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Novel immunologic mechanisms in eosinophilic esophagitis

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Introduction

Eosinophilic esophagitis (EoE), a chronic, immune/antigen-driven disease, specifically affects the esophagus with eosinophil-predominant inflammation. The disease clinically features esophageal dysfunction and frequently associates with atopic conditions [1]. The incidence and prevalence of EoE are increasing [2], and the disease negatively impacts patients' quality of life [3]. Although EoE is well established to be a type 2 cytokine-associated disease, novel innate and adaptive immunological mechanisms underlying the disease continue to be uncovered.

Environmental factors influencing EoE pathogenesis

The well-established association of EoE with food antigen and aeroallergen exposure suggests a critical environmental component of the disease [1]. Animal models support a mechanistic link between antigen exposure and EoE [4]. Moreover, epidemiological studies implicate environmental factors as a strong contributor to EoE. For example, a twin and family study suggests EoE heritability can be attributed to both genetic and environmental factors [5]. Environmental factors contributing to EoE risk include seasonal variation, geographic differences [5], and several factors related to microbial dysbiosis. For example, gastric *Helicobacter pylori* infection is inversely associated with esophageal eosinophilia [6]. Early life exposures such as antibiotic use in infancy and Caesarian delivery are associated with increased odds for developing EoE [7,8,9]. These exposures impact the intestinal and potentially the esophageal microbiome, both of which have recently come under intensive study. Alterations in the esophageal microbiome have been observed in EoE, including increased total bacterial load and increased levels of proteobacteria [10,11]. Interestingly, preclinical studies in mice suggest that specific probiotics alleviate experimental EoE [12]. These studies collectively suggest that multiple environmental exposures contribute to EoE pathogenesis, including a critical role for antigen exposure but underscoring the importance of other potential modifying factors.

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Conflict of interest statement

M.E.R. is a consultant for NKT Therapeutics, Pulm One, Spoon Guru, Celgene, Shire, Astra Zeneca and Novartis and has an equity interest in the first three listed and Immune Pharmaceuticals and royalties from reslizumab (Teva Pharmaceuticals). J.M.C. and M.E.R. are co-inventors of a patent application, owned by Cincinnati Children's Hospital.

Esophageal epithelial barrier in EoE

The esophageal lining consists of non-keratinized stratified squamous epithelium that responds to and regulates access of antigens, pathogens, and other environmental factors to the underlying tissue. The esophageal epithelium of patients with EoE exhibits features of impaired barrier function (IBF) measured at histological (e.g., increased numbers of epithelial dilated intercellular spaces and decreased numbers of desmosomes [13]), functional [14^{**}], and molecular levels (e.g., decreased levels of proteins that comprise adherens junctions [E-cadherin] [15^{*}], desmosomes [DSG1] [14^{**}], and tight junctions [claudin-1] [16] or are otherwise important in epithelial barrier integrity maintenance [e.g., FLG] [17,18^{*}]). Appropriate epithelial differentiation likewise promotes establishment of the esophageal barrier. In EoE, marked expansion of the basal epithelium occurs concurrent with dysregulation of genes related to epithelial cell differentiation, including downregulation of specific epithelial differentiation complex (EDC) genes (e.g., *FLG* and *SPRR3*) [17]. Associated with this is the observation that esophageal tissue of patients with EoE exhibits characteristics of epithelial mesenchymal transition [15^{*}]. Moreover, a substantial number of genes specifically expressed in the esophagus are dysregulated in EoE; of these, a striking majority are downregulated, suggesting a profound loss of esophageal tissue identity. This includes dysregulation of epithelial differentiation genes (e.g., keratins, cornulin) but also additional classes of genes likely involved in maintaining esophageal tissue integrity such as proteases, protease inhibitors, and IL-1 family members [19^{**}]. These significant alterations in esophageal epithelial structure likely contribute to the initiation and propagation of EoE and constitute a mechanism accounting for the tissue specificity of the disease.

