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Molecular, Genetic, and Cellular Bases for Treating Eosinophilic Esophagitis

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Abstract

Eosinophilic esophagitis (EoE) was historically distinguished from gastroesophageal reflux disease on the basis of histology and lack of responsiveness to acid suppressive therapy, but it is now appreciated that esophageal eosinophilia can respond to proton pump inhibitors. Genetic and environmental factors contribute to risk for EoE—particularly early-life events. Disease pathogenesis involves activation of epithelial inflammatory pathways (production of eotaxin-3 [encoded by *CCL26*]), impaired barrier function (mediated by loss of desmoglein-1), increased production and/or activity of transforming growth factor- β , and induction of allergic inflammation by eosinophils and mast cells. Susceptibility has been associated with variants at 5q22 (*TSLP*) and 2p23 (*CAPN14*), indicating roles for allergic sensitization and esophageal specific protease pathways. We propose that EoE is a unique disease characterized by food hypersensitivity, strong heritability influenced by early-life exposures and esophageal specific genetic risk variants, and allergic inflammation and that the disease is remitted by disrupting inflammatory and T-helper type 2 cytokine-mediated responses and through dietary elimination therapy.

Keywords

Allergy; Barrier; Cytokines; Eosinophils; Epithelium; Esophagitis; Diagnostics; Genetics; Therapy

Eosinophilic esophagitis (EoE) is a chronic, immune-mediated disease. Diagnostic criteria include symptoms of esophageal dysfunction; eosinophilic inflammation localized to the esophagus, with at least 15 eosinophils per high-power field (eos/hpf) in an esophageal mucosal biopsy; and exclusion of other recognized causes of esophageal eosinophilia, including proton pump inhibitor (PPI)–responsive esophageal eosinophilia (REE).^{1–3} A PPI trial is required before a diagnosis of EoE can be made; patients with esophageal

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eosinophilia that responds to PPIs do not have EoE. However, a substantial fraction of patients with esophageal eosinophilia (>15 eos/hpf) have PPI-REE. Although EoE and PPI-REE are considered to be separate disorders, they overlap in clinical and histologic features, so they might be different stages of a single disorder. Figure 1A summarizes the clinical presentation of EoE and the endoscopic approach and microscopic requirements.

EoE was rarely recognized prior to scattered reports in the 1990s, when the presence of intraepithelial eosinophils in the esophagus was thought to indicate reflux esophagitis.^{4, 5} In 2004, researchers reported that the disease could be inherited, that symptoms varied with age, and that there were 1/10,000 new cases each year, with a prevalence of ~1/2000 individuals.⁶ The first consensus guidelines for EoE were published in 2007,⁷ with revisions in 2011 and 2013.^{1, 2} The first animal model of the disease was described in 2001,⁸ and the EoE transcriptome was identified and findings from the first controlled clinical trial were reported in 2006.^{9, 10} Results from genome-wide susceptibility studies were reported in 2010 and 2014,^{11, 12} and the effects of biologic therapies, including antibodies against interleukin-5 (IL5) and IL13, were reported in 2006 and 2014, respectively.^{13, 14} The contribution of genetic and environmental factors to risk of EoE was reported from a study of twins published in 2014.¹⁵ We review the molecular, cellular, and immune mechanisms involved in the pathogenesis of EoE, interactions between genetic and environmental factors in disease susceptibility and manifestations, and the implications of these findings for current and developing treatments.

Histopathology

In addition to high numbers of eosinophils, which are detected in all regions of the esophagus (proximal and distal), features of EoE include a thickened mucosa with basal layer hyperplasia and papillary lengthening.¹⁶ Furthermore, esophageal biopsies from patients with EoE may have eosinophil surface layering and eosinophilic microabscesses, and increased levels of dendritic cells and degranulating mast cells (generally found at levels higher than those of patients with gastroesophageal reflux disease [GERD]). The biopsies also have higher numbers of CD8⁺ T cells and lower numbers of FOXP3⁺ T cells, proposed to be T-regulatory cells, than esophageal tissues from patients without EoE. Dilated intercellular spaces, particularly in the epithelium, are readily observed. Extracellular deposition of eosinophil granule proteins, such as eosinophil peroxidase (EPO, also abbreviated as EPX), is present in the esophagus of patients with EoE, and it has been proposed that EPO correlates with clinical features even better than eosinophil levels.¹⁷ A large subset of patients with EoE have fibrostenotic complications associated with radiographic and endoscopic findings, including strictures and mucosal rings. In histologic analyses, these have been associated with collagen deposition—primarily in the lamina propria, where mast cells accumulate¹⁸ processes at rates proportional to the length of time that EoE has been left untreated.¹⁹

Heritability

Familial Clustering, Environmental Factors, and Twin Studies

Elucidating the complex inheritance of EoE involves identifying unique features of disease etiology and pathogenesis. Among 914 pediatric probands (within 2192 first-degree family members), relative risk ratios for EoE in family members range from 10 to 64, depending on the relationship, with higher values for brothers (64 fold), fathers (43 fold), and men (51 fold) compared to sisters, mothers, and women, respectively.¹⁵ Overall, EoE is observed in 1.8%–2.4% of relatives, depending upon their relationship and sex.¹⁵ This is a substantial rate of heritability, especially since relative risk ratios in related diseases such as asthma and food allergy are typically only about 2 fold.¹⁵ Analyses of EoE concordance in twins have provided some important information about the genetic basis of EoE. There is approximately 40% concordance between monozygotic twins and, notably, 30% concordance between dizygotic twins (approximately 10-fold higher than for non-twin siblings).¹⁵ These findings indicate a role for the environment in EoE risk (an estimated 80% contribution)—the high concordance of EoE in dizygotic twins implicates early-life exposures in susceptibility.

