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IL-13 Induces Esophageal Remodeling and Gene Expression by an Eosinophil-Independent, IL-13Rα2–Inhibited Pathway

Li Zuo,* Patricia C. Fulkerson,* Fred D. Finkelman,† Melissa Mingler,* Christine A. Fischetti,* Carine Blanchard,* and Marc E. Rothenberg*

Eosinophilic esophagitis (EE) is an emerging disease associated with both food and respiratory allergy characterized by extensive esophageal tissue remodeling and abnormal eosinophil gene expression, including increased IL-13. We investigated the ability of increased airway IL-13 to induce EE-like changes. Mice with pulmonary (but not esophageal) overexpression of IL-13 evidenced esophageal IL-13 accumulation and developed esophageal remodeling with epithelial hyperplasia, angiogenesis, collagen deposition, and increased circumference. IL-13 induced notable changes in esophageal transcripts that overlapped with the human EE esophageal transcriptome. IL-13–induced esophageal eosinophilia was dependent on eotaxin-1 (but not eotaxin-2). However, remodeling occurred independent of eosinophils as demonstrated by eosinophil lineage-deficient, IL-13 transgenic mice. IL-13–induced remodeling was significantly enhanced by IL-13Rα2 deletion, indicating an inhibitory effect of IL-13Rα2. In the murine system, there was partial overlap between IL-13–induced genes in the lung and esophagus, yet the transcriptomes were divergent at the tissue level. In human esophagus, IL-13 levels correlated with the magnitude of the EE transcriptome. In conclusion, inducible airway expression of IL-13 results in a pattern of esophageal gene expression and extensive tissue remodeling that resembles human EE. Notably, we identified a pathway that induces EE-like changes and is IL-13–driven, eosinophil-independent, and suppressed by IL-13Rα2. The Journal of Immunology, 2010, 185: 660–669.

The etiology of EE is poorly understood, but allergy has been implicated. In fact, the majority of EE patients have evidence of food and aeroallergen hypersensitivity as defined by skin prick testing and in vitro lymphocyte responses (4). Previous studies have shown that repeated challenge of mice with intranasal Aspergillus fumigatus Ag induces lymphocyte-dependent EE changes, supporting the association between airway allergy and the development of EE (5). In addition, eosinophilic inflammation in the esophagus can be induced directly by intranasal or intratracheal human or murine IL-13 (6) and can be blocked by anti-human IL-13 Ab (6). In addition, aeroallergen-induced EE is dependent on IL-13, as assessed by failure of IL-13–gene-targeted mice to develop esophageal eosinophilia and epithelial hyperplasia (7). Based on these findings in mice, IL-13 may have a role in EE induction in humans. Indeed, IL-13 is overproduced in the esophagus of EE patients and is capable of inducing a gene expression profile in esophageal epithelial cells that partially overlaps with the esophageal transcriptome present in EE patients (8). The IL-13–induced, epithelial cell-produced gene that is most overexpressed in the esophagus of EE patients is eotaxin-3, a potent eosinophil chemokine and activating factor (8). Whether IL-13 directly contributes to esophageal tissue pathology (independent of eosinophils) has not been determined. Although IL-13 has been shown to be involved in the development of tissue remodeling in the lung (9) and intestine (10), its role in the development of esophageal remodeling has not been examined. Experimental models of aeroallergen and IL-13 delivery to the lung have shown that eosinophils contribute, at least in part, to esophageal epithelial hyperplasia and collagen levels in the esophagus. Yet, IL-13 has also been shown to induce tissue remodeling by eosinophil-independent effects under a variety of conditions within multiple tissues (9–12). As such, it is relevant to determine whether IL-13 is capable of directly or indirectly inducing esophageal remodeling typical of EE and the relevance of this process to human EE. To test these possibilities, we used a murine, inducible, lung-specific IL-13 transgenic model, wherein IL-13 is selectively overexpressed in the lungs in an externally regulated fashion after transgene induction with dietary doxycycline (9). We aimed to determine the ability of chronic airway IL-13 stimulation to induce experimental EE with tissue remodeling, the mechanism and receptors involved in disease development, and the applicability of our results to human EE.
**Materials and Methods**

**Inducible, lung-specific IL-13 transgenic mice**

Bitransgenic mice (CC10-IL-13) were generated in which IL-13 was expressed in a lung-specific manner that allowed for external regulation of the transgene expression as previously described (13). CC10-IL-13 mice deficient in eosinophils, eotaxin-1, and eotaxin-2 were generated by breeding the CC10-IL-13 (FVB/N) with the Δdbl-GATA (BALB/c), eotaxin-1 (SVEV), and eotaxin-2 (SVEV) gene-targeted mice for three generations. Δdbl-GATA mice were generously provided by Drs. Alison Humble and Craig Gerard (Children’s Hospital, Boston, MA). For all experiments, wild-type mice with the appropriate mixed backgrounds (FVB/N) were used as controls. Transgene expression was induced by feeding bitransgenic mice doxycycline-impregnated food (DOX) (625 mg/kg; Purina Mills, Richmond, IN). Animals were housed under specific pathogen-free conditions in accordance with institutional guidelines.

