



Pathophysiology of Eosinophilic Esophagitis

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Abstract

Eosinophilic esophagitis (EoE) is a chronic inflammatory disease of the esophagus associated with an atopic predisposition which appears to be increasing in prevalence over the last few decades. Symptoms stem from fibrosis, swelling, and smooth muscle dysfunction. In the past two decades, the etiology of EoE has been and is continuing to be revealed. This review provides an overview of the effects of genetics, environment, and immune function including discussions that touch on microbiome, the role of diet, food allergy, and aeroallergy. The review further concentrates on the pathophysiology of the disease with particular focus on the important concepts of the molecular etiology of EoE including barrier dysfunction and allergic hypersensitivity.

Keywords Aeroallergy · Basophil · Epithelial-to-mesenchymal transition · Eosinophilic esophagitis · Eosinophil · Esophagus · Esophagitis · Food allergy · Innate lymphoid cells · Invariant natural killer T cells · Microbiome · Squamous epithelium

Abbreviations

AD	Atopic dermatitis
AEC	Absolute eosinophil count
ALI	Air-liquid interface
ALOX15	Arachidonate-15 lipooxygenase
APCs	Antigen-presenting cells
APT	Atopy patch test
CAPN14	Calpain 14
CDH26	Cadherin-like 26
CPA3A	Carboxypeptidase 3A
<i>CRLF2</i>	Cytokine receptor-like factor 2
CRS	Chronic rhinosinusitis
CRTH2	Chemoattractant receptor expressed on Th2 cells
CTDs	Connective tissue disorders
DOCK8	Dedicator Of cytokinesis 8
<i>DSG1</i>	Desmoglein 1
ECP	Eosinophil cationic protein
EDC	Epidermal differentiation complex
EDP	EoE diagnostic panel
EMR,	Electronic medical record
EMT	Epithelial-to-mesenchymal transition
EoE	Eosinophilic esophagitis

EPO	Eosinophil peroxidase
EPX	Eosinophil peroxidase
EST	Esophageal string test
FCERI	IgE Fragment crystallizable epsilon receptor 1
FLG	Filaggrin
GERD	Gastroesophageal reflux disease
GWAS	Genome-wide association studies
HPF	High-power field
IBD	Inflammatory bowel disease
Ig	Immunoglobulin
IL	Interleukin
ILCs	Innate lymphoid cells
iNKT	Invariant natural killer T cells
IVL	Involucrin
LDS	Loeys-Dietz syndrome
MBP	Major basic protein
MIF	Macrophage migration inhibitory factor
MIP-1	Macrophage inflammatory protein 1
miRNAs	MicroRNAs
OIT	Oral immunotherapy
PAI-1	Plasminogen activator inhibitor-1
PGD2	Prostaglandin D2
PHTS	PTEN hamartoma tumor syndrome
POSTN	Periostin
PPI-REE	PPI-responsive esophageal eosinophilia
PPI	Proton pump inhibitor
PR	Pathogenesis-related
RANTES	Regulated on activation, normal T cells expressed and secreted

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SAM	Severe dermatitis, multiple allergies, and metabolic wasting
SERPINS	Serine protease inhibitors
SPINKs	Serine protease inhibitors, Kazal type
SPRR	Small proline-rich repeat
SPT	Skin prick test
STAT	Signal transducer and activator of transcription
TGF- β	Transforming growth factor beta
T _H	T helper type
TNF- α	Tumor necrosis factor- α
TPSAB1	Tryptase
TRAIL	TNF-related apoptosis-inducing ligand
Tregs	T regulatory cells
TSLP	Thymic stromal lymphopoietin
TTG	Tissue transglutaminase
TYK2	Tyrosine-protein kinase-2
UC	Ulcerative colitis
UPK1B	Uroplakin 1B

Introduction

Eosinophilic esophagitis (EoE) has been reported worldwide, with prevalence rates that continue to increase and reach 1:1000 approaching the prevalence of inflammatory bowel disease [1]. In fact, cost associated with EoE in the USA are in the range of \$1 billion per year, again on par with inflammatory bowel disease [2]. This increase in prevalence follows the trend of a general increase in prevalence of allergic diseases over the last 50 years [3]. Though, some of this increase in prevalence appears to be due to improved recognition of this disorder [4]. EoE occurs in both the pediatric and adult populations, though more commonly in atopic, male patients. Presenting symptoms progress with age with difficulty eating, failure to thrive, and vomiting occurring more during early childhood and chest and/or abdominal pain, dysphagia, and food impaction occurring more in adolescents and adulthood [5].

The endoscopic appearance of the esophagus in EoE can show linear furrows with loss of vascularity, mucosal rings (trachealization), a small-caliber lumen, strictures, mucosal exudates, polyps, and ulcerations [6], though up to one-third of patients with EoE have a normal endoscopy [7]. The esophagus is normally devoid of eosinophils, so any number of eosinophils on biopsy indicates an abnormality, though, the primary histologic diagnostic criteria for EoE is 15 eosinophils per high-power field (HPF) on at least one of four biopsies taken at different levels of the esophagus. Other histologic changes seen in EoE include basal layer hyperplasia, papillary lengthening, and lamina propria fibrosis [6].

The diagnosis of EoE also includes exclusion of other eosinophilic diseases of the esophagus, including gastroesophageal reflux disease (GERD). Though, EoE and GERD may coexist in the same patient. Currently, as part of the process to exclude GERD, it is recommended that a proton pump

inhibitor (PPI) trial of 8 weeks is performed [7]. Although a disease entity called PPI-responsive esophageal eosinophilia (PPI-REE), which appears to be a subtype more closely aligned with EoE [8], will also respond to this PPI trial. Despite this, in patients suspected of having EoE, the esophageal eosinophilia response to PPI is still important to evaluate as it may save patients from the long-term effects of fibrotic disease.

Symptomatic and histologic remission typically occurs with either diet elimination or topical steroid treatment with a response rate of 70–80% with either option [9]. Elemental formulas represent the most effective therapy both in terms of histologic and symptomatic response, with a response rate of >90% [10]. The empiric elimination diet eliminates foods commonly associated with immunoglobulin E (IgE)-mediated food allergy (typically the top 6 foods which include milk, egg, wheat, soy, fish, and nuts) regardless of allergen test results. Skin tests directed diets appear to be as effective as the empiric elimination diet. Though, due to the high variability and low predictive value of skin prick tests, serum IgE, and atopic patch tests measurements [9] to identify causative foods in EoE, there appears to be low general clinical utility for this type of assessment of food-specific reactivity in patients. Yet, antigen testing may retain its value to specific patients as both a means to design patient-specific elimination diets and as a guide for the food reintroduction process [9]. Finally, esophageal dilation, a mechanical procedure has proven efficacy in improving symptoms related to strictures and luminal narrowing (though does not address the underlying disease process) in the majority of patients with EoE [11].

EoE is a chronic disease, with relapse often occurring quickly after the discontinuation of diet or drug therapy [12] and if left untreated, EoE is likely to progress to esophageal fibrosis and dysfunction. As such, patients with EoE are more likely to have a parent with a history of esophageal strictures and if biopsied are often found to have EoE. Additionally, patients diagnosed with EoE by retrospective biopsy analysis are at increased risk of developing dysphagia, food impaction, and a need for esophageal dilation [4, 13].

Epidemiology

The etiology of EoE is beginning to be understood on multiple levels including genetics, environment, and its association with an allergic phenotype. Many prior studies have demonstrated a high prevalence of Caucasian patients with EoE, as high as 90% in some studies. More recently though, multiple studies have found an equal prevalence of EoE in the Caucasian and African-American populations [14]. Approximately 3/4ths of all EoE cases are male patients. Notably, a male-specific EoE risk is conferred by a genetic variant in the cytokine receptor-like factor 2 (*CRLF2*) gene,

which encodes for the thymic stromal lymphopoietin (TSLP) receptor and is located on the X and Y chromosomes [15].

Genetics

Three main methods have been utilized to study the genetics of EoE including candidate gene studies [16–19], genome-wide association studies (GWAS) [20–22], and association with other monogenic disorders. More recently, a modified GWAS using electronic medical record (EMR) phenotypic data identified additional EoE-risk loci [23]. Table 1 outlines these risk loci and their corresponding function. Additionally, functions of these gene products are discussed further in subsequent sections.