Interestingly, antibiotic use in infancy, cesarean delivery, preterm birth, season of birth, birth weight, and breastfeeding have all been identified as factors that affect the development of EoE.^{15, 20} Though we do not know the specific mechanisms by which these and other early-life exposures increase or decrease risk, all affect the microbiome, which influences the developing immune system and development of atopy.²¹ For example, bacterial products stimulate production of the innate cytokines thymic stromal lymphopoietin (TSLP) and IL33, which induce and activate eosinophils to contribute to development of atopy.²²

Association with Mendelian Disorders

A small fraction of patients develop EoE in association with a genetic syndrome. The most studied is the association of EoE with inherited connective tissue disorders (CTD) that involve hypermobility syndromes (e.g., Loery-Dietz syndrome [LDS], Marfan syndrome Type II [MF] and Ehlers-Danlos syndrome). The co-morbidity of EoE with these disorders is now called EoE-CTD.^{23, 24} Although only 1% of patients with EoE have EoE-CTD, EoE increases the risk for CTD approximately 8 fold. Patients with inherited connective tissue hypermobility syndromes are at increased risk for cardiovascular complications, so patients with EoE should be screened for hypermobility and receive appropriate care if they are found to also have a CTD.

EoE and CTD share excessive production of transforming growth factor- β (TGF β) and TGF β signaling. LDS is caused by gain-of-function mutations in the TGF β receptors, whereas MF is caused by mutations in connective tissue proteins that bind to TGF β , such as fibrillin 1 (Type I). Ehlers-Danlos syndrome is caused by mutations in collagens.^{23, 24} Interestingly, patients with EoE have increased levels of collagen 8A2 in the esophagus, whereas patients with EoE-CTD have reduced expression of collagen 8A2 mRNA in the esophagus.²³ Preliminary analyses, focused primarily on patients with LDS, indicate that these changes promote development of T-helper type 2 (Th2) cells from naive lymphocytes in a cell-autonomous manner.²⁴ Th2 cells produce large amounts of specific cytokines (such

as IL4, IL5, and IL13) that are involved in development of allergic disorders (such as allergic rhinitis, atopic dermatitis, and asthma).

The finding that LDS is caused by mutation in a single gene shows that activation of TGF β signaling is sufficient to cause allergic phenotypes. It also demonstrates the role for TGF β in directing immune responses to antigens in a manner that promotes development of EoE.²⁴ Patients with non-syndromic EoE produce increased amounts of eosinophil- and mast cell-derived TGF β —particularly in the esophageal muscularis mucosa. This process has been proposed to contribute to smooth muscle hypercontractility.¹⁸

In addition to inherited CTDs, EoE has been associated with other autosomal dominant Mendelian diseases, such as PTEN hamartoma tumor syndromes (PHTS). Although patients with PHTS have a more than 200-fold increase in risk for EoE and other eosinophil-associated gastrointestinal disorders, there is only a 4% to 9% prevalence of eosinophil-associated gastrointestinal disorders among pediatric patients with PHTS and a *PTEN* mutation.²⁵ The mechanism connecting PHTS and EoE has not been agreed upon. However, transduction of PTEN into eosinophils reduces their survival and chemotaxis; furthermore, PTEN and TGF β have been shown to cooperate to induce colon cancer.²⁵

Other EoE-associated, inherited diseases include severe dermatitis, multiple allergies, and metabolic wasting (SAM) syndrome. SAM syndrome is caused by homozygous mutations in *DSG1*, which encodes desmoglein 1. DSG1 is a major constituent of desmosomes, which connect the cell surface to the keratin cytoskeleton to help maintain epidermal integrity and barrier function.²⁶ SAM syndrome is a rare disorder only reported in 3 consanguineous families. However, studies of these families have provided information about EoE pathoetiology, since acquired loss of DSG1 and impaired barrier function in the esophagus are general features of non-syndromic EoE.^{26, 27} Furthermore, EoE has been reported in 2 other atopy-associated Mendelian disorders: autosomal dominant hyper-IgE syndrome, caused by loss-of-function mutations in *STAT3*²⁸, and a syndrome characterized by increased levels of mast cell tryptase in the blood and associated with CTD²⁹. Figure 2 contains a Table that summarizes the genes and Mendelian disorders associated with EoE.

