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Esophageal Remodeling Develops as a Consequence of Tissue Specific IL-5-Induced Eosinophilia

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Abstract

Background & Aims—Eosinophilic esophagitis (EE) is an increasingly recognized disease that mimics gastro-esophageal reflux disease. Recently, EE has been associated with esophageal remodeling, but the mechanisms involved are poorly understood. We hypothesized that the development of EE in patients and in an experimental murine model would be associated with eosinophil-mediated tissue remodeling.

Methods—Histopathologic analysis of basal layer thickness and collagen accumulation was performed on the biopsy specimens of normal individuals, EE patients, and mouse esophageal tissue sections following experimental induction of EE in wild-type, eosinophil lineage-deficient, interleukin (IL)-5-deficient, and IL-5 transgenic mice, with the latter 2 mice groups having decreased and increased esophageal eosinophilia, respectively.

Results—An impressive accumulation of collagen in the epithelial mucosa and lamina propria, as well as basal layer thickening, was observed in the esophagus of patients with EE as well as in mice with experimental EE compared with controls. Significantly reduced lamina propria collagen and basal layer thickness were observed in IL-5-deficient mice and eosinophil lineage-deficient mice compared with wild-type mice following the induction of experimental EE. Furthermore, the esophagus of CD2-IL-5 transgenic mice showed increased basal layer thickness and collagen accumulation compared with nontransgenic mice, yet IL-5 intestine transgenic mice did not have EE-like esophageal changes. Additional analysis revealed increased IL-5 levels in the esophagus of EE patients, allergen-challenged wild-type mice, and CD2-IL-5 transgenic mice but not in IL-5 intestine transgenic mice.

Conclusions—These findings provide evidence that local IL-5-mediated eosinophilia is essential in the induction of esophageal remodeling.

Tissue remodeling is the result of an imbalance in tissue regeneration and repair,^{1,2} and leukocyte infiltration is believed to contribute, at least in part.³ In the case of asthma, tissue

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remodeling involves epithelial cell hyperplasia and thickening of the epithelial mucosa with the development of subepithelial fibrosis from deposition of extracellular matrix proteins (collagen) beneath the basement membrane.^{4,5} Some of these characteristics have been reported recently in the esophageal biopsy specimens of patients with eosinophilic esophagitis (EE)^{6,7} and in a murine model of experimental EE.^{8,9} EE is a chronic disease characterized by eosinophil infiltration into the esophageal mucosa.^{10–14} Although EE clinically mimics gastroesophageal reflux disease, it is distinguished by the magnitude of eosinophils and basal layer hyperplasia and the lack of response to antireflux medication.^{15,16} Evidence from animal models suggests that eosinophils may have a role in tissue remodeling through the release of lipid mediators, cytokines, and cytotoxic proteins,^{17–19} yet their role in esophageal remodeling has been largely unexplored. Therefore, we focused the present study on understanding the role of eosinophils in the induction of esophageal tissue remodeling such as basal layer thickening and collagen accumulation.

To dissect the mechanism responsible for the induction of collagen deposition and basal layer thickening, we investigated mice genetically deficient in interleukin (IL)-5 and transgenic for IL-5 under the control of the T-cell CD2 promoter because we have previously shown that these genetic events induce and reduce EE,^{20,21} respectively. The levels of eosinophils, lamina propria collagen, and basal layer thickness in the esophagus following induction of experimental EE were assessed. Furthermore, we analyzed EE patient biopsy specimens for the degree of eosinophils, lamina propria collagen, and basal layer thickness. Our results demonstrate that esophageal biopsy specimens of EE patients and the esophagus of mice with experimental EE have multiple features of tissue remodeling. Notably, mice deficient in IL-5 were protected from the development of esophageal remodeling compared with wild-type (WT) mice. In contrast, transgenic mice that overexpress IL-5 (specifically in T cells) had increased esophageal collagen and basal layer thickness. Taken together, we provide evidence that eosinophils and local expression of IL-5 in the esophagus have a crucial role in esophageal remodeling.