Twin Studies

EoE occurs in family members in a non-Mendelian pattern, indicating the heritability in EoE is complex. Straumann et al. [24] recently reported an EoE-like disease without tissue eosinophilia in EoE families. The frequency of first-generation offspring of patients affected by EoE or EoE-like disease was 40%. There was an almost complete absence of eosinophils in the esophageal tissues of patients with EoE-like disease, but there was a considerable T cell infiltration, comparable to

EoE. In contrast to EoE, eotaxin-3 mRNA and protein were markedly reduced in EoE-like disease ($P < 0.05$). The mRNA expression levels of three selected EoE genes (eotaxin-3, MUC4, and CDH26) showed an intermediate expression level compared to high levels in EoE and low levels in normal epithelium. Recently, Alexander et al. studied heritability in a large familial cohort [25]. The study analyzed 914 pediatric EoE patients (within 2192 first-degree family members) and reported relative risk ratios for EoE in family members, which ranged from 10 to 64 depending on the relationship, with higher values among male relatives [25]. Overall, EoE is observed in approximately 2% of relatives [25].

Co-morbid Disease

While it has been thoroughly reported that other atopic disorders co-occur with EoE in approximately 70–80% of patients, there has been increasing recognition of EoE in association with several genetic disorders [26]. These EoE-associated diseases highlight important candidate genes and their corresponding pathways related to EoE, and interestingly, often have other co-occurring atopic conditions.

The most studied of these disease associations with EoE are inherited connective tissue disorders (CTDs) (e.g., Loeyz-Dietz syndrome [LDS], Marfan syndrome type II, and

Table 1 Genetic association in eosinophilic esophagitis

Gene	Approach	Known function/possible pathogenic mechanisms
CAPN14	GWAS	Epithelial homeostasis and repair
CCL26	Candidate gene	Effects eosinophil chemotaxis
CRLF2	Candidate gene	TSLP receptor/signaling/Th2 skewing
DOCK8	Phenotype association	Dysregulated T cells
DSG1	Mapping/sequencing/phenotype association	Barrier function
FLG	Candidate gene	Barrier function
IL-5/IL-13	PheWAS	Th2 signaling
LRRC32	GWAS	Possibly TFG- β signaling/epithelial protease function/barrier function
PTEN	Phenotype association	Regulation of eosinophil response
STAT3	Phenotype association	Dysregulated cytokine response
STAT6	GWAS	IL-13 responsivity
SPINK5	Phenotype association	Barrier function
TGF β 1	Candidate gene	Th2 skewing and fibrosis
TFG β R1/TFG β R2/FBN	Phenotype association	Th2 skewing and fibrosis
TSLP	GWAS	Potent Th2 skewing

IL interleukin, *TGF β 1* tissue growth factor beta 1, *CAPN14* calpain-14, *FLG* filaggrin, *DSG1* desmoglein-1 *TSLP* thymic stromal lymphoprotein, *CCL26* C-C motif chemokine ligand 26, *CRLF2* cytokine receptor-like factor 2, *DOCK8* dedicator of cytokinesis 8, *LRRC32* leucine-rich repeat-containing protein 32, *PTEN* phosphatase and tensin homolog, *STAT* signal transducer and activator of transcription, *SPINK5* serine protease inhibitor Kazal-type 5, *FBN* fibrillin, *GWAS* genome-wide association study, *Th2* T helper cell type 2, *PheWAS* phenome-wide association studies

Ehlers-Danlos syndrome). The odds ratio for the co-occurrence of EoE and CTD is approximately eight [14]. EoE and CTD have in common both increased production of transforming growth factor beta (TGF- β) and TGF- β signaling. LDS is caused by gain-of-function mutations in the TGF- β receptors, and Marfan syndrome type II is caused by mutations in connective tissue proteins that bind to TGF- β , such as fibrillin 1 (type I). Fibrillin proteins have complex regulatory interactions with TGF- β receptors. In mice, fibrillin deficiency leads to excessive TGF- β signaling [27]. Additionally, patients with LDS have elevated IgE levels, eosinophil counts, and T helper type 2 (T_H2) cytokines. Ehlers-Danlos syndrome is caused by mutations in collagens. Recently, TGF- β dysregulation has been reported in EDS [28]. Additionally, direct interactions between TGF- β and collagen have also been reported [26].

Another Mendelian disease that has been reported to co-occur with EoE is severe dermatitis, multiple allergies, and metabolic wasting (SAM) syndrome. SAM syndrome is a rare disorder reported in only two consanguineous families and one non-consanguineous family [29, 30]. It is an autosomal recessive disease associated with mutations in desmoglein 1 (*DSG1*). *DSG1* is a major constituent of desmosomes, which connect the cell surface to the keratin cytoskeleton to help maintain epidermal integrity and barrier function in the esophagus. *DSG1* is decreased in EoE and is associated with an impaired barrier phenotype [31].

Associations of EoE with a few other diseases have also been noted. These include autosomal dominant hyper-IgE syndrome, caused by mutations in signal transducer and activator of transcription (STAT) 3, tyrosine-protein kinase-2 (TYK2), and dedicator of cytokinesis 8 (DOCK8), a syndrome characterized by hypertryptasemia and associated with CTD [32]. PTEN hamartoma tumor syndrome (PHTS) notably carries a >200-fold increase in risk for EoE and other eosinophil-associated gastrointestinal disorders [37]. Additionally, celiac disease co-occurs with EoE [33–37]; celiac disease occurs at an increased rate (~14%) in the EoE population compared to that of ~1% in the general population [33]. In one study, serum tissue transglutaminase (TTG) Ab was elevated in almost a quarter of the total EoE cohort, and at least 20% of these patients did not have potential CD [38].

Environment

In the same twin study mentioned above, Alexander et al. revealed environment attributes to 81% of phenotype variance among twins [25]. Esophageal eosinophilia was also found to be more common in rural and suburban areas [39] than in urban zones [40–42]. Interestingly, studies in asthma and atopic dermatitis have found the opposite relationship. In contradiction to this, Spergel et al. [42] found that in the USA, EoE is diagnosed more often in urban areas. Two studies

showed that the prevalence of esophageal eosinophilia occurs more commonly in cold and dry climates [42, 43], a finding that has also been reported in other allergic and autoimmune disorders.

Dysbiosis, or dysregulation of the microbiome, may contribute to the etiology of a variety of diseases including inflammatory bowel disease, asthma, eczema, and irritable bowel disease [44, 45]. Several early-life exposures which may contribute to dysbiosis have been linked to increased risk of EoE including cesarean birth, antibiotics, and formula feeding [46]. Additionally, *Helicobacter pylori* infection appears to be inversely related to an EoE diagnosis [47].

Immune System

It is not fully understood how allergic sensitization occurs in EoE, though it appears to be a non-IgE-mediated process, because an anti-IgE treatment (omalizumab) is not effective in humans with EoE [48]. IgE- and B cell-deficient mice can develop an EoE-like disease including esophageal eosinophilia [49] and IgE-based skin testing does not consistently identify food antigen triggers [50]. Delayed-type, T cell-mediated reactions are increasingly understood to participate in EoE. This delayed-type hypersensitivity is characterized by T_H2 responses involving interleukin 4 (IL-4), IL-5, and IL-13 and the associated eosinophilic infiltrate [51].

Atopy

The majority (50–80%) of patients with EoE have an atopic phenotype. This phenotype includes other allergic diseases, such as atopic dermatitis (AD), asthma, oral allergy syndrome, allergic rhinitis, and food allergy, or elevated IgE to foods and aeroallergens [15]. These clinical findings have been supported through multiple murine studies demonstrating that skin, lung, and intranasal sensitization to various known food and aeroallergens can induce EoE-like symptoms [52–55].

EoE is a disease that is associated with food antigen-driven hypersensitivity, as evidenced by its response to dietary therapy. Hypersensitivity reactions can occur via multiple immune mechanisms including IgE (immediate type) and/or T cell mediated (delayed type). Most patients with EoE develop sensitivity to foods, based on food-specific serum IgE or skin prick test (SPT) [56–58]. Yet, the detection of these specific IgEs does not appear to be highly reliable to identify causative foods in EoE [12, 59–63]. In fact, empiric removal of foods appear to be equally or more effective than test-directed elimination [7, 50, 62–64]. Additionally, testing of delayed-type hypersensitive T cell response via the atopy patch test (APT) has limited value in the diagnosis of food allergy in EoE [62]. This may be partly due to performing the test on the skin and not the esophagus, thus not targeting the appropriate tissue-

specific T cell compartment. Though, recent studies have shown that epicutaneous immunotherapy in mice [65] and pigs [66] leads to Treg-dependent, long-term protection from esophageal and gastric eosinophilia respectively and that this protection is not necessarily limited only to the antigen administered in the epicutaneous immunotherapy. Studies on the safety and efficacy of epicutaneous immunotherapy for EoE appear are underway. These findings may demonstrate that skin T cells do circulate with the esophageal environment and thus may support more in-depth study of patch testing.