Genetic Variants

There have been 3 basic approaches to identify the genetic factors associated with EoE: association of EoE with Mendelian disorders, candidate-gene identification, and genome-wide association studies (GWASs). Using the candidate-gene approach, researchers identified a common single-nucleotide polymorphism (SNP) in the 3' untranslated region of *CCL26* (encodes eotaxin-3)—the most highly overexpressed esophageal transcript in the EoE transcriptome.⁹ Expression of *CCL26* is induced by IL13 in esophageal epithelial cells, and *CCL26* mRNA and protein are overexpressed in significantly more patients with EoE than controls.⁹

EoE risk has also been associated with coding variants (2282del4) in *FLG*, which encodes the epidermal barrier protein filaggrin. Expression of *FLG* is negatively regulated by IL13 and decreased in the esophageal mucosa of patients with EoE.³⁰ In a small cohort of patients with EoE who had received treatment with steroids, a variant in the promoter of the *TGFB*

gene was associated with steroid unresponsiveness. The variant also correlated with increased numbers of TGF β -positive cells in the esophagus.³¹

To identify disease risk variants in a more unbiased fashion, researchers performed a GWAS, genotyping 351 patients with EoE and 3104 healthy controls and evaluating 550,000 common variants. On chromosome 5q22, a single locus spanning the *TSLP* and *WDR36* repeat domain 36 (*WDR36*) genes showed a significant association with EoE.¹¹ *TSLP* is a cytokine that has been shown to be produced by keratinocytes and to promote the development of Th2 cells. *TSLP* mRNA was increased in esophageal tissues from patients with EoE, compared with controls. The most strongly associated variant was found to increase *TSLP* expression.¹¹ *TSLP* risk genotypes correlated with increased numbers of basophils, which promote EoE-like disease in mice, and of granulocyte-monocyte progenitor-like cells in the esophagus.³²

A separate candidate-gene approach also associated variants in *TSLP* with EoE risk.³³ In an analysis of more than 700 variants in epithelial-derived genes linked to atopy, those in *TSLP* were most strongly associated with EoE.³³ Moreover, a coding variant in the gene encoding cytokine receptor–like factor 2 (*CRLF2*), which encodes for the receptor for *TSLP*, was associated with EoE risk in only men.³³ *TSLP* activates basophils, which are increased in the esophagus of patients with EoE and required in a mouse model of EoE.³² These findings indicate that the *TSLP* pathway is contributory in patients with EoE. However, many studies have associated the locus that contains *TSLP* and *WDR36*, as well as *TSLP* protein, with other atopic diseases and responses.³⁴

A deeper GWAS was performed, focusing on approximately 2.5 million genetic variants in 736 individuals with EoE and 9246 controls (without EoE).¹² In addition to identifying the 5q22 locus (meta-analysis $P=1.9\times10^{-16}$), this analysis made 4 other genome-wide significant associations; the strongest was located at 2p23 (encoding *CAPN14*, $P=2.5\times10^{-10}$).¹² Remarkably, *CAPN14* was specifically expressed in the esophagus, in comparison to 130 other tissues.¹² This finding was recently independently replicated.³⁵ It was also upregulated with disease activity and in patients with the EoE-associated genetic haplotypes; *CAPN14* mRNA levels and calpain protein activity were also shown to be increased in esophageal epithelial cells incubated with IL13.¹²

CAPN14 is located in an epigenetic hotspot modified by IL13. IL13 induces histone 3 lysine 27 (H3K27) acetylation in the *CAPN14* promoter, and the disease-associated risk haplotype promotes binding of nuclear proteins expressed by esophageal epithelial cells.¹² *CAPN14* belongs to the classical calpain sub-family of proteolytic systems (in addition to the proteasomal, lysosomal, and caspase systems). Classical calpains are calcium-dependent proteases. Their substrates include structural proteins, signaling molecules, transcription factors, cell adhesion molecules, and inflammatory mediators of allergic responses, such as STAT6 and IL33. IL33 has been associated with EoE.¹²

Collectively, these findings support a 2-hit mechanism of EoE susceptibility. The first hit occurs at 5q22 (leading to *TSLP*-induced allergic sensitization) and the second occurs at 2p23 (leading to activation of esophageal-specific protease *CAPN14*). Consistent with this

concept, there is increased esophageal expression of the genes neighboring the top 208 EoE-associated sequence variants.¹² Therefore, the tissue specificity of EoE appears to be manifested, at least partially, by esophageal-specific pathways. See Figure 2 for a summary of the genetic variants associated with EoE.¹²

