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TGFβ1: Mediator of a feedback loop in eosinophilic esophagitis ... or should we really say mastocytic esophagitis?1

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Although eosinophilic esophagitis (EE) is associated with eosinophils by definition, several recent studies have demonstrated the occurrence of esophageal mastocytosis in patients with EE, raising questions about the etiology and pathogenesis of this newly emerging enigmatic disease. The first report on this subject (2001) demonstrated increased levels of tryptase- and/or IgE-positive cells in the esophageal mucosa of EE patients compared to normal controls¹. Subsequent reports demonstrated that mast cell numbers correlate with basal cell hyperplasia in EE patients², that increased mast cell numbers closely correlate to eotaxin-3 and KIT ligand expression^{3,4}, and that mast cell numbers and a recently described mast cell-related transcriptome normalize following the usage of swallowed steroid therapy^{5–8}. Notably, esophageal mast cells are readily identified by tracking the mRNA expression of their specific proteases tryptase and carboxypeptidase A3 (CPA3) but not chymase⁴, a transcript signature that appears to be a surrogate marker for human mast cells in other Th2-associated processes⁹.

In spite of these findings, it appears that we are only beginning to scratch the surface with regards to the involvement of mast cells in EE. In this issue of the *Journal*, Aceves et al. demonstrate that the deeper-lying smooth muscle layer of the esophagus of EE patients, not often sampled during endoscopic procedures, has an increased level of tryptase-positive mast cells. In addition, while transforming growth factor β1 (TGFβ1) is already known to be increased in EE patients and localized to eosinophils within the inflamed and fibrotic esophageal lamina propria, these investigators demonstrate that the deeper-lying mast cells also express TGFβ1 as assessed by immunofluorescent co-staining for tryptase and TGFβ1^{10–12}. Further, TGFβ1 promoted direct effects on cultured human smooth muscle

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cells derived from the esophagus. Following treatment with TGF β 1, these esophageal smooth muscle cells (impregnated into collagen gels) contracted in size by nearly 50%; this effect was abrogated by the use of actinomycin. Further, contraction was also induced by treatment of these gels with histamine, suggesting that multiple mast cell mediators could promote abnormal smooth muscle function. Perhaps dysphagia, a common symptom in EE, could be related to the acute release of histamine and the *de novo* production of TGF β 1.

TGF β 1 has been associated with numerous processes relevant to allergic inflammation including direct regulation of pro-fibrotic processes^{13;14}, and has been noted to promote the generation of periostin which leads to eotaxin-3-mediated eosinophil adhesion and recruitment, along with tissue remodeling¹⁵, and likely amplified by periostin-induced activation of TGF β 1¹⁶. In addition, TGF β 1 is expressed by T regulatory cells, and an increased level or imbalance of these cells has been shown to occur in EE patients and in animal models of EE^{17;18}. Furthermore, TGF β 1 in conjunction with Th2 cytokines (IL-4) has been shown to induce IL-9-producing T helper cells (Th9 cells) in mice, suggesting a potential feedback mechanism wherein mast cell- and eosinophil-derived TGF β 1 may promote an IL-9-driven mastocytosis in EE^{19;20}. Indeed, mice with defective TGF β 1 signaling mount a rather poor inflammatory mastocytosis and retain a high parasitic worm burden relative to controls, presumably due to low IL-9 production following infection¹⁹. Notably, the esophagus of EE patients overexpresses IL-9 compared with control individuals, and IL-9 is a cytokine that was originally isolated as a potent mast cell growth factor^{21;22}. Genetic variants in TGF β 1 signaling molecules and excess TGF β 1 expression have been associated with connective tissue disorders such as Duchenne's muscular dystrophy and Marfan's syndrome, and these disorders are often associated with dysphagia^{23;24}. Evidence exists that TGF β 1 may drive changes in tissue biophysical properties; perhaps this could be related to the mechanical esophageal dysfunction in EE^{25–29}. However, as eosinophilia does not appear to be regularly reported in these connective tissue disorders, it is unclear if this disconnect could be more closely linked to a non-clonal mastocytosis in these disorders. Accordingly, perhaps inhibition of the TGF β 1 pathway through the blockade of the renin-angiotensin system with an angiotensin receptor inhibitor could lead to improvement in the dysphagia seen in these varied syndromes²⁸.

Collectively, these findings place TGF β 1, expressed by both eosinophils and mast cells, as a potential central molecular mediator of EE. TGF β 1 may be involved in the chronic problems of fibrosis as well as in the acute smooth muscle contraction associated with immediate symptoms of dysphagia (Figure 1) and the mechanical abnormalities seen in EE. It is notable that thymic stromal lymphopoietin, the protein product of the main EE associated gene (*TSLP*) and genetic susceptibility locus (5q22), has been shown to cooperate with TGF β 1 in regulating tolerance mechanisms^{30–32}. As a consequence, TGF β 1 and mast cells represent a unique and exciting pharmacologic target with the potential to modulate varied features of EE via a single major pathway.

Abbreviations used in this paper

CPA3	Carboxypeptidase A3
EE	eosinophilic esophagitis
TGFβ1	transforming growth factor β 1

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Figure 1.

A proposed model to explain molecular and cellular mechanisms involved in EE pathogenesis, TGF β 1-associated pathology, eosinophil recruitment and treatments. Aeroallergen and food allergen sensitization have been implicated in EE pathogenesis. Elemental diet, glucocorticoids, and anti-IL-5 treatments have been noted to improve the microscopic features of EE acting at different levels on the disease pathogenesis. Hyperplasic epithelial cells of the esophagus overexpress eotaxin-3 likely in response to IL-13. Eotaxin-3 overexpression promotes chemoattraction of CCR3⁺ eosinophils. EE disease susceptibility is genetically defined by strong association to SNPs in the TSLP gene with lesser contribution of SNPs in the FLG gene. TGF β 1 and Th2 cytokines promote IL-9 generation which promotes mastocytosis. Th2 cytokines (e.g. IL-13 and IL-4) drive eotaxin-3 production, as well as synergize with TGF β 1 to promote Th9 cells which drive mastocytosis, thereby resulting in a positive feedback loop as mast cells and eosinophils produce TGF β 1. TGF β 1 has been shown to induce several processes that promote the EE disease pathogenesis including fibrosis, smooth muscle contractility, and periostin expression, which further promotes tissue remodeling and regulates eotaxin-mediated eosinophil adhesion and migration.