Food

Food elimination or elemental diet therapy can reduce or eliminate disease symptoms in 70–99% of patients [9] and relapse almost universally occurs after reintroduction of food allergens. This striking response to food elimination and reintroduction suggests that chronic food antigen hypersensitivity is a fundamental feature of EoE. Additionally, a subset of patients with EoE (15%) have a history of food anaphylaxis [5, 12, 57, 67] and there appears to be a progression from IgE-mediated food allergy to later development of EoE to the same food [68]. This progression may also occur in the other direction, that is, loss of tolerance with EoE elimination diet can lead to food allergy [69].

More recently, there has been evidence of oral immunotherapy (OIT) for food allergy inducing the development of EoE and in some cases resolving after the discontinuation of oral immunotherapy. It occurs in 2 to 10% of treated patients [70–72]. This has been reported with OIT for egg, milk, and peanut [73]. Interestingly, OIT is known to enable the production of immunoglobulin (Ig) IgG4 and there is some recent evidence that IgG4 responses are important in EoE [74, 75].

Aeroallergens

While food allergens appear to play a central role in EoE pathogenesis, aeroallergens may also play an important role. Aeroallergens exposure is associated with development of EoE in humans [76–78] and mouse models of EoE [79, 80]. There are also reports of EoE diagnosis varying by season, with highest rates during times of high aeroallergens, such as summer or fall [81–86]. Additionally, there are case reports of large allergen exposure just prior to the onset of EoE [78]. Recently, OIT to dust mite has also been associated with the development of EoE [87]. In adult patients with EoE, airway allergy precedes EoE [88]. Aeroallergens may also contribute to food hypersensitivity due to cross-reactivity or cross-sensitization. Food-specific cross-reactive responses to aeroallergen IgE are common [89, 90]. The predominant allergen families responsible for this cross-reactivity are profilins, pathogenesis-related (PR)-10, and lipid transfer proteins. These proteins remain intact until they are degraded in

the stomach [89, 91], giving a mechanistic explanation for hypersensitivity responses limited to the esophagus.

Though the role of aeroallergens was not specifically studied, Padia et al. [92] found a striking association of chronic rhinosinusitis (CRS) and EoE. They showed probands with CRS demonstrated a 3.4-fold increased risk of having EoE. First-degree relatives of CRS probands had a 1.5-fold increased risk of having EoE. Ninety-four percent of the study population were adults.

Comparison with Similar Immune Disorders

Two other immune-mediated diseases share similarities to EoE, atopic dermatitis (AD) and inflammatory bowel disease (IBD). AD is a chronic inflammatory Th2-mediated response associated with food antigen occurring in a stratified squamous epithelium. IBD is a chronic inflammatory response of the gastrointestinal mucosa involving a complex mechanism of food antigen exposure and dysbiosis.

Similar to EoE, over 80% of patients with AD have increased IgEs to foods and inhalant allergens in the peripheral blood [93] and the positive predictive value of IgE specific to food allergens is low in both EoE and AD [94]. In 32% of AD patients undergoing food allergen challenge, delayed eczematous reactions occurred likely via T cell-mediated response [94]. This is in agreement with the fact that in patients with food-triggered AD flares, pertinent food allergen-specific T cells can be detected in the peripheral blood as well as the skin [95, 96]. Also, similar to EoE, positive APT to inhalant and food allergens can occur in the absence of corresponding positive serum-specific IgE tests [97]. Like EoE, AD often has associated high total serum IGE and tissue eosinophilia, but despite this, patients have poor response to both anti-IgE and anti-IL-5 therapy [98, 99]. Additionally, common gene products have been implicated in both AD and EoE including filaggrin, DSG-1, and SPINK5 [100].

Similar to EoE, IBD pathogenesis is determined by genetic, environmental, and microbial factors [101]. Additionally, IBD patients will respond to food elimination therapy (i.e., total parental nutrition). The susceptibility to IBD is determined by genetic variants related to innate immunity, autophagy, and phagocytosis in Crohn's disease and to barrier function in ulcerative colitis (UC) [102]. Whereas, barrier function is a major theme in EoE. Also, similar to EoE, via increased epithelial permeability, food antigens and microbes may activate an immune response. In IBD, this response includes a release of pro-inflammatory cytokines such as TNF- α , IL-1, IL-18, and IL-33 eventually leading to a chronic inflammatory Th1 and Th17 response [101]. While both EoE and IBD have in common an impaired epithelial barrier, there are differences including genetic factors, different epithelial tissue types (columnar epithelium versus stratified squamous epithelium), and differential microbial

environment which together likely lead to different types of immune responses in these two diseases.

Immune Cells

Eosinophil

The relative importance of the eosinophil in EoE has been extensively studied in both mouse and human investigations (Table 2). In mouse models of EoE, mice genetically engineered to lack eosinophils or selectively targeted via antibody treatment have a decrease in many, but not all, features of the disease suggesting that in mice an EoE-like disease is not completely dependent on eosinophils [103–105] (Table 3). Eosinophils have been shown to have direct effects on immune function and tissue damage [1]. The eosinophil cationic proteins major basic protein (MBP), eosinophil peroxidase (EPX), and eosinophil cationic protein (ECP) are cytotoxic to epithelium at physiologic concentrations. ECP can damage cellular membranes barriers. MBP increases smooth muscle reactivity and can provoke mast cell and basophil degranulation. Evidence of release of eosinophil granule components has been directly observed in EoE biopsies; therefore, eosinophils likely effect epithelial damage in EoE. Eosinophils may also act as antigen-presenting cells (APCs). Eosinophils express major histocompatibility complex class II molecules as well as the co-stimulatory molecules (CD40, CD28, CD86, B7) and secrete cytokines (IL-2, IL-4, IL-6, IL-10, IL-12) which together can activate T cells [106]. Additionally, experimental adoptive transfer of antigen-pulsed eosinophils produces antigen-specific T cell responses in vivo. Eosinophils also have the potential for diverse cytokine production including IL-1, IL-3, IL-4, IL-5, and IL-13; GM-CSF; TGF- β and TGF- β ; tumor necrosis factor- α (TNF- α); RANTES

(regulated on activation, normal T cells expressed and secreted); macrophage inflammatory protein 1 (MIP-1); and eotaxin [1]. Thus, eosinophils have the ability to impact multiple aspects of the immune response, tissue damage, as well as repair.

Mast Cell

There are significant increases in mast cell degranulation and mastocytosis within the epithelium, lamina propria, and smooth muscle layer [107, 108] observed in EoE. Notably, the presence of mast cells is more abundant in EoE compared to PPI-REE and GERD [47]. Mast cells, which are classically primed with membrane-bound IgE via the FcR ϵ -I receptor, normally reside in the mucosa and submucosa of the esophagus, which likely contributes to a local IgE-mediated immediate hypersensitivity response in the esophagus in EoE, similar to what is observed in oral allergy syndrome. Mast cells in EoE can decline with steroid therapy [107] and maybe partially related to the response seen with steroids in EoE. Mast cells generate numerous products including cytokines, proteases, and bioactive compounds. Many of these products can activate eosinophils and cause fibrosis [108–111]. Multiple studies have demonstrated a role of mast cells in regulating smooth muscle contractility and vagal nerve activity [108] [112, 113]. In murine models of EoE, mice with depleted IgE and devoid of mast cells still can develop esophageal inflammation and food impaction [68, 114], though they have reduced muscle cell hyperplasia and hypertrophy consistent with a role for mast cells in remodeling (Table 3). Additionally, mast cell-specific genes, carboxypeptidase 3A (CPA3), IgE receptor (FCER1), and tryptase (TPSAB1), are highly expressed in the EoE transcriptome. Two unique mast cell-related transcriptomes have been identified on the basis of counts of biopsy mast cells [107]. Interestingly, IL-5 appears to lead to increases of mast cells in the tissue, as IL5 transgenic mice had

Table 2 Extensively studied factors in eosinophilic esophagitis

Factors	Relevant downstream effects
Eosinophils	• Number correlates with degree of EMT and fibrosis • Number correlates with response to steroid and elimination diet therapy
IL-13	• Anti-IL-13 therapy normalizes EoE related transcripts • IL-13 treatment of biopsy-derived primary epithelial cells can closely replicate EoE transcriptome • Induces barrier dysfunction likely via induction of proteases (CAPN14) and downregulation of barrier proteins (EDC complex genes, DSG1, FLG) and tissue remodeling (collagen and periostin deposition, epithelial hyperplasia) • Anti-IL-13 therapy improves eosinophil number • IL-13 induces eotaxin-3 and periostin
TGF- β	• Induces myofibroblasts expression of collagen and periostin • Induces smooth muscle contractility.

