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## Relationships Between Eosinophilic Inflammation, Tissue Remodeling and Fibrosis in Eosinophilic Esophagitis\*

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### SYNOPSIS

The clinical and pathologic features of Eosinophilic Esophagitis (EE) include extensive tissue remodeling. Increasing evidence supports a key role for the eosinophil in multiple aspects of the esophageal remodeling and fibrosis seen in this allergic disease, including epithelial hyperplasia, subepithelial fibrosis, smooth muscle hyperplasia, and angiogenesis. These structural changes contribute to the endoscopic findings of esophageal thickening, luminal narrowing, furrowing, transient and fixed rings (trachealization) and stricture, as well as the clinical features of dysmotility, dysphagia and food impactions in pediatric and adult EE. This chapter reviews the clinical implications of esophageal remodeling and fibrosis in EE and discusses the possible pathogenic mechanisms inducing and regulating these responses. We focus specifically on eosinophil and cytokine interactions with the esophageal epithelium, vascular endothelium, resident fibroblasts, and smooth muscle. Current and potential therapeutic interventions are discussed that may impact the development or resolution of chronic esophageal remodeling and fibrosis in EE.

### Keywords

eosinophilic esophagitis; eosinophils; inflammation; remodeling; fibrosis; angiogenesis; transforming growth factor  $\beta$

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## INTRODUCTION

Eosinophilic esophagitis (referred to herein as EE) is a disease of increasing prevalence and/or detection.<sup>13,78,94,98</sup> Its pathogenesis relies in part on an allergic immune reaction that can involve both IgE and T cell mediated hypersensitivity to inhaled aeroallergens and ingested food allergens. The clinical manifestations of EE include vomiting, abdominal pain, regurgitation, heartburn, and failure to thrive, especially in young children. In adolescents and adults, the symptoms can progress to odynophagia and dysphagia that can be associated with the clinical complications of food impactions with or without concurrent esophageal strictures.<sup>59,68,95</sup> EE appears to be a chronic disease with persistent dysphagia when left untreated in adults.<sup>90</sup> In children, the disease remits with therapies, including systemic or topical esophageal corticosteroids, and elimination or elemental diets, but recurs in the majority of patients when the therapeutic intervention is removed.<sup>6</sup>

The histopathologic changes that occur in EE traverse the depths of the esophageal wall.<sup>28,89</sup> The mucosa becomes infiltrated with eosinophils, mast cells, and T cells, and active proliferation of the epithelium leads to the histologic finding of basal zone hyperplasia.<sup>32,67</sup> The submucosal lamina propria also becomes infiltrated by inflammatory cells and demonstrates increased collagen deposition that provides an extracellular matrix for the capture of cells and their cytokine, interleukin, and chemokine<sup>4,14,64,93</sup> products. The muscularis mucosa, as well as the circular and longitudinal muscle layers have reported abnormalities with hypertrophy and dysfunction.<sup>28</sup> In combination, the multiple facets of esophageal remodeling and subepithelial fibrosis that occur during the instigation and propagation of EE could explain many of the clinical symptoms. In this chapter, we review the known aspects of esophageal remodeling, the basic molecular mechanisms utilized by eosinophils to promote tissue remodeling and fibrosis, and the clinical correlates of esophageal remodeling in EE.

Based on the paradigms of other eosinophil-mediated diseases, the role of tissue remodeling in the pathogenesis and clinical manifestations of EE are beginning to be investigated (Figure 1). Tissue remodeling in response to Th2 and eosinophil-associated diseases was first characterized in the hypereosinophilic syndrome (HES) and asthma.<sup>48,54,58,96</sup> In HES, eosinophil activation and degranulation causes target organ fibrosis. Significant patient mortality and morbidity is related to the development of endomyocardial fibrosis and subsequent cardiac failure associated with eosinophilic infiltration.<sup>87,96</sup> Another Th2 and eosinophil-associated disease, asthma, is characterized by tissue remodeling consisting of airway epithelial cell transformation to mucous production, smooth muscle hyperplasia and hypertrophy, subepithelial fibrosis, and angiogenesis.<sup>54</sup> These histologic and structural changes cause clinical disease manifestations of bronchial hyperreactivity, airway edema and mucous plugging with subsequent airway lumen narrowing. In a subset of patients, this airways obstruction becomes irreversible. While the role of the eosinophil in HES is clear, its role in asthma is beginning to be resolved.<sup>48,49</sup>

