Esophageal functional impairments in experimental eosinophilic esophagitis

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Mavi P, Rajavelu P, Rayapudi M, Paul RJ, Mishra A. Esophageal functional impairments in experimental eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol 302: G1347–G1355, 2012. First published February 23, 2012; doi:10.1152/ajpgi.00013.2012.—Eosinophilic esophagitis (EoE) is an emerging chronic esophageal disease. Despite the increasing diagnosis of EoE globally, the causes of EoE and other esophageal eosinophilic disorders are not clearly understood. Esophageal pathology includes accumulation of inflammatory cells (e.g., eosinophils, mast cells), characteristic endoscopic features (e.g., furrows, the formation of fine concentric mucosal rings, exudates), and functional impairments (e.g., esophageal stricture, dysmotility). We hypothesized that the esophageal structural pathology and functional impairments of EoE develop as a consequence of the effector functions of the accumulated inflammatory cells. We analyzed eosinophils (anti-major basic protein immunostaining), esophageal stricture (X-ray barium swallowing), and esophageal motility (isometric force) in two established transgenic murine models of EoE (CD2-IL-5 and rtTA-CC10-IL-13) and a novel eosinophil-deficient model (ΔdblGATA/CD2-IL-5). Herein, we show the following: 1) CD2-IL-5 and doxycycline (DOX)-induced rtTA-CC10-IL-13 mice have chronic eosinophilic and mast cell esophageal inflammation; 2) eosinophilic esophageal inflammation promotes esophageal stricture in both transgenic murine models; 3) the eosinophil-deficient ΔdblGATA/CD-2-IL-5 mice were protected from the induction of stricture, whereas the eosinophil-competent CD2-IL-5 mice develop esophageal stricture; 4) esophageal stricture is not reversible in DOX-induced rtTA-CC10-IL-13 mice (8 wk DOX followed by 8 wk no-DOX); and 5) IL-5 transgene-induced (CD2-IL-5) EoE evidences esophageal dysmotility (relaxation and contraction) that is independent of the eosinophilic esophageal inflammation. CD2-IL-5 and ΔdblGATA/CD2-IL-5 mice have comparable esophageal dysmotility. Collectively, our present study directly implicates chronic eosinophilic inflammation in the development of the esophageal structural impairments of experimental EoE.

eosinophils; esophagus; interleukin; mast cells; motility; stricture

EOSINOPHILIC ESOPHAGITIS (EoE) is an emerging chronic disease characterized by esophageal mucosal eosinophilia that exhibits a lack of response to acid repletion (4, 32) and whose prevalence is rising throughout the world (18). Patients with EoE typically suffer from dysphagia, food impaction (29, 37, 39), and esophageal dysmotility and stricture (29, 37, 39); however, the etiology of the esophageal pathology and dysfunction in EoE is unclear. Radiographic and endoscopic studies of patients with EoE have shown many structural pathologies, including small-caliber esophagi, strictures, mucosal rings, ulcers, whitish papules, and polyps (3, 14, 15, 34, 40, 42). Esophageal stricture is an abnormal narrowing of the esophagus and may be associated with esophageal rings, mucosal inflammation, or tissue remodeling, such as collagen deposition and esophageal fibrosis (13, 14, 25, 34, 42). Diseases that evidence esophageal stricture can be grouped into two categories: 1) intrinsic diseases that narrow the esophageal lumen through inflammation, fibrosis, and neoplasia; 2) extrinsic diseases that compromise the esophageal lumen by direct invasion, such as lymph node enlargement or neoplasia. We and others (23, 43) have previously established murine models of EoE in which transgenic IL-5 (CD-IL-5) or IL-13 (rtTA-CC10-IL-13) overexpression promotes esophageal eosinophilia, mastocytosis, and tissue remodeling. Other studies have shown that eosinophil and mast cell activation and degranulation promote tissue damage, fibrosis, epithelial cell hyperplasia (14, 19, 34), and notably, muscle cell dysfunction (1, 11, 38). We therefore hypothesize that the chronic esophageal inflammation of EoE causes esophageal stricture and dysmotility. To dissect the role of eosinophils in the development of esophageal stricture and dysmotility in EoE, we bred a novel model of mice (ΔdblGATA/CD2-IL-5) that is both deficient in the eosinophil lineages (ΔdblGATA) (41) and overexpresses transgenic IL-5 under the control of the T cell-specific CD2 promoter (CD2-IL-5). The previously established CD2-IL-5 and rtTA-CC10-IL-13 murine models of EoE overexpress transgenic IL-5 and IL-13, respectively, and have high levels of esophageal eosinophilia (19, 20, 43). Herein, we provide the evidence that chronic eosinophilic inflammation of IL-5- and IL-13-induced EoE has a critical role in development of esophageal stricture.