IL-13 interleukin-13, *TGF- β* tissue growth factor beta *CAPN14* calpain-14, *FLG* filaggrin, *DSG1* desmoglein-1, *EMT* epithelial-to-mesenchymal transition, *EoE* eosinophilic esophagitis, *EDC* epidermal differentiation complex

Table 3 Necessary and dispensable factors in eosinophilic esophagitis

Important factor	Findings	Required	Not required
iNKT	• Rag1 KO mice are protected from experimental EoE	x	
IgE	• CD1-deficient mice are protected from experimental EoE • IgE deficient mice are not protected from experimental EoE • Anti-IgE therapy does not completely reverse histologic disease		x
Mast cell	• Food and Aeroallergen-Specific IgE have poor predictive value Mast cell deficient mice are not protected from experimental EoE		x
Basophils	Basophil depletion mice are protected from experimental EoE	x	
TSLP	TSLP KO mice are protected from experimental EoE	x	
CD8 T cells	CD8 KO mice are not protected from experimental EoE		x
B cells	B cell deficient mice are not protected from experimental EoE		x
IL-13	IL-13 transgenic mice have experimental EoE	x	
IL-33	Basophil IL-33 receptor KO mice are protected from experimental EoE	x	
Eosinophil	• Familial reports of EoE patient with family member who have eosinophil negative EoE-like disease • Eosinophil deficient mice are not protected from experimental EoE		?

TSLP thymic stromal lymphoprotein, *IgE* immunoglobulin E, *IL* interleukin, *EoE* eosinophilic esophagitis, *KO* knockout, *CD* cluster of differentiation

increased levels of esophageal mast cells [103]. Moreover, anti-IL-5 therapy in clinical trials found reduced esophageal mast cells in treated patients [115]. The anti-IL-5 clinical trial also showed that the severity of EoE symptoms correlated with mast cell number, but not eosinophil numbers [115]. IL-9, an elevated cytokine in EoE biopsies [116], can promote the activation and maturation of mast cells [117]. It is possible that the activated mast cells in the esophagus are central to the pathophysiology of EoE in a subgroup of patients.

Basophil

Basophils are known to be important in allergic inflammation, but until recently there has been a paucity of data on basophil function in EoE. Multiple studies have demonstrated increased basophil levels in EoE biopsies [54, 118]. In one of these studies, Siracusa et al. [118] showed that TSLP can affect basophil hematopoiesis. In the other study, Noti et al. [54] found that sensitization to egg or peanut protein could occur during skin inflammation or injury (tape stripping) in a TSLP-dependent, basophil-dependent, and IgE-independent manner (Tables 3 and 4).

Dendritic Cell

The understanding of antigen presentation in EoE is limited, but there is evidence that dendritic cells as well as nonprofessional APCs, such as epithelial cells [119] and eosinophils, may present antigens [49, 120, 121]. Langerhans cell, a type of dendritic cell found in all squamous epithelia [90], is found in the esophagus. Allergens can induce a T_H2 -mediated

response, either alone (with a self-adjuvant effect) or in combination with other environmental adjuvants (viral or bacterial infections or air pollution) [122], and this effect likely occurs via communication of resident stromal cells and dendritic cells [123, 124]. Importantly, the stimulation of dendritic cells via environmental adjuvants may be one direct mechanism by which environment is contributing to EoE.

T Cell

Lymphocytes represent another component of the inflammatory infiltrate that is consistently elevated in patients with active EoE relative to both normal controls and inactive EoE [16, 120, 125, 126]. The B cell role will be discussed in the IgE section below. Similar to other atopic diseases, tissue inflammation in EoE is characterized by a T_H2 -type inflammatory response [116, 120, 127, 128]. This immune response includes an increase in both $CD4^+$ and $CD8^+$ T cells and an increase in the $CD8^+$ T cell/ $CD4^+$ T cell ratio in the esophagus [125, 126]. Studies of different T cell populations in murine EoE models tell an interesting story. Rag1 knockout mice are completely protected from experimental EoE, whereas CD4 knockout mice are partially protected, and CD8 knockout mice confer no protection [49] (Table 3). Regarding T regulatory cells (Tregs), studies in pediatric and adult EoE populations regarding Treg numbers in EoE biopsies have opposite results. Increased numbers of Tregs were seen in pediatric cases, whereas there was a relative lack of Tregs in the adult cases [129]. This may suggest an inherent difference in pediatric versus adult cases of EoE.

Table 4 Recent advances in the study of eosinophilic esophagitis

Important factor/concept	Recent findings
CAPN14	Affects barrier dysfunction likely through a homeostatic mechanism of barrier repair
IL-15	- IL-15 supports and skews CD4 T cells and iNKT cells in EoE - IL-15 induces IL-5 and IL-13 in EoE
iNKT	- Rag1 KO and CD1-deficient mice are protected from experimental EoE - iNKs are increased in EoE and produce more IL-13 compared to controls - Activation of iNKT is sufficient to induce experimental EoE and neutralization of iNKT cells protects against experimental EO - iNKT cells from peripheral blood of EoE patients can be activated by milk sphingolipids
Basophil	Experimental EoE was basophil dependent
IL-33	- IL-33 is upregulated in EoE - IL-33 regulates IL-5 production and eosinophil commitment and homeostasis - IL-33 is required for basophil-dependent experimental EoE
TSLP	- TSLP effects basophil hematopoiesis - Experimental EoE was TSLP dependent
Esophageal tissue identity	There is substantial loss of tissue identity in EoE
ILCs	ILCs are increased in EoE biopsies and correlate with eosinophil number
IL-18	IL-18 plays a role in promoting IL-5 and iNKT-dependent EoE
TRAIL	TRAIL regulates MID1 and TSLP, inflammation, fibrosis, smooth muscle hypertrophy, and expression of including MID1, TSLP, CCL11, CCL20, CCL24, IL-5, IL-13, IL-25, and TGF- β in experimental EoE
IgG4	High-titer IgG4 in EoE biopsies to common EoE food triggers
Protease and protease inhibitor family members	Most dysregulated esophagus-specific genes
Microbiome	Microbiome is dysregulated in the esophagus in EoE and correlates with activity of disease
EMT	- Important tissue remodeling process - EMT correlates with eosinophils, TGF-B, and fibrosis

IL interleukin, *CAPN14* calpain-14, *EMT* epithelial-to-mesenchymal transition TSLP thymic stromal lymphoprotein, *IgG4* immunoglobulin G4, *KO* knockout, *CD* cluster of differentiation iNKT invariant natural killer T cells, *ILC* innate lymphoid cells, *EoE* eosinophilic esophagitis, *CCL* C-C motif ligand, *MID1* midline-1, *TRAIL* TNF-related apoptosis-inducing ligand, *TGF- β* , tissue growth factor beta

iNKT Cells

Recent data suggest the effects seen in the RAG1 knockout experiments mentioned above may likely reflect contribution of invariant natural killer T (iNKT) cells in the pathophysiology of EoE. EoE patients have reduced peripheral blood iNKs, and increased esophageal iNKs compared to controls. Additionally, iNKs from patients with active EoE expand more readily and produce more IL-13 in response to stimulation when compared to controls [130]. In animal models, activation of iNKT is sufficient to induce EoE and neutralization of iNKT cells protects against experimental EoE [131] (Table 3). iNKT cells recognize lipid and glycolipid antigens presented by CD1d molecules [132, 133]. iNKT cells from peripheral blood of EoE patients can be activated by milk sphingolipids [130]. Furthermore, CD1d-deficient mice are protected from experimental

EoE [134]. It has been previously shown that mucosal iNKT cell tolerance to environmental antigens is formed at an early age [135] and these cells can mediate allergic sensitization and tissue inflammation in the absence of tolerance [136]. Insufficient immune imprinting by environmental microorganisms in early-onset EoE results in an esophageal increase of CXCL16, an iNKT cell chemokine [137] (Table 4).

