

Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF- β 1, and increase esophageal smooth muscle contraction

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Background: Increased numbers of mast cells are present in the esophageal epithelium in patients with eosinophilic esophagitis (EE). However, mast cell infiltration into the esophageal lamina propria (LP) and smooth muscle (SM) and the effects of their products on SM function has not been determined.

Objective: We investigated mast cell localization and characterization in esophageal SM, the functional significance of mast cell TGF- β 1 expression to contraction of human esophageal smooth muscle (HESM) cells *in vitro*, and the effect of topical corticosteroids on the number of tryptase-positive (MC_T) and chymase-positive (MC_C) mast cells in patients with EE.

Methods: MC_T- and MC_C-positive mast cell numbers were quantitated in the epithelium, the LP before and after topical corticosteroid therapy, and the muscularis mucosa in patients with EE and control subjects by using immunohistology. Double immunofluorescence was used to assess mast cell production of TGF- β 1. The ability of TGF- β 1 to influence HESM cell contractility was assessed *in vitro*.

Results: In the SM in patients with EE, significantly increased numbers of MC_T- and TGF- β 1-positive cells (but only low levels of eosinophils) were detected compared with those seen in control subjects. MC_T expressed TGF- β 1, which increased the contractility of cultured primary HESM cells *in vitro*. Topical corticosteroid therapy in patients with EE significantly reduced epithelial MC_T numbers but not LP tryptase-chymase-positive mast cell numbers.

Conclusions: MC_T numbers, rather than eosinophil numbers, are increased in the SM in patients with EE, express TGF- β 1, and increase the contractility of HESM cells *in vitro*. As such, mast cells localized to SM in patients with EE might modulate esophageal contractility. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Eosinophilic esophagitis, mast cell, TGF- β 1, smooth muscle, pediatric, topical corticosteroids, tryptase, chymase, eosinophil, remodeling

Eosinophilic esophagitis (EE) is a disease of increasing recognition and prevalence.¹⁻³ EE is defined by both clinical and pathologic criteria of greater than 15 esophageal epithelial eosinophils per high-powered field (hpf). The inflammatory infiltrate in patients with EE includes mast cells, T cells, and B cells.^{4,7} Recent studies with manometry have demonstrated that EE is associated with dysmotility of the esophagus in pediatric and adult patients with EE.⁸⁻¹⁰ Episodes of dysmotility correlate with clinical complaints of dysphagia, an important distinguishing feature of pediatric and adult EE.^{2,8,12,13} Esophageal epithelial tryptase-positive mast cells (MC_T) are degranulated in patients with EE, and there is a significant increase in mast cell gene expression in esophageal biopsy specimens from patients with EE, such as tryptase α , carboxypeptidase A, and CXCL1.^{4,7,14} However, the presence of mast cells in esophageal smooth muscle (SM) in patients with EE, the effect of mast cell products on esophageal SM contractility, or the distribution of mast cell subtypes (MC_T vs chymase-tryptase-positive mast cells [MC_{TC}]) in the subepithelium has not yet been reported.

Studies in asthmatic patients demonstrate increased mast cell infiltration into the airway SM. Mast cell products, such as TGF- β 1, can influence SM contractility and might cause airway hyperreactivity.^{15,16} Although TGF- β 1 mRNA and protein levels are increased in patients with EE, its relationship with esophageal SM has not been previously described.^{17,18} Based on previous observations that there are both increased mast cells and TGF- β 1 levels in patients with EE, we hypothesized that mast cell-derived TGF- β 1 might influence esophageal SM function. In this study we have examined whether mast cells are localized to SM in patients with EE and whether TGF- β 1 can influence esophageal SM contraction *in vitro*. In addition, we examined the distribution of mast cell subtypes (MC_T vs MC_{TC}) in different esophageal compartments (epithelium and lamina propria [LP]) and examined the effect of topical corticosteroid therapy on the resolution, persistence, or both of MC_T and MC_{TC} in these esophageal tissue compartments in patients with EE.

METHODS

Esophageal biopsy specimens in patients with EE and control subjects

Esophageal epithelium and LP. Esophageal biopsy specimens from patients with EE and control subjects were retrospectively selected by

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Abbreviations used

EE:	Eosinophilic esophagitis
HESM:	Human esophageal smooth muscle
hpf:	High-powered field
LP:	Lamina propria
MC _C :	Chymase-positive mast cells
MC _T :	Tryptase-positive mast cells
MC _{TC} :	Tryptase-chymase-positive mast cells
SM:	Smooth muscle

using a database of patients with EE evaluated in the Eosinophilic Gastrointestinal Disorders Clinic at Rady Children's Hospital, San Diego, from 2004-2006 using defined inclusion criteria. All patients with EE in the database from 2004-2006 who met the following inclusion criteria were included: (1) histologic evidence of isolated EE defined as a peak esophageal eosinophil count of 20 or more eosinophils per hpf at $\times 400$ light microscopy and absence of eosinophilia in the stomach and duodenum; (2) adequate LP from baseline and second biopsy (after topical budesonide for 3-6 months) defined as at least 3 to 5 hpf for analysis to determine the effect of topical corticosteroids on LP mast cell levels; (3) treatment with the same topical corticosteroid (ie, swallowed budesonide [Pulmicort; AstraZeneca, Wilmington, Del], 1 mg daily, mixed with sucralose, dextrose, and maltodextrin [Splenda; McNeils Nutritionals, Washington, Pa] during routine clinical care; and (4) no change in diet, environment, or medications between the baseline and follow-up biopsies. By using these inclusion criteria, 17 patients with EE were identified (see Table E1 in this article's Online Repository at www.jacionline.org). Control subjects ($n = 9$) were defined as children in our esophageal biopsy histology database who underwent endoscopy for clinical complaints but had 0 eosinophils per hpf and no histologic or endoscopic evidence of EE and who had adequate LP (defined as above), SM (see below), or both for analysis (see Table E1). Patients were treated with budesonide for a median of 4 months (mean, 4.7 months); 19 of 21 patients with EE failed proton pump inhibitor or H2 blockade before the diagnosis of EE (see Table E1).

