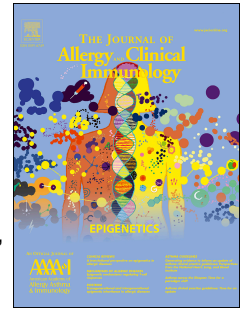


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Editorial, JACI

Bagels and LOX in Eosinophilic Esophagitis

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M.E.R. is a consultant for Pulm One, Spoon Guru, ClostraBio, Celgene, Shire, Astra Zeneca,
Allakos, Adare, and Regeneron and has an equity interest in the first three listed, and royalties
from reslizumab (Teva Pharmaceuticals), PEESSv2 (Mapi Research Trust) and UpToDate.
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24 **Key words:** *LOX, TGF- β , TNF- α , fibroblasts, epithelial cells, fibrosis.*

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Eosinophilic esophagitis (EoE) is a chronic, allergen-induced inflammatory disease of the esophagus characterized by marked esophageal eosinophilia and progressive esophageal dysfunction due to tissue inflammation and variable fibrosis.¹ EoE was first recognized decades ago in scattered reports describing patients having refractory reflux symptoms with esophageal eosinophilia. More recently, it has been noted that there are different clinical, histologic, and endoscopic manifestations of EoE and that patients fall into three distinct endotypes, including a fibrostenotic group.² Evidence is mounting that the natural history of EoE includes progression from a more benign inflammatory phenotype to fibrostenosis characterized by a more difficult-to-treat narrowing of the esophagus (e.g., rings and stricture).

Fibrosis is a highly orchestrated process determined by a sequence of cellular and molecular mechanisms involving ineffective repair following chronic injury, resulting in extensive formation and accumulation of excess fibrous connective tissue and deposition of extracellular matrix components leading to progressive architectural remodeling. Previous studies in EoE suggest that uncontrolled T helper type 2 (TH2) eosinophilic esophageal inflammation leads to esophageal rigidity through tissue remodeling characterized by histologic changes including basal zone hyperplasia, fibrosis, angiogenesis, and smooth muscle hyperplasia with hypertrophy. The TH2 cell product IL-13³ is overproduced in the esophagus of patients with EoE and chronic overexpression of IL-13 in mice is sufficient to induce extensive esophageal remodeling, through a pathway dependent upon IL-13R α 1, inhibited by IL-13R α 2, and independent of the marked eosinophilia elicited by IL-13 overproduction.¹ These histologic changes are closely related to the formation of fibrostenosis that can be captured by endoscopic monitoring, radiographic imaging (e.g., barium swallow), and functional lumen imaging.

There is mounting evidence that the inflammatory process promotes fibrogenesis in EoE. For example, following impaired barrier function mediated by a number of pathways, including lost expression of the homeostatic anti-protease SPINK7,⁴ the epithelium releases the pro-TH2 cytokine thymic stromal lymphopoietin (TSLP), which is encoded for by the chief EoE susceptibility locus on 5q22, as well as other pro-inflammatory cytokines including the alarmin IL-33.¹ This initiates the TH2 inflammatory cascade by promoting secretion of the TH2 cell-derived inflammatory cytokines, including IL-5 and IL-13.¹ IL-13 stimulates the esophageal epithelium to secrete chemokines, such as CCL26 (eotaxin-3), and disrupts the epithelial barrier by downregulating the expression of the adhesion molecule desmoglein-1 (DSG-1) and simultaneously upregulating the protease calpain 14 (CAPN14), which is the gene product encoded for by one of the chief EoE susceptibility loci (2p23).^{1, 5} These processes promote the infiltration of immune cells, predominantly eosinophils, mast cells, and basophils that secrete tumor growth factor- β (TGF- β), a main cytokine in the fibrotic network.¹ TGF- β is also generated by T regulatory cells that are over-represented in the esophagus of patients with EoE.¹ TGF- β acts on fibroblasts and stimulates their trans-differentiation into activated myofibroblasts that proliferate, migrate, and secrete collagen and other matrix component. Activated myofibroblasts also secrete tumor necrosis factor- α (TNF- α), which together with TGF- β has been shown to stimulate epithelial cells to undergo epithelial mesenchymal transition (EMT) and to secrete collagen, migrate, and contract. Additionally, TGF- β induces tissue remodeling factors, such as matrix metalloproteinases (MMPs), plasminogen activator inhibitor 1 (PAI-1), and periostin (POSTN), which have all been implicated in EoE pathogenesis.¹ Thus, TGF- β promotes multiple mechanisms that likely contribute to the tissue stiffness and esophageal stricture in EoE (summarized in **Fig 1**).