Innate Lymphoid Cells

Innate lymphoid cells (ILCs) are tissue resident immune cells that can serve as reservoirs for robust production of IL-5 and IL-13 [138–140]. These cells are important in mouse models of infection, inflammation, and tissue repair [138]. Group 2 ILCs (ILC2s) express CCR2 and are induced by IL-33 and TSLP to produce substantial quantities of Th2 cytokines. Doherty et al. [138]

recently showed that ILC2s are present in EoE biopsies, increased in active vs inactive EoE, increased compared to PPI-REE and normal controls, and correlate with the number of eosinophils in the biopsy (Table 4).

Soluble Immune Effectors

Cytokines

A number of cytokines are elevated in both patients with EoE [116, 120] and in murine models of EoE [141] including the T_H2 cytokines (IL-4, IL-5, and IL-13), as well as alarmin molecules TSLP and IL-33 and others.

IL-4

IL-4 is known to induce skewing of naive T_H cells into T_H2 cells and activate B cell class switching to produce IgE, initiating or supporting a T_H2 -mediated immune response. There is evidence of numerous cellular sources of IL-4 in EoE including TSLP-elicited basophils, T_H2 cells, and iNKT [120, 142–144].

IL-5

IL-5, produced by T_H2 cells, mast cells, and eosinophils, regulates eosinophil expansion and survival and primes eosinophils to respond to activating signals [145]. In EoE, IL-5 overexpression induces disease and IL-5 neutralization completely blocks allergen- or IL-13-induced EoE in mice [79]. However, current studies of anti-IL-5 therapy in humans have not been shown to be effective at improving symptoms although esophageal eosinophilia does improve [146].

IL-13

IL-13 is one of the most studied cytokines in EoE (Table 2). IL-13 is highly upregulated in the esophagus of patients with EoE. IL-13 is produced by T_H2 cells and activated eosinophils in EoE [120, 147, 148]. In humans, a study of an anti-IL-13 therapy showed improvement in both esophageal eosinophilia and disease-related transcripts, as well as a trend for improved symptoms [98]. IL-13 treatment of biopsy-derived primary esophageal epithelial cells can largely replicate the EoE transcriptome [127]. In murine models, IL-13 induces EoE and tissue remodeling [149] whereas inhibiting IL-13 lead to improvement in esophageal inflammation [150]. IL-13 also recruits eosinophils via upregulation of an eosinophil chemokine, eotaxin-3, and by promoting fibroblasts to produce periostin, which increases eosinophil chemotaxis [151]. Additionally, IL-13 plays a role in barrier function. IL-13 has been shown to downregulate DSG-1, filaggrin, and

involucrin, proteins important in epithelial integrity and barrier function [17, 152]. IL-13 induces tissue remodeling by promoting collagen deposition, angiogenesis, and epithelial hyperplasia [149] which occurs independently of eosinophilia [149]. Finally, STAT6, a downstream transcription factor of the IL-13 signaling pathway has recently been found to have a major genetic association in EoE [20]. In support of this, JAK-STAT6 pathway inhibitors, ruxolitinib, AS1517499, leflunomide, inhibit IL-13 induced eotaxin-3 expression both in fibroblasts and in epithelial cells, suggesting a potential role for JAK-STAT inhibitors in treating both epithelial inflammation and subepithelial fibrosis in EoE [153].

IL-15

IL-15 has been demonstrated to be elevated in patients with EoE, as well as in murine models of EoE. IL-15 appears to be important in supporting and skewing of $CD4^+$ T and iNKT cells [144] including the synthesis of IL-5 and IL-13 in EoE [17, 104, 127, 144].

TGF- β

TGF- β is a key cytokine for epithelial growth, fibrosis, and tissue remodeling and is generated by mast cells, eosinophils, and esophageal epithelial cells (Table 2). Its expression is elevated in esophageal biopsy samples of patients with active EoE relative to control patients or patients with GERD [104, 111, 146, 154]. TGF- β stimulates myofibroblast differentiation [108] and, more specifically, expression of smooth muscle actin, collagen, and periostin [151]. Additionally, TGF- β has been shown to induce esophageal smooth muscle contractility and thus may potentially contribute to esophageal dysmotility in patients with EoE [108]. Also, a genetic variant in the promoter of TGFB1 was associated with increased TGF- β -positive cells in the esophagus and with steroid unresponsiveness in EoE patients [18]. Interestingly, there is a high rate of EoE with CTDs [14], such as Marfan syndrome, Ehlers-Danlos syndrome, and Loeys-Dietz syndrome, which has been associated with variants in the TGF- β receptors 1 and 2 [155]. Recently, it has been shown that plasminogen activator inhibitor-1 (PAI-1) induces TGF- β -induced gene expression, is significantly increased in the EoE esophageal epithelium and is associated with fibrosis [156]. Additionally, a TGF β 1 promoter SNP C509T was associated with significantly more TGF β 1 and tryptase positive cells and poorer epithelial remodeling scores. These findings were significantly increased when there was concurrent food sensitization [157].

Multiple recent studies have identified additional cytokines that are important in EoE. Collison et al. [158] found that TNF-related apoptosis-inducing ligand (TRAIL) regulates MID1 and TSLP, inflammation, fibrosis, smooth muscle hypertrophy, and expression of inflammatory effector

chemokines and cytokines of experimental EoE including TSLP, CCL11, CCL20, CCL24, IL-5, IL-13, IL-25, and TGF- β . De Souza et al. [159] showed that macrophage migration inhibitory factor (MIF) induces eosinophil infiltration and remodeling in a mouse model of EoE. Dutt et al. [160] demonstrated evidence that allergen-induced IL-18 has a significant role in promoting IL-5- and iNKT-dependent EoE pathology.

Prostaglandins

Prostaglandins can affect eosinophil infiltration into the esophagus while prostaglandin inhibitors protect against EoE-like inflammation [161]. Chemoattractant receptor expressed on Th2 cells (CRTH2) is the receptor for Prostaglandin D2 (PGD2) and mediates chemotaxis of Th2 cells, eosinophils, and basophils [162]. Recently, a CRTH2 inhibitor was studied in EoE. In steroid resistant disease, anti-CRTH2 therapy was associated with reduced esophageal eosinophils and symptoms compared to placebo [163].

Antibodies

IgE

The generation of antigen-specific IgE via T_H2 cell-mediated class switching of B cells is a process central to the pathogenesis of many atopic disorders. This IgE can then bind the FcRI receptor on mast cells and basophils to induce degranulation leading to the downstream effects of anaphylaxis, allergic bronchospasm and urticaria. Allergen-specific serum IgE and SPT is commonly abnormal, providing evidence of immediate hypersensitivity in EoE, but the role of IgE in EoE is not clear. B cells have been shown to perform class switching and generation of IgE locally within the esophagus of both atopic and nonatopic patients with EoE [128, 164, 165]. However, in murine models of EoE, B cell-deficiency (IgH6) [49], IgE-deficiency (IgH7) [54] mice and utilization of an IgE-independent aeroantigen (*Aspergillus*) do not affect the ability to induce EoE, supporting the dispensability of IgE in the pathogenesis of disease [80]. Studies of anti-IgE therapy in EoE demonstrated clinical but not histologic or endoscopic improvement [48], suggesting that some acute symptoms in EoE may be associated with IgE but that the chronic inflammation may not be as dependent [49, 54].

Recently, it has been shown that EoE patients with low titers of IgE to milk can respond well to milk protein elimination diet [166]. These patients often have high-titer IgG4 ab levels to cow's milk [74]. A recent study showed that esophageal tissues from patients with EoE had a 45-fold increase in IgG4 compared with controls and that patients with EoE had increased food-specific serum IgG4 to the foods that are most associated with EoE (milk, egg, wheat, nuts) [74]. In support

of this, two additional smaller studies have also demonstrated elevated food-specific IgG4 in EoE [167, 168]. Interestingly, OIT is known to enable the production of immunoglobulin (Ig) IgG4 as well as induce EoE. [169]. As Aalberse et al. has suggested, the high IgG4 antibody levels in patient serum may prevent sIgE binding to its relevant antigens in serum-specific IGE assays causing the falsely negative or low titer results. Thus, the IgG4 antibodies may reveal the milk proteins that are relevant to the pathology, though it is still possible that a minor allergen that fails to induce a protective IgG4 may be more relevant.

Esophagus

Epithelium

Transcriptome

The EoE transcriptome, a gene expression profile signature of 574 genes, distinguishes patients with EoE from healthy control subjects and from patients with GERD [16]. Ninety-eight percent of this transcriptome reverses with topical steroid therapy. More recently, a more sensitive method of whole-transcriptome sequencing (RNA-seq) of biopsy transcripts identified over 1000 new EoE-dysregulated genes (66% of the RNA-seq profile) not noted in the previous microarray data [170]. Using the same RNA-seq profiling method, KC et al. found that 286 genes that were modified by IL-13 in an air-liquid interface (ALI) system overlapped with the gene signature present within the inflamed esophageal tissue from patients with EoE [171].