MATERIALS AND METHODS

Mice. The wild-type (Balb/c), CD2-IL-5 transgenic (20), rtTA-CC10-IL-13 transgenic (43), and ΔdblGATA/CD2-IL-5 transgenic mice (developed in our laboratory) were used to understand the development of esophageal functional abnormalities in experimental EoE. 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.

Generation of GATA-1 gene-deficient (ΔdblGATA), IL-5 transgenic mice. The CD2-IL-5 transgenic mice were crossed with ΔdblGATA mice, and their F1 generation was further mated to generate ΔdblGATA/CD2-IL-5 transgenic mice. The F2 progeny were screened for the IL-5 transgene and ΔdblGATA. The generated ΔdblGATA/CD2-IL-5 transgenic mice are composed of a mixed background; therefore, experiments were performed with age- and gender-matched littermate controls.

IL-5 and IL-13 protein analysis in the esophagus. Cytokine protein levels of esophageal homogenate were determined using a Duoset ELISA set (R&D Systems, Minneapolis, MN) as per the manufacturer’s protocol. Briefly, the homogenate was applied to cytokine-specific monoclonal antibody-precoated, 96-well ELISA plates after blocking nonspecific protein binding with 10% FBS. The plates were incubated...
for 2 h at room temperature and washed with PBS-0.05% Tween-20. Biotinylated cytotoxic-specie monoclonal antibody was applied to each well followed by streptavidin-horseradish peroxidase conjugate reagent. Finally, 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (BD Biosciences Pharmingen) was added to each well, and the color was developed in the dark at room temperature. The reaction was stopped using 2N-H2SO4; the optical density was immediately read at 450 nm. The cytokine concentration of each sample was calculated by using a standard curve.

Analysis of esophageal eosinophils and mast cells in the murine models of EoE. The 5-μm esophageal paraffin tissue sections of wild-type, CD2-IL-5 transgenic, and ΔdbGATA/CD2-IL-5 transgenic mice were immunostained with antiserum against mouse eosinophil major basic protein (anti-MBP) as described (17, 21). In brief, endogenous peroxide in the tissue was quenched with 0.3% hydrogen peroxide in methanol followed by nonspecific protein blocking with normal goat serum. Tissue sections were then incubated with rat anti-MBP (1:2,000) primary antibody overnight at 4°C, followed by a 1:200 dilution of biotinylated anti-rat IgG secondary antibody and avidin-peroxidase complex. The slides were further developed with diaminobenzidine–cobalt chloride solution to form a black precipitate and counterstained with hematoxylin. Nonnegative samples included replacing the primary antibody with normal rat serum. Further, the esophageal paraffin tissue sections of mice were deparaffinized, stained with hexazonized new fuchsin (Sigma Aldrich) with 4% sodium nitrate in naphthol-AS-D chloroacetate (Sigma Aldrich), and washed in PBS solution for 30 min, and counterstained with hematoxylin. Nonhexazonized new fuchsin was used as a negative control. Eosinophils and mast cells were quantified by counting the anti-MBP-positive immunostained cells and chloroacetate esterase-stained cells in the epithelial mucosa and lamina propria; combined cell numbers in each esophageal tissue section were quantified with the assistance of digital morphometry using the Metamorph Imaging System (Universal Imaging, West Chester, PA) (eosinophils/mm2 or mast cells/mm2) as described previously (20, 22).