Murine models of asthma have demonstrated a significant contribution of the eosinophil to disease pathogenesis. Double transgenic mice with airway eotaxin-2 and systemic IL-5 have severe asthma and collagen deposition that is significantly diminished if the animals lack eosinophils.<sup>58,70</sup> The best human correlate to absent eosinophils is treatment with anti-IL-5 antibodies. Through the reduction in IL-5, the major eosinophilopoietic stimulus is lost. As compared to control patients, asthmatics treated with a humanized, monoclonal anti-IL-5 antibody demonstrate decreased levels of extracellular matrix proteins such as lumican, tenascin, and pro-collagen III.<sup>25</sup> Studies on the role of tissue remodeling in eosinophilic gastrointestinal diseases is beginning to evolve and are delineated below.

## CLINICAL IMPLICATIONS OF TISSUE REMODELING

### Epithelial Changes in Relation to Fibrosis

The non-keratinized squamous epithelial cells of the esophagus are important mediators of inflammation in EE. When in a Th2 milieu, the esophageal epithelium becomes an immunologically active tissue that expresses chemotactic factors for eosinophils, including eotaxin-3 and periostin.<sup>7,10</sup> In addition, both an aeroallergen driven murine model of EE and human biopsy specimens demonstrate the induction of mucin genes such as muc-5 in the epithelium.<sup>65</sup>

The Th2 cytokine, interleukin-13 (IL-13) is pivotal for airway remodeling. Studies in murine asthma models over-expressing airway epithelial IL-13 demonstrate robust tissue fibrosis and airway mucous production.<sup>31,103</sup> IL-13 also appears to be an important inflammatory mediator in EE with IL-13 mRNA levels induced 16-fold in epithelial biopsies from pediatric EE patients as compared with normal controls.<sup>8</sup> Esophageal epithelial cells increase their production of eotaxin-3 in a STAT-6 dependent manner when cultured with IL-13, providing a potential positive feedback loop for eosinophil recruitment.<sup>8,64</sup> The increased bulk of epithelial cells in EE patients, reflected in the histologic finding of basal zone hyperplasia and the endoscopic finding of epithelial mucosal thickening with luminal narrowing, may further enhance eosinophil recruitment and, hence, eosinophil-mediated esophageal remodeling and fibrosis (Figure 1). Although the eosinophil can be a significant cellular source of IL-13 in other disease states,<sup>86</sup> the source of IL-13 in EE remains to be clearly identified.

### Clinical Implications of Smooth Muscle Hyperplasia

Dysphagia, in part a reflection of esophageal dysmotility, is a cardinal and distinguishing clinical feature in both pediatric and adult EE.<sup>5,52,73,78</sup> Pediatric studies<sup>59</sup> have shown that EE patients complain of dysphagia at significantly higher rates than their normal, allergic, or gastroesophageal reflux disease (GERD) counterparts.<sup>1,3,5</sup> Whereas young children with EE have vomiting, heartburn, and poor growth, older children and adults often complain of persistent or recurrent dysphagia.<sup>69,90</sup> In one adult series, 10% of patients who complained of solid food dysphagia, met histologic criteria (defined as >20 eosinophils per hpf) for EE, and adults with EE are 2.6 times more likely to complain of dysphagia.<sup>79</sup>

Studies of esophageal dysmotility in EE have occurred exclusively in adult patients. To date, 61 adult patients have had published motility studies.<sup>60</sup> Of these patients, 60% had abnormal motility, mainly categorized as spastic or hypercontractile.<sup>60,83</sup> More recently, Hariprasad and colleagues have reported that the longitudinal muscle contractions in EE patients are abnormal while circular muscle contractions are normal, leading to the dissociation of coordinated muscle contraction.<sup>39</sup> The eosinophil, with granule products such as major basic protein-1 (MBP-1) that are known to alter smooth muscle contractility through inhibition of M2 muscarinic receptors,<sup>50</sup> may contribute to the motor dysfunction of the esophagus, and topical steroid therapy with resultant resolution of eosinophilia is associated with the resolution of esophageal dysmotility.<sup>60</sup>

Muscular hypertrophy and hyperplasia may exacerbate the contractile abnormalities seen in EE. Although analyses of the smooth muscle in EE are limited to date, endoscopic ultrasound studies in pediatric EE patients have demonstrated thickening through the entire esophagus wall, including the mucosa and submucosa as well as the muscularis propria.<sup>28</sup> Esophageal involvement in eosinophilic gastroenteritis is also reportedly associated with smooth muscle hypertrophy and infiltration of the muscularis propria with eosinophils.<sup>89</sup> Lastly, an EE model using inhaled *Aspergillus* demonstrates an increase in muscularis mucosa thickness.<sup>65</sup> Taken together, these data demonstrate that aeroallergen driven EE can cause muscle hypertrophy/

hyperplasia with eosinophilic inflammation. Resultant discordant hypercontractility, likely driven in part by eosinophil granule products such as MBP-1, could explain the endoscopic finding of transient concentric rings and the clinical symptom of food impaction without strictures (Figure 1).

