

Esophageal remodeling in pediatric eosinophilic esophagitis

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Background: Eosinophils are associated with airway remodeling in asthma, but studies have not yet determined whether eosinophilic esophagitis (EE) is associated with esophageal remodeling.

Objective: We performed quantitative immunohistochemical analysis of remodeling changes in esophageal biopsy specimens from children with and without EE to evaluate if there were changes in the esophagus of pediatric patients with EE akin to airway remodeling. In addition, we determined whether the esophagus of patients with EE had increased levels of expression of TGF- β_1 and its signaling molecule, phosphorylated SMAD2/3 (phospho-SMAD2/3).

Methods: To determine esophageal levels of eosinophilic inflammation, fibrosis, and vascular activation, endoscopically obtained esophageal biopsy specimens from 7 patients with EE (5 strictured, 2 nonstrictured), 7 with gastroesophageal reflux disease, and 7 normal patients were processed for immunohistology, trichrome staining, and assessment of levels of expression of TGF- β_1 , phospho-SMAD2/3, and vascular cell adhesion molecule 1.

Results: Esophageal biopsies in patients with EE demonstrated increased levels of subepithelial fibrosis and increased expression of TGF- β_1 and its signaling molecule phospho-SMAD2/3 compared with gastroesophageal reflux disease and normal control patients. In addition, esophageal biopsies in patients with EE demonstrated an increased vascular density and an increased expression of the vascular endothelial adhesion molecule, vascular cell adhesion molecule 1.

Conclusion: Previously unrecognized esophageal remodeling changes analogous to aspects of airway remodeling are detectable in the subepithelial region of the esophagus in pediatric patients with EE.

Clinical implications: Pediatric patients with EE demonstrate increased fibrosis, vascularity, and vascular activation in the esophagus that may contribute to stricture formation and

potentially provide a basis for stratifying patients with EE on the basis of disease severity and/or prognosis. (J Allergy Clin Immunol 2007;119:206-12.)

Key words: Eosinophilic esophagitis, children, vascularity, fibrosis, strictures

Eosinophilic esophagitis (EE) is a disease of increasing incidence in both the pediatric and adult population.¹⁻³ EE is a patchy panesophagitis, and the diagnosis is based on esophageal histologic findings of greater than 20 to 24 eosinophils per high-power field (hpf) at $\times 400$ light microscopy. Clinical symptoms of dysphagia, vomiting, and abdominal pain are common.³⁻⁸ Typical endoscopic findings include pallor, linear furrows, concentric rings, white exudates, and strictures.^{3,7,9-13} The immune mechanism mediating EE appears to involve both immediate and delayed hypersensitivity to inhaled and ingested allergens.¹⁴⁻¹⁷ Patients are more commonly male and have a greater prevalence of allergies to foods and aeroallergens than the general population.^{18,19} To date, the most severe reported complication of long-standing EE is stricture formation.^{3,7,20}

Esophageal biopsy specimens from patients with EE demonstrate a mixed T_H2-T_H1 cytokine profile with increased expression of T_H2 cytokines (for example, IL-5 as well as intracellular IL-4 and IL-13 in eosinophils) and T_H1 cytokines (IFN- γ).²¹⁻²³ There is also increased expression of the eosinophil chemoattractants eotaxin-1 and eotaxin-3 as well as TNF- α .^{23,24} Animal models have demonstrated that IL-5 and IL-13 are both important cytokines for driving esophageal eosinophilia.²⁵⁻²⁷ Treatment with anti-IL-5 therapy can result in disease resolution in adults with EE.²⁸

Airway remodeling in asthma refers to structural changes that include subepithelial fibrosis and angiogenesis. Implicated growth factors include the profibrotic molecule, TGF- β_1 , and its downstream transcription factors, phosphorylated SMAD2/3 (phospho-SMAD2/3).²⁹⁻³³ Activation of the TGF- β_1 pathway in the cell results in the phosphorylation and nuclear translocation of SMAD complexes, thus allowing changes in specific gene transcription. Patients with asthma have elevated levels of TGF- β_1 , and mice deficient in SMAD-3 have decreased fibrosis, implicating these factors in the genesis of airway fibrosis.³⁰⁻³² An increase in proangiogenic factors leads to greater vascular density in the bronchial subepithelium, potentially resulting both in increased airway edema and an increased number of vascular conduits for inflammation.

