CD4⁺ T cell-dependent airway mucus production occurs in response to IL-5 expression in lung

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CD4⁺ T cell-dependent airway mucus production occurs in response to IL-5 expression in lung. Am J Physiol Lung Cell Mol Physiol 282: L1066–L1074, 2002. First published December 21, 2001; 10.1152/ajplung.00195.2001.—The potential role of airway interleukin-5 (IL-5) expression in eliciting mucus production was demonstrated in a pulmonary IL-5 transgenic mouse model (NJ.1726) in which naive transgenic mice display comparable levels of airway mucus relative to allergen-sensitized and -challenged wild-type mice. The intrinsic mucus accumulation of NJ.1726 was abolished in compound transgenic-gene knockout mice deficient of either CD4⁺ cells [NJ.1726/CD4⁻/CD4⁻] or αβ T cell receptor-positive (TCR⁺) cells [NJ.1726/αβ TCR⁻/αβ TCR⁻]. In addition, mucus production in naive NJ.1726 was inhibited by >90% after administration of the soluble anti-IL-4 receptor α-subunit antagonist. The loss of mucus production in NJ.1726/CD4⁻/CD4⁻, NJ.1726/αβ TCR⁻/αβ TCR⁻, and anti-IL-4 receptor α-subunit antagonist-treated mice occurred notwithstanding the significant pulmonary eosinophilia and expansion of airway B cells induced by ectopic IL-5 expression. Furthermore, the loss of mucus accumulation occurred in these mice despite elevated levels of airway and peripheral IL-5, indicating that IL-5 does not directly induce goblet cell metaplasia and mucus production. Thus pulmonary expression of IL-5 alone is capable of inducing CD4⁺ T cell-dependent goblet cell metaplasia, apparently mediated by IL-4 receptor α-subunit-ligand interactions, and represents a previously unrecognized novel pathway for augmenting allergen-induced mucus production.

Increased mucus production and secretion is a common characteristic of human asthma and is believed to be partly responsible for the development of airway obstruction, a pathophysiological manifestation associated with this disease (23). Although mice do not have epithelium-associated mucous glands in the lung and thus mucus plugging of bronchioles is infrequently observed, allergen-mediated inflammation is associated with the induction of goblet cell metaplasia regardless of the specific model utilized (e.g., see Refs. 2, 14). The proliferation of these mucus-containing epithelial cells has been shown (1) to be intimately linked to T helper type 2 (Th2)-mediated inflammation in response to aeroallergen exposure, and studies have implicated specific roles for the Th2 cytokines interleukin-4 (IL-4) (7), IL-9 (40), and IL-13 (18) as proinflammatory mediators linked to the induction of mucus production. Moreover, recent studies (5) have also implicated the Th1 cytokine interferon-γ (IFN-γ) as an important downregulator of allergen-induced mucus overproduction in the lung, suggesting that this response results from a complex interplay of multiple inflammatory signals.

Transgenic/gene knockout mice have been particularly instrumental to the characterization of the specific signaling pathways contributing to increased mucus production after allergen challenge. Overexpression of either IL-9 (12) or IL-13 (44) in the lungs of transgenic mice elicits allergen-independent goblet cell metaplasia and mucus overproduction. Adoptive transfer studies (5, 6) using gene knockout mice and ovalbumin (OVA) T cell receptor (TCR) transgenic animals have demonstrated that CD4⁺ T cells and IL-4 receptor expression are each necessary (and possibly sufficient) for allergen-induced mucus overproduction. In addition, studies utilizing IL-5-deficient mice showed that allergen-induced mucus production can occur in the absence of this cytokine and extensive pulmonary eosinophilia (6). Collectively, however, these earlier studies demonstrate only that mucus production is causatively linked to IL-4/IL-13 expression or can occur in the absence of IL-5 and pulmonary eosinophils. The unresolved issue is whether IL-5 and/or eosinophils themselves are capable of eliciting pulmonary mucus overproduction. Indeed, constitutive ectopic expression of IL-5 from Clara cells in naive transgenic mice (line NJ.1726; Ref. 26) is sufficient to induce a pulmonary eosinophilia. More significantly, these naive animals exhibit mucus production approaching levels observed in wild-type allergen-challenged mice. However, the mech-
IL-5-ASSOCIATED PULMONARY MUCUS OVERPRODUCTION

