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Expanding the paradigm of eosinophilic esophagitis: mast cells and IL-9

Yui-Hsi Wang, Simon P. Hogan, Patricia C. Fulkerson, J. Pablo Abonia, and Marc E. Rothenberg

Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center

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Eosinophilic esophagitis (EoE) is a disease characterized by the aberrant infiltration of eosinophils into the esophageal mucosa. Symptoms of EoE vary by patient age, are typically refractory to acid suppressive therapy, and include difficulties with swallowing, vomiting, failure to thrive, abdominal pain, and dysphagia¹. The severity of EoE correlates positively with the esophageal eosinophil level and the degree of epithelial cell hyperplasia, substantiating that eosinophils are likely the primary effector cells involved in the pathogenesis of EoE. While the etiology of EoE remains unclear, it has been hypothesized that an uncontrolled immune response to food allergens and aeroallergens triggers the development of EoE².

Several clinical and animal studies have addressed the potential immunological mechanisms that underlie the recruitment and accumulation of esophageal eosinophils in EoE. Using a whole-genome expression analysis approach, the transcription of *CCL26* (*eotaxin-3*), a potent eosinophil chemoattractant in humans, was revealed to be highly dysregulated in the esophageal tissue of patients with EoE. Notably, the loss of the CCL26 receptor (CCR3) attenuated eosinophil recruitment into the esophagus in an experimental model of EoE³. In addition to *CCL26*, experimental studies and clinical reports support a role of interleukin (IL)-5, a CD4⁺ T cell-derived TH2 cytokine, in contributing to the accumulation of esophageal eosinophils in the pathogenesis of EoE. Mice overexpressing IL-5 were shown to be prone to develop experimental EoE^{4, 5}, whereas the neutralization of IL-5 blocked allergen- and IL-13-induced EoE pathogenesis in mouse models^{6, 7}. In humans, the expression level of *IL5* transcript in the esophageal biopsies of patients with EoE was positively associated with esophageal eosinophil numbers and the disease activity⁸. The role of IL-5 in regulating EoE pathogenesis was further evaluated in patient studies using the humanized monoclonal antibody against IL-5, which was originally designed to specifically target eosinophils for the treatment of asthma. Clinical trials conducted in patients with EoE treated with either mepolizumab (Bosatria®, GlaxoSmithKline) or reslizumab (Cinquil®,

Cephalon) demonstrated that humanized anti-IL-5 antibody could effectively attenuate eosinophils in the esophagitis or peripheral blood^{9–12}. However, while the mepolizumab-treated patients experienced improvements in clinical symptoms and epithelial hyperplasia, there were no significant differences between the mepolizumab- and placebo-treated groups. The ambiguous results may be due to the usage of non-standardized patient-reported outcome metrics and to a subset of patients with EoE failing to respond to anti-IL-5 antibody treatment^{9, 11–13}. Thus, alternative measurements of EoE-specific clinical outcomes need to be identified, and additional research to elucidate other inflammatory mechanisms involved in EoE pathogenesis in patients who fail to respond to anti-IL-5 treatments is required.

In this report¹⁴, Otani *et al.* performed a detailed analysis of the biopsy specimens from a previous trial using a quantitative immunochemistry approach. Among the studied 43 patient biopsies, 40% of the subjects that showed reduced esophageal eosinophil numbers (<15 eosinophils per high power field (hpf)) after anti-IL-5 antibody treatment were defined as “responders”. Notably, the effect of anti-IL-5 antibody treatment in reducing esophageal eosinophil number was most pronounced in a subgroup of responders that also displayed a marked reduction of tryptase-positive mast cell numbers (pretreatment 62 vs. post treatment 19 per hpf). Interestingly, these esophageal mast cells were found adjacent to eosinophils, and the frequency of these mast cell/eosinophil couplets in the esophagus of the responders was reduced significantly 12 weeks after anti-IL-5 antibody treatment. Several lines of evidence from previous studies suggest the involvement of mast cells in EoE pathogenesis^{15, 16}. First, the presence of activated mast cells in the intraepithelial layer of the esophagus was found to be a useful diagnosis marker that differentiates the pathology of EoE from that of gastroesophageal reflux disease (GERD)¹⁵. In a later transcriptome expression profile analysis, the expression of carboxypeptidase A3 (*CPA3*) and tryptase, but not chymase transcript, were found to be elevated in esophagus of patients with EoE¹⁶. Notably, the expression levels of these mast cell-associated transcripts correlated positively with that of KIT ligand (*KITLG*), a surrogate marker for the degranulation and activation of mast cells¹⁶, suggesting that esophageal mastocytosis and degranulation can occur in patients with EoE. Consistent with this possibility, a recent experimental model of IL-13-driven EoE established that esophageal pathology could occur independent of eosinophils¹⁷. Thus, in addition to eosinophils, esophageal mast cells may be critical for disease manifestations, and the complex interplay between these different infiltrating cell types, through their cytokine production, may be involved in the immunopathogenesis of EoE.

