Taylor & Francis
Taylor & Francis Group

Expert Review of Gastroenterology & Hepatology

ISSN: 1747-4124 (Print) 1747-4132 (Online) Journal homepage: https://www.tandfonline.com/loi/ierh20

Molecular basis and cellular mechanisms of eosinophilic esophagitis for the clinical practice

Ángel Arias & Alfredo J. Lucendo

To cite this article: Ángel Arias & Alfredo J. Lucendo (2019) Molecular basis and cellular mechanisms of eosinophilic esophagitis for the clinical practice, Expert Review of Gastroenterology & Hepatology, 13:2, 99-117, DOI: 10.1080/17474124.2019.1546120

To link to this article: https://doi.org/10.1080/17474124.2019.1546120

Accepted author version posted online: 08 Nov 2018.
Published online: 15 Nov 2018.

EXPERT REVIEW OF GASTROENTEROLOGY & HEPATOLOGY
2019, VOL. 13, NO. 2, 99–117
https://doi.org/10.1080/17474124.2019.1546120

Taylor & Francis
Taylor & Francis Group

REVIEW

Molecular basis and cellular mechanisms of eosinophilic esophagitis for the clinical practice

Ángel Arias [a,b] and Alfredo J. Lucendo [b,c]

[a]Research Unit, Hospital General La Mancha Centro, Alcázar de San Juan, Spain; [b]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain; [c]Department of Gastroenterology, Hospital General de Tomelloso, Ciudad Real, Spain

ABSTRACT

Introduction: Eosinophilic esophagitis (EoE) is a chronic, allergen-driven inflammatory esophageal disease characterized by predominantly eosinophilic inflammation leading to esophageal dysfunction. Recent efforts to understand EoE have increased our knowledge of the disease.

Areas covered: Multiple cells, molecules, and genes interplay with early life environmental factors in the pathophysiology of EoE to converge in the esophageal epithelium at the center of disease pathogenesis. Epithelial cells constitute a mayor cytokine source for TSLP and Calpain-14; an impaired epithelial barrier function allowing penetration of food and microbiota-derived antigens is involved in triggering and maintaining inflammation. Eosinophil and mast cell-derived products, including TGFβ, together with IL-1β and TNFα, promote epithelial mesenchymal transition in EoE, contributing to tissue remodeling by synthetizing and depositing extracellular matrix in subepithelial layers. This article aims to provide a state-of-the-art update on the pathophysiology of EoE applied to clinical practice, and latest research and developments with potential interest to improve the diagnosis and treatment of patients with EoE are revised.

Expert commentary: Preliminary approaches have provided promising results toward incorporating minimally invasive methods for patient diagnosis and monitoring in clinical practice. Early diagnosis and optimized therapies will allow for personalized medicine in EoE.

ARTICLE HISTORY
Received 6 September 2018
Accepted 6 November 2018

KEYWORDS
Diagnosis; eotaxin-3; eosinophilic esophagitis; eosinophils; food allergy; interleukin-13; inflammation; immunopathogenesis; microbiota; mast cells; remodeling; therapy; thymic stromal lymphopoietin

1. Introduction

Eosinophilic esophagitis (EoE) is a chronic immune-mediated inflammatory disorder of the esophagus, defined symptomatically by esophageal dysfunction and histologically by eosinophil predominant inflammation restricted to this organ [1]. Initially characterized as a distinct clinicopathological disorder in the early 1990s [2,3], the incidence and prevalence of EoE have rapidly increased in children and adults in the last two decades to constitute a common cause of esophageal symptoms in clinical practice [4]. EoE is the most prevalent cause of chronic or recurrent esophageal symptoms after gastroesophageal reflux disease (GERD) and the main cause of dysphagia and food impaction in children, adolescents, and young adults in Europe and North America [5], where it affects 1-to-2 per 2000 inhabitants [6,7]. It is also emerging in other regions, including developing countries. As a result, EoE represents a large financial burden to the health care systems, with an estimated annual health-care cost of up to $1.4 billion in the United States [8]. The continually developing epidemiology of the disease, its chronic nature and the need to involve multidisciplinary teams in its management, demand the need for further research to understand the ultimate causes of the disease [9], to optimize the cost-effectiveness of the interventions, and finally, to plan preventive strategies.

Efforts to understand EoE have sharply increased in recent years, making it one of the topics of greatest interest among gastroenterologists and allergists. Research papers addressing the many aspects of EoE have increased almost exponentially as the disease is being recognized in multiple settings. In addition, pharmaceutical and biotechnological companies have acknowledged the unmet needs of EoE patients and are currently allocating resources to the potentially expanding market for EoE diagnosis and therapeutics. After 20 years of research on the causes of this disorder, large-scale epidemiological studies to define potential risk factors are still needed however. Integrating knowledge from genetic susceptibility loci proposed for EoE with environmental factors is required, and efforts should be made to develop non or minimally invasive tests for EoE diagnosis and monitoring. In addition, the optimal management of EoE patients remains controversial and widely variable [10–14] and treatment in clinical practice varies more than any other aspect related to the disease [11,13].

This article aims to provide a state-of-the-art update on the pathophysiology of EoE applied to clinical practice, and an updated review of the latest research and developments with potential interest to improve the diagnosis and treatment of patients with EoE.

CONTACT Alfredo J. Lucendo ajlucendo@hotmail.com Department of Gastroenterology, Hospital General de Tomelloso, Vereda de Socuéllamos, s/n, 13700, Tomelloso, Spain

2. Esophageal eosinophils: from the cell to the histopathology

2.1. Eosinophils in the gastrointestinal tract and its trafficking to the esophagus

Eosinophils are granulocytes of myeloid lineage produced in the bone marrow and traditionally considered to be IgE-dependent effector cells that arise in inflammatory processes in response to allergic hypersensitivity and parasitosis. In normal conditions they are present in many tissues, including the mucosa of most segments of the gastrointestinal (GI) tract where they are extremely common, except in the esophagus, which is the only digestive organ that does not normally contain eosinophils. The ubiquity of these cells have led some authors to consider eosinophils to be regulatory cells involved in the maintenance of intestinal homeostasis [15] as opposed to the more conventionally active role they play in several intestinal diseases -including ulcerative colitis or EoE- and similar which occurs in bronchial asthma [16].

In order to achieve the high numbers of eosinophils that are detected in all layers of the esophagus in patients with EoE, they need to have first proliferated and matured in the bone marrow under the regulatory effect of several cytokines and growth factors. Among these Th2-cytokine interleukin (IL)-5 is the most specific and better studied for the selective expansion of eosinophils and their further release into the circulating blood [17] and was one of the first proposed therapeutic targets in EoE [18]. Research in murine models of the disease showed that transgenic mice with overproduction of IL-5 suffered from blood eosinophilia and intense eosinophils accumulation in the esophageal tissues, including the lamina propria, as well as in the small bowel after inhaled [19,20] or epicutaneous [21] stimulation with allergens, which was proportional to the serum concentration of IL-5 [16]. Deletion in the *IL-5* gene however protected mice from developing tissue eosinophilia after allergen stimulation [20]. The *IL-5* gene and its protein are upregulated in esophageal biopsies from active EoE patients [22,23]; the blood lymphocytes of EoE patients produce significantly higher levels of IL-5 following *in vitro* stimulation compared to normal controls [24] and the percentage of blood-circulating IL-5+CD4 T cells correlates with the severity of esophageal tissue eosinophilia [25]. Assessing the effectiveness of blocking IL-5 with monoclonal antibodies was, therefore, predictable.

Trafficking of eosinophils to the esophagus is accounted for the effect of several activation signals released from the inflamed tissue, which first induce the acquisition of tissue-specific functional properties in blood eosinophils. These differ not only depending on the tissue they exert inflammatory functions on (such as the esophageal, bronchial or colonic mucosa) [16], but also according to patients' age [26] and the disease status activity [25]. Despite the effect of homing molecules in the recruitment of eosinophils toward the esophageal mucosa having not yet been assessed, preliminary research, mainly with flow cytometry, has begun to delineate specific peculiarities of blood eosinophils which are able to lead them toward an inflamed esophagus: circulating blood eosinophils in EoE exhibit an enhanced expression of the CC chemokine receptor CCR3 common for eotaxins [25], the low-affinity receptor for IgE (CD23), the intercellular adhesion molecule (ICAM)-1 (or CD54) [16,26], integrin CD11c, the receptor for prostaglandin D2 CRTH2 [16,26] and FOXP3 mRNA [26]. Some of these have been assessed as potential therapeutic targets for EoE.

2.2. Therapeutic interventions for esophageal trafficking of eosinophils

The IL-5 blocker mepolizumab was tested in randomized controlled trials (RCT)s involving children [27] and adults [28], while reslizumab was evaluated in children only [29], neither of them demonstrating significant differences between the active and placebo groups in terms of symptom relief nor histological remission. Reslizumab has been suggested as being effective in children with EoE when used in the long term [30].

A selective CRTH2 antagonist (OC000459) with proven efficacy against eosinophilic asthma was assessed in a double-blind, placebo-controlled RCT in adult patients with EoE [31]: the drug induced a significant decrease in both esophageal eosinophilia and symptoms, with a trend toward improvement in endoscopic abnormalities compared with a placebo. However, esophageal mucosa did not revert to normal.

Selective, competitive antagonists of CCR3 are potentially promising drugs that are being investigated in bronchial asthma (an eosinophilic inflammation in the airways). As yet, no studies in EoE with these drugs have been proposed.

3. The epithelial cell: a central player in the pathophysiology of EoE

Epithelial cells are increasingly recognized as major components of the innate immune system that play a role in defensive functions of the GI mucosa [32]. The intestinal epithelium is crucial for preserving gut homeostasis and acts both as a physical barrier and as a coordinating hub for immune defense and crosstalk between bacteria and immune cells. If deregulated, the immunomodulatory function of epithelial cells may contribute to the development of intestinal inflammation. Cumulative research data are placing the esophageal epithelium in the center of the pathogenesis of EoE. As previously described with epithelial cells from various tissues including nasal, airway and intestinal mucosa [33,34], the esophageal mucosa is able to express major histocompatibility complex (MHC) class II molecules during inflammation [35,36] and thus behave as non-professional antigen-presenting cells [25,26].

The esophageal epithelium is a relatively impermeable surface unable to be passed through by medium and large-size molecules. It has also been demonstrated that superficial layers, but not basal and suprabasal ones, are those involved in establishing the esophageal epithelial barrier [37]. The eosinophilic infiltration in EoE is usually organized in a density gradient toward the more superficial layers and is more abundant on the strata in contact with the esophageal lumen (the contact point with swallowed allergens) [38]. In fact, eosinophils frequently cluster to form microabscesses within these superficial strata [39,40].

There is evidence that active EoE is characterized by an impaired barrier function caused by epithelial barrier defects [41], with reduced expression of E-cadherin, desmoglein-1,

involucrin and filaggrin, all being structural proteins involved in maintaining mucosal integrity. Tight junctions (TJ) are multiprotein junctional complexes that prevent leakage of transported solutes and water by sealing the paracellular pathway. The expression of some of their components (as claudin-1, claudin-4, claudin-7, occludin, and zonula occludin-1 proteins) has also shown alteration in patients with active EoE [42,44]. In addition, active eosinophilic inflammation alters the expression of the cytoskeletal protein synaptopodin in the esophageal epithelium [45].

Very recently, the origin of all these changes has been related with a depletion of the serine protease inhibitor, kazal type (SPINK) 7, a antiprotease, which is part of the differentiation program of the esophageal epithelium. SPINK7 was practically absent in esophageal biopsies taken from adults and children with active EoE but was prevalent in biopsies from healthy people. To demonstrate the role of SPINK7 in the pathophysiology of EoE, *SPINK7* expression was silenced in an esophageal epithelial cell line and primary esophageal epithelial cells, which lead to barrier dysfunction and transcriptional changes, characterized by loss of cellular differentiation and altered gene expression able to stimulate allergic responses with production of proinflammatory cytokines. Changes associated with SPINK7 silencing were reversed after treating the culture with antiserine protease α1-antitrypsin [46].

As a consequence of the above, an increased permeability has been demonstrated in patients with active EoE [47,48], which is translated at a tissue level by dilated intercellular spaces, an usual finding repeatedly reported in EoE patients of all ages [44,49]. This impaired barrier function might allow pathogens to invade the esophagus, and facilitate antigen penetration in active EoE patients. In fact, biopsy samples from active EoE are characterized by overexpression of epithelial antimicrobial peptides (mainly beta-defensins, cathelicidin LL-37, and psoriasin) [41] and upregulation of bacterial pattern recognition Toll-like receptors (TLR) [50]. Differences in anti-gliadin staining among patients with active and inactive EoE also suggest presence of intraepithelial food antigens in patients with active disease [51]. Both facts potentially contribute to perpetuate the inflammatory condition in EoE (Figure 1).

The esophageal epithelium is also the main source for thymic stromal lymphopoietin (TSLP), a cytokine with a central role in EoE. TSLP is mainly produced by non-hematopoietic cells such as epithelial cells, fibroblasts, and different types of stromal cells and its expression is linked to many allergic and immune-mediated diseases including asthma [52], atopic dermatitis [53], inflammatory bowel disease [54] and EoE. The factors inducing the release of TSLP are not clearly defined, but it plays an important role in the activation of antigen-presenting cells, including the food antigen-presenting dendritic cells in the esophageal mucosa, to promote maturation of T cell populations and inducing Th2 polarization of naïve CD4 + T cells [55,56]. These Th2 cells then secrete Th2 cytokines, including IL-13. IL-13 is a Th2-type cytokine with pleiotropic effects that play a key role in EoE. *IL-13* gene expression is upregulated in the blood eosinophils of patients with several eosinophilic inflammatory disorders including EoE [57] and especially in the esophageal epithelium of EoE patients compared with healthy controls [58]. The key role of IL-13 in the pathophysiology of EoE is supported by the fact that human esophageal cell cultures stimulated with IL-13 selectively induce the expression and secretion of the eosinophil-activating chemoattractants eotaxin-1/CCL11 and eotaxin-3/CCL26 [59], operating through the nuclear transcription factor STAT6 (which plays a central role in Th2 cell differentiation) [58], and are capable of partially reproducing the characteristic EoE transcriptome. This can then be reversed after topical steroid treatment in parallel with a significant reduction in *IL-13* mRNA expression levels [58]. In murine models, intratracheal delivery of IL-13 induces experimental EoE, whereas IL-13-deficient mice and those with a targeted deletion of STAT6 have attenuated degrees of allergen-induced experimental EoE and are partially protected from allergen- and IL-13-induced experimental EoE, respectively [21].

IL-13 promotes epithelial dysfunction in EoE: A decreased expression in filaggrin (*FLG*) and involucrin (*IVL*) genes is documented in IL-13-stimulated esophageal epithelial cells and that obtained from EoE patients compared with normal biopsy specimens [60]; IL-13 also reduces the adhesion molecule desmoglein-1 [61], inhibits the expression of filaggrin and involucrin, and alters the expression pattern of TJ-associated proteins [37]. The disruptive effects of IL-13 on the esophageal epithelium are regulated through the *CAPN14* gene, which is encoded in the EoE-susceptibility locus 2p23 and codified for Calpain-14 (CAPN14), an esophageal-specific protease with a role in protecting the integrity of esophageal tissue [62]. The *CAPN14* gene is dynamically upregulated by both IL-4 and IL-13 and exerts a gatekeeper role in EoE. Upregulation of CAPN14 is linked to impairment of the epithelial barrier, partially mediated by loss of DSG1, whereas its down regulation leads to failure in repairing IL-13–induced epithelial changes [63].