Using translational studies of esophageal biopsies, there has been recent interest in investigating whether unique molecular profiles are involved in the fibrostenosis-related disease group in EoE on the basis of a subtype approach (e.g., phenotype and endotype). A disease phenotype defines any observable characteristic of a disease without any implication of a mechanism, whereas an endotype is a subtype of a disease condition, which is defined by a distinct pathophysiologic mechanism. Focusing on the association between gene expression and phenotype, one study showed that esophageal narrowing was associated with increased expression of *CCL26*, *ALOX15*, *GRK5*, *CPA3*, and *TRIM2* in adult EoE,⁶ whereas another study showed that pediatric patients with EoE with food impaction had distinct expression of regulators of esophageal motility, mast cell markers, and TH2-associated transcripts.⁷ Regarding endotypes, three clusters associate with distinct endotypes [i.e., EoE endotype 1-3 (EoEe1-3)] despite similar eosinophil levels.⁴ Interestingly, EoEe3 was associated with the narrow-caliber esophagus and enriched for epithelial genes that lose expression, particularly *ACPP*, *CITED2*, *CTNNAL1*, *EML1*, *FLG*, *GRPEL2*, *MT1M*, *PNLIPPR3*, and *TSPAN12*. Although these molecular analyses of the fibrostenosis-related disease group were restricted to limited genes, the approach has the potential to provide pathogenic insight, candidate biomarkers, and points of therapeutic intervention for this group with difficult-to-treat EoE. However, the molecular features and critical pathways responsible for the fibrostenosis seen in EoE remain largely unknown.

In this issue of the journal, Kasagi *et al.*⁸ provide evidence that the enzyme lysyl oxidase (LOX), which promotes the formation of extracellular collagen fibers, may serve as a biomarker for fibrostenotic disease in EoE. Previous publications revealed that *LOX* expression was upregulated in biopsies from patients with EoE and in an epithelial cell line.^{9, 10} Kasagi *et al.* found that *LOX* expression correlated with clinical and endoscopic fibrostenotic disease. This

upregulation was linked to epithelial cells stimulated by TNF- α produced by fibroblasts. This observation was reinforced using 3-dimensional organoid models that were grown from epithelial cell components. Furthermore, anti-TNF- α antibody in human fibroblast and epithelial co-cultures suppressed *LOX* expression. Additionally, the investigators demonstrated that fibroblast-derived TNF- α induced epithelial production of TGF- β and this contributed to the epithelial *LOX* expression by means of an autocrine stimulation loop. *LOX* in turn, cross linked the collagen secreted by activated fibroblasts, likely contributing to the esophageal stiffness via lamina propria fibrosis (**Fig 1**).

The work presented in the study by Kasagi *et al.* focuses on the need for better diagnostic methods to evaluate fibrostenotic EoE. Currently, the gold standard for fibrostenosis diagnosis is based on the evaluation of the lamina propria from patient's biopsies. However, up to 50% of patients may not even undergo evaluation because biopsy samples are not deep enough to contain sufficient amount of lamina propria. Thus, there is a need for better biomarkers for the fibrostenotic component of this disease. Although this work provides correlative evidence that *LOX* upregulation occurs in fibrostenotic EoE, as defined by clinical and endoscopic manifestations, histologic and molecular verification would advance these findings. Additionally, it would be interesting to correlate *LOX* with pro-fibrotic genes and epithelial mesenchymal transformation genes, as well as additional histologic manifestations. The use of *LOX* as a biomarker may not be clinically practical as the reported sensitivity of *LOX* as marker of fibrostenotic EoE is only moderate (~50%). *LOX* expression was also upregulated in response to a number of pro-inflammatory and pro-fibrotic cytokines known to be involved in EoE.¹ Thus, *LOX* may already be upregulated at the inflammatory stage of the disease rather than limited to

the fibrostenotic phase. This does not, however, take away from the possibility that interfering with LOX could be a therapeutic entry point for fibrosis.

