EXPERT REVIEWS

Cellular and molecular immunological mechanisms in eosinophilic esophagitis: an updated overview of their clinical implications

Expert Rev. Gastroenterol. Hepatol. Early online, 1–17 (2014)

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Eosinophilic esophagitis (EoE) is a pathophysiologically complex disorder driven by distinct, multiple mechanisms involving a large number of cells, molecules, and genes. Associated with food allergy from its initial descriptions, a key role for the Th2-type cytokines IL-5 and IL-13 in recruiting and activating eosinophils has been described. Epithelial cells have been recognized as major effectors in initiating EoE, both through their recruitment of iNKT cells towards the esophageal epithelium, which constitutes a major cytokine source, and through the release of eotaxin-3 and other chemoattractants. Epithelial and mesenchymal-released TSLP is a key regulator for which a connecting role between the adaptive and innate mucosal-associated immune response has been suggested. Finally, activated eosinophil- and mast cell-derived TGF β1 secretion is crucial in EoE-associated tissue remodeling.

KEYWORDS: diagnosis • eosinophilic esophagitis • eosinophils • eotaxin-3 • food allergy • IL-13 • IL-5 • immunopathogenesis • inflammation • mast cells • remodeling • therapy • TSLP

Eosinophilic esophagitis (EoE) has emerged over the past few years as a relevant, chronic esophageal disorder affecting patients worldwide. Far from being considered a rare disorder, as was the case several years ago, EoE today represents the second most common cause of chronic esophageal symptoms after gastroesophageal reflux disease (GERD) in developed countries and is the main cause of esophageal dysfunction among children and young adults [1,2]. In fact, an increasing prevalence for EoE has been observed, with the disorder currently affecting up to 43–56/100,000 inhabitants, both children and adults, in Europe and the USA [3–6]. As a result, EoE poses a large burden to the health care system, involving multidisciplinary teams that include gastroenterologists, dietitians and allergists in the management of the disease. This is in addition to the costs derived from frequent emergency room visits due to food impaction or after complications [7]. Given the high prevalence of the disease, the cost–effectiveness of performing esophageal biopsies in patients with upper GI symptoms to rule out EoE has been demonstrated [8].

After having been defined as a particular form of food allergy from its early descriptions in pediatric patients [9], researchers have only recently begun to unravel the pathogenesis of EoE, and a full explanation of its origins has yet to be elucidated. The frequent association of EoE with allergies has also contributed to the consensual definition of EoE as an immune/antigen-mediated disorder [10], promoting its treatment with drugs used effectively in other allergic conditions.

The control of exposure to dietary antigens through various dietary modifications has also been demonstrated to reverse the histopathological lesions and derived symptoms of the disease [11]. Moreover, fibrotic phenomena promoted by the chronic esophageal inflammation marking the disorder often lead to reductions in esophageal caliber in a time-dependent manner [12], a consequence that

 10.1586/17474124.2014.909727 ISSN 1747-4124

may be ameliorated through endoscopic dilatations. Despite these findings, a commonly accepted treatment algorithm is currently lacking, and the optimal management of EoE patients remains controversial [13]. As a result, treatment of EoE in clinical practice varies more than any other aspect related to the disease [14].

After 20 years of research on the causes of this disorder, EoE today represents one of the most relevant topics in gastroenterology and allergology, with patient management constituting an intriguing challenge. In addition, having recognized the unsatisfied needs of EoE patients, pharmaceutical and biotechnological companies are allocating more resources to EoE diagnosis and therapeutics, which is emerging as an area of increased interest. This article aims to provide a state-of-the-art update on the pathophysiology of EoE and the potential applications of this knowledge in clinical practice.

Gastrointestinal eosinophils

The esophagus is the only organ of the GI tract that does not normally contain eosinophils, granulocytes of myeloid lineage traditionally considered to be IgE-dependent effector cells that arise in inflammatory processes in response to allergic hypersensitivity and parasitosis. In contrast, eosinophils are extremely common in the remaining intestinal mucosa under normal conditions, an observation that has led some authors to consider them to be regulatory cells involved in the maintenance of intestinal homeostasis [15] as opposed to the more conventional active role played by these cells in several intestinal diseases, including ulcerative colitis or EoE, similar to what occurs in bronchial asthma [16].

Eosinophil trafficking to the esophagus

Eosinophils are derived in the bone marrow, where they proliferate and mature under the regulatory effect of several cytokines and growth factors. Of these, IL-5 is the most specific to the eosinophil lineage and is responsible for the selective expansion of eosinophils and their release into the circulating blood [17]. From there, these cells finally traffic to specific peripheral tissues, predominantly the gastrointestinal tract, where they reside for at least 1 week [18]. IL-5 has also been clearly implicated in the physiopathology of allergic asthma [19,20] and the fibrous remodeling phenomena present in bronchial [21] and cutaneous [22] inflammation. The critical role of IL-5 in the production, migration and tissue accumulation of eosinophils in EoE has been demonstrated most compellingly through genetic manipulation in experimental murine models, which has shown that an overactive Th2-mediated response is required to induce the disorder. In fact, when stimulated with inhaled [23,24] or epicutaneous allergens [25], transgenic mice with an overproduction of IL-5 showed an increase of these granulocytes in circulating blood along with an intense accumulation of eosinophils in the esophageal lamina propria and small intestine proportional to the serum concentration of IL-5 [17]. In contrast, deletion in the *IL-5* gene caused a marked reduction of eosinophils in the blood, lungs and GI tract after allergen challenge [24]. Human studies have documented that the blood lymphocytes of EoE patients produce significantly higher levels of IL-5 following stimulation *in vitro* compared with normal controls [26] and that the percentage of blood-circulating IL-5+ CD4 T cells correlates with the severity of esophageal tissue eosinophilia [27].

The effect of additional chemoattractants on circulating eosinophils has recently been assessed for several diseases. In fact, distinct patterns of activation signals released from the inflamed tissues may induce the acquisition of tissue-specific functional properties in blood eosinophils [16], with these properties being different for EoE, bronchial asthma and ulcerative colitis. Although the effect of homing molecules in the recruitment of eosinophils toward the esophageal mucosa has not been assessed to date, we already know that in EoE, circulating eosinophils exhibit an enhanced expression of the low-affinity receptor for IgE (CD23), the intercellular adhesion molecule (ICAM)-1 (or CD54), integrin CD11c and the receptor for prostaglandin D_2 CRTH2 [16].