L1067

anism responsible for this IL-5-dependent, allergen-independent mucus production is currently undefined. NJ.1726 mice were observed to exhibit significant expansion of B cell and the CD4+ and CD8+ T cell populations independent of allergen exposure. Therefore, we assessed the potential role of IL-5 and/or pulmonary eosinophils in eliciting mucus overproduction in mice alone or synergistically with individual lymphocyte subsets in allergen-challenged wild-type mice and naive IL-5 transgenic mice, as well as compound IL-5 transgenic/gene knockout animals deficient of either CD4 or IL-5 transgenic mice. Surprisingly, the mucus production observed in naive IL-5 transgenic mice was also abolished in mice that were deficient of either CD4 or αβ TCR+ T cells. However, in the absence of CD4 or αβ TCR+ T cells, mucus production was obviated, confirming earlier studies (5, 6) showing the necessity of these cells. Interestingly, the mucus production observed in naive IL-5 transgenic mice was also abolished in mice that were deficient of either CD4 or αβ TCR+ T cells. The elimination of mucus production in these compound transgenic-gene knockout mice occurred despite a profound pulmonary eosinophilia and elevated levels of peripheral and lung IL-5. This suggests that a previously unknown IL-5-mediated effect on T cells exists that is capable of eliciting mucus production independent of IL-5 activities on eosinophils and potential effects on airway epithelium.

METHODS

Mice. Transgenic mice constitutively expressing murine IL-5 from the lung epithelium (line NJ.1726) were generated as previously described (26) and maintained by continual backcross to C57BL/6J. NJ.1726 animals were bred with gene knockout mice (Jackson Laboratories, Bar Harbor, ME) lacking either B cells [C57BL/6-Igh-6tm1Mom (24)], T cells (αβ/γδ) [C57BL/6-Tcrβtm1Mom Tcrδtm1Mom (31)], αβ TCR+ cells [C57BL/6-Tcrβtm1Mom (31)], γδ TCR+ cells [C57BL/6-J/Tcrγδtm1Mom (22)], CD4+ cells [C57BL/6-J/Cd4tm1Kow (30)], or CD8+ cells [C57BL/6-Cd8αtm1Mak (13)] to generate compound IL-5 transgenic-gene knockout mice deficient in B, T, αβ TCR+, γδ TCR+, CD4+, and CD8+ cells, respectively. Genotypes of mice derived from these crosses were determined by the presence of the IL-5 transgene (PCR of tail DNA) and loss of B cells or T cells or T cell subtypes as assayed by flow cytometry on peripheral blood using conjugated antibodies against B220 (B cells), TCR-β, TCR-δ, CD4, and CD8, as previously described (3). Control C57BL/6J mice were obtained from Jackson Laboratories, and all procedures were conducted on mice 8–12 wk of age maintained in microisolation cages housed in a specific pathogen-free animal facility. The sentinel cages within this animal colony were negative for viral antibodies and the presence of known mouse pathogens. Protocols and studies involving animals were conducted in accordance with National Institutes of Health and Mayo Clinic Foundation guidelines.

OVA sensitization and challenge. Mice were sensitized and challenged with chicken OVA as previously described (11). Briefly, mice were sensitized by an intraperitoneal injection (100 μl) of 20 μg chicken OVA (Sigma, St. Louis, MO) emulsified in 2 mg Imject Alum [Al(OH)3/Mg(OH)2; Pierce, Rockfield, IL] on days 0 and 14. Mice were subsequently challenged with an aerosol generated from 1% OVA in saline or saline alone for 20 min by ultrasonic nebulization (Devilbiss, Somerset, PA) on days 24, 25, and 26. Assessment of goblet cell metaplasia and mucus overproduction and parenchymal eosinophils were performed on day 28. All NJ.1726 strains were placed on a similar protocol with the exception of intra-peritoneal saline injection (without Imject Alum) on days 0 and 14 and administration of nebulized saline on days 24, 25, and 26.