Materials and Methods

Patient Biopsy Specimens

Formalin-fixed, paraffin-embedded biopsy samples from the esophagus of normal or EE patients were obtained as per an institutional review board-approved protocol. A total of 32 esophageal biopsy specimens from patients was initially selected, and 8 EE and 6 normal biopsy specimens were subsequently selected on the basis of the presence of esophageal lamina propria to evaluate the presence of esophageal remodeling.⁷ EE patients were defined histologically as having >24 eosinophils/high-power field (hpf); normal individuals were defined as having 0 eosinophils/hpf and no esophageal abnormality.

Normal and EE Patient's Characteristics

The normal individuals and EE patients were selected without regard for age, atopic status, or gender. All EE patients' biopsy samples selected for esophageal remodeling studies had endoscopic signs of EE. Diagnosis was established based on the maximum eosinophil counts per hpf (magnification, 400×) and basal layer expansion. Normal was defined as having 0 eosinophils per hpf and no basal layer expansion. The normal biopsy specimens were obtained from patients with symptoms typical of gastroesophageal reflux disease and EE but were found to have completely normal esophageal endoscopic and microscopic analyses. Patients with EE were defined by more than 24 esophageal eosinophils/hpf and extensive basal layer hyperplasia. Some of the patients also had allergic diseases, and 2 normal individuals had asthma treated with leukotriene receptor antagonists. The detailed summary of the normal

individuals' and EE patients' characteristics including eosinophil levels in the esophageal epithelium, disease status, therapy, and trichrome staining scores are shown in Table 1.

Mice

BALB/c mice were obtained from Taconic Farms, Inc, New York, NY, and housed under specific pathogen-free conditions. The CD2-IL-5 transgenic,²¹ intestine fatty acid-binding protein promoter (FABPi)-IL-5 transgenic (iIL-5),²² and Δ dbl-GATA and IL-5-deficient mice (all BALB/c) were used as described previously.^{21,23} All mice were maintained in a barrier facility, and the animals were handled under Institutional Animal Care and Use Committee approved protocols. There was no serologic evidence of pathogens in sentinel mice maintained with the colony.

Allergen-Induced Experimental EE

A mouse model of allergic esophageal inflammation was established using methods described previously.²⁰ In brief, mice were lightly anesthetized with isoflurane (Iso-Flo; Abbott Laboratories, North Chicago, IL), and 100 μ g (50 μ L) *Aspergillus fumigatus* (Bayer Pharmaceuticals, Spokane, WA) or 50 μ L normal saline alone was applied to the nares using a micropipette with the mouse held in the supine position. After instillation, mice were held upright until alert. After 3 treatments per week for 3 weeks, mice were killed between 18 and 20 hours after the last intranasal challenge.

Collagen Staining

Esophageal tissue samples from mice and patients were fixed with 4% paraformaldehyde, embedded in paraffin, cut into 5- μ m sections, and fixed to positively charged slides. Collagen staining was performed on the tissue sections using Masson's trichrome (Poly Scientific R&D Corporation, Bay Shore, NY) for the detection of collagen fibers according to the manufacturer's recommendations.

Collagen Quantification on Tissue Sections

Multiple tissue pieces of the esophagus from 0.5 cm ascending to the stomach were taken from each mouse, and at least 4 or 5 random Masson's trichrome-stained sections/mouse were analyzed. Using digital image capture, tissue lamina propria regions of the esophagus were quantified for the thickness of the total accumulated collagen with the assistance of digital morphometric analysis using the Metamorph Imaging System (Universal Imaging Corporation, West Chester, PA), as described previously.^{9,21} The morphometric analysis was performed on a total of 10–12 mice/group in 3 experiments. Approximately 50–60 representative sections were analyzed, and results report the average of all experiments. The total area of lamina propria collagen was measured and normalized to the tissue length using the computerized software that provides both collagen area and length of the tissue measured and is expressed as collagen thickness in micrometers.