Contribution of genetic variation and inflammation to epithelial dysfunction

IBF and loss of appropriate tissue differentiation in EoE result from genetic predisposition and inflammatory mediators present during active disease. Remarkably, certain genetic loci associated with increased EoE risk (e.g., *FLG*, *CAPN14*) [17,20^{**},21^{*}] encode proteins that mediate epithelial barrier function [22,23]. Furthermore, certain rare, damaging genetic variants observed in affected individuals exhibiting a familial pattern of EoE inheritance occur in esophagus-specific genes [19^{**}]. Certain Mendelian diseases associated with squamous epithelial cell barrier defects exhibit EoE (e.g., Netherton's Syndrome [*SPINK5*] and severe atopy syndrome associated with metabolic wasting [SAM syndrome] [*DSG1*, *DSP1*] [24,25,26]). The association of EoE risk with polymorphisms in genes specifically expressed in the esophagus, including the cysteine protease CAPN14, which is known to regulate barrier function, points to a likely mechanism accounting for the tissue specificity of EoE. In terms of the inflammatory environment present in the esophageal tissue, the type 2 cytokine IL-13, present at elevated levels in patients with active EoE, is sufficient to induce IBF and in large part to recapitulate *in vitro* the epithelial gene expression changes observed in EoE, including effects on genes encoding proteins related to cellular junctions, barrier integrity maintenance, and epithelial differentiation [14^{**},17,27,28]. Notably, a polymorphism in *STAT6*, which encodes a transcription factor activated in response to IL-13 signaling, is associated with both EoE and food allergy risk [29]. In EoE, IBF may promote increased antigen exposure to elicit immune responses either in the esophagus or other

distant sites (e.g., skin), initiate pro-inflammatory signaling responses, and affect leukocyte migration.

Epithelium-derived cytokines

The esophageal epithelium likely has an important role in the initiation of EoE via production of the largely epithelium-derived cytokines TSLP and IL-33, which can be induced by proteases or mechanical damage and promote type 2 inflammation [30]. In EoE, increased levels of each protein have been observed in the esophageal epithelium [18*,31*,32]. Furthermore, genetic association studies have identified epithelium-derived cytokines and components of their signaling pathways with EoE risk. In particular, genome-wide association studies (GWAS) of EoE have consistently linked EoE susceptibility with the *TSLP* locus at 5q22 [20**,21*,33]. Furthermore, polymorphisms in a gene encoding a component of the TSLP receptor, *CRLF2*, exhibit association with EoE risk [34]. Interestingly, although *TSLP* polymorphisms are associated with other allergic disorders that often occur as EoE comorbidities, a subset of *TSLP* variants associate with EoE risk independent of these conditions [20*,34]. Additionally, suggestive genetic association of IL-33 variants with EoE exists [20*]. Finally, preclinical animal models support a role for TSLP and IL-33 signaling in EoE initiation. Mice genetically deficient in TSLP receptor or with TSLP neutralized are protected from experimental EoE [31*]. Additionally, leukocyte-intrinsic, competent IL-33 signaling is necessary for esophageal eosinophilia and type 2 cytokine induction [35], and intraperitoneal IL-33 administration is sufficient to induce esophageal eosinophilia and epithelial hyperplasia [36]. Collectively, the observation of their elevated epithelium-localized expression in EoE, genetic variant association with disease risk, and their requirement for select pathologic aspects of experimental EoE suggest that these cytokines likely have a key role in the initiation of EoE pathogenesis.