### Pathogenic Mechanisms of Eosinophil – Smooth Muscle Interactions

Evidence for the pathophysiologic participation of eosinophils in smooth muscle hypertrophy and hyperplasia, contraction and hyperreactivity to cholinergic agents comes from *in vitro*, animal model, and human studies of eosinophil-derived TGF- $\beta$  and the eosinophil granule cationic proteins, particularly MBP-1, on airway bronchial smooth muscle in asthma. In chronic murine allergic asthma models, eosinophil-deficient mice show significant decreases in airway smooth muscle hyperplasia in association with decreased numbers of TGF- $\beta$  positive cells, primarily eosinophils, and myofibroblasts in the airways.<sup>15,47,70</sup> Likewise, treatment of mild to moderate asthmatics with anti-IL-5 antibody (Mepolizumab<sup>TM</sup>) significantly decreases the numbers of TGF- $\beta$  positive cells, primarily eosinophils, in the airways, with concomitant decreases in airways remodeling in terms of the deposition of extracellular matrix proteins and numbers of myofibroblasts.<sup>25,54</sup>

A connection between eosinophils and smooth muscle contractility was initially demonstrated by the ability of eosinophil granule cationic proteins such as MBP-1 to directly induce airway smooth muscle contraction, bronchoconstriction and airways hyperreactivity in rat,<sup>19,20</sup> guinea pig<sup>21</sup> and primate<sup>38</sup> asthma models. The mechanism by which eosinophils increase (airway) smooth muscle contractility and hyperresponsiveness to cholinergic stimulation was initially highlighted by studies demonstrating that MBP-1 is a potent antagonist of inhibitory M2 muscarinic receptors.<sup>51</sup> Studies in the Guinea pig asthma model followed showing that parasympathetic neurons in the airways secrete eotaxin, which recruits eosinophils to the nerves, resulting in eosinophil secretion of MBP and inhibition of M2 muscarinic receptors, leading to airways hyperreactivity.<sup>50</sup> Importantly, pre-treatment of allergen-challenged Guinea pigs with a neutralizing antibody to eosinophil MBP was shown to prevent airway hyperresponsiveness by protecting neuronal M2 muscarinic receptors.<sup>24</sup> As well, hyperreactivity to histamine in this model was vagally mediated and dependent on MBP.<sup>16</sup> Loss of M2 receptor function leads to increased acetylcholine release from cholinergic nerves, providing a mechanism for the vagally-mediated airway hyperreactivity seen in this model.<sup>17</sup> Studies also showed that eosinophils localize to the airway nerves of sensitized animals after antigen challenge, and that inhibiting this localization with an antibody to IL-5<sup>23</sup> or the eosinophil adhesion molecule VLA-4,<sup>29,101</sup> or with an eotaxin receptor (CCR3) antagonist,<sup>30</sup> prevents airway hyperreactivity subsequent to the loss of inhibitory M2 muscarinic receptor function. Although this mechanism remains to be confirmed in human asthma, loss of function of lung neuronal M2 muscarinic receptors may also occur, and neurons in human airways have been shown to secrete eotaxin<sup>30</sup> and to be infiltrated by eosinophils in fatal human asthma.<sup>18</sup> The rapid reappearance of both eosinophils and concentric rings (trachealization) in the esophagus of some EE patients within 2–3 days of re-introducing an offending food allergen into their diet is entirely consistent with eosinophil-mediated effects on smooth muscle and/or neurons through these types of mechanisms.<sup>37</sup> Whether eosinophils contribute to the discordant hypercontractility of esophageal smooth muscle directly in EE, or whether eosinophil-neuronal cell interactions contribute to the endoscopic finding of trachealization and clinical symptoms of food impaction in the absence of strictures remains to be determined.