Esophageal SM. Approximately 15% of our esophageal biopsy specimens contain SM. We reviewed the database to detect subjects who had sufficient SM (defined as 3-5 hpf at $\times 400$) in their baseline biopsy specimen for analysis based on notations in the database made by a pediatric pathologist (R. N.) regarding the presence or absence of SM in each biopsy specimen. We identified 4 additional patients with EE and 6 control subjects in the database who met all of the inclusion criteria listed above except adequate posttherapy LP who had sufficient SM for study (see Table E1, patients with EE 18-21). All subjects who were identified in the database and met the entry criteria were included in the analysis. The 8 patients with EE in the SM studies included 4 from the initial EE cohort described above and these 4 additional subjects with EE. The 6 control subjects in the SM studies were all from the initial control cohort described above (see Table E1). To ensure that equivalent amounts of SM area was analyzed in each patient, the numbers of cells per square millimeter at $\times 400$ magnification in the SM was quantitated by using image analysis (ImagePro; Media Cybernetics, Bethesda, Md). SM bundles did not express fibroblast markers (data not shown) and thus represent pure SM cells.

The study was approved by the Institutional Review Boards from the University of California and Rady Children's Hospital, San Diego.

Immunostaining

Five-micrometer sections of formalin-fixed, paraffin-blocked esophageal specimens were used for all immunostaining studies. Esophageal biopsy specimens were immunostained by using the Vectastain ABC system (Vector Laboratories, Burlingame, Calif) with primary antibodies specific for either human tryptase, chymase (both from Abcam, Boston, Mass) or TGF- $\beta 1$ (Santa Cruz Biochemicals, Santa Cruz, Calif), as previously described.¹⁷ The numbers of either MC_T or chymase-positive mast cell (MC_C) and TGF- $\beta 1$ -positive cells were enumerated in 3 to 5 separate fields in the epithelium, LP, and muscularis mucosa at $\times 400$ magnification by an observer blinded

to the diagnosis and therapy. Results are expressed as the number of cells per hpf (epithelium and LP) and per square millimeter (SM) at $\times 400$ light microscopy. Double-label immunofluorescence was used to colocalize tryptase and chymase, as well as tryptase and TGF- $\beta 1$, as previously described.¹⁷ All biopsy specimens with adequate residual LP (21/43: 8 patients with EE before budesonide, 8 patients with EE after budesonide, and 5 control subjects) were used for confirmation of mast cell phenotyping by tryptase-chymase double immunofluorescence. Isotype control antibodies and skin biopsy specimens were used as negative and positive controls, respectively, and demonstrated appropriate antibody specificity (data not shown).

Quantitation of eosinophils in the esophagus: Definition of response to corticosteroid therapy

The peak number of eosinophils was counted in 5 hpf at $\times 400$ light microscopy with hematoxylin and eosin staining, as previously described.¹⁷ We have previously defined a histologic response to corticosteroid therapy in patients with EE as "responders" or "nonresponders" based on posttherapy maximal epithelial eosinophil counts of 7 or less per hpf (responder) and 20 or more eosinophils per hpf (nonresponders) after at least 3 months of swallowed viscous budesonide.¹⁹

Esophageal SM cell *in vitro* contraction assay

Primary human esophageal smooth muscle (HESM) cells were cultured according to the manufacturer's instructions for use in an *in vitro* SM gel contraction assay (ScienCell, San Diego, Calif), which we have adapted from studies of human airway SM.¹⁵ HESM cells were cultured in basal medium without growth factors for 24 hours before seeding in collagen gels free of LPS (Advanced BioMatrix, San Diego, Calif). After overnight incubation in collagen gels, HESM cells were cultured in the presence or absence of TGF- $\beta 1$ (10 ng/mL for 2-24 hours; R&D Systems, Minneapolis, Minn). To determine whether TGF- $\beta 1$ -induced HESM contraction was transcription dependent, HESM cells were incubated simultaneously with TGF- $\beta 1$ and actinomycin D (5 μ g/mL; Sigma-Aldrich, St Louis, Mo). Control mediators used in the gel contraction assay included histamine (100 μ mol/L). With agonist-induced HESM contraction, the area of the gel decreases significantly, as described in studies of airway SM.¹⁵ The area of the gels was quantitated by using a Bio-Rad ImageDR transilluminator and Versadoc scanner (Bio-Rad Laboratories, Hercules, Calif) with an accompanying image-capture and analysis program to generate area in square millimeters. Experiments were done in triplicate on at least 3 separate days. HESM cells in culture expressed significant levels of SM markers (α -smooth muscle actin) and low levels of fibroblast markers on quantitative PCR (collagen I and fibronectin), as noted by other investigators who culture SM *in vitro*.²⁰

Statistical analysis

Statistical analysis was done with the NCSS statistical package. Comparisons between groups were made by using the Mann-Whitney *U* test, pre-post comparisons were made with a Wilcoxon test, and Spearman *r* used for non-parametric data was used for correlations. Graphs were created with GraphPad Prism software (GraphPad Software, Inc, La Jolla, Calif). A 2-tailed *P* value of less than .05 was considered statistically significant.

RESULTS

MC_T numbers are increased in the epithelium and SM but not in the LP in patients with EE

Patients' characteristics are described in Table E1. Disease duration did not correlate with the degree of epithelial eosinophil ($r = 0.17$, $P = .52$) or MC_T ($r = -0.026$, $P = .82$) infiltration.