Examination for esophageal stricture following barium swallowing in murine model of EoE. 5- to 6-mo-old wild-type, CD2-IL-5 transgenic, and ΔdbGATA/CD2-IL-5 transgenic mice were orally given the barium solution with a 5-ml syringe; this was done slowly to allow for swallowing of the barium suspension. After administration of ~4–5 ml of suspension, wild-type and transgenic mice were examined by X-ray analysis for presence of esophageal stricture; the complete mouse esophagus and gastrointestinal tract were examined. The barium swallowing technique was validated by performing the technique in different age groups of wild-type and transgenic mice. Our observation indicated that the younger transgenic mice (4–6 wk old) do not show esophageal stricture; stricture is only detected in the older mice. This indicates that stricture is progressive in chronic esophageal inflammation.

Analysis of the contractility of the esophageal circular and longitudinal muscle layer in experimental EoE. Contractility of esophageal circular and longitudinal muscles in CD2-IL-5 transgenic mice, along with respective wild-type mice, was examined as per the protocol developed by us in Dr. Richard Paul’s laboratory in the University of Cincinnati College of Medicine’s Division of Physiology. In brief, the mouse’s esophagus was removed, and a circular, 1-mm esophageal segment was taken for studies of contractile function in circular muscle. The rest of the esophagus was used for testing contractility of the longitudinal muscles. From the time of the dissection, the esophagus was maintained in physiological salt solution (PSS). PSS contained (mmol/L) 118 NaCl, 4.73 KCl, 1.2 MgSO4, 0.026 EDTA, 1.2 NaH2PO4, 2.5 CaCl2, 5.5 glucose, was buffered with 25 NaHCO3, and had a pH of 7.4 when bubbled with 95% O2/5% CO2 at 37°C. The esophageal ring and longitudinal segment were attached to the Differential Capacitor Force Transducer (Harvard Apparatus, Holliston, MA) so that isometric force in the circumferential and longitudinal directions could be measured. Experiments were conducted at optimal tension for force production, which was established by adjusting the length of the esophagi to a point where maximum isometric contractions were observed. Basal measurements were taken after 1 h of equilibration following exposure of the esophagi to at least two stimulation/relaxation cycles with 15 mM KCl to ensure reproducible contractions. Concentration-isometric force relations were generated in response to cumulative additions of carbachol (carbachol, 1 nM to 10 μM) at 5-min intervals. Concentration-relaxation relations were undertaken at 80% of the maximum isometric force as determined from the concentration-force relation. This is to avoid saturated concentrations of the agonist and to compare all esophagi at the same level of activation. Relaxation was measured at 5-min intervals with cumulative addition of isoproterenol (1 nM to 10 μM). Measurements were sampled at 100 Hz using a model MI100 Data Acquisition System (Biopac Systems, Goleta, CA). Data analysis was completed using the Biopac AcqKnowledge (Biopac Systems) software program. Force was normalized by dividing by the cross-section area, which was estimated as weight/length for longitudinal preparations or 2*(wet weight/circumference) for rings. Concentration-response relations were fitted with a logistic function using Origin software; and differences in parameters of transgenic and wild-type mice were analyzed by t-test. Data were expressed as means ± standard deviation (SD).

Analysis of the relaxation of the esophageal circular and longitudinal muscle layer in experimental EoE. Relaxation of esophageal circular and longitudinal muscles following IL-5 transgene-induced esophageal inflammation was examined in the mice. Circular and longitudinal muscle relaxation was examined following a similar experimental protocol as the one described for the contractility experiment. Briefly, the basal measurement was sampled for 100 s following exposure of the esophageal segments to two cycles of stimulation with 15 mM KCl. The peak contraction force response was generated in response to CCh (1 μM), and the subsequent concentration-dependent relaxation of muscles was generated by using cumulative addition of isoproterenol (1 nM to 1 μM) to determine the relaxation force of the circular and longitudinal muscle layers of control and inflamed esophagi. The data were analyzed using the companion Biopac AcqKnowledge software.

Statistical analysis. The nonparametric Mann-Whitney U-test was employed for comparison of data between two groups, and the Kruskal-Wallis test was used for comparison of more than two groups. The concentration-isometric force and -relaxation data was fitted to a logistic equation using Origin software. Differences in the maximum forces and EDSO values were tested using Student’s t-test. Parametric data were compared using t-tests or ANOVA. Values are reported as means ± SD or means ± SE. P values < 0.05 were considered statistically significant.