3.1. Epithelial products as diagnostic markers of EoE

The histologic method is the gold standard of an EoE diagnosis in patients with suggestive symptoms. However, EoE clinical symptoms do not always correlate with histology [64], and the patchy distribution of EoE limits the proper assessment of the disease if a minimum of 5 to 6 biopsies are not obtained [65]. EoE is characterized by a well-preserved genetic transcriptome, which was first discovered in 2006 [66], and led to the development of the EoE diagnostic panel (EDP), a novel molecular tool built on a Taqman®-qPCR-based low-density array system, which has the additional advantage of identifying histologically ambiguous subjects who may later develop active EoE [67]. By combining expression levels of 77 genes, the EDP identified adult and pediatric patients with EoE with approximately 96% sensitivity and 98% specificity, and distinguished patients with EoE in remission from controls, as well as identified patients exposed to swallowed glucocorticoids. A large prospective study validated the EDP, additionally demonstrating its feasibility from a single paraffin-embedded esophageal biopsy [68]. Among genes represented in the EDP, the epithelial-related ones were an essential component, with those codifying for filaggrin (*FLG*), Uroplakin-1a (*UPK1A*), serine peptidase inhibitor kazal-type (*SPINK*)7, cysteine-rich secretory protein (*CRISP*)3 and mucin (*MUC*)4 as the major representatives.

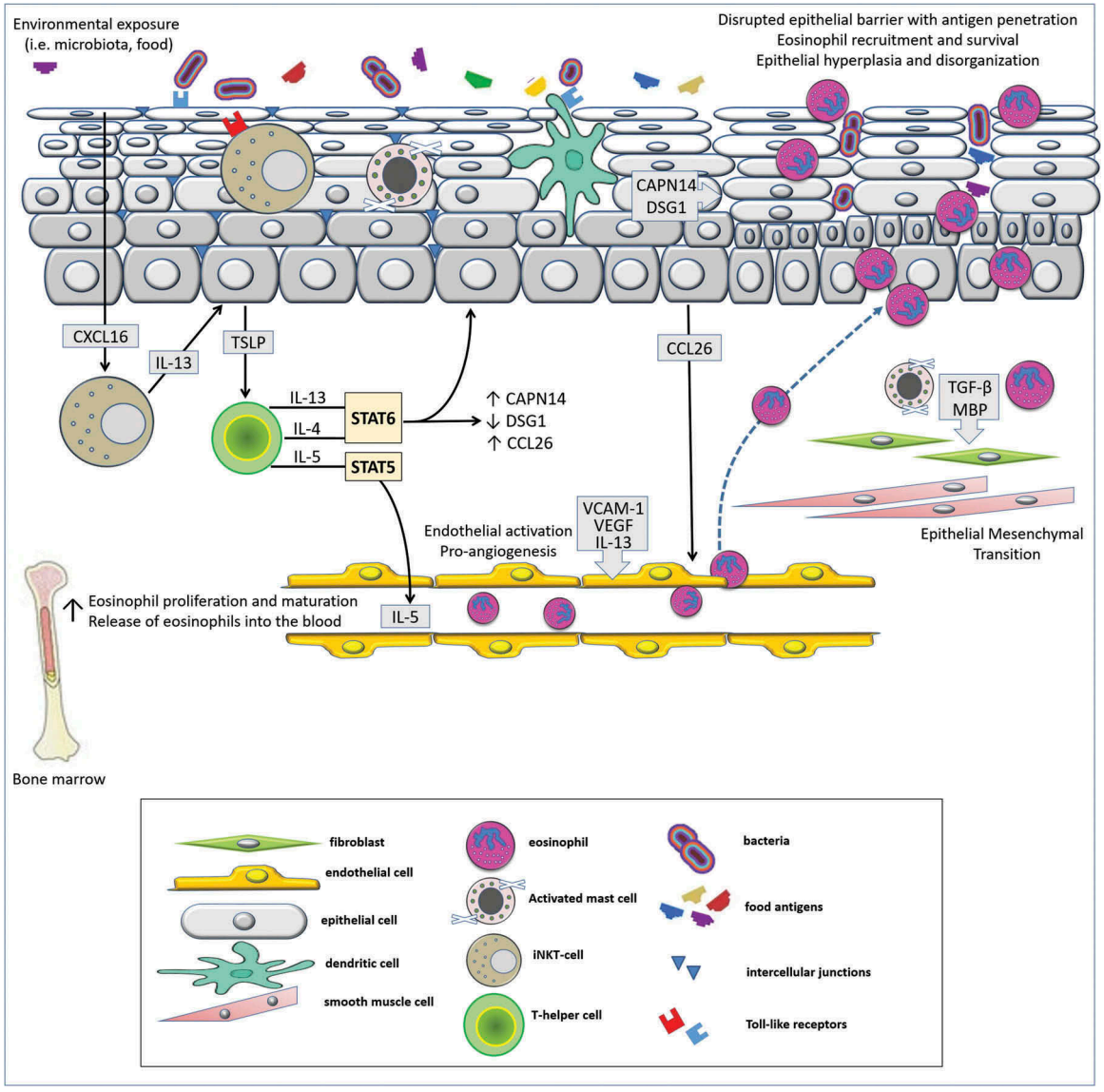

Figure 1. The esophageal epithelium in eosinophilic esophagitis as an immunologically active surface, which initiates and perpetuates inflammatory and structural changes characterizing eosinophilic esophagitis (EoE).

The activation of epithelial and dendritic cells after exposure (or lack of exposure) to components of the esophageal lumen (i.e., bacteria and food antigens) induce CXCL16 expression, which directly promotes invariant natural killer T (iNKT) cells recruitment. iNKT cells are the major source of Th2 cytokines, including IL-13, which directly induces changes in the gene expression pattern on epithelial cells, leading to thymic stromal lymphopoietin (TSLP) secretion. TSLP act on T-helper cells, promoting secretion of Th2 cytokines IL-13, IL4, and IL-5. IL-13, primarily acting together with IL-4 through signal transducer and activator of transcription 6 (STAT6) promotes the transcription of calpain-14 (CAPN14) and C-C Motif Chemokine Ligand 26 (CCL26 or eotaxin-3). While the first contributes to disrupt the epithelial surface which increases its permeability by decreased expression of the tight junction protein desmoglein 1 (DSG1) among others, CCL26 is a potent chemoattractant for eosinophils and mast cells. IL-5 also promotes tissue recruitment and survival of eosinophils signaling primarily through STAT5. Th2 cytokines also trigger the production of IgE by plasma cells. Activated eosinophils are multifunctional cells that regulate diverse processes including angiogenesis and endothelial activation by releasing vascular cell adhesion molecule 1 (VCAM-1), and vascular endothelial growth factor (VEGF) which are needed for recruiting inflammatory cells toward the esophagus. The effects of transforming growth factor 1 (TGF-1) and other activated eosinophil and mast cell-derived mediators on smooth muscle fibers (as major basic protein or MBP) lead to hyperplasia and hypercontractility. At the same time, they are key mediators for activation and proliferation of fibroblasts and for the subsequent synthesis of extracellular matrix components. Eosinophils themselves regulate the process of epithelial–mesenchymal transition, acting in a paracrine environment characterized by the presence of Th2 cytokines and eotaxins.

3.2. Therapeutic targets focused on epithelial function in EoE

Several studies have demonstrated that well-established therapies for EoE are able to restore the impaired esophageal barrier by improving epithelial integrity and reducing its permeability. This has been shown for elemental diet [69], proton pump inhibitors (PPIs) [70] and topic swallowed steroids [44,71]. The esophageal expression of gene encoding for several barrier integrity proteins -filaggrin, desmoglein-1, zonula occludin-3, and claudin-1, was impaired at baseline and restored after diet or steroids to similar levels to subjects with no esophageal disease [69,71,72]. This was manifested by normalization of esophageal impedance and transepithelial small molecule flux [69,72].

With regard to investigational products, anti-TSLP antibodies have been assessed in murine models of atopy, including asthma and EoE. TSLP antibodies or antibodies that inhibit its receptor TSLPR block CD4 Th2 development in asthma or allergic rhinitis in mice [73,74,75], and were shown to block the development of esophageal eosinophilia and food-related symptoms in experimental EoE [55]. As for human research, a fully human anti-TSLP monoclonal antibody that specifically

binds human TSLP (tezepelumab or AMG 157), preventing interaction with its receptor, has been tested in a phase IIb trial in adult patients with uncontrolled asthma with favorable effects [76]. TSLP is also a potent chemoattractant for eosinophils, thus reinforcing the activity of this drug [77], making this product a promising pharmacological target also for EoE [78].

Anti-IL-13 antibodies have also been assessed in EoE patients in clinical trials. The first one investigated QAX576 as a potential treatment of adult EoE and was published in 2015. Patients were randomly assigned to QAX576 (6 mg/kg) or placebo every 28 days for 3 doses with 6-month follow-up. QAX576 led to a decrease in mean intraepithelial eosinophil counts but reached no histologic remission, and a non-significant trend toward improvement in dysphagia severity, as measured by the Mayo Dysphagia Questionnaire, was documented. In addition, QAX576 normalized the expression levels of some EoE-related genes, including *eotaxin-3/CCL26, periostin* (*POSTN), carboxypeptidase A3* (*CPA3*), and *desmoglein-1* (*DSG1*). Transcriptional changes differed between responders and nonresponders to QAX576 [79].

IL-13 exhibits a 30% sequence similarity with IL-4 and both share similar structures. *IL-13* (but not *IL-4*) gene expression is upregulated in the esophageal epithelium of EoE patients compared with healthy controls [58]. However, both cytokines mediate downstream effects via a common heterodimeric receptor, IL-4Ra and IL-13Ra1. It has been proposed that therapies targeting IL-4 and IL-13 separately may be ineffective because IL-4 and IL-13 have overlapping downstream effects [80]. Dupilumab, a monoclonal antibody against IL-4Ra, is the most promising IL-4/IL-13-targeted therapy to date. After demonstrating effectiveness in asthma [81] and atopic dermatitis [82], ongoing trials are now assessing dupilumab in EoE [83]. A phase II, randomized, double-blind, placebo-controlled clinical trial (NCT02379052) was carried out with 47 participants to assess the clinical efficacy of a 12-week treatment period with dupilumab for relieving symptoms in adult patients with active, moderate-to-severe EoE [84,85]. Patients received either dupilumab 300 mg weekly following a 600-mg loading dose or placebo. At week 10, patients who received dupilumab reported a significant improvement in the ability to swallow compare to placebo (45% *vs*. 19% improvement from baseline in the Straumann's Dysphagia Symptoms Score). Esophageal eosinophil counts significantly reduced by 93% from baseline in patients who received dupilumab weekly compared with an increase of 14% in those who received placebo. Long term assessment of the effectiveness of dupilumab in the sustained control of EoE is required.

4. EoE as an allergic disease

EoE constitutes a particular allergic condition triggered and maintained by food allergens [86–88], with a potential role for aeroallergen exposure in the genesis and exacerbations of EoE which is not supported by most of the current evidence [87,88]. Atopy has been linked to EoE since the initial reports of the disease, with most patients having a family history of bronchial asthma or allergic rhinitis; atopic dermatitis; hypersensitivity to drugs, blood eosinophilia; or elevated serum total and specific IgE levels [89]. IgE-mediated food allergy is also common among EoE patients and alters its clinical presentation during childhood [90]. Overall, atopic manifestations are 3 to 5 times more common among patients with EoE compared with control subjects [91]. The definitive demonstration of EoE as a food allergy came in 1995, when Kelly *et al*. provided firm evidence of resolution of histological lesions and symptoms in pediatric patients following elemental amino acid-based diets lacking antigenic capacity [92]. In children, retrospective cohort analyses have suggested that EoE is a late manifestation of the allergic march in some individuals, with a peak of incidence which appears after that of atopic dermatitis, IgE-mediated food allergy and bronchial asthma. There was also a cumulative effect of multiple preceding allergic conditions in the rate of subsequent EoE diagnosis, which was higher in individuals with more than one preceding allergic condition [93].

4.1. iNKT lymphocyte responses as initiators of allergy and EoE

Inflammatory responses in food allergy, including EoE and atopic dermatitis, occurs on the epidermal border and are closely related to the microbiota and its metabolites able to modulate host immune responses [94], leading to the development of both tolerance or allergy. The global increase in all kind of allergies and immune-mediated diseases, especially in industrialized countries to represent a major health concern has been interrelated through the hygienic hypothesis. That is, reduced exposure to microorganisms during childhood has modified the patterns of gut microflora, leading to a change in the fine tuning of Th1, Th2 and regulatory T-lymphocytes (Treg) responses [95]. A lack of appropriate immune stimulation during early childhood leads to disturbed alignment in the sequence of encountering self- or non-self-antigens and accounts for the rise of atopy and autoimmune disease. A central role of 'training' regulatory T-cells through sufficient microbial exposure, leading to a robust, healthy balance between inflammation and anti-inflammation or immune tolerance has been recognized in the so call 'early immune challenge hypothesis' [96].

Invariant natural killer T (iNKT) cells are innate-like T cells that recognize glycolipid antigens rather than protein antigens via the MHC class I-like protein, CD1d, which is involved in the initial phases of a great variety of immune responses from oral tolerance to autoimmunity [97]. iNKT cells rapidly produce Th2-type cytokines (IL4, IL5 and IL13), as well as eotaxins; this leads to IgE production and subsequent sensitization to protein antigens [98]. Thus, iNKT cells play an important role in affecting the pathogenesis of allergic diseases. An age-sensitive contact with commensal microbes is critical for establishing mucosal iNKT cell tolerance to later environmental exposures [99,100]. When early-life microbial signals are not provided to mucosal tissues that are usually exposed to commensal microbiota, such as the intestine and airways (either by restricting microbial exposition or by using antibiotics during the first year of life [101], an excessive and persistent accumulation of iNKT cells occurs [99]. Consequently, these mucosal tissues are rendered more susceptible to later-

life environmental triggers of iNKT cells, which will mediate allergic sensitization and tissue inflammation [102].

iNKT lymphocytes are recognized as the major source for pro inflammatory cytokines in EoE [103,104]. Thus, although iNKT cells primarily recognize glycolipid structures located in pathogenic bacteria [105,106] and presented by CD1d, they can also be activated by sphingolipids found in food. For example, milk sphingolipids were shown to activate peripheral blood iNKTs in EoE-active children, producing Th2 cytokines [104]. Sphingolipids are present in many other common foods, with the foods richest in these components (i.e., milk and eggs) being the major common triggers of food allergies and EoE [86] (Figure 1).

The contribution of iNKT cells to the pathophysiology of EoE have been recently demonstrated: In animal models, activation of iNKT is sufficient to induce EoE, while neutralization of iNKT cells protects against experimental EoE [107,108]. CD1-deficient mice are protected from experimental EoE [109]. EoE patients have reduced peripheral blood iNKTs, and increased esophageal iNKTs compared to controls. Additionally, iNKTs from patients with active EoE expand more readily and produce more IL-13 in response to stimulation when compared to controls [104]. A study on children with EoE provided compelling evidence of insufficient immune imprinting by environmental microorganisms resulting in esophageal upregulation of epithelial and dendritic cell-derived CXCL16 [103], a chemokine that induces chemotaxis of iNKT cells into the esophagus. Esophageal samples from children with EoE show an increase in iNKT cells and components that regulate its chemotaxis and activity. iNKT cells activity was more pronounced in patients with early-onset EoE, who also had high levels of sensitization to food allergens. The elimination of allergens from the diet normalized cellular markers of iNKT activity. The modulation of the CXCL16–iNKT–CD1d axis remains a challenging therapeutic target to be investigated not only for allergic disorders such as EoE, but also in inflammatory bowel disease, celiac disease and cancer therapy.