Quantification of Basal Layer Thickness

Multiple tissue pieces of the esophagus from 0.5 cm above the stomach were taken from each mouse, and at least 4 or 5 random H&E-stained sections/mouse were analyzed. Using digital image capture, the basal layer area of each esophageal section (1 to 5 layers thick) was measured by digital morphometric analysis using the Metamorph Imaging System computerized software (Universal Imaging Corporation, West Chester, PA), as described previously.^{9,21} The morphometric analysis was performed on a total 10–12 mice/group in 3 experiments. Approximately 50–60 representative sections were analyzed, and results report the average of all experiments. The system provides total selected area and length of the tissue measured.

The total area of the basal layer was normalized with the length measured and expressed as basal layer thickness in micrometers.

Semiquantitative Analysis of Collagen Accumulation in Patient Biopsy Specimens

Masson's trichrome-stained sections of normal individuals and EE patients were blindly examined under a light microscope for the semiquantitative collagen accumulation analysis. Absence of collagen in the tissue sections was graded as negative (–), and increased collagen staining was graded as positive (+) on a scale of 1–3.

Real-Time Polymerase Chain Reaction Analysis

The RNA samples (500 ng) were subjected to reverse-transcription analysis using Bioscript reverse transcriptase (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. IL-5, transforming growth factor (TGF)- β , and MUC5AC was quantified by real-time polymerase chain reaction (PCR) using the LightCycler instrument and LightCycler FastStart DNA master SYBR green I as a ready-to-use reaction mix (Roche, Indianapolis, IN). Results were then normalized to GAPDH amplified from the same complementary DNA (cDNA) mix and expressed as fold induction compared with the controls. cDNA was amplified using the mouse and human IL-5, TGF- β 1, and MUC5AC primers obtained from SuperArray Bioscience Corporation (Frederick, MD) and GAPDH by using the following primers: tggaatcccatcaccatct and gtcttctgggtggcagtgat.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation (SD). Statistical significance comparing different sets of mice was determined by unpaired InStat GraphPad *t* test (San Diego, CA).

Results

Esophageal Tissue Remodeling in EE Patients

The esophageal basal layer thickness and collagen accumulation in the lamina propria were examined in biopsy samples from pediatric individuals with EE and in normal individuals who had no abnormal gastrointestinal pathology. EE patient biopsy specimens from the proximal and/or distal esophagus demonstrated >24 eosinophils/hpf. Histopathologic analysis revealed a prominent accumulation of collagen in the lamina propria and in the papillae of EE patient biopsy specimens, whereas low levels of collagen were detected in the esophageal biopsy specimens of normal individuals (Figure 1A and B). A large increase of the lamina propria collagen thickness was observed in the EE patients' esophageal biopsy specimens compared with normal individuals' biopsy specimens. Morphometric analysis showed a thickened basal layer in EE patients' biopsy specimens compared with normal individuals' (Figure 1C). Normal individuals and EE patients had a basal layer thickness of $3.0 \pm 1.2 \mu\text{m}$ and $11.0 \pm 2.6 \mu\text{m}$, respectively (mean $\pm$ SD, $n = 12$ – 16 individuals, $P < .001$).

Lamina Propria Fibrosis in Allergen-Induced Experimental EE

Lamina propria collagen accumulation and basal layer thickness in the esophageal tissue sections were examined in allergen- and saline-challenged mice. Histopathologic analysis of Masson's trichrome-stained esophageal tissue sections revealed an impressive collagen accumulation in the lamina propria and a thickened basal layer in allergen-challenged mice compared with saline-challenged mice (Figure 2A–D). In addition, we also observed increase of muscularis mucosa thickness (Figure 2A–C) and impressive accumulation of trichrome-positive material in the elongated stromal papillae and into the muscularis mucosa (Figure 2C and D) of allergen-challenged mice. An increased thickening of the basal layer was observed in allergen-challenged mice compared with saline-challenged mice (Figure 2E and F).

