Epithelial origin of eosinophilic esophagitis

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Eosinophilic esophagitis (EoE) is a chronic, allergen-driven inflammatory disease of the esophagus characterized predominantly by eosinophilic inflammation, leading to esophageal dysfunction. Converging data have placed the esophageal epithelium at the center of disease pathogenesis. In particular, the main EoE disease susceptibility loci at 2p23 and 5p22 encode for gene products that are produced by the esophageal epithelium: the intracellular protease calpain 14 and thymic stromal lymphopoietin, respectively. Furthermore, genetic and functional data establish a primary role for impaired epithelial barrier function in disease susceptibility and pathoetiology. Additionally, the EoE transcriptome, a set of genes dysregulated in the esophagi of patients with EoE, is enriched in genes that encode for proteins involved in esophageal epithelial cell differentiation. This transcriptome has a high proportion of esophagus-specific epithelial genes that are notable for the unexpected enrichment in genes encoding for proteases and protease inhibitors, as well as in IL-1 family genes, demonstrating a previously unappreciated role for innate immunity responses in the esophagus under homeostatic conditions. Among these pathways, basal production of the serine protease inhibitor, Kazal-type 7 (SPINK7) has been demonstrated to be part of the normal differentiation program of esophageal epithelium. Profound lost expression of *SPINK7* occurs in patients with EoE and is sufficient for unleashing increased proteolytic activity (including urokinase plasminogen activator), impaired barrier function, and production of large quantities of proinflammatory and proallergic cytokines, including thymic stromal lymphopoietin. Collectively, we put forth a model in which the esophagus is normally equipped as an anti-inflammatory sensing organ and that defects in this pathway, mediated by epithelial protease/protease inhibitor imbalances, unleash inflammatory responses resulting in disorders, such as EoE. (J Allergy Clin Immunol 2018;142:10-23.)

Key words: *Eosinophilic esophagitis, eosinophilic oesophagitis, esophageal epithelium, epithelial differentiation, epithelial barrier, proteases, protease inhibitors, serine protease inhibitors, Kazal type*

Eosinophilic esophagitis (EoE) is a chronic, allergen-driven eosinophilic inflammatory disease of the esophagus. The disease often coexists with other atopic disorders; has a growing prevalence reaching nearly 1:2000 subjects, indicating that it is considered a rare orphan disease; and is associated with an annual cost of $1 billion in the United States alone.[1,2] Symptoms include failure to thrive, vomiting, chest and abdominal pain, dysphagia, and food impaction, progressing in this order from childhood to adulthood.[3] Compared with other chronic diseases of childhood, EoE has one of the lowest qualities of life, likely because of the restricted diets, chronic pain, relapsing nature, and need for recurrent endoscopies.[4,5]

The pathogenesis of EoE involves a complex interplay of genetic, environmental, and immunologic components.[6-8] Prior studies have demonstrated a high prevalence of white patients with EoE, and approximately 70% of the patients are male.[9] A genetic component is supported by twin studies[6] because monozygotic twins have 41% disease concordance compared with 22% in dizygotic twins and 2.4% in nontwin siblings of patients with EoE. These results highlight a critical contribution of genetics, as well as early-life environmental factors that account for more than 80% of the phenotypic variation of the disease.[7,10-12] Perinatal risk factors for EoE include maternal or newborn fever, antibiotic use, proton pump inhibitor use, and admission into the neonatal intensive care unit. Interestingly, exposure to furry pets in the first year of life protects against EoE.[13] Evidence is also emerging that implicates microbiota as a potentially critical factor in esophageal development, epithelial barrier function, and induction of EoE.[14-16]

EoE is considered to be a type 2 immune disease that often co-occurs with atopic diseases, including atopic dermatitis (AD), asthma, and food allergies.[17-20] As such, EoE is characterized by increased levels of the type 2 cytokines, which are critical for promoting cellular responses in patients with EoE. Early mouse studies revealed IL-5 as an important contributing factor for eosinophilic development and tissue infiltration and for remodeling.[21,22] Similarly, IL-13 is highly upregulated in

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Terms in boldface and italics are detailed in the glossary on page 12.

Abbreviations used

AD: Atopic dermatitis
ALI: Air-liquid interface
BMP: Bone morphogenetic protein
CAPN14: Calpain 14
CST: Cystatin
DSC: Desmocollin
DSG1: Desmoglein 1
DSP: Desmoplakin
EDC: Epidermal differentiation complex
EMSY (C11ORF30): Chromosome 11 open reading frame 30
EMT: Epithelial-mesenchymal transition
EoE: Eosinophilic esophagitis
FA: Food allergy
FLG: Filaggrin
FST: Follistatin
GWAS: Genome-wide association study
IBF: Impaired barrier function
IBL: Interpapillary basal layer
IVL: Involucrin
KLK: Kallikrein
KRT: Keratin
LRRC32: Leucine-rich repeat-containing 32
NKX2.1: NK2 homeobox 1
PAR2: Protease-activated receptor 2
PBL: Papillary basal layer
SERPIN: Serine protease inhibitor
SNP: Single nucleotide polymorphism
SOX2: SRY-box-2
SPINK: Serine protease inhibitor, Kazal type
SPRR: Small proline-rich repeat
STAT: Signal transducer and activator of transcription
SYNPO: Synaptopodin
TAC: Transit-amplifying cell
TGM: Transglutaminase
TSLP: Thymic stromal lymphopoietin
uPA: Urokinase plasminogen activator
uPAR: Urokinase plasminogen activator receptor
WES: Whole-exome sequencing