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Supported by the American Academy of Allergy, Asthma & Immunology Education and Research Trust (ERT) Clinical Research Grant (S.S.A.), National Institutes of Health T32 Training award AI 007469 (S.S.A.), and National Institutes of Health grant AI 38425 (D.H.B.).

Disclosure of potential conflict of interest: S. S. Aceves has received grant support from the American Academy of Allergy, Asthma and Immunology Clinical ERT. D. H. Broide has received grant support from the National Institutes of Health. The other authors have declared that they have no conflict of interest.

Received for publication June 15, 2006; revised October 5, 2006; accepted for publication October 10, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.10.016

Abbreviations used

BZH:	Basal zone hyperplasia
EE:	Eosinophilic esophagitis
EGD:	Esophagogastroduodenoscopy
GERD:	Gastroesophageal reflux disease
hpf:	High-power field
LP:	Lamina propria
Phospho-SMAD2/3:	Phosphorylated SMAD2/3
VCAM-1:	Vascular cell adhesion molecule 1
vWF:	von Willebrand Factor

The concept that esophageal stricture formation is akin to allergen-induced airway remodeling remains to be evaluated. In this study, we demonstrate that esophageal tissue from children with EE demonstrates several features of structural remodeling found in allergen-induced airway remodeling, including increased subepithelial fibrosis and vascular density. In addition, we elucidate potential mechanisms for fibrosis, because children with EE have increased expression of TGF- β_1 and its downstream signaling molecule, phospho-SMAD2/3. We also demonstrate that the increased vascularity in patients with EE is associated with endothelial activation with increased expression of vascular cell adhesion molecule 1 (VCAM-1), which is likely to play an important role in the accumulation of eosinophils into the esophagus of patients with EE.

METHODS

Patient selection

An EE database was compiled at Children's Hospital San Diego of all patients meeting criteria for EE and an equal number of patients with gastroesophageal reflux disease (GERD).³ The biopsy used in this study was the first biopsy that demonstrated EE in the patient. EE was defined histologically as greater than 20 eosinophils per hpf on $\times 400$ light microscopy. Patients with GERD were children who underwent diagnostic endoscopic esophageal biopsy for complaints of vomiting and pain consistent with gastroesophageal reflux and who did not meet histologic criteria for EE. Normal patients were defined as children who underwent diagnostic esophagogastroduodenoscopy (EGD) with biopsy, had normal EGD, and had no eosinophilic or other inflammation and no basal zone hyperplasia; therefore, "normal" refers to the state of the child's esophagus. Medical records were reviewed for all patients, and histologic, pathologic, endoscopic, and clinical information was compiled into the database. The diagnosis of an esophageal stricture was based on barium swallow and/or endoscopy.

Esophageal biopsy specimens from 7 patients with EE from the EE database were studied on the basis of the presence of an adequate amount of esophageal lamina propria (LP) being available for immunohistochemistry studies and the presence of stricture on the basis of review of medical records. A similar number of esophageal biopsy specimens from patients with GERD and normal patients were randomly chosen from the database on the basis of an adequate amount of LP being available for immunohistochemistry studies, and the subject being similar in age to patients with EE. All studies were approved by the combined Institutional Review Board of University of California, San Diego, and Children's Hospital, San Diego.

Histologic evaluation

Fibrosis. Levels of esophageal fibrosis were assessed by Masson trichrome staining, hematoxylin-eosin, or both on light microscopy. A subepithelial region from 70 to 150 microns immediately beneath the epithelium was assessed for fibrosis in each biopsy specimen. Fibrosis was scored by a blind investigator from 0 to 3 on the basis of the number of fibroblasts, thickness of collagen bundles, and collagen accumulation.