**Quantification of tissue eosinophils**

Eosophageal eosinophils were detected using an immunohistochemical stain against murine eosinophilic major basic protein (MBP) as reported (9). Endogenous peroxidase activity was quenched using a 0.3% hydrogen peroxide in methanol solution. Tissue was then subjected to pepsin for 10 min at 37°C (DIGEST-ALLTM 3, Zymed Laboratories, San Francisco, CA). Non-specific binding was blocked using 5% goat serum in PBS for 2 h at room temperature, followed by the addition of rabbit anti-murine MBP (1:8000) primary Ab (kindly provided by Dr. Jamie Lee, Mayo Clinic, Scottsdale, AZ), which was allowed to incubate overnight at 4°C. Slides were incubated with biotinylated goat anti-rabbit (1:250) secondary Ab for 40 min at room temperature, and then incubated with an avidin-peroxidase complex for 30 min (Vector Laboratories, Burlingame, CA). Development of peroxidase reaction was achieved by incubating slides with nickel diaminobenzidine-cohult chloride solution (Vector Laboratory) for 4 min at room temperature. Slides were then counterstained with nuclear fast red. Quantification of positive cells was performed using ImagePro Plus imaging software and results were reported as immunoreactive cells per square millimeter.

**Epithelial thickness and collagen quantitation**

Epithelial thickness was determined by staining cross-sectional eosophageal samples with H&E. Quantitation of thickness was performed using Image-Pro Plus imaging software by taking three to six lengthwise measurements per slide from the lumen to the basement membrane of each esophagus. Collagen deposition was determined by staining esophageal samples with trichrome and quantitated using ImagePro Plus imaging software. Collagen measurements are recorded as area of collagen staining per length of basement membrane.

**Analysis of epithelial cell proliferation**

To determine the degree of epithelial cell proliferation, 5′-BrdU (Zymed Laboratories) incorporation analysis was performed according to previously reported methods (6). In brief, DOX and untreated (NO-DOX) mice were injected i.p. with 0.25 ml 5′-BrdU solution (0.75 μg BrdU) 2 h before death. The esophagus was fixed with 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO) for 24 h. After fixation, the tissue was embedded in paraffin, and 5-μm sections were processed using standard histologic approaches. Tissue was digested with trypsin (0.125%) for 3 min at 37°C, followed by incubation for 30 min at room temperature. Sections were washed with PBS three times for 2 min and further incubated with monoclonal biotinylated anti-BrdU Ab for 60 min at room temperature. Negative controls included replacing the primary Ab with PBS, and positive controls were provided by the manufacturer. Cells with nuclear staining for BrdU were detected with streptavidin-peroxidase and DAB substrate (Zymed Laboratories), followed by counterstaining with H&E. The BrdU+ cell quantitation was carried out with the assistance of digital morphometry as described previously for eosinophils.

**Assessment of angiogenesis**

Tissues were fixed, embedded, sectioned, and prepared as described previously. Slides were trypsinized with 0.1% trypsin at 37°C for 5 min (BD Difco, Franklin Lakes, NJ), incubated with 180 ml methyl alcohol and 3 ml 30% hydrogen peroxide, blocked with 2% rabbit serum/PBS/Triton for 2 h, and incubated overnight with rat anti-mouse CD31 (PECAM-1) Ab (BD Pharmingen, 01951D, San Diego, CA) at 4°C. Slides were then incubated with biotinylated rabbit anti-rat Ab (Vector Laboratory, BA-4001) for 30 min, developed with ABC complex and DAB substrate, and counterstained with 0.1% nuclear fast red in 5% aluminum sulfate for 2 min. Morphometry was used to determine the average number of PECAM-1 positive vessels per high-power field in each group.

**Determination of esophageal circumference**

The esophagus was isolated by cutting the proximal end at the level of the cricoid ring and the distal end 2–5 mm above the stomach. It was then suspended in HBSS and longitudinally exposed on an agarose gel for the measurement of esophageal circumference.