Epithelium. Pediatric patients with EE have statistically higher MC_T numbers in the epithelium (median, 23 cells/hpf; 95% CI, 12-42 cells/hpf) compared with healthy control patients (median, 1 cell per hpf; 95% CI, 0.3-4 cells/hpf; $P < .001$; Fig 1, A,

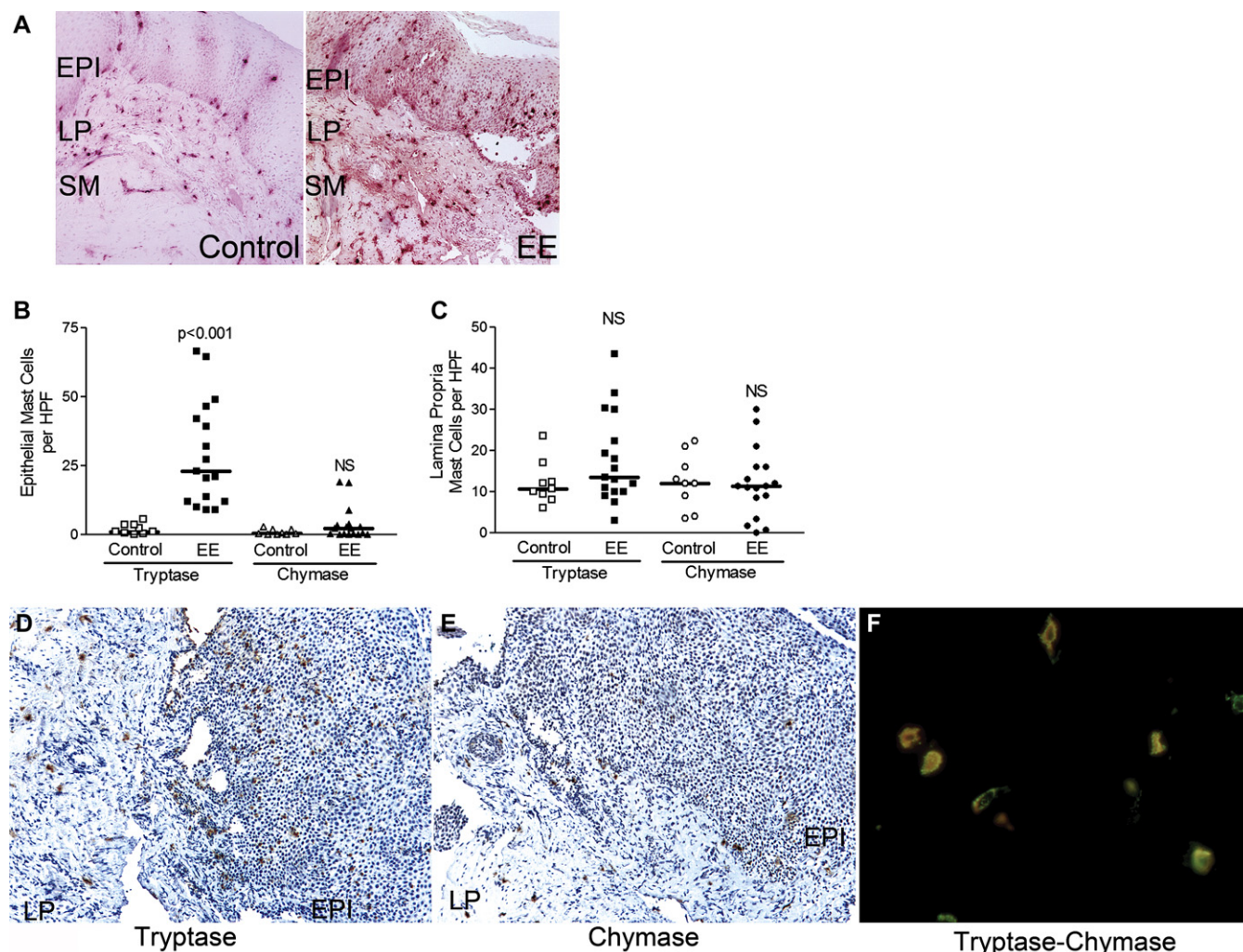


FIG 1. Mast cells in the epithelium (EPI) and LP. **A**, MC_T infiltrating the epithelium, LP, and SM in control subjects and patients with EE (magnification $\times 100$). **B** and **C**, Tryptase (**Fig 1, B**)– and chymase (**Fig 1, C**)–positive cell numbers. Bars represent medians. **D–F**, Epithelial and LP MC_T (**Fig 1, D**), LP MC_C (**Fig 1, E**; magnification $\times 100$), and LP MC_{TC} (tryptase, green; chymase, red; overlap, yellow; magnification $\times 400$; **Fig 1, F**) from patients with EE.

B, and **D**; see [Table E2](#) in this article's Online Repository at www.jacionline.org. MC_T numbers in the epithelium correlated with epithelial eosinophil numbers before and after budesonide ($r = 0.6$, $P = .003$), as well as MC_C numbers ($r = 0.6$, $P = .004$).

LP. There was no significant increase in MC_T numbers in patients with EE in the LP (median, 14 cells/hpf; 95% CI, 10–22 cells/hpf) compared with those seen in control subjects' biopsy specimens (median, 11 cells/hpf; 95% CI, 8–17 cells/hpf; $P = .17$; **Fig 1, C** and **E**). In contrast to the epithelium and SM, LP MC_T numbers did not correlate with LP eosinophil numbers before budesonide, after budesonide, or both ($r = 0.18$, $P = .31$) but did correlate with LP MC_C numbers ($r = 0.50$, $P = .01$; see [Table E2](#)).

SM. Patients with EE had significantly higher MC_T numbers in the SM (median, 261 cells/mm²; 95% CI, 190–375 cells/mm²) compared with healthy patients (median, 85 per mm²; 95% CI, 55–160 cells/mm²; $P = .002$); **Fig 2, A–C**, and see [Table E3](#) in this article's Online Repository at www.jacionline.org. Mast cells were located between the muscle bundles in the muscularis mucosa (**Fig 2, A** and **B**). MC_T numbers significantly exceeded the

numbers of eosinophils in the SM (median, 261 vs 48 cells/mm²; $P = .01$; **Fig 2, C** and **D**). Patients with EE had increased eosinophil numbers in the SM compared with control subjects (median, 48 vs 0 cells/mm²; $P = .009$; **Fig 2, D**). SM MC_T cell numbers correlated with SM eosinophil numbers ($r = 0.55$, $P = .04$). Interestingly, SM MC_T numbers correlated with the clinical complaint of dysphagia ($r = 0.72$, $P = .04$).