Eosinophils. The number of eosinophils per hpf in the LP and epithelium was quantitated in 3 separate hpf on hematoxylin-eosin light microscopy (at $\times 400$), and the maximum number of eosinophils per hpf was entered into a database. Only intact eosinophils were counted to determine the eosinophil number. Each esophageal specimen was evaluated by a blind investigator for the presence of degranulated eosinophils (defined as free eosinophil granules in the esophageal epithelium), basal zone hyperplasia (BZH; normal basal zone was defined as less than 25% of the total esophageal epithelium thickness in well oriented sections), eosinophil clusters (defined as 4 or more eosinophils clustered together), and epithelial desquamation (defined as degenerative squamous epithelial cells and edema surrounded by a superficial infiltrate of eosinophils). The basal zone was graded as normal for <25%, mild for 26% to 50%, moderate for 51% to 75%, and severe hyperplasia for >75% of the total esophageal epithelial thickness.³⁴

Immunohistochemistry

Esophageal biopsies were fixed in 10% formalin and processed for immunohistochemistry as previously described.²⁹ In brief, sections were deparaffinized, hydrated, and immunostained with primary antibodies to detect features of esophageal fibrosis and vascular changes. To detect cytokines and signaling molecules associated with fibrosis, an anti-TGF- β_1 antibody and an anti-pSMAD2/3 antibody (detects nuclear, phosphorylated SMAD2/3; both 1:500 dilution, both from Santa Cruz Biochemicals, Santa Cruz, Calif) were used.²⁹ To detect vascular changes, we used either an anti-von Willebrand Factor (vWF, detects endothelial cells, 1:200; DAKO, Carpinteria, Calif) or an anti-VCAM-1 (detects activated endothelium, 1:400; R&D, Minneapolis, Minn) antibody. For studies using fluorescence microscopy, sections were first incubated with the primary antibody (anti-TGF- β_1 , anti-VCAM-1, or anti-vWF) and then with the appropriate directly conjugated secondary fluorescent antibody, or developed using a TSA-ALEXA fluorescence amplification system (Invitrogen, Carlsbad, Calif). For double-labeling fluorescence, esophageal sections were incubated with anti-MBP (detects eosinophils, 1:10; Biotex, Woburn, Mass) or MIB-1 (also known as anti-Ki67, detects proliferating epithelium, 1:100; DakoCytomation, Carpinteria, Calif) followed sequentially by a secondary fluorescent antibody and a second primary antibody (anti-phospho-SMAD2/3 or anti-TGF- β_1) and appropriate fluorescent secondary antibody. Specimens were mounted in Vectashield (Vector Labs, Burlingame, Calif) mounting media with or without a fluorescent nuclear stain (DAPI, DAPI Vector Labs, Burlingame, Calif; or Sytox, Invitrogen, Carlsbad, Calif) and analyzed by using standard fluorescence microscopy. For studies using nonfluorescent detection methods, sections were first incubated with a primary antibody (anti-TGF- β_1 , or anti-pSMAD2/3), and then with the appropriate secondary antibody in conjunction with an ABC signal amplification signal (Vector Labs) as previously described.²⁹ Images were captured by using Microfire (Optronics, Goleta, Calif) or ImagePro (Media Cybernetics, Silver Spring, Md). Immunostained slides were all quantified under identical light or fluorescence microscope conditions, including magnification ($\times 400$), gain, camera position, and background illumination. Cells were enumerated at $\times 400$, and vessel density in the LP was quantitated at

