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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.

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*, *DSP*]) [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