MC_C numbers are similar in patients with EE and control subjects

To evaluate whether the mast cells in patients with EE had a mucosal (MC_T) versus connective tissue (MC_{TC}) phenotype, we stained biopsy specimens with a chymase-specific antibody. Although MC_C numbers were highest in the LP (median, 11 cells/hpf) and lower in the epithelium and SM (median, 2 and 6 cells/hpf, respectively), the differences were statistically significant only in the epithelium (MC_C LP vs epithelium, $P = .01$; MC_C LP vs SM, $P = .27$; **Fig 1, B, C**, and **E**, and see [Tables E2](#) and [E3](#)). Within the EE group, there were greater epithelial

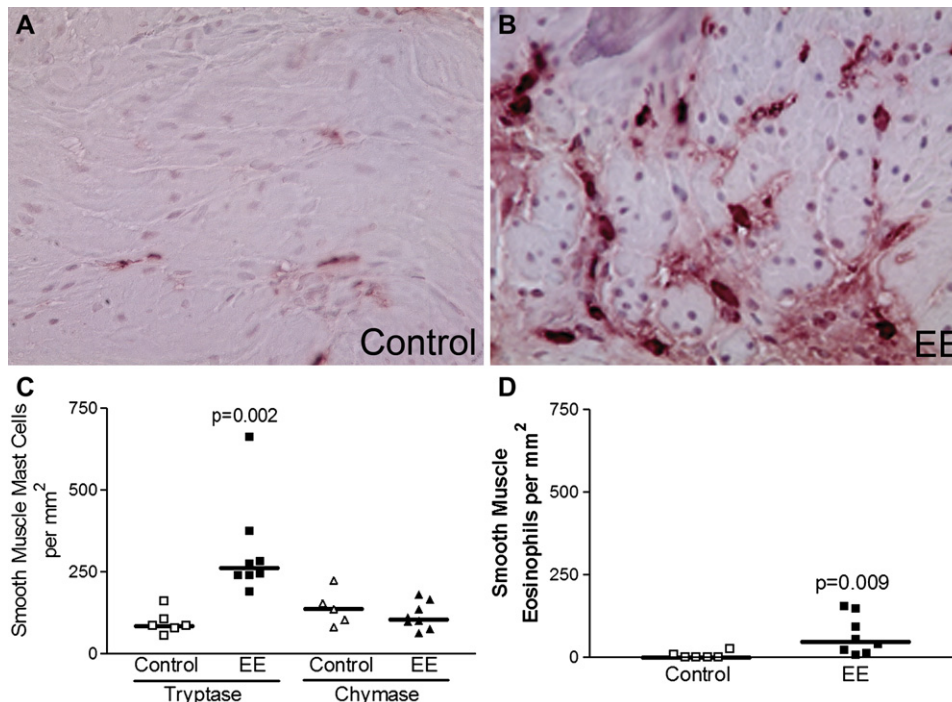


FIG 2. Esophageal SM is infiltrated by MC_T. **A** and **B**, Representative image of SM MC_T in control subjects (Fig 2, A) and patients with EE (Fig 2, B). **C** and **D**, Quantitation of MC_T and MC_C (Fig 2, C) and eosinophil (Fig 2, D) numbers in SM. Bars represent medians.

MC_T numbers (median, 23 cells/hpf) than MC_C numbers (median, 2 cells/hpf; $P = .0002$). There were also greater MC_T than MC_C numbers in the LP in patients with EE (median, 14 and 11 cells/hpf, respectively; $P = .01$). There were no differences in MC_C numbers in patients with EE versus control subjects in the epithelium (median, 2 cells/hpf [95% CI, 0.3-3 cells/hpf] in patients with EE vs 0.33 cells/hpf [95% CI, 0-1.7 cells/hpf] in control subjects, $P = .13$), the LP (median, 11 cells/hpf [95% CI, 9-16 cells/hpf] in patients with EE versus 12 cells/hpf [95% CI, 8-18 cells/hpf] in control subjects; $P = .42$), and the SM (median, 105 cells/mm² [95% CI, 64-166 cells/mm²] in patients with EE versus 137 cells/mm² [95% CI, 71-208 cells/mm²] in control subjects; $P = .38$; Fig 1, B, C, and E; Fig 2, C; and see Tables E2 and E3). Patients with EE had higher SM MC_T than MC_C numbers (median, 261 [MC_T] vs 105 [MC_C] cells/hpf, $P = .02$), whereas control subjects had higher SM MC_C than MC_T numbers (median, 137 [MC_C] vs 85 [MC_T] cells/hpf, $P = .04$), potentially reflecting a shift in mast cell profile in the SM of patients with EE (Fig 2, C, and see Table E3).

MC_{TC} numbers are similar in patients with EE and control subjects

Based on the increased ratio of MC_C- to MC_T-positive cells (in our single-labeling studies for chymase or tryptase), it appeared that the mast cells in the LP had a connective tissue phenotype. A subset of biopsy specimens ($n = 21$) was used for double immunofluorescence with tryptase- and chymase-specific antibodies to verify this observation. These double-label studies confirmed that whereas the epithelium in patients with EE was infiltrated by mucosal MC_T, the LP contained mainly MC_{TC} double-positive connective tissue-type mast cells (Fig 1, F).

MC_T in patients with EE express TGF- β 1

Given the increased mast cell infiltration of the muscularis mucosa and the known effects of TGF- β 1 on SM, such as hypertrophy, we were interested in determining whether esophageal mast cells expressed TGF- β 1. Double-immunofluorescence studies showed that MC_T in patients with EE produce TGF- β 1 (Fig 3, A-C) and that there were greater numbers of TGF- β 1-positive cells in the muscularis mucosa of patients with EE compared with those seen in control subjects ($P = .005$; Fig 3, D and E). The numbers of TGF- β 1-positive cells in the SM correlated with both SM MC_T numbers ($r = 0.70$, $P = .01$) and clinical dysphagia ($r = 0.48$, $P = .05$).