### Clinical Implications of Fibrosis

Fibrosis is defined histologically by increased collagen content of the subepithelial tissue. Although the exact collagen subtypes that are elevated in esophageal remodeling in EE remain to be elucidated, both adult and pediatric patients have increased subepithelial fibrosis.<sup>4,14,</sup>

<sup>95</sup> Fibrosis likely contributes to multiple clinical aspects of EE, including dysphagia symptoms, disease chronicity, and stricture formation.

Among pediatric EE patients with increased subepithelial fibrosis, 42% complain of dysphagia, often with concurrent food impaction.<sup>14</sup> Pediatric EE patients with long-standing or stricture-associated disease have increased subepithelial collagen deposition as compared with their normal or gastroesophageal reflux disease counterparts. It is likely that both TGF $\beta$  and the eosinophil play important roles in the mechanism of fibrosis in EE since pediatric EE patients have increased numbers of TGF $\beta$ <sub>1</sub> producing cells and increased activation of the TGF $\beta$  signaling pathway as reflected by the increased numbers of cells expressing the nuclear phosphorylated Smad2/3 complex.<sup>4</sup> The eosinophil is one cellular source of TGF $\beta$  in EE patients and animals that lack eosinophils have diminished subepithelial fibrosis in response to aeroallergen challenge.<sup>4,65</sup>

Interleukin-5 (IL-5) is a master regulator of eosinophilopoiesis, trafficking, survival, and activation. Biopsies from both adult and pediatric EE patients demonstrate elevated IL-5 levels<sup>91</sup> and murine allergen driven EE requires IL-5.<sup>65</sup> IL-5, together with the eotaxins, activates eosinophils to release their inflammatory products. IL-5 deficient EE mice lack esophageal subepithelial fibrosis<sup>65</sup> and patients with both asthma and atopic dermatitis have decreased tissue remodeling of the airways and skin, respectively, when treated with a humanized monoclonal antibody that blocks IL-5.<sup>25,76</sup> Adult EE patients treated with anti-IL-5 (Mepolizumab<sup>TM</sup>) have been reported to have decreased dysphagia and improvement in EE associated strictures following therapy in a small open-label study,<sup>88</sup> suggesting the role of eosinophils and/or IL-5 in the pathogenesis of human esophageal narrowing. In contrast, a recent placebo-controlled study of anti-IL-5 in adult EE patients showed ~55% decreases in esophageal eosinophils without improvements in clinical disease.<sup>92</sup> It is possible that, as suggested by studies with topical corticosteroids and food elimination or elemental diets, esophageal eosinophils may need to be reduced to near normal levels (i.e. essentially no eosinophils) to reverse clinical symptoms, and anti-IL-5 alone may therefore not be sufficient to induce significant clinical and pathologic remissions. Current clinical trials ongoing in EE should contribute to our understanding of the role of IL-5 and the eosinophil in esophageal remodeling and fibrosis.

### Pathogenic Mechanisms of Eosinophil-mediated Fibrosis

In addition to EE,<sup>28,85</sup> eosinophils are considered a major effector cell of tissue fibrosis<sup>35</sup> in a variety of eosinophil-associated allergic diseases and hypereosinophilic syndromes including asthma,<sup>53,63</sup> eosinophil myalgia syndrome,<sup>100</sup> eosinophilic endomyocardial fibrosis,<sup>87</sup> idiopathic pulmonary fibrosis,<sup>34</sup> scleroderma,<sup>100</sup> and eosinophilic esophagitis. Eosinophils are implicated in fibrogenesis through these clinical disease associations, their elaboration of fibrogenic growth factors such as TGF- $\beta$ ,<sup>33,72</sup> PDGF-BB,<sup>71</sup> IL-1 $\beta$ <sup>36</sup> and secretion of their granule cationic proteins, particularly major basic protein (MBP)<sup>84</sup> and eosinophil peroxidase (EPO).<sup>74</sup> The association of degranulating eosinophils and deposition of their granule cationic proteins in tissues with pathological fibrosis is a recurrent finding in a broad group of eosinophilic illnesses including EE.<sup>59</sup> Eosinophils have been identified as the major TGF- $\beta$  producing cell in the lungs of asthmatics<sup>63</sup> and in the esophagus in pediatric EE.<sup>4</sup>

Both human and animal model studies provide compelling evidence for eosinophils as effectors of tissue remodeling and fibrosis. Reduction in bronchial eosinophils induced by treatment of asthmatics with anti-IL-5 antibody (Mepolizumab<sup>TM</sup>) decreases the expression of ECM proteins in the reticular basement membrane,<sup>25</sup> and anti-IL-5 similarly decreases both eosinophils and deposition of ECM proteins in allergen-induced late-phase skin reactions in atopic subjects.<sup>76</sup> Direct evidence for eosinophil induction of remodeling and fibrosis comes from studies in eosinophil-deficient mice, demonstrating their essential role in the development

of airway remodeling, including mucus (goblet) cell metaplasia, smooth muscle cell hyperplasia, and subepithelial fibrosis.<sup>15,47,58</sup>

