Tissue remodeling in eosinophilic esophagitis

Edaire Cheng,1 Rhonda F. Souza,2 and Stuart J. Spechler2

From the Departments of 1Pediatrics and 2Internal Medicine, Children’s Medical Center and the VA North Texas Health Care System, Harold C. Simmons Comprehensive Cancer Center, and the University of Texas Southwestern Medical Center, Dallas, Texas

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Cheng E, Souza RF, Spechler SJ. Tissue remodeling in eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol 303: G1175–G1187, 2012. First published September 27, 2012; doi:10.1152/ajpgi.00313.2012.—Eosinophilic esophagitis (EoE) is a recently recognized, immune-mediated disease characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation. The chronic esophageal eosinophilia of EoE is associated with tissue remodeling that includes epithelial hyperplasia, submucosal fibrosis, and hypertrophy of esophageal smooth muscle. This remodeling causes the esophageal rings and strictures that frequently complicate EoE and underlies the mucosal fragility that predisposes to painful mucosal tears in the EoE esophagus. The pathogenesis of tissue remodeling in EoE is not completely understood, but emerging studies suggest that secretory products of eosinophils and mast cells, as well as cytokines produced by other inflammatory cells, epithelial cells, and stromal cells in the esophagus, all contribute to the process. Interleukin (IL)-4 and IL-13, Th2 cytokines overproduced in allergic disorders, have direct profibrotic and remodeling effects in EoE. The EoE esophagus exhibits increased expression of transforming growth factor (TGF)-β1, which is a potent activator of fibroblasts and a strong inducer of epithelial-mesenchymal transition. In addition, IL-4, IL-13, and TGF-β all have a role in regulating periostin, an extracellular matrix protein that might influence remodeling by acting as a ligand for integrins, by its effects on eosinophils or by activating fibrogenic genes in the esophagus. Presently, few treatments have been shown to affect the tissue remodeling that causes EoE complications. This report reviews the potential roles of fibroblasts, eosinophils, mast cells, and profibrotic cytokines in esophageal remodeling in EoE and identifies potential targets for future therapies that might prevent EoE complications.

Eosinophilic esophagitis (EoE) recently has been defined as a chronic, immune/antigen-mediated disease characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation (89). EoE affects both children and adults of all ages and of all racial and ethnic groups, and EoE can severely impair the quality of life. The first report describing EoE as a distinct clinicopathological disorder was published in 1993 (14), and the incidence of this disorder has increased dramatically ever since. In EoE, food and aeroallergens appear to activate a Th2 immune response, resulting in the production of Th2 cytokines such as interleukin (IL)-13 and IL-4 (142, 147). These cytokines stimulate the esophagus to express eotaxin-3, a potent eosinophil chemoattractant thought to play a key role in drawing eosinophils to the esophagus in EoE (18, 19). Esophageal epithelial injury results from the release of toxic eosinophil degranulation products. Prolonged eosinophil-predominant inflammation also can result in tissue remodeling characterized by hyperplasia of the epithelium, submucosal fibrosis (Fig. 1), angiogenesis, and hypertrophy of esophageal smooth muscle (3, 4, 7, 93, 103, 107, 120, 126, 148, 171). This remodeling can cause serious complications including esophageal ring and stricture formation, which are responsible for the dysphagia and episodes of food impaction typical of EoE (45, 135, 141). Tissue remodeling also can result in esophageal dysmotility, which might contribute to the dysphagia and chest pain of EoE, and in fragility of the esophageal mucosa, which predisposes to painful mucosal tears that can occur spontaneously or with instrumentation of the esophagus. The study of tissue remodeling in EoE is especially challenging because the process largely involves the deep layers of the esophageal mucosa, as well as the submucosa and muscularis propria, and techniques for sampling and imaging these layers with microscopic precision are limited. Typical endoscopic biopsies of the esophageal mucosa are usually limited to just the epithelium. In a recent study in which the investigators evaluated the depth of 1,692 endoscopic esophageal biopsy tissue pieces (53), for example, fewer than 11% were found to contain submucosal lamina propria. Thus the large majority of endoscopic biopsy specimens provide little information on tissue remodeling, and very few full-thickness esophageal...
Fibroblasts and Fibrogenesis