Pathogenesis

Allergic Sensitization

EoE pathogenesis is highly linked with atopy on the basis of disease co-occurrence, the success of allergen avoidance (primarily dietary control), animal models, and genetic linkage. Most patients with EoE have evidence of food and aeroallergen hypersensitivity¹ and a concurrent history of respiratory allergy.^{36, 37} Food anaphylaxis occurs in about 15% of patients with EoE.¹ Unlike patients with food anaphylaxis, most patients with EoE are sensitive to a variety of foods, as assessed by skin-prick tests, serum levels of food-specific immunoglobulin (Ig)Es, and dietary add-back studies.³⁶ The role of food antigen sensitization has been demonstrated by the success of reducing specific food exposures (selected by skin and patch tests), empiric avoidance of the 6 most common allergenic food types, and use of amino acid-based formula, all of which are capable of inducing disease remission.^{37, 38} EoE flares upon reintroduction of the eliciting foods, which are most commonly milk-, wheat-, and/or egg-containing foods³⁹. Food-specific IgE is clearly present but not likely to be required for disease induction and/or maintenance, because IgE-deficient, B cell-deficient mice develop experimental EoE;^{32, 40} Administration of humanized anti-IgE to patients with EoE is ineffective.⁴¹ Interestingly, food-specific IgG4 is locally produced in the esophagus and might block IgE, yet propagate EoE in a food-specific manner.⁴¹ It has been proposed that birch pollen sensitization may lead to cross reactivity to a variety of foods and be partially responsible for the prevalence of food-specific IgE in patients with EoE.⁴²

Experimental EoE can be induced in mice by allergen exposure through the skin,^{32, 43} respiratory,⁸ or gastrointestinal tract,^{44, 45} as well as by overexpression of select Th2 cytokines (e.g., IL5 and IL13,⁴⁶⁻⁴⁸ and IL15⁴⁹). For example, repeated intranasal exposure to aeroallergens (e.g., *Aspergillus fumigatus*) or food allergens⁵⁰ induces simultaneous eosinophilic airway and esophageal inflammation (without inducing lower gastrointestinal eosinophilia).⁸ Furthermore, IL13 is overexpressed in the esophagus of patients with EoE and selectively induces expression of *CCL26* in esophageal epithelial cells.⁵¹ Transgenic overexpression of IL13 in mice induces an EoE-like disease with molecular features of human EoE.⁴⁸ Intra-tracheal delivery of human or mouse IL13 induces dose-dependent experimental EoE⁴⁷; this process can be blocked with an antibody against human IL13.⁵² Consistent with this, STAT6-deficient mice are partially protected from allergen- and IL13-induced experimental EoE.^{43, 47} Further, IL13-deficient mice have impaired allergen-induced EoE in some models.^{43, 53}

Epicutaneous allergen sensitization is a strong primer for respiratory allergen-induced experimental EoE.^{32, 43} This finding may be particularly important for understanding EoE because a large fraction of patients with EoE have preceding allergic skin disease (atopic dermatitis). Also, allergy-inducing innate cytokines such as TSLP are derived from the

skin,⁵⁴ and genetic studies have linked EoE susceptibility to *TSLP*.^{11, 12} These findings indicate that sensitization could occur via cutaneous antigen exposure and that there is a connection between eosinophil-mediated inflammation of the respiratory tract and esophagus in response to not only external allergic triggers, but also intrinsic Th2 cytokines. Notably, patients with allergic rhinitis have seasonal increases in esophageal eosinophils,⁵⁵ patients with EoE have seasonal variations in their symptoms,⁵⁶ and birth season has been associated with EoE susceptibility. These observations support the roles of environmental factors and allergen-induced, eosinophil-associated responses in the esophagus. Importantly, the relationship between EoE and allergic sensitization is supported by the association of EoE susceptibility with 9 of 22 allergic sensitization loci.¹² Although only some individuals without atopy develop EoE, the mechanisms of allergic inflammation are likely to still operate, regardless of the initial triggering stimuli. Figure 3 summarizes key steps involved in eosinophil development at baseline and during the development of EoE.

The EoE Transcriptome

There is marked overexpression of approximately 1% of the human genome in the esophagus of patients with active EoE compared to individuals without any esophageal pathology, individuals with chronic esophagitis without eosinophilia, or individuals with symptomatic GERD (confirmed by multi-channel intraluminal impedance testing).^{9, 57, 58} This EoE transcriptome is highly conserved among patients regardless of sex, age, history of atopy, or genetic variant, and *CCL26* is consistently the most highly induced gene.^{9, 59, 60} Levels of *CCL26* mRNA in paraffin-embedded esophageal tissues have been proposed to distinguish EoE from GERD.⁶¹ Figure 1B summarizes the EoE transcriptome and its potential usage for diagnosing and characterizing EoE.

Comparing patients with EoE who did or did not have co-occurring allergies revealed that the gene transcript signature is markedly conserved across these 2 major EoE phenotypes.⁹ This demonstrates that the effector phase of the disease is conserved between individuals with EoE, irrespective of the inflammatory trigger. Interestingly, a marked portion of the EoE transcriptome is directly induced by exposure of primary esophageal epithelial cells to IL13 (including *CCL26*, which is the gene most strongly induced by IL13)⁵¹ or overexpression of transgenic IL13 in mice.⁴⁸ IL13-regulated genes in the EoE transcriptome include those encoding periostin (markedly induced by IL13 and overexpressed in EoE)⁶² and FLG (markedly downregulated by IL13 and decreased in EoE).⁹ Periostin is a fascilin domain-containing extracellular matrix molecule that regulates eosinophil adhesion and promotes eotaxin-induced eosinophil recruitment.⁶² Its levels correlate with esophageal and pulmonary eosinophilia,⁶³ and it is a marker used to predict response to anti-IL13 drugs in patients with asthma.⁶⁴