Therapeutic interventions for esophageal trafficking of eosinophils

The efficacy of two anti-IL-5-humanized monoclonal antibodies has been assessed in pediatric and adult EoE patients in various randomized clinical trials (RCTs) with concordant results. After having demonstrated some benefit in patients with hypereosinophilic syndrome and severe eosinophilic asthma, mepolizumab was assessed in adult patients, while reslizumab was tested in two pediatric series [28,29]. Both drugs significantly reduced eosinophil numbers in esophageal biopsies, albeit not to normal, and reverted the expression of molecules associated with esophageal remodeling. Unfortunately, clinical improvement was minimal.

A selective CRTH2 antagonist (OC000459) with proven efficacy against eosinophilic asthma was recently assessed in a double-blind, placebo-controlled RCT in adult patients with steroid-dependent or steroid-refractory EoE [.30] The drug induced a significant decrease in both esophageal eosinophilia (114.83–70.26 eos/high power field) and symptoms, with a trend toward improvement in endoscopic abnormalities compared with a placebo, but esophageal mucosa did not revert to normal.

Epithelial function in EoE

The active role that the epithelium of the GI tract plays in defensive functions of the mucosa is increasingly recognized [31]. In fact, although the integrity of the junctions between intestinal epithelial cells and the differentiation of some of them into mucus-producing Globet cells are both of vital importance for defending the intestinal mucosa, a growing body of evidence shows that enterocytes contribute to a much more complex immune response than simple regulation of bowel permeability. Epithelial cells of the GI tract are crucial for integrating external and internal signals and for coordinating the ensuing immune response, harmonizing information that comes from inflammatory and noninflammatory components of the GI lumen (i.e., food antigens and microbiota) to preserve intestinal homeostasis [32]. If dysregulated, the immunomodulatory function of epithelial cells may contribute to the development of intestinal inflammation [33].

The histological structure of the esophageal epithelium is different than that of other organs of the digestive tract. For example, instead of a single cuboid cell stratus, its flat epithelial cells are arranged in various layers; moreover, it lacks specific secretory or absorptive functions. However, resident cell components of the innate mucosal surveillance immune system are also present within the esophageal epithelium [34], and its role in regulating inflammatory responses has been recognized in eosinophilic gastrointestinal diseases [35]. In EoE, the inflammatory response is restricted to the esophagus with no involvement of more distal GI segments; this implies an organ-specific homing signal that attracts eosinophils toward the epithelium.

Eotaxins are a subfamily of chemokines that act through the chemokine receptor CCR3 which is expressed predominately in eosinophils [36]. Eotaxin-1/CCL11 is the most widely studied eosinophil-attracting chemokine in the digestive tract, where it is expressed ubiquitously [37,38]. However, *eotaxin-3/CCL26* is the most highly upregulated gene in EoE [39] and the single most overexpressed gene in the esophageal epithelial cells of patients with the disease. Patients with EoE showed higher eotaxin-3 plasma levels than control subjects, and the gene expression of *eotaxin-3* and its protein in esophageal tissue is directly and closely related to tissue eosinophils and mast cell densities. Furthermore, a single-nucleotide polymorphism (SNP; +2496T>G, rs2302009) in the *eotaxin-3* gene has been associated with disease susceptibility. This SNP is located at the 3′-untranslated region of the *eotaxin-3* gene and may participate in maintaining mRNA stability [39].

IL-13, another Th2-type cytokine with pleiotropic effects, is implicated in parasite expulsion, asthma pathophysiology and the natural history of cancer and other human pathologies; it has also been demonstrated to play a key role in EoE. It is secreted by many types of cells, but especially by Th2 cells. In fact, 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 [40] and also expressed in the blood eosinophils of patients with several eosinophilic inflammatory disorders including EoE [41]. 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 [42], operating through the nuclear transcription factor STAT6 (which plays a central role in Th2 cell differentiation) [40]. Thus, intratracheal delivery of IL-13 in a murine model of EoE induced experimental EoE [43], whereas *IL-13*-deficient mice and those with a targeted deletion of STAT6 exhibited attenuated degrees of allergen-induced experimental EoE and were partially protected from allergen- and IL-13-induced experimental EoE, respectively [25]. All of these results confirm the key role played by this cytokine in the development of the disease. The importance of the human esophageal epithelium in the development of EoE has been demonstrated in additional studies; thus, human esophageal epithelial cell cultures stimulated with IL-13 were shown to be capable of partially reproducing the characteristic EoE transcriptome [40], which could then be reversed after topical steroid treatment in parallel with a significant reduction in *IL-13* mRNA expression levels.

Several morphological features exhibited by the epithelial layers in EoE samples are directly related to the effects of IL-13. This cytokine contributes to the esophageal hyperplasia observed in EoE, as shown by the strong decrease observed in the expression of the epidermal differentiation genes filaggrin and involucrin in IL-13-stimulated esophageal epithelial cells and EoE compared with normal biopsy specimens [44]. Finally, IL-13 also alters epithelial integrity in EoE patients by reducing the adhesion molecule desmosomal cadherin desmoglein-1, which results in cell separation and impaired barrier function with increasing mucosal permeability [45]. In fact, dilated intercellular spaces have been repeatedly described in EoE samples [46,47] to which the potent mediators released by eosinophils act on epithelial cells also contribute [48]. This epithelial dysfunction may facilitate the uptake of undegraded allergens and perpetuate the inflammatory condition (Figure 1).

Therapeutic targets focused on epithelial function in EoE

Blocking the biological effects of IL-13 has been recognized as a putative therapeutic target in several eosinophil-related inflammatory disorders and in inflammatory bowel disease [49]. For example, lebrikizumab, a humanized IgG4 anti-IL-13 monoclonal antibody, has proved beneficial in asthmatic patients [50,51]. In the case of EoE, pretreatment of experimental mice with a human antihuman IL-13 IgG4 monoclonal antibody (CAT-354) significantly attenuated respiratory and esophageal inflammation induced by intratracheal human IL-13 [52], providing evidence for the need of further research on this potential benefits. In fact, a Phase II, double-blind RCT has ascertained the safety and efficacy of an intravenously administered anti-IL-13 antibody (QAX576, Novartis, Switzerland) against the frequency and severity of symptoms in EoE patients [53] although the results have yet to be published.

The inhibition of the CCL26–CCR3 ligand–receptor system by blocking the effects of eotaxin-3 through either anti-eotaxin-3 or anti-CCR3 antibodies may be an attractive target for developing therapies to limit the progress of inflammation in airway diseases and EoE and has been proposed as a separate line of research [13]. To date, however, the efficacy of these antibodies has only been assessed *in vitro* [54,55].