Fibrogenesis (the development of fibrous tissue) is part of the normal repair process triggered by epithelial injury. Injured epithelial cells and infiltrating immune cells release mediators that can initiate and regulate fibrogenesis. Profibrotic cytokines like IL-13 and profibrotic growth factors, such as TGF-β and platelet-derived growth factor, can activate quiescent fibroblasts to transdifferentiate into myofibroblasts. These myofibroblasts play key roles in the synthesis, deposition, organization, and degradation of extracellular matrix (ECM) proteins including collagen, fibronectin, tenascin-C, periostin, and matrix metalloproteinases (MMPs). Myofibroblasts have features of both fibroblasts and smooth muscle cells, as they express large amounts of vimentin, an intermediate filament protein typically expressed by fibroblasts, as well as α-smooth muscle actin (α-SMA), a microfilament protein characteristic of smooth muscle cells. By virtue of their contractile phenotype, myofibroblasts can cause wound contraction.

Myofibroblasts typically are derived from local fibroblasts in the mesenchyme of the injured tissue. In some circumstances, however, fibrocytes can be recruited from the bone marrow through the blood to peripheral sites of injury, where those inactive fibrocytes differentiate into active fibroblasts (44). Recent studies also suggest that fibroblasts can be derived directly from epithelial and endothelial cells through the processes of epithelial-mesenchymal transition (EMT) (70) and EMT (124), respectively. Thus epithelial and endothelial cells can actively contribute to tissue remodeling with fibrogenesis. Although limited fibrogenesis is a normal response to tissue injury, severe injury or chronic inflammation can cause the deposition of large quantities of ECM protein and the excess matrix contraction that characterize fibrosis. Extensive subepithelial fibrosis often is observed in the esophagus of adult (107, 120, 148) and pediatric patients (7, 28) with EoE.

Eosinophils and Mast Cells

Both eosinophils and mast cells infiltrate the esophagus in EoE, and both are considered major effector cells of tissue fibrosis and remodeling (74, 86, 117). There are complex interactions among eosinophils, mast cells, fibroblasts, and other cell types that might influence tissue remodeling in the esophagus. Eosinophils and mast cells release a wide array of biologically active cytokines that can regulate fibroblast function. Conversely, fibroblasts can regulate the function of eosinophils and mast cells (84–86, 158), and eosinophils and mast cells can affect each other’s activation, signal trafficking, and proliferation (88, 110, 116, 121, 161). In addition, eosinophil- and mast cell-derived proteins can cause epithelial cells, endothelial cells, and smooth muscle cells to produce substances that also can regulate tissue remodeling.

There is abundant evidence supporting the role of eosinophils in the fibrosis and airway remodeling that occurs in asthma (73). Studies in asthma and other fibrotic disorders have implicated a number of eosinophil protein products, including TGF-β, IL-13, IL-4, vascular endothelial growth factor (VEGF), angiogenin, IL-8, and major basic protein (MBP), in the regulation of tissue remodeling (Table 1) (61, 62, 74). Angiogenic eosinophil products such as VEGF, angiogenin, and IL-8 recently have been identified in greater levels in esophageal mucosal biopsies from patients with EoE than from control patients (122). In esophageal epithelial cells,
MBP has been shown to induce the production of fibroblast growth factor (FGF9), which promotes cell proliferation, and high levels of FGF9 have been found in esophageal biopsy specimens from patients with EoE (108).

Immunohistochemical staining has revealed increased numbers of mast cells in the esophagus of patients with EoE, and elevated esophageal levels of mast cell genes such as carboxypeptidase A3, tryptase, histidine decarboxylase, and FcεRI have been documented as well (1, 5, 19, 64, 142). However, the precise distribution of these mast cells in the layers of the esophageal wall is not clear, largely because few full-thickness esophageal specimens from patients with EoE have been available for examination. Studies of esophageal biopsy specimens from patients with EoE have revealed mast cells in the epithelium, lamina propria, and muscularis mucosae (1, 5, 142). A report of a patient with idiopathic eosinophilic esophagitis who had an esophagectomy described mast cells in the muscularis propria as well (111).

Mast cell activation (with degranulation and mediator release) can be initiated by a number of factors including cross-linking of IgE antibodies, stem cell factor, complement fragments, neuropeptides, adenosine, bacteria cell wall components, eosinophil-derived proteins, acid refluX, and bile refluX (162). The specific mast cell proteins released depend in part on the factors that initiate mast cell activation. Mast cells and eosinophils share a number of the same protein products (Table 1). However, some mast cell-specific proteins such as histamine, tryptase, and chymase are known to be involved in smooth muscle contraction and hypertrophy (5, 26, 117). The observation that mast cells are found in the muscle layers of the esophagus in EoE suggests that mast cells might contribute to the smooth muscle hypertrophy and dysmotility that occurs in this disorder (5).