TABLE I. Clinical, endoscopic, and histologic characteristics

	Diagnosis	Age (y)	Sex	Symptom	Atopy	Endoscopy	Eosinophil/hpf	BZH	Fibrosis
EE									
1	EE	14	M	D	None	S, Pa, F	25	Severe	2
2	EE	13	M	D, V	A, AR	S, Pa, F	40	Mod	3
3	EE	10	M	D	AR, Urt	S, Pa, Pl, F	75	Severe	2
4	EE	14	M	D	ND	S	80	Mod	3
5	EE	12	M	D	A, AR, FA	S, Pa, F	60	Severe	3
6	EE	5	M	D, V	AR	Pa, Pl	50	Severe	3
7	EE	6	M	D, V, P	Urt	Pa, Pl, F	45	Mild	2
GERD									
1	GERD	11	M	V	None	Normal	1	No	1
2	GERD	3	F	P	ND	Normal	3	No	1
3	GERD	15	F	V, P	None	Erosions	1	No	0
4	GERD	14	M	V	None	Normal	13	No	0
5	GERD	4	M	V, P	A	Normal	2	No	2
6	GERD	11	F	V, P	None	Normal	1	No	2
7	GERD	16	M	V	A, AR	Normal	1	Mild	2
Normal									
1	Nausea	14	F	P	None	Normal	0	No	0
2	Dyspepsia	16	M	P	A	Normal	0	No	0
3	Lactic acidemia	13	M	V	None	Normal	0	No	1
4	Constipation	10	M	P	AR	Normal	0	No	1
5	Diarrhea	2	M	Dia	None	Normal	0	No	1
6	Dyspepsia	16	M	P	None	Normal	0	No	1
7	Constipation	7	M	V, P	None	Normal	0	No	1

Symptom: *D*, Dysphagia; *Dia*, diarrhea; *P*, pain; *V*, vomiting; atopy: *A*, asthma; *AR*, allergic rhinitis; *FA*, food allergy; *ND*, not determined; *Urt*, urticaria; endoscopy: *F*, furrows; *Pa*, pallor; *Pl*, white plaques; *S*, stricture. *M*, Male; *F*, female.

×400 fluorescence microscopy in 2 to 5 separate fields depending on the abundance of LP. Results are expressed as the number of cells/hpf or vessels/hpf.

Statistical analysis

Statistical analysis was performed by using the NCSS statistical package (NCSS, Kaysville, Utah). Two-tailed *P* values were generated using an unpaired Student *t* test for comparison of means. For data on the noncontinuous variable of fibrosis, a Mann-Whitney *U* test was used to compare between groups. Correlation coefficients (Spearman *r*) were calculated using GraphPad Prism (GraphPad software, San Diego, Calif). A *P* value < .05 was considered statistically significant.

RESULTS

Patient characteristics

The mean age of patients with EE (*n* = 7), patients with GERD (*n* = 7), and patients with a normal esophagus (*n* = 7) was 10.5, 8.4, and 11.2 years, respectively (Table I). All children with EE were male, whereas 57% of children with GERD and 86% of normal children were male. All of the patients with EE complained of dysphagia, whereas none of the patients with GERD or normal patients complained of dysphagia. Five of 7 patients with EE were known to be atopic (71%), 2 of 7 patients with GERD were atopic (28%), and 2 of 7 normal children were atopic (28%; Table I). Two patients with EE (numbers 1 and 3) were on omeprazole 20 mg twice daily.

The average duration of symptoms among patients with EE was 3.2 years before correct diagnosis. There was no correlation between symptom duration and any markers of remodeling.

Endoscopic characteristics

Five of 7 patients with EE had esophageal strictures detected at the time of EGD. Three of the patients with EE had known strictures from previous upper gastrointestinal barium swallow testing. None of the patients with EE had a normal endoscopy. Endoscopic features noted in patients with EE included strictures (71%), fissures (71%), plaques (41%), and/or pallor (86%). All of the normal controls had a normal endoscopy, and 1 patient with GERD had distal erosions.

Histologic characteristics

The mean number of eosinophils in the esophageal epithelium was 52 eosinophils per hpf (range, 25-80) in patients with EE, 3 per hpf (range, 1-13) in patients with GERD, and 0 per hpf (range, 0-0) in normal patients. Eosinophils were diffusely scattered through the epithelium, and in those specimens with epithelial desquamation, eosinophils were also layered at the luminal surface. In the LP, patients with EE had a mean of 17 eosinophils per hpf (range, 4-58), whereas patients with GERD had 2 (range, 0-4). Degranulated eosinophils were found only in the patients with EE. All of the patients with EE had BZH, 1 patient with GERD had mild BZH, and none of the