Assessment of goblet cell metaplasia and mucus overproduction. On day 25 of the OVA sensitization and challenge protocol, mice were euthanized with ketamine (2 mg/kg body wt). Lungs were perfused with 10% formalin (Biochemical Sciences, Swedensboro, NJ), excised, and bathed in 10% formalin overnight before embedding in paraffin. Mucous cell development along the airway epithelium was quantified in paraffin-embedded tissue sections (4–8 μm) stained with periodic acid-Schiff’s reagent (PAS). Parasagittal sections (n = 5 mice/group) were analyzed by bright-field microscopy using an image analysis software program (ImagePro Plus, Media Cybernetics, Silver Spring, MD) to derive an airway mucus index (MI) reflective of both the amount of mucus per airway and the number of airways affected. The mucus content of all the airways per section (20–30, proximal to distal) was measured from groups of four to five animals. An imaging program (Image ProPlus, Media Cybernetics) was used to quantify the area and intensity of PAS staining per airway. The data were quantified as follows: MI = (average PAS staining intensity of airway epithelium) × (area of airway epithelium staining with PAS)/(total area of conducting airway epithelium) × (total number of airways assessed).

Flow cytometry of leukocytes recovered from total lung digests. Leukocytes within the lung parenchyma were assessed by collagenase digestion of perfused lungs. Isolation of lung cells was performed as previously described (3). Briefly, perfused lungs were removed and diced into pieces <300 μl in volume. Hanks’ balanced salt solution (HBSS; 4 ml; Gibco, Gaithersburg, MD) containing 175 U/ml collagenase (Sigma), 10% FCS (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin was added to the tissue and incubated for 26 min at 37°C in an orbital shaker. The digested lungs were sheared with a 20-gauge needle and filtered through 45- and 20-μm filters. Cells were washed three times and resuspended in HBSS before counting with a hemacytometer. Lymphocyte populations in the lung were subsequently identified and expressed as the product of the total cell count and the percentage of total cells analyzed (1 × 106) by flow cytometry. CD3+CD4+ double-positive, CD3+CD8+ double-negative, and B cells were identified or quantified by staining with the following conjugated antibodies: phycoerythrin-anti-mouse CD3 (Caltag, Burlingame, CA), FITC-anti-mouse CD4 (Pharmingen, San Diego, CA), FITC-anti-mouse CD8 (Pharmingen), and phycoerythrin-anti-mouse B220 (Caltag). Flow cytometry was performed on a FACSscan flowcytometer (Becton Dickinson, Franklin Lakes, NJ). Data acquisition and analysis were performed using CellQuest software (Becton Dickinson).

Immunocytochemistry of lung sections and detection of airway eosinophils. Eosinophils within the lung parenchyma were identified by immunohistochemistry using a rabbit polyclonal antibody against mouse major basic protein (MBP). MBP antigen-antibody complexes were detected in 4-μm sections of formalin-fixed, paraffin-embedded sections of mouse lungs as previously described (11). Tissue eosinophils were assessed by determining the total number of MBP-positive cells in five randomly selected high-power
fields, with data expressed as eosinophils per square millimeter of lung tissue (n = 4–5 mice/group).

**Treatment of NJ.1726 mice with IL-4 receptor antagonist.** The “QY” IL-4 mutant binds the IL-4 receptor with an affinity similar to wild-type IL-4. However, unlike IL-4, the QY mutant is unable to mediate receptor signaling (16). NJ.1726 mice were administered QY according to a previously published protocol (17). Briefly, NJ.1726 mice (~2 mo of age) were injected intraperitoneally two times on day 0 with 50 μg of QY. On days 1 through 10 of this protocol, mice were injected one time intraperitoneally with 10 μg of QY. Control mice received injections of diluent (i.e., PBS) throughout the protocol. On day 10, the lungs from experimental and control groups were removed and prepared for histology and mucus quantitation as described in Assessment of goblet cell metaplasia and mucus overproduction.

**Statistical analysis.** Data are presented as means ± SE. Statistical analysis was performed on parametric data using t-tests with differences between means considered to be significant at P < 0.05.