As shown in Table 1, there are numerous eosinophil- and mast cell-derived cytokines and chemokines with the potential to participate in tissue remodeling in EoE. Furthermore, other cell types also may express some of these molecules. For example, Straumann et al. (142) have demonstrated that tumor necrosis factor (TNF-α) is highly expressed by epithelial cells in EoE. It is not clear whether TNF-α exerts direct profibrotic effects, but studies on Crohn’s disease (155) and pulmonary fibrosis (119) have suggested that TNF-α might contribute to fibrosis by upregulating tissue inhibitor of metalloproteinase (TIMP)-1, which can increase collagen accumulation. Furthermore, angiogenic effects have been described for TNF-α (168), and activation of the TNF-α-αNF-κB pathway has been implicated as a cause for angiogenic remodeling in EoE (122).

### TGF-β

TGF-β is a multifunctional cytokine that clearly plays a key role in fibrosis and has been extensively studied in a number of fibrotic diseases (166). Although there are three isotypes of TGF-β, tissue fibrosis is associated primarily with the TGF-β1 isoform produced in large part by monocytes and macrophages, but also by eosinophils and mast cells. TGF-β is synthesized and secreted as part of a large latent complex in which it is bound to a latency-associated protein (LAP) in a complex with a latent-TGF-β-binding protein (LTBP) (8). TGF-β remains inactive in this complex, and dissociation of the LAP and LTBP renders TGF-β active. This dissociation and activation is induced by a number of agents such as plasmin, thrombospondin-1, integrins, MMPs, reactive oxygen species, and acid (8, 109). The activated TGF-β binds its receptors (TGF-βR1 and TGF-βR2) and exerts its effects via both Smad-dependent and Smad-independent pathways (Fig. 2) (32).

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**Table 1. Eosinophil- and/or mast cell-derived proteins involved in tissue remodeling**

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Eosinophil-Derived</th>
<th>Mast Cell-Derived</th>
<th>Fibrosis (Fibroblast Activation and Proliferation)</th>
<th>Epithelial Hyperplasia (Epithelial Cell Activation and Proliferation)</th>
<th>Smooth Muscle Hypertrophy and Contraction (Smooth muscle cell Activation and Proliferation)</th>
<th>Angiogenesis (Endothelial Cell Activation and Proliferation)</th>
<th>ECM Protein</th>
</tr>
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<tr>
<td>TGF-β (23, 68, 113, 159)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>TNF-α (24, 95, 113, 155)</td>
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<td>VEGF (113, 122)</td>
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<td>IL-8 (113)</td>
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<td>IL-13 (21, 92, 105, 151)</td>
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<td>MMP-9 (71, 115)</td>
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<td>Angiogenin (122)</td>
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<td>MBP (66, 108, 132)</td>
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<td>Chymase (82, 129)</td>
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<td>Tryptase (26, 49, 129, 163)</td>
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<td>Histamine (5, 58)</td>
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<td>Heparin (49)</td>
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</table>
Smad proteins are a family of transcription factors that mediate TGF-β signals. In Smad-dependent signaling, TGF-β is activated when it dissociates from LAP and LTBP. The active TGF-β binds its receptor to initiate Smad-dependent and independent signaling. Smad-dependent signaling regulates fibrogenic target genes such as α-smooth muscle actin (α-SMA), collagen, connective tissue growth factor (CTGF), tissue inhibitor of metalloprotease (TIMP-1), and periostin. TGF-β can also induce a number of Smad-independent pathways such as Ras, TGF-β-activated kinase (TAK), RhoA, and phosphatidylinositol-3-kinase (PI3K), thereby adding to its pleiotropic effects. *Protein has been linked to EoE.

The predominant cellular source of TGF-β in EoE is not clear and may vary with the layer of the esophageal wall. In the lamina propria, for example, TGF-β is found largely in eosinophils (7), whereas mast cells appear to be the predominant producers of TGF-β in the muscularis mucosae (5). It is also possible that T cells contribute to TGF-β production in EoE.

