate content per data set was equated to the mean pixel intensity for all mucus-identified pixels.

ASM measurement

ASM band width and thickness were assessed using OR-OCT as described (24). Regions 6 mm in length and of comparable radius (±0.1 mm) were analyzed for both ASM thickness and band width. To compare the measured ASM thickness between data sets, the measured ASM area was normalized to the local basement membrane perimeter.

Statistical analysis

Data are means $\pm$ SEM, unless otherwise noted. χ^2 test or Fisher's exact test was used to evaluate categorical values, and the nonparametric *t* test was used for continuous data. Median allergen doses were not normally distributed, and thus, comparison was performed with the Mann-Whitney test. Two-way ANOVA was used to compare mean differences between groups. Tukey's or Sidak's test was used to correct for multiple comparisons. Because the distribution was skewed, cytokine data were log-transformed for analysis. Pearson's correlation coefficients were used for assessing the strength of association between pairs of predefined variables. In all cases, differences in results were considered to be statistically significant when *P* was less than 0.05 based on a two-sided test.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/8/359/359ra132/DC1

Methods

Fig. S1. Gene expression in conventional CD4⁺ T cells and T_{regs}.

Fig. S2. Sample gating for peptide-MHC class II tetramers.

Fig. S3. Additional cytokine and chemokine analysis.

Fig. S4. Cytokine response to allergen.

Table S1. Characteristics of the subjects at baseline.

Table S2. Summary of peptide-MHC class II tetramers.