4.2. Immunoglobulin involvement in EoE

The generation of antigen-specific IgE induced by a Th2 cell-mediated class switching of plasma cells is a central process to the pathophysiology of multiple allergic disorders. The effect IgE over FcRI receptors to induce degranulation from mast cells and basophils leads to immediate responses, anaphylaxis representing the clearest and most severe example [110]. Total and food-specific serum IgE levels are usually increased in patients with EoE, who frequently show allergen-specific skin prick test (SPT) responses [111], providing evidence of an immediate hypersensitivity in EoE. B cells have been identified within the inflammatory infiltrate of EoE [38,112], which have been shown to perform class switching and generation of IgE locally within the esophagus of both atopic and non-atopic patients with EoE [112], similarly to shown in IgE-mediated conditions as bronchial asthma [113] and allergic rhinitis [114]. The esophageal lining acquires the characteristic elements of an IgE-mediated response such as dendritic cells [38,115], class-switched B-cells [112], tryptase-positive mast cells [55] and Th2-cytokines [23,58]. However, the role of IgE in EoE is not still clear.

Evidence points to the independent evolution of EoE and concurrent atopies in the same patients. The elimination of foods that give positive results on skin prick tests usually fails to achieve disease remission [116,117] even though positive skin prick testing (SPT) results are observed in more than 80% of adult patients [89]. Atopic features and allergy sensitization patterns in EoE appear to be no different from those in atopic individuals without EoE living in the same geographic area and exposed to common allergens [118] with no significant differences regarding history of allergic rhinitis, atopic dermatitis, IgE-mediated food allergy, sensitization to aeroallergens, and family history of atopy [119]. Demographic, clinical, and histopathologic esophageal features were identical in patients with EoE who did not present with other atopic manifestations. Serum levels of allergen-specific IgE and the results from SPT correlate poorly with the food trigger(s), with the response to food elimination diets being equally effective in patients with EoE with negative allergy test results [120]. Food reintroduction in EoE does not determine immediate responses such as anaphylaxis. IgE-deficient [55], and B-cell deficient [109] mice are able to develop experimental EoE, as well as those exposed to the IgE-independent aeroantigen Aspergillus [19], which supports the dispensability of IgE in the pathogenesis of EoE. Collectively, these observations suggest there are other non-IgE-mediated pathways important in the EoE pathogenesis. Common genetic and environmental etiologic factors that contribute to the independent development of atopy and EoE might explain the association of both entities [101,121].

Recently, an increasing role for IgG4 in EoE is been recognized, after a seminal study which demonstrated a 45-fold increase in IgG4 concentration compared to controls in the esophageal tissues of adult EoE patients with active disease, as well as increased food-specific serum IgG4 to the foods that are most associated with EoE: milk, wheat, egg and nuts [122]. Additional studies in children [123–125] and adults [126] confirmed these results; tissue IgG4 levels correlated with esophageal peak eosinophil count, degree of histological features, *IL-4, IL-10* and *IL-13* gene expression level in subjects with EoE [125], thus supporting the potential role of IgG4 in EoE.

IgE and IgG4 are the most prominent isotypes of Ig in human immune responses to allergens. Similarities in allergen specificity patterns of IgE and IgG4 are due to their common dependence on IL-4 as a switching factor [127]. Upon natural exposure, IgE antibodies appear earlier, but exposure to most if not all allergens will induce substantial amounts of IgG4 antibodies [128]. Only upon frequent exposure the plasma IgG4 level rises and IgG4 becomes the dominating antibody [129], suggesting that IgG4 antibodies are associated with prolonged exposure to antigens, including food antigens. As a result, IgG4 has been involved in allergen-specific immunotherapy (AIT) in the treatment of IgE-mediated food allergy. In AIT, incrementally increasing doses of inciting allergen are given with the aim of increasing tolerance, initially through desensitization, which relies on regular exposure to allergen. With prolonged therapy in some subjects, AIT may induce sustained unresponsiveness, in which tolerance is retained after a period of allergen

avoidance [130]. Due to its poor capacity to activate effector cells or complement, IgG4 has been commonly associated with 'tolerance' and its appearance during the treatment of food allergy through oral immunotherapy (OIT) for food (one the methods of AIT) has been related to the protective role played by IgG4 in avoiding IgE-mediated responses after exposure to culprit antigens. Interestingly, together with enabling the production of IgG4, OIT is known to induce *de novo* EoE after being used to treat food specific IgE-mediated food allergy in up to 4% of patients [131]. The reasons why Ig4 seems to lose its tolerogenic capacity in these circumstances have not been clarified, but it has been proposed that T cells that home toward the esophagus in EoE enhance IgG4 antibody local production [128] or the role of eosinophils to support plasma cell survival [132] maybe also relevant in this condition.

4.3. Steroid treatment for EoE patients

As in other atopic disorders, topical steroids currently constitute the prevailing therapeutic option for EoE; the development of new formulations targeted to provide an optimal esophageal coverage suppose that they will probably continue to do so in the near future. Several RCTs summarized in sequential meta-analyses [133–136] have demonstrated that topically administered fluticasone propionate and budesonide are highly effective in children and adults, significantly superior to a placebo and comparable to oral prednisone [137] in inducing histological and symptomatic disease remission. However, despite the efficacy of steroids in treating the symptoms of EoE, their action is not sustained after discontinuation of medication. The ability of topical steroids to reverse EoE has been repeatedly demonstrated at a gene expression and molecular level [58,138], exerting their actions through a variety of mechanisms including transcriptional inhibition of specific promoter response elements, destabilization of cytokine mRNA and direct induction of cellular apoptosis. In the specific case of EoE, swallowed steroid therapy has been demonstrated to act topically and mediates its effects by directly regulating gene expression in esophageal epithelial cells [139]; thus, after binding to the glucocorticosteroid receptor, steroids repress IL-13-induced eotaxin-3 expression while induce FK506-binding protein 5 (*FKBP51*) gene expression. This inhibits glucocorticoid receptor-mediated signaling, which in turn represses IL-13-induced *eotaxin-3* promoter activity [139].

4.4. The anti-inflammatory effects of PPIs in EoE

The consideration of proton pump inhibitor (PPI) therapy within the diagnostic and/or therapeutic algorithm has been the most evolving topic in EoE over the past decade. As for patients with PPI-responsive esophageal eosinophilia (PPI-REE) , it was demonstrated that baseline expression of markers of Th2-mediated and eosinophilic inflammation (including *CCL26, IL-13, TSLP* and *POSTN*) in esophageal tissue largely overlaps in non-responders and responders to PPI therapy [22,70]. Patients with PPI-REE also showed a transcriptome that almost completely overlapped with non-responders to PPIs, including the hallmark EoE gene for eosinophil chemotaxis (*CCL26*), barrier molecules (*DSG1*), tissue remodeling (*POSTN*), and mast cells (*CPA3*) [140,141], constituting a genetic profile that was radically different from that observed in patients with GERD and control subjects. PPI monotherapy in PPI-REE patients can almost completely reverse the Th2 signature and normalize the EoE diagnostic panel expression [22,140], similar to other anti-inflammatory drugs, like topical steroids or anti IL-13 blockers. The molecular mechanisms whereby PPIs blocks Th2 cytokine-driven esophageal eosinophilia *in vitro*, independently of effects on gastric acid secretion, include its ability to inhibit IL-4 and IL-13-stimulated eotaxin-3 expression in esophageal cells and block STAT6 by binding the promoter [142,143].

5. Mast cells and other components of the inflammatory infiltration in EoE

Mast cells are mesenchymal bone marrow-derived myeloid cells widely distributed in vascular connective tissues. As a part of the innate immunity, they act against parasites and bacteria. In humans, mast cells are classified into two types depending on their granule content [144,145]: MC_T (mast cells with tryptase) and MC_{TC} (mast cells with tryptase and chymase). The mast cell population within the esophageal epithelium predominantly consists of MC_{TC} cells, both under normal conditions and in EoE [146]. This phenotypic diversity is not only a descriptor of tissue location, but also of the regulation of cytokine gene expression and, as such, is associated with functional differences [147–149].

A role for mast cells in the pathogenesis of EoE was proposed after studies demonstrated both their activation [150] and increased density in the esophageal mucosa of experimental [150,151] and human EoE in adults [23,38,146,152] and children [66,149,153–156]. These increases were significant compared with healthy controls as well as with patients with GERD; in fact, mast cell density has been proposed as a marker to distinguish GERD from EoE [153,157]. Several pieces of research have supported the potential role played by mast cells in EoE: Its density correlates with eosinophilic infiltration within the esophageal epithelium [158], with a reduction in both cell types after treatment with topical steroids [159,160] or anti-IL-5 [161], anti-IL-13 [79] or 6-food elimination diet [146], in association with clinical remission [146,152,160,162].

Mast cell infiltration, together with eosinophils, is directly associated and significantly correlated with symptoms in adult patients with EoE [146]: The peak number and activation of mast cells, and the expression of major mast cell proteases (including *CPA3, chymase/CMA* and *tryptase/TPSB2*) in the esophageal mucosa directly and significantly correlated with symptom scores in adult patients with EoE. Mast cell-mediators have been shown to be upregulated in EoE in several reports.

The expression of specific mast cell-mediators has also been shown to be upregulated in several reports [66,149,155], with mast cell-derived TGF-b1 contributing to esophageal dysmotility in both human [155] and murine experimental EoE [150] through

the induction of smooth muscle hypertrophy and hyperplasia, thus contributing to esophageal symptoms.

5.1. Activation of mast cells in EoE

Antigen cross-linking of IgE antibodies on the mast cell surface is the most extensively studied mechanism leading to mast cell activation and degranulation. This results in a rapid release of autacoid mediators and a sustained synthesis and release of cytokines, chemokines and growth factors [163] and leads to anaphylaxis as its most characteristic consequence. However, immediate systemic reactions to the foods responsible for EoE are not described in these patients, despite the fact that local IgE production has been demonstrated in the esophageal mucosa of patients with EoE regardless of their atopic background [112]. No differences in esophageal mast cell densities were shown between EoE patient with and without an atopic background [146], despite IgE-bearing mast cells being described in the esophageal epithelium of the former [164,165]. This suggests that IgE is not the main trigger of mast cell activation in EoE, with other IgE-independent mechanisms playing the principal roles. In fact, MC_{TC} are also strong responders to non-IgE -mediated regulation including the activation of toll-like receptors [166], exposure to gastric reflux [167,168], bile acids [169], the enteric nervous system [170], and certain eosinophil-derived proteins, mainly major basic protein [171]. In any case, the definitive exclusion of a putative role for IgE-promoting, mast cell-dependent, immediate reactions would require evidence of mast cell activation just after challenging a patient with a known food trigger for EoE, and this has yet to be demonstrated.

5.2. Treatments acting on mast cell activation in EoE

Cromolyn, as a mast cell stabilizer, is a first-line agent to treat GI symptoms of systemic mastocytosis with a poor absorption and almost nonexistent side effects. When used in patients with asthma it is able to significantly decrease activated eosinophils in bronchial mucosa, similarly to fluticasone propionate and superiorly to placebo or beta-2 agonists [172,173]. Early case reports in children with EoE failed to demonstrate a beneficial effect for cromolyn on symptoms and inflammation [174]. A very recent randomized placebo-controlled trial has structurally assessed viscous oral cromolyn for EoE in 16 pediatric patients [175]. Esophageal peak eosinophil counts and blood eosinophilia did not change after an 8-week treatment. A non-significant trend to symptoms improvement was documented in the intervention arm. It should be noted that MC_{TC} cells do not specifically respond to mast cell-stabilizer drugs such as cromolyn in the same way as MC_T cells, which are predominant in the bronchial mucosa and alveolar wall, a finding which explains the documented lack of efficacy of these drugs in treating EoE.

Montelukast, a leukotriene D4 receptor antagonist, is used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Montelukast also inhibits mast cell degranulation in the skin [176] and gastrointestinal tract mucosa [177] and has been assessed as a potential therapy for EoE. Used at standard doses in children [178] led to some symptomatic improvement in an open-label trial, with no patients achieving histologic response. Montelukast did not demonstrate superiority over placebo in maintaining remission in adult patients with EoE [179,180].

Omalizumab is an anti-IgE monoclonal antibody effective in controlling asthma in severely allergic asthmatic patients. It has also been assessed as a treatment for EoE in short case pediatric series [181] and recently in an open-label trial on 15 adolescents and young adults [182]. After 12 weeks, histological and clinical remission of EoE was documented in one third of participants, who were those with low peripheral blood absolute eosinophil counts. Finally, 30 adults with EoE were randomly assigned to receive omalizumab or placebo in a double-blind trial in which omalizumab did not alter esophageal symptoms or eosinophil counts in biopsy samples compared with placebo [122]. Despite these disappointing results, this trial observed granular deposits of IgG4, abundant IgG4-containing plasma cells, and serum levels of IgG4 reactive to specific foods in patients with EoE, indicating that, in adults, eosinophilic esophagitis is IgG4-associated, and not an IgE-induced allergy. Similar findings have been recently reproduced in children [123].

6. Genes and environment in EoE

As in other immunoallergic diseases, EoE arises from the interaction of environmental, host immunologic and genetic components [183,184]. The relative weight of each one in the final result of the disease has just begun to be defined. The contribution of genetic heritability to EoE has been defined in two family-based studies. In the first one, concordances for EoE among nonrelated individuals, siblings, dizygotic twins, and monozygotic twins were assessed. While the prevalence of EoE in the general population (or its general risk) was estimated in about 0.05% (1/2,000 inhabitants), it increased to 2,4% in siblings, 22% in dizygotic twins and 41% in monozygotic twins, despite the last sharing 100% of their genetic identity [183]. Since dizygotic twins and siblings have the same genetic relatedness, the authors used this difference to determine that environmental factors contributed 81% toward the phenotypic variance in the development of EoE. The contribution of genetic risk variants accounted for only 15% of the phenotypic variation of disease risk. More recently, a population-based genealogy resource linked to electronic medical records for health care systems across the state of Utah was used to estimate familial aggregation and risk of EoE in extended relatives to clarify the contribution of genetic factors to the disease [184]. Risks of EoE increased among first-degree relatives (OR 7.19), especially if they were diagnosed <18 years of age (OR, 16.3). In second-degree relatives and first cousins, the risk was also significant (OR 1.99 and 1.03, respectively). However, spouses of EoE patients were observed to be also at increased risk of EoE (OR 2.86), which suggested a shared environmental exposition leading to the disease.

To identify genes providing susceptibility to EoE, candidate gene approaches and genome-wide association studies (GWAS) were developed [185]. Microarray analysis of RNA

expression (or transcriptome) in EoE patients compared with control subjects shows significant changes in 1% of the human genome, which are remarkably conserved across sex, age and allergic status [66]. Eotaxin-3/*CCL26* is by far the most highly expressed gene in the EoE transcriptome, with a 53-fold increase compared with the controls. Both the TSLP receptor and its ligand seem to be implicated in the genetic links in EoE, especially after 5q22 (which contains the TSLP gene) was identified as a susceptibility locus for pediatric EoE through genome-wide association studies [186]. Single nucleotide polymorphisms (SNP) in *CCL26, TGFβ* and its binding protein LRRC32, *FLG, TSLP, DSG1, CRLF2* and *TLR3* genes have been described as risk factors for EoE [56,61,185–189]. The male predominance (~70%) traditionally described in EoE [4], implying that currently unidentified sexual chromosome-related genes or hormonal factors may be involved in the development of the disease, have been explained by a mutation in the X chromosome affecting two chains for the IL-13 receptor (IL-13 Ra 1 and 2 located in position Xq13.1–q28), which would remain uncorrected by the Y chromosome genes in males [189]. More recently, an SNP in the gene encoding for the TSLP receptor (*TSLPR*) located in the pseudoautosomal region on Xp22.3 and Yp11.3 has been shown to be directly involved in the male predominance of EoE [184]. The comorbidity of EoE with other allergic diseases and the involvement of some of the genetic variants in other diseases have given rise to the identification of specific EoE risk and esophageal tissue-related loci by GWAS, which was significant, independent of the sensitization status of the patients [190]. Among them *CAPN14* (located in 2p23), *TSLP* and *WDR36* (the second coding for a protein involved in facilitating multiprotein complexes) (located in 5q22), *LRRC32* and *C11orf30* (11q13) and the downstream primary mediator for IL-13 and IL-14 signaling *STAT6* (12q13) were the most relevant. However, the extent of the association with disease susceptibility for the currently described gene variants is modest (<2 fold), similar to the magnitude described in other allergic and immunologic diseases.