Development of the type 2 inflammatory milieu in EoE

Multiple cellular sources likely contribute to elevated local levels of type 2 cytokines, including IL-5 and IL-13 [37,38,39], which propagate downstream pathological consequences in EoE. The type 2 milieu development is orchestrated in part by adaptive immune-mediated processes. Dendritic cells (DCs) normally reside in the esophageal epithelium [40] and are present in increased numbers in patients with EoE [41]. TSLP, IL-25, and IL-33, expressed in the esophageal epithelium, promote DC-mediated Th2 cell polarization [30]; thus, these molecules may be involved in promoting Th2 cell accumulation in the esophagus. Consistently, elevated numbers of CD3⁺, CD4⁺, and CD8⁺ T cells have been reported in the esophageal tissue of patients with EoE. Furthermore, increased numbers of activated CD3⁺ esophageal T cells [42] and a specific subset of CRTH2⁺, PGD2-expressing memory effector Th2 cells that express IL-5 and IL-13 are detected [43**]. Animal models of EoE recapitulate the observation of increased numbers of activated T cells in the esophagus [44], and experimental EoE is T cell dependent and to a lesser extent CD4⁺ T cell dependent [45]. Interestingly, FoxP3⁺ regulatory T cells (Tregs) are increased in EoE [46,47], although fewer esophageal Tregs are observed in experimental EoE [44]. The FoxP3⁺ cells observed in EoE could represent an activated memory T cell population [48]. In summary, evidence of increased numbers of activated T cells in the

esophagus suggests that they serve as a major local source of type 2 cytokines in EoE, but this has not been definitively proven.

Several types of innate or innate-like cells in the esophageal mucosa may serve as sources of type 2 cytokines. Group 2 innate lymphoid cells (ILC2s), lineage-negative innate immune cells that are observed at elevated levels in the esophagus of patients with EoE [49^{*}], secrete large quantities of type 2 cytokines in response to IL-25, IL-33, and TSLP [50]. Invariant natural killer T (iNKT) cells, a lineage of innate-like T cells responsive to lipid antigens, have the capacity to produce type 2 cytokines [51]. The presence of fewer iNKT in the peripheral blood of patients with active EoE [52,53] occurs concurrently with an increased number of esophageal iNKT cells, iNKT-specific transcripts, and iNKT chemotactic and growth factors (CXCL16, IL-15, and IL-18) [53,54]. Models of aeroallergen- and food allergen-induced experimental EoE display a dependence on iNKT cells, and the iNKT ligand α GalCer is sufficient to induce esophageal eosinophilia and esophageal type 2 cytokine production in an iNKT cell-dependent manner [53]. Basophils are observed to be increased in EoE [31^{*}], colocalize with TSLP [32], and have increased ST2 expression [55]. In addition, an increased number of basophils are recruited to the esophagus in an ST2-dependent manner in a TSLP-dependent experimental EoE model, suggesting IL-33 may drive basophil recruitment to the esophagus [35]. Depletion of basophils prevents esophageal eosinophilia and induction of genes related to type 2 cytokine responses; furthermore, TSLP and basophils are required after disease establishment to maintain esophageal eosinophilia [31^{*}]. Basophils have the capacity to act as a source of type 2 cytokines or act as antigen-presenting cells that induce Th2 cells [56]. Taken together, several types of innate or innate-like cells present at elevated levels in EoE may potentially serve as sources of type 2 cytokines or critically regulate production of type 2 cytokines by other cell types (Figure 1).

Effector cells in EoE

In response to the inflammatory environment established during EoE, effector cell populations are recruited, activated, and promote downstream pathological consequences (summarized in Figure 2). Esophageal B lymphocytes and B cell-specific transcripts are elevated in patients with EoE [39,57]. In EoE, the esophagus has the pre-requisites for *in situ* IgE synthesis, including expression of IL-4 and IL-13, the presence of mature (CD20⁺) B cells, expression of cytidine deaminase, detection of epsilon germline transcripts, and detection of IgE in the esophageal tissue [58], which could promote local IgE-mediated mast cell (MC) or basophil activation. Interestingly, although EoE is often associated with elevated serum IgE levels [1], experimental EoE is not dependent on IgE [45] and patients do not respond to omalizumab therapy [59], suggesting that the disease may not be exclusively IgE mediated. Interestingly, IgG4 is elevated at both the local and systemic level in EoE, along with increased numbers of IgG4-positive plasma cells in the esophageal lamina propria [60^{*}]; furthermore, elevated levels of food-specific IgG4 are detected in esophageal biopsies, and serum IgG4 levels decline upon diet-mediated remission [60^{*},61]. Future studies will likely focus on further understanding the role of these immunoglobulins in EoE pathogenesis and directing effective diet therapy.