Morphometric analysis indicated ~2-fold increase in basal layer thickness (Figure 2G) and >2-fold increase in lamina propria collagen thickness in allergen-challenged mice compared with saline-challenged mice (Figure 2H). The basal layer and lamina propria collagen thickness in the esophagus of saline- and allergen-challenged mice was $3.8 \pm 0.9 \mu\text{m}$ vs $9.2 \pm 2.3 \mu\text{m}$ (mean $\pm$ SD, $n = 12$) and $7.4 \pm 1.2 \mu\text{m}$ vs $19.9 \pm 2.2 \mu\text{m}$ (mean $\pm$ SD, $n = 12$), respectively.

Transcript Levels of TGF- β 1 and MUC5AC Are Induced in EE

TGF- β and mucin MUC5AC genes have a significant role in the induction of tissue remodeling in the lung^{24,25}; therefore, we examined transcript levels of TGF- β 1 and MUC5AC in the esophageal biopsy specimens of normal individuals and EE patients as well as in the murine system following saline and allergen challenge. Real-time quantitative PCR analysis indicated that both TGF- β 1 and MUC5AC messenger RNA (mRNA) levels were significantly induced in the esophagus of human EE patients (Figure 3A and B) and experimental EE (Figure 3C and D) compared with respective controls.

Esophageal Tissue Remodeling in Allergen-Induced EE Is Dependent on IL-5

We next tested the hypothesis that allergen-induced esophageal tissue remodeling was dependent on IL-5-mediated tissue eosinophilia. To test this, experimental EE was induced in WT and IL-5 gene-deficient mice, and esophageal tissue remodeling was determined by analyzing the lamina propria fibrosis and basal layer thickness. The IL-5 gene-deficient mice demonstrated no induction of collagen in the lamina propria, stromal papillae, and muscularis mucosa compared with WT mice following allergen challenge (Figure 4A and data not shown). A significant reduction in basal layer thickness in allergen-challenged IL-5 gene-deficient mice compared with allergen-challenged WT mice was also observed (Figure 4B). The basal layer thickness of allergen-challenged WT and IL-5 gene-deficient mice was $10.4 \pm 1.9 \mu\text{m}$ and $4.7 \pm 1.0 \mu\text{m}$ (mean $\pm$ SD, $n = 8$, $P < .01$), respectively, and the lamina propria collagen thickness of allergen-challenged WT and IL-5 gene-deficient mice was $21.1 \pm 2.2 \mu\text{m}$ and $5.2 \pm 1.0 \mu\text{m}$ (mean $\pm$ SD, $n = 8$, $P < .001$), respectively.

Lymphocyte-Derived IL-5 Promotes Esophageal Tissue Remodeling

We were next interested in establishing whether IL-5 overexpression in lymphocytes promoted esophageal tissue remodeling. Therefore, esophageal tissue sections from WT and CD2-IL-5 transgenic mice were analyzed for lamina propria collagen and basal layer thickness. The esophagus of CD2-IL-5 transgenic mice showed a thickened epithelium, expansion of the connective tissue, and collagen accumulation in the lamina propria and the extended stromal papillae (Figure 5A–C). Representative photomicrographs show increased basal layer and lamina propria collagen in CD2-IL-5 transgenic mice compared with WT mice (Figure 5A–C). The significant accumulation of collagen in the muscularis mucosa was also observed in IL-5 transgenic mice (Figure 5C). Morphometric analysis indicated ~2.5-fold increase in basal layer thickness (Figure 5D) and ~4-fold increase in lamina propria collagen thickness in IL-5 transgenic mice compared with WT mice (Figure 5E). Furthermore, we examined the contribution of eosinophils by examining IL-5 transgenic mice that were deficient in eotaxin-1. We have previously reported that these mice have decreased esophageal eosinophils compared with CD2-IL-5 transgenic mice.²¹ Morphometric quantitative analysis revealed a significant reduction in the basal layer and lamina propria collagen thickness in eotaxin-1-deficient CD2-IL-5 transgenic mice compared with CD2-IL-5 transgenic mice (Figure 5D and E). The basal layer and lamina propria collagen thickness in CD2-IL-5 transgenic mice was $11.0 \pm 1.8 \mu\text{m}$ and $25.5 \pm 4.7 \mu\text{m}$ (mean $\pm$ SD, $n = 10$), respectively, and in eotaxin-1-deficient CD2-IL-5 transgenic mice $6.7 \pm 1.4 \mu\text{m}$ and $16.3 \pm 3.3 \mu\text{m}$ (mean $\pm$ SD, $n = 8$), respectively, compared with $4.1 \pm 0.9 \mu\text{m}$ and $6.2 \pm 1.2 \mu\text{m}$ (mean $\pm$ SD, $n = 10$), respectively, in WT mice.