The capacity of steroids to reverse the IL-13-induced EoE transcriptome in esophageal epithelial cell cultures correlates with the widely demonstrated ability of these drugs to reverse EoE-associated histopathological features back to normal in EoE patients. The efficacy of the various available steroids assayed in EoE will be discussed below.

EoE as a digestive allergic condition

EoE likely arises from an immunoallergic response to environmental factors in susceptible individuals. In fact, allergies have been linked to its origin 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, food or airborne allergens; blood eosinophilia; or elevated serum total

doi: 10.1586/17474124.2014.909727

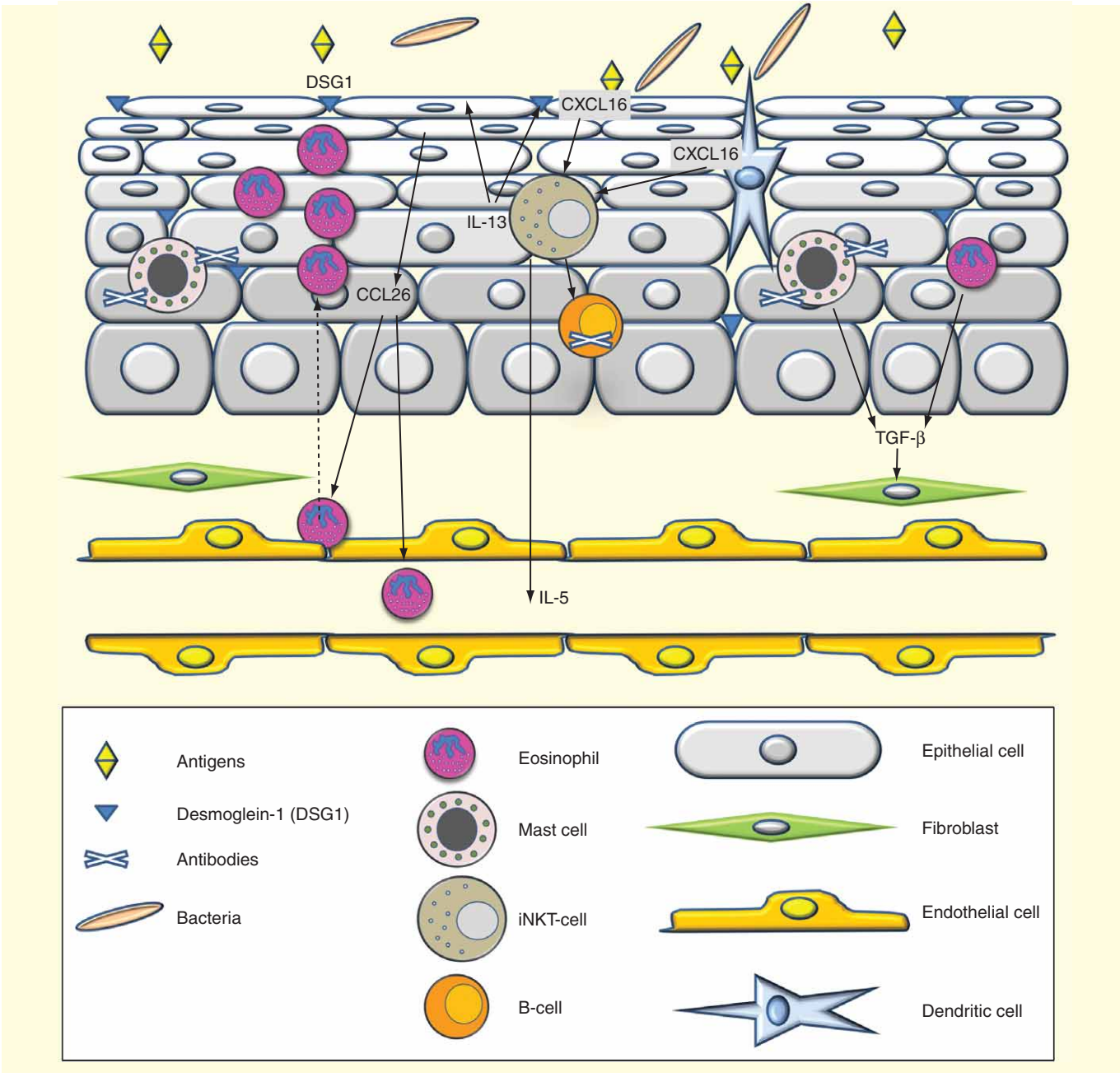

Figure 1. The epithelial surface in eosinophilic esophagitis is an active cellular and molecular complex in which a series of changes leading to the accumulation of inflammatory cells occur. The activation of epithelial and dendritic cells after exposure (or lack of exposure) to components of the esophageal lumen (i.e., bacteria and food antigens) induce the CXCL16 expression, which directly promotes iNKT cells recruitment. iNKT cells are the major source of Th2 cytokines, including IL-5, with a pro-eosinophilic effect at the distance, and IL-13, which directly induces changes in the gene expression pattern on epithelial cells, including the upregulation, synthesis and secretion of eotaxin-3/CCL26. This is a potent chemoattractant for eosinophils and mast cells. IL-13 and other Th2 cytokines trigger the production of IgE by B cells. Activated mast cells and eosinophils are important sources of TGF-β1, a key activator of fibrous remodeling in the lamina propria.
iNKT: Invariant natural killer T.

and specific IgE levels [56]. Positive skin prick responses and radioallergosorbent test results are also generally observed in patients with EoE. However, 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 [9]. Since then, the ability of food avoidance diets to induce and maintain remission of EoE has been repeatedly demonstrated [57,58]. The potential role of airborne allergens in triggering the disease has also been suggested

after observing seasonal variations in the incidence of EoE [59] and correlations with the seasonal pollen count [60].

iNKT lymphocyte responses & EoE

From an epidemiological point of view, the rising incidence of EoE over the past few decades along with that of many other allergic and immune-mediated diseases in common geographical areas has been interrelated through the hygienic hypothesis [48]. This hypothesis provides a general explanation for the increase of immunoallergic disorders together with a decrease in infectious diseases. 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 Treg responses. As a result, there is an imbalance in the immune system and a predisposition to develop allergic and autoimmune disorders triggered by altered or missing innate immune cell activation [61].