The potential role of environmental exposure in the etiology of EoE has been assessed in retrospective cohort studies and case-control designs. Despite appropriate inference, the overall risk of bias of these studies was high, with selection of patients being limited to single centers for the most part [191,192]. Available research showed that prenatal and early life factors seems essential to determine risk of EoE, including exposure to antibiotics during childhood [101,193,194], cesarean delivery [101,192–194], maternal fever, and preterm labor [192]. All these factors have been associated with dysbiosis in gut colonization in early life [195,196]. In contrast, having a furry pet in infancy has been proposed as providing a protective role [192]. Population density (rural versus urban) [197,198], aeroallergen exposition [116,199] and pollen season were also described as risk factors. For the later, a systematic review with meta-regression found no significant variations in the seasonal distribution of either the diagnosis or clinical recrudescence of EoE throughout the year [87]. A supposed inverse relationship between EoE and *Helicobacter pylori* infection [200,201] has been also excluded by a recent large case-control study [202].

The interplay between genes and environmental factors in EoE has only been assessed very recently in a preliminary study. Interactions between EoE-predisposing polymorphisms (within *TSLP, LOC283710/KLF13, CAPN14, CCL26*, and *TGFβ*) and early-life factors (antibiotic use in infancy, cesarean delivery, breast-feeding, neonatal intensive care unit admission, and absence of pets in the home) were tested in a case-control study recently published [203]. Interactions between rs6736278 (*CAPN14*) and breast-feeding ($p = 0.02$) and rs17815905 (*LOC283710/KLF13*) and neonatal intensive care unit admission ($p = 0.02$) were demonstrated, but not with the remaining factors examined. In addition, the authors found that breast-feeding had a strong protective effect in those with the susceptibility genotype in *CAPN14* gene, suggesting for the first time in the literature that risk of EoE disease might be modifiable in subjects with certain environmental exposures and gene variants.

Taken together, the evidence supports that EoE is a multifactorial and genetically complex disease, which involves an interplay between genetic predisposition and environmental factors, among which early life exposure likely to affect esophageal/gut microbiome content and diversity appear to be the most relevant.

7. Fibrous remodeling in EoE patients

Subepithelial fibrous remodeling as a consequence of chronic esophageal inflammation has been demonstrated in children and adults with EoE, and reproduced in animal models [204]. Eosinophil-associated tissue remodeling is a common process found in several conditions in which chronic eosinophilic inflammation is the common hallmark, including bronchial asthma [205], hypereosinophilic syndrome [206], eosinophilic gastroenteritis [207], and lastly, EoE [204]. All share structural changes within the affected tissue, including subepithelial fibrosis, which ultimately alter the functionality of the affected organs. Uncontrolled remodeling due to ongoing inflammation in EoE may adversely affect esophageal function, leading to dysmotility [208], esophageal rigidity [209], progressive dysphagia and food impaction and, finally, stricture formation.

Esophageal strictures constitute one of the most severe complications of EoE that develop as a result of a long-standing untreated eosinophilic inflammation. Despite patient age and delayed diagnosis being recognized as determining factors for fibrotic esophageal strictures [210–212], not every patient with prolonged EoE evolution develops such strictures. Esophageal strictures are less commonly found in pediatric cases of EoE, likely due to the limited progression of the disease.

7.1. Cellular & molecular basis of tissue remodeling in EoE

Several mediators released from inflammatory cells are involved in driving esophageal remodeling in EoE, with a particular role for transforming growth factor (TGF)-β1 [213], analogous to the one observed in airway remodeling associated with asthma [214]. In addition to TGF-β1 signaling, other mechanisms involved in EoE remodeling include epithelium-mesenchymal transition and angiogenesis [215].

Research conducted in a murine model [216] and on esophageal cell cultures [217] has shown that subepithelial fibrosis in EoE develops as a consequence of IL-5, IL-4 and IL-13-promoted tissue eosinophilia [218,219]; blocking its respective activation pathways represents potential therapeutic targets. The esophageal tissue of EoE patients shows higher levels of angiogenic factors compared with control samples including CD31, von Willebrand factor, VEGF-A and vascular cell adhesion molecule-1, all of which promote neovascularization and angiogenic remodeling [220]. An activated endothelium facilitates the arrival of bone marrow-derived inflammatory cells into the esophagus, which are activated to release their granule proteins locally. Eosinophils and other proinflammatory cells interface with mesenchymal cell components in the deep esophageal layers, affecting fibroblasts and muscle cells by making them direct targets of activated eosinophils and their products [217]. Fibrosis in EoE has been related with eosinophil activation [221] which can be determined by immunohistochemical staining for eosinophilic major basic protein (MBP) [158]. Eosinophil-released MBP increases the expression of *FGF-9* in biopsies of EoE patients [222], correlates with the basal cell hyperplasia in the esophageal epithelium, and directly promotes both fibroblast activation and deposition of extracellular matrix (ECM) (Figure 1). Eosinophils also produce and secrete high amounts of CCL18, a type 2 chemokine implicated in fibrous remodeling of the lungs, through fibroblast proliferation and collagen deposition. High expression levels of this chemokine have been shown in EoE [223].

7.2. Epithelial mesenchymal transition in EoE

Epithelial mesenchymal transition (EMT), a process characterized by activating quiescent epithelial cells and fibroblasts, causing them to transdifferentiate into myofibroblasts, and defined by gain of mesenchymal markers (such as α-*smooth muscle actin* and *vimentin*) and loss of epithelial (*E-cadherin*) gene expression, has been recognized as a key process in all models of fibrosis [224]. TGF-β released from activated eosinophils and mast cells [225] strongly induces EMT in the esophageal epithelium [215] and is the most extensively analyzed cytokine in EoE-associated fibrous remodeling. In addition, EMT in EoE can also occur independently of TFG-β but mediated by IL-1β and TNFα as previously implicated in other models of cross-talk and fibrosis [226].

Myofibroblasts share features of both fibroblasts and smooth muscle cells and simultaneously participate in the synthesis, deposition and degradation of ECM along with the contraction of wound tissue [227]. Tissue remodeling also involves morphological and functional changes in smooth muscle components. In fact, esophageal muscle cells respond to various profibrogenic stimuli and eosinophil products. Thus, while MBP is a strong agonist of the M2-type receptors of acetylcholine, which governs smooth muscle function [228], at the same time, eosinophil-derived mediators affect the release of acetylcholine from the neuromuscular junction [217]. Hypertrophy of the muscularis mucosa along with the circular and longitudinal muscle layers has also been reported in patients with EoE [229], contributing to the esophageal dysfunction repeatedly demonstrated in EoE patients of all ages.

7.3. Clinical assessment of esophageal remodeling in EoE

As a result of fibrous remodeling, alterations in the biomechanical properties of the esophageal wall are common features of EoE [230]. The distensibility of the esophageal body was significantly reduced compared to controls in patients with EoE when assessed using the EndoFLIP system (Crospon Medical Devices, Galway, Ireland) [231], which uses impedance planimetry to calculate multiple adjacent cross-sectional areas within a cylindrical bag while simultaneously measuring intraluminal pressure during controlled volumetric distension [232]. EndoFLIP research in EoE has shown that a reduced esophageal distensibility predicts the risk of food impaction [233] and correlates with endoscopically-identified ring severity [234]. Improvements in esophageal body distensibility are achieved with medical and dietary therapies without dilation [235]. However, a lack of correlation between eosinophil counts and esophageal distensibility has been shown with EndoFLIP [233], partially explaining the dissociation between inflammatory activity and symptoms in EoE. Whether the addition of the EndoFLIP system to patient reported outcome measures can enhance the accuracy of predicting the biological activity of EoE and improve results of EoE therapies, including endoscopic dilation, warrants further investigation [236].

7.4. Therapeutic interventions for EoE-associated fibrous remodeling

Mechanical dilation with through-the-scope hydropneumatic balloons and Maloney or Savary bougies constitutes a preferred treatment option for EoE patients with esophageal strictures or a narrow-caliber esophagus, which improves dysphagia in 95% of patients, according to a recent meta-analysis including 27 studies assessing 845 individual patients undergoing 1,820 dilation procedures [237–239]. Because endoscopic dilation is a mechanical procedure with no effect on the underlying inflammatory process [238], its efficacy is limited over time, with duration of the effect ranging from 1 to 36 months [237].

Swallowed topical steroids have been demonstrated effective to reverse fibrous remodeling in children and adults with EoE, as well as in reducing the consequences of fibrosis in the esophageal distensibility. Research in children documented first that collagen deposition was a reversible phenomenon [188,239,240]. Reduction in epithelial eosinophils was a predictor of resolution of remodeling that accounted, in parallel, for the reduction in TGF-b and pSmad 2/3-positive cells and decrease in vascular activation, as determined by reduced expression of vascular cell adhesion molecule-1 [188]. Subsequent research in adult patients showed that fluticasone propionate use for one year were also able to non-significantly reduce collagen deposits in the esophageal subepithelium despite the treatment induced down regulation of profibrogenic cytokine gene expression [223]. In contrast no changes were noted with low doses of budesonide [241]. The

fact that the drug formulas used were not designed for esophageal targeting or insufficient amounts for esophageal covering were applied might explain the difference among ages. The effectiveness of novel formulas of budesonide specifically developed for EoE [242] in reducing subepithelial fibrosis is yet to be determined.

As for dietary therapy, studies in adult patients have shown its effectiveness in reversing clinical, endoscopic, and histologic features in EoE [120,243,244], but suggest that fibrostenotic phenotype may be less likely to respond [243].

Both elimination diet and topical steroid therapy may improve esophageal distensibility using FLIP together with reducing esophageal eosinophilia [235]. The lasting effect on esophageal distensibility to a complete esophageal recovery is yet to be determined.

Among the investigational therapies in fibrous remodeling, losartan, an angiotensin II receptor blocker approved to treat high blood pressure in children and adults, which has proven safe when administered to patients with normal blood pressure, is currently been tested for EoE. Losartan may reduce the amount of TGF-β thus constituting a potential treatment for fibrosis in EoE. A Phase II trial with increasing doses of losartan is currently underway to evaluate endoscopic, histological and symptomatic improvement [245]. An additional open-label study will assess changes from baseline in peak esophageal eosinophil count and in blood and esophageal TGFβ levels at the end of treatment [246].

8. Expert commentary

Early diagnosis of patients with EoE, providing them with effective therapies and developing non-invasive monitoring methods are currently the most relevant goals for clinicians. Identifying the specific risk factors for developing EoE and defining their relative weight is key to proposing future preventive strategies in populations at risk.

The relative contribution of genes and the environment in the origin of EoE has been analyzed by some studies with different approaches, all assigning a predominant role to the latter [180,184]. The environmental risk factors leading to EoE and the way they interact with the host toward losing immunological tolerance in the esophageal mucosa are still to be revealed [192,203]; its discovery is essential to propose preventive strategies for EoE. The underexplored potential role of esophageal microbiota in mediating the interplay between the environment and the esophageal mucosal surveillance system appears as one the most promising approaches. Changes in the esophageal microbiome composition in adult and pediatric EoE patients compared to non-EoE controls have also been recently described [247,248] while antibiotic-induced changes in the microbiota represents an early life risk factor for developing EoE [192]. Biopsy samples from adults with active EoE have increased bacterial load by 16S expression and upregulation of several TLRs compared to controls which reverse after dietary therapy. Mediators of inflammation in the TLR signaling pathways were also upregulated. Finally, innate immune effector proteins also showed increased activity. All of these corrected after disease remission induced by a dietary intervention [50]. Genotyping of nucleotide polymorphisms (tSNPs) revealed TLR3 as a novel genetic susceptibility locus for developing EoE, with independent effects of TSLP [187].

The discrepancy between symptoms and histopathologic features is one of the major challenges in patients with EoE. Significant esophageal eosinophilia can be present in many patients with minimal symptoms due to food behavior adaptations, and some patients under histologic remission may suffer food impaction episodes due to a reduced esophageal caliber. Endoscopy with biopsies is essential for the initial diagnosis of EoE and the only accurate method for disease monitoring [1]. Identifying reliable non- or minimally invasive markers for EoE is, therefore, urgently required. Several candidate single molecules obtained mainly from blood have been studied in patients with EoE, none of them having provided enough accuracy to be incorporated into clinical practice [249]. However, efforts to identify new EoE biomarkers have rapidly expanded to include complex combination of molecules which could provide a reliable distinction of active EoE from inactive EoE, and both from normal controls and atopic subjects. In fact, a well-preserved EoE transcriptome has facilitated the development of an EoE diagnostic panel that provides the additional advantage of identifying histologically ambiguous subjects who may later develop active EoE [67]. The utility of such a panel to elucidate key elements in EoE, including the potential responsiveness to drug-based or dietary therapies, predicting the disease course, or in identifying atopic patients or relatives at risk of developing EoE, is a potential utility that should be assessed [250].

9. Five-year view

The expansion of EoE and its wide recognition across multiple settings will undoubtedly facilitate in coming years significant advances in the knowledge of the intimate mechanisms of the disease, in the optimization of diagnostic and disease monitoring methods to make them less dependent on endoscopy, and in its therapeutic approach toward personalized medicine.

Minimally invasive methods for patient diagnosis and monitoring are urgently needed in clinical practice; and some preliminary approaches have provided promising results. Among them, substituting endoscopy with biopsies by cytology have been assessed recently. The cytosponge consists of an ingestible gelatin capsule comprising compressed mesh attached to a string, able to obtain cells from the esophageal surface when removed. Its accuracy compared to endoscopy with biopsies has been recently assessed in a multicenter study, which provided a sensitivity and specificity of 75% and 86%, respectively (AUC 0.87) for disease activity, defined by a cutoff of 15 eos/HPF. No complications were reported, and patients preferred cytosponge to endoscopy as a monitoring method [251].

An alternative approach to cytology is to retrieve eosinophil-derived proteins obtained from esophageal exudates. A minimally invasive string-based technology composed of a capsule filled with 10 cm of string, derived from the Enterotest (HDC Corporation, Pilpitas, CA, USA) originally designed to detect gastric and small intestine pathogens, sample bile and assess for GERD was assessed in pediatric patients [252]. The quantities of eosinophil granule proteins in esophageal luminal samples obtained with the esophageal string test significantly correlated with eosinophil counts and granule protein levels in esophageal biopsies; MBP1 and Charcot-Leyden crystal protein indicated a high predictive

power with AUC of 0.97 and 0.97, respectively, compared to biopsies. More recently, the esophageal mucosa was sampled with a cytology brush inserted through a nasogastric tube. Eosinophil-derived neurotoxin (EDN) was measured by ELISA from the samples obtained with the brush, in the samples extracted from brushes and its diagnostic accuracy validated against endoscopic biopsies. A sensitivity of 0.98 and specificity of 0,89 was found, overall providing an AUC of 0.99 [253]. These novel methods suggest that eosinophil-derived proteins are superior to cytology in monitoring esophageal inflammation in patients with EoE.