Esophageal Remodeling in Eosinophil-Deficient Δ dbl-GATA Mice

Next, we were interested in further establishing whether eosinophils had an essential role in promoting esophageal remodeling following allergen challenge using an independent approach. We addressed this by inducing experimental EE in eosinophil-deficient Δ dbl-GATA and WT mice. Following allergen challenge, Δ dbl-GATA mice had significantly reduced basal layer and lamina propria collagen thickness compared with WT mice. The saline-challenged Δ dbl-GATA and WT mice had comparable lamina propria collagen and basal layer thickness (Figure 6A and B). The basal layer thickness of allergen-challenged WT and Δ dbl-GATA mice was $11.4 \pm 2.9 \mu\text{m}$ and $7.1 \pm 2.2 \mu\text{m}$ (mean $\pm$ SD, $n = 10-12$, $P < .01$), respectively, and the lamina propria collagen thickness of allergen-challenged WT and Δ dbl-GATA mice was $22.8 \pm 5.9 \mu\text{m}$ and $9.9 \pm 2.7 \mu\text{m}$ (mean $\pm$ SD, $n = 10-12$, $P < .001$), respectively. Notably, exposure of Δ dbl-GATA mice to intranasal allergen did not induce any esophageal eosinophilia (data not shown).

FABPi-IL-5 Transgenic Mice Do Not Have Esophageal Tissue Remodeling

We examined whether ectopic overexpression of IL-5 in the intestine induced esophageal tissue remodeling. To test this, we examined basal layer thickness and collagen accumulation in mice that overexpressed IL-5 under the control of the rat FABPi intestinal promoter. The transgene is specifically expressed in the small intestine.²² The basal layer and lamina propria collagen thickness in iIL-5 transgenic mice were comparable with that in WT mice (Figure 7A and B). Basal layer thickness in WT and iIL-5 transgenic mice was $4.26 \pm 0.87 \mu\text{m}$ and $4.77 \pm 0.9 \mu\text{m}$ (mean $\pm$ SD, $n = 8$), respectively, and lamina propria collagen thickness was $5.84 \pm 1.17 \mu\text{m}$ and $6.59 \pm 1.36 \mu\text{m}$ (mean $\pm$ SD, $n = 8$, $P > .05$), respectively.

Interestingly, CD2-IL-5 transgenic mice and iIL-5 transgenic mice both demonstrated increased levels of eosinophilia in the esophagus (Figure 7C), yet the CD2-IL-5 transgenic mice had substantially higher levels. The eosinophil levels in the esophagus of WT and CD2-IL-5 transgenic mice were $1.9 \pm 0.9/\text{mm}^2$ and $121 \pm 14/\text{mm}^2$ (mean $\pm$ SD, $n = 10$, $P < .0001$), respectively, whereas WT and iIL-5 transgenic had $1.26 \pm 0.85/\text{mm}^2$ and $14.5 \pm 3.2/\text{mm}^2$ (mean $\pm$ SD, $n = 8$, $P < .001$), respectively. These data suggest that systemic overexpression of IL-5 is not sufficient to induce esophageal remodeling, implying that local effects of IL-5 on esophageal eosinophils are required.