The EoE transcriptome also contains a series of non-coding RNAs including microRNAs (miRs) and long non-coding RNAs, which regulate transcription and translation.^{58, 65} Levels of the BRAF-activated non-protein coding RNA (*BANCR*) are increased in esophageal tissues of patients with EoE and in primary esophageal epithelial cells exposed to IL13. Reducing levels of *BANCR* with small hairpin RNAs alters the expression of IL13-induced genes that promote inflammation.⁵⁸ hsa-miR-21 is the most strongly induced miR in human

EoE samples.⁶⁵ It stimulates Th2-associated hypersensitivity by down-regulating *IL12p35* mRNA in dendritic cells⁶⁶ and promotes induction of experimental EoE in mice.⁶⁷ Levels of hsa-miR-375 are reduced in human EoE tissues; hsa-miR-375 is expressed by esophageal epithelial cells and involved in an IL13-induced auto-inflammation.⁶⁸

Impaired Barrier Function

Biopsy specimens from patients with EoE show dilated intercellular spaces and impaired barrier function (see Figure 4 for a conceptual and microscopic view of impaired barrier function); these observations are supported by ex vivo measurements of permeability and resistance.²⁷ The epidermal differentiation complex locus, at 1q21, has the highest density of genes with altered expression in EoE tissues, as measured by transcriptome analysis.³⁰ Interestingly, most of these genes are downregulated and encode factors that regulate formation of structured epithelium and barrier function, such as involucrin, small proline-rich protein, and FLG. FLG is a structural barrier protein in the skin; its loss of function is associated with increased permeability and susceptibility to atopic dermatitis in humans⁶⁹ and atopic sensitization in mice.⁷⁰ The impaired barrier function observed in esophageal tissues of patients with EoE can be explained, at least in part, by loss of expression of the desmosomal cadherin DSG1. Silencing of *DSG1* weakens esophageal epithelial integrity, induces separation of epithelial cells, and impairs barrier function independent of changes in levels of FLG and the other major epithelial cadherin, DSG3.²⁷ Notably, *DSG1* deficiency induces transcriptional changes in esophageal epithelial cells that partially overlap with the transcriptome of inflamed esophageal mucosa; periostin is the most highly induced overlapping gene.²⁷ Considering the ability of IL13 to strongly downregulate *DSG1*, and that EoE is associated with homozygous disruption of *DSG1*²⁶, we propose that loss of DSG1 propagates allergic esophageal inflammation. Loss of DSG1 provides a mechanism by which food antigen-induced Th2 cell-mediated adaptive immunity leads to impaired barrier function, propagation of local inflammatory processes (including sensitivity to acid), and increased antigen uptake in the esophagus (Figure 4).

Immune Cells

Manipulation of eosinophil numbers in mice by overexpression of IL5, disruption of the gene encoding the chemokine receptor CCR3, or lineage ablation leads to reduced esophageal remodeling, including epithelial cell hyperplasia and collagen deposition.⁷¹ Intestinal eosinophils regulate production of secretory IgA (sIgA), and eosinophil-deficient mice have markedly reduced levels of sIgA^{72, 73} (for review of eosinophils in inflammation see^{74 and 75}). Figure 3 summarizes eosinophil development and regulation.

Eosinophil granules contain a crystalloid core composed of major basic proteins (MBPs) 1 and 2, and a matrix composed of cytotoxic proteins including eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and EPO.⁷⁴ Additionally, ECP and EDN belong to the ribonuclease A superfamily and have anti-viral and ribonuclease activities. EDN is an endogenous ligand of Toll-like receptor 2 (TLR2); EDN activates myeloid dendritic cells by activating a signaling pathway that includes the TLR2–myeloid differentiation factor 88.⁷⁶ Importantly, EDN provides dendritic cells with the ability to promote Th2-cell responses. EDN therefore provides a potential endogenous signal that

primes the adaptive immune system toward an antigen-specific Th2-cell-mediated immune response. ECP can insert voltage-insensitive, ion-nonselective toxic pores into the membranes of target cells, which facilitates the entry of other toxic molecules. MBP directly increases smooth muscle reactivity by causing dysfunction of vagal muscarinic M2 receptors and also induces degranulation of mast cells and basophils.