**RESULTS**

**Allergen-induced mucus production is dependent on αβ TCR+ T cells and not B cells or γδ TCR+ T cells.** OVA challenge of C57BL/6J mice induces airway inflammation characterized by increases in CD3+, CD4+, and B220+ cell populations (Table 1). Furthermore, OVA sensitization and aerosol challenge elicits a pronounced increase in airway eosinophils 48 h after the final allergen challenge (data not shown). Figure 1, A, B, and F, shows that the sensitization and challenge regime also results in goblet cell metaplasia and the production of mucus relative to saline-treated control groups (MI = 9.9 ± 1.1 vs. 0.4 ± 0.02, respectively). OVA sensitization and aerosol challenge of mice deficient in specific lymphocyte cell types demonstrated that allergen-induced mucus production was independent of B cells (Fig. 1, C and F; MI = 9.7 ± 0.8) and γδ TCR+ T cells (Fig. 1, E and F; MI = 8.7 ± 1.7). In contrast, goblet cell metaplasia and mucus production in OVA-treated αβ TCR+ T cell-deficient mice was abolished (Fig. 1, D and F; MI = 1.2 ± 0.98).

**OVA-induced mucus production requires CD4+ T cells.** A comparison of mucus production in OVA-sensitized and aerosol-challenged CD4+ T cell-deficient and CD8+ T cell-deficient mice demonstrates that CD4+ and not CD8+ T cells are required for OVA-induced goblet cell metaplasia (Fig. 2; MI = 0.69 ± 0.39 vs. 8.1 ± 1.1, respectively).

**Constitutive ectopic expression of IL-5 in respiratory epithelial cells results in mucus production in naive transgenic mice.** Pulmonary IL-5 expression induces an inflammatory-type condition in naive NJ.1726 mice, including the accumulation of inflammatory leukocytes (i.e., eosinophils, T cells, and B cells) in the lung and changes in the airway epithelium (26; Table 1). Among the changes occurring in the airways of these transgenic mice is a significant goblet cell metaplasia and accompanying mucus production. The MI of naive (i.e., saline-challenged) NJ.1726 mice is more than 10-fold higher relative to saline-challenged wild-type mice (Fig. 3, A and E, vs. Fig. 1, A and F, MI = 5.9 ± 1.5 vs. 0.4 ± 0.02, respectively). Moreover, this naive transgenic MI is only nominally larger than the MI observed in OVA-sensitized and aerosol-challenged wild-type mice (Fig. 1F, MI = 9.9 ± 1.1), demonstrating that pulmonary IL-5 expression alone (i.e., independent of allergen-induced Th2 inflammation) is capable of eliciting airway mucus production.

**IL-5-associated mucus production in naive transgenic mice is dependent on presence of CD4+ T cells.** In addition to IL-5 expression and airway inflammation (Table 1), the intrinsic mucus production in naive NJ.1726 mice was also dependent on the presence of CD4+ αβ TCR+ T cells (Fig. 3). Specifically, despite similar levels of serum IL-5 (3), the MI of naive compound transgenic-knockout mice deficient in CD4+ T cells [NJ.1726/CD4(−/−)] was reduced by 80% relative to naive NJ.1726 mice (Fig. 3E, MI = 2.03 ± 0.58 and 5.9 ± 1.5, respectively). Interestingly, despite the dramatic increase in both pulmonary CD4+ T cells and B cells associated with these IL-5 transgenic mice, mucus expression remained elevated in B cell-deficient NJ.1726 animals (Fig. 3, C and E, MI = 5.2 ± 0.35).

**Increased mucus production in NJ.1726 mice requires IL-4 receptor α-subunit-mediated signaling events.** We used the IL-4 receptor antagonist QY (16) to block IL-4 and IL-13 signaling through this receptor to assess the potential contribution these cytokines have in mucus production associated with naive NJ.1726 mice. Blockade of IL-4 receptor α-subunit demonstrated that signaling events through this receptor were necessary for the mucus production associated with naive NJ.1726 mice (Fig. 4). Treatment of NJ.1726 mice with QY reduced the MI to levels comparable with naive wild-type animals (0.27 ± 0.17 vs. 0.40 ± 0.02, respectively).