RESULTS

CD2-IL-5 and rtTA-CC10-IL-13 transgenic mice show induced levels of esophageal eosinophils and mast cells. We hypothesized that transgenic IL-5- or IL-13-induced chronic esophageal eosinophil and mast cell inflammation in mice lead to the development of esophageal stricture and peristaltic dysfunction. Accordingly, we first analyzed the IL-5 and IL-13 protein and eosinophil levels in the esophagus of wild-type, CD2-IL-5, no-doxycycline (no-DOX) and DOX-exposed rtTA-CC10-IL-13 transgenic mice. We observed an approximately tenfold increase in IL-5 protein (~0.65 to 1.35 μg/mg; P < 0.01) and an approximately sixfold increase in IL-13 protein (~0.2 to 0.5 μg/mg; P < 0.01). We observed an approximately tenfold increase in IL-5 protein (~0.65 to 1.35 μg/mg; P < 0.01) and an approximately sixfold increase in IL-13 protein (~0.2 to 0.5 μg/mg; P < 0.01). We observed an approximately tenfold increase in IL-5 protein (~0.65 to 1.35 μg/mg; P < 0.01) and an approximately sixfold increase in IL-13 protein (~0.2 to 0.5 μg/mg; P < 0.01). We observed an approximately tenfold increase in IL-5 protein (~0.65 to 1.35 μg/mg; P < 0.01) and an approximately sixfold increase in IL-13 protein (~0.2 to 0.5 μg/mg; P < 0.01).
1.92 ± 1.9/mm² and 121.3 ± 21.4 (means ± SD), respectively, and of 6-wk no-DOX and DOX-exposed rTA-CC-10-IL-13 mice was 2.1 ± 1.8/mm² and 76.3 ± 19.2/mm² (means ± SD), respectively. Interestingly, no esophageal eosinophilia was detected in ΔdblGATA/CD2-IL-5 mice. Furthermore, we also examined mast cell numbers in wild-type, CD2-IL-5, no-DOX and DOX-exposed rTA-CC10-IL-13, and ΔdblGATA/CD2-IL-5 mice. We observed an approximately twofold increase in mast cell number in CD2-IL-5 and ΔdblGATA/CD2-IL-5 mice compared with wild-type mice (Fig. 1C), indicating that the IL-5-induced increase in mast cells is independent of eosinophilia as the number of mast cells in CD2-IL-5 (has eosinophilia) and ΔdblGATA/CD2-IL-5 (lacks eosinophilia) was comparable. Of note, the number of mast cells in no-DOX and DOX-exposed rTA-CC10-IL-13 mice was also found comparable (Fig. 1D), supporting that the increase in esophageal mast cells in these EoE murine models is independent of the presence of esophageal eosinophilia.

**Esophageal stricture develops in transgenic IL-5-induced chronic inflammation.** Esophageal stricture is reported in a number of esophageal diseases including EoE (33, 40). Therefore, we tested the hypothesis that chronic eosinophilic and mast cell inflammation in the esophagus leads to esophageal stricture. To detect the presence or absence of esophageal stricture, barium-swallowing experiments were performed in 5- to 6-mo-old wild-type and CD2-IL-5 mice. Esophageal stricture was present in all examined CD2-IL-5 mice. Notably, no esophageal stricture was observed in any of the age- and sex-matched wild-type mice. The X-ray photographs of the barium-filled esophagi of wild-type mice (Fig. 2, A and B) and CD2-IL-5 mice (Fig. 2, C–E) are shown. In addition, we performed morphometric analysis in formalin-fixed, hematoxylin and eosin-stained cross-sections of these same wild-type and CD2-IL-5 mice to measure for lumen narrowing. We observed a reduction in lumen area in the esophagi of CD2-IL-5 mice compared with wild-type mice, with lumens measuring 4.8 ± 0.9 × 10⁴ μm² in wild-type mice compared with 2.6 ± 0.7 × 10⁴ μm² in CD2-IL-5 mice (means ± SD, n = 6, P < 0.01).