**Measurement of IL-13 and IL-13/soluble IL-13Ra2 complex**

Protein content of IL-13 and IL-13/soluble IL-13Ra2 complex was determined by ELISA (14). For IL-13, an IL-13-specific ELISA kit (R&D Systems, Minneapolis, MN) was used to measure the protein level from homogenized tissue solutions. IL-13/soluble IL-13Ra2 complex was quantified using a capture ELISA method. Briefly, 96-well plates were coated with anti–IL-13 Ab (R&D Systems) overnight at 4°C. Samples of either blood serum or soluble extract of homogenized esophagus were applied to the plate and incubated at room temperature for 2 h. Wells were washed and then incubated at room temperature with biotinylated anti-mouse IL-13Ra2 Ab (R&D Systems) for 2 h, streptavidin-HRP conjugate (R&D Systems) for 20 min, and substrate solution (R&D Systems) for 20 min. Absorbance was read at 450 nm, and OD readings were converted to nanograms per milliliter. To determine the total IL-13Ra2 level, samples were presaturated with IL-13 prior to being added to the wells. A saturation percentage was calculated using the ratio of IL-13/soluble IL-13Ra2 complex divided by total IL-13Ra2.

**Microarray analysis**

RNA from the esophagus or lung of inducible IL-13 transgenic mice obtained after 30 d of DOX or NO-DOX treatment was subjected to gene chip analysis using MOE 430 2.0 chips as previously reported (15). Gene transcript levels were determined using algorithms in the Microarray Analysis Suite and GeneSpring software (Silicon Genetics, Redwood City, CA). For comparison with human EE, murine genes were translated to human homologs represented on the human U133 chip using GeneSpring software and compared with microarray results previously described (8, 15). Welch’s t test and fold change cutoff were performed. The correlation between IL-13 expression and levels of dysregulated genes were also analyzed using GeneSpring software. Raw microarray data in GEO format is available electronically with GEO Submission number GSE21267 at www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE21267.

**Real-time quantitative PCR analysis**

RNA samples from the whole esophagus were subjected to reverse transcription analysis using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) according to manufacturer’s instructions. Real-time PCR analysis of transgenic IL-13 and rtTA levels was performed using the LightCycler 480 system in conjunction with the ready-to-use LightCycler 480 SYBR Green I Master reaction kit (Roche Diagnostic Systems, Branchburg, NJ). Results were normalized to GAPDH cDNA. Amplification of cDNA was achieved using the following primers: transgenic IL-13 (205 bp), 5′-ATGCCTGCACTA-GAAGGC-3′ and 5′-CATCTACAGGACCAGAGGA-3′; rtTA (200 bp), 5′-GCCATTAGCTGCTTATAG-3′ and 5′-AAAATCTGAGCATTT-CTCCC-3′; eosin 1 (87 bp), 5′-CCAACACTAAGAGGACAGCT-CAAAC-3′ and 5′-TTTGGCCAAAACCCTGTGCTT-3′; eosin 2 (280 bp), 5′-TGTGACCATCCCTACATCTGTTG-3′ and 5′-AAACATGCTGGTGATCTTCCAGC-3′; human IL-13 (125 bp), 5′-ACAGCCCTTCAGGGACAGCT-3′ and 5′-TCAGTTGTAGTCTCCATACC-3′.

**Statistical analysis**

Statistical significance comparing different sets of mice was determined by Student t test. The p values <0.05 were considered statistically significant. SPSS software was used for the statistical analysis.

**Results**

**Eosophageal eosinophilia induced by pulmonary IL-13 overexpression**

Dietary DOX exposure of ill-13 transgenic mice increased eosophageal eosinophilia ~47-fold in ill-13 transgenic mice (Fig. 1). Representative photomicrographs of untreated and DOX-treated mice stained with anti-MBP Ab to detect eosinophils are shown in Fig. 1A and 1B, respectively. Eosinophils infiltrated all layers of the esophagus, including the muscularis mucosa, lamina propria,
and epithelium. A kinetic analysis revealed that eosinophilic epithelium peaked after 10 d of DOX and remained elevated during the 30-d experiment (Fig. 1C). The kinetics of eosinophil and pulmonary eosinophilia differed, but both returned to baseline levels by 3 wk after DOX withdrawal. Airway IL-13 did not increase eosinophil levels in gastrointestinal segments distal to the esophagus. For example, stomach eosinophil levels in untreated and DOX-treated mice were 12.4 and 7.6/mm², respectively ($p = 0.7$).