TGF-β is both a potent activator of fibroblasts and a strong inducer of EMT, the process whereby epithelial cells assume morphological and phenotypical properties of fibroblasts (70). Recently, Kagalwalla et al. (68) found evidence of EMT by immunostaining for vimentin (an intermediate filament protein expressed by mesenchymal cells) and cytokeratins (proteins of keratin-containing intermediate filaments expressed by epithe-
Th2 Cytokines

Studies have indicated that IL-4 and IL-13, Th2 cytokines that are often overproduced in allergic disorders, have direct profibrotic and remodeling effects in a number of diseases including asthma, atopic dermatitis, schistosomiasis, and chronic colitis (15, 27, 41, 47, 55, 72, 166). IL-4 and IL-13 induce the expression of activated fibroblast markers (α-SMA, fibronectin, CTGF), and, in human fibroblasts and stellate cells, IL-4 and IL-13 regulate the expression of matrix proteins (collagen, MMP, periostin) (9, 92, 105, 125, 151). In addition to these profibrotic effects, IL-13 and IL-4 also can induce fibroblasts to produce proinflammatory substances, including the eosinophil chemotactant eotaxin (59, 60).

IL-13 and IL-4 share many of the same biological effects, probably because both cytokines bind the Type II IL-4 receptor, which is comprised of IL-4Rα and IL-13Rα1 subunits (Fig. 3) (67, 75, 79). The cytoplasmic tails of these subunits are associated with tyrosine kinases of the Janus family (Jak 1–2 and Tyk2). When activated by either IL-13 or IL-4 ligands, the receptor subunits heterodimerize and enhance Jak activity. IL-13Rα1 activates Jak2 and/or Tyk2. IL-4Rα activates Jak1. Subsequently, phosphorylation of signaling molecules such as signal transducer and activator of transcription (STAT)3, STAT6, and insulin receptor substrate (IRS) occurs. Once phosphorylated, STAT6 dimerizes and translocates to the nucleus to initiate transcription of its target genes such as eotaxin-3, which is highly upregulated in EoE (18). Similarly, STAT3 dimerizes after phosphorylation and translocates to the nucleus to initiate transcription. Periostin, which is discussed further below, may be a potential target gene because its promoter contains putative STAT3 binding sites. IL-4Rα can also heterodimerize with a γ-chain (γC) subunit to form the Type I IL-4 receptor, whereas IL-13Rα1 cannot (75). The γC subunit is associated with Jak3, which becomes activated upon IL-4 receptor binding. Thus variability in the activation of these receptor subunits may account for some of the disparate functions of IL-13 and IL-4 (39, 79, 99).

IRS proteins comprise a family of cytoplasmic proteins that function as signaling intermediates when their tyrosine residues are phosphorylated by activated cell surface receptors. The IRS cascade engages two pathways, PI3K/Akt and Ras/MAPK, whose downstream proteins are many and beyond the scope of this review. In general, the PI3K/Akt pathway regulates cell growth, survival, and protein synthesis, whereas the Ras/MAPK pathway mediates cell proliferation and differentiation. IRS proteins were first identified for their role in insulin signaling, and they are major downstream effectors of the insulin-like growth factor-1 receptor (IGF-1R). IGF-1 can stimulate collagen synthesis and influence myofibroblast activation, proliferation, and survival (46, 137, 164), and IGF-1 signaling appears to play an important role in fibrogenesis in the lungs and intestines (42, 154, 165). Thus there is reason to suspect that IRS signaling might mediate some profibrotic effects of IL-4 and IL-13 in EoE. Interestingly, studies on intestinal and pulmonary fibrosis have suggested that IL-4 and IL-13 also can induce IGF-I expression although the mechanisms underlying this expression appeared to be cell-type dependent. In colonocytes, IL-13 stimulates IGF-I expression via IL-13Rα2 and TGFβ1 signaling (42), whereas IGF-I expression induced by IL-4 and IL-13 in alveolar macrophages seems to be STAT6 dependent (165). Little is known about the role of IGF-1 signaling in EoE fibrogenesis, and, clearly, studies on this issue are warranted.