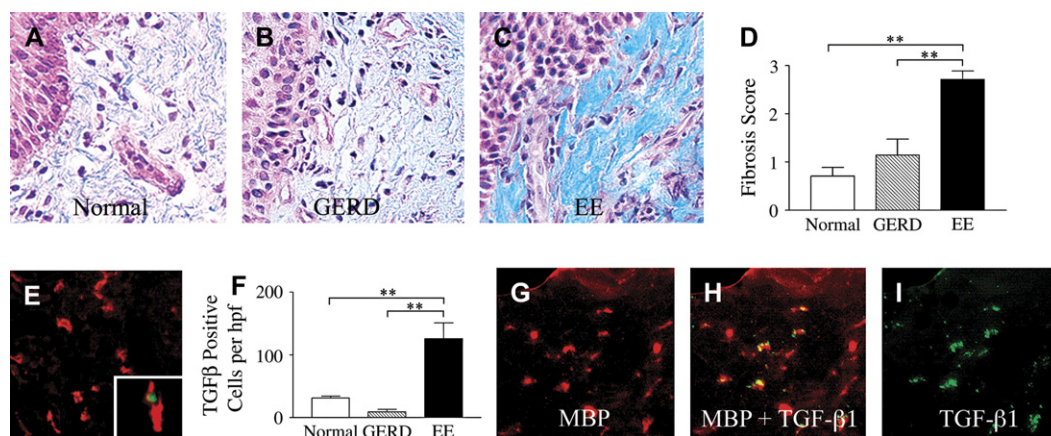


FIG 1. Pediatric patients with EE have increased subepithelial fibrosis and TGF- β_1 . **A-D**, Trichrome stain of normal, GERD, EE esophageal biopsies and fibrosis score. **E**, Representative image of EE biopsy stained for TGF- β_1 (red; inset shows green nucleus). **F**, Quantitated TGF- β_1 -positive cells in normal patients, patients with GERD, and patients with EE. **G-I**, Eosinophils express MBP (red), TGF- β_1 (green), or both (yellow). ** $P < .005$.

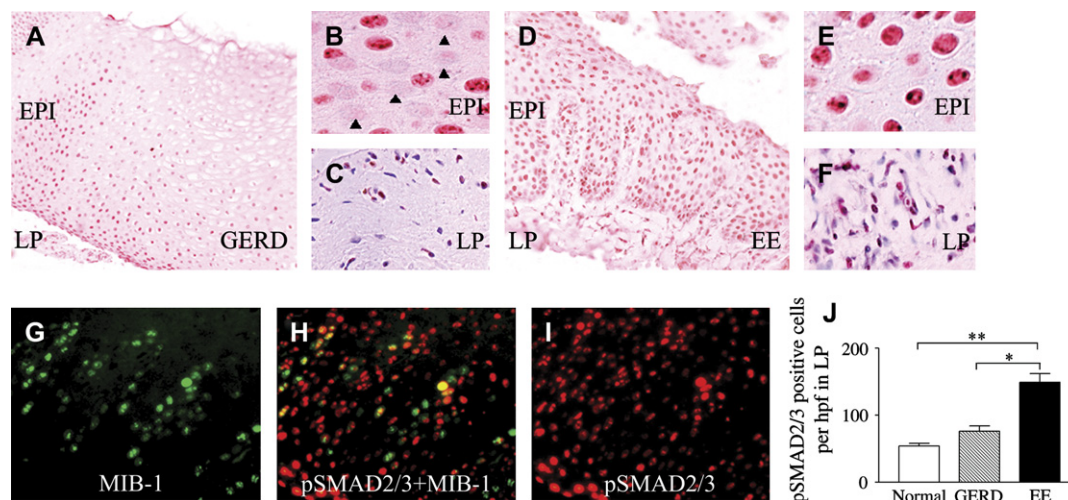


FIG 2. Increased nuclear phospho-SMAD2/3 in the esophagus of patients with EE. **A-F**, Representative image of phospho-SMAD2/3 staining in epithelial (EPI) and lamina propria cells in patients with GERD (arrows show phospho-SMAD2/3-negative nuclei) and patients with EE. **G-I**, A subset of actively proliferating epithelial cells coexpress (yellow) MIB-1 (green) and phospho-SMAD2/3 (red). **J**, Quantitation of phospho-SMAD2/3-positive cells in normal, GERD, and EE. * $P < .05$; ** $P < .005$.

normal patients had BZH. Four patients with EE had histologic evidence of epithelial desquamation that was associated with eosinophil clusters in the epithelium. None of the patients with GERD or normal patients demonstrated either of these histologic changes.