Multiple growth factors and cytokines expressed by eosinophils<sup>56</sup> are implicated in tissue remodeling and fibrosis. TGF- $\beta$ , the most widely studied and potently fibrogenic, regulates the expression of the pro-fibrogenic cytokine IL-6, the myofibroblast marker  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), and other ECM proteins such as the collagens. TGF- $\beta$  expression is correlated with bronchial airway fibrosis and asthma severity,<sup>62</sup> and its over-expression in the lung in rodent animal models induces pulmonary fibrosis.<sup>33</sup>

Eosinophil-fibroblast interactions have been implicated in the generation of subepithelial fibrosis and airway remodeling characteristic of human asthma in murine allergic asthma models.<sup>42,43</sup> However, mechanistic assessments of eosinophil-fibroblast interactions that may lead to fibrosis are still limited. Ackerman and colleagues reported that eosinophil granule MBP synergizes with TGF- $\beta$  or IL-1 $\beta$  primed lung fibroblasts to induce significant increases in gene transcription and secretion of the IL-6 family of inflammatory and fibrogenic cytokines, including IL-6 and IL-11.<sup>84</sup> TGF- $\beta$  induced fibroblast secretion of IL-6 is implicated in the overproduction of collagens, tissue inhibitor of metalloproteinases (TIMPs), and glycosaminoglycans in fibrogenesis.<sup>22,99</sup> Eosinophil-lung fibroblast co-culture in the presence of IL-5 induces fibroblasts to transdifferentiate into myofibroblasts with increased expression of  $\alpha$ -SMA and ECM proteins.<sup>77</sup> Eosinophils may indirectly impact fibroblast phenotype and fibrogenesis through activation of the epithelial-mesenchymal trophic unit,<sup>75</sup> e.g. through secretion of MBP and EPO (Figure 1).<sup>74</sup> Alternatively, eosinophils may induce fibrogenesis through TGF- $\beta$  induction of the epithelial to mesenchymal transition (EMT) as shown to occur in the kidney<sup>102</sup> and lung.<sup>55</sup>

Subepithelial fibrosis, a component of airway remodeling in asthma pathogenesis, is initiated by insults that include Th2-mediated allergic responses. Eosinophilic inflammation is thought to drive the differentiation of airway fibroblasts to myofibroblasts as characterized by the expression of myofibroblast-specific markers such as  $\alpha$ -SMA, the deposition of ECM proteins such as collagens, fibronectin, and other ECM constituents such as tenascin and lumican.<sup>25, 75</sup> Eosinophils recruited to the lung in asthma likely interact with fibroblasts beneath the reticular basement membrane, become activated to release their fibrogenic growth factors such as TGF- $\beta$ , driving fibroblasts to differentiate into myofibroblasts, which then deposit pathologic amounts of collagens and other ECM proteins contributing to airway subepithelial fibrosis.<sup>54</sup> A report showing correlations between pulmonary expression of eotaxin-1, expression of eotaxin-1 receptor (CCR3), TGF- and pulmonary fibrosis in a bleomycin mouse model  $\beta_1$  support this general mechanism.<sup>46</sup>

Studies of eosinophil-mediated tissue remodeling and fibrosis in EE are still limited to date, principally due to the difficulties inherent in obtaining sufficient biopsies containing esophageal lamina propria below the stiffened hyperplastic epithelium. For this reason, evidence for progressive remodeling and fibrosis of the esophagus has been derived principally from endoscopic and radiologic features of the disease.<sup>95</sup> However, the recent study by Aceves et al demonstrated that pediatric EE esophageal biopsies showed increased levels of subepithelial fibrosis and increased expression of TGF- $\beta_1$  by eosinophils and its signaling molecule phospho-SMAD2/3 compared with gastroesophageal reflux disease and normal controls.<sup>4</sup> Beyond this report, the mechanisms regulating esophageal remodeling and fibrosis in chronic EE have not been systematically studied to define the changes in epithelial cell and fibroblast phenotype, the role of eosinophil-fibroblast interactions, or the contribution of EMT to this process.