REFERENCES AND NOTES

1. T. J. Craig, T. S. King, R. F. Lemanske Jr., M. E. Wechsler, N. Icitovic, R. R. Zimmerman Jr., S. Wasserman; Blood Institute's Asthma Clinical Research Network, Aeroallergen sensitization correlates with PC₂₀ and exhaled nitric oxide in subjects with mild-to-moderate asthma. *J. Allergy Clin. Immunol.* **121**, 671–677 (2008).
2. R. Shaaban, M. Zureik, D. Soussan, J. M. Antó, J. Heinrich, C. Janson, N. Kunzli, J. Sunyer, M. Wjst, P. G. Burney, F. Neukirch, B. Leynaert, Allergic rhinitis and onset of bronchial hyperresponsiveness: A population-based study. *Am. J. Respir. Crit. Care Med.* **176**, 659–666 (2007).
3. R. M. Locksley, Asthma and allergic inflammation. *Cell* **140**, 777–783 (2010).
4. D. A. Kuperman, X. Huang, L. L. Koth, G. H. Chang, G. M. Dolganov, Z. Zhu, J. A. Elias, D. Sheppard, D. J. Erle, Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat. Med.* **8**, 885–889 (2002).
5. J. G. Martin, A. Duguet, D. H. Eidelman, The contribution of airway smooth muscle to airway narrowing and airway hyperresponsiveness in disease. *Eur. Respir. J.* **16**, 349–354 (2000).
6. G. Zhen, S. W. Park, L. T. Nguyen, M. W. Rodriguez, R. Barbeau, A. C. Paquet, D. J. Erle, IL-13 and epidermal growth factor receptor have critical but distinct roles in epithelial cell mucin production. *Am. J. Respir. Cell Mol. Biol.* **36**, 244–253 (2007).
7. S. S. An, T. R. Bai, J. H. Bates, J. L. Black, R. H. Brown, V. Brusasco, P. Chitano, L. Deng, M. Dowell, D. H. Eidelman, B. Fabry, N. J. Fairbank, L. E. Ford, J. J. Fredberg, W. T. Gerthoffer, S. H. Gilbert, R. Gosens, S. J. Gunst, A. J. Halayko, R. H. Ingram, C. G. Irvin, A. L. James, L. J. Janssen, G. G. King, D. A. Knight, A. M. Lauzon, O. J. Lakser, M. S. Ludwig, K. R. Lutchen, G. N. Maksym, J. G. Martin, T. Mauad, B. E. McParland, S. M. Mijailovich, H. W. Mitchell, R. W. Mitchell, W. Mitzner, T. M. Murphy, P. D. Paré, R. Pellegrino, M. J. Sanderson, R. R. Schellenberg, C. Y. Seow, P. S. Silveira, P. G. Smith, J. Solway, N. L. Stephens, P. J. Sterk, A. G. Stewart, D. D. Tang, R. S. Tepper, T. Tran, L. Wang, Airway smooth muscle dynamics: A common pathway of airway obstruction in asthma. *Eur. Respir. J.* **29**, 834–860 (2007).
8. S. T. Holgate, The sentinel role of the airway epithelium in asthma pathogenesis. *Immunol. Rev.* **242**, 205–219 (2011).
9. R. Afshar, J. P. Strassner, E. Seung, B. Causton, J. L. Cho, R. S. Harris, D. L. Hamilos, B. D. Medoff, A. D. Luster, Compartmentalized chemokine-dependent regulatory T-cell inhibition of allergic pulmonary inflammation. *J. Allergy Clin. Immunol.* **131**, 1644–1652 (2013).
10. S. Y. Thomas, A. Banerji, B. D. Medoff, C. M. Lilly, A. D. Luster, Multiple chemokine receptors, including CCR6 and CXCR3, regulate antigen-induced T cell homing to the human asthmatic airway. *J. Immunol.* **179**, 1901–1912 (2007).
11. S. Y. Thomas, C. M. Lilly, A. D. Luster, Invariant natural killer T cells in bronchial asthma. *N. Engl. J. Med.* **354**, 2613–2616 (2006).
12. D. Hartl, B. Köller, A. T. Mehlhorn, D. Reinhardt, T. Nicolai, D. J. Schendel, M. Griese, S. Krauss-Etschmann, Quantitative and functional impairment of pulmonary CD4⁺CD25^{hi} regulatory T cells in pediatric asthma. *J. Allergy Clin. Immunol.* **119**, 1258–1266 (2007).
13. M. Miyara, Y. Yoshioka, A. Kitoh, T. Shima, K. Wing, A. Niwa, C. Parizot, C. Taffin, T. Heike, D. Valeyre, A. Mathian, T. Nakahata, T. Yamaguchi, T. Nomura, M. Ono, Z. Amoura, G. Gorochov, S. Sakaguchi, Functional delineation and differentiation dynamics of human CD4⁺ T cells expressing the FoxP3 transcription factor. *Immunity* **30**, 899–911 (2009).
14. S. T. Holgate, Innate and adaptive immune responses in asthma. *Nat. Med.* **18**, 673–683 (2012).
15. B. N. Lambrecht, H. Hammad, The immunology of asthma. *Nat. Immunol.* **16**, 45–56 (2015).
16. J. V. Fahy, B. F. Dickey, Airway mucus function and dysfunction. *N. Engl. J. Med.* **363**, 2233–2247 (2010).
17. A. G. Henderson, C. Ehre, B. Button, L. H. Abdullah, L. H. Cai, M. W. Leigh, G. C. DeMaria, H. Matsui, S. H. Donaldson, C. W. Davis, J. K. Sheehan, R. C. Boucher, M. Kesimer, Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure. *J. Clin. Invest.* **124**, 3047–3060 (2014).
18. C. M. Evans, K. Kim, M. J. Tuvim, B. F. Dickey, Mucus hypersecretion in asthma: Causes and effects. *Curr. Opin. Pulm. Med.* **15**, 4–11 (2009).
19. P. G. Woodruff, B. Modrek, D. F. Choy, G. Jia, A. R. Abbas, A. Ellwanger, L. L. Koth, J. R. Arron, J. V. Fahy, T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am. J. Respir. Crit. Care Med.* **180**, 388–395 (2009).
20. P. A. Keane, M. Karampelas, D. A. Sim, S. R. Sadda, A. Tufail, H. N. Sen, R. B. Nussenblatt, A. D. Dick, R. W. Lee, P. I. Murray, C. E. Pavesio, A. K. Denniston, Objective measurement of vitreous inflammation using optical coherence tomography. *Ophthalmology* **121**, 1706–1714 (2014).
21. B. J. Vakoc, R. M. Lanning, J. A. Tyrrell, T. P. Padera, L. A. Bartlett, T. Stylianopoulos, L. L. Munn, G. J. Tearney, D. Fukumura, R. K. Jain, B. E. Bouma, Three-dimensional microscopy of the tumor microenvironment in vivo using optical frequency domain imaging. *Nat. Med.* **15**, 1219–1223 (2009).
22. S. H. Yun, G. J. Tearney, B. J. Vakoc, M. Shishkov, W. Y. Oh, A. E. Desjardins, M. J. Suter, R. C. Chan, J. A. Evans, I.-K. Jang, N. S. Nishioka, J. F. de Boer, B. E. Bouma, Comprehensive volumetric optical microscopy in vivo. *Nat. Med.* **12**, 1429–1433 (2006).
23. S. Al-Muhsen, J. R. Johnson, Q. Hamid, Remodeling in asthma. *J. Allergy Clin. Immunol.* **128**, 451–462 (2011).
24. D. C. Adams, L. P. Hariri, A. J. Miller, Y. Wang, J. L. Cho, M. Villiger, J. A. Holz, M. V. Szabari, D. L. Hamilos, R. S. Harris, J. W. Griffith, B. E. Bouma, A. D. Luster, B. D. Medoff, M. J. Suter, Birefringence microscopy platform for assessing airway smooth muscle structure and function in vivo. *Sci. Transl. Med.* **8**, 359ra131 (2016).
25. S. K. Huang, H. Q. Xiao, J. Kleins-Tebbe, G. Paciotti, D. G. Marsh, L. M. Lichtenstein, M. C. Liu, IL-13 expression at the sites of allergen challenge in patients with asthma. *J. Immunol.* **155**, 2688–2694 (1995).
26. J. L. Brown, A. F. Behndig, B. E. Sekerel, J. Pourazar, A. Blomberg, F. J. Kelly, T. Sandström, A. J. Frew, S. J. Wilson, Lower airways inflammation in allergic rhinitis: A comparison with asthmatics and normal controls. *Clin. Exp. Allergy* **37**, 688–695 (2007).
27. E. A. Becky Kelly, W. W. Busse, N. N. Jarjour, A comparison of the airway response to segmental antigen bronchoprovocation in atopic asthma and allergic rhinitis. *J. Allergy Clin. Immunol.* **111**, 79–86 (2003).
28. S. Lam, S. Al-Majed, H. Chan, K. Tse, J. C. LeRiche, M. Chan-Yeung, Differences in mediator release between allergic rhinitis and asthma. *J. Allergy Clin. Immunol.* **87**, 842–849 (1991).
29. J. R. Shaver, J. J. O'Connor, M. Pollice, S. K. Cho, G. C. Kane, J. E. Fish, S. P. Peters, Pulmonary inflammation after segmental ragweed challenge in allergic asthmatic and nonasthmatic subjects. *Am. J. Respir. Crit. Care Med.* **152**, 1189–1197 (1995).
30. S. E. Wenzel, A. A. Fowler III, L. B. Schwartz, Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. *Am. Rev. Respir. Dis.* **137**, 1002–1008 (1988).
31. S. E. Wenzel, G. L. Larsen, K. Johnston, N. F. Voelkel, J. Y. Westcott, Elevated levels of leukotriene C4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am. Rev. Respir. Dis.* **142**, 112–119 (1990).
32. S. E. Wenzel, J. Y. Westcott, G. L. Larsen, Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: Relationship to the development of late asthmatic responses. *J. Allergy Clin. Immunol.* **87**, 540–548 (1991).
33. J. G. Zangrilli, J. R. Shaver, R. A. Cirelli, S. K. Cho, C. G. Garlisi, A. Falcone, F. M. Cuss, J. E. Fish, S. P. Peters, sVCAM-1 levels after segmental antigen challenge correlate with eosinophil influx, IL-4 and IL-5 production, and the late phase response. *Am. J. Respir. Crit. Care Med.* **151**, 1346–1353 (1995).
34. M. Noval Rivas, O. T. Burton, P. Wise, L. M. Charbonnier, P. Georgiev, H. C. Oettgen, R. Rachid, T. A. Chatila, Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* **42**, 512–523 (2015).
35. J. J. Moon, H. H. Chu, J. Hataye, A. J. Pagán, M. Pepper, J. B. McLachlan, T. Zell, M. K. Jenkins, Tracking epitope-specific T cells. *Nat. Protoc.* **4**, 565–581 (2009).
36. M. Bonvalet, E. Wambre, H. Moussu, S. Horiot, W. W. Kwok, A. Louise, D. Ebo, C. Hoarau, L. Van Overtvelt, V. Baron-Bodo, P. Moingeon, Comparison between major histocompatibility complex class II tetramer staining and surface expression of activation markers for the detection of allergen-specific CD4⁺ T cells. *Clin. Exp. Allergy* **41**, 821–829 (2011).

