

Eosinophilic esophagitis: Shifting immune complexity beyond the eosinophil



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Eosinophilic esophagitis (EoE) is an antigen-driven type 2 inflammatory eosinophil prominent disease with increasing prevalence. It is the most common cause of dysphagia and carries a substantive risk for tissue remodeling–driven strictures. Its diagnosis requires the presence of more than 15 eosinophils per high power field (hpf) and relies on repeated tissue biopsy for management.¹ Although expert opinion has been the foundation for the diagnostic and management criteria, recent studies are driving an evolving consensus that the eosinophil serves as a biomarker for disease activity but is not solely responsible for pathogenesis.

One approach to identifying involved novel mechanistic pathways is to utilize unbiased experimental approaches that involve bulk or single-cell sequencing. In EoE, there is the investigational good fortune that these experiments can be performed using tissue. This is not usually the case in other type 2 diseases such as asthma and atopic dermatitis because biopsy is not needed for diagnosis or management. Approaches that capture the whole biopsy transcriptome (ie, bulk RNA sequencing [RNA-Seq]) or individual cells (ie, single-cell RNA sequencing [scRNA-Seq]) are hypothesis-generating for molecular pathways that then require validation through experimentation. Although powerful, sequencing approaches have their limitations because transcript levels are an imperfect proxy for protein levels and also because of the high per-sample cost—especially for scRNA-Seq, which limits the numbers of samples that can be analyzed. This inherently limits the completion of subgroup analyses by patient phenotype. Further, there can be sampling bias; that is, the possibility that the limited numbers of patients analyzed does not represent the disease pathogenesis in the entire population and/or that the subset of patients who agree to research studies do not represent the whole. Despite these limitations, modern science can leverage large sequencing data sets to generate innovative ideas and understanding of disease mechanisms.

In this issue of the *Journal of Allergy and Clinical Immunology*, Jacobse et al begin to overcome the issue of small sample sizes in any EoE scRNA-seq or bulk RNA-seq study by computationally

integrating 9 data sets.² They used data sets from the Gene Expression Omnibus database combined with 2 of their own unpublished data sets. The integrity of the integration method was supported by appropriate use of an established batch correction using the limma software package and subsequent statistical validation via principal component analysis. After finding the differentially expressed genes in the integrated data set, Jacobse et al² used multiple standard computational tools to hypothesize and emphasize potential pathways. Importantly, by combining the data sets, the group was able to perform previously inaccessible subgroup analyses, specifically between children and adults and in males versus in females.

Using their approach, Jacobse et al² completed cell-type analyses that are important to hypothesize shifts in the immune-type composition and posit a more immunologically rich milieu than is generally appreciated in EoE (Fig 1). The investigators binned their data into findings that had already been discovered and validated, those that were noted previously but not yet validated, and those that were novel and yet to be verified experimentally. For example, they found dysregulation in CCL26/eotaxin-3,³ TSPAN12,⁴ filaggrin,³ POSTN,³ and SPINK7,⁵ all of which have been previously reported and functionally dissected using murine and *in vitro* disease models. Jacobse et al² also discovered genes that may be important in disease pathogenesis or may serve as disease markers such as transglutaminase 6 (TGM6), IL-17 receptor- β (IL-17R β), and glycine decarboxylase, which could play a role in EoE phenotypes and/or metabolic derangements.² The investigators then used the pattern of differentially expressed genes in standard pathway analyses, including Gene Set Enrichment Analysis and Ingenuity Pathway Analysis. These analyses verified prior data supporting a robust interferon response. In addition, they elucidated previously defined pathways for proliferation; epithelial mesenchymal transition; and IL-4, IL-5, IL-6, IL-13, and TNF- α signaling.⁶ However, less appreciated pathways, including IL-1b and colony-stimulating factor, were also predicted. Together, these data suggest a rich immunologic cytokine environment that extends beyond canonic type 2 inflammation.

Predictions for pathogenic cells included the finding of non-clonal B cells as well as mast cells. Potential new avenues for study were further revealed by a comparison of adult versus pediatric EoE bulk transcriptomes. The prediction that mastocytosis was prominent in children but macrophages were common in adults was validated by Jacobse et al² using immunostaining of biopsy samples for tryptase and CD68. Representative images suggest that tryptase-positive mast cell infiltration occurs in the epithelium of children, whereas CD68⁺ macrophages are in the lamina propria and vascular papillae, the elongated extensions of the lamina propria into the epithelial space. Consistent with the distribution of macrophages versus that of mucosal mast cells, pathway analyses suggest a prominence of potential signals from the lamina propria in adults, including IL-6, which can be derived from activated