Recently, the EoE diagnostic panel (EDP), a set of 94 genes differentially expressed in the esophagus of EoE patients measured by microarray, was introduced [172]. The EDP identifies EoE with ~96% sensitivity and ~98% specificity in adult and pediatric patients and distinguishes patients with EoE in remission from non-EoE controls, as well as identifies patients who have been exposed to swallowed glucocorticoids. The EDP demonstrates predicative capacity for patients with subclinical histology (1–14 eosinophils per HPF). Furthermore, the EDP has the capacity to reveal EoE pathogenesis on a molecular level that could vary from patient to patient. Notably, the EDP has a high performance using only one distal esophageal biopsy compared with conventional histology, which currently requires at least four biopsies according to consensus recommendations [7, 172]. Using this panel two studies have demonstrated that there are no major differences between EoE biopsies and PPI-REE prior to PPI therapy. While this suggests that a PPI trial may not distinguish between EoE and GERD, knowing that a patient has PPI-REE is important clinical information to avoid the long-term effects of fibrosis in EoE. [173, 174]

A select set of microRNAs (miRNAs), short, noncoding RNAs that repress the expression of target genes by binding

complementary sequences in the 3' untranslated region of target mRNAs [175], has been shown to be dysregulated in the esophageal mucosa of patients with EoE. The EoE miRNA transcriptome includes 21 upregulated and 11 downregulated genes. This dysregulation is mostly reversed with steroid therapy [176, 177]. Some of these miRNAs (miR-21, miR-223, and miR-375) have been shown to correlate with esophageal eosinophil levels in patients with EoE [176] and regulation of IL-13-induced transcriptional responses [178]. Thus, miRNAs may act as potential biomarkers for EoE diagnosis and steroid responsiveness.

Barrier

The epithelial layer of the esophagus is a stratified squamous epithelium. The human esophagus lacks a cornified layer but does have a mucus layer that provides additional protection. Of note, the mouse esophagus, a common experimental system for EoE, has a cornified epithelium. This esophageal epithelium is composed of a proliferating basal layer of 1 to 3 cells in depth and a differentiating suprabasal layer which migrates apically to the esophageal lumen [179]. When the barrier of this epithelium is broken, it can lead to a foreign antigen hypersensitive immune responses [180]. In EoE, the epithelium demonstrates basal cell hyperplasia, dilated intracellular spaces, and impaired barrier function [181–183] and a similar disruption occurs in an in vitro esophageal epithelium model when stimulated with IL-13. These changes are likely linked to known IL-13-mediated effects in processes such as epithelial differentiation and epithelial barrier formation, including cellular junction formation. In line with these epithelial changes in EoE, recently, Rockman et al. [184] revealed a profound loss of esophageal tissue differentiation (identity) occurring in EoE. They reported that approximately 40% of esophageal specific genes are downregulated in EoE. Within this set of esophageal specific genes, keratinization and differentiation were the most affected biological processes.

The epidermal differentiation complex (EDC) on human chromosome 1q21 is a cluster of genes that regulates terminal differentiation [185] and it contains the largest number of dysregulated genes in the EoE transcriptome [17]. EDC genes including filaggrin (FLG), involucrin (IVL), and several small proline-rich repeat (SPRR) family members (2C, 2D, and 3) are expressed in esophageal epithelial cells and are downregulated in response to IL-13 [17]. FLG dysfunction has been associated with defects in epidermal barrier function in patients with atopic dermatitis patients [186, 187], a disease which frequently co-occurs with EoE. FLG also represents another genetic locus linked with EoE. FLG is decreased in the esophageal mucosa of patients with EoE, and two coding variants (R501X and 2282del4) in FLG associate with EoE risk irrespective of atopic status [17]. FLG is downregulated in

the esophageal mucosa of patients with active EoE, and this change is reversed with treatment [188].

Other junctional proteins, including E-cadherin and claudin-1, are also reduced in EoE [189]. It is important to note that 2% of the EoE transcriptome is not fully reversible after disease remission [127]. Interestingly, these transcripts include genes that are involved in regulating epithelial homeostasis, such as cadherin-like 26 (CDH26), uroplakin 1B (UPK1B), periostin (POSTN), and DSG1 [127].

DSG1 is a transmembrane desmosomal cadherin component which facilitates the calcium-dependent interactions between adjacent cells that give both structure and mechanical strength to the epithelia. DSG1 autoantibodies are found in pemphigus foliaceus and pemphigus vulgaris, squamous epithelial diseases that demonstrate decreased cellular adhesion, resulting in epidermal blistering, with some patients demonstrating eosinophilic infiltration [190]. Sherrill et al. [152] reported that expression of DSG1 is decreased in active EoE (22 fold) and that DSG1 deficiency in esophageal epithelial system in vitro leads to histologic changes including acantholysis and intraepidermal clefting as well as an impaired barrier function. This impaired barrier function is also seen with EoE patient biopsies. Additionally, two recent reports have shown that families with recessive mutations in DSG1 have a severe allergy phenotype, including one patient with EoE [29, 30].

Keratin genes have been identified to be dysregulated by pathway analysis of RNA-seq data in two separate and different studies. KC et al. [171] found an association studying overlapping genes of EoE biopsies and an in vitro esophageal epithelium model. Rochman et al. [184] identified a set of esophageal identity genes that are lost in EoE biopsies which include the keratin family.

Recently, calpain 14 (CAPN14), a member the calpain family, a group of intracellular, calcium-activated proteases was found to be the most highly associated gene with EoE [191]. This finding was soon replicated in a separate GWAS [20]. CAPN14 was shown to be specifically expressed in the esophagus, dynamically regulated as a function of disease activity and genetic haplotype and after exposure of esophageal epithelial cells to IL-13, and was located in an epigenetic hotspot modified by IL-13 [191]. Recently, it has also been shown that CAPN14 overexpression and silencing has a significantly disruptive effect on esophageal epithelial barrier and its expression and activity appear to lead to cleavage of DSG1 [192]. Together, this supports a role for CAPN14 in homeostasis and repair of the esophageal epithelium and provides a unifying mechanism that connects genetics with an essential disease susceptibility mechanism.

Serine peptidase pathways have also been recently linked to EoE. Serine protease inhibitors (SERPINs) and serine protease inhibitors, Kazal type (SPINKs) were the most dysregulated peptidase families in EoE [184]. Kallikrein serine peptidases have been also shown to be

dysregulated in EoE [193]. Given that the esophagus loses its tissue identity and its structural integrity which leads to an impaired barrier function and that proteases play a major role in these processes, proteases appear to play a key role in the pathophysiology of EoE.

Thus, in EoE, the mucosal integrity appears altered due to defects in desmosomal and tight junction adhesion proteins and proteases acting on these structural units. These findings place the esophageal epithelium as a central location for the basic defect in EoE.

Epithelial Inflammatory Response

Under attack from environmental toxins or microbes, the esophageal epithelium can induce inflammation. The epithelium is a reservoir of cytokines and lipid mediators [194]. Esophageal epithelial cells also express toll-like receptors [195, 196] and produce pro-inflammatory cytokines in response to both pathogen-associated and danger-associated molecular patterns [120, 195]. EoE-derived epithelial cells can produce RANTES (CCL5), a chemotactic factor for T cells, eosinophils, and basophils [130], and CXCL16 [137], which may play a role in the migration and activation of iNKT cells in EoE, as well as eotaxin-3, TSLP, and IL-33. In addition, others have found that esophageal epithelial cells may also function as nonprofessional APCs [119]. Resident ILCs are also present within the esophageal epithelium at baseline [126, 137], and their roles in EoE are being actively investigated [137]. Like other allergic disorders, it is very likely that barrier impairment leads to alarmin production and release.

Blanchard et al. [16] was the first to identify an EoE risk variant; the variant was within the eotaxin-3 gene. This observed association was independent of atopic status, indicating a direct link with EoE susceptibility. IL-13 stimulates esophageal epithelial cells to produce eotaxin-3 [16], which signals eosinophils to traffic to the esophagus. The CCL26 gene, coding for eotaxin-3, is the most upregulated chemokine gene in the esophagus of patients with EoE [16]; eotaxin-3 belongs to the eotaxin family of CC chemokines and is the only family member that is upregulated in EoE. The esophageal epithelium is the main source of eotaxin-3 production [16]. Its expression is increased 53-fold in esophageal biopsy specimens from patients with EoE and is upregulated by 279-fold after IL-13 stimulation of primary esophageal epithelial cells grown in culture *ex vivo* [127]. Mice lacking the eotaxin receptor, CCR3, are protected from developing experimental EoE [16]. Additionally, PPIs have been suggested to decrease eotaxin-3 expression [197], giving a potential mechanism of action for PPI therapy in EoE.