Mast cell products affect esophageal SM function

To assess the effect of TGF- β 1 on HESM cells, we used an esophageal SM contraction collagen gel assay system.¹⁶ Cell viability and quantitative PCR assays showed that cells remained viable and functional for the treatment time period (data not shown). Collagen gels impregnated with HESM cells cultured with TGF- β 1 demonstrated significantly increased contractility, as reflected in the decreased size of the collagen gel, compared with that seen in cells cultured without TGF- β 1 (Fig 3, F and G). Contraction was dose dependent, with the maximal contraction occurring at a TGF- β 1 concentration of 10 ng/mL compared with 2 and 5 ng/mL (data not shown). Histamine also induced significant contraction, but TGF- β 1 and histamine were not synergistic (Fig 3, H, and data not shown).

Studies of the time course of TGF- β 1-induced HESM cell contraction demonstrated that in the presence of TGF- β 1, the size of the collagen gels was 85% the size of those cultured without TGF- β 1 at 2 hours (ie, TGF- β 1 had induced 15% contraction),

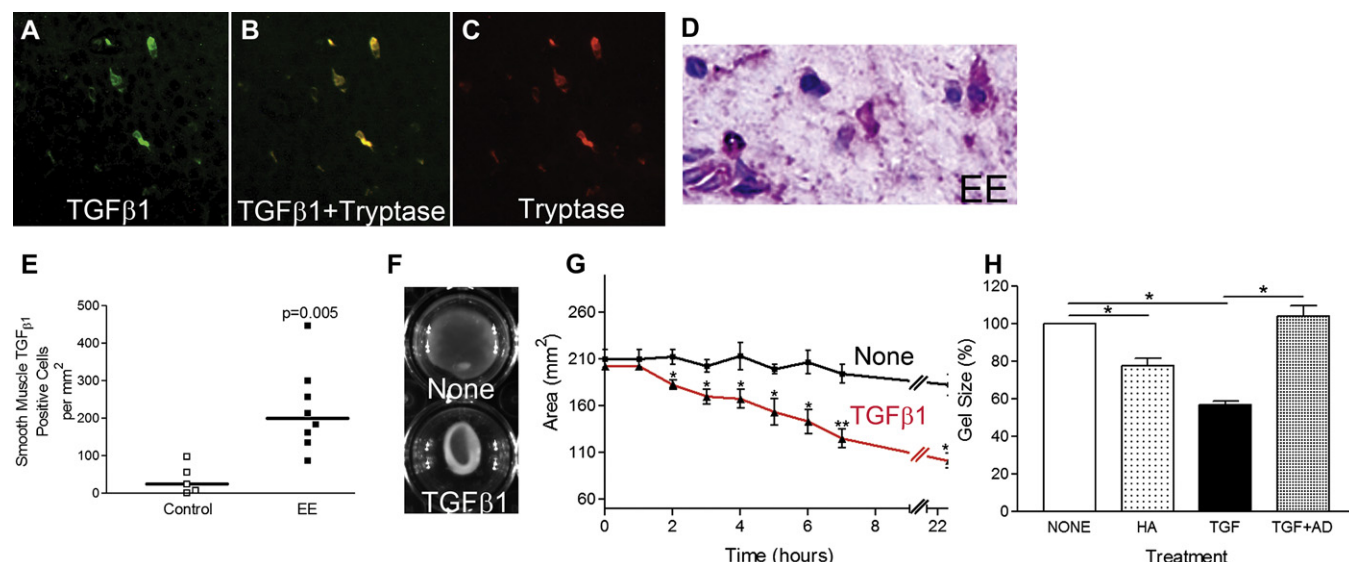


FIG 3. Mast cells express TGF- β 1, which increases esophageal SM contraction. **A–C**, TGF- β 1–positive cells (green, Fig 3, A) coexpress tryptase (red, Fig 3, C; overlap, yellow, Fig 3, B; magnification $\times 200$). **D** and **E**, Representative image (Fig 3, D) and numbers (Fig 3, E) of TGF- β 1–positive cells (magnification $\times 400$) in SM from patients with EE and control subjects. Bars represent medians. **F** and **G**, TGF- β 1 treatment of esophageal SM cells decreases collagen gel size. **H**, Collagen gel size after histamine (HA), TGF- β 1, and actinomycin D (AD). Bars represent means $\pm$ SEs. * $P < .05$.

78% at 4 hours (ie, TGF- β 1 had induced 22% contraction), 69% at 6 hours (ie, TGF- β 1 had induced 31% contraction), and 55% at 24 hours (ie, TGF- β 1 had induced 45% contraction) compared with the size of those HESM cells cultured without TGF- β 1 (Fig 3, G). The relatively delayed time course for the onset of HESM contraction (onset of approximately 2 hours) in the presence of TGF- β 1 suggested a potential transcriptional mechanism. Indeed, the addition of the transcription inhibitor actinomycin D completely blocked TGF- β 1–induced HESM contraction (Fig 3, H), and phosphorylated Smad3 was found in the nucleus of HESM cells only after TGF- β 1 treatment (see Fig E1 in this article's Online Repository at www.jacionline.org), suggesting that TGF- β 1–induced HESM cell contraction was dependent on gene transcription.

MC_T but not MC_{TC} numbers are decreased with topical corticosteroid therapy

Prior studies have not examined whether epithelial eosinophil or MC_T responses to topical corticosteroids in patients with EE predict reductions in LP mast cells.

Epithelial mast cells. Swallowed budesonide caused a significant decrease in epithelial MC_T numbers in those patients with EE who had significant reductions in epithelial eosinophil numbers (Fig 4, A and C). In contrast, epithelial MC_T numbers were persistent in those patients who did not respond to budesonide treatment (defined as persistent eosinophilic inflammation of more than 20 cells/hpf; Fig 4, A). MC_C numbers in the epithelium did not change after therapy in either group but were much lower than MC_T numbers in the epithelium (Fig 4, B, and see Table E2).

LP mast cells. LP MC_T and MC_C numbers were not decreased in patients with EE, even in patients who had significant reductions in epithelial eosinophil numbers after topical

corticosteroid therapy (Fig 4, D and E). This persistence of LP mast cell numbers was in marked contrast to LP eosinophil numbers, which were also significantly reduced by topical corticosteroid therapy in responders to topical corticosteroid therapy (Fig 4, F, and see Table E2). As such, normal numbers of LP mast cells remain static, regardless of response to therapy.