Key issues

- EoE is a particular form of food allergy associated with a Th2-type inflammatory response that shares common molecular pathways with atopic diseases characterized by IL-5, IL-13 and eotaxins expression.
- The esophageal epithelium is being placed at the center of the pathogenesis of EoE: an impaired barrier function related to a depletion of SPINK7 determines an increased permeability, allowing an enhanced contact between mucosal immune system and component of the diet or microbiota.
- Epithelial cell-derived TSLP activates antigen presenting cells in EoE to polarize T cells toward a Th2-type response with secretion of IL-13.
- IL-13 upregulates CAPN1 in the esophageal epithelium, a protease with an important role for epithelial barrier function which is involved in repairing IL-13-induced epithelial changes. CAPN14 is also implicated in the down-regulation of DSG1.
- Several genes and variants providing susceptibility to EoE have been identified, overall contributing modestly to disease susceptibility. In contrast, environmental factors including perinatal and early life exposures are mainly involved in determining risk for EoE.
- Interactions of epithelial cells with components of the esophageal microbiota modulate the expression of CXCL16 and recruit invariant natural killer T (iNKT) cells toward the esophageal epithelium, in an early stage of EoE development.
- Mediators released from activated mast cells and eosinophils induce epithelial mesenchymal transition leading to esophageal remodeling by subepithelial deposition of collagen and other extracellular matrix components, the reversion of which is being increasingly recognized as a clinically relevant target for therapy.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

ORCID

Ángel Arias http://orcid.org/0000-0003-1006-0958
Alfredo J. Lucendo http://orcid.org/0000-0003-1183-1072

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Lucendo AJ, Molina-Infante J, Arias A, et al. Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. United Eur Gastroenterol J. 2017;5(3):335–358.
•• **First evidence-based guidelines for the diagnosis and management of eosinophilic esophagitis (EoE) patients developed following GRADE methodology, which cover topics related with EoE concept and epidemiology, diagnosis, natural history and treatment.**
2. Attwood SE, Smyrk TC, Demeester TR, et al. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. Dig Dis Sci. 1993;38(1):109–116.
3. Straumann A, Spichtin HP, Bernoulli R, et al. Idiopathic eosinophilic esophagitis: a frequently overlooked disease with typical clinical aspects and discrete endoscopic findings. Schweiz Med Wochenschr. 1994;124 (33):1419–1429.
4. Arias A, Pérez-Martínez I, Tenías JM, et al. Systematic review with meta-analysis: the incidence and prevalence of eosinophilic oesophagitis in children and adults in population-based studies. Aliment Pharmacol Ther. 2016;43(1):3–15.
5. Dellon ES, Epidemiology HI. Natural history of eosinophilic esophagitis. Gastroenterology. 2018;154(2):319–332.
6. Molina-Infante J, Gonzalez-Cordero PL, Ferreira-Nossa HC, et al. Rising incidence and prevalence of adult eosinophilic esophagitis in midwestern Spain (2007–2016). United Eur Gastroenterol J. 2018;6(1):29–37.
7. Á A, Lucendo AJ. Incidence and prevalence of eosinophilic oesophagitis increase continiously in adults and children in Central Spain: A 12-year population-based study. Dig Liver Dis. 2018. DOI:10.1016/j.dld.2018.07.016
8. Jensen ET, Kappelman MD, Martin CF, et al. Health-care utilization, costs, and the burden of disease related to eosinophilic esophagitis in the United States. Am J Gastroenterol. 2015;110(5):626–632.
9. Spechler SJ. Speculation as to why the frequency of eosinophilic esophagitis is increasing. Curr Gastroenterol Rep. 2018;20(6):26.
10. Schoepfer AM, Panczak R, Zwahlen M, et al. How do gastroenterologists assess overall activity of eosinophilic esophagitis in adult patients? Am J Gastroenterol. 2015;110(3):402–414.
11. Lucendo AJ, Arias T, Molina-Infante J, et al. Diagnostic and therapeutic management of eosinophilic oesophagitis in children and adults: results from a Spanish registry of clinical practice. Dig Liver Dis. 2013;45(7):562–568.
12. Harris BR, Hon E, Gupta SK. Implementation of guidelines in eosinophilic esophagitis at an academic pediatric practice. Dis Esophagus. 2018. DOI:10.1093/dote/doy029
13. Peery AF, Shaheen NJ, Dellon ES. Practice patterns for the evaluation and treatment of eosinophilic oesophagitis. Aliment Pharmacol Ther. 2010;32(11–12):1373–1382.
14. Wallach T, Genta RM, Lebwohl B, et al. Adherence to celiac disease and eosinophilic esophagitis biopsy guidelines is poor in children. J Pediatr Gastroenterol Nutr. 2017;65(1):64–68.
15. Svensson-Frej M. Immunobiology of intestinal eosinophils - a dogma in the changing? J Innate Immun. 2011;3(6):565–576.
16. Johnsson M, Bove M, Bergquist H, et al. Distinctive blood eosinophilic phenotypes and cytokine patterns in eosinophilic

esophagitis, inflammatory bowel disease and airway allergy. J Innate Immun. 2011;3(6):594–604.
• **Blood circulating eosinophils acquire varying functional properties, manifested by a differential phenotype, in Eosinophilic esophagitis (EoE), bronchial asthma and ulcerative colitis, as consequence of different plasmatic patterns of proeosinophilic cytokines.**
17. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). J Allergy Clin Immunol. 2004;113(1):11–28.
18. Hogan SP, Rothenberg ME. Review article: the eosinophil as a therapeutic target in gastrointestinal disease. Aliment Pharmacol Ther. 2004;20(11–12):1231–1240.
19. Mishra A, Hogan SP, Brandt EB, et al. An etiological role for aeroallergens and eosinophils in experimental esophagitis. J Clin Invest. 2001;107(1):83–90.
20. Mishra A, Hogan SP, Brandt EB, et al. IL-5 promotes eosinophil trafficking to the esophagus. J Immunol. 2002;168(5):2464–2469.
21. Akei HS, Mishra A, Blanchard C, et al. Epicutaneous antigen exposure primes for experimental eosinophilic esophagitis in mice. Gastroenterology. 2005;129(3):985–994.
22. Molina-Infante J, Rivas MD, Hernandez-Alonso M, et al. Proton pump inhibitor-responsive oesophageal eosinophilia correlates with downregulation of eotaxin-3 and Th2 cytokines overexpression. Aliment Pharmacol Ther. 2014;40(8):955–965.
• **Fifty-three consecutive patients with symptoms of esophageal dysfunction and tissue eosinophilia were defined as EoE or proton pump inhibitor-responsive esophageal eosinophilia (PPI-REE) diagnoses. Baseline cytokine gene expression in PPI-REE was nearly indistinguishable from EoE. PPI therapy significantly downregulated oesophageal eotaxin-3/Th2-cytokine gene expression in PPI-REE, similarly to that seen in steroid-responsive EoE.**
23. Straumann A, Bauer M, Fischer B, et al. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. J Allergy Clin Immunol. 2001;108(6):954–961.
24. Yamazaki K, Murray JA, Arora AS, et al. Allergen-specific in vitro cytokine production in adult patients with eosinophilic esophagitis. Dig Dis Sci. 2006;51(11):1934–1941.
25. Bullock JZ, Villanueva JM, Blanchard C, et al. Interplay of adaptive Th2 immunity with eotaxin-3/c-C chemokine receptor 3 in eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2007;45(1):22–31.
• **This research explains the development of EoE as cooperation between systemic CD4+ Th2-cell-mediated immunity and an enhanced eosinophil-CCR3/eotaxin3 pathway and demonstrates the reversibility of increased CCR3 expression after disease remission.**
26. Lingblom C, Wallander J, Ingelsten M, et al. Eosinophils from eosinophilic oesophagitis patients have T cell suppressive capacity and express FOXP3. Clin Exp Immunol. 2017;187(3):455–465.
27. Assa'ad AH, Gupta SK, Collins MH, et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. Gastroenterology. 2011;141(5):1593–1604.
28. Straumann A, Conus S, Grzonka P, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: A randomised, placebo-controlled, double-blind trial. Gut. 2010;59(1):21–30.
29. Spergel JM, Rothenberg ME, Collins MH, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol. 2012;129(2):456–463.
30. Markowitz JE, Jobe L, Miller M, et al. Safety and efficacy of reslizumab for children and adolescents with eosinophilic esophagitis treated for 9 years. J Pediatr Gastroenterol Nutr. 2018;66(6):893–897.
• **An open-label extension of a trial with reslizumab in children, showing that symptoms and eosinophil count improved considerably during treatment despite a relatively unrestricted diet.**
31. Straumann A, Hoesli S, Bussmann C, et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. Allergy. 2013;68(3):375–385.
32. Allaire JM, Crowley SM, Law HT, et al. The intestinal epithelium: central coordinator of mucosal immunity. Trends Immunol. 2018. DOI:10.1016/j.it.2018.04.002
33. Arebro J, Tengroth L, Razavi R, et al. Antigen-presenting epithelial cells can play a pivotal role in airway allergy. J Allergy Clin Immunol. 2016;137(3):957–960.
34. Goto Y, Ivanov II. Intestinal epithelial cells as mediators of the commensal-host immune crosstalk. Immunol Cell Biol. 2013;91(3):204–214.
35. Mulder DJ, Pooni A, Mak N, et al. Antigen presentation and MHC class II expression by human esophageal epithelial cells: role in eosinophilic esophagitis. Am J Pathol. 2011;178(2):744–753.
36. Sayej WN, Foster C, Jensen T, et al. Expanding and characterizing esophageal epithelial cells obtained from children with eosinophilic esophagitis. Pediatr Res. 2018;84(2):306-313.
37. Wu L, Oshima T, Li M, et al. Filaggrin and tight junction proteins are crucial for IL-13-mediated esophageal barrier dysfunction. Am J Physiol Gastrointest Liver Physiol. 2018;315(3):G341-G350.
• **The permeability of tight junctions (TJ) in cultured primary human esophageal epithelial cells was assayed by biotinylation. Transepithelial electrical resistance was measured after stimulation with IL-13 and after silencing the expression of of *FLG* gene. IL-13 reduced the esophageal barrier by causing deficiency of FGL. An altered expression pattern, but not reduction in the levels of TJ-associated proteins, was documented.**
38. Lucendo AJ, Navarro M, Comas C, et al. Immunophenotypic characterization and quantification of the epithelial inflammatory infiltrate in eosinophilic esophagitis through stereology: an analysis of the cellular mechanisms of the disease and the immunologic capacity of the esophagus. Am J Surg Pathol. 2007;31(4):598–606.
39. Rusin S, Covey S, Perjar I, et al. Determination of esophageal eosinophil counts and other histologic features of eosinophilic esophagitis by pathology trainees is highly accurate. Hum Pathol. 2017;62:50–55.
40. Straumann A, Spichtin H-P, Bucher KA, et al. Eosinophilic esophagitis: red on microscopy, white on endoscopy. Digestion. 2004;70(2):109–116.
41. Simon D, Radonjic-Hosli S, Straumann A, et al. Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation. Allergy. 2015;70(4):443–452.
•• **Esophageal samples from EoE patients showed a reduced expression of filaggrin and lympho-epithelial Kazal-type-related inhibitor, together with elevated leves of epithelial antimicrobial peptides (human beta-defensin-2, human beta-defensin-3, cathelicidin LL-37, psoriasin) and cytokines (TSLP, IL-25, IL-32, IL-33) as compared to normal esophageal tissues.**
42. Nguyen N, Fernando SD, Biette KA, et al. TGF-β1 alters esophageal epithelial barrier function by attenuation of claudin-7 in eosinophilic esophagitis. Mucosal Immunol. 2018;11(2):415–426.
• **The effect of TGF-β1 on the esophageal epithelial barrier was assessed in a model of EoE based in immortalized esophageal cell cultures. In vitro, TGF-β1 participates in esophageal epithelial barrier dysfunction through CLDN7 dysregulation.**
43. Abdulnour-Nakhoul SM, Al-Tawil Y, Gyftopoulos AA, et al. Alterations in junctional proteins, inflammatory mediators and extracellular matrix molecules in eosinophilic esophagitis. Clin Immunol. 2013;148(2):265–278.
44. Katzka DA, Tadi R, Smyrk TC, et al. Effects of topical steroids on tight junction proteins and spongiosis in esophageal epithelia of patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2014;12(11):1824–1829.
• **Treatment with topic fluticasone propionate was able to restore expression of Filaggrin, ZO-3, and claudin-1 (but not ZO-1 or ZO-2) to the levels shown by healthy donors, improving spongiosis and dilated intercellular spaces.**
45. Rochman M, Travers J, Abonia JP, et al. Synaptopodin is upregulated by IL-13 in eosinophilic esophagitis and regulates esophageal epithelial cell motility and barrier integrity. JCI Insight. 2017;2:20.