TSLP has been termed a “master regulator” of T_H2 responses [198] and has been shown to target dendritic cells and promote their T_H2 -skewing ability [199]. It also exerts effects on nearly every cell type involved in T_H2

inflammation, including eosinophils [200] and mast cells [201]. The TSLP locus, which encodes an epithelial protein expressed in response to cytokines [202], noxious substances [203], and mechanical stress [204], is highly associated with EoE in multiple GWAS studies [20, 191, 205]. TSLP is increased in the esophageal tissue of patients with EoE [16, 21]. Patients carrying the risk allele for the variant most associated with EoE have elevated TSLP expression [21]. In addition, TSLP risk genotypes correlated with increased levels of basophils [54]. As mentioned in the basophil section, Siracusa et al. [118] showed that TSLP can affect basophil hematopoiesis. Noti et al. [54] found that experimental EoE was induced in a TSLP-dependent, basophil-dependent, and IgE-independent manner. Together, it seems very likely that risk variants in the TSLP signaling pathway could potentially amplify innate inflammatory responses to food and aeroallergen antigens.

IL-33 has recently arisen to a level of highly active area of research. IL-33 expression has been shown to be upregulated in pediatric EoE and it promotes EoE development in mice [206]. IL-33 in EoE esophagus appears to be expressed in both the endothelium and the basal layer of the epithelium [206, 207]. Additionally, IL-33 upregulates IL-5 production and regulates eosinophil commitment and is required for eosinophil homeostasis [208]. Venturelli et al. [209] recently showed that wild-type, but not IL-33 receptor-deficient, basophils restored esophageal eosinophil accumulation in basophil-depleted mice indicating that basophils are critical in IL-33-mediated EoE. Other IL-1 cytokines family members including IL36A, IL36RN, IL1A, and IL1RN are all downregulated in EoE, though, the significance of this finding is poorly understood [184].

Lamina Propria

Fibrosis

In line with other eosinophilic inflammatory conditions, EoE has been described as a progression from an inflammatory to a fibrotic disease [210]. Esophageal remodeling is associated with stricture formation resulting in the symptoms of dysphagia and food bolus impaction [210, 211]. In fact, EoE is currently the leading cause of emergency endoscopy for esophageal food impaction in adults [212]. Both histopathologic fibrosis [213] and gross fibrostenotic disease increase with age [154]. Fibrotic remodeling, including metaplasia of the mucosal glands, smooth muscle hypertrophy, angiogenesis, and subepithelial collagen deposition or fibrosis, occurs in pediatric [110, 111] and adult [154, 213] patients with EoE. The myofibroblast, which shares properties of smooth muscle cells and fibroblasts, is the key effector cell in fibrosis. The myofibroblast contracts the extracellular matrix and secretes extracellular matrix components, including type 1 collagen [214].

A number of effector molecules have been studied in the context of the fibrotic process in EoE. FGF9 and CCL18 are important in fibroblast activation and extracellular matrix deposition. Periostin, which functions as a cell adhesion molecule that regulates extracellular matrix deposition [215, 216], is dramatically upregulated (approximately 52-fold) in patients with EoE and reverse with treatment [188]. Both TGF- β and IL-13 are able to induce periostin expression in both primary esophageal fibroblasts and epithelial cells. Periostin has been shown to facilitate eosinophil recruitment into the esophagus [151]. Matrix metalloproteases (MMPs) are also known to play a role in remodeling. Recently, Beppu et al. [217] found MMP-2 and MMP-14 are elevated in pediatric EoE subjects and significantly decrease following topical corticosteroid therapy. They also found that TGF β 1 increases MMP-2 in esophageal epithelial cells.

Similar to the esophageal epithelium, the lamina propria and muscularis mucosa both undergo eosinophilic infiltration [218, 219]. Esophageal inflammation leads to deposition of subepithelial fibrous tissue and tissue remodeling correlated with eosinophil degranulation [110]. In fact, eosinophil-released MBP has been found to increase the expression of fibroblasts growth factor 9 (FGF9) in biopsies of patients with EoE [220]. Additionally, eosinophils secrete high levels of CCL18, and its expression is highly increased in EoE [213].

Epithelial-to-Mesenchymal Transition

Epithelial-to-mesenchymal transition (EMT) is a process in which epithelial cells lose epithelial markers (e.g., E-cadherin, keratins 8 and 14) and acquire markers of mesenchymal cells (e.g., smooth muscle actin, vimentin, fibronectin, N-cadherin), which share features of both fibroblasts and smooth muscle cells (myofibroblasts). These cells then participate in the synthesis, deposition, and degradation of extracellular matrix along with the contraction of wound tissue to facilitate tissue repair [221–223]. TGF- β , MMP-9, IL-13, and MBP released by eosinophils or damaged epithelium may induce EMT and contribute to fibrosis [222, 224]. In EoE, the degree of EMT correlates with eosinophils, TGF- β , and fibrosis [224]. Another TGF superfamily member, bone morphogenetic protein (BMP), and one of its inhibitors, follistatin, also appear to play a role in the EMT process in EoE [225]. In fact, follistatin is increased in EoE, increased with IL-13, and correlates with basal cell hyperplasia and loss of differentiation markers [226].

It appears that both swallowed topical corticosteroids [18, 227] and elimination diets [228] can prevent and even reverse this esophageal remodeling process in children; however, there is conflicting evidence in adults [213]. This difference may be due to the populations studied, the differences in topical steroid formulations (budesonide in children vs fluticasone in adults), or the strictness of the fibrosis scoring system used, as the adult study used a more stringent scoring

system. Thus, untreated eosinophilic inflammation leads to esophageal remodeling with stricture formation, and the use of early, consistent anti-inflammatory therapy may protect against this outcome. Losartan, an angiotensin II receptor blocker and FDA-approved antihypertensive medication, may reduce the amount of TGF- β and thus is a potential treatment for EoE. Currently, there are two active phase II trials evaluating losartan therapy in patients with EoE [229].

Smooth Muscle

EoE is associated with esophageal dysmotility and dysphagia. While these symptoms can be related to inflammation, edema, or stricture formation, which physically narrow the lumen of the esophagus, it may also be related to smooth muscle motor dysfunction. In fact, EoE patients who respond endoscopically and histologically to therapy may still have symptoms. These continued symptoms may be attributed to muscle dysfunction not appreciated via endoscope or biopsy. Esophageal ultrasound has demonstrated dysfunctional muscularis mucosa in EoE [230]. Both eosinophils and mast cells infiltrate the submucosa, myenteric neuronal plexus and smooth muscle in EoE [108, 231] and affect smooth muscle function and their innervation [219, 231, 232]. Specifically, TGF- β has been shown to increase smooth muscle contractibility over time [108] but decrease its force of contraction [219].

Microbiome

Commensal bacteria that colonize human epithelium are now appreciated as fundamental components of normal human physiology including the immune system and they have been identified as important variables in allergic disease [233]. Investigations into how commensals contribute to EoE is just beginning. It is known that patients with EoE have dysregulated microbial populations compared to healthy controls [234]. Additionally, the total microbial load is increased in patients with EoE compared to healthy subjects [235]. Together, these findings indicate that EoE is associated with microbial dysbiosis.

Whether this dysbiosis is simply serving as a biomarker of inflammation in EoE or is a direct inducer to it is unknown. Two independent groups have evaluated the microbiome of the esophagus in both control and EoE patients. In both studies, *Streptococcus* is the predominate genus in the esophagi of control patients. Harris et al. [235], using an esophageal string to capture samples, found that the EoE esophagus had significantly increased *Haemophilus* species and that, with remission, the streptococcal population increased. Benitez et al. [234] found *Firmicutes* as the predominate genus in the normal esophagus biopsies, and an increase in *Neisseria* and *Corynebacterium* in patients with active EoE. Harris et al. [235] showed that subjects in Aurora, Colorado and Chicago, Illinois, had distinct

esophageal microbiota, whereas the Benitez study [234] was performed in Philadelphia, PA, which could account for the differences between the two studies.