In summary, this study identifies *LOX* as an esophageal epithelial cell gene that is upregulated in fibrostenotic EoE and may have a role in the development of fibrosis. It will be of interest to examine *LOX* correlation with other TNF-mediated fibrotic markers (i.e., α -SMA, collagen 1, vimentin, and PAI-1) and to examine its specificity for the fibrotic stage in EoE and potential value as a therapeutic target. It is hopeful that better deciphering LOX expression and activity may ultimately lead EoE patients to better enjoy their “Bagel and LOX”.

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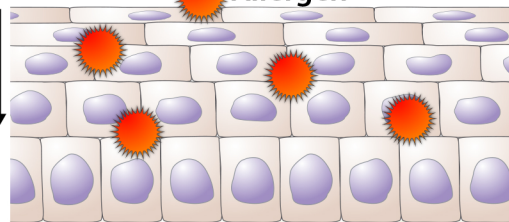
FIGURE LEGENDS**FIG 1. Proposed model for the development of fibrosis and esophageal stiffness in EoE.**

Following injury and/or loss of barrier function, such as downregulation of homeostatic anti-proteases (e.g., SPINK7), the epithelium releases the TH2-promoting cytokines thymic stromal lymphopoietin (TSLP) and IL-33. This promotes the secretion of the Th2-derived inflammatory cytokines IL-5, IL-4, and IL-13. IL-13 promotes granulocyte infiltration by stimulating the esophageal epithelium to secrete CCL26 and disrupt epithelial barrier by downregulating the expression of desmoglein-1 (DSG-1) and upregulating that of Calpain-14 (CAPN14.) The granulocytes secrete tumor growth Factor- β (TGF- β), which acts on fibroblasts and stimulates their trans-differentiation into collagen-secreting, activated myofibroblasts. Also, TGF- β induces the upregulation of plasminogen activator inhibitor 1 (PAI-1) and periostin (POSTN). Fibroblasts secrete tumor necrosis factor- α (TNF- α), which induces epithelial production of TGF- β , which in turn contributes to epithelial LOX expression by means of autocrine stimulation. LOX in turn, cross links collagen secreted by activated fibroblasts. TSLP and CAPN14 are highlighted because they are in genetic susceptibility loci 5q22 and 2p23 and variants in TSLP and CAPN14 promote EoE susceptibility.

Esophageal Epithelial Cells

SPINK7

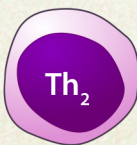
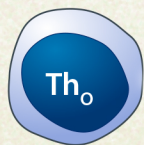
Allergen



Lamina Propria

TSLP

IL-33



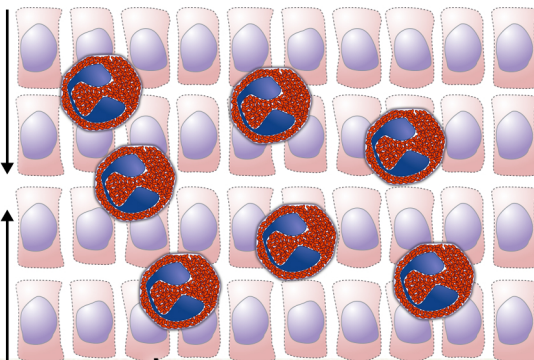
IL-4

IL-13

IL-5

DSG-1

CAPN14



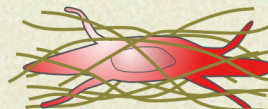
CCL-26



TGF β



Fibroblast

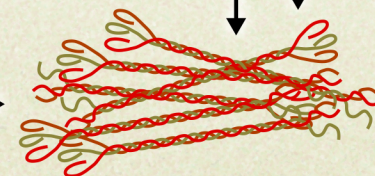


Myofibroblast

EMT

TGF β

TNF α



Collagen

LOX

PAI-1
POSTN