A cardinal finding in EoE and a prerequisite for clinical diagnosis is the presence of 15 eosinophils per 400× high-power microscopic field in the esophageal epithelium, which is normally devoid of eosinophils [1]. The type 2 cytokines IL-5 and IL-13 cooperate to promote esophageal eosinophilia in EoE; IL-5 promotes eosinophil survival and activation, and IL-13 drives altered gene expression including epithelial induction of the eosinophil chemoattractant CCL26 (eotaxin-3) [38] and fibroblast induction of periostin, which promotes eosinophil adhesion [62]. The inhibitory receptor PIRB negatively regulates eosinophil migration into the esophagus in an IL-13-driven model of experimental EoE [63]. Thus, eosinophil migration into the tissue is intricately mediated by eosinophil-intrinsic and -extrinsic factors.

Eosinophils secrete numerous biologically active molecules, including cytokines, chemokines, lipid mediators, and granule proteins. During active EoE, intraepithelial esophageal eosinophils undergo piecemeal degranulation and cytolysis, concomitant with the observation of granule deposition into extracellular sites [64]. Extracellular granules retain the capacity to secrete individual granule proteins after stimulation [65]. Eosinophil extracellular traps composed of eosinophil-derived DNA, which are formed by primed eosinophils following exposure to factors including bacterial products or TSLP, are observed in active EoE [18*]. The presence of activated esophageal eosinophils in EoE is consistent with their involvement in attracting, sustaining, and activating other cell types, as well as directly promoting pathological consequences.

Likely supported in part by increased levels of the MC growth and survival factor IL-9 [37], MCs are present at elevated numbers in the esophageal tissue of patients with EoE [39] and are evident by a characteristic transcript signature [66]. Specifically, mucosal MCs are increased in the esophageal epithelium and muscularis mucosa, whereas connective tissue MCs are present but not different between control and EoE tissue [67]. In EoE, intraepithelial eosinophils expressing IL-9 are located in close proximity to MCs [68], suggesting these cells impact the other's recruitment, viability, or activation. MC degranulation is detected in nearly all patients with EoE [66]. IgE or other factors secreted by local immune cells may regulate this activation, which can result in secretion of biologically active molecules including histamine, tryptase, lipid mediators, and cytokines. Although MCs are not required for esophageal eosinophilia in experimental EoE, they are required for increased muscularis mucosa thickness [69]. MCs thus have the capacity to influence diverse cell types present in EoE and to drive esophageal remodeling and dysmotility.

Remodeling in EoE

EoE likely affects the entire esophageal wall, featuring epithelial hyperplasia, subepithelial fibrosis [70,71], increased muscularis mucosa thickness [72], increased angiogenesis, and endothelial activation [70]. Although properties of remodeling are observed even in pediatric EoE, it is thought to progress throughout the course of the disease. Studies suggest symptoms and remodeling can persist even when eosinophilia has been corrected [73]. Moreover, a unique fibrostenotic phenotype of EoE doubles in incidence every 10 years of

having the disease [74], suggesting chronic inflammation drives the evolution of these characteristics as part of the natural history of EoE.

Esophageal remodeling in EoE likely depends on inflammatory cell infiltration [67,69,70,71,75]. Eosinophils constitute a major source of TGF- β 1 to drive subepithelial fibrosis by acting on fibroblasts and epithelial cells [70,76]. In experimental EoE, animals protected from eosinophil accumulation in the esophagus are likewise protected from features of remodeling including epithelial hyperplasia, subepithelial fibrosis, and increased muscularis mucosa thickness [71]. In the muscularis mucosa, MCs are a source of TGF- β 1, which is sufficient to promote contraction of human esophageal smooth muscle; therefore, this mechanism may contribute to esophageal dysmotility observed in EoE [67]. Additional pathways are likely involved; for example, IL-13 drives esophageal remodeling independent of eosinophils in experimental EoE [77]. In summary, remodeling in EoE results from the complex interplay of multiple mediators that are derived from and impact diverse cell types within the esophagus.