IL-9, a pleiotropic cytokine, can promote the activation and maturation of mast cells¹⁸. Notably, the expression of the *IL9* gene transcript is elevated in the esophagus of EoE patients with EoE⁸. The finding that the reduction of esophageal mast cell numbers could occur primarily in a subgroup of patients EoE who respond to the anti-IL-5 antibody treatment raises the question of the cellular source of IL-9. In a double-immunofluorescence analysis, the authors show that activated MBP⁺ eosinophils and other unidentified cells that are adjacent to the tryptase⁺ mast cells in the esophagus produced IL-9. This intriguing observation suggests that the esophageal eosinophils represent only one of the IL-9-producing immune cell types involved in the pathogenesis of EoE. One study identified an IL-9-producing CD4⁺ cell subset, termed TH9 cells¹⁹. Distinct from classical CD4⁺TH2

cells that can produce IL-9 and other TH2 cytokines (IL-4, IL-5, and IL-13), TH9 cells that expressed the transcription factors GATA3, PU.1, and IRF4 primarily produced IL-9 but few of the other TH2 cytokines¹⁸. Moreover, a recently discovered type-2 cytokine-producing innate lymphoid cell (ILC2), also called natural helper cells or nuocytes, also transiently produced IL-9 that facilitated IL-5 and IL-13 production in an autocrine manner²⁰. Whether CD4⁺TH2 or TH9 cells or ILC2 represent the unidentified esophageal IL-9 producers that are involved in the pathogenesis of EoE remains to be determined (Figure 1). Since TGF- β and IL-4 together can induce IL-9 production¹⁹, it will be interesting to determine whether these two cytokines, produced by esophageal mast cells¹⁶ and CD4⁺TH2 cells, respectively, may induce the adjacent eosinophils to produce IL-9, which in turn, activates the accompanying mast cells to release TGF- β and other inflammatory mediators, thus amplifying the pathogenesis of EoE (Figure 1).

One of the significant findings of this report is that the interplay between esophageal eosinophils and mast cells can be associated with the severity of EoE symptoms. On the basis of a severity scale from 1–6 for reported symptoms employed in the clinical trial, the authors reported that the esophageal mast cell numbers correlated with the severity of EoE symptoms only in a subgroup of patients who exhibited a >70% decrease of esophageal mast cells after anti-IL-5 antibody treatment. In contrast, the reduction of eosinophil numbers did not correlate with symptom severity. Since many studies have demonstrated a role of mast cells in regulating smooth muscle contractile and vagal nerve activity^{21–23}, it is reasonable to speculate that the activated mast cells in the esophagus are the primary drivers of the pathophysiology of EoE in a subgroup of patients. Thus, among patients with EoE who respond to anti-IL-5 antibody treatment, only the subset of responders who exhibit significant reduction of esophageal mastocytosis may experience diminished clinical symptoms of EoE. In summary, the findings in this study revealed an additional pathological function of esophageal eosinophils, providing IL-9 that promotes esophageal mastocytosis, and that the interactions between mast cells and eosinophils may regulate the severity of EoE symptoms.

References

1. Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *The Journal of allergy and clinical immunology*. 2011; 128:3–20. e6. quiz 1–2. [PubMed: 21477849]
2. Abonia JP, Rothenberg ME. Eosinophilic esophagitis: rapidly advancing insights. *Annu Rev Med*. 2012; 63:421–34. [PubMed: 22034864]
3. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *The Journal of clinical investigation*. 2006; 116:536–47. [PubMed: 16453027]
4. Mishra A, Hogan SP, Brandt EB, Rothenberg ME. IL-5 promotes eosinophil trafficking to the esophagus. *Journal of immunology*. 2002; 168:2464–9.
5. Masterson JC, McNamee EN, Hosford L, Capocelli KE, Ruybal J, Fillon SA, et al. Local hypersensitivity reaction in transgenic mice with squamous epithelial IL-5 overexpression provides a novel model of eosinophilic oesophagitis. *Gut*. 2012
6. Mishra A, Hogan SP, Brandt EB, Rothenberg ME. An etiological role for aeroallergens and eosinophils in experimental esophagitis. *The Journal of clinical investigation*. 2001; 107:83–90. [PubMed: 11134183]