A very recent study on children with early-onset EoE has provided compelling evidence of insufficient immune imprinting by environmental microorganisms resulting in esophageal upregulation of epithelial and dendritic cell-derived CXCL16 [62]. This molecule induces chemotaxis of invariant natural killer T (iNKT) cells into the esophagus [63]. iNKT cells are a subset of T lymphocytes responsive to lipidic or glucolipidic rather than protein antigens when presented by CD1d molecules [31,64] involved in the initial phases of a great variety of immune responses from oral tolerance to the development of autoimmunity [31]. iNKTs, when appropriately stimulated, promote a potent and rapid Th2 response with increased expression and release of IL-4, IL-5, IL-13 and eotaxins [65–67]. This leads to IgE production and subsequent sensitization to protein antigens [68,69].

An age-sensitive contact with commensal microbes is critical for establishing mucosal iNKT cell tolerance to later environmental exposures 70. 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 [71]), an excessive and persistent accumulation of iNKT cells occurs [70]. Consequently, these mucosal tissues are rendered more susceptible to later-life environmental triggers of iNKT cells, which will mediate allergic sensitization and tissue inflammation [61].

These iNKT lymphocytes are increasingly being recognized as the major source for proinflammatory cytokines in EoE (Figure 1) [62,72]. Thus, although iNKT cells primarily recognize glucolipidic structures located in pathogenic bacteria [73,74] 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 [72]. 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 [75]. Indeed, a recent study has shown that CD1d-deficient mice were protected from disease induction [76].

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.

Steroid treatment for EoE patients

As in other atopic disorders, topical steroids currently constitute the prevailing off-label therapeutic option for EoE and will probably continue to do so in the near future. Topically administered (i.e., swallowed rather than inhaled), fluticasone propionate (FP) has been demonstrated in RCTs to be highly effective in children and adults, significantly superior to a placebo and comparable to oral prednisone in inducing histological and symptomatic disease remission. A viscous suspension of budesonide in sucralose has recently emerged as an alternative to FP in order to improve proper esophageal delivery of swallowed nebulized formulations [77]. Another alternative is ciclesonide, a prodrug that becomes active after being converted by esterases from the esophageal epithelial cells. Its advantages over FP and budesonide include much higher glucocorticoid receptor binding (up to 100 times greater) and fewer systemic side effects because of its low systemic bioavailability due to a high first-pass hepatic metabolism [78]. 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 [66,79]. Glucocorticoids exert their action 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 [80]; thus, after binding to the glucocorticosteroid receptor, FP represses IL-13-induced *eotaxin-3* expression while inducing FK506-binding protein 5 (*FKBP51*) gene expression. This inhibits glucocorticoid receptor-mediated signaling, which in turn represses IL-13-induced *eotaxin-3* promoter activity [80].

Allergy immunotherapy for EoE

Allergy immunotherapy (AIT), a well established and effective treatment for various allergic diseases, has been shown in some cases to induce tolerance to environmental allergens. As EoE patients frequently present aeroallergen sensitization [56], AIT has been proposed as a possible treatment modality. In one ongoing, off-label, interventional study, AIT is being offered to patients in order to evaluate its effectiveness with the aid of a questionnaire, laboratory tests, endoscopies and biopsies [81].

Mast cell functions in EoE

Mast cells are mesenchymal bone marrow-derived myeloid cells that are widely distributed in all vascular connective tissues as part of the innate immunity against parasites and bacteria. As they are the main effector cells in IgE-associated responses, mast cells play a key role in various types of allergies [82] including EoE [34,83–85]. The potential role played by mast cells in EoE is supported mostly by indirect evidence; for example, the observation that mast cell density within the esophageal epithelium parallels that of

doi: 10.1586/17474124.2014.909727

eosinophils in EoE patients [39], with both being reduced after topical steroid [34,86] and anti-IL-5 [87] treatment. In addition, the expression of mast cell-related genes has been shown to be upregulated in several reports [39,88], with mast cell-derived TGF-1 contributing to esophageal dysmotility in human [86] and experimental murine EoE [89] through induction of smooth muscle hypertrophy and hyperplasia.

IgE involvement in EoE

The most extensively studied mechanism involved in mast cell activation and degranulation is antigen cross-linking of IgE antibodies on the cell surface, which results in the rapid release of autacoid mediators and the sustained synthesis and release of cytokines, chemokines and growth factors [90]. The most characteristic consequence is anaphylaxis. Still, although IgE-bearing mast cells have been repeatedly observed in mucosal biopsies [34,91] and IgE is locally produced in the esophageal mucosa of EoE patients [92], immediate reactions to foods responsible for the disease are not usually described in these patients. This suggests that mast cell activation in EoE is not predominantly mediated by IgE. In fact, while the specific role played by IgE in the pathophysiology of EoE has yet to be defined, the repeatedly documented absence of a relationship between serum-specific IgEs and foods responsible for EoE [58,93,94] leads us to hypothesize that the pathogenic role of IgE in EoE must be exercised at the local level through as of yet undefined mechanisms.

Human mast cells are classified into two types depending on the content of their granules [95]: mast cells with tryptase and mast cells with tryptase and chymase (MC_{TC}). This phenotypic diversity both constitutes a descriptor of tissue location [96] and is associated with functional differences [97]. MC_{TC}, which comprises 90% of the mast cell population in the esophageal epithelium [Arias Á, *et al.* data not published yet], is strong responders to non-IgE-mediated regulatory stimuli that comprise from activation of toll-like receptors (TLRs) [82] to nonimmunological mechanisms, for example, after exposure to acid GER [98,99] or bile acids [100] or due to the action of the enteric nervous system.

Treatments acting on mast cell activation in EoE

Although mast cell stabilizers (disodium cromoglycate and ketotifen) have been used for eosinophilic gastroenteritis due to their resistance to gastric acidity [101–105], no clinical or histological improvement was observed in a study of 14 children with EoE who were given doses of 100 mg/day (divided into four doses) over 1 month [106]. Accordingly, the use of this drug for treating EoE has not been recommended [10]. Indeed, unlike mast cells with tryptase cells, which are predominant in the bronchial mucosa, alveolar wall and the small intestine mucosa [95], MC_{TC} cells do not specifically respond to mast cell stabilizer drugs; this may explain the lack of efficacy documented for this drug in EoE patients.

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 series and in an RCT with disappointing results regarding both esophageal eosinophilic infiltration and symptomatic improvement [78,107].