Eosinophils are activated via receptors for cytokines, Igs, and complement, leading to production of inflammatory cytokines such as IL1, IL3, IL4, IL5, IL13, GM-CSF, TGF α , TGF β , TNF, RANTES, MIP1 α , and eotaxin-1. Eosinophils therefore have the potential to modulate multiple aspects of the immune response. Notably, eosinophil-derived TGF β is linked with epithelial growth, fibrosis, and tissue remodeling—processes that are associated with the fibrostenotic complications of EoE.¹ Eosinophils are a source of TGF β in EoE tissues, and TGF β levels correlate with esophageal fibrosis.⁷⁷ Interestingly, eosinophils rapidly release mitochondrial DNA following exposure to bacteria, C5a, or CCR3 ligands.⁷⁸ Eosinophil DNA traps contain the granule proteins ECP and MBP and display antimicrobial activity.⁷⁸ Further, eosinophils cause damage via toxic hydrogen peroxide and halide acids, generated by EPO, and by superoxide, generated by the respiratory burst oxidase enzyme pathway. Eosinophils also generate large amounts of cysteinyl leukotriene C₄, which increases vascular permeability and mucus secretion and is a potent stimulator of smooth muscle contraction, which might contribute to dysmotility in EoE tissues. However, agents designed to inhibit cysteinyl leukotriene C₄ have not shown efficacy in patients.^{7, 74}

Immune cells other than eosinophils also appear to be involved in EoE. Analyses of lymphocyte-deficient mice have indicated a role for T cells in EoE pathogenesis; B-cell-deficient, but not T-cell-deficient, mice develop antigen-induced EoE.⁷⁹ Notably, CD8⁺ and CD4⁺ cells are not required for induction of allergen-induced experimental EoE.⁷⁹ Invariant natural killer T cells may be involved in EoE pathogenesis—their levels are increased in the esophageal tissues of patients with EoE, and milk-derived sphingolipids cause them to produce Th2 cytokines.⁸⁰ Increased levels of circulating CD4⁺FOXP3⁺ T cells produce high levels of IL13 and contribute to the increased numbers of Th2 cells observed in patients with LDS.²⁴ However, it is not yet clear whether these cells are involved in non-syndromic EoE.

Numbers of mast cells and mast-cell degranulation are markedly increased in the epithelium and smooth muscles of patients with EoE. Mast cells are leading sources of TGF β , which has been proposed to stimulate smooth muscle contractility and thereby contribute to esophageal dysfunction.^{81, 82} Mast cell-deficient mice have impaired development of muscle cell hyperplasia and hypertrophy.⁴⁰ Additionally, TSLP-regulated basophils contributed to experimental EoE in mice. These findings might apply to human EoE, because a gain-of-function polymorphism in *TSLP* is associated with increased basophil responses in patients with EoE.³²

Dietary, Anti-inflammatory, and Biologic Therapies

One key feature of EoE is its reversibility with appropriate treatment and its nearly universal recrudescence after withdrawal of therapy. Even the fibrostenotic complications can reverse with treatment in some patients. However, there are a few caveats that are worth

emphasizing. Although dietary therapy can be highly effective and is considered the most upstream therapy, it typically requires removal of a number of food groups or the strict usage of amino acid–based formulas (e.g., Neocate or Elecare). Dietary elimination therapy is thought to target the adaptive immune system, presumably by suppressing antigen-driven T-cell responses, although this has not been empirically demonstrated.

Anti-inflammatory agents have substantial rates of failure. For example, 30% of patients do not respond to topical delivery of high-dose fluticasone.⁸³ Swallowed glucocorticoids exert local anti-inflammatory effects in the esophagus, indicated by esophageal induction of steroid-responsive genes (e.g., FK506-binding protein)⁸⁴ and miRs (e.g., hsa-miR-675),⁶⁵ and partially reverse the EoE transcriptome.^{83, 84} However, despite complete histologic resolution, transcripts of many genes that regulate epithelial differentiation remain abnormally expressed,⁵¹ indicating their roles in disease predisposition and/or relapse.

There is often a discrepancy between improvement of clinical symptoms and tissue histology.¹ A small subset of patients has continued symptoms despite complete resolution of pathologic features in tissue. Clinical trials have been limited because we do not understand the causes of these continued symptoms and also because we lack of validated clinical outcome metrics, which have only been recently generated.⁸⁵

Identification of the EoE transcriptome and elucidation of EoE immunopathogenesis have provided opportunities to develop mechanism-based therapeutics. Humanized antibodies against IL5 (mepolizumab, reslizumab) and IL13 (QAX576) reduced numbers of esophageal eosinophils and mast cells, albeit modestly compared with dietary and glucocorticoid therapy. However, these humanized antibodies did not yield robust clinical effects, perhaps because of the lack of validated, disease-specific clinical outcome metrics.^{14, 86, 87} It is encouraging that inhibiting IL13 improves indices of barrier function, tissue remodeling, and inflammation on the basis of changes in the EoE transcriptome (Figure 5).¹⁴ Findings from these early-stage clinical trials have prompted further studies of these and related biological agents.