DISCUSSION

In this study we demonstrate a number of novel findings related to mast cell SM interactions in patients with EE that have potential implications for the pathogenesis of hypercontractility of the esophagus, a consistent physiologic finding among both adult and pediatric patients with EE.^{8–11} We demonstrate for the first time that in patients with EE the esophageal SM is infiltrated by MC_T, that esophageal MC_T express TGF- β 1, and that TGF- β 1 increases the contractility of primary HESM cells *in vitro*. In addition, we demonstrate that the absolute number of mast cells in the SM in patients with EE is significantly higher than the number of eosinophils in the SM. Whether increased mast cell density in the muscularis mucosa reflects increased mast cell numbers in the longitudinal and circular muscle remains to be evaluated. However, our observations suggest a potential mechanistic link between the mast cell and esophageal SM hypercontractility in patients with EE similar to that seen in patients with asthma. The numbers of biopsy specimens with adequate SM and LP are the minority (15% and 40%, respectively), but because our population is reflective of the general pediatric EE population in terms of ethnicity, age, sex, steroid response, and fibrosis, our findings are likely clinically relevant. SM in patients with EE is thickened on ultrasonography, a finding that our current study did not evaluate.²¹ In addition, studies with esophageal manometry in pediatric and adult patients have demonstrated that patients with EE have increased high-amplitude contractions with resulting abnormal peristalsis.^{8–10} Episodes of abnormal motor

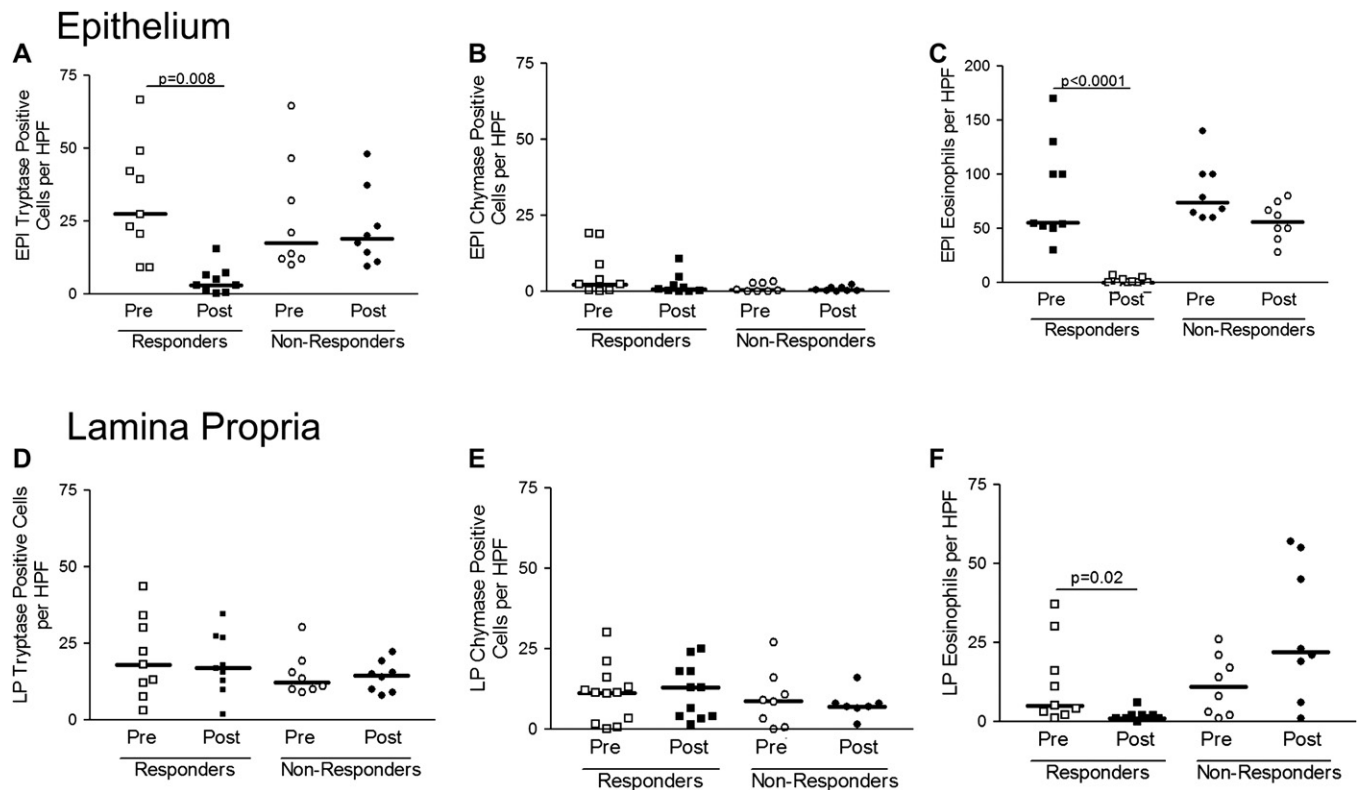


FIG 4. Effects of topical corticosteroid therapy on MC_T and MC_C numbers in the epithelium (EPI) and LP. Epithelial (A–C) and LP (D–F) tryptase- and chymase-positive cells and eosinophils before (*pre*) and after (*post*) budesonide in responders (7 or fewer epithelial eosinophils per hpf after therapy) and nonresponders (>20 epithelial eosinophils per hpf after therapy). Bars represent medians.

function coincide simultaneously with complaints of dysphagia, which is a clinical hallmark of EE.⁸ In adult patients with EE, the lack of longitudinal muscle relaxation contributes to SM hypercontractility.¹¹ Given that we have demonstrated that mast cells, but not eosinophils, are the predominant cell type increased in the SM of patients with EE, SM mast cells might be more important than SM eosinophils in mediating esophageal dysmotility and, potentially, the clinical complaint of dysphagia in patients with EE. In addition, the shift in the SM mast cell profile from predominantly MC_T in patients with EE but MC_C in control subjects might be of pathogenic importance. The effect of topical corticosteroids on SM mast cell numbers and phenotype remains to be studied.