Genetic profiling in EoE

As described in other Th-2-associated atopic disorders, the onset of EoE may be predisposed by genetic factors, which have just recently begun to be defined. Association of EoE cases within a single family has been described in approximately 7% of patients [108], with the majority being affected siblings [109]. Even though no differentiating parameters to distinguish between sporadic and familiar EoE presentations have been elucidated to date [109], it seems clear that certain inherited genes could increase susceptibility to the disease. In fact, a very high-risk recurrence ratio of up to 80% for EoE among siblings has been described [110,111], in contrast, with approximately 2% estimated for bronchial asthma [112,113]. Still, while the environmental and epigenetic factors determining the onset of EoE have yet to be clearly defined, some recent findings have started to shed light on this subject. Thus, several types of miRNA have been found to be upregulated or downregulated in the esophageal mucosa of EoE patients, distinguishing them from healthy control subjects [114]. Moreover, these alterations were largely reversible in response to steroid treatment.

A polygenic inheritance pattern has thus been proposed for EoE, and genetic susceptibility has begun to be defined through a candidate gene approach [111]. 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 [39]. *Eotaxin-3* is by far the most highly expressed gene in the EoE transcriptome, with a 53-fold increase compared with the controls.

A male predominance (~70%) has been traditionally described in EoE [115], implying that currently unidentified sexual chromosome-related genes or hormonal factors may be involved in the development of the disease. A mutation in the X chromosome affecting two chains for the IL-13 receptor (*IL-13 Rα* 1 and 2 located in position Xq13.1–q28), which would remain uncorrected by the Y chromosome genes in males has been proposed as a hypothetical explanation [116]. More recently, an SNP in the gene encoding for the thymic stromal lymphopoietin (TSLP) receptor located in the pseudoautosomal region on Xp22.3 and Yp11.3 has been shown to be directly involved in the male predominance of EoE [117].

TSLP in the pathophysiology of EoE

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 [118]. TSLP is produced mainly by nonhematopoietic cells such as fibroblasts, epithelial cells and different types of stromal or stromal-like cells. It activates professional antigen-presenting cells, including dendritic cells, which initiate Th2-type allergic responses [119] inducing pre-B-cell differentiation and proliferation. TSLP has previously been implicated in various atopic responses, and studies have also identified increased *TSLP* gene expression in the esophagi of EoE patients compared with controls [117].

The importance of TSLP as a link connecting the innate and adaptive mucosal immune system has also been demonstrated. Thus, *TSLP* mRNA expression was shown to be induced in primary esophageal epithelial cells after activation of the TLR3 pathway [116]. TLR3 recognizes double-stranded RNA [119], a form of genetic information carried by some viruses such as retroviruses, and therefore its activation plays a role in host defense against viruses. As a consequence, an alternative hypothesis has been proposed for the onset of EoE, namely that it occurs after a viral infection deregulates the esophageal mucosal-associated immune system.

Diagnostic applications of EoE characteristic genetic profiling

A molecular diagnostic test has been developed to identify patients with EoE in a fast, objective and mechanistic manner by means of a Taqmanq PCR-based low-density array system [120]. The utility of such testing in providing a differential diagnosis between active EoE, inactive EoE, GERD and healthy subjects has been demonstrated with RNA obtained from both fresh and formalin-fixed, paraffin-embedded esophageal tissue. The potential of molecular diagnostic tests to overcome the limitations of histological evaluation should be further assessed.

In the absence of responsive/sensitive serological markers of active esophageal inflammation for noninvasive monitoring of EoE [121], the expression of certain types of miRNA in the plasma (MiR-146a, miR-146b and miR-223) has been suggested as noninvasive biomarkers of EoE [114]. The clinical utility of this strategy should be assessed in future research.

Proton pump inhibitor responsive EoE

The first cases of EoE appeared in the literature in the late 1970s [122,123]. After these initial reports, mistaken interpretations of esophageal eosinophilia as a pathognomonic sign of GERD [124,125] hindered the proper diagnosis of many patients and contributed to the perception of EoE as a rare disorder [126], mostly documented among children and manifested by esophageal strictures [127]. Therapies based on controlling acid exposure were usually ineffective and affected children generally reappeared years later in adult clinics [128]. Patient symptoms were attributed to GERD even though most patients had normal esophageal acid exposure as assessed with 24-h pH monitoring [129] and failed to respond to standard antireflux therapies including Nissen fundoplication [9]. Eventually, after nearly simultaneous reporting of an American and a European adult case series [129,130], EoE was redefined as a distinct clinicopathological disorder.

Current diagnostic criteria for EoE incorporate the exclusion of GERD through a proton pump inhibitor (PPI) trial, since pH monitoring might not adequately distinguish GERD from EoE [10] in patients who share both conditions. This is especially relevant after the identification of a new disease phenotype of eosinophilic infiltration of the esophagus, which achieves clinical–histological remission upon PPI therapy [131]. In fact, it is now estimated that PPI responsive esophageal eosinophilia (PPI-REE) presents in at least one-third of patients with suspected EoE [132].

Besides the major effects that all PPI drugs exert in blocking gastric acid secretion, new anti-inflammatory properties for omeprazole and lansoprazole have recently been described. These PPIs inhibited both Th2 cytokine-stimulated increases in *eotaxin-3* mRNA and protein expression in cultures of esophageal squamous cells from GERD and EoE patients [133] by blocking the binding of STAT6 to the *eotaxin-3* gene promoter [134]. These effects were observed at drug concentrations achieved in the blood with conventional doses and provide a link between EoE and PPI-REE. Indeed, although EoE and PPI-REE are currently considered to be mutually exclusive disorders [10], an increasing body of knowledge underscores the many similarities shared by both entities, which are indistinguishable in their demographic, clinical and endoscopic characteristics [135,136] and for which pH monitoring allows no reliable differentiation [131,132]. Furthermore, two RCTs comparing an 8-week course with either swallowed aerosolized FP or oral esomeprazol in adult EoE patients showed no difference in the degree of improvement in dysphagia and eosinophilic infiltration between both comparison groups [137,138]. In fact, esomeprazole was superior to fluticasone, particularly in patients with established GERD [138].

Additional proof of the similarities between EoE and PPI-REE has recently been provided at the molecular level in that patients with PPI-REE exhibited basal *eotaxin-3*, *IL-13* and *IL-5* gene expression that was nearly indistinguishable from that observed in EoE. Histological remission of PPI-REE was correlated with downregulation of *eotaxin-3* and Th2 cytokines, similar to remission in EoE after treatment with topical steroids [136].

Despite the finding that PPIs appear to be capable of resolving multiple cell subsets within the EoE-associated inflammatory infiltrate (including eosinophils, mast cells and CD45RO cells) [139] in PPI-REE patients, the durability of their effects has not been fully assessed; thus, while a transient histological remission has been documented in children [139], the effect may be more durable among adults.