7. Mishra A, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. *Gastroenterology*. 2003; 125:1419–27. [PubMed: 14598258]
8. Blanchard C, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, et al. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *The Journal of allergy and clinical immunology*. 2011; 127:208–17. 17 e1–7. [PubMed: 21211656]
9. Spergel JM, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G 3rd, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *The Journal of allergy and clinical immunology*. 2012; 129:456–63. 63 e1–3. [PubMed: 22206777]
10. Stein ML, Collins MH, Villanueva JM, Kushner JP, Putnam PE, Buckmeier BK, et al. Anti-IL-5 (mepolizumab) therapy for eosinophilic esophagitis. *The Journal of allergy and clinical immunology*. 2006; 118:1312–9. [PubMed: 17157662]
11. Straumann A, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut*. 2010; 59:21–30. [PubMed: 19828470]
12. Assa'ad AH, Gupta SK, Collins MH, Thomson M, Heath AT, Smith DA, et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. *Gastroenterology*. 2011; 141:1593–604. [PubMed: 21835135]
13. Rothenberg ME, Aceves S, Bonis PA, Collins MH, Gonsalves N, Gupta SK, et al. Working with the US Food and Drug Administration: progress and timelines in understanding and treating patients with eosinophilic esophagitis. *The Journal of allergy and clinical immunology*. 2012; 130:617–9. [PubMed: 22935588]
14. Otani IM, Anilkumar AA, Newbury RO, Bhagat M, Beppu LY, Dohil R, et al. Anti-IL-5 Therapy Reduces Mast Cells and IL-9 Cells in Pediatric Eosinophilic Esophagitis. *Journal of Allergy and Clinical Immunology*. 2013
15. Kirsch R, Bokhary R, Marcon MA, Cutz E. Activated mucosal mast cells differentiate eosinophilic (allergic) esophagitis from gastroesophageal reflux disease. *Journal of pediatric gastroenterology and nutrition*. 2007; 44:20–6. [PubMed: 17204948]
16. Abonia JP, Blanchard C, Butz BB, Rainey HF, Collins MH, Stringer K, et al. Involvement of mast cells in eosinophilic esophagitis. *The Journal of allergy and clinical immunology*. 2010; 126:140–9. [PubMed: 20538331]
17. Zuo L, Fulkerson PC, Finkelman FD, Mingler M, Fischetti CA, Blanchard C, et al. IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway. *Journal of immunology*. 2010; 185:660–9.
18. Goswami R, Kaplan MH. A brief history of IL-9. *Journal of immunology*. 2011; 186:3283–8.
19. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity*. 2006; 24:179–89. [PubMed: 16473830]
20. Wilhelm C, Hirota K, Stieglitz B, Van Snick J, Tolaini M, Lahl K, et al. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nature immunology*. 2011; 12:1071–7. [PubMed: 21983833]
21. Bara I, Ozier A, Tunon de Lara JM, Marthan R, Berger P. Pathophysiology of bronchial smooth muscle remodelling in asthma. *The European respiratory journal: official journal of the European Society for Clinical Respiratory Physiology*. 2010; 36:1174–84.
22. Van Nassauw L, Adriaensen D, Timmermans JP. The bidirectional communication between neurons and mast cells within the gastrointestinal tract. *Auton Neurosci*. 2007; 133:91–103. [PubMed: 17169619]
23. Aceves SS, Chen D, Newbury RO, Dohil R, Bastian JF, Broide DH. Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF-beta1, and increase esophageal smooth muscle contraction. *The Journal of allergy and clinical immunology*. 2010; 126:1198–204. e4. [PubMed: 21047675]

Figure 1.

A potential immunological mechanism involved in the pathogenesis of EoE. An uncontrolled TH2 immune response initiated by an allergic insult results in the transition of the esophagus from a normal (NL) to EoE phenotype through enhanced IL-13 production that induces highly elevated CCL26 (eotaxin-3) expression by esophageal epithelium. Dysregulated TH2 immune response and enhanced CCL26 secretion together promote the infiltration of CD4⁺TH2 cells, eosinophils, and mast cells, and potentially, type-2 innate lymphoid cells (ILC2) and CD4⁺TH9 cells; into the esophagus. TGF-β and IL-4 produced by the activated mast cells and CD4⁺TH2 cells may induce eosinophils, ILC2, and/or CD4⁺TH9 cells to produce IL-9, which in turn, promotes esophageal mastocytosis that contributes to the development of EoE pathophysiology.