Studies in asthmatic patients have previously demonstrated increased numbers of mast cells in airway SM compared with those seen in control subjects.¹⁶ In this regard EE resembles asthma in that both conditions are associated with the localization of increased numbers of mast cells in SM. We have phenotyped the mast cells in patients with EE in SM as being MC_T, which is similar to those identified in SM in patients with asthma.¹⁶ We also see MC_{TC} in the SM, but further studies in larger numbers of patients are required to make definitive conclusions. Our current data suggest that because MC_C numbers are not increased in the SM that MC_{TC} numbers also are not increased. TGF- β 1 has also been implicated in asthma in contributing to the increased reactivity of airway SM but through mechanisms that differ from that which we observed for TGF- β 1's effect on esophageal SM. TGF- β 1 can affect the contractility of airway SM by increasing

SM actin production.¹⁶ However, distinct from our observations in HESM cells, airway SM cells require histamine for TGF- β 1-dependent contraction.¹⁵ Our data suggest that although histamine can be sufficient to cause esophageal SM cell contraction *in vitro*, it is not required when TGF- β 1 is present. Thus TGF- β 1 has an isolated procontractile effect on esophageal SM cells *in vitro*. Indeed, using antihistamines in our contraction assay did not block the effect of TGF- β 1 (data not shown). Although we have made the novel observation that TGF- β 1 induces transcription-dependent HESM cell contraction *in vitro*, further studies are needed to determine the precise mechanism by which TGF- β 1 exerts this effect on HESM cells.

The importance of TGF- β 1 to patients with EE is suggested from studies demonstrating increased levels of TGF- β 1 protein and mRNA in both pediatric^{17,18} and adult²² patients. We have previously demonstrated that increased levels of TGF- β 1 are associated with esophageal remodeling in patients with EE.¹⁷ Our current study shows that TGF- β 1 has the potential not only to contribute to remodeling and fibrosis in patients with EE but also to contribute to the functional abnormality of SM evident in patients with EE. These data provide mounting evidence that TGF- β 1 might function as a potential therapeutic target that is involved in both esophageal remodeling and dysmotility.

In this study we detected increased MC_T numbers not only in the SM of patients with EE but also in the epithelium. Although the epithelial mast cells have a MC_T mucosal phenotype, LP mast cells are primarily MC_{TC}. Interestingly, although MC_T in the epithelium are decreased after successful topical

corticosteroid therapy, mast cell numbers in the LP remain unchanged and are no different than the number of mast cells found in control subjects. In asthmatic patients the effects of topical corticosteroids on tissue mast cells can be variable, with some reports showing resolution and others showing continued mast cell infiltration.^{23,24} MC_{TC} can be long lived in tissues and can retain their proliferative capacity while keeping a mature phenotype.²⁵ Although MC_{TC} numbers in the LP were unchanged by topical budesonide, there were significant reductions in LP eosinophil numbers in the same corticosteroid-responsive patients. Whether the activation of the state of LP mast cells is changed by topical steroids is currently unclear. Our data suggest that in the LP topical corticosteroids have differential effects on various inflammatory cell types. Whether nonresponders failed therapy because of pharmacogenetic, compliance, or other reasons are unclear. Interestingly, we have noticed that the TGF- β 1 CC genotype at C-509T is associated with response to topical corticosteroids.¹⁹ Our current study cannot determine the effects of corticosteroids on esophageal SM mast cells because of the paucity of paired specimens with SM.

In summary, in this study we have demonstrated novel observations regarding a mast cell–SM cell interaction in patients with EE, which might have implications for the pathogenesis of esophageal dysmotility and dysphagia in these patients. Increased numbers of MC_T infiltrate the esophageal SM in patients with EE. The expression of TGF- β 1 by MC_T in esophageal SM has the potential to provide a local significant source of TGF- β 1 that can influence esophageal SM contractility. Topical corticosteroids effectively reduce epithelial MC_T and the numbers of cells expressing TGF- β 1 in the LP.^{4,19} Given that successful EE treatment with swallowed fluticasone is associated with resolution of dysmotility in adults,^{9,10} one mechanism of topical corticosteroid action might be through decreasing esophageal TGF- β 1 levels. Further studies are needed to determine whether topical corticosteroid–induced reductions in MC_T numbers and TGF- β 1 expression are associated with improved peristalsis.

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Clinical implications: Patients with EE have significant dysmotility, but the effect of inflammation on esophageal SM contraction is unclear. Mast cells and TGF- β 1 might provide therapeutic targets for alleviating dysmotility and dysphagia in patients with EE.

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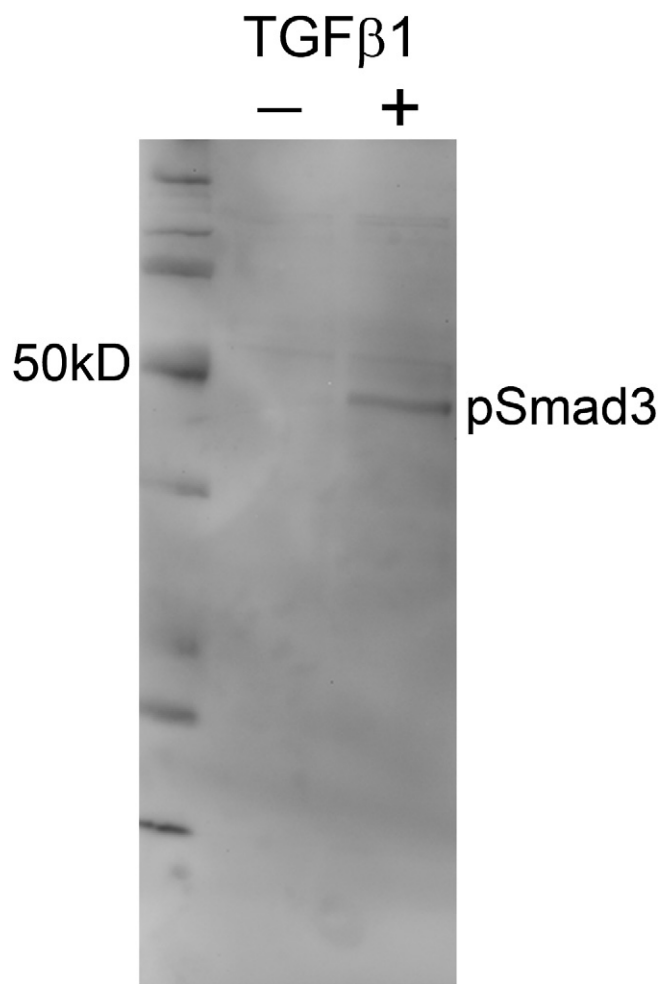