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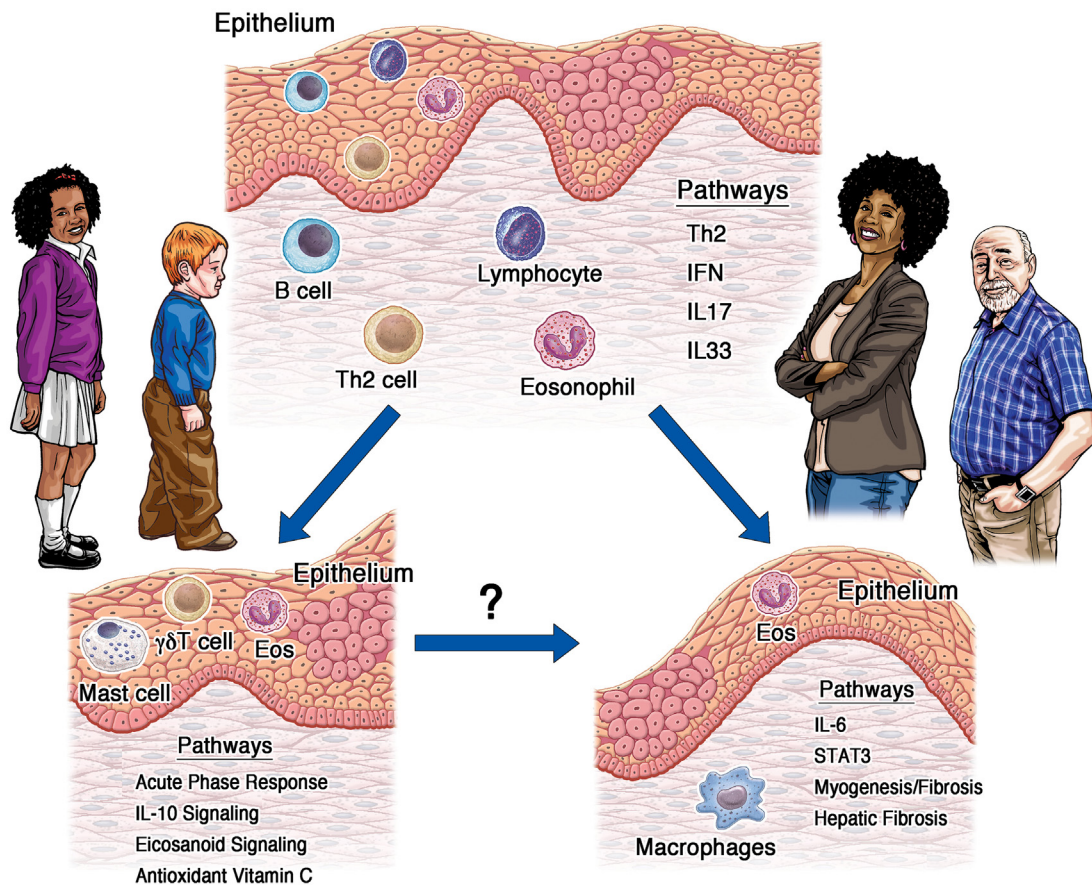


FIG 1. Immunologic complexity in EoE. Predicted common immune cells and pathways in children and adults include classic T_H2 cell inflammation and innate immune activation with IL-33 and eosinophils (Eos), as well as less studied pathways of IgG and IL-17 (top). Biopsy bulk sequence and computational integration suggest a cellular shift from mast cell to macrophage predominance in children versus in adults, as well changes in predicted pathways from acute-phase and potentially protective (eg, IL-10 and antioxidant) in children to fibrotic and myogenic in adults (bottom). $\gamma\delta$ T, $\gamma\delta$ cell; LP, lipoprotein lipase; STAT3, signal transducer and activator of transcription 3.

monocytes and macrophages, and collagen gene–predominant myogenesis pathways. In contrast, children had predominant TNF- α /nuclear factor- κ B and damage-mitigating pathways, such as antioxidant vitamin C and G2M checkpoint. These data have interesting potential implications for a shift in the principal pathogenic pathways in children versus in adults. Future investigations to delineate whether there is a longitudinal shift during EoE from the pathways seen in children to those predicted in adults during disease progression will be of interest. It should be noted that some of the prognosticated pathways have already been experimentally dissected; this provides additional validation of the analyses completed by Jacobse et al.² For example, release of TNF- α from esophageal fibroblasts cocultured with TGF- β 1 expressing epithelial cells induces the collagen cross-linking agent LOX, thereby inducing profibrotic changes.⁶ A recent proteomic study also predicted IL-6 signaling in EoE.⁷ In addition, the TNF superfamily member TNFSF14/LIGHT uses noncanonical nuclear factor- κ B via its receptors HSV entry mediator (HVEM) and lymphotoxin- β receptor to induce proinflammatory fibroblasts.⁸

These pathway analyses may also provide some insight into the lack of uniform therapeutic response in patients with EoE. Topical corticosteroids that diminish inflammation in an esophagus-targeted but immunologically untargeted fashion can induce

histologic remission in 50% to 90% of patients. Anti-IL-5 and anti-IL-5 receptor mAbs reduce tissue eosinophilia but not symptoms or histologic remodeling.⁹ Although the absence of predicted eosinophil pathways does not obviate the eosinophil as pathogenic given the notoriously elusive nature of granulocyte transcriptomes, it does suggest that the isolated removal of eosinophils does not address the immune or structural cell complexities that occur in EoE. The success of dupilumab, which targets the IL-4 receptor- α (IL-17R α), and that of cendakimab, which targets IL-13 (NCT04753697), speak to type 2 inflammation as being critical in many patients.¹⁰ The failure rate of any of these therapies in about 40% of patients or more aligns with the predicted pathways that support a complex pathogenesis to EoE. Certainly, any targeted strategy can create a lacunar defect for which the immune system can compensate through the activation of an alternate pathogenic mechanism. Certainly, the experience of the EoE practitioner who struggles to find the ideal treatment strategy aligns with the utilization of multiple disease mechanisms in EoE.

Lastly, the studies from Jacobse et al.² suggest that the longitudinal trajectory from childhood inflammatory to adult fibrostenotic EoE may rely on a shift in disease mechanisms (Fig 1). Alternatively, the predicted pathways may simply reflect distinct EoE endotypes. Future studies should aim to carefully evaluate

each of the hypothesized pathways because even with the best batch correction methods, biases from different compiled studies and imbalances between covariates will lead to false-positive results and many true positives will be lost. In either case, if validated, targeting different disease pathways in EoE could provide personalized or synergistic therapeutics. In the end, this study provides a valuable hypothesis set and a strong motivator for stakeholders to support and execute large, well-controlled cohort and longitudinal studies that include innovative techniques, such as single-cell and spatial studies to clearly define human phenotypes and molecular tissue cell neighborhoods during esophageal health and disease.

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