37. S. A. Islam, A. D. Luster, T cell homing to epithelial barriers in allergic disease. *Nat. Med.* **18**, 705–715 (2012).
38. L. Guo, Y. Huang, X. Chen, J. Hu-Li, J. F. Urban Jr., W. E. Paul, Innate immunological function of T_H2 cells in vivo. *Nat. Immunol.* **16**, 1051–1059 (2015).
39. D. J. Erle, D. Sheppard, The cell biology of asthma. *J. Cell Biol.* **205**, 621–631 (2014).
40. C. L. Ordoñez, R. Khashayar, H. H. Wong, R. Ferrando, R. Wu, D. M. Hyde, J. A. Hotchkiss, Y. Zhang, A. Novikov, G. Dolganov, J. V. Fahy, Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am. J. Respir. Crit. Care Med.* **163**, 517–523 (2001).
41. H. W. J. Young, O. W. Williams, D. Chandra, L. K. Bellinghausen, G. Pérez, A. Suárez, M. J. Tuvim, M. G. Roy, S. N. Alexander, S. J. Moghaddam, R. Adachi, M. R. Blackburn, B. F. Dickey, C. M. Evans, Central role of Muc5ac expression in mucous metaplasia and its regulation by conserved 5' elements. *Am. J. Respir. Cell Mol. Biol.* **37**, 273–290 (2007).
42. C. M. Evans, D. S. Raclawska, F. Ttofali, D. R. Liptzin, A. A. Fletcher, D. N. Harper, M. A. McGing, M. M. McElwee, O. W. Williams, E. Sanchez, M. G. Roy, K. N. Kindrachuk, T. A. Wynn, H. K. Eltzschig, M. R. Blackburn, M. J. Tuvim, W. J. Janssen, D. A. Schwartz, B. F. Dickey, The polymeric mucin Muc5ac is required for allergic airway hyperreactivity. *Nat. Commun.* **6**, 6281 (2015).
43. W. C. Moore, J. D. Hasday, S. S. Meltzer, P. L. Wisniewski, B. White, E. R. Bleeker, Subjects with mild and moderate asthma respond to segmental allergen challenge with similar, reproducible, allergen-specific inflammation. *J. Allergy Clin. Immunol.* **108**, 908–914 (2001).
44. T. S. C. Hinks, X. Zhou, K. J. Staples, B. D. Dimitrov, A. Manta, T. Petrossian, P. Y. Lum, C. G. Smith, J. A. Ward, P. H. Howarth, A. F. Walls, S. D. Gadola, R. Djukanović, Innate and adaptive T cells in asthmatic patients: Relationship to severity and disease mechanisms. *J. Allergy Clin. Immunol.* **136**, 323–333 (2015).
45. M. Raundhal, C. Morse, A. Khare, T. B. Oriss, J. Milosevic, J. Trudeau, R. Huff, J. Pilewski, F. Holguin, J. Kolls, S. Wenzel, P. Ray, A. Ray, High IFN- γ and low SLPI mark severe asthma in mice and humans. *J. Clin. Invest.* **125**, 3037–3050 (2015).
46. C. M. Lilly, H. Nakamura, O. I. Belostotsky, K. J. Haley, E. A. Garcia-Zepeda, A. D. Luster, E. Israel, Eotaxin expression after segmental allergen challenge in subjects with atopic asthma. *Am. J. Respir. Crit. Care Med.* **163**, 1669–1675 (2001).
47. F. P. Legoux, J. J. Moon, Peptide: MHC tetramer-based enrichment of epitope-specific T cells. *J. Visualized Exp.* 4420 (2012).
48. F. P. Legoux, J.-B. Lim, A. W. Cauley, S. Dikiy, J. Ertelt, T. J. Mariani, T. Sparwasser, S. S. Way, J. J. Moon, CD4⁺ T cell tolerance to tissue-restricted self antigens is mediated by antigen-specific regulatory T cells rather than deletion. *Immunity* **43**, 896–908 (2015).
49. G. K. Smyth, Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* **3**, 1–25 (2004).
50. R. Cao, T. T. Wang, G. DeMaria, J. K. Sheehan, M. Kesimer, Mapping the protein domain structures of the respiratory mucins: A mucin proteome coverage study. *J. Proteome Res.* **11**, 4013–4023 (2012).
51. M. E. Sardi, M. P. Washburn, Enriching quantitative proteomics with SLN. *Nat. Biotechnol.* **28**, 40–42 (2010).