**Esophageal stricture does not develop in eosinophil-deficient CD2-IL-5 mice.** To test whether the development of esophageal stricture was dependent on transgenic IL-5-induced esophageal eosinophilia, we generated ΔdblGATA/CD2-IL-5 transgenic mice in our laboratory by cross-breeding CD2-IL-5 mice with ΔdblGATA mice. The 5- to 6-mo-old ΔdblGATA/CD2-IL-5 mice lacked the esophageal eosinophilia (Fig. 3A) and esophageal stricture that was observed in the CD2-IL-5 mice (Fig. 3, B–D). The lumen area in the esophagus of 5- to 6-mo-old ΔdblGATA/CD2-IL-5 mice was greater than the lumen area of CD2-IL-5 mice. The lumens measuring 5.1 ± 1.2 × 10⁴ μm² in ΔdblGATA/CD2-IL-5 mice compared with 3.1 ± 1.2 × 10⁴ μm² in CD2-IL-5 mice (means ± SD, n = 6, P < 0.01).

**Esophageal stricture is not reversible in experimental EoE.** To determine whether esophageal stricture is reversible following reduced eosinophilic inflammation, we employed a DOX-regulated transgenic IL-13-induced murine model of EoE, rTA-CC10-IL-13. As previously reported, these mice develop esophageal fibrosis after DOX food exposure (43). rTA-CC10-IL-13 mice examined for esophageal stricture and eosinophilia after 8 wk of DOX food evidenced esophageal

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*Fig. 1. IL-5, IL-13, and mast cell induction in the esophagus. Protein levels of IL-5 and IL-13 from esophageal tissue of wild-type (WT), CD2-IL-5, and uninduced and doxycycline (DOX)-induced rTA-CC10-IL-13 mice were measured by performing ELISA. Levels of IL-5 in 12-wk-old WT and in CD2-IL-5 mice and of IL-13 in 8-wk-old WT and in rTA-CC10-IL-13 mice are shown (A and B). The level of mast cells in the esophagus of WT, CD2-IL-5, ΔdblGATA/CD2-IL-5, and DOX- and no-DOX-treated rTA-CC10-IL-13 mice were quantified by performing morphometric analysis following chloroacetate tissue staining (C and D). The results are the summary of 3 independent experiments, reported as means ± SE with n = 6 mice for each group. The statistical P values are provided in each figure.*
Esophageal stricture and eosinophilia (Fig. 4, A, B, and E). rtTA-CC10-IL-13 mice given DOX food for 8 wk and subsequently switched to a regimen of no-DOX for another 8 wk showed comparable esophageal stricture (Fig. 4, C and D), but significantly reduced esophageal eosinophilia (Fig. 4E), compared with the rtTA-CC10-IL-13 mice assessed immediately after 8 wk of DOX food.

IL-5-induced EoE has impaired longitudinal esophageal contractility. It has been shown that expression of the IL-5 transgene in the esophagus promotes fibrotic responses in the
esophagus (23). Therefore, we investigated whether the esophageal remodeling induced by the chronic esophageal inflammation affects esophageal motility. We examined the esophageal circular and longitudinal muscle contraction activity of CD2-IL-5 mice in response to different concentrations of carbachol, which induces contraction of smooth muscle. Our analysis indicated that the esophagus of CD2-IL-5 mice developed more force in response to increased carbachol concentration compared with wild-type mice (Fig. 5, A–B). The concentration-force relation for the longitudinal muscle is shown in Fig. 5B. The maximum CD2-IL-5 isometric force of 5.45 ± 0.15 mN/mm² was significantly (P < 0.005) greater than that of the wild-type, 3.90 ± 0.37; however, the sensitivity in response to carbachol was not statistically significant (CD2-IL-5 = 0.40 ± 0.04 μM; wild-type = 0.60 ± 0.20 μM). Esophageal circular muscle contractility in response to increased carbachol concentration was qualitatively similar to the longitudinal preparation. These data indicate that the chronic esophageal inflammation of CD2-IL-5 mice affects the contractility of the esophageal smooth muscles.