The relationship between IL-13 and TGF-β is complex, and there appear to be both TGFβ-dependent and TGFβ-independent pathways by which IL-13 exerts its profibrotic effects. IL-13 can induce the production of latent TGF-β (i.e., TGF-β bound to LAP) in macrophages, and IL-13 also can upregulate MMP- and plasmin protease-mediated activation of latent TGF-β, thereby enabling TGF-β to exert its profibrotic effects (81, 83). In IL-13 transgenic mice, the development of subepithelial fibrosis in the lungs is reduced significantly by treatment with TGF-β antagonists (83). Thus the profibrotic effects of IL-13 in this model are TGF-β dependent. However, in a mouse model of IL-13-dependent liver fibrosis caused by Schistosoma mansoni infection, disruption of all or part of the TGF-β cascade still results in liver fibrosis (72). In this model,
therefore, the profibrotic effects of IL-13 are TGF-β independent.

Interestingly, IL-13 has another receptor subunit, IL-13Rα2, which binds IL-13 exclusively and with high affinity (Fig. 3). This receptor appears to lack a signaling motif and exists in both membrane-bound and soluble forms. Thus, IL-13Rα2 initially was thought to be a “decoy” receptor for IL-13 (i.e., one that binds IL-13 without producing biological effects) (35, 169), and researchers studying models of pulmonary and hepatic fibrosis suggested that IL-13Rα2 might function to abrogate the profibrotic effects of IL-13 (30, 170). In colitis models, however, IL-13Rα2 has been implicated as an inducer of TGF-β-dependent fibrosis (41, 42). Therefore, the function of IL-13Rα2 is still unclear and may be organ specific.

IL-5, another Th2 cytokine, appears to promote fibrosis primarily through its effects on eosinophils (31, 128). IL-5 is known to play key roles in the differentiation, activation, and recruitment of eosinophils, and, as discussed above, eosinophils are important producers of profibrotic cytokines such as TGF-β1 and IL-13. Most studies suggest that IL-5 does not increase fibrosis directly, but rather indirectly by increasing the levels of profibrotic effector molecules (e.g., TGF-β, IL-13) secreted by eosinophils (20, 31, 43, 65, 157).

In animal models, fibrosis and other features of remodeling can be prevented by IL-5 deficiency or by treatment with anti-IL-5 antibodies (20, 31, 43, 65, 157). For example, wild-type mice administered Aspergillus fumigatus intranasally develop eosinophilic esophagitis with deposition of collagen in the esophageal lamina propria and thickening of the esophageal basal cell layer. In IL-5-deficient mice, however, intranasal administration of Aspergillus fumigatus results in significantly less collagen deposition and less basal layer thickening (103). CD2-IL-5 transgenic mice, which overexpress IL-5 in lymphocytes, develop esophageal eosinophilia and remodeling. However, CD2-IL-5 transgenic mice that are also deficient in the eosinophil chemoattractant eotaxin-1 exhibit substantially less esophageal eosinophilia and remodeling (103). Furthermore, an eosinophil-deficient CD2-IL-5 transgenic mouse does not develop esophageal strictures (98). These studies demonstrate that IL-5 overproduction alone is not sufficient to induce esophageal remodeling in the absence of eosinophils.

Unlike IL-5, IL-13 appears to induce esophageal remodeling through effects that are independent of eosinophils. An interesting animal model of EoE involves rtTA-CCL10-IL-13 mice that overexpress IL-13 in the lung and esophagus when induced with doxycycline (171). This IL-13 overexpression causes esophageal remodeling with fibrosis and stricture formation, even in rtTA-CC10-IL-13 mice that are deficient in eosinophils (98, 171). In addition, the IL-13-induced remodeling is significantly enhanced by deletion of IL-13Rα2, suggesting that IL-13Rα2 inhibits remodeling induced by this Th2 cytokine. Thus IL-13 appears to induce fibrosis directly, whereas IL-5 promotes fibrosis indirectly through its effects on eosinophils. These findings challenge the notion that therapies directed only at eosinophils will be sufficient to prevent fibrosis and remodeling in EoE.