**Table 1. T and B cell populations significantly expand in lungs of naive NJ.1726 mice**

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Aerosol Challenge</th>
<th>Total Cells</th>
<th>CD4+/CD3+ Cells</th>
<th>CD8+/CD3+ Cells</th>
<th>B220+ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>Saline</td>
<td>9.7 ± 1.9</td>
<td>0.16 ± 0.04</td>
<td>0.04 ± 0.01</td>
<td>0.19 ± 0.003</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>Ovalbumin</td>
<td>12.4 ± 1.1*</td>
<td>0.89 ± 0.01*</td>
<td>0.07 ± 0.01*</td>
<td>0.85 ± 0.01*</td>
</tr>
<tr>
<td>NJ.1726</td>
<td>Saline</td>
<td>46.0 ± 2.4†</td>
<td>2.2 ± 0.2†</td>
<td>0.93 ± 0.17†</td>
<td>8.9 ± 1.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE given as no. of cells × 10⁶. Ectopic expression of interleukin-5 (IL-5) in the airways mediates expansion of CD4+ T cell, CD8+ T cell, and B cell populations in naive NJ.1726 mice. Total cells and specific lymphocyte populations from collagenase-digested perfused lungs from saline-sensitized wild-type mice (C57BL/6J), ovalbumin-challenged wild-type mice, and saline-challenged naive NJ.1726 mice were dispersed into single cell suspensions before assessment of lung parenchymal cellularity. *P < 0.05, significantly different from saline-challenged wild-type mice; †P < 0.05, significantly different from saline-challenged wild-type mice.
T cell-mediated production of mucus induced by ectopic IL-5 expression occurs independent of airway eosinophils. In addition to increased mucus production in naive NJ.1726 mice, ectopic expression of IL-5 in the airways elicits eosinophil recruitment to the lung parenchyma (26), raising the possibility that the induced eosinophilia was responsible for the intrinsic goblet cell metaplasia and mucus production associated with these mice. Eosinophil infiltration of the lung was assessed in naive NJ.1726 mice and compound transgenic-gene knockout animals to determine if a correlative relationship exists in naive NJ.1726 mice between goblet cell metaplasia and mucus production and the development of a pulmonary eosinophilia. Both parameters were assessed in cohorts of animals representing naive NJ.1726 mice and compound transgenic-gene knockout mice deficient in B cells [NJ.1726/B(−/−)] or CD4+ T cells [NJ.1726/CD4(−/−)]. Goblet cell metaplasia and mucus production was observed only in naive NJ.1726

Fig. 1. Mucus production after ovalbumin (OVA) challenge is dependent on αβ T cell receptor-positive (TCR) but not γδ TCR expression by T cells. Mucus expression was visualized by tissue staining of formalin-fixed, paraffin-embedded lung sections with periodic acid-Schiff’s (PAS) stain. The mucus index was calculated for each lung section as described in METHODS. A–E: representative photographs of PAS-stained lung sections from saline-challenged (Sal) wild-type mice (A), OVA-challenged wild-type mice (B), OVA-challenged B cell-deficient mice (C), OVA-challenged αβ TCR-deficient mice (D), and OVA-challenged γδ TCR-deficient mice (E). F: histograms represent the average mucus index for each cohort. Values are means ± SE; n = 4 mice/group. *P < 0.05.

Fig. 2. Mucus overproduction after OVA challenge is dependent on CD4 but not CD8 expression by T cells. The histograms represent the average mucus index for each cohort. Values are means ± SE; n = 4 mice/group. ND, nondetectable levels of mucus. *P < 0.05.
and NJ.1726/B(−/−) mice, yet no differences were observed in the number of eosinophils per square millimeter of basement membrane found in all three naive cohorts (Fig. 5). Interestingly, each group of naive animals had tissue eosinophil numbers equivalent to OVA-sensitized and aerosol-challenged C57BL/6J mice (a cohort with elevated mucus production).

**DISCUSSION**

Airway inflammation characterized by infiltration by eosinophils and T cells and an accompanying increase in expression of Th2 cytokines (e.g., IL-4, IL-5, and IL-13) are common characteristics of both asthma patients and mouse respiratory inflammation models (27, 42). One particularly striking feature common to both humans and mice exposed to aerosolized allergens is the development of goblet cell metaplasia and the concomitant expression of mucus glycoproteins (14). The observed mucus production after allergen challenge can lead to narrowing of midsized airways and in severe cases precipitate mucus-plugging of the airways (23), which undoubtedly leads, in part, to airway narrowing and subsequent dyspnea.