An orally active small-molecule inhibitor of the eosinophil and Th2-cell chemoattractant receptor prostaglandin D2 receptor 2 (also known as CRTH2) modestly reduces esophageal eosinophilia and warrants further study.⁸⁸ Additional agents in development for treatment of EoE include inhibitors of TSLP (e.g., AMG 157), CCR3, eotaxin (e.g., bertilimumab), IL4R α (the common receptor for IL4 and IL13; e.g., duplibumab). Other agents include eosinophil-depleting antibodies, such as those against IL5R α (e.g., benralizumab) or sialic acid-binding Ig-like lectin 8.⁸⁹ Losartan has been shown to reduce levels of TGF β and connective tissue complications in patients with MF, so it might also be effective for patients with EoE, with or without CTD.⁹⁰

Molecular Diagnostics and Personalized Medicine

Molecular analysis of esophageal biopsies provides not only an opportunity to probe EoE pathogenesis, but also the ability to extend diagnosis and monitoring beyond visual inspection of endoscopic and microscopic findings (Figure 1B). Transcriptome analysis of a single biopsy can identify patients with EoE with 96% sensitivity and 98% specificity vs the

standard of histologic analysis. These values might not be 100% because of limitations of histology, rather than transcriptome analysis.⁵⁷ Transcriptome assays might also be used to predict clinical outcomes, such as responsiveness to glucocorticoids.⁸³ A set of 15 genes has been reported to predict which patients will and will not respond to glucocorticoids.⁸³

Levels of specific biomarkers may predict responsiveness to drugs that target specific pathways. For examples, levels of periostin expression might identify patients most likely to respond to anti-IL13. Residual gene expression after initial treatment provides an opportunity for targeted add-on therapy, directed at correcting the remaining activated inflammatory pathways. Molecular profiling of esophageal tissue for genes that distinguish EoE from PPI-REE provides an opportunity to definitively differentiate esophageal eosinophilia subtypes and thereby promptly direct therapy accordingly (Figure 1C).⁹¹ For example, esophageal levels of the potassium inwardly-rectifying channel, subfamily J, member 2 (encoded by *KCNJ11*) have been proposed to distinguish patients with EoE from those with PPI-REE before PPI therapy.⁹¹

Patient care could be transformed by non-invasive biomarkers that can provide definitive diagnoses and/or be used to monitor disease status. Though circulating levels of miRs (hsa-miR-146a, hsa-miR-146b and hsa-miR-223) can distinguish patients with EoE from controls, their predictive value is not yet of clinical relevance.⁶⁵ Semi-invasive tests that measure eosinophils or eosinophil-derived products from a retrieved swallowed string or cytosponge are promising and in development.^{92, 93}

Conclusion and Future Directions

Immunophenotype and genome-wide expression analyses of esophageal biopsies, animal models, translational medicine systems focused on ex vivo culture of cells procured during endoscopic biopsy, GWASs, and now proof-of-concept clinical intervention trials have elucidated key features of EoE (Figure 4). On the basis of these collective approaches, we conclude that EoE is characterized by immune hypersensitivity to food, heritability composed of genetic susceptibility elements interacting with early-life exposures, activation of innate epithelial inflammatory pathways (resulting in eotaxin-3 production), impaired barrier function (mediated by loss of DSG1), augmented TGF β production and signaling, and allergic inflammation mediated by eosinophils and mast cells. We propose a 2-hit mechanism involving allergic sensitization and esophageal specific pathways, in combination with genetic risk factors at 5q22 (*TSLP*) and 2p23 (*CAPN14*), respectively. Although the distinction between EoE and PPI-REE has been emphasized, these disorders have overlapping molecular patterns of pathogenesis. We might therefore need to reconsider the classification of esophageal eosinophilia into allergic EoE or PPI-REE, as these might be different stages of the same disease.⁹¹ It is notable that PPIs reduce IL13-induced signaling, STAT6 activation, and eotaxin-3 production in esophageal epithelial cells. This could be the mechanism by which PPIs block components of allergic inflammation.^{94, 95} Future studies are needed to identify the genetic and environmental factors that contribute to PPI-REE, as well as its mechanisms of induction and progression—especially in comparison to EoE.

Notably, EoE susceptibility genes do not overlap with those of other gastrointestinal diseases but instead cluster with atopic diseases. Therapies that target allergic inflammation, already in development for other allergic diseases (e.g., anti-IL5 and anti-IL13) are therefore being actively developed for EoE. A better understanding of EoE-specific pathways (e.g., calpain proteases) and how early-life exposures increase susceptibility to EoE could transform treatment. Therapeutic agents such as drugs and microorganisms are easy to deliver to the esophagus, so it might be possible to prevent EoE in individuals with high risk for this disease.

Molecular analyses of esophageal tissues could improve diagnosis and clinical monitoring, patient-specific therapy, and predictive and personalized medicine approaches to EoE. For example, a molecular diagnostic test for EoE is now available in the clinic (Figure 1B). Notably, development of EoE is now recognized as an adverse event of oral desensitization treatment for food anaphylaxis, indicating the intimate relationship between IgE-mediated food allergy and the chronic, Th2 cell-associated immunity in EoE.⁹⁶ A better understanding of the balance between IgE-mediated anaphylaxis and EoE pathogenesis may lead to tolerance induction protocols for treatment and to an eventual cure of EoE.

In conclusion, EoE is a unique disease characterized by hypersensitivity to food, strong heritability influenced by early-life exposures and genetic variants, and allergic inflammation that is reversible by anti-inflammatory, anti-Th2 cytokine, and/or dietary elimination therapy. Improvement of barrier function and modification of environmental exposures might also benefit patients with this disease.