IL-5-induced EoE has altered esophageal muscle relaxation. We examined the effects of chronic inflammation in relaxation of esophageal smooth muscle. Longitudinal preparations of esophageal muscles were precontracted with carbachol to achieve 80% of the maximal response. The relaxation of isometric force in esophageal smooth muscle from wild-type and CD2-IL-5 mice was measured in response to cumulative additions of isoproterenol. Both CD2-IL-5 and wild-type muscles responded in a concentration-dependent manner and were completely relaxed in response to 10⁻⁶ M isoproterenol (Fig. 6A). The concentration dependence of the isoproterenol-induced relaxation of smooth muscles from the CD2-IL-5 and wild-type mice is shown in Fig. 6B. In Fig. 6B, the initial force of the IL-5 transgenic mice was greater than the wild-type mice, consistent with Fig. 5B. The sensitivity of the CD2-IL-5 mice was lower, as expressed in the ED₅₀ of the response (CD2-IL-5 = 1.68 ± 0.11 × 10⁻⁸ M vs. wild-type = 0.94 × 10⁻⁸ ± 0.06 × 10⁻⁸ M; P < 0.001). These data indicate that chronic inflammation decreases the isoproterenol sensitivity of CD2-IL-5 mice compared with wild-type
mice. Relaxation of the esophageal circular muscle of CD2-IL-5 mice was also more with isoproterenol than that of wild-type mice (data not shown). Esophageal motility dysfunction is independent of eosinophilic inflammation.

To determine whether esophageal muscle contractility and relaxation is dependent on transgenic IL-5-induced esophageal eosinophilic inflammation, we examined carbachol-induced esophageal muscle contractility and isoproterenol-induced relaxation of carbachol-precontracted muscles in wild-type, CD2-IL-5, and \( \Delta dbiGATA/CD2-IL-5 \) mice. Notably, both CD2-IL-5 and \( \Delta dbiGATA/CD2-IL-5 \) mice developed more force in response to carbachol and were more sensitive to isoproterenol-induced relaxation than wild-type mice (Fig. 7, A and B). Although the \( \Delta dbiGATA/CD2-IL-5 \) transgenic mice lacked eosinophilic esophageal inflammation, the alterations in carbachol-induced esophageal longitudinal muscle contractility and isoproterenol-induced relaxation and in sensitivity to carbachol and isoproterenol were comparable to those of CD2-IL-5 mice. Notably, we also observed comparable carbachol-induced esophageal muscle contractility and isoproterenol-induced relaxation between uninduced (no esophageal eosinophilia) and induced (6-wk DOX; high degree of eosinophilia) rTATCC10-IL-13 mice (data not shown), indicating independence of esophageal motility dysfunction from eosinophilic inflammation in two transgenic EoE models.

**DISCUSSION**

EoE is a chronic inflammatory disease associated with marked eosinophil and mast cell accumulation in the esophageal mucosa (24, 30, 31). Previous reports indicate that macrophages, lymphocytes, and granulocytes possess the ability to stimulate fibrogenesis (6, 7, 16, 27, 35), and we recently reported esophageal remodeling in experimental and human EoE (23). Several clinical reports also indicate that esophageal dysmotility and stricture develop in patients in a variety of esophageal disorders including EoE (3, 14, 15, 34, 40, 42). Herein, we show that esophageal stricture develops in chronic EoE and is dependent on eosinophilic inflammation; however,
esophageal motility dysfunction is independent of eosinophilic inflammation in experimental EoE.