IL-5 Expression in the Esophagus

To test further the hypothesis that local expression of IL-5 is required for induction of esophageal remodeling, we performed quantitative real-time PCR analysis for IL-5 mRNA expression in the esophagus of WT, CD2-IL-5 transgenic, iIL-5 transgenic, and saline- or *Aspergillus*-challenged mice. CD2-IL-5 transgenic mice had ~20-fold relative increase in expression of IL-5 mRNA in the esophagus compared with iIL-5 transgenic and WT mice (Figure 8A). Both WT and iIL-5 transgenic mice expressed comparable levels of IL-5 mRNA in the esophagus. The allergen-challenged mice showed ~7-fold relative increase in the expression of esophageal IL-5 mRNA compared with the saline-challenged mice (Figure 8B). Furthermore, a similar observation of induced IL-5 mRNA was observed in EE patients' esophageal biopsy specimens compared with the normal individuals' (Figure 8C).

Discussion

EE is a chronic inflammatory disease associated with marked eosinophil infiltration in the esophageal epithelial mucosa.^{12,26,27} Several clinical reports indicate that esophageal remodeling including basal layer thickening and collagen accumulation can develop in EE patients even in pediatric individuals.^{6,28-30} Previous reports indicate that macrophages, lymphocytes, and granulocytes possess the ability to stimulate fibrogenesis^{3,17,19,31,32};

therefore, we investigated whether infiltrative cells, especially eosinophils, were necessary to induce esophageal tissue remodeling in EE. Previously, we showed that eosinophil infiltration induces esophageal epithelial hyperplasia in experimental EE,^{9,20} consistent with the basal layer thickening observed in human EE.²⁶ Now, we present evidence that IL-5-mediated eosinophilia promotes esophageal tissue remodeling in mice. Translational studies first showed that, indeed, human esophageal biopsy specimens from EE patients had a thickened basal layer, elongated papillae filled with trichrome-positive materials, lamina propria fibrosis, and induced transcript levels of inflammatory mediators such as IL-5, TGF- β , and MUC5AC, consistent with previous studies.^{6,7} The identification of increased MUC5AC distinguishes EE from Barrett's esophagus³³ and further supports an esophageal T helper cell type-2 environment in EE. Furthermore, we observed basal layer thickening and increased lamina propria collagen accumulation in association with a large influx of eosinophils in the esophagus and induced expression of inflammatory cytokines (IL-5 and TGF- β) and mucin MUC5AC genes in an experimental EE model of mice. Both TGF- β 1 and MUC5AC have an important role in promoting tissue remodeling,^{7,25,34–36} and both of these genes are induced in EE patients as well as in experimental EE. We previously showed that IL-5 has a central role in regulating esophageal eosinophilia^{8,21}; therefore, we further examined the role of IL-5 in allergen-induced esophageal tissue remodeling. Importantly, no induction of basal layer thickening and lamina propria collagen accumulation was observed in allergen-challenged, IL-5 gene-deficient mice. To support the theory that IL-5-mediated eosinophilia promotes esophageal tissue remodeling, we examined basal layer thickness and collagen accumulation in mice that overexpress IL-5. A thickened basal layer and densely positive trichrome-stained material were observed in the lamina propria of esophageal tissue sections of CD2-IL-5 transgenic mice. Furthermore, to evaluate the role of eosinophils in mediating esophageal remodeling, we examined CD2-IL-5 transgenic mice that were genetically deficient in eotaxin-1. We previously showed that eotaxin-1 had a significant role in IL-5-mediated esophageal eosinophilia.²¹ Our analysis indicated a significant reduction in both basal layer and lamina propria collagen thickness in the esophagus of eotaxin-1-deficient CD2-IL-5 transgenic mice compared with CD2-IL-5 transgenic mice. In addition, we also examined esophageal remodeling in allergen-challenged WT and Δ dbl-GATA mice. Δ dbl-GATA mice are totally deficient in eosinophils at baseline^{23,37,38} and do not induce eosinophilia following allergen challenge.²³ The Δ dbl-GATA mice following allergen challenge showed a significantly reduced induction of esophageal remodeling compared with WT mice. Collectively, these studies highlight the importance of eosinophils in the induction of esophageal remodeling. Furthermore, to determine whether esophageal remodeling was mediated by the local effect of IL-5, we also examined iIL-5 transgenic mice, in which IL-5 was overexpressed in the small intestine.²² Our data revealed that iIL-5 transgenic mice had unaltered basal layer thickening and lamina propria collagen accumulation in the esophagus. These data suggest that proper expression of IL-5 in a particular cell type is necessary for the induction of esophageal remodeling. Indeed, EE patients and CD2-IL-5 transgenic and allergen-challenged mice had local overexpression of IL-5 in the esophagus, consistent with prior studies demonstrating the presence of lymphocytes in the esophagus.²⁶ Collectively, these results support a role for IL-5 and eosinophils in the development of collagen accumulation and basal layer thickening in the esophagus. It is noteworthy that T helper cell type-2 cytokines are strongly implicated in tissue remodeling and fibrosis in other tissues.^{39–41}