**Esophageal tissue remodeling induced by increased pulmonary IL-13 expression**

DOX ingestion for 30 d induced marked inflammatory and remodeling changes in the esophagus of iIL-13 transgenic mice (Fig. 2). Microscopically, DOX increased epithelial thickness (Fig. 2A, 2B, Supplemental Fig. 1A) after 30 d of DOX and remained elevated until 3 wk after DOX withdrawal. Eosinophil levels in the lamina propria in DOX-treated mice were 2.6 times higher in the esophagus than in the lung (Fig. 2C). The kinetics of transgenic IL-13–induced genes, such as cathepsin C (17), suggest a role for IL-13 in esophageal remodeling in mouse and man.

Morphometric analysis also showed that the esophageal circumference was increased in DOX-treated mice (Fig. 3A, 3B). DOX treatment increases esophageal IL-13 protein, but not IL-13 mRNA

DOX treatment for 4 wk increased IL-13 protein nearly 1000-fold in the lungs and ~8-fold in the esophagus of iIL-13 transgenic mice (Fig. 4A). Esophageal IL-13 peaked after 1 wk of DOX treatment and plateaued over the next 3 wk; whereas, pulmonary IL-13 continued to increase. IL-13 mRNA (measured using transgene-specific primers) increased in the lung but not the esophagus during the same period (Fig. 4B). DOX did not increase the expression of endogenous IL-13 mRNA (data not shown). The finding of increased transgenic IL-13 mRNA in the lung but not in the esophagus is consistent with the lung specificity of this IL-13 transgenic model.

**IL-13 induces marked in situ eosinophilic gene expression**

To define the molecular pathways induced by IL-13 in the esophagus, we conducted a genome-wide microarray expression profile analysis of RNA isolated from the esophagus of IL-13 transgenic mice that were exposed to DOX for 30 d and compared the gene transcript levels with age- and gender-matched transgenic mice that were not exposed to DOX, using methods previously reported (9). DOX-induced increases in IL-13 protein were associated with a significant difference in 767 genes ($p < 0.05$) in the esophagus of transgenic mice. This amount constitutes 1.7% of the total transcriptome (45,101 transcripts). Among those 767 genes, 80 genes were modified ≥ 2 fold (Supplemental Table I). These genes encode cell-signaling proteins, cellular adhesion proteins, and cell surface glycoproteins. Eosinophil-associated RNase, IL-1R–like 1, MAPK-1, cholinergic receptor, leukotriene C4 synthase, C3a receptor 1, and IFN-induced transmembrane protein 6 were included in the IL-13–induced EE transcriptome.

**Comparison of human EE and IL-13–induced murine EE transcriptomes**

There were 283 genes in the murine IL-13–induced esophageal eosinophilia that overlapped with the human EE transcriptome ($p < 0.05$) (Supplemental Table II). The genes significantly conserved between experimental EE and human EE are presented in a heat diagram (Fig. 5A). Functional analysis of genes that have >5-fold changes in both the human or murine system (Table I) revealed their involvement in cell communication, cytokine–cytokine receptor interaction, calcium signaling, histidine metabolism, complement and coagulation cascades, JAK-STAT signaling, and arachidonic acid metabolism. Notably, three CXC chemokines (CXCL1, 2, and 6) were also among those genes that overlapped. Both CXCL1 and CXCL2 have been shown to mediate IL-13–induced remodeling and inflammatory processes in the lung (12, 16).

In the human system, 343 genes correlated with IL-13 mRNA levels (Fig. 5B, Supplemental Table III). These included known IL-13–induced genes, such as eotaxin-3, as well as genes involved in tissue remodeling, such as collagen type VIII alpha2, cathepsin S, cathepsin C, and fibrinogen g-chain. Taken together, these results suggest a role for IL-13 in esophageal remodeling in mouse and man.

**IL-13 induces esophageal eotaxin-1 and eotaxin-2, which are required for esophageal eosinophilia**

To investigate the mechanism of IL-13–induced eosinophilia in this experimental EE model, we examined eotaxin production in the lung and esophagus. Esophageal and pulmonary protein levels of eotaxin-1 (Fig. 6A) increased after 1 wk of DOX administration and remained elevated at 4 wk of DOX. The level of eotaxin-1 in
the lung peaked by 4 wk of DOX administration. The level of eotaxin-2 (Fig. 6B) increased after 1 wk of DOX administration and remained elevated at 4 wk in the lung and esophagus. The level of eotaxin-1 and eotaxin-2 mRNA in the esophagus increased by 3.8- and 1.48-fold, respectively, between NO-DOX and DOX mice using gene chip analysis. To investigate if eotaxins were mediating IL-13–driven esophageal eosinophilia, we generated IL-13 transgenic mice that were deficient in eotaxin-1 or eotaxin-2. These studies revealed that IL-13–driven esophageal eosinophilia was primarily eotaxin-1 dependent (Fig. 6C).