We show that esophageal stricture is associated with a large influx of eosinophils in the esophagus in murine models of EoE. We have previously shown that IL-5 and IL-13 have central roles in regulating esophageal eosinophilia (19, 20); therefore, we first examined CD2-IL-5 and rtTA-CC10-IL-13 mice for the development of esophageal stricture. We show that both CD2-IL-5 transgenic and DOX-treated rtTA-CC10-IL-13 mice induce esophageal IL-5 and IL-13. We also show that CD2-IL-5 transgenic mice have an increased number of mast cells in the esophagus that is independent of the recruitment of eosinophils. The eosinophil-deficient ΔdblGATA/CD2-IL-5 transgenic mice show a comparable number of mast cells with the CD2-IL-5 transgenic mice, indicating that mast cells may not have an important role in promoting esophageal stricture and that IL-5- and IL-13-mediated eosinophilia is only responsible for promoting esophageal stricture in mice. Eosinophil granules contain a crystallloid core composed of MBP-1 and MBP-2 and a matrix composed of eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase (5). These toxic mediators are capable of promoting scarring in the tissue and accelerating the remodeling process. We earlier showed that esophageal remodeling develops in these transgenic mice (23). Additionally, the data obtained from the eosinophil-deficient ΔdblGATA/CD2-IL-5 mice support our theory that chronic esophageal eosinophilic inflammation is needed to promote esophageal stricture. Of note, the ΔdblGATA/CD2-IL-5 mice do not develop stricture in the esophagus. Additionally, we show that CD2-IL-5 transgenic and ΔdblGATA/CD2-IL-5 mice have a comparable number of mast cells; therefore, it may be possible that mast cells may not have a role in the development of esophageal stricture. Furthermore, to determine whether esophageal stricture is a reversible process following reduction of esophageal eosinophilic inflammation, we used DOX-inducible IL-13 overexpression mice (rtTA-CC10-IL-13). Our data revealed that 8 wk of DOX exposure promotes IL-13-induced esophageal eosinophilia and esophageal stricture but that the esophageal stricture is not reversed following an 8-wk cessation of DOX food even though the eosinophilia diminishes within this time period. Taken together, these data denote that eosinophils and their mediators have a significant role in inducing stricture; however, these results also emphasize that once the stricture has developed, it is not completely reversible even if esophageal eosinophilia is reduced in the mice. Furthermore, isometric force in the CD2-IL-5 mice was greater than that in the wild-type mice. Since these force values were normalized to a calculated cross-sectional area, this may suggest an increase in force-generating capacity due to increasing cellularity or hypertrophy within the esophageal wall. Relaxation to isoproterenol, on the other hand, was less sensitive in CD2-IL-5 mice. Potentially, both could be responses to inflammation. At this stage, speculation is premature, as there is little data in the literature to this effect.

Furthermore, our data indicate that chronic esophageal inflammation induced by local IL-5 expression in the esophagus leads to altered esophageal motility in the CD2-IL-5 transgenic mice. It has been shown that the eosinophilic protein MBP directly increases smooth muscle reactivity by causing dysfunction of vagal muscarinic M2 receptors (10). In addition, MBP also triggers degranulation of mast cells and basophils (26). The clinical evidence is emerging that esophageal mast cell numbers increase in human EoE and possibly are also involved in the pathogenesis of the disease (2, 8, 36). These mast cells also contain a number of toxic proteins that may alter the esophageal pathophysiology, including esophageal motility dysfunction. Notably, our present data indicate that motility dysfunction is not dependent on eosinophilic inflammation, as carbachol-induced esophageal muscle contractile and isoproterenol-induced relaxation properties were comparable between CD2-IL-5 and ΔdblGATA/CD2-IL-5 transgenic mice. Therefore, the possibility is that mast cells may be involved in the development of esophageal motility dysfunction in experimental EoE. The comparable mast cell induction in the esophagus of CD2-IL-5 and ΔdblGATA/CD2-IL-5 transgenic mice support the possible role of mast cells in the development of motility dysfunction in the esophagus.

In conclusion, we show that esophageal stricture develops in EoE and is dependent on chronic eosinophilic inflammation.
Second, IL-13 transgene-induced stricture is an irreversible process. Third, we show that both local IL-5- and IL-13-induced inflammation promote longitudinal esophageal muscle motility dysfunction that is independent of eosinophilic inflammation but that may be dependent on mast cells, as these cells are also induced in EoE and possess highly toxic granules. Taken together, these studies are the first to directly implicate eosinophils in promoting esophageal stricture in experimental EoE and, thus, will potentially provide critical insight in the development of human esophageal pathophysiological disorders such as EoE.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

P.M. and R.J.P. performed experiments; P.M., P.R., M.R., R.J.P., and A.M. approved the final version of the manuscript; P.R. analyzed data; P.R. and M.R. prepared figures; M.R. and R.J.P. edited and revised the manuscript; R.J.P. and A.M. drafted the manuscript; A.M. contributed the conception and design of the research; A.M. interpreted results of the experiments.

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