Finally, recent genome-wide expression profiling studies of EE esophageal tissue that identified increased expression of eotaxin-3 as the principal mediator of eosinophil recruitment,<sup>11</sup> interestingly did not identify many genes known to participate in tissue remodeling and fibrosis, perhaps because the biopsy specimens analyzed were sufficiently superficial to include mainly hyperplastic epithelium and not sub-epithelial fibrotic tissue. However, one of the identified genes, periostin, expressed predominantly in collagen-rich fibrous connective tissues subject to mechanical stresses and in wound healing, has been reported to participate in the development of subepithelial fibrosis in bronchial asthma downstream of the IL-4 and IL-13 signals.<sup>97</sup> Primary esophageal fibroblasts have recently been shown to release periostin when cultured with IL-13 and TGF $\beta$ .<sup>7</sup> Periostin, found mainly in the vascular papillae (projections of subepithelial lamina propria into the epithelium) in the esophagus, could contribute to eosinophil trafficking by increasing eosinophil adhesion to fibronectin.<sup>7</sup> Therefore, eosinophil-derived TGF $\beta$ , through induction of fibroblast periostin expression might provide an amplification loop for eosinophilic inflammation and its consequent induction of the remodeling and fibrosis characteristic of EE.

### Clinical Implications of Angiogenesis

Angiogenesis, the formation of new blood vessels, has a number of implications in inflammatory diseases such as EE. Increased vasculature increases the density of conduits for inflammatory cell trafficking, thus propagating inflammation. In addition, interleukins and histamine can modulate vascular permeability and lead to enhanced tissue edema. One of the histologic features of EE is dilated intercellular spaces,<sup>82</sup> which may be a reflection of increased tissue edema and, ultimately, increased mucosal and submucosal thickness when compared to control patients.<sup>27,66</sup> Linear furrowing, caused by a thickened esophagus folding on itself, could be an endoscopic finding related to esophageal edema. Ultimately, an edematous esophagus will decrease luminal size and predispose to clinical complications such as food impaction.

Pediatric EE patients have an increased vascular density as compared to their age-matched counterparts with either a normal esophagus or esophagitis associated with gastroesophageal reflux disease.<sup>4</sup> Blood vessels from EE patients also demonstrate an activated endothelial phenotype with increased expression of vascular cell adhesion molecule-1 (VCAM-1).<sup>4</sup> VCAM-1 interactions with its cognate ligand very late activation-4 molecule (VLA-4) allows leukocytes, particularly eosinophils, to selectively traffic to Th2 activated tissues. Interestingly, IL-13 and TNF $\alpha$  induce vascular endothelial VCAM-1 expression, and both are present at elevated levels in the esophageal mucosa in patients EE.<sup>8,93</sup>

Recent EE animal model studies demonstrate that IL-13 also increases vessel density in EE in a manner dependent upon IL-13 interaction with IL-13R $\alpha$ 2.<sup>104</sup> Mice with increased expression of airway Clara cell CC-10-driven IL-13 have increased esophageal vascular density and increased esophageal circumference,<sup>104</sup> consistent with the linear furrowing and luminal narrowing seen in the human disease; this IL-13 effect is dependent on an intact IL-13R $\alpha$ .<sup>104</sup> Since pediatric EE patients demonstrate an elevated expression of IL-13 mRNA,<sup>8</sup> IL-13 is also implicated in the generation of new blood vessels in the human disease. Animal models have also shown that the induction of EE via IL-13 is dependent on both IL-5 and Signal Transducer Activation Transcription-6 (STAT-6),<sup>8,64</sup> and thus therapy with either IL-5 or IL-13 blocking agents<sup>9,88</sup> may remediate not only esophageal fibrosis, but also the increased angiogenesis associated with EE.<sup>4</sup>

### Pathogenic Mechanisms of Eosinophil-Induced Angiogenesis

Eosinophils express a number of angiogenic factors, not the least of which is vascular endothelial growth factor (VEGF),<sup>40</sup> and they are therefore implicated in the increased

angiogenesis seen in many eosinophil-associated allergic inflammatory diseases such as asthma.<sup>2,12,81</sup> The expression of VEGF, basic fibroblast growth factor (bFGF), angiogenin and the VEGF receptors (flt-1 and flk-1) is increased in asthmatic airways, and the numbers of cells including eosinophils expressing these factors and receptors is correlated with measurements of increased lung vascularity.<sup>41,44</sup> As well, increased expression of VEGF has been demonstrated in the induced sputum of asthmatic children, supporting the concept that it participates in the pathophysiology of augmented angiogenesis seen in bronchial asthma.<sup>45</sup>