Fig. 3. Interleukin (IL)-13 and IL-4 signaling pathway. When the receptor subunits IL-4Rα and IL-13Rα1 bind to their respective ligands, heterodimerization occurs (IL-4Rα-IL-13Rα1 or IL-4Rα-γC), which enhances Janus kinase (JAK) activity. Subsequently, signaling molecules such as signal transducer and activator of transcription (STAT)6, STAT3, and insulin receptor substrate (IRS) are phosphorylated and activated. STAT6 and STAT3 are transcription factors that can initiate transcription of target genes including eotaxin-3 and, potentially, periostin. IRS can initiate other pathways such as PI3K/Akt and Ras/mitogen-activated protein kinase (MAPK), which can regulate survival and proliferation. The function of IL-13Rα2 is unclear but may operate as a decoy or inhibitor. *Protein has been linked to EoE.
Periostin

A role for the ECM protein periostin has been established in a number of conditions characterized by tissue remodeling including asthma, pulmonary fibrosis, myocardial infarction, valvular heart disease, bone development, and certain cancers (56, 80, 100, 106, 114). Periostin is a secreted, 90-kDa, disulfide-linked protein that was formerly known as osteoblast-specific factor 2 (152). Periostin has the ability to bind to itself and to other matrix proteins including tenasin-C, fibronectin, and collagen (56, 80, 151). In particular, periostin facilitates collagen cross-linking by enhancing the proteolytic activation of lysyl oxidase, an enzyme responsible for cross-link formations (97). Periostin also can bind integrins in the cell membrane, an interaction that has the potential to initiate a variety of biological effects including cell proliferation, adhesion, migration, and differentiation (25, 37, 51, 114, 118). For example, binding of periostin to the integrins αvβ3, αvβ5, and α6β4 has been shown to trigger crosstalk with epidermal growth factor receptor, which initiates the Akt/protein kinase B (PKB) and focal adhesion kinase-mediated signaling pathways (106). These pathways are involved in cell migration/invasion and EMT.

Some studies in cancer cells have explored a role for periostin as an inducer of EMT that upregulates mesenchymal markers such as vimentin, fibronectin, and MMP-9 and downregulates epithelial markers such as E-cadherin (77, 167). It is conceivable that periostin might also induce EMT in EoE, thereby contributing to tissue remodeling. In some tissues, periostin has been shown to induce profibrotic effects such as increased collagen matrix contraction and increased expression of α-SMA, collagen, and fibronectin (38, 160). In addition, periostin has been implicated in upregulating TGF-β activation in epithelial cells, an effect that might perpetuate a fibrogenic signaling cycle (136). Although TGF-β, IL-13, and IL-4 all appear to have a role in regulating periostin, the transcriptional regulation of this matrix protein is incompletely understood (63, 151).

In the esophagus of patients with EoE, periostin has been shown to be one of the most highly upregulated (46-fold) genes, second in upregulation magnitude only to the eotaxin-3 gene (19). Blanchard et al. (17) have shown that TGF-β1 and IL-13 can induce periostin expression in esophageal fibroblasts and that only IL-13 can induce periostin in epithelial cells. They also have demonstrated that eosinophil recruitment is significantly decreased in allergen-challenged, periostin-null mice and that periostin increases eosinophil adhesion to fibronectin. These observations suggest that periostin plays a role in eosinophil recruitment and trafficking. Thus periostin appears to play an important role in EoE remodeling (Table 2).

However, further studies are needed to determine whether periostin influences remodeling primarily by acting as a ligand for integrins in the esophagus, by its effects on eosinophils, or by activating fibrogenic genes in other cells.

EoE Therapies and Remodeling

Studies on EoE treatments have been hampered by a paucity of appropriate animal models for the disease. Animal models that have been used to study EoE remodeling include the IL-5 transgenic mouse, the IL-13 transgenic mouse, and the ovalbumin-challenged mouse models. The IL-5 transgenic mouse model involves intranasal administration of a fungal allergen (Aspergillus fumigatus) to anesthetized mice that overexpress IL-5 under the control of a T cell (CD2) promoter (103). In this model, intranasal administration of Aspergillus to anesthetized mice (a technique that delivers the allergen to both the lungs and the esophagus) appears to be required, as oral or intragastric administration alone does not elicit eosinophilia (102). The IL-13 transgenic mouse model involves doxycycline-inducible overexpression of IL-13 under the promoter control of Clara cells (CC10), which are found in the small airways of the lungs (171). These mice develop both pulmonary and esophageal eosinophilia. The IL-5 and IL-13 transgenic models involve genetically engineered overexpression of individual Th2 cytokines, which results in eosinophilic esophagitis and remodeling. However, eosinophilic esophagitis is not required for tissue remodeling in the IL-13 transgenic mouse. The ovalbumin-challenged mouse model does not involve genetic manipulations. Rather, BALB/c mice are sensitized to ovalbumin by intraperitoneal administration of the egg allergen, followed by the chronic intraesophageal administration of ovalbumin (133). These animals also develop eosinophilic esophagitis and remodeling. Although the eosinophilic changes described in these animal models resemble those seen in patients with EoE, it is not clear how closely these models recapitulate the human disease.