Esophageal fibrosis and expression of TGF- β_1 in EE

There was increased trichrome staining in esophageal biopsies from pediatric patients with EE compared with both patients with GERD and normal control patients (Fig 1, A-C). All 7 patients with EE demonstrated subepithelial fibrosis (4 severe, 3 moderate) on hematoxylin-eosin staining and/or trichrome staining. The esophageal

fibrosis score was significantly higher in patients with EE (mean, 2.7) compared with patients with GERD (mean, 1.1; $P = .004$) or normal patients (mean, 0.7; $P = .001$; Fig 1, D). Given the increase in total collagen deposition noted on trichrome staining, we assessed molecules that may be involved in the fibrotic process occurring in pediatric patients with EE.

Esophageal biopsy specimens from pediatric patients with EE had increased numbers of TGF- β_1 -positive cells in the LP (mean, 126 per hpf; 95% CI, 61-191 per hpf) compared with patients with GERD (mean, 9 per hpf; 95% CI, -2-24 per hpf; $P = .002$) and normal patients (mean, 31 per hpf; 95% CI, 24-38; $P = .004$; Fig 1, E and F). Cytoplasmic TGF- β_1 expression was primarily

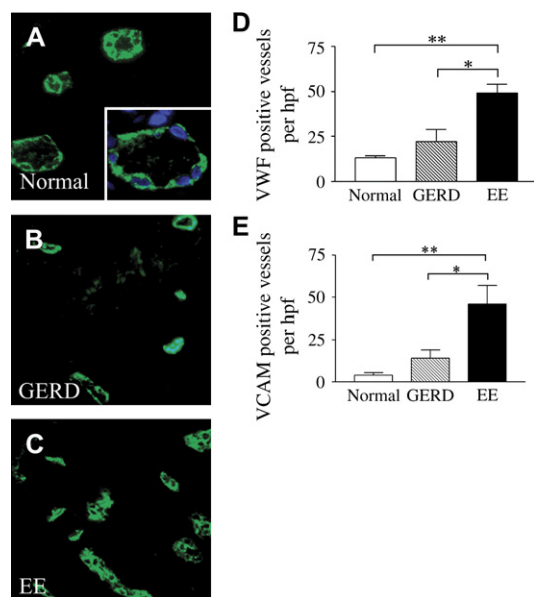


FIG 3. Increased vascularity and VCAM-1 in the subepithelial esophagus of patients with EE. **A-C**, Representative images of vWF-positive vessels in the lamina propria of normal patients, patients with GERD, and patients with EE (inset shows blue nuclei). **D and E**, Quantitation of vWF and VCAM-positive vessels. * $P < .05$; ** $P < .005$.

restricted to the LP because in neither patient population did the epithelium stain consistently for TGF- β_1 (Fig 1, E). Within the LP, many of the TGF- β_1 -positive cells were eosinophils (Fig 1, G-I). The number of eosinophils was positively correlated with the number of TGF- β_1 -expressing cells ($r = 0.74$; $P = .01$). Double-labeling studies using antibodies to detect myocytes, fibroblasts, or macrophages demonstrated that these cellular populations did not express TGF- β_1 (data not shown).

Increased phospho-SMAD2/3 nuclear expression in esophageal basal epithelium and LP in subjects with EE

To evaluate whether the SMAD signaling pathway was activated by the increased expression of TGF- β_1 detected in patients with EE, we used an antibody specific for phospho-SMAD2/3. Patients with EE had a mean of 148 (95% CI, 116-181) phospho-SMAD2/3-positive cells per hpf at $\times 400$ in the LP compared with 76 (95% CI, 54-98) positive cells per hpf in GERD ($P = .002$) and 54 (95% CI, 45-63) in normal children ($P < .00001$; Fig 2, C, F, J). Both the basal layer of the epithelium and the LP demonstrated significantly increased activation of pSMAD2/3 in patients with EE compared with those without EE (Fig 2, A-F). Double immunofluorescence studies using MIB-1 antibody demonstrated that a subpopulation of actively proliferating epithelial cells had nuclear phospho-SMAD2/3 (Fig 2, G-I). Double-labeling studies with MBP and phospho-SMAD2/3 antibodies demonstrated no overlapping expression, indicative that eosinophils do not have nuclear phospho-SMAD2/3