EoE therapies

Removal of the antigenic trigger for EoE by restricting the dietary regimen to an amino acid-based elemental formula or removing trigger foods represents an effective therapy for EoE [76,78]. Likewise, topical glucocorticoid therapy is effective in reducing eosinophil counts, epithelial hyperplasia, and subepithelial fibrosis [79]. Several rational, targeted therapies show promising results. Anti-IL-5 treatment lowers esophageal eosinophil counts despite variable reports about resolution of symptoms or other associated histopathologic characteristics [68,80,81,82]. The key function of IL-13 in EoE pathogenesis is supported by the fact that anti-IL-13 treatment reduces esophageal eosinophilia and normalizes associated mucosal gene expression [83]. The CRTH2 antagonist OC000459, which targets cells expressing this PGD2 receptor including Th2 cells, eosinophils, basophils, and a subset of iLC2s, reduces esophageal eosinophil counts [84]. These promising results will likely spur additional study of these therapies, and advancing knowledge of EoE pathogenesis will provide the potential for new rational therapeutic interventions.

Conclusion

EoE is a chronic, tissue-specific, type 2 cytokine-associated inflammatory disease characterized by epithelial barrier defects and esophageal dysfunction. Both genetic and environmental factors contribute to EoE pathogenesis. Studies have continually discovered novel cell types, molecules, and processes associated with the disease. Ultimately, it is hoped that this will result in improved strategies for the prevention, diagnosis, and treatment of EoE and other type 2 inflammation-associated conditions.

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Figure 1. Model for initiation of cytokine production in EoE

In EoE, the esophageal epithelium, which may exhibit intrinsic defects in barrier function due to certain genetic polymorphisms, responds to mechanical damage or environmental factors such as proteases and food antigens by induction of cytokines including TSLP, IL-33, and IL-25. These cytokines target multiple cell types present in the esophageal epithelium, including dendritic cells, basophils, iLC2s, and iNKT cells either to promote their production of type 2 cytokines or to influence those cells to drive the development of Th2 cells. These leukocytes secrete type 2 cytokines including IL-4, IL-5, IL-13, and IL-9, which are observed to be elevated in the esophageal mucosa of patients with active EoE.

Figure 2. Cellular targets and processes impacted by type 2 cytokines

Locally elevated levels of IL-4, -5, -9, and -13 target multiple structural and immune cells in the esophageal mucosa to influence various processes involved in EoE pathogenesis. This schematic summarizes the impact of these cytokines on epithelial cells, fibroblasts, smooth muscle cells, and infiltrating leukocytes within the esophagus. IL-13 is sufficient to alter epithelial gene expression, including induction of *CCL26* and *CAPN14* and downregulation of *DSG1* and epithelial differentiation complex (EDC) genes. Additionally, IL-13 induces fibroblast periostin production and stimulates smooth muscle hypertrophy. IL-5 and IL-9 promote eosinophil and MC survival and activation, respectively, in the esophageal tissue. Both cells act as sources of TGF- β 1 in distinct regions of the esophageal mucosa; TGF- β 1 promotes epithelial mesenchymal transition (EMT) and esophageal smooth muscle contraction. IL-4 directs B cell class switch recombination to IgE and IgG4, which are elevated locally in EoE. Collectively, evidence suggests that these type 2 cytokines in large part promote the clinical, histological, and molecular phenotypes associated with EoE including basal zone hyperplasia, impaired epithelial cell differentiation, impaired epithelial barrier function, subepithelial fibrosis, and esophageal dysmotility.