Increasing evidence of the multiple common aspects shared by both phenotypes associated with esophageal eosinophilia should lead us to reconsider whether they are actually a single entity and whether the real difference between EoE and PPI-REE lies in the genetically determined metabolic capacity of PPIs by the cytochrome P450 polymorphisms exhibited in each individual patient. The influence of the PPI metabolism phenotype (i.e., CYP2C19 and CYP3A4 rapid/medium/slow metabolizers) [140] in the short- and long-term PPI response should be further addressed in EoE/PPI-REE patients.

Fibrous remodeling in EoE patients

Chronic eosinophil-mediated inflammation has been associated with the promotion of structural changes in the affected tissues, leading to functional impairment. These changes are grouped under the common term of *fibrous remodeling* [141] and have been studied extensively in the airways of asthmatic patients [142]. They include metaplasia of the mucosal glands, smooth muscle hypertrophy, angiogenesis and subepithelial collagen deposition or fibrosis, all of which have been shown to impair respiratory function.

doi: 10.1586/17474124.2014.909727

Based on the paradigm of other eosinophilic inflammatory conditions, the natural history of EoE has been described as a progression from an inflammatory to a fibrostenotic disease [143], with the pathogenesis and clinical implications of this progression receiving renewed attention in recent years. In EoE, the inflammatory infiltration traverses the depth of the entire esophageal wall from the epithelial stratum to the muscular layers [144], contributing to structural changes that have been recognized from the very first descriptions of the disease [126,128,145]. In fact, fibrotic changes promoted by the inflammatory infiltrate characterizing EoE (which includes eosinophils, mast cells and T lymphocytes) have been repeatedly documented in children [146,147] and adults [148,149] suffering from the disease. Since collagen deposition in lamina propria has been shown to correlate directly and significantly with patient age [148], the likelihood of a fibrostenotic disease also increases markedly with age [149]. In fact, a recent retrospective study found that the duration of untreated disease was directly correlated to the prevalence of esophageal strictures in a time-dependent manner [143,150], underscoring the importance of early diagnosis and effective therapy for preventing this disease-related complication.

Cellular & molecular basis of tissue remodeling in EoE

Tissue remodeling and chronic eosinophilic infiltration in EoE seem to represent two sides of the same coin (Figure 2). In fact, research conducted in a murine model [151] and on esophageal cell cultures [152] has shown that subepithelial fibrosis in EoE develops as a consequence of IL-5, IL-4 and IL-13-promoted tissue eosinophilia. 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 [153]. 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 [152].

EoE-associated fibrosis has been related to esophageal eosinophil activation, as shown by eosinophil degranulation, which can be determined by immunohistochemical staining for eosinophilic major basic protein (MBP) [146]. In fact, eosinophil-released MBP has been found to increase the expression of FGF-9 in biopsies of EoE patients [154]. This cytokine, which participates in the proliferative response to injury, correlates with the basal cell hyperplasia documented in the esophageal epithelium of EoE patients and directly promotes both fibroblast activation and deposition of extracellular matrix (ECM). However, the most extensively analyzed cytokine in EoE-associated fibrous remodeling is TGF-β, a potent activator of fibrobasts and a strong inducer of epithelial–mesenchymal transition, which is released from activated eosinophils and mast cells [155]. In an epithelial–mesenchymal transition process that has already been demonstrated for EoE [156], TGF-β can activate quiescent epithelial cells and fibroblasts, causing them to transdifferentiate into myofibroblasts. These cells 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 [157].

Th2-cytokines IL-4 and IL-13 may also induce the expression of activated fibroblast markers, secretion of ECM components and proinflammatory molecules such as eotaxins [158,159]. 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. Indeed, the expression levels of this chemokine have been shown to be highly increased in EoE [148].

Tissue remodeling also involves morphological and functional changes in smooth muscle components. In fact, esophageal muscle cells respond to various profibrogenic stimuli and eosinophilic products. Thus, while MBP is a strong agonist of the M2-type receptors of acetylcholine, which governs smooth muscle function [160], at same time, eosinophil-derived mediators affect the release of acetylcholine from the neuromuscular junction [152]. Mast cells may also play an important role in EoE-associated motor disturbances. In fact, rather than exhibiting an increase in eosinophils, the smooth muscle of EoE patients presents an increase in mast cell numbers, with the TGF-β1 expressed by them possibly increasing the contractility of smooth muscle fibers [86], thereby modulating esophageal contractility. Hypertrophy of the muscularis mucosa along with the circular and longitudinal muscle layers has also been reported in patients with EoE [161], contributing to the esophageal dysfunction repeatedly demonstrated in pediatric [162] and adult EoE patients [163–165]. Finally, eosinophilic infiltration of the submucosal and myenteric neuronal plexus has also been documented in EoE [144].

Esophageal remodeling & EoE symptoms

The severity of epithelial eosinophilia has not been shown to be predictive of the severity of symptoms in EoE patients; in contrast, esophageal symptoms in EoE are attributable to dysfunction in deep esophageal structures resulting from the combination of fibrotic structural changes and motor disturbances. High-resolution manometry in EoE patients has helped demonstrate pressurization patterns consistent with reduced esophageal compliance and functional outflow obstruction [157]. Esophageal distensibility has also been demonstrated to be reduced in EoE patients and has been proposed as a measure of disease severity, predicting which patients require esophageal dilation [166].

Therapeutic interventions for EoE-associated fibrous remodeling

The effect of swallowed topical steroids in reversing esophageal remodeling in EoE has been assessed in several studies. While collagen deposition seems to be a reversible phenomenon in children, it tends to persist among adults. Aceves *et al.*, who