46. Azouz NP, Ynga-Durand MA, Caldwell JM, et al. The antiprotease SPINK7 serves as an inhibitory checkpoint for esophageal epithelial inflammatory responses. Sci Transl Med. 2018;10:444.
•• **First characterization of serine peptidase inhibitor, kazal type 7 (SPINK7) as the homeostatic mechanism that controls barrier function and inflammatory responses in EoE. This disease is shown to be characterized by a nearly absent expression of SPINK7, leading to barrier dysfunction and increased tissue permeability.**
47. Katzka DA, Geno DM, Blair HE, et al. Small intestinal permeability in patients with eosinophilic oesophagitis during active phase. Gut. 2015;64(4):538–543.
48. Van Rhijn BD, Kessing BF, Smout AJ, et al. Oesophageal baseline impedance values are decreased in patients with eosinophilic oesophagitis. United Eur Gastroenterol J. 2013;1(4):242–248.
49. Ravelli A, Villanacci V, Cadei M, et al. Dilated intercellular spaces in eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2014;59 (5):589–593.
50. Arias A, Vicario M, Bernardo D, et al. Toll-like receptors-mediated pathways activate inflammatory responses in the esophageal mucosa of adult eosinophilic esophagitis. Clin Transl Gastroenterol. 2018;9(4):147.
•• **First study examining the potential role of TLRs in the pathophysiology of EoE, demonstrating that active EoE characterizes by upregulated esophageal expression of TLRs compared to healthy controls, as well as that of transcription factors and subsequent effectors of the TLRs signaling pathway. Changes were restored to control levels after effective dietary treatment.**
51. Marietta EV, Geno DM, Smyrk TC, et al. Presence of intraepithelial food antigen in patients with active eosinophilic oesophagitis. Aliment Pharmacol Ther. 2017;45(3):427–433.
• **First results suggesting that an impaired epithelial integrity in active EoE might facilitate antigen penetration. Patients with active EoE had significantly greater epithelial density of antigliadin staining when compared to inactive EoE and patients on a gluten-free diet.**
52. Redhu NS, Gounni AS. Function and mechanisms of TSLP/TSLPR complex in asthma and COPD. Clin Exp Allergy. 2012;42(7):994–1005.
53. Mu Z, Zhao Y, Liu X, et al. Molecular biology of atopic dermatitis. Clin Rev Allergy Immunol. 2014;47(2):193–218.
54. Park JH, Jeong DY, Peyrin-Biroulet L, et al. Insight into the role of TSLP in inflammatory bowel diseases. Autoimmun Rev. 2017;16 (1):55–63.
55. Noti M, Wojno EDT, Kim BS, et al. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. Nat Med. 2013;19 (8):1005–1013.
56. Rothenberg ME. Molecular, genetic, and cellular bases for treating eosinophilic esophagitis. Gastroenterology. 2015;148(6):1143–1157.
• **A review on the molecular and genetic aspects of EoE, considered as a food hypersensitivity with a strong hereditability influenced by early-life exposures and esophageal-specific genetic risk variants, among whom those identified at 5q22 (TSLP) and 2p23 (CAPN14), have essential roles for allergic sensitization and esophageal specific protease pathways.**
57. Schmid-Grendelmeier P, Altznauer F, Fischer B, et al. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. J Immunol. 2002;169(2):1021–1027.
58. Blanchard C, Mingler MK, Vicario M, et al. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. J Allergy Clin Immunol. 2007;120(6):1292–1300.
•• **Confirms the central role played by IL-13 in the development of EoE. Furthermore, gene expression changes were reversed after topical steroid treatment in parallel to a significant reduction in IL-13 mRNA expression levels.**
59. Neilsen CV, Bryce PJ. Interleukin-13 directly promotes oesophagus production of CCL11 and CCL24 and the migration of eosinophils. Clin Exp Allergy. 2010;40(3):427–434.
• **By using isolated esophageal rings treated in vitro with IL-4 or IL-13, the authors demonstrated that esophageal production of CCL11 and CCL24 upon IL-13 stimulation is sufficient to promote eosinophil migration.**
60. Blanchard C, Stucke EM, Burwinkel K, et al. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. J Immunol. 2010;184(7):4033–4041.
61. Sherrill JD, Kc K, Wu D, et al. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. Mucosal Immunol. 2014;7(3):718–729.
• **This study identifies a dysregulation of intercellular adhesion molecule desmoglein-1 caused by IL-13 in patients with EoE. The loss of desmoglein-1 contributes to potentiate allergic inflammation through the induction of proinflammatory mediators.**
62. Litosh VA, Rochman M, Rymer JK, et al. Calpain-14 and its association with eosinophilic esophagitis. J Allergy Clin Immunol. 2017;139 (6):1762–1771.
• **A thorough review on the genetic, structural, and biochemical properties of *CAPN14* and its gene product CAPN14, and its emerging role in patients with EoE.**
63. Davis BP, Stucke EM, Khorki ME, et al. Eosinophilic esophagitis-linked calpain 14 is an IL-13-induced protease that mediates esophageal epithelial barrier impairment. JCI Insight. 2016;1(4):e86355.
•• **Cultured epithelial cell treated with IL-13 overexpressed CAPN14 displayed impaired epithelial architecture and epithelial barrier function, as well as loss of DSG1 expression. The IL-13-induced loss of DSG1 was normalized by *CAPN14* gene silencing.**
64. Lucendo AJ, Molina-Infante J. Limitation of symptoms as predictors of remission in eosinophilic esophagitis: the need to go beyond endoscopy and histology. Gastroenterology. 2016;150(3):547–549.
65. Nielsen JA, Lager DJ, Lewin M, et al. The optimal number of biopsy fragments to establish a morphologic diagnosis of eosinophilic esophagitis. Am J Gastroenterol. 2014;109(4):515–520.
66. Blanchard C, Wang N, Stringer KF, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest. 2006;116(2):536–547.
•• **A seminal research that led to the development of an EoE-specific transcriptome in which eotaxin-3 was the most upregulated gene.**
67. Wen T, Stucke EM, Grotjan TM, et al. Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. Gastroenterology. 2013;145 (6):1289–1299.
•• **The development of a new molecular diagnostic test capable of identifying patients with EoE and distinguishing them from other conditions through a fast, objective and mechanistic method.**
68. Dellon ES, Veerappan R, Selitsky SR, et al. A gene expression panel is accurate for diagnosis and monitoring treatment of eosinophilic esophagitis in adults. Clin Transl Gastroenterol. 2017;8(2):e74.
69. Warners MJ, Vlieg-Boerstra BJ, Verheij J, et al. Esophageal and small intestinal mucosal integrity in eosinophilic esophagitis and response to an elemental diet. Am J Gastroenterol. 2017;112(7):1061–1071.
70. van Rhijn BD, Weijenborg PW, Verheij J, et al. Proton pump inhibitors partially restore mucosal integrity in patients with proton pump inhibitor-responsive esophageal eosinophilia but not eosinophilic esophagitis. Clin Gastroenterol. 2014;12 (11):1815–1823.
71. van Rhijn BD, Verheij J, van Den Bergh Weerman MA, et al. Histological response to fluticasone propionate in patients with eosinophilic esophagitis Is associated with improved functional esophageal mucosal integrity. Am J Gastroenterol. 2015;110 (9):1289–1297.
72. Katzka DA, Ravi K, Geno DM, et al. Endoscopic mucosal impedance measurements correlate with eosinophilia and dilation of intercellular spaces in patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2015;13(7):1242–1248.
73. Zhang F, Huang G, Hu B, et al. A soluble thymic stromal lymphopoietin (TSLP) antagonist, TSLPR-immunoglobulin, reduces the severity of allergic disease by regulating pulmonary dendritic cells. Clin Exp Immunol. 2011;164(2):256–264.

74. Shi L, Leu S-W, Xu F, et al. Local blockade of TSLP receptor alleviated allergic disease by regulating airway dendritic cells. Clin Immunol. 2008;129(2):202–210.
75. Al-Shami A, Spolski R, Kelly J, et al. A role for TSLP in the development of inflammation in an asthma model. J Exp Med. 2005;202 (6):829–839.
76. Corren J, Parnes JR, Wang L, et al. Tezepelumab in adults with uncontrolled asthma. N Engl J Med. 2017;377(10):936–946.
77. Wong CK, Hu S, Cheung PFY, et al. Thymic stromal lymphopoietin induces chemotactic and prosurvival effects in eosinophils: implications in allergic inflammation. Am J Respir Cell Mol Biol. 2010;43(3):305–315.
78. Cianferoni A, Spergel J. The importance of TSLP in allergic disease and its role as a potential therapeutic target. Expert Rev Clin Immunol. 2014;10(11):1463–1474.
79. Rothenberg ME, Wen T, Greenberg A, et al. Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis. J Allergy Clin Immunol. 2015;135(2):500–507.
80. Vatrella A, Fabozzi I, Calabrese C, et al. Dupilumab: a novel treatment for asthma. J Asthma Allergy. 2014;7:123–130.
81. Wenzel SE, Wang L, Pirozzi G. Dupilumab in persistent asthma. N Engl J Med. 26 de. 2013;369(13):1276.
82. Beck LA, Thaçi D, Hamilton JD, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. N Engl J Med. 2014;371 (2):130–139.
83. Sastre J, Dávila I. Dupilumab: A new paradigm for the treatment of allergic diseases. J Investig Allergol Clin Immunol. 2018;28(3):139–150.
84. Sanofi. Study of Dupilumab in Adult Patients With Active Eosinophilic Esophagitis (EoE), Identifier: NCT02379052. ClinicalTrials.gov; [cited 2017 Dec 3]. Available from: https://clinicaltrials.gov/.
85. Sanofi-Regeneron. Sanofi and Regeneron Announce Positive Phase 2 Study Results for Dupilumab in Patients With Active Moderate-to-Severe Eosinophilic Esophagitis. Press Release. 2017 Oct 16.
86. Molina-Infante J, Lucendo AJ. Dietary therapy for eosinophilic esophagitis. J Allergy Clin Immunol. 2018;142(1):41–47.
87. Lucendo AJ, Arias A, Redondo-Gonzalez O, et al. Seasonal distribution of initial diagnosis and clinical recrudescence of eosinophilic esophagitis: a systematic review and meta-analysis. Allergy. 2015;70(12):1640–1650.
88. Guajardo JR, Zegarra-Bustamante MA, Brooks EG. Does aeroallergen sensitization cause or contribute to eosinophilic esophagitis? Clin Rev Allergy Immunol. 2018;55(1):65–69.
89. Simon D, Marti H, Heer P, et al. Eosinophilic esophagitis is frequently associated with IgE-mediated allergic airway diseases. J Allergy Clin Immunol. 2005;115(5):1090–1092.
90. Pelz BJ, Wechsler JB, Amsden K, et al. IgE-associated food allergy alters the presentation of paediatric eosinophilic esophagitis. Clin Exp Allergy. 2016;46(11):1431–1440.
91. González-Cervera J, Arias Á, Redondo-González O, et al. Association between atopic manifestations and eosinophilic esophagitis: A systematic review and meta-analysis. Ann Allergy Asthma Immunol. 2017;118(5):582–590.
92. Kelly KJ, Lazenby AJ, Rowe PC, et al. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. Gastroenterology. 1995;109(5):1503–1512.
93. Hill DA, Grundmeier RW, Ramos M, et al. Eosinophilic esophagitis is a late manifestation of the allergic march. J Allergy Clin Immunol Pract. 2018.DOI:10.1016/j.jaip.2018.05.010
• **The authors used primary care birth cohort of 130,435 children to determine the natural histories of atopic dermatitis, IgE-mediated food allergy, asthma, EoE, and allergic rhinitis in individual patients. A case-control analysis found that allergic comorbidities were positively associated with EoE diagnosis, suggesting that EoE could be a late manifestation of the allergic march.**
94. Park HJ, Lee SW, Hong S. Regulation of allergic immune responses by microbial metabolites. Immune Netw. 2018;18(1):e15.
95. Okada H, Kuhn C, Feillet H, et al. The «hygiene hypothesis» for autoimmune and allergic diseases: an update. Clin Exp Immunol. 2010;160(1):1–9.
96. Kramer A, Bekeschus S, Bröker BM, et al. Maintaining health by balancing microbial exposure and prevention of infection: the hygiene hypothesis versus the hypothesis of early immune challenge. J Hosp Infect. 2013;83(Suppl 1):S29–S34.
97. La Cava A, Van Kaer L, Fu-Dong-Shi. CD4+CD25+ Tregs and NKT cells: regulators regulating regulators. Trends Immunol. 2006;27 (7):322–327.
98. Monteiro M, Graca L. iNKT cells: innate lymphocytes with a diverse response. Crit Rev Immunol. 2014;34(1):81–90.
99. Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science. 2012;336(6080):489–493.
100. Wingender G, Stepniak D, Krebs P, et al. Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. Gastroenterology. 2012;143(2):418–428.
101. Jensen ET, Kappelman MD, Kim HP, et al. Early life exposures as risk factors for pediatric eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2013;57(1):67–71.
102. Garn H, Renz H. Epidemiological and immunological evidence for the hygiene hypothesis. Immunobiology. 2007;212(6):441–452.
103. Lexmond WS, Neves JF, Nurko S, et al. Involvement of the iNKT cell pathway is associated with early-onset eosinophilic esophagitis and response to allergen avoidance therapy. Am J Gastroenterol. 2014;109(5):646–657.
•• **This study highlights the central place of invariant natural killer T in the allergic inflammation associated with EoE, providing evidence of the involvement of an early restriction to the exposition to microbial factors in determining EoE susceptibility.**
104. Jyonouchi S, Smith CL, Saretta F, et al. Invariant natural killer T cells in children with eosinophilic esophagitis. Clin Exp Allergy. 2014;44 (1):58–68.
• **An ex-vivo study that suggests that sphingolipids contained in milk may drive the development of EoE by promoting an iNKT-cell-mediated Th2-type cytokine response and facilitate eosinophil-mediated allergic inflammation.**
105. Kinjo Y, Tupin E, Wu D, et al. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat Immunol. 2006;7(9):978–986.
106. Wieland Brown LC, Penaranda C, Kashyap PC, et al. Production of α-galactosylceramide by a prominent member of the human gut microbiota. PLoS Biol. 2013;11(7):e1001610.
107. Rayapudi M, Rajavelu P, Zhu X, et al. Invariant natural killer T-cell neutralization is a possible novel therapy for human eosinophilic esophagitis. Clin Transl Immunol. 2014;3(1):e9.
108. Rajavelu P, Rayapudi M, Moffitt M, et al. Significance of para-esophageal lymph nodes in food or aeroallergen-induced iNKT cell-mediated experimental eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol. 2012;302(7):G645–G654.
109. Mishra A, Schlotman J, Wang M, et al. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. J Leukoc Biol. 2007;81(4):916–924.
110. Garman SC, Wurzburg BA, Tarchevskaya SS, et al. Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha. Nature. 2000;406(6793):259–266.
111. Philpott H, Thien F. The role of allergy testing in eosinophilic esophagitis: an update of the evidence. Curr Treat Options Gastroenterol 2017;15 (1):26–34.
112. Vicario M, Blanchard C, Stringer KF, et al. Local B cells and IgE production in the oesophageal mucosa in eosinophilic oesophagitis. Gut. 2010;59 (1):12–20.
•• **Demonstrates local immunoglobulin class switching to IgE and IgE production in the esophageal mucosa of EoE patients as a significant mechanism contributing to disease pathogenesis. IgE-bearing cells were not only mast cells, but others, a fact that suggest that new IgE-mediated regulatory effects aside of anaphylactic mast cell degranulation should be further studied in EoE.**
113. Takhar P, Corrigan CJ, Smurthwaite L, et al. Class switch recombination to IgE in the bronchial mucosa of atopic and nonatopic patients with asthma. J Allergy Clin Immunol. 2007;119(1):213–218.
114. Takhar P, Smurthwaite L, Coker HA, et al. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. J Immunol. 2005;174 (8):5024–5032.