For patients with EoE, treatment options include proton pump inhibitors (PPIs), diet therapy, systemic and topical corticosteroids, anti-IL-5 antibodies, and esophageal dilation (89). However, authorities disagree on the issue of what is the appropriate endpoint for assessing the efficacy of these treatments. Some feel that symptom relief alone is an adequate endpoint, whereas others insist that, in addition to relieving symptoms, an effective treatment should cause resolution of eosinophilic esophagitis. In most published studies on EoE therapies, the endpoints assessed have been symptoms and esophageal eosinophil levels (52, 69, 76, 78, 90, 96, 134,
138–140). Given the difficulty in procuring deep (lamina propria-containing) mucosal biopsies, relatively few studies have addressed the effects of EoE treatments on esophageal fibrosis and remodeling (2, 6, 33, 68, 93, 144–146).

In regard to the endpoint of remodeling, topical corticosteroids have been the most extensively studied of the EoE therapies. One study found that patients with EoE who responded to treatment with swallowed fluticasone propionate exhibited reduced IL-13 mRNA levels and reversal of the EoE transcriptome (18). Several reports have suggested that TGF-β1 expression is reduced by corticosteroid treatment. In a retrospective study of 16 children with EoE who had been treated with oral budesonide 1 mg daily for at least 3 mo, for example, Aceves and her colleagues (6) assessed fibrosis, TGF-β1 activation, and vascular activation (VCAM-1) in pre- and posttreatment esophageal biopsy specimens. They concluded that, in the 9 patients for whom budesonide reduced eosinophil density to ≤7 eosinophils/hpf, there was an accompanying reduction in esophageal remodeling as evidenced by significantly lower fibrosis scores, significant reductions in the numbers of TGF-β1-positive and pSmad2/3-positive cells, and decreased number of VCAM-1-positive vessels. Straumann and his colleagues (144) randomized 36 adolescent or adult patients with EoE to receive either high-dose (2 mg daily) budesonide or placebo for 15 days. They found significant reductions in esophageal fibrosis scores, TGF-β1 staining, and tenascin-C staining in the group treated with budesonide. In a subsequent randomized, placebo-controlled trial conducted by the same investigators, patients who received 50 wk of treatment with low-dose (0.25 mg twice daily) budesonide exhibited no reductions in those same parameters (145). However, the overall extent of esophageal remodeling was significantly less in the treated group, and endoscopic ultrasonography of the treated patients showed a significant reduction in their esophageal wall thickness. Finally, Lucendo et al. (93) studied 10 adult patients treated with fluticasone (400 µg twice daily) for 1 yr and found only insignificant reductions in subepithelial fibrosis and in the levels of profibrogenic genes for IL-5, TGF-β1, and FGF9 quantified by real-time PCR. However, they did note a significant decrease in levels of CCL18, which is an eosinophil-derived chemokine that induces collagen production in fibroblasts (12).

IL-5 plays key roles in eosinophil production, differentiation, recruitment, activation, and survival, and the elimination of IL-5 has been shown to improve esophageal eosinophilia in animal models of EoE (103). Several studies in patients with EoE have explored treatment with anti-IL-5 antibodies (11, 139, 140, 146), but these studies have focused primarily on effects on symptoms and esophageal eosinophilia, not on remodeling. Overall, anti-IL-5 antibody therapy appears to decrease esophageal eosinophilia somewhat but has only inconsistent effects on EoE symptoms. Stein et al. (140) noted that three of four adults with EoE exhibited a twofold decrease in epithelial hyperplasia after 3 mo of treatment with mepolizumab (humanized monoclonal IL-5 antibody) in an open-label trial (140). In contrast, a multicenter, double-blind, placebo-controlled, prospective study of 59 children who received monthly mepolizumab infusions found no significant improvement in epithelial hyperplasia or lamina propria fibrosis (11). Finally, Straumann and colleagues (146) detected decreases in

![Diagram of tissue remodeling in EoE](https://example.com/diagram.png)