Acknowledgments: We thank C. Leary, L. Tracy, L. Liang, M. Brevard, and the Pulmonary Special Procedures Unit for their assistance with this study. Statistical analysis was conducted with support from Harvard Catalyst and the Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, NIH Award UL1 TR001102) and financial contributions from Harvard University and its affiliated academic healthcare centers. The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the NIH. **Funding:** This work was supported by NIH grants U19AI095261, R37AI040618, and T32HL116275 (to A.D.L.); R01CA167827 (to M.J.S.); K08AI113083 (to J.L.C.); and R01HL103940 (to M.K.). **Author contributions:** J.L.C.: collection and assembly of data, data analysis and interpretation, and writing of the paper; M.F.L.: collection and assembly of data, data analysis and interpretation, and writing of the paper; D.C.A., L.F., S.A.I., and M.J.S.: collection and assembly of data as well as data analysis and interpretation; R.A., J.W.G., R.S.H., G.R., A.A.F., M.K., and R.B.: collection and assembly of data; D.L.H.: conception and design, subject recruitment and enrollment, and writing of the paper; A.N. and R.X.: data analysis and interpretation; W.W.K. and A.K.H.: provision of reagents; J.J.M.: provision of reagents as well as data analysis and interpretation; B.D.M.: conception and design, collection and assembly of data, data analysis and interpretation, and writing of the paper; A.D.L.: conception and design, data analysis and interpretation, and writing of the paper. **Competing interests:** R.B. reports a financial interest in Parion Sciences. M.J.S. and D.C.A. are authors on patent WO2015084964 A1 ("Compensating for input polarization mode variations") and Patent Cooperation Treaty Application No. 15/100,815 ("Apparatus and method to compensate for input polarization mode variation"). M.J.S. has received sponsored research support from NinePoint Medical and Boston Scientific. M.J.S. is a consultant for NinePoint Medical.