The strong correlation between mucus overproduction and Th2 inflammation after allergen exposure has led to the common speculation that there is a cause and effect relationship in which T cells are responsible for the observed changes in mucus levels in the airways in asthma. However, Blyth et al. (2) have demonstrated that induction of mucus expression and goblet cell metaplasia precedes both lymphocytic and eosinophilic inflammation and can persist for 7 days after airway inflammation has returned to resting levels. Although the increase in mucus expression does not correlate with the timing of the inflammatory cell infiltrate, it does correlate with the observed time course of Th2 cytokine production after a single exposure to allergen challenge (32). In addition, Henderson and colleagues...
cells are the predominant cell type, comprising naive mice and epithelial-associated mucous glands producing cells (i.e., goblet cells) are rarely observed in allergen exposure. Secretory serous cells and mucus-producing cells of the airway epithelium in the mouse (34, 39). After exposure to allergens and/or toxicants, the secretory cells undergo metaplasia/hyperplasia and concurrently accumulate mucus within cytoplasmic granules. However, the time course of these events is quite prolonged. It has been shown that increased expression of MUC2 mucin transcripts takes ~24 h, whereas the presence of the fully glycosylated protein requires at least 48 h after toxicant exposure (38). A similar time course has been suggested for the other major respiratory mucin MUC5A/C (41). Therefore, the anatomic data from mice concerning mucus-producing cell types and the biochemical data concerning the rate of mucus production suggest that this phenomenon is associated with events that occur over a period of days, much like the time course of Th2 inflammation in the airways after allergen exposure.

Previous studies (35) in the mouse have shown that IL-5 effector functions are predominantly exerted on eosinophils and B cells. In both leukocytes, IL-5 is primarily a proliferative signal for lineage-committed precursors; however, substantive effects have also been demonstrated on mature cells in the periphery. For example, B cell differentiation into plasma cells appears to be mediated, in part, by IL-5 effector function(s) (36), and IL-5 has been shown (4, 9, 37) to enhance eosinophil chemotaxis and survival. IL-5 has also been shown (29) to elicit bone deposition in the spleen, indicating that IL-5 has broad effects on various cell types previously not associated with IL-5 effector functions. The data presented in this study suggest that IL-5 has additional activities that are mediated by (through?) CD4⁺ T cells as pulmonary expression of IL-5, in the absence of allergen sensitization and aerosol challenge, induces goblet cell metaplasia and mucus production.

The necessity of CD4⁺ T cells in an IL-5-dependent model system complements previous studies that have implicated CD4⁺ T cells and Th2 inflammatory responses as critical components of this phenomenon. For example, OVA-induced mucus production is absent in RAG(-/-) mice (T cell and B cell deficient) and adoptive transfer of wild-type CD4⁺ T cells into these mice reconstitutes this parameter (10). In addition, Cohn et al. (6, 7) have demonstrated, through a series of adoptive transfer studies, that CD4⁺ T cells of the Th2 subset and signaling through IL-4 receptor α-subunit have significant roles in mucus production, although IL-4 expression is not required. Likewise, Gavett et al. (15) have demonstrated that the administration of an IL-4 receptor antagonist blocks allergen-induced mucus production, suggesting a role for IL-13 in this process (6, 20). Consistent with this finding, Grunig and colleagues (18) have demonstrated that selective neutralization of IL-13 inhibits allergen-induced mucus overproduction and expression of IL-13 from the lung epithelium of transgenic mice induces goblet cell metaplasia and mucus overproduction in the absence of an allergen challenge (44). Additional studies (8), however, have also demonstrated that adoptively transferred Th1 polarized T cells are as efficient as Th2 committed cells at inducing mucus production in IFN-γ receptor-deficient recipient mice. These data suggest that mucus production after allergen exposure is complex and is likely a consequence of interactions between several T cell-derived factors.

Previous studies (e.g., see Ref. 6) have demonstrated that goblet cell metaplasia and mucus production can occur in the absence of IL-5 and significant airway eosinophilia. Our data support this conclusion by demonstrating that increased IL-5 levels and robust airway eosinophilia are insufficient to induce mucus. This indicates that IL-5 does not have a direct effect on airway...

Fig. 4. Mucus production in interleukin-5 (IL-5) transgenic mice is dependent on signaling through IL-4 receptor α-subunit. Naive NJ.1726 mice were administered the IL-4 receptor antagonist QY for 10 consecutive days before assessment of mucus production. Wild-type saline- and OVA-treated mice are shown for comparison purposes. The histograms represent the average mucus index for each cohort. Values are means ± SE; n = 5–8 mice/group. *P < 0.05.