(data not shown). There was a positive correlation between the number of eosinophils and phospho-SMAD2/3-positive cells in the epithelium ($r = 0.69$; $P = .02$) and LP ($r = 0.78$; $P = .01$).

Esophageal vascularity in the LP in patients with EE

Patients with EE had a significantly increased number of esophageal blood vessels in the subepithelial region, assessed by immunostaining for vWF, compared with patients with GERD and normal patients. Whereas patients with EE had a mean of 49 vessels per hpf (95% CI, 37-60), patients with GERD had 22 vessels per hpf (95% CI, 4-39; $P = .007$), and normal patients had 13 vessels per hpf (95% CI, 10-16; $P = .00002$; Fig 3, A-D). The number of eosinophils per hpf correlated significantly with the number of vWF-stained vessels ($r = 0.66$; $P = .01$), demonstrating a positive correlation between eosinophils and vascularity.

To evaluate the activation state of the vascular endothelium, we stained esophageal biopsy specimens with an antibody specific for VCAM-1. Compared with patients with GERD and normal patients, patients with EE had a significantly greater number of blood vessels that were positive for VCAM-1 staining (Fig 3, E). The mean number of VCAM-1-positive vessels per hpf in the subepithelial region of patients with EE was 47 (95% CI, 27-66) compared with 14 VCAM-1-positive vessels per hpf (95% CI, 1-28; $P = .01$) in patients with GERD and 4 (95% CI, 1-8; $P = .001$) in normal children (Fig 3, E). VCAM-1-positive vessels were found both in the LP and in the vascular papillae extending into the epithelium, and there was a positive trend between the number of eosinophils per hpf and the number of VCAM-1-positive vessels ($r = 0.61$; $P = .07$).

DISCUSSION

In this study we have demonstrated the novel finding that several structural features associated with allergen-induced airway remodeling in patients with asthma are also occurring in the esophagus of pediatric patients with EE, especially in the subset of patients who have esophageal strictures. However, even nonstrictured patients with EE had increased fibrosis and vascularity compared with patients with GERD and normal patients, suggesting that the remodeling process is occurring even in the absence of frank stricture formation. We chose to concentrate on patients with stricture formation because this is the most severe complication reported to occur with EE. We elucidate a potential mechanism of fibrosis through the observation that there is increased expression of TGF- β_1 and activation of its downstream signaling molecule, phospho-SMAD2/3. In addition, we demonstrate that the increased vascularity in patients with EE is likely an important component of eosinophil recruitment to the esophagus given the increased expression of the vascular adhesion molecule, VCAM-1. Although it has been previously noted

that there is increased collagen deposition in the esophagus of patients with EE,^{7,20,35} the increase in profibrotic cytokines, signaling molecules, vascularity, and vascular activation have not been previously demonstrated in EE.