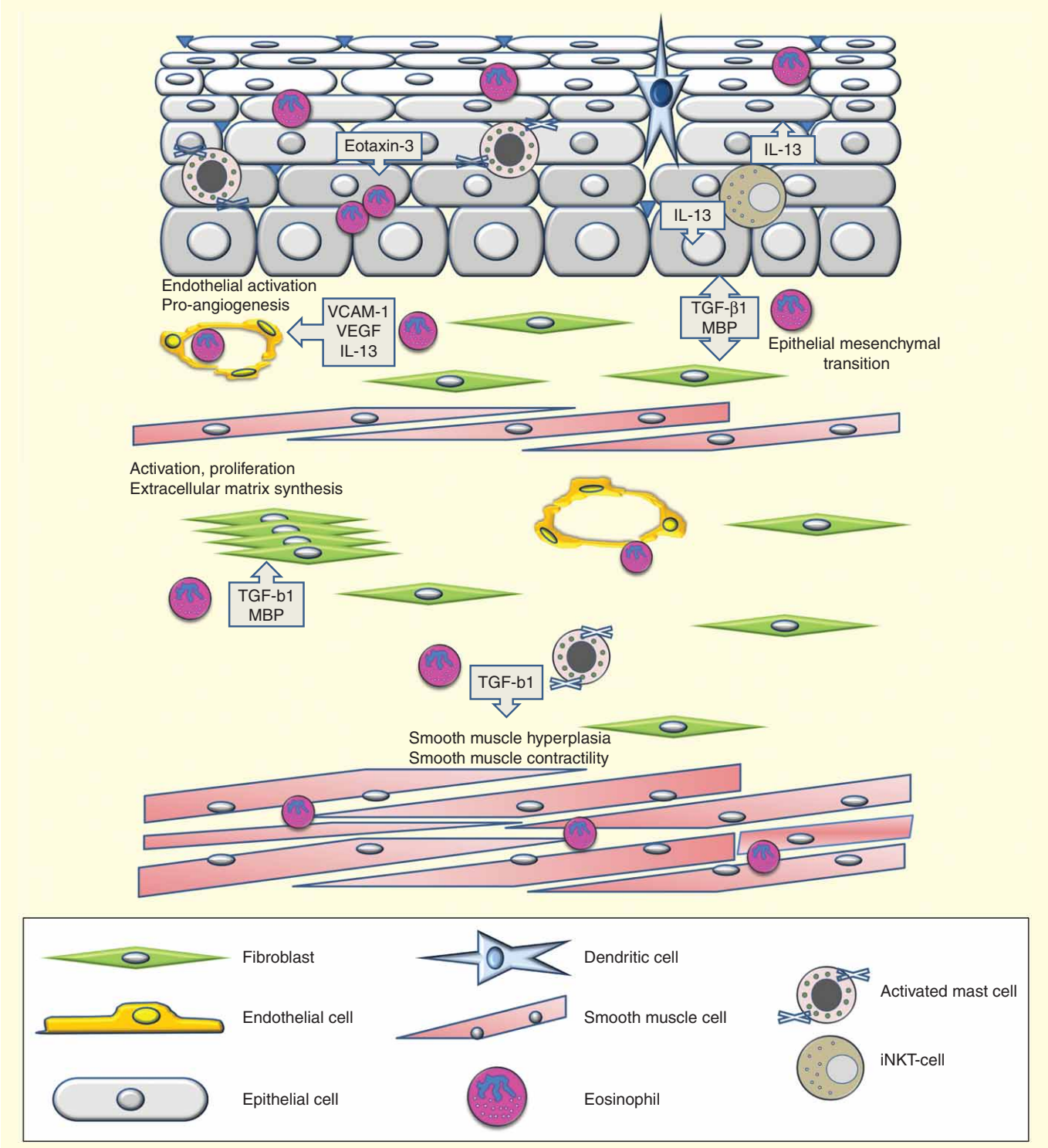

Figure 2. An outline of the cellular and molecular esophageal fibrous remodeling-associated processes in EoE. Activated eosinophils are multifunctional cells that regulate diverse processes including angiogenesis and endothelial activation, which are necessary for recruiting inflammatory cells within the esophageal tissue. The effects of TGF-1 and other activated eosinophil and mast cell-derived mediators on smooth muscle fibers 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.
iNKT: Invariant natural killer T; MBP: Major basic protein; VCAM: Vascular cell adhesion molecule.

doi: 10.1586/17474124.2014.909727

retrospectively assessed the efficacy of budesonide in reversing structural changes in a pediatric EoE series [167], found that responder patients showed significantly reduced esophageal remodeling with decreased fibrosis, TGF-β and pSmad 2/3-positive cells and decreased vascular activation as determined by reduced expression of vascular cell adhesion molecule-1. In contrast, Lucendo *et al.* observed no significant reductions in collagen deposition in the lamina propria of 10 adult patients prospectively treated with FP for 1 year, despite the fact that the treatment induced downregulation of profibrogenic cytokine gene expression [148]. These contradictory results may be attributed to the distinct tissue penetration capacities of different steroids into the lamina propria or the different mechanisms behind pediatric versus adult fibrosis. At any rate, a C/C polymorphism at the -509 position in the TGF-β1 gene promoter has recently been related to reduce remodeling after steroid treatment.

Reversion of subepithelial fibrosis after dietary therapy has also been demonstrated in a retrospective study involving pediatric EoE patients [168]. In contrast, no data for adults have been provided to date.

The paradigm of fibrous remodeling in EoE is represented by the rigid fibrous stenosis frequently observed in EoE patients [169]. Esophageal dilation is a mechanical procedure with proven efficacy in improving symptoms in the majority of EoE patients, at least in the short term, according to a recent meta-analysis [170]. Endoscopic dilation has mostly been used in adult EoE patients among whom esophageal stenosis with a reduction in esophageal caliber is common. Because early studies on dilation treatment of EoE reported a higher than expected complication rate, it came to be considered a risky procedure [171]. However, when performed by an experienced specialist, esophageal dilation is generally safe as demonstrated by case series-based studies [172,173] and a meta-analysis [174]. There are, however, several proven risk factors for complications including a long history of dysphagia, high eosinophil density, younger patient age, repeated procedures and luminal narrowing in the upper and middle esophageal thirds [173,175]. Since endoscopic dilation has no effect on the underlying inflammatory process [172], patients must undergo repeated procedures. Because of this, many authors consider dilation to be an ancillary therapy for EoE that allows the resolution of strictures that cannot be managed with steroids or diet-based interventions.

Investigational therapies in fibrous remodeling

Losartan, an angiotensin II receptor blocker, has been approved for the treatment of high blood pressure in children and adults and has proven safe when administered to patients with normal blood pressure. Losartan may reduce the amount of TGF-β and thus constitutes a potential treatment for EoE. A Phase II trial administering increasing doses of losartan to pediatric and adult EoE patients is currently underway to evaluate endoscopic, histological and symptomatic improvement [176].

Expert commentary

Noteworthy advances in our understanding of the molecular pathophysiology of EoE have been made over the last decade, along with increased insight into its proper diagnosis and treatment. While theories contending that the onset of EoE results from the interplay between exposure to external (mainly food) antigens and the esophageal surface are widely accepted, a definitive etiology of EoE remains elusive.