In conclusion, our study demonstrates that eosinophil recruitment to the esophagus is associated with tissue remodeling. First, we show impressive collagen accumulation in the epithelial and subepithelial mucosa of esophageal biopsy specimens from human EE patients. Second, we report esophageal collagen accumulation and basal layer thickening in a murine model of experimental EE. Third, we demonstrate that IL-5 is required for allergen-induced esophageal collagen accumulation and basal layer thickening, at least in the setting of

experimental EE. Finally, we demonstrate that IL-5 overexpression in lymphocytes is sufficient to induce eosinophil-associated esophageal remodeling. Taken together, these studies suggest a critical role for eosinophils and IL-5 in promoting esophageal tissue remodeling. These data support the potential clinical utility of anti-IL-5 in patients with EE, supporting our early clinical data with such an approach.⁴²

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Abbreviation used in this paper

EE
eosinophilic esophagitis

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Figure 1.

Esophageal lamina propria collagen accumulation and basal layer thickness in EE and normal individuals. A representative photomicrograph of a normal esophageal biopsy specimen shows low levels of collagen (A, original magnification, 100×). Representative photomicrograph of an EE patient's biopsy specimen shows impressive collagen in the lamina propria and papillae (B, original magnification, 100×). The morphometric measurements of basal layer thickness in EE patients compared with normal individuals are shown in C. The data are expressed as mean ± SD. EP, epithelium; LP, lamina propria.

Figure 2.

Collagen accumulation and basal layer thickening in the esophagus following allergen-induced EE. Representative photomicrograph of Masson's trichrome-stained tissue sections of a saline-challenged mouse shows normal lamina propria collagen (A, original magnification, 100 $\times$; and B, 400 $\times$) and induced collagen accumulation in lamina propria and muscularis mucosa and extended stromal papillae following allergen challenge (C, original magnification, 100 $\times$; and D, 400 $\times$). The photomicrograph of the H&E-stained tissue section shows single or 2 cell layers thickening of basal layer in saline-challenged mice (E, original magnification, 400 $\times$) compared with 4 or 5 cell thickening in allergen-challenged mice (F, original magnification, 400 $\times$). The thickness area of basal layer is identified with the representative

line in the photomicrograph (*E* and *F*). Morphometric analysis was performed to quantify basal layer and collagen thickness and is shown in *G* and *H*. The data are expressed as mean $\pm$ SD, $n = 10\text{--}12$ mice. EP, epithelium; LP, lamina propria; MS, muscularis mucosa; LU, lumen.

Figure 3.

MUC5AC and TGF- β 1 mRNA in the esophagus of human and experimental EE. Esophageal expression of TGF- β 1 and MUC5AC mRNA was measured by performing real-time PCR analysis on normal and EE patients' esophageal biopsy RNA (A and B) and RNA from the saline and allergen challenge WT mice (C and D). The data are expressed as mean $\pm$ SD, n = 7–9 mice/group.