**Fig. 4. Pathogenesis of tissue remodeling in EoE.** In the epithelium, eosinophils, Th2 cells and, possibly, mast cells can secrete IL-13 and IL-4. Epithelial cells respond by expressing eotaxin-3, which recruits more eosinophils. Eosinophils (and possibly mast cells) also secrete TGF-β1, which stimulates epithelial cells to undergo epithelial-mesenchymal transition (EMT) and acquire fibroblast-like characteristics. TGF-β1, IL-13, and IL-4 also can induce fibroblast activation with myofibroblast transdifferentiation and production of ECM proteins (collagen, fibronectin, tenascin-C, and peristin), resulting in subepithelial fibrosis. TGF-β1, IL-13, and IL-4 all have potential angiogenic properties. Lastly, mast cells in the muscularis mucosae can secrete TGF-β1. The exact nature of TGF-β1 effects on smooth muscle is still unclear.
tenascin-C and TGF-β1 expression in the esophageal epithelium of adult patients with EoE after 13 wk of mepolizumab therapy (146).

Dietary therapies including amino-acid-based elemental diets, allergy-testing-directed diets, and empiric six-food-elimination diets, have demonstrated efficacy in reducing esophageal eosinophil density and symptoms in children and adults with EoE, but few data are available regarding dietary effects on tissue remodeling (52, 76, 96, 138). Recently, one retrospective study explored whether dietary therapy could reverse esophageal subepithelial fibrosis in children with EoE (91). The investigators performed trichrome stains to evaluate fibrosis on pre- and posttreatment esophageal biopsy specimens and found that diet therapy (elemental and elimination diets) resulted in resolution of fibrosis in 3 of 17 patients (18%). Complete resolution of fibrosis also was observed in five of nine patients (56%) treated with corticosteroids. Another retrospective study assessed treatment effects on esophageal remodeling by quantifying EMT scores in esophageal biopsy specimens from 18 children before and after diet or corticosteroid therapy (68). Elemental diet, six-food-elimination diet, and topical corticosteroids all decreased EMT scores significantly by 81.2%, 72.8%, and 68.2%, respectively.

Gastroesophageal reflux disease (GERD) and EoE can have similar symptoms and histological findings, including dense esophageal eosinophilia, and a trial of PPI therapy has been recommended to distinguish the two disorders (48). The premise underlying this recommendation has been that inhibition of gastric acid secretion is the only important effect of PPIs, and therefore only an acid-peptic disorder like GERD can respond to PPI treatment. This assumption has been challenged by a recent study (29) showing that PPIs inhibit Th2 cytokine-stimulated secretion of eotaxin-3 by esophageal squamous epithelial cells. Thus PPIs might have beneficial effects in EoE that are independent of their effects on gastric acid secretion, and PPI-induced inhibition of eotaxin-3 secretion might be expected to alter tissue remodeling. A number of reports have described resolution of esophageal eosinophilia and/or EoE symptoms with PPI therapy, but few studies have explored PPI effects on remodeling (34, 104, 123). One prospective study examined this issue and found no effects of PPI therapy on subepithelial fibrosis in EoE, even though the PPIs caused symptomatic improvement (87). Further studies to address this issue are warranted.

Reports of small studies have described limited efficacy for anti-TNFα antibodies, anti-IgE antibodies, and leukotriene antagonists in symptom relief for patients with EoE, but these reports also have not described effects on tissue remodeling (13, 54, 94, 131, 143). New therapeutic agents for EoE unreported also have not described effects on tissue remodeling antagonists in symptom relief for patients with EoE, but these anti-TNF antibodies (AMG 317), anti-IL-4 antibodies (pascolizumab, Nuvance), anti-IL-13 antibodies (tralokinumab), and anti-TGF-β1 antibodies (GC1008) are currently under investigation in clinical trials for airway remodeling in asthma and idiopathic pulmonary fibrosis, and these agents might be expected to have beneficial effects in EoE as well.

This review has identified a number of molecular mechanisms that contribute to esophageal remodeling in EoE (summarized in Fig. 4). Proteins in these pathways are potential targets for novel treatments that might be used to prevent the troublesome complications of the disease. Well-designed studies that focus specifically on therapeutic effects on remodeling in EoE are sorely needed. Furthermore, the mechanisms underlying this remodeling are incompletely understood. Studies designed to elucidate these mechanisms might reveal useful therapeutic targets and might even suggest a means to prevent the development of EoE altogether.

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DISCLOSURES

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