Experience has shown that EoE is a complex disease involving a large number of cells, molecules and genes. Rather than being unique, EoE may actually be a clinical and evolutionary spectrum in each individual patient. Eosinophilic infiltration of the esophagus could also represent the ultimate common phenotype resulting from the convergence of different activation forms of inflammation, which cannot be identical in every case [48], thus representing different stages of a progressive disorder. In fact, the EoE phenotypes that have already been defined with regard to endoscopic appearance should be further investigated from a molecular point of view. Most of the genetic and molecular knowledge about EoE comes from research carried out in pediatric forms of the disease, which may represent its initial stages, but up to now, little information on the evolutionary consequences at a basic level has been provided.

As proof of the complex origin of the disease and its varied onset patterns, EoE has been repeatedly described after the induction of tolerance in patients with IgE-mediated allergies. Thus, besides a strong genetic background providing susceptibility to the disease, the *de novo* onset of EoE after milk [177] and egg [178], oral immunotherapy or after sublingual pollen immunotherapy [179] among patients with immediate IgE-dependent reactions should lead us to reconsider food allergy as a dynamic concept. This opens up the possibility of a change in the immune response pattern from a Th2-type to a predominantly Th1 type of esophageal allergy. This hypothesis would, in turn, admit the possibility that the importance of Th2 cytokines in some secondary forms of EoE could be diminished in favor of a Th1 profile involving TNF-α and IFN-, which have already been demonstrated to be overexpressed in some EoE patients [180,181].

Apart from the exact proinflammatory mechanisms leading to EoE, the specific reasons for its explosive epidemiological rise in the last few decades must be elucidated. Incidence of EoE is growing faster than that of any other forms of allergy; even when a higher awareness may partially explain its increased diagnosis, other epidemiological factors apart from the hygienic hypothesis are needed to explain why certain common foods that have been repeatedly consumed for thousands of years are now inducing a hypersensitivity response isolated to the esophageal mucosa while apparently leaving the oral and GI mucosa unaffected. The relationship between the appearance of EoE and food processing changes, genetic modification of crops and altered composition of the human GI tract microbiota through exposure to antibiotics in developed societies all constitute important research topics.

Five-year view

The definition of EoE as a fibrostenotic progressive disorder [149] with potential deleterious consequences in the long term, and the recognition of the many unsatisfied needs of EoE patients will impact the management of the disease in the coming years.

A well-preserved genetic EoE transcriptome has facilitated the development of an EoE diagnostic panel, which has the additional advantage of identifying histologically ambiguous subjects who may later develop active EoE. Given the high genetic load of EoE, the usefulness of this panel in identifying atopic patients or relatives at risk of developing EoE should be assessed. Minimally invasive methods for patient diagnosis and monitoring are urgently needed in clinical practice; research assessing the applicability of the esophageal string test [182], among others, is thus of vital importance.

Drugs and dietary interventions constitute the two major therapy modalities for EoE. Still, novel formulations of steroids, which are meant to be concentrated over the esophageal mucosal surface should give way in future to individualized therapies specifically targeted to the key molecules altered in each individual patient; to this end, the identification of molecular EoE phenotypes will facilitate personalized medicine and improve on the disappointing results obtained with biological therapies up to now. In parallel, ongoing research focused on less restrictive dietary approaches and the implementation of dietary support for EoE patients will improve the acceptance of food restrictions as highly effective, drug-free therapies.

Though still far from being able to 'reprogram' dendritic cells to restore the mucosal tolerance to common food antigens in EoE patients, promising results in ulcerative colitis point to the possibility of restoring the regulatory properties of gut dendritic cells through probiotic bacteria-derived peptides [183]. Integrative research in the coming years will further define the individual factors that lead to EoE to provide each patient with therapeutic interventions adapted to specific targets in the near future.

Financial & competing interests disclosure

The author had no conflict of interest or affiliation with any of the institutions, organizations or companies that might be mentioned in the manuscript. The authors have no other 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 apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Eosinophilic esophagitis (EoE) is a pathophysiologically complex food allergy-related disorder in which not only eosinophils, but also a large number of cells, molecules and genes are involved. A tissue-specific functional phenotype has been recognized for blood circulating eosinophils in EoE.
- EoE has been associated with a Th2-type inflammatory response that shares common molecular pathways with bronchial asthma and other atopic diseases characterized by *IL-5*, *IL-13* and *eotaxins* expression.
- The strong genetic load of EoE has facilitated the identification of an EoE characteristic genetic profile, which affects 1% of the human genome and is remarkably conserved across gender, age and allergic status. From this, a PCR-based array molecular diagnostic test has been developed to identify patients with EoE in a fast, objective and mechanistic manner, which may overcome the limitations of histological evaluation.
- A key role for esophageal epithelial cells has been recognized in the past few years; their interaction with the normal microbiota modulates the expression of CXCL16 to recruit T lymphocytes (especially invariant natural killer T cells) toward the esophageal epithelium. IL-13 derived from Th-2 cells induces *eotaxin-3* expression in epithelial cells; this chemoattractant then recruits eosinophils from the circulating blood.
- An increasing role for invariant natural killer T cells as the major source of proinflammatory Th2 cytokines in EoE has recently been recognized. These are activated after exposure to glycolipids (including those contained in some common food allergens), and their modulation is proposed as a potential therapeutic target in EoE.
- Epithelial and other mesenchymal cells also produce thymic stromal lymphopoietin, which has been shown to be a key regulating factor in EoE, acting as a link between the mucosa-associated innate immune system and adaptive responses.
- Topical steroids are the most commonly used treatment for EoE patients; their predominant effect over epithelial cells is able to reverse the EoE transcriptome *in vitro*, repressing the activity of the *eotaxin-3* gene promoter induced by IL-13.
- Recognized only a few years ago, PPI-responsive esophageal eosinophilia constitutes a differential diagnosis of EoE. Although considered mutually exclusive disorders, they are indistinguishable in their demographic, clinical, endoscopic and even molecular characteristics. In fact, the effect of PPI drugs in reducing *eotaxin-3* gene expression by acting on the gene promoter provides additional proof of the close relationship between both disorders.
- The molecular basis and clinical consequences of esophageal remodeling in EoE have been increasingly recognized in recent years. These complex phenomena appear to be a direct consequence of the Th2 cytokine-promoted tissue eosinophilia and involve mast cells, fibroblasts and smooth muscle cells.
- TGF-β1 released by activated eosinophils and mast cells directly activates epithelial cells and fibroblasts, which transdifferentiate into myofibroblasts capable of synthesizing and depositing extracellular matrix components in an epithelial–mesenchymal transition that leads to tissue remodeling. This phenomenon underlies the symptoms and fibrotic stenosis observed in EoE patients, and its reversibility has not yet been fully demonstrated.

doi: 10.1586/17474124.2014.909727

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doi: 10.1586/17474124.2014.909727