

Eosinophilic esophagitis: unclear roles of IgE and eosinophils

■ C. Blanchard¹, D. Simon², A. Schoepfer³, A. Straumann⁴ & H.-U. Simon⁵

From the ¹Nestlé Research Center, Lausanne; ²Department of Dermatology, Inselspital, Bern University Hospital, Bern; ³Division of Gastroenterology, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne; ⁴Swiss EoE Clinic and EoE Research Network, Olten; and ⁵Institute of Pharmacology, University of Bern, Bern, Switzerland

[Content List](#) – Read more articles from the symposium: Nobel Conference: Cell Cycle and Cell Death in Disease.

Abstract. Blanchard C, Simon D, Schoepfer A, Straumann A, Simon H-U (Nestlé Research Center, Lausanne; Bern University Hospital, Bern; Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne; Swiss EoE Clinic and EoE Research Network, Olten; University of Bern, Bern; Switzerland). Eosinophilic esophagitis: unclear roles of IgE and eosinophils (Review Symposium). *J Intern Med* 2017; **281**: 448–457.

Eosinophilic esophagitis (EoE) is a chronic inflammatory disease of the oesophagus. Recognized as a distinct entity only two decades ago, the emergence of the disease along with the availability of new technologies has rapidly opened new research avenues and outlined the main features of the pathogenesis of EoE. Yet, each advance in our understanding of the disease has raised new questions about the previous consensus. Currently, new subsets of the disease challenge our diagnostic

criteria. For instance, it was believed that EoE did not respond to proton pump inhibitor (PPI) therapy; however, it has now been shown that a substantial proportion of EoE patients indeed respond to PPIs. In addition, a new subset of patients not even presenting eosinophil infiltrates in the oesophagus has also been described. Moreover, approaches for better understanding the heritability of the disease bring into question the dogma of predominant genetic involvement. Furthermore, the specificity and sensitivity of allergy testing for targeted food avoidance is highly controversial, and the production of specific antibodies in EoE now includes IgG4 in addition to IgE. In conclusion, EoE is perceived as ‘a moving target’ and the aim of this review was to summarize the current understanding of EoE pathogenesis.

Keywords: Eosinophilic esophagitis, eosinophils, immunoglobulin E, pathogenesis.

Introduction

Eosinophilic esophagitis (EoE) is a relatively new disease. EoE was first recognized in the scientific literature in 1977 and 1978 by Dobbins *et al.* and Landres *et al.*, [1, 2] respectively, and initially characterized by Attwood *et al.* in 1993 and Straumann *et al.* in 1994 [3, 4]. Consensus guidelines have only recently outlined diagnostic criteria and treatments for the disease. EoE is presently defined as a chronic inflammatory condition of the oesophagus characterized clinically by symptoms of oesophageal dysfunction – in particular dysphagia to food impaction in adults and food refusal, failure