Future Directions

A number of recent studies in EoE have shown promise for improvements in the areas of diagnosis and treatments. Biomarkers have become a hot topic to improve diagnosis, phenotyping, and endotyping, as well as to predict and follow response to therapy. Schlag et al. [236] demonstrated the use of peripheral blood analysis to aid in following response to therapy in EoE. They showed absolute eosinophil count (AEC), serum-CCL17, CCL26, and ECP, and mast cell tryptase significantly decreased under budesonide but not under placebo. In support of this, Min et al. subsequently showed AEC were higher in EoE subjects compared with controls and

correlated with degree of esophageal eosinophilia and it predicted post-treatment eosinophilia, suggesting a potential role in monitoring EoE disease activity. Hui et al. [237] showed arachidonate-15 lipooxygenase (ALOX15) immunohistochemistry facilitates the diagnosis of EoE in situations with strong clinical suspicion where no biopsy specimens reach 15 eos/HPF. ALOX15 is a non-heme iron-based enzyme involved in di-oxygenation of polyunsaturated fatty acids to form lipid-based cytokines.

Multiple new esophageal sample collection methods have also been studied.

Saffari et al. [238] described a simple, quick method to measure an eosinophil granule protein, eosinophil peroxidase (EPO), as a marker of eosinophil activity, in EoE along the entire length of the esophagus using an esophageal brush during endoscopy to collect sample tissue. Cytosponge is a non-invasive method and is mesh attached to a string that is swallowed in a capsule that dissolves in the stomach. As the

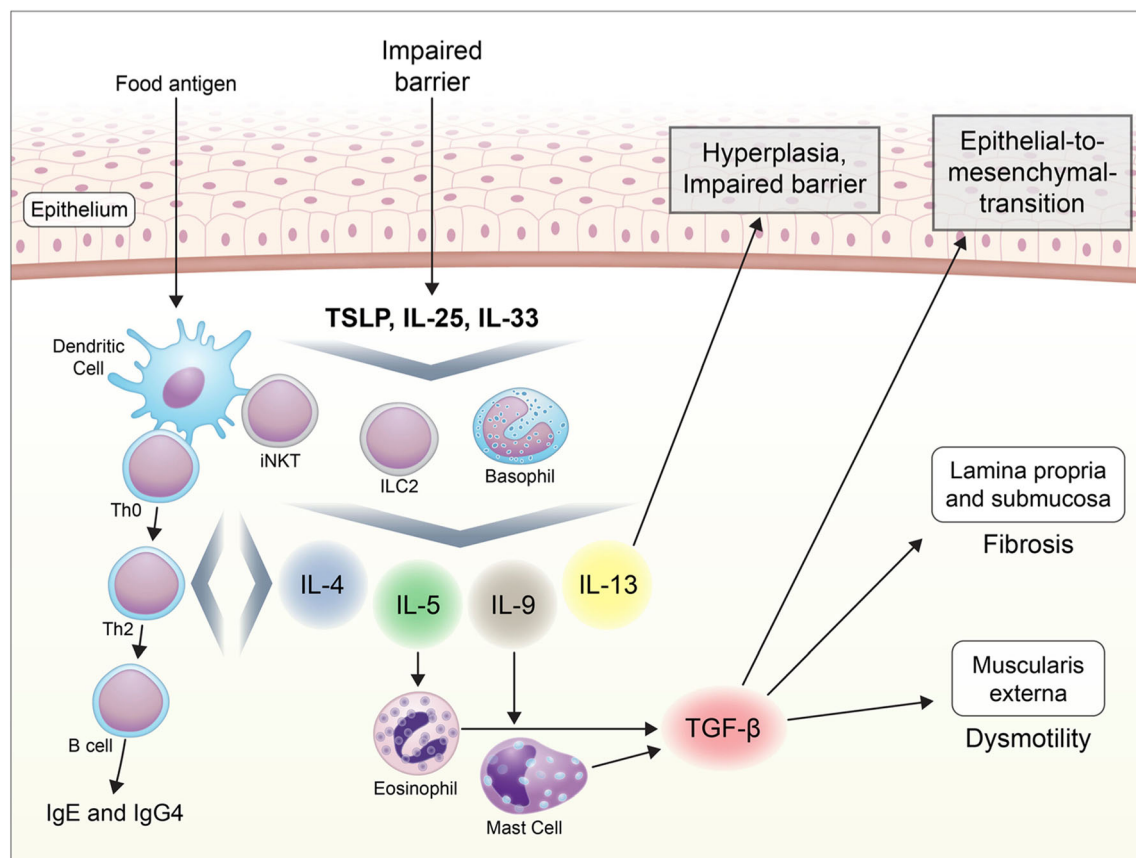


Fig. 1 Mechanistic model of eosinophilic esophagitis (EoE). Impaired barrier function appears to be central to the pathophysiology of EoE. This impaired barrier likely leads to alarmin (TSLP, IL-15, IL-33) release. Alarmins then initiate, via ILC2s and basophils, a coordinated immune response orchestrated by the cytokine milieu (IL-4, IL-5, IL-9, IL-13). In parallel to this process is antigen sensitization, occurring through dendritic cell processing and presentation leading to activated Th2 and iNKT cells which further contribute to the cytokine milieu and skew B cell and antibody development. The downstream effects of cytokines

effect the epithelium, lamina propria, submucosa, and smooth muscle remodeling and dysfunction (hyperplasia, impaired barrier, EMT in the epithelium; fibrosis in the lamina propria and submucosa; and smooth muscle dysmotility within the muscularis interna and externa). EMT, epithelial-to-mesenchymal transition; IgE, immunoglobulin E; IgG4, immunoglobulin G4; IL, interleukin; ILC, innate lymphoid cells; iNKT, invariant natural killer T cells; TGF-β, tissue growth factor beta; Th, T helper cells; TSLP, thymic stromal lymphoprotein

sponge is pulled up through the esophagus, it collects esophageal mucosa for histologic evaluation. Histologic evaluation of the sponge tissue accurately diagnosed EoE 83% of the time in a small cohort of adult patients [239]. Another non-invasive method called the esophageal string test (EST) is an absorbent string with the proximal end taped to the cheek and the distal end is attached to a capsule which is swallowed and dissolved [240]. After the string has been in the esophagus overnight, it is removed and then analyzed. With this method, active EoE patients were able to be distinguished from inactive, GERD, and normal patients. These newer collection methods could be combined with other methods of analysis, for example the EDP test mentioned above which analyzes by microarray 96 EoE-relevant transcripts, to potentially improve diagnosis and clinical monitoring as well as patient-specific therapy.

Therapy with biologic modifiers has also gained traction. Though the findings from the early-stage clinical trials for IL-5 (mepolizumab, reslizumab) and IL-13 (QAX576) mentioned in the review did not meet primary endpoints, they did demonstrate tissue responses which suggest avenues for additional research. Notably, oral desensitization trials for food anaphylaxis have demonstrated that a major side effect of therapy includes the possible development of EoE [70]. In mice, epicutaneous immunotherapy (EPIT) can prevent esophageal eosinophilia and in pigs, EPIT can reverse food induced GI eosinophilia. Currently, there is a phase II trial underway to evaluate the safety and effectiveness of EPIT in EoE [45]. These findings demonstrate an entangled relationship between IgE-mediated food allergy and the chronic, T_H2 cell-associated immunity in EoE as well as the modes of antigenic exposure in relationship to disease induction and resolution.

Conclusion

In summary, the cumulative research to date on the basic pathologic mechanisms in EoE support a number of genetic links connecting EoE risk and the epithelium including filaggrin, eotaxin-3, TSLP, CAPN14, DSG1, and SPINK5. These findings place the esophageal epithelium as a central location for the basic defect in EoE. Studies linking these risk loci to impaired barrier function provide a unifying mechanism that connects genetics with a likely pathophysiologic mechanism. This barrier defect then likely leads to allergic hypersensitivity, likely via secretion of TSLP and IL-33 by the epithelium leading to a basophil-dependent allergic response to food, demonstrated most strikingly by response to food elimination diets. This allergic inflammation may start with sensitization via iNKT cells and includes elevated IgE and IgG4 to foods and infiltration of eosinophils and mast cells and ultimately

leads to increased TGF- β which can effect fibrosis and dysmotility that together contribute to the major symptom of dysphagia (Fig. 1). Both heritability via genetic risk variants (e.g., 5q22 [TSLP] and 2p23 [CAPN14]) and early-life environmental risk factors leading to activation of innate epithelial inflammatory pathways together contribute to the predisposition of an individual to this likely pathologic mechanism.

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