115. Teitelbaum JE, Fox VL, Twarog FJ, et al. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. Gastroenterology. 2002;122(5):1216–1225.
116. Simon D, Straumann A, Wenk A, et al. Eosinophilic esophagitis in adults–no clinical relevance of wheat and rye sensitizations. Allergy. 2006;61(12):1480–1483.
117. Arias A, Gonzalez-Cervera J, Tenias JM, et al. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: a systematic review and meta-analysis. Gastroenterology. 2014;146(7):1639–1648.
118. Castro Jimenez A, Gomez Torrijos E, Garcia Rodriguez R, et al. Demographic, clinical and allergological characteristics of eosinophilic esophagitis in a Spanish central region. Allergol Immunopathol (Madr). 2014;42(5):407–414.
119. Vernon N, Shah S, Lehman E, et al. Comparison of atopic features between children and adults with eosinophilic esophagitis. Allergy Asthma Proc. 2014;35(5):409–414.
120. Lucendo AJ, Arias A, Gonzalez-Cervera J, et al. Empiric 6-food elimination diet induced and maintained prolonged remission in patients with adult eosinophilic esophagitis: a prospective study on the food cause of the disease. J Allergy Clin Immunol. 2013;131(3):797–804.
121. Miller RL, Peden DB. Environmental effects on immune responses in patients with atopy and asthma. J Allergy Clin Immunol. 2014;134(5):1001–1008.
122. Clayton F, Fang JC, Gleich GJ, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. Gastroenterology. 2014;147(3):602–609.
•• **A prospective trial with the anti-IgE blocker omalizumab did not reduce symptoms or tissue eosinophil counts in adults with EoE compared with placebo. More interestingly, granular deposits of IgG4, abundant IgG4-containing plasma cells, and serum levels of IgG4 reactive to specific foods were observed in patients with EoE, indicating that EoE is IgG4-associated, and not an IgE-induced allergy.**
123. Mohammad N, Avinashi V, Chan E, et al. Pediatric eosinophilic esophagitis is associated with increased lamina propria immunoglobulin G4-positive plasma cells. J Pediatr Gastroenterol Nutr. 2018;67(2):204–209.
124. Schuyler AJ, Wilson JM, Tripathi A, et al. Specific IgG4 antibodies to cow's milk proteins in pediatric patients with eosinophilic esophagitis. J Allergy Clin Immunol. 2018;142(1):139–148.
125. Rosenberg CE, Mingler MK, Caldwell JM, et al. Esophageal IgG4 levels correlate with histopathologic and transcriptomic features in eosinophilic esophagitis. Allergy.2018;73(9):1892-1901.
• **A research on pediatric esophageal samples that shows IgG4 levels closely related to esophageal eosinophil counts, type 2 immunity and T regulatory cytokines.**
126. Wright BL, Kulis M, Guo R, et al. Food-specific IgG4 is associated with eosinophilic esophagitis. J Allergy Clin Immunol. 2016;138(4):1190–1192.
127. Aalberse RC, Stapel SO, Schuurman J, et al. Immunoglobulin G4: an odd antibody. Clin Exp Allergy. 2009;39(4):469–477.
128. Aalberse RC, Platts-Mills TA, Rispens T. The developmental history of IgE and IgG4 antibodies in relation to atopy, eosinophilic esophagitis, and the modified TH2 response. Curr Allergy Asthma Rep. 2016;16(6):45.
129. van der Zee JS, van Swieten P, Aalberse RC. Serologic aspects of IgG4 antibodies. II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency. J Immunol. 1986;137(11):3566–3571.
130. Feuille E, Nowak-Wegrzyn A. Allergen-specific immunotherapies for food allergy. Allergy Asthma Immunol Res. 2018;10(3):189–206.
131. Lucendo AJ, Arias A, Tenias JM. Relation between eosinophilic esophagitis and oral immunotherapy for food allergy: a systematic review with meta-analysis. Ann Allergy Asthma Immunol. 2014;113(6):624–629.
132. Chu VT, Berek C. The establishment of the plasma cell survival niche in the bone marrow. Immunol Rev. 2013;251(1):177–188.
133. Sawas T, Dhalla S, Sayyar M, et al. Systematic review with meta-analysis: pharmacological interventions for eosinophilic oesophagitis. Aliment Pharmacol Ther. 2015;41(9):797–806.
134. Murali AR, Gupta A, Attar BM, et al. Topical steroids in eosinophilic esophagitis: systematic review and meta-analysis of placebo-controlled randomized clinical trials. J Gastroenterol Hepatol. 2016;31(6):1111–1119.
135. Tan ND, Xiao YL, Chen MH. Steroids therapy for eosinophilic esophagitis: systematic review and meta-analysis. J Dig Dis. 2015;16(8):431–442.
136. Chuang M-YA, Chinnaratha MA, Hancock DG, et al. Topical steroid therapy for the treatment of eosinophilic esophagitis (EoE): a systematic review and meta-analysis. Clin Transl Gastroenterol. 2015;6:e82.
137. Schaefer ET, Fitzgerald JF, Molleston JP, et al. Comparison of oral prednisone and topical fluticasone in the treatment of eosinophilic esophagitis: a randomized trial in children. Clin Gastroenterol Hepatol. 2008;6(2):165–173.
138. Lucendo AJ, De Rezende L, Comas C, et al. Treatment with topical steroids downregulates IL-5, eotaxin-1/CCL11, and eotaxin-3/CCL26 gene expression in eosinophilic esophagitis. Am J Gastroenterol. 2008;103(9):2184–2193.
139. Caldwell JM, Blanchard C, Collins MH, et al. Glucocorticoid-regulated genes in eosinophilic esophagitis: a role for FKBP51. J Allergy Clin Immunol. 2010;125(4):879–888.
140. Wen T, Dellon ES, Moawad FJ, et al. Transcriptome analysis of proton pump inhibitor-responsive esophageal eosinophilia reveals proton pump inhibitor-reversible allergic inflammation. J Allergy Clin Immunol. 2015;135(1):187–197.
•• **An evaluation of the EoE diagnostic panel signature in biopsy samples obtained from adult and pediatric patients with PPI-REE and compared the pre- and post-PPI therapy expression profiles of these subjects with those of patients with active EoE provides definitive evidence that PPI-REE exhibits a significant molecular overlap with EoE, suggesting that both entities represent a continuum of the same pathogenic allergic mechanisms.**
141. Shoda T, Matsuda A, Nomura I, et al. Eosinophilic esophagitis versus proton pump inhibitor-responsive esophageal eosinophilia: transcriptome analysis. J Allergy Clin Immunol. 2017;139(6):2010–2013.
142. Zhang X, Cheng E, Huo X, et al. Omeprazole blocks STAT6 binding to the eotaxin-3 promoter in eosinophilic esophagitis cells. PloS One. 2012;7(11):e50037.
143. Cheng E, Zhang X, Huo X, et al. Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. Gut. 2013;62(6):824–832.
•• **Eotaxin-3 protein secretion stimulated by Th2 cytokines IL-4 and IL-13 was measured in the presence or absence of omeprazole. Esophageal squamous cells from GERD and EoE patients expressed similar levels of eotaxin-3 when stimulated by Th2 cytokines, and omeprazole blocked that eotaxin-3 expression. These findings suggest that PPIs might have eosinophil-reducing effects independent of effects on acid reflux and that response to PPIs might not distinguish EoE from GERD.**
144. Weidner N, Austen KF. Heterogeneity of mast cells at multiple body sites. Fluorescent determination of avidin binding and immunofluorescent determination of chymase, tryptase, and carboxypeptidase content. Pathol Res Pract. 1993;189(2):156–162.
145. Gri G, Frossi B, D'Inca F, et al. Mast cell: an emerging partner in immune interaction. Front Immunol. 2012;3:120.
146. Arias Á, Lucendo AJ, Martínez-Fernández P, et al. Dietary treatment modulates mast cell phenotype, density, and activity in adult eosinophilic oesophagitis. Clin Exp Allergy. 2016;46(1):78–91.
• **A paper that defining that the predominant phenotype of mast cells the esophageal mucosa was MC_{TC} subtype, and they were modulated through dietary therapy. Epithelial densities of mast cells and eosinophils were significantly associated and correlated with the symptom score, as well as gene expression of mast cell proteases.**
147. Schwartz LB. Mast cells and basophils. Clin Allergy Immunol. 2002;16:3–42.

148. Austen KF, Boyce JA. Mast cell lineage development and phenotypic regulation. Leuk Res. 2001;25(7):511–518.
149. Abonia JP, Blanchard C, Butz BB, et al. Involvement of mast cells in eosinophilic esophagitis. J Allergy Clin Immunol. 2010;126(1):140–149.
150. Niranjan R, Mavi P, Rayapudi M, et al. Pathogenic role of mast cells in experimental eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol. 2013;304(12):G1087–G1094.
151. Mavi P, Rajavelu P, Rayapudi M, et al. Esophageal functional impairments in experimental eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol. 2012;302(11):G1347–G1355.
152. Martín-Muñoz MF, Lucendo AJ, Navarro M, et al. Food allergies and eosinophilic esophagitis–two case studies. Digestion. 2006;74(1):49–54.
153. Kirsch R, Bokhary R, Marcon MA, et al. Activated mucosal mast cells differentiate eosinophilic (allergic) esophagitis from gastroesophageal reflux disease. J Pediatr Gastroenterol Nutr. 2007;44(1):20–26.
154. Gupta SK, Fitzgerald JF, Kondratyuk T, et al. Cytokine expression in normal and inflamed esophageal mucosa: a study into the pathogenesis of allergic eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2006;42(1):22–26.
155. Aceves SS, Chen D, Newbury RO, et al. Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF-β1, and increase esophageal smooth muscle contraction. J Allergy Clin Immunol. 2010;126(6):1198–1204.e4.
156. Lomazi EA, Brandalise NA, Servidoni MFPC, et al. Mast cells distinguish eosinophilic esophagitis in pediatric patients. Arq Gastroenterol. 2017;54(3):192–196.
157. Mueller S, Neureiter D, Aigner T, et al. Comparison of histological parameters for the diagnosis of eosinophilic oesophagitis versus gastro-oesophageal reflux disease on oesophageal biopsy material. Histopathology. 2008;53(6):676–684.
158. Tappata M, Eluri S, Perjar I, et al. Association of mast cells with clinical, endoscopic, and histologic findings in adults with eosinophilic esophagitis. Allergy. 2018;73(10):2088-2092.
159. Chehade M, Sampson HA, Morotti RA, et al. Esophageal subepithelial fibrosis in children with eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2007;45(3):319–328.
160. Konikoff MR, Noel RJ, Blanchard C, et al. A randomized, double-blind, placebo-controlled trial of fluticasone propionate for pediatric eosinophilic esophagitis. Gastroenterology. 2006;131(5):1381–1391.
161. Otani IM, Anilkumar AA, Newbury RO, et al. Anti-IL-5 therapy reduces mast cell and IL-9 cell numbers in pediatric patients with eosinophilic esophagitis. J Allergy Clin Immunol. 2013;131(6):1576–1582.
162. Boldorini R, Mercalli F, Oderda G. Eosinophilic oesophagitis in children: responders and non-responders to swallowed fluticasone. J Clin Pathol. 2013;66(5):399–402.
163. Holgate ST. The role of mast cells and basophils in inflammation. Clin Exp Allergy. 2000;30(Suppl 1):28–32.
164. Mulder DJ, Mak N, Hurlbut DJ, et al. Atopic and non-atopic eosinophilic oesophagitis are distinguished by immunoglobulin E-bearing intraepithelial mast cells. Histopathology. 2012;61(5):810–822.
• **Mast cells were increased in the LP of patients with EoE, but IgE-bearing mast cells were only found in the subgroup of patient with an atopic background according to clinical history.**
165. Mulder DJ, Justinich CJ. B cells, IgE and mechanisms of type I hypersensitivity in eosinophilic oesophagitis. Gut. 2010;59(1):6–7.
166. Krishnaswamy G, Ajitawi O, Chi DS. The human mast cell: an overview. Methods Mol Biol. 2006;315:13–34.
167. Paterson WG. Role of mast cell-derived mediators in acid-induced shortening of the esophagus. Am J Physiol. 1998;274(2 Pt 1):G385–G388.
168. Feldman MJ, Morris GP, Dinda PK, et al. Mast cells mediate acid-induced augmentation of opossum esophageal blood flow via histamine and nitric oxide. Gastroenterology. 1996;110(1):121–128.
169. Quist RG, Ton-Nu HT, Lillienau J, et al. Activation of mast cells by bile acids. Gastroenterology. agosto de. 1991;101(2):446–456.
170. Undem BJ, Taylor-Clark T. Mechanisms underlying the neuronal-based symptoms of allergy. J Allergy Clin Immunol. 2014;133(6):1521–1534.
171. Mann NS, Leung JW. Pathogenesis of esophageal rings in eosinophilic esophagitis. Med Hypotheses. 2005;64(3):520–523.
172. Diaz P, Galleguillos FR, Gonzalez MC, et al. Bronchoalveolar lavage in asthma: the effect of disodium cromoglycate (cromolyn) on leukocyte counts, immunoglobulins, and complement. J Allergy Clin Immunol. 1984;74(1):41–48.
173. Lindqvist A, Karjalainen E-M, Laitinen LA, et al. Salmeterol resolves airway obstruction but does not possess anti-eosinophil efficacy in newly diagnosed asthma: a randomized, double-blind, parallel group biopsy study comparing the effects of salmeterol, fluticasone propionate, and disodium cromoglycate. J Allergy Clin Immunol. 2003;112(1):23–28.
174. Liacouras CA, Spergel JM, Ruchelli E, et al. Eosinophilic esophagitis: a 10-year experience in 381 children. Clin Gastroenterol Hepatol. 2005;3(12):1198–1206.
175. Lieberman JA, Zhang J, Whitworth J, et al. A randomized, double-blinded, placebo-controlled study of the use of viscous oral cromolyn sodium for the treatment of eosinophilic esophagitis. Ann Allergy Asthma Immunol. 2018;120(5):527–531.
176. Cikler E, Ersoy Y, Cetinel S, et al. The leukotriene D4 receptor antagonist, montelukast, inhibits mast cell degranulation in the dermis induced by water avoidance stress. Acta Histochem. 2009;111(2):112–118.
177. Ersoy Y, Cikler E, Cetinel S, et al. Leukotriene D4 receptor antagonist montelukast alleviates water avoidance stress-induced degeneration of the gastrointestinal mucosa. Prostaglandins Leukot Essent Fatty Acids. 2008;78(3):189–197.
178. Stumphy J, Al-Zubeidi D, Guerin L, et al. Observations on use of montelukast in pediatric eosinophilic esophagitis: insights for the future. Dis Esophagus. 2011;24(4):229–234.
179. Lucendo AJ, De Rezende LC, Jimenez-Contreras S, et al. Montelukast was inefficient in maintaining steroid-induced remission in adult eosinophilic esophagitis. Dig Dis Sci. 2011;56(12):3551–3558.
180. Alexander JA, Ravi K, Enders FT, et al. Montelukast does not maintain symptom remission after topical steroid therapy for eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2017;15(2):214–221.
181. Rocha R, Vitor AB, Trindade E, et al. Omalizumab in the treatment of eosinophilic esophagitis and food allergy. Eur J Pediatr. 2011;170(11):1471–1474.
182. Loizou D, Enav B, Komlodi-Pasztor E, et al. A pilot study of omalizumab in eosinophilic esophagitis. PloS One. 2015;10(3):e0113483.
183. Alexander ES, Martin LJ, Collins MH, et al. Twin and family studies reveal strong environmental and weaker genetic cues explaining heritability of eosinophilic esophagitis. J Allergy Clin Immunol. 2014;134(5):1084–1092.
•• **A seminal study that quantified the risk associated with genes and environment on familial clustering of EoE by analyzing nuclear-family-based and twins' cohorts. In addition to estimate the rate of EoE in different degrees of relatives, this research revealed a powerful role for common environment (81.0%) compared with additive genetic heritability (14.5%).**
184. Allen-Brady K, Firszt R, Fang JC, et al. Population-based familial aggregation of eosinophilic esophagitis suggests a genetic contribution. J Allergy Clin Immunol. 2017;140(4):1138–1143.
• **By analyzing EoE cases identified in the population-based genealogy resource linked to electronic medical records for health care systems across the state of Utah, the population-attributable risk of EoE was estimated to be 31%, thus supporting a significant genetic contribution to EoE as evidenced by increased risk of the disease in distant relatives.**
185. Kottyan LC, Rothenberg ME. Genetics of eosinophilic esophagitis. Mucosal Immunol. 2017;10(3):580–588.
• **A review on the contribution of genetic variation to EoE risk and the experimental tools and statistical methodology used to identify EoE risk loci. The opportunities provided by genetic analyses for developing decision support tools, molecular diagnostics, and novel therapeutic approaches in EoE are also discussed.**
186. Rothenberg ME, Spergel JM, Sherrill JD, et al. Common variants at 5q22 associate with pediatric eosinophilic esophagitis. Nat Genet. 2010;42(4):289–291.