Eosinophils, through expression of VEGF, have been shown experimentally by Puxeddu, Levi-Schaffer and colleagues to induce new vessel formation in chick embryo models.<sup>81</sup> Blood eosinophil extracts were found to induce rat aortic endothelial cell proliferation *in vitro*, rat aorta sprouting *ex vivo*, and angiogenesis in the chick embryo chorioallantoic membrane *in vivo*.<sup>80</sup> These pro-angiogenic effects were mediated principally by eosinophil-expressed VEGF, since neutralizing antibodies to VEGF could significantly inhibit them.<sup>80</sup> As well, intact eosinophils were found to induce VEGF mRNA expression and increased VEGF receptor density expression on endothelial cells, to enhance endothelial cell proliferation and to augment angiogenic responses in aorta rings and chorioallantoic membranes.<sup>80</sup> Thus, eosinophils are capable of inducing angiogenesis, in part by their secretion of pre-formed VEGF.<sup>81</sup> Whether eosinophils, through their expression of angiogenic factors such as VEGF (Figure 1), contribute to increased vascularization and activated VCAM-1 positive vascular endothelium in the esophagus, as shown by one of us in children with EE,<sup>4</sup> remains to be determined.

### Therapeutics and Tissue Remodeling

Currently, there are no large clinical trials that demonstrate the efficacy of EE therapies on reducing tissue remodeling and fibrosis. One case report demonstrates that inhaled budesonide in a patient with stricture associated EE and concurrent asthma was associated with decreased subepithelial fibrosis following 2 months of therapy.<sup>61</sup> Our current observations demonstrate that a subset of children with EE will have reversal of deep tissue remodeling following topical esophageal corticosteroid therapy (Aceves and Broide, unpublished). Successful EE therapy with swallowed fluticasone results in the normalization of an EE-specific transcriptome, including IL-13.<sup>8</sup> As such, topical corticosteroids could diminish esophageal remodeling by their effects on the esophageal levels of interleukins such as IL-13. As noted above, anti-IL-5 therapy has also been reported to result in the improvement of EE-associated strictures/esophageal narrowing and improvement of basal zone hyperplasia and eosinophilic inflammation in an open-label trial in a small number of patients.<sup>88</sup> In contrast, a second placebo-controlled study in adults with severe EE concluded that anti-IL-5 therapy, though effective in reducing the numbers of esophageal eosinophils on average by ~55% (but not less than 10 eosinophils/hpf), showed little efficacy in reducing patient's clinical symptom scores.<sup>92</sup> Whether this is sufficient to result in histologic remission of the remodeling and fibrosis of the subepithelial tissue in EE remains to be determined. Of note, this finding is reminiscent of the first studies of anti-IL-5 therapy in asthmatic subjects, in which reductions of pulmonary tissue eosinophils by ~55% did not significantly impact pulmonary function or airway hyperreactivity,<sup>26,57</sup> but did significantly reduce the deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics.<sup>25</sup> These results suggest that an additional therapy, e.g. one targeting eosinophil recruitment through antagonism of eotaxin-3 or its receptor on eosinophils (CCR3), may need to be combined with anti-IL-5 to achieve a therapeutic reduction in eosinophils with complete endoscopic and histologic remission of esophageal remodeling in EE.

## CONCLUSIONS AND FUTURE DIRECTIONS

The central role of the eosinophil in esophageal remodeling and subepithelial fibrosis, and the relationships of this remodeling to the clinical signs, symptoms and pathogenesis of EE, are now beginning to be defined at the cellular and molecular levels, but clearly warrant further study. However, the natural history of EE, the time frame from disease onset to the development of epithelial hyperplasia, thickening of the muscularis propria and esophageal wall, and subepithelial deposition of collagens and other ECM constituents that contribute to esophageal remodeling and fibrosis in EE are still not well defined. Also unclear are the relationships of esophageal remodeling to disease severity and duration, and to what extent esophageal remodeling and fibrosis are reversible with treatments that significantly reduce tissue eosinophils in the esophagus. Better understanding of the mechanisms by which eosinophils promote tissue remodeling and fibrogenesis in the esophagus should contribute to the development of novel therapeutic approaches for blocking eosinophil recruitment to the esophagus and/or reversing the debilitating consequences of esophageal remodeling in EE, and the tissue remodeling and fibrosis seen in many other eosinophil-associated allergic diseases and hypereosinophilic syndromes.