Airway remodeling in asthma, which can be considered a form of airway injury and repair occurring in a T_H2 cytokine milieu, is composed of several pathologic features, including subepithelial fibrosis and angiogenesis. The eosinophil has been noted to play a prominent role in allergen-induced airway remodeling in mice as well as in airway remodeling in human beings with asthma.^{30,31} Interestingly, our pediatric patients with EE with prominent esophageal eosinophilia also demonstrated features of remodeling, supporting the notion that eosinophilic inflammation may be associated with features of remodeling in the esophagus. Consistent with this, the degree of eosinophilia was positively correlated with the degree of expression of TGF- β ₁, phospho-SMAD2/3, vWF, and VCAM-1 expression. TGF- β ₁, a profibrotic molecule, likely plays an important role in the subepithelial remodeling occurring in patients with EE given its increased expression in the esophagus in patients with EE. Because TGF- β ₁ signals through SMAD2/3, our demonstration of increased activation of phospho-SMAD2/3 in the esophagus would predict that the TGF- β ₁ signaling pathway is indeed activated in subjects with EE. The increased expression of TGF- β ₁ was greater than the degree of nuclear phospho-SMAD2/3 that was detected (4-fold to 14-fold vs 2-fold to 3-fold), which may indicate that there is not a 1:1 stoichiometry between TGF- β ₁ and phospho-SMAD2/3, that there are greater stores of intercellular than secreted TGF- β ₁, or that TGF- β ₁ is using additional transcription pathways. Although eosinophils appear to be a significant source of TGF- β ₁ in EE, it is likely that other cell types are also producing TGF- β ₁ because not all TGF- β ₁-positive cells are eosinophils. These TGF- β ₁-positive cells, however, seem not to be macrophages, myocytes, or fibroblasts (data not shown). Because the majority of inflammation in EE is a result of eosinophils that express TGF- β ₁, and because eosinophil number correlates with TGF- β ₁, eosinophils appear to be the major source of TGF- β ₁ in these children. This observation directly implicates the eosinophil in EE disease pathogenesis, because stricture formation is a fibrotic process. Because increased nuclear phospho-SMAD2/3 was detected in both the epithelium and LP, TGF- β ₁ may be acting in its capacity as a diffusible molecule to affect the esophageal epithelium, or additional signaling molecules may be partially responsible for the increased epithelial phospho-SMAD2/3. Increased levels of TGF- β ₁ have been noted in the remodeled airways of mice and humans with asthma,^{30,31} and anti-TGF- β ₁ antibodies inhibit allergen-induced airway remodeling in mice.³² Thus, a similar TGF- β ₁-mediated pathway to remodeling may be activated in the esophagus in EE. The importance of SMAD3 signaling to TGF- β ₁-mediated fibrosis is suggested from studies of mice deficient in SMAD3 that have significantly reduced levels of TGF- β ₁ signaling and significantly reduced levels of fibrosis.³³

By using an antibody specific for the vascular endothelial protein, vWF, we demonstrate that there is an increase in esophageal subepithelial vascular density in patients with EE compared with patients with GERD and normal patients. In addition, we demonstrate that esophageal blood vessels have an activated phenotype and express the adhesion molecule VCAM-1. Endothelial expressed VCAM-1 binds to very late antigen 4 expressed by eosinophils and thus may provide an important pathway for eosinophil adhesion to esophageal blood vessel, promoting eosinophil tissue recruitment in patients with EE. In murine models of allergic inflammation, studies have revealed important roles for TNF- α , IL-1, and IL-4 in eosinophil adhesion to inflamed blood vessels as well as to eosinophil tissue recruitment.³⁶ Previous studies of human EE biopsy specimens have also demonstrated an increase in the tissue expression of TNF- α but not IL-4 in EE.^{22,23} It is possible that the increased expression of TNF- α in EE is at least partially responsible for vascular activation with facilitated eosinophil transmigration in the presence of CC chemokines such as eotaxin-1 and eotaxin-3 and the cytokine IL-5, which have previously been detected in EE.²²⁻²⁴

In summary, we have demonstrated that esophageal biopsies in pediatric patients with EE demonstrate many of the structural remodeling features noted in the airway in asthma, including fibrosis and increased vascularity. Because the endoscopically obtained esophageal biopsies do not contain significant amounts of smooth muscle, this study is unable to address whether smooth muscle remodeling changes also occur in EE. The increased levels of TGF- β ₁ and phospho-SMAD2/3 expression noted in EE suggest that activation of this signaling pathway may be important in esophageal stricture formation. Because not all patients with EE develop esophageal strictures, a subset of patients with EE may have a genetic predisposition to stricture formation. Further prospective studies will help to determine whether detection of TGF- β ₁ and phospho-SMAD2/3 expression in esophageal biopsies from subjects with EE is able to stratify subjects on the basis of disease severity and prognosis.

We thank Drs J. Y. Cho and Paul Grimm for helpful discussions regarding immunohistochemistry studies and fibrosis and S. McElwain for technical assistance.

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