Submitted 13 May 2016

Accepted 19 August 2016

Published 5 October 2016

10.1126/scitranslmed.aag1370

Citation: J. L. Cho, M. F. Ling, D. C. Adams, L. Faustino, S. A. Islam, R. Afshar, J. W. Griffith, R. S. Harris, A. Ng, G. Radicioni, A. A. Ford, A. K. Han, R. Xavier, W. W. Kwok, R. Boucher, J. J. Moon, D. L. Hamilos, M. Kesimer, M. J. Suter, B. D. Medoff, A. D. Luster, Allergic asthma is distinguished by sensitivity of allergen-specific CD4⁺ T cells and airway structural cells to type 2 inflammation. *Sci. Transl. Med.* **8**, 359ra132 (2016).



Allergic asthma is distinguished by sensitivity of allergen-specific CD4⁺ T cells and airway structural cells to type 2 inflammation

Josalyn L. Cho, Morris F. Ling, David C. Adams, Lucas Faustino, Sabina A. Islam, Rosh Afshar, Jason W. Griffith, Robert S. Harris, Aylwin Ng, Giorgia Radicioni, Amina A. Ford, Andre K. Han, Ramnik Xavier, William W. Kwok, Richard Boucher, James J. Moon, Daniel L. Hamilos, Mehmet Kesimer, Melissa J. Suter, Benjamin D. Medoff and Andrew D. Luster (October 5, 2016) *Science Translational Medicine* **8** (359), 359ra132. [doi: 10.1126/scitranslmed.aag1370]

Editor's Summary

Advanced analysis of asthma

Not all individuals who have respiratory reactions to allergens progress to asthma. In this issue, Cho *et al.* found that although allergic asthmatics and allergic nonasthmatics both experienced inflammation after allergen challenge, asthmatics had more mucin and type 2 cytokines, and allergen-specific T cells sampled from the airspace had increased innate type 2 receptors. Using orientation-resolved optical coherence tomography, described by Adams *et al.*, they demonstrated that allergic asthmatics also had increased airway smooth muscle mass. This technique allows for in vivo imaging of airway smooth muscle structure and function, which could shed light on the pathogenesis of many respiratory diseases.

The following resources related to this article are available online at <http://stm.sciencemag.org>.
This information is current as of November 20, 2016.

Article Tools	Visit the online version of this article to access the personalization and article tools: http://stm.sciencemag.org/content/8/359/359ra132
Supplemental Materials	"Supplementary Materials" http://stm.sciencemag.org/content/suppl/2016/10/03/8.359.359ra132.DC1
Related Content	The editors suggest related resources on <i>Science's</i> sites: http://stm.sciencemag.org/content/scitransmed/8/359/359ra131.full
Permissions	Obtain information about reproducing this article: http://www.sciencemag.org/about/permissions.dtl

Science Translational Medicine (print ISSN 1946-6234; online ISSN 1946-6242) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Copyright 2016 by the American Association for the Advancement of Science; all rights reserved. The title *Science Translational Medicine* is a registered trademark of AAAS.