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Figure 1. Diagnostic Evaluation of EoE, Including Next-generation Molecular Analysis

A, EoE should be considered for patients with characteristic symptoms of upper gastrointestinal diseases (note the age dependence) that are refractory to PPI treatment, and for patients with typical features of EoE (male, atopic, family history of esophageal dysfunction, and family history of EoE; note the high relative risk Ratio¹⁵). Patients with these features should undergo esophagogastroduodenoscopy; a diagnosis of EoE can be made for those with at least 15 eosinophils/hpf in esophageal biopsies. B, diagnosis and assessment of disease activity is facilitated by molecular analysis of esophageal tissue for the expression of 94 EoE-related transcripts (via the EoE Diagnostic Panel [EDP]). The EDP involves panel expression acquisition, signature analysis, and quantitative scoring for EoE-related processes (a Molecular Diagnostic Report). Recent attention has focused on analysis of PPI-responsive esophageal eosinophilia (PPI-REE), which has a transcript profile that overlaps with that of EoE but normalizes following PPI treatment. C, representative heat diagrams of the esophageal transcript profiles in EoE, PPI-REE before and after PPI therapy, as well as control normal (NL) and GERD patients. Figure is adapted from a recent publication.⁵⁷

Figure 2. Genetic Associations in EoE
A, a Manhattan Plot with data from 736 subjects with EoE and 9246 controls (1,468,075 genetic variants) with minor allele frequencies greater than 1% in the subjects with EoE. The $-\log$ of the probability is shown as a function of the genomic position of the autosomes. Genome-wide significance (red dotted line, $p = 5 \times 10^{-8}$) and suggestive significance (solid blue line, $P = 10^{-7}$) are shown. The figure is adopted from a recent publication.¹² B, a summary of the specific genes implicated in EoE susceptibility. The putative genes, the approach by which they were discovered, the type of identified genetic modifications, and plausible genetic mechanisms are listed. Mendelian inherited diseases associated with EoE are indicated.

Figure 3. Eosinophil Development and Tissue Localization

Hematopoietic progenitor cells expand in response to FLT3 ligand (FLT3L) and stem cell factor (SCF); the eosinophil lineage is regulated by IL5 and the GATA1 transcription factor. IL5 subsequently promotes eosinophil migration from the bone marrow into the blood and maintains circulating levels of eosinophils. Eosinophil adhesion molecules (β7 and VLA4) interact with their respective endothelium receptors MAdCAM1 and VCAM1. Under baseline conditions, eosinophils migrate into all segments of the gastrointestinal tract (except the esophagus) in response to tissue eotaxin1. Intestinal eosinophils regulate sIgA production and intestinal microbiota composition. Innate lymphoid type 2 cells (ILC2) produce high levels of IL5 and IL13 in response to IL33 (via its receptor ST2). IL13 subsequently binds to its receptor IL13Rα1 on epithelial cells, which then produce eotaxin. In the esophagus, eosinophils accumulate in response to local production of eotaxin-3 and promote esophageal dysfunction and tissue remodeling.

Figure 4. Pathogenesis and Mechanism-based Therapy for EoE

A model of eosinophil development in the bone marrow, transit into the peripheral blood, trafficking to the esophagus, and the subsequent tissue changes, including impaired barrier function. In brief, allergens (aeroallergens and food allergens) induce Th2-associated inflammation, with a primary IL13-induced tissue response that involves modification of an extensive set of esophageal transcripts, including eotaxin3, which recruits eosinophils. Impaired barrier function is mediated by a pathogenic cycle induced by IL13 that results in decreased expression of desmoglein-1 (DSG1) (also associated with SAM) and subsequent induction of periostin. These changes promote tissue remodeling and eosinophil adhesion, as well as production of TSLP, which propagates the allergic inflammatory cascade. Local IgE and IgG4 are produced and mast cells are sensitized. Certain genetic elements increase susceptibility to EoE, including 2p23 (CAPN14), 5q22 (TSLP), and genes encoding the TSLP receptor and filaggrin. Their expression is modulated by miR-21 and miR-223. Therapies for EoE include PPI agents, topical glucocorticoids, elimination diets, and anti-cytokine humanized antibodies (e.g. anti-IL5, anti-IL13, anti-IL4R α).

Figure 5. Molecular Evidence for the Efficacy of Anti-IL13 and Identification of IL13-associated Molecular Nodes

Transcriptome profile of esophageal biopsies at baseline and following 3 months of treatment with humanized anti-IL13 or placebo. A shows the heat map of the top differentially modified genes. B shows the quantitative levels of representative genes. Note the increased expression of genes involved in eosinophil chemoattraction (*CCL26*), tissue remodeling (*POSTN*), mast cell activity (*CPA3*), and barrier dysfunction (*DSG1*). C, the comprehensive pathways, focused on protein-protein interactions, shows the in vivo IL13 networks. Several communication centers (shown as blue nodes) contribute to most of the organization and regulating functions. The different levels of red indicate the statistical strength of the interactions. Figures are adapted from a recent publication.¹⁴