Figure 4.

Morphometric quantification of basal layer and lamina propria collagen thickness in allergen-challenged WT and IL-5 gene-deficient mice. The basal layer and lamina propria collagen thickness in WT and IL-5 gene-deficient mice were measured by performing morphometric analysis on the esophageal tissue sections following 9 intranasal saline and allergen challenges. The basal layer thickness is shown in *A* and lamina propria collagen thickness in *B*. The data are expressed as mean $\pm$ SD, $n = 12$ mice.

Figure 5.

IL-5 transgene-induced esophageal tissue remodeling. Histopathologic analysis was performed on the esophageal tissue sections of WT and IL-5 transgenic mice following Masson's trichrome staining as shown in A–C. A representative photomicrograph of WT esophagus highlighting its basal layer thickness and lamina propria collagen level (A, original magnification, 100 $\times$). A representative photomicrograph of CD2-IL-5 transgenic mice demonstrate thickened basal layer and collagen accumulation in the lamina propria and muscularis mucosa (B, original magnification, 100 $\times$). Accumulated collagen in the lamina propria and elongated papillae is shown (C, original magnification, 400 $\times$). Morphometric analysis of basal layer thickness is shown in D, and lamina propria collagen thickness is shown in E. The data are expressed as mean $\pm$ SD, n = 12 mice. EP, epithelium; LP, lamina propria; MS, muscularis mucosa; LU, lumen.

Figure 6.

Morphometric quantification of basal layer and lamina propria collagen thickness in allergen-challenged WT and GATA-1 gene-deficient mice. The basal layer and lamina propria collagen thickness in WT and GATA-1 gene-deficient mice were measured by performing morphometric analysis on the esophageal tissue sections following 9 intranasal saline and allergen challenges. The basal layer thickness is shown in *A* and lamina propria collagen thickness in *B*. The data are expressed as mean $\pm$ SD, $n = 10$ –12 mice.

Figure 7.

Esophageal tissue remodeling in iIL-5 transgenic mice. The levels of esophageal basal layer thickness (A) and lamina propria collagen thickness (B) of WT and iIL-5 transgenic mice are shown. The levels of eosinophils in the esophagus of CD2-IL-5 transgenic and iIL-5 transgenic mice compared with their respective control WT mice are shown in C. The data are expressed as mean $\pm$ SD, $n = 12$ mice.

Figure 8.

IL-5 mRNA expression in the esophagus. Esophageal expression of IL-5 mRNA was measured by performing real-time PCR analysis on the esophageal RNA from CD2-IL-5, iIL-5, and WT mice (A), saline- and allergen-challenged mice (B), and normal individuals and EE patients (C). The data are expressed as mean $\pm$ SD, $n = 6-8$ individuals/group.

Table 1
Clinical and Pathologic Characteristics

Patients	Age, y	Sex	Esophageal disease	Allergic disease	Eosinophil (maximum/hpf)	Collagen score	Treatment
1	11	M	NL	Rhinitis	0	-	None
2	11	M	NL	Asthma	0	+/-	LTRA
3	9	F	NL	Unknown	0	-	None
4	14	M	NL	None	0	+/-	None
5	7	F	NL	Unknown	0	+	LTRA
6	13	F	NL	None	0	-	None
7	15	M	EE	Asthma	30	++	None
8	15	M	EE	Rhinitis/asthma	31	+	None
9	3	M	EE	None	56	++	PPI
10	2	M	EE	None	83	++	None
11	4	F	EE	Rhinitis	218	+++	PPI
12	12	M	EE	Asthma	25	++	None
13	14	F	EE	Asthma, Atopic dermatitis	41	+++	PPI
14	9	M	EE	Unknown	35	+++	None

NOTE. Absence of collagen in the tissue sections was graded as negative (-), and increased collagen staining was graded as positive (+) on a scale of 1–3.
LTRA, leukotriene receptor antagonist; NL, normal; PPI, proton-pump inhibitor.