esophageal biopsy specimens from patients with EoE,[23] and IL-13 overexpression in mice is sufficient to induce esophageal eosinophilia and other structural esophageal changes that are reminiscent of those in patients with EoE.[24] In addition, IL-13 stimulation in esophageal epithelial cells induces a molecular transcript signature that overlaps with the esophageal genes that are altered in patients with EoE, known as the EoE transcriptome.[23,25] The critical role of IL-13 signaling in EoE pathogenesis is further supported by successful clinical trials of anti–IL-13 antibodies for the treatment of EoE.[26]

Analysis of gene expression in human tissues revealed that the esophageal mucosa has a unique transcription signature of approximately 250 esophagus-specific genes (https://www.proteinatlas.org/humanproteome/esophagus).[27] Esophageal genes are considered to be enriched in the esophagus if their expression is at least 5-fold greater in the esophagus than in any other interrogated tissue. Nearly 40% of the esophagus-specific genes have dysregulated expression in patients with EoE, designated as Eso-EoE genes, with the majority being downregulated.[28] Strikingly, Eso-EoE genes were highly enriched in protease-related activities, uncovering proteolytic activity as an intrinsic property of the esophageal tissue and a putative operational pathway in EoE pathogenesis. These genes included serine peptidase inhibitors from the serpin family (serine protease inhibitors ***[SERPINs]***); serine protease inhibitors, Kazal-type ***(SPINKs)***; and the calpain protease calpain 14 (CAPN14), the gene for which is located in the 2p23 locus that is strongly genetically linked to increased EoE risk.[29,30] Additionally, whole-exome sequencing (WES) analysis identified potentially damaging rare mutations in 18 Eso-EoE genes in patients with active EoE, some carrying more than 1 mutation.[28,31] Collectively, these findings uncover potentially critical contributions of the esophageal epithelial responses to maintaining the epithelial barrier in the homeostatic esophagus and to the initiation and propagation of EoE.

Several recent reviews focused on EoE genetics and pathophysiology and involvement of multiple cells, including T and B cells, mast cells, basophils, eosinophils, and dendritic cells, in disease pathogenesis.[32-35] In this review we will explore our understanding of the esophageal epithelium in the pathogenesis of EoE, focusing on epithelial genes, inflammatory pathways, barrier function, and the balance of proteases and protease inhibitors.

PROPERTIES OF THE ESOPHAGEAL EPITHELIUM

The esophagus is classically considered a simple organ involved in transferring food and liquids from the oral cavity to the stomach. The embryonic foregut gives rise to the esophagus, as well as the respiratory tract, stomach, liver, and pancreas. After expression of the transcription factors ***NK2 homeobox 1 (NKX2.1) and SRY-box-2 (SOX2)***, the anterior region of the foregut is differentiated into the trachea and esophagus, respectively.[36] Bone morphogenetic protein ***(BMP)*** and the ***WNT signaling pathway*** have been implicated in tracheal-esophageal separation. High levels of WNT signaling molecules preferentially induce NKX2.1 expression and thereby tracheal development, whereas BMP inhibitory molecules maintain high expression of SOX2, leading to esophageal epithelial stratification.[37-39] Expression of the transcriptional factors SOX2 and ***p63*** is critical for proper esophageal epithelial stratification during development and maintaining esophageal homeostasis.[36] Notably, in the developed esophagus, inhibition of BMP signaling is required to keep basal layer progenitors at an undifferentiated stage, and increased expression of the BMP antagonist follistatin (FST) leads to hyperproliferation of the esophageal epithelium in patients with EoE.[40,41]

The mucosa of the developed human esophagus is lined by the multilayer squamous nonkeratinized epithelium, which serves as a protective barrier against environmental insults, such as microorganisms, foods, and acid exposure.[42] Histologically, the esophageal epithelium can be divided into 2 morphologically distinct regions: (1) the basal zone, with undifferentiated and proliferating cells, and (2) the suprabasal zone, consisting of progressively flattened cells with an increased degree of differentiation as they move closer to the lumen. The basal zone comprises the basal layer of cells in direct contact with the lamina propria and a few layers of dividing cells above the basal layer, which were defined as transit-amplifying cells (TACs). Notably and unlike the human esophagus, TACs in the mouse esophagus are localized to the basal layer of the esophageal epithelium.[43,44] Basal layer cells can either overlay the epithelial papillae of the esophagus to form the papillary basal layer (PBL) or cover the