(21) have demonstrated that inhibition of either 5-lipoxygenase or the 5-lipoxygenase-activating gene blocks airway mucus overproduction after intranasal OVA challenge. Given the rapid manner in which leukotriene synthesis and release occurs (19), the aforementioned data suggest that cells associated with the acute response to allergen exposure (i.e., macrophages or airway epithelium) are critical for the induction of mucus production. These seemingly contrary reports raise the question as to whether cells of the acquired immune response (i.e., CD4⁺ T cells) and the cytokines associated with a Th2 immune reaction (i.e., IL-4, IL-5, and IL-13) are the sole inflammatory pathways leading to allergen-induced mucus overproduction.

Assessments of airway anatomy and the rate of metaplastic airway epithelial cell responses in the mouse, however, argue against an acute response to allergen exposure. Secretory serous cells and mucus-producing cells (i.e., goblet cells) are rarely observed in naive mice and epithelial-associated mucous glands are virtually nonexistent (33). Instead, airway Clara cells are the predominant cell type, comprising ~60% of the airway epithelium in the mouse (34, 39). After exposure to allergens and/or toxicants, the secretory cells undergo metaplasia/hyperplasia and concurrently accumulate mucus within cytoplasmic granules. However, the time course of these events is quite prolonged. It has been shown that increased expression of MUC2 mucin transcripts takes ~24 h, whereas the presence of the fully glycosylated protein requires at least 48 h after toxicant exposure (38). A similar time course has been suggested for the other major respiratory mucin MUC5A/C (41). Therefore, the anatomic data from mice concerning mucus-producing cell types and the...
epithelium to induce goblet cell metaplasia and mucus production. However, the ability of IL-5 to elicit mucus production through a CD4+ T cell-dependent mechanism suggests that pulmonary expression of IL-5 represents an additional mechanism during an allergen provocation capable of eliciting mucus production. The demonstration that this IL-5-mediated effect is dependent on the presence of CD4+ T cells suggests a previously unknown activity associated with this cytokine. In particular, does IL-5 mediate the proliferation of specific T cell subpopulations and/or does IL-5 signaling activate T cells in such a way as to elicit, among other events, goblet cell metaplasia and mucus production? The dramatic increase in pulmonary CD4+ T cells in naive NJ.1726 mice, as well as the dramatic increase in circulating T cells associated with another IL-5 transgenic line (28), implies a previously unknown IL-5 effector function on T cells, although an indirect mechanism mediated by IL-5 effects on a third cell population cannot be ruled out.

It is possible that IL-5 may induce T cell expression of various factors (e.g., release of eicosanoids directly by T cells or indirectly by T cell activation of eosinophils), leading to increased mucus production independent of Th2-associated inflammatory signals. However, IL-5-mediated secretion of IL-4/IL-13 by T cells would be a parsimonious explanation linking IL-5 effector function and goblet cell metaplasia and mucus production. Alternatively, because eosinophils are capable of releasing Th2 cytokines (43), the pulmonary eosinophilia induced by IL-5 expression may play a significant role via T cell-mediated activation. Although IL-4 and IL-13 expression was not elevated above basal levels in the NJ.1726 mice (data not shown), this does not rule out the possibility that IL-5 may lead to the release of these cytokines at low (i.e., undetectable) concentrations.
levels or in a time-dependent fashion by activating T cells and/or, in turn, possibly eosinophils. Indeed, the administration of an IL-4 receptor antagonist inhibited the mucus production apparently mediated by IL-5, demonstrating a role for IL-4/IL-13 in the observed mucus production associated with NJ.1726 mice. Provocatively, similar data are found in a mouse study of OVA-induced respiratory inflammation utilizing anti-IL-5 antibody (25). In this study, administration of an anti-IL-5 monoclonal (TRFK-5) to OVA-treated wild-type mice led to a significant decrease in mucus production relative to control antibody-treated mice. These data suggest that IL-5 expression in allergen-treated wild-type mice also uniquely augments mucus production and may represent an important component of signaling pathways effecting the absolute levels of mucus production in the lung. Thus in addition to effects primarily on eosinophils and eosinophil progenitor cells, IL-5 apparently has a novel function(s) in allergic pulmonary inflammation that leads to mucus production. This conclusion would imply the existence of a previously unrecognized IL-5 activity (activities) on T cells critical to the development of allergen-induced pulmonary pathology.

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