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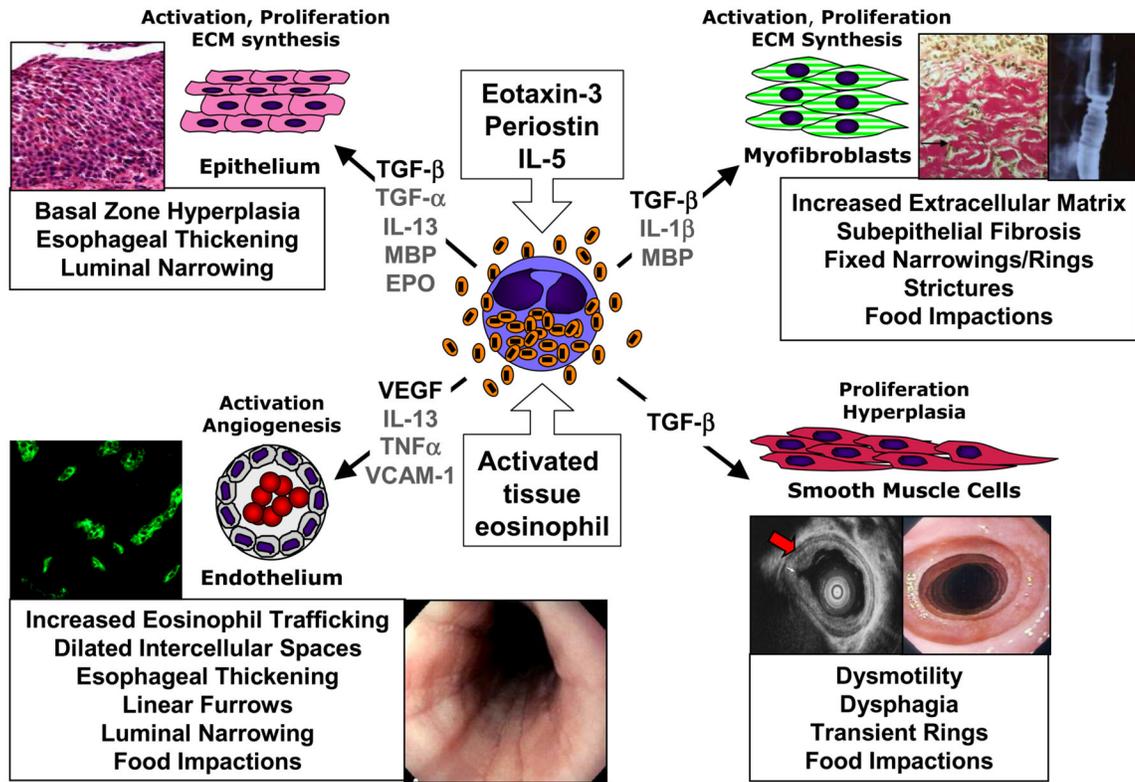
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**Figure 1. Eosinophil induction of esophageal remodeling and fibrosis in EE: relationships to endoscopic and histologic pathologies**

Eosinophil activation during recruitment to the esophagus occurs in response to eotaxin-3, periostin, IL-5 and interactions with vascular endothelium, epithelium and fibroblasts, leading to their expression of fibrogenic factors such as TGF- $\beta$ . Eosinophil-expressed TGF- $\beta$  and granule proteins (MBP, EPO) induce epithelial basal zone hyperplasia, contributing to esophageal thickening and luminal narrowing. Eosinophil-derived TGF- $\beta$  induces fibroblast activation, with transdifferentiation to myofibroblasts and consequent over-production of ECM leading to subepithelial fibrosis, fixed narrowings/rings, strictures and food impactions. Alternatively, TGF- $\beta$  expressed by eosinophils or MBP/EPO damaged epithelium itself may induce epithelial to mesenchymal (myofibroblast) transition (EMT) contributing to subepithelial fibrosis. Eosinophil-expressed TGF- $\beta$  may induce smooth muscle cell hypertrophy/hyperplasia leading to thickening of the esophageal muscularis propria, contributing to dysmotility, dysphagia, transient rings and non-stricture food impactions. Eosinophil expression of VEGF likely supports increased angiogenic responses of vascular endothelium with VCAM-1 activation by IL-13 and TNF- $\alpha$ , contributing to increased eosinophil trafficking, dilated intercellular spaces, esophageal thickening, furrowing, luminal narrowing and non-stricture food impactions.