**IL-13–induced esophageal remodeling is independent of eosinophils**

To determine the role of eosinophils in promoting the tissue remodeling seen in the IL-13 transgenic mice, we examined triple transgenic mice [CC10/IL-13/GATA-1 knockout (KO)]. These mice express IL-13 but lack the eosinophil lineage because of deletion of the double palindromic site in the GATA1 promoter (ΔdblGATA). These triple transgenic mice had no esophageal eosinophilia after DOX induction for 4 wk (Fig. 7A) but still developed the same degree of tissue remodeling, including epithelial thickness (Fig. 7B), collagen deposition (Fig. 7C), and cellular hyperplasia (Fig. 7D).

These data indicate that IL-13–induced esophageal tissue remodeling occurs independent of eosinophils in this animal model.

**Increased accumulation of IL-13Rs**

To further investigate the mechanism of IL-13–induced EE, we examined the accumulation of IL-13Rα2 as well as IL-13/IL-13Rα2 protein complexes because IL-13Rα2 has been shown to have a “decoy” effect on IL-13 function. Protein levels of free and IL-13–bound sIL-13Rα2 were increased in both the blood (Fig. 8A) and the esophagus (Fig. 8B) after 4 wk of DOX. The data showed that essentially all the sIL-13Rα2 is complexed with IL-13 in the esophagus, suggesting that free IL-13 may also be present. We also tested the mRNA expression of IL-13Rα2 using real-time PCR.

**FIGURE 2.** IL-13 transgene-induced esophageal tissue remodeling. The esophagus of lung-specific IL-13 transgenic mice without (NO-DOX) (A, C, E, G) and after (B, D, F, H) 4 wk of DOX exposure was stained with H&E (A, B), for BrdU incorporation (C, D), trichrome (E, F), and anti–PECAM-1 (G, H). Representative images are shown of three independent experiments with n = 6 mice in each experiment. Original magnification for A, B, and E–H, ×100; C and D, ×400. EP, epithelial; L, lumen; LP, lamina propria; MM, muscularis mucosa.

**FIGURE 3.** Esophageal circumference. The excised esophagus without (NO-DOX) and after DOX exposure is shown in (A). Quantitative assessment of the esophageal circumference is shown in (B). The results are presented as mean ± SD, n = 6 mice and the experiment was repeated independently at least three times.

**FIGURE 4.** IL-13 accumulation and expression in the esophagus and lung. Protein levels of IL-13 from pulmonary and esophageal tissue (n = 8 mice for each group) following a time course of DOX exposure were measured by ELISA (A). The expression of IL-13 mRNA in the esophagus and lung after 4 wk of DOX versus NO-DOX exposure is shown. Real-time PCR analysis of IL-13 expression level is performed and results are normalized to GAPDH cDNA (B). The results are the summary of three independent experiments, reported as mean ± SD with n = 6 mice for each group or time point in each experiment.
Although DOX ingestion significantly increased pulmonary IL-13Ra2 expression ($p < 0.05$), it had no significant effect on IL-13Ra2 expression in the esophagus ($p = 0.15$) (data not shown).

Inhibitory effect of IL-13Ra2 on esophageal tissue remodeling in lung-specific, IL-13 transgenic mice

IL-13Ra2 has been described as both an IL-13 antagonist and a mediator of IL-13–induced fibrosis (17, 18). To investigate the role of IL-13Ra2 in EE, we generated triple transgenic mice that overexpressed CC10/IL-13 and were genetically deficient in IL-13Ra2. DOX-induced IL-13 stimulated more severe esophageal remodeling in IL-13Ra2–deficient mice than in IL-13Ra2–sufficient mice (Fig. 9); there was increased epithelial thickness (Fig. 9B), collagen deposition (Fig. 9C), and esophageal circumference (Fig. 9D). Esophageal tissue eosinophilia, however, was similarly induced by IL-13 in IL-13Ra2–deficient and IL-13Ra2–sufficient mice (Fig. 9A). The increased esophageal remodeling, lung inflammation, and tissue necrosis induced by transgenic IL-13 in IL-13Ra2–deficient mice (not shown) was accompanied by increased weight loss and death (Fig. 10).