187. Ávila-Castellano R, García-Lozano J-R, Cimbollek S, et al. Genetic variations in the TLR3 locus are associated with eosinophilic esophagitis. United Eur Gastroenterol J. 2018;6(3):349–357.
• **The identification of *TLR3* as a novel novel genetic susceptibility locus for developing EoE, with effects that would be independent of *TSLP*.**
188. Aceves SS, Newbury RO, Chen D, et al. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. Allergy. 2010;65(1):109–116.
189. Sherrill JD, Gao P-S, Stucke EM, et al. Variants of thymic stromal lymphopoietin and its receptor associate with eosinophilic esophagitis. J Allergy Clin Immunol. 2010;126(1):160–165.
•• **After defining a disease susceptibility locus on 5q22, this study identified the TSLP gene harbored in this region as candidate gene critically involved in EE susceptibility beyond its role in promoting Th2 responses.**
190. Kottyan LC, Davis BP, Sherrill JD, et al. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. Nat Genet. 2014;46(8):895–900.
•• **The identification of CAPN14 associated at 2p23 locus a susceptibility gene for EoE, specifically expressed in the esophagus, and dynamically upregulated as a function of disease activity and after exposure of epithelial cells to IL-13. This finding provides a model to explain the tissue-specific nature of EoE.**
191. Green DJ, Cotton CC, Dellon ES. The role of environmental exposures in the etiology of eosinophilic esophagitis: a systematic review. Mayo Clin Proc. 2015;90(10):1400–1410.
192. Jensen ET, Kuhl JT, Martin LJ, et al. Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. J Allergy Clin Immunol. 2018;141(1):214–222.
•• **Analyses of large registries of patients with a EoE and population-based community control subjects provided evidence that early-life factors, including maternal fever, preterm labor, cesarean delivery, and antibiotic or acid suppressant use in infancy, were associated with risk of pediatric EoE, while having a pet in the home was protective.**
193. Slae M, Persad R, Leung AJ-T, et al. Role of environmental factors in the development of pediatric eosinophilic esophagitis. Dig Dis Sci. 2015;60(11):3364–3372.
194. Radano MC, Yuan Q, Katz A, et al. Cesarean section and antibiotic use found to be associated with eosinophilic esophagitis. J Allergy Clin Immunol Pract. 2014;2(4):475–477.
195. Patel AL, Mutlu EA, Sun Y, et al. Longitudinal survey of microbiota in hospitalized preterm very-low-birth-weight infants. J Pediatr Gastroenterol Nutr. 2016;62(2):292–303.
196. van Nimwegen FA, Penders J, Stobberingh EE, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J Allergy Clin Immunol. 2011;128(5):948–955.
197. Lee Y-J, Redd M, Bayman L, et al. Comparison of clinical features in patients with eosinophilic esophagitis living in an urban and rural environment. Dis Esophagus. 2015;28(1):19–24.
198. Jensen ET, Hoffman K, Shaheen NJ, et al. Esophageal eosinophilia is increased in rural areas with low population density: results from a national pathology database. Am J Gastroenterol. 2014;109(5):668–675.
199. Moawad FJ, Veerappan GR, Lake JM, et al. Correlation between eosinophilic oesophagitis and aeroallergens. Aliment Pharmacol Ther. 2010;31(4):509–515.
200. Von Arnim U, Wex T, Link A, et al. Helicobacter pylori infection is associated with a reduced risk of developing eosinophilic oesophagitis. Aliment Pharmacol Ther. 2016;43(7):825–830.
201. Dellon ES, Peery AF, Shaheen NJ, et al. Inverse association of esophageal eosinophilia with Helicobacter pylori based on analysis of a US pathology database. Gastroenterology. 2011;141(5):1586–1592.
202. Molina-Infante J, Gutierrez-Junquera C, Savarino E, et al. Helicobacter pylori infection does not protect against eosinophilic esophagitis: results from a large multicenter case-control study. Am J Gastroenterol. 2018;113(7):972-979.
• **A prospective, multicenter case-control study involving children and adults naïve to eradication therapy for *H. pylori* demonstrated that this infection was not inversely associated with EoE, asthma of food allergy neither in children nor in adults, thus questioning its protective role of *H. pylori* infection against allergic disorders.**
203. Jensen ET, Kuhl JT, Martin LJ, et al. Early-life environmental exposures interact with genetic susceptibility variants in pediatric patients with eosinophilic esophagitis. J Allergy Clin Immunol. 2018;141(2):632–637.
•• **This first case-control study tested for gene-environment interaction between EoE-predisposing polymorphisms and implicated early-life factors in the risk of developing EoE. The interplay of gene (CAPN14 and LOC283710/KLF13) and early-life environment factors (breast-feeding and neonatal intensive care unit admission) was found to contributed to EoE susceptibility, thus open a new avenue of research in the epidemiology of the disease.**
204. Aceves SS. Remodeling and fibrosis in chronic eosinophil inflammation. Dig Dis. 2014;32(1–2):15–21.
205. Minshall EM, Leung DY, Martin RJ, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. Am J Respir Cell Mol Biol. 1997;17(3):326–333.
206. Alizadeh-Sani Z, Vakili-Zarch A, Kiavar M, et al. Eosinophilic endomyocardial fibrosis and strongyloides stercoralis: a case report. Res Cardiovasc Med. 2013;2(2):104–105.
207. Bramuzzo M, Martelossi S, Villanacci V, et al. Ileoileal intussusceptions caused by eosinophilic enteropathy. J Pediatr Gastroenterol Nutr. 2016;62(6):e60.
208. Colizzo JM, Clayton SB, Richter JE. Intrabolus pressure on high-resolution manometry distinguishes fibrostenotic and inflammatory phenotypes of eosinophilic esophagitis. Dis Esophagus. 2016;29(6):551–557.
209. Hirano I, Pandolfino JE, Boeckxstaens GE. Functional lumen imaging probe for the management of esophageal disorders: expert review from the clinical practice updates committee of the AGA Institute. Clin Gastroenterol Hepatol. 2017;15(3):325–334.
210. Schoepfer AM, Safroneeva E, Bussmann C, et al. Delay in diagnosis of eosinophilic esophagitis increases risk for stricture formation in a time-dependent manner. Gastroenterology. 2013;145(6):1230–1236.
• **The first demonstration of the impact of duration of untreated diseasea on the prevalence of esophageal strictures, through a retrospective study using the Swiss EoE Database.**
211. Dellon ES, Kim HP, Sperry SLW, et al. A phenotypic analysis shows that eosinophilic esophagitis is a progressive fibrostenotic disease. Gastrointest Endosc. 2014 abril de;79(4):577–585.e4.
212. Lipka S, Kumar A, Richter JE. Impact of diagnostic delay and other risk factors on eosinophilic esophagitis phenotype and esophageal diameter. J Clin Gastroenterol. 2016;50(2):134–140.
213. Rawson R, Yang T, Newbury RO, et al. TGF-β1-induced PAI-1 contributes to a profibrotic network in patients with eosinophilic esophagitis. J Allergy Clin Immunol. 2016;138(3):791–800.
214. Yang Z-C, Yi M-J, Ran N, et al. Transforming growth factor-β1 induces bronchial epithelial cells to mesenchymal transition by activating the Snail pathway and promotes airway remodeling in asthma. Mol Med Rep. 2013;8(6):1663–1668.
215. Kagalwalla AF, Akhtar N, Woodruff SA, et al. Eosinophilic esophagitis: epithelial mesenchymal transition contributes to esophageal remodeling and reverses with treatment. J Allergy Clin Immunol. 2012;129(5):1387–1396.
• **A study assessing epithelial mesenchymal transition in EoE patient and its contribution to subepithelial fibrosis in subjects with EoE, and its resolution with treatments that decrease esophageal inflammation (i.e., diet and topic steroids).**
216. Mishra A, Wang M, Pemmaraju VR, et al. Esophageal remodeling develops as a consequence of tissue specific IL-5-induced eosinophilia. Gastroenterology. 2008;134(1):204–214.
217. Rieder F, Nonevski I, Ma J, et al. T-helper 2 cytokines, transforming growth factor beta1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. Gastroenterology. 2014;146(5):1266–1277.

•• **In an analysis of tissues samples from patients with EoE and healthy donors, the presence and activation state of eosinophils was linked with altered fibrogenesis and motility of esophageal fibroblasts and muscle cells, thus providing an explanation of dysphagia in EoE.**

218. Hoeck J, Woisetschläger M. Activation of eotaxin-3/CCLl26 gene expression in human dermal fibroblasts is mediated by STAT6. J Immunol. 2001;167(6):3216–3222.
219. Hoeck J, Woisetschläger M. STAT6 mediates eotaxin-1 expression in IL-4 or TNF-alpha-induced fibroblasts. J Immunol. 2001;166(7):4507–4515.
220. Persad R, Huynh HQ, Hao L, et al. Angiogenic remodeling in pediatric EoE is associated with increased levels of VEGF-A, angiogenin, IL-8, and activation of the TNF-α-NFκB pathway. J Pediatr Gastroenterol Nutr. 2012;55(3):251–260.
221. Aceves SS, Newbury RO, Dohil R, et al. Esophageal remodeling in pediatric eosinophilic esophagitis. J Allergy Clin Immunol. 2007;119 (1):206–212.
222. Mulder DJ, Pacheco I, Hurlbut DJ, et al. FGF9-induced proliferative response to eosinophilic inflammation in oesophagitis. Gut. 2009;58(2):166–173.
223. Lucendo AJ, Arias A, De Rezende LC, et al. Subepithelial collagen deposition, profibrogenic cytokine gene expression, and changes after prolonged fluticasone propionate treatment in adult eosinophilic esophagitis: a prospective study. J Allergy Clin Immunol. 2011;128(5):1037–1046.
224. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol. 2008;214(2):199–210.
225. Cheng E, Souza RF, Spechler SJ. Tissue remodeling in eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol. 2012;303(11): G1175–G87.
226. Muir AB, Lim DM, Benitez AJ, et al. Esophageal epithelial and mesenchymal cross-talk leads to features of epithelial to mesenchymal transition in vitro. Exp Cell Res. 2013;319(6):850–859.
227. Mack M, Yanagita M. Origin of myofibroblasts and cellular events triggering fibrosis. Kidney Int. 2015;87(2):297–307.
228. Lucendo AJ, Castillo P, Martín-Chávarri S, et al. Manometric findings in adult eosinophilic oesophagitis: A study of 12 cases. Eur J Gastroenterol Hepatol. 2007;19(5):417–424.
229. Fox VL, Nurko S, Teitelbaum JE, et al. High-resolution EUS in children with eosinophilic «allergic» esophagitis. Gastrointest Endosc. 2003;57(1):30–36.
230. Kwiatek MA, Hirano I, Kahrilas PJ, et al. Mechanical properties of the esophagus in eosinophilic esophagitis. Gastroenterology. 2011;140 (1):82–90.
231. Lin Z, Kahrilas PJ, Xiao Y, et al. Functional luminal imaging probe topography: an improved method for characterizing esophageal distensibility in eosinophilic esophagitis. Ther Adv Gastroenterol. 2013;6 (2):97–107.
232. McMahon BP, Frøkjaer JB, Kunwald P, et al. The functional lumen imaging probe (FLIP) for evaluation of the esophagogastric junction. Am J Physiol Gastrointest Liver Physiol. 2007;292(1):G377–G84.
233. Nicodème F, Hirano I, Chen J, et al. Esophageal distensibility as a measure of disease severity in patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2013;11(9):1101–1107.
234. Chen JW, Pandolfino JE, Lin Z, et al. Severity of endoscopically identified esophageal rings correlates with reduced esophageal distensibility in eosinophilic esophagitis. Endoscopy. 2016;48(9):794–801.
235. Carlson DA, Hirano I, Zalewski A, et al. Improvement in esophageal distensibility in response to medical and diet therapy in eosinophilic esophagitis. Clin Transl Gastroenterol. 2017;8(10):e119.
• **Patients with EoE were evaluated with FLIP during endoscopy at baseline and following therapy without interval dilatation. Improvement in esophageal body distensibility was achieved with medical and dietary therapies. Improved distensibility plateau appeared to be better indicator of symptomatic improvement than eosinophil count, supporting FLIP as a valuable outcome measure in EoE.**
236. Ahuja NK, Agnihotri A, Lynch KL, et al. Esophageal distensibility measurement: impact on clinical management and procedure length. Dis Esophagus. 2017;30(8):1–8.
237. Moawad FJ, Molina-Infante J, Lucendo AJ, et al. Systematic review with meta-analysis: endoscopic dilation is highly effective and safe in children and adults with eosinophilic oesophagitis. Aliment Pharmacol Ther. 2017;46(2):96–105.
• **An updated meta-analysis that summarizes results from 27 studies demonstrates that endoscopic dilation is consistently effective in children and adults with EoE, and results in improvement in 95% of patients with very low rates (<1%) of major complications.**
238. Schoepfer AM, Gonsalves N, Bussmann C, et al. Esophageal dilation in eosinophilic esophagitis: effectiveness, safety, and impact on the underlying inflammation. Am J Gastroenterol. 2010;105(5):1062–1070.
239. Andreae DA, Hanna MG, Magid MS, et al. Swallowed fluticasone propionate is an effective long-term maintenance therapy for children with eosinophilic esophagitis. Am J Gastroenterol. 2016;111 (8):1187–1197.
240. Abu-Sultaneh SMA, Durst P, Maynard V, et al. Fluticasone and food allergen elimination reverse sub-epithelial fibrosis in children with eosinophilic esophagitis. Dig Dis Sci. 2011;56(1):97–102.
241. Straumann A, Conus S, Degen L, et al. Long-term budesonide maintenance treatment is partially effective for patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2011;9(5):400–409.
242. Lucendo AJ, Miehlke S, Vieth M. Budesonide or dispersible tablets are highly effective for treatment of active eosinophilic esophagitis: results from a randomized, double-blind, placebo-controlled, pivotal multicenter trial (EOS-1) [abstract]. Gastroenterology. 2017;152 (Suppl 1):A1118:S207.
243. Gonsalves N, Yang G-Y, Doerfler B, et al. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. Gastroenterology. 2012;142(7):1451–1459.
244. Wolf WA, Jerath MR, Sperry SLW, et al. Dietary elimination therapy is an effective option for adults with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2014;12(8):1272–1279.
245. Testing Effectiveness of Losartan in Patients With EoE With or Without a CTD. ClinicalTrials.gov (internet). [cited 2018 Aug 8]. Available from: https://clinicaltrials.gov/ct2/show/NCT01808196?term=losartan&cond=Eosinophilic+Esophagitis&rank=2
246. An Open-Label Trial of Losartan Potassium in Participants With Eosinophilic Esophagitis (EoE). ClinicalTrials.gov (internet). [cited 2018 Aug 8]. https://clinicaltrials.gov/ct2/show/NCT03029091?term=losartan&cond=Eosinophilic+Esophagitis&rank=1
247. Harris JK, Fang R, Wagner BD, et al. Esophageal microbiome in eosinophilic esophagitis. PloS One. 2015;10(5):e0128346.
248. Benitez AJ, Hoffmann C, Muir AB, et al. Inflammation-associated microbiota in pediatric eosinophilic esophagitis. Microbiome. 2015;3:23.
249. Hines BT, Rank MA, Wright BL, et al. Minimally-invasive biomarker studies in eosinophilic esophagitis: a systematic review. Ann Allergy Asthma Immunol. 2018;121(2):218–228.
250. Wen T, Rothenberg ME. Clinical applications of the eosinophilic esophagitis diagnostic panel. Front Med. 2017;4:108.
251. Katzka DA, Smyrk TC, Alexander JA, et al. Accuracy and safety of the cytosponge for assessing histologic activity in eosinophilic esophagitis: a two-center study. Am J Gastroenterol. 2017;112 (10):1538–1544.
• **A prospective study enrolling patients with known EoE demonstrated a sensitivity and specificity of the cytosponge compared to endoscopic biopsies of 75% and 86%, respectively.**
252. Furuta GT, Kagalwalla AF, Lee JJ, et al. The oesophageal string test: A novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. Gut. 2013;62(10):1395–1405.
• **This minimally invasive clinical device was compared with esophageal mucosal biopsies obtained from children undergoing upper endoscopy with active EoE, in remission and GERD. The presence of eosinophil-derived proteins in luminal secretions was reflective of mucosal inflammation in children with EoE.**
253. Smadi Y, Deb C, Bornstein J, et al. Blind esophageal brushing offers a safe and accurate method to monitor inflammation in children and young adults with eosinophilic esophagitis. Dis Esophagus. 2018. DOI:10.1093/dote/doy056