FIG E1. Immunoblot for phosphorylated Smad3 using nuclear extracts from esophageal SM cells cultured in the absence (–) or presence (+) of TGF-β1 shows pSmad3 in the nucleus only after TGF-β1 treatment.

TABLE E1. Clinical characteristics of patients with EE and control subjects

	Age	Sex	Symptoms	PPI	Endoscopy	Eosinophils per hpf	
						Before	After
Patients with EE							
1	2 y, 10 mo	M	HB, V	Yes	Pa, Fu, Plq	100	0
2	17 y, 9 m	F	HB, P	Yes	Pa, Fu, Plq	130	0
3	3 y, 5 mo	M	HB, V	Yes	Normal	55	0
4	1 y, 1 mo	M	HB, V	Yes	Fu, Pl	52	0
5	1 y, 5 mo	M	P, V	Yes	Fu, Pl	54	1
6	7 y, 6 mo	F	HB, P, V, AE	No	Fu, Pl	170	3
7	2 y, 8 mo	M	HB, P, V, AE	Yes	Pa	100	5
8	3 y, 9 mo	M	HB, P, V, AE	Yes	Fu	30	7
9	9 y, 4 mo	M	P, AE	Yes	Fu	50	0
10	7 y, 4 mo	M	HB, P, V, Dy	Yes	Pa, Fu	60	80
11	2 y, 3 mo	M	Dy, AE	Ranitidine	Pa, Fu	68	67
12	5 y, 6 mo	M	HB, P, AE, Dy	Yes	Plq	60	50
13	6 y, 9 mo	M	P, V, Dy	No	Pa, Fu, Plq	65	62
14	7 y, 10 mo	M	HB, V, Dy	Yes	Pa	100	75
15	2 y, 8 mo	M	HB, P, V	Yes	Pa, Pl	100	50
16	5 y, 8 mo	F	P, V	Yes	Pa, Fu, Plq	79	28
17	13 y, 11 mo	F	P, V, HB	Yes	Fu	140	40
18*	4 y, 5 mo	F	Dy, AE	Yes	Fu, Pa	40	
19*	5 y, 7 mo	M	Dy, V, HB	Yes	Fu, Pa, Plq	100	
20*	3 y, 7 mo	M	V	Yes	Fu, Pa, Plq	70	
21*	1 y, 9 mo	F	V, FTT	Ranitidine	Fu, Pa	40	
Control subjects							
1	6 y	M	P, N, diarrhea		Normal	0	
2	16 y	M	P		Normal	0	
3	2 y	M	Diarrhea		Normal	0	
4	10 y	M	Constipation		Normal	0	
5	14 y	F	N		Normal	0	
6	13 y	M	HB		Normal	0	
7	7 y	M	P		Normal	0	
8	16 y	F	P, V		Normal	0	
9	9 y	M	V		Normal	0	

Dy, Dysphagia; F, female; Fu, furrows; HB, heartburn; M, male; N, nausea; P, pain; Pa, pallor; Plq, plaques; PPI, proton pump inhibitor; FTT, failure to thrive; V, vomiting.

*Biopsy specimens used for SM studies only.

TABLE E2. MC_T and MC_C in the epithelium and LP

	MC _T number per hpf				MC _C number per hpf			
	Epithelium		LP		Epithelium		LP	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Patients with EE								
1	9	0.7	34	17	2	0	13	3
2	23	0.3	3	10	0.3	0.2	21	4
3	21	7	22	16	0.3	0.7	11	13
4	42	5	13	13	4	2	11	13
5	49	3	18	27	19	5	11	18
6	39	7	12	18	19	1	12	18
7	67	16	30	35	9	11	30	25
8	27	3	44	27	2	0.3	16	24
9	9	2	8	2	0	0	2	4
10	12	10	16	15	0.7	0.3	16	16
11	21	20	10	14	0	0.3	0.7	2
12	14	14	10	8	3	1	9	7
13	10	11	11	10	0	2	3	7
14	32	37	19	16	0	1	11	7
15	47	23	9	19	3	0.3	9	8
16	65	18	30	22	3	0.5	27	8
17	12	48	14	9	0.3	0	0	NA
Median	23	10	14	16	2	1	11	8
Control subjects								
1	4		10		2		4	
2	4		8		0		12	
3	0		6		0.3		4	
4	6		11		3		12	
5	2		12		0.7		21	
6	1		9		0		9	
7	1		12		0		13	
8	0.3		17		0.2		22	
9	0.7		24		2		16	
Median	1		11		0.3		12	

NA, Not available; *Post*, after at least 3 months of swallowed topical budesonide therapy; *Pre*, before budesonide.

TABLE E3. SM MC_T and MC_C numbers

	Cells per hpf		Cells per mm ²	
	Tryptase	Chymase	Tryptase	Chymase
Patients with EE				
1	27	20	282	180
8	41	2	662	166
14	10	6	242	136
16	22	6	276	136
18*	10	12	240	64
19*	11	3	246	76
20*	14	10	190	110
21*	21	6	375	97
Median	17	6	261	105
Control subjects				
1	3	13	55	82
4	5	7	77	102
6	5	10	105	136
7	5		85	
8	4	9	84	152
9	3	19	160	225
Median	4	10	85	137

*Patients' biopsy specimens were used only for SM studies.