Comparison of the IL-13–induced pulmonary and esophageal transcriptomes

We aimed to define molecular pathways that would distinguish pulmonary and esophageal responses induced by IL-13, especially because IL-13–induced remodeling is eosinophil-dependent in the human EE. The 283 genes presented in the heat diagram (standard correlation) correspond to genes significantly different in experimental EE ($p < 0.01$) and human EE ($p < 0.01$) compared with their respective controls (NO-DOX and normal [NL] patient esophageal biopsies). Each line represents a separate individual. Upregulated and downregulated genes are shown in red and blue, respectively. B, The correlation between IL-13 expression and dysregulated genes. The 343 genes correlated with eosinophil levels (based on Spearman correlation) are shown in the gene tree format. Samples are organized in the order of increased IL-13 expression (from left to right) with each sample normalized for GAPDH expression. This data represents samples from a single patient, but it was repeatable in different patient samples.

Table I. Comparison of human EE and IL-13–induced murine EE transcriptomes

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Functional analysis of genes that have >5-fold changes in either the human or murine system revealed their involvement in cell communication, cytokine–cytokine receptor interaction, calcium signaling, histidine metabolism, complement and coagulation cascades, JAK-STAT signaling, and arachidonic acid metabolism. Those genes are among genes in the murine IL-13–induced esophageal transcriptome that overlapped with the human EE transcriptome ($p < 0.05$). DOX, mice treated with Dox for 4 wk; EE, biopsy samples from EE patients; NL, biopsy samples from non-EE patients; NO DOX, untreated mice.
lung (11), but not in the esophagus according to our current study. There were 836 genes modified by ≥2-fold in the lungs or esophagus. Cluster analysis stratified IL-13–induced genes into conserved (clusters 2 and 4) and discordant (clusters 1 and 3) clusters between the lungs and the esophagus (Fig. 11). In cluster 2, 126 genes increased in both the lung and esophagus by 5-fold (Supplemental Table IV). Notably, these gene families are involved in chemotaxis (C3a, CCL6, CCL8, CCL9, and CCL11), cytokine signaling (IL-27Rα, IL-7R, and CSFRs), and other immune and inflammatory functions (chitinase 3, platelet selectin, and serum amyloid). Consistent with a role for TGF-β and MMP-12 in promoting IL-13-induced esophageal fibrosis, DOX significantly increased levels of esophageal TGF-β1 and MMP-12 mRNA by 2.0- and 2.7-fold as assessed by gene chip analysis (data not shown). These two genes are increased in both the lung and esophagus (cluster 2 above). In cluster 4, 67 genes decreased in both the lung and esophagus by ≥5-fold (Supplemental Table V). Among these genes were the somatotropin hormone gene family (such as prolactin-like protein E) and ion binding protein genes (zinc finger protein 64 and carbonic anhydrase 3). Cluster 4 also included three

FIGURE 6. Eotaxin production in the lung and esophagus. Protein levels of eotaxin 1 (A) and eotaxin-2 (B) in the esophagus are shown after 1–4 wk of DOX administration. Results were normalized to the esophageal protein content and are expressed as mean ± SD; p < 0.01 for week 1 and 4 lung and esophageal time points compared with DOX on week 0. The esophageal eosinophil count from IL-13 transgenic mice was compared with IL-13/eotaxin-1 and IL-13/eotaxin-2 KO mice (C). *p < 0.001 wild-type versus KO. The figures are the summary of three independent experiments with n = 4 for each group per experiment.

FIGURE 7. Effect of Δdbl-GATA (GATA-1 KO) on IL-13 transgene-induced esophageal tissue remodeling. The esophagi of lung-specific IL-13 transgenic mice and IL-13 transgenic/GATA-1 KO mice after 4 wk of DOX exposure were stained with anti-MBP (A), H&E (B), trichrome staining (C), and BrDU (D). Morphometric analysis was shown for each of those measurements. The figures are the summary of three independent experiments with n = 4 for each group per experiment.
genes involved in sensory perception (tectorin β, sry-box containing gene, and cadherin 23) and two genes involved in neurogenesis (zinc finger protein of the cerebellum 2 and paired box gene 5). Other genes in this cluster included IL-12β, adrenergic receptor α2c, and keratin complex 2. In cluster 3, 52 genes decreased in the lung but increased in the esophagus (Supplemental Table VI). In cluster 1, 39 genes increased in the lung but decreased in the esophagus (Supplemental Table VII). Clusters 1 and 3 are particularly interesting because these transcriptomes may be associated with tissue-specific function. Taken together, these results indicate that IL-13 induces a set of shared and tissue-specific genes in the lung and esophagus.

Discussion

Esophageal eosinophilia is the characteristic finding of human EE but the mechanisms involved in tissue pathology, including the role of eosinophils, remain poorly understood (19). Among environmental factors, both food and aeroallergen hypersensitivity have been implicated in the induction of EE, suggesting the involvement of Th2 immunity. Indeed, the Th2 cytokine IL-13 has been shown to be markedly overexpressed in the esophagus of EE patients and to induce eotaxin-3 in esophageal epithelial cells, yet the involvement of IL-13 with other aspects of disease remains uncertain. In this study, inducible expression of IL-13 in the lung resulted in extensive esophageal eosinophilia and tissue remodeling. Although pulmonary eosinophilia was also observed, the kinetics of eosinophilia was different between the lung and esophagus. In fact, esophageal eosinophilia peaked around day 10 after DOX induction, which is earlier than the peak of pulmonary eosinophilia after DOX induction. Interestingly, both pulmonary and esophageal eosinophilia returned to near baseline levels 3–4 wk after withdrawal of DOX, suggesting that IL-13–induced eosinophilia is reversible in this animal model, similar to eosinophilia’s reversibility in patients after therapy.

In asthma patients, tissue remodeling has an important role in chronic inflammation and is part of the mechanism of airway obstruction (20). In EE patients, esophageal remodeling, including increased tissue fibrosis and angiogenesis, occurs even in pediatric patients (3); however, the mechanisms that promote these changes remain unclear. Our study establishes that overexpressing pulmonary IL-13 induces extensive esophageal tissue remodeling, including increased epithelial thickness, collagen deposition, epithelial cell proliferation, angiogenesis, and esophageal circumference. The increased esophageal circumference, which is visible to the naked eye, may be responsible for the esophageal furrowing notable by endoscopic analysis in patients (21). After lung-specific IL-13 transgene induction, a significant accumulation of IL-13 protein was observed in both the lung and the esophagus. The content of IL-13 in the lung was much higher than that in the esophagus. The content of IL-13 in the lung was much higher than that in the esophagus. In addition to the accumulation of IL-13 protein, both IL-13/IL-13Rα2 complex and total IL-13Rα2 were increased after IL-13 transgene induction.

FIGURE 8. Accumulation of IL-13/sIL-13Rα2 complexes. Protein levels of blood IL-13/sIL-13Rα2 complex (A) after a time course of DOX exposure were measured using ELISA. Protein content of esophageal IL-13/sIL-13Rα2 complex and total sIL-13Rα2 after 4 wk of DOX exposure were also measured by the same method (B). The figures are the summary of three independent experiments with n = 4 for each group per experiment.

FIGURE 9. Effect of IL-13Rα2 deficiency on IL-13 transgene-induced esophageal tissue remodeling. The esophagus of lung-specific IL-13 transgenic mice with or without IL-13Rα2 deletion were studied for esophageal eosinophilia (A), epithelial thickness (B), collagen deposition (C), and esophageal circumference (D). The figures are the summary of three independent experiments with n = 4 for each group per experiment.
IL-13 mRNA in situ hybridization failed to detect IL-13–positive cells in the esophagus of the IL-13–expressing mice (data not shown). Perhaps IL-13 protein accumulation in the esophagus may be derived from swallowing pulmonary-derived IL-13. Free IL-13 that is produced in the lung and either swallowed or carried from the lung to the esophagus via the blood likely binds to the type 2 IL-4R in the esophagus. The lack of gastric and intestinal eosinophilia distal to the esophagus is more supportive of the swallowing theory. Regardless, tissue eosinophilia did not occur distal to the esophagus, highlighting the esophageal tissue-specific effect of IL-13, at least in this experimental system.

In this study, we observed the coexistence of esophageal eosinophilia and tissue remodeling. Moreover, we demonstrated that eotaxin-1 (but not eotaxin-2) is required for esophageal eosinophilia. Notably, murine eotaxin-3 is a pseudogene in mice, and mouse eotaxin-1 appears to be the functional homolog of human eotaxin-3, which is the main chemokine operational in human EE (22, 23). Furthermore, we demonstrated that esophageal remodeling is eosinophil-independent, as much as we observed the same degree of tissue remodeling in eosinophil-sufficient and -deficient IL-13 transgenic mice. Thus, we have identified an IL-13–driven pathway that mediates EE-like changes independent of eosinophils, suggesting that some aspects of the disease may occur independent of eosinophils in humans. In our prior studies, we have demonstrated a role of IL-5–driven eosinophils in aeroallergen-induced epithelial proliferation (as measured by BrdU and basal layer thickness) and collagen layer thickness (5). In contrast, the current study is the first to identify eosinophil-independent responses in EE. Preliminary

**FIGURE 10.** Effect of IL-13Ra2 deficiency on death rate and weight loss. The death rate (A) and weight loss (B) were shown for IL-13 transgenic mice with or without IL-13Ra2 deficiency after 4 wk of DOX exposure. B, Clear bars indicate groups without IL-13Ra2 deficiency and dark bars indicate groups with IL-13Ra2 deficiency. The figures are the summary of three independent experiments with n = 4 for each group per experiment.

**FIGURE 11.** Esophageal transcript profile of lung-specific IL-13 transgenic mice. Comparison of transcript profiles of CC10-rtTa (tet on)-IL-13 transgenic mice in the lungs and esophagus is shown. The genes (2-fold cutoff) in lungs of mice treated with doxycycline (+, DOX 30 d) or left untreated (−, NO-DOX) were compared with the genes significantly different (p < 0.01) in the esophagus of mice treated with DOX (30 d) or left untreated (NO-DOX). The overlap of the two signatures, composed of 836 genes, is presented in a heat diagram and is clustered using gene tree analysis (standard correlation). Cluster 1 contains genes upregulated (red) in the lung and downregulated (blue) in the esophagus. Cluster 2 contains genes upregulated in the lung and the esophagus. Cluster 3 contains genes downregulated in the lung and upregulated in the esophagus. Cluster 4 contains genes downregulated in the lung and the esophagus.
studies in humans have suggested that humanized anti–IL-5 is a potentially useful therapy for lowering esophageal eosinophilia, although an effect on remodeling has not yet been proven; in fact, the limited patients examined still have extensive tissue remodeling (22, 24). We recognize that our system involves supraphysiological levels of IL-13; therefore, the direct action of IL-13 (not requiring eosinophils) may not apply to an Ag-driven process in patients. Nevertheless, our study provides a rationale for therapeutic intervention focused on the noneosinophilic components of the disease. Furthermore, because IL-13–induced pulmonary remodeling (using the same transgenic system) is eosinophil-dependent (11), whereas, the remodeling in the esophagus was not, our study highlights distinctive pathways for disease induction between the lung and esophagus even when disease is driven by the same atopic trigger (in this case IL-13). Notably, concurrent photomicrographs from the lung of IL-13 transgenic mice deficient in eosinophils revealed significantly less remodeling compared with IL-13 transgenic mice (data not shown). This is consistent with our prior studies (11), which incidentally used experimental lung tissue that overlapped in part with the exact mice studied in this report. Indeed, transcript expression profile analysis readily identified shared and unique molecular events between the lung and esophagus.

To further explore the mechanisms of IL-13–mediated tissue remodeling, we used a triple transgenic CC10/IL-13/IL-13Ra2 deletion mouse model. The triple transgenic with IL-13Ra2 deletion showed significantly enhanced remodeling including increased esophageal mucosal thickening and collagen deposition as well as clinical features (weight loss and death) compared with the double transgenic CC10/IL-13 mice. The enhanced esophageal remodeling after IL-13Ra2 deletion indicates that IL-13Ra2 inhibits IL-13–induced remodeling, consistent with a “decoy” effect of IL-13Ra2 that prevents IL-13 from binding to the stimulatory type 2 IL-4R (17, 25) and with recent pulmonary results from IL-13 transgenic IL-13Ra2–deficient mice (26). These findings indicate that IL-13Ra2–mediated TGF-β–dependent fibrotic effect (27) is not likely operational in EE. Taken together, our study establishes that (1) pulmonary IL-13 expression induces extensive esophageal tissue eosinophilia and remodeling, including fibrosis, angiogenesis, and epithelial hyperplasia (2); IL-13 promotes increased esophageal circumference (3); esophageal eosinophilia is mediated by eotaxin-1 (and not eotaxin-2), which appears to be the functional homolog of human eotaxin-3, a cytokine considered to have an important role in human EE (4); tissue remodeling occurs largely independent of eosinophils (5); IL-13Ra2 dampens IL-13–mediated esophageal pathology and clinical features (6); in vivo, IL-13 induces a marked esophageal transcriptome in mice that overlaps, at least in part, with the human EE transcriptome; and (7) IL-13 induces a set of shared genes in the lung and esophagus. Additional translational studies demonstrated that IL-13 levels directly correlate with relevant gene expression in esophageal tissue from EE patients. Thus, we establish that IL-13 is sufficient to induce significant esophageal pathology and remodeling in vivo, that this process is likely operational in human EE, and that disease pathology of EE occurs largely independent of eosinophils (under these experimental conditions) and is inhibited by IL-13Ra2. Consequently, pharmacological targeting of IL-13 production and signaling may be a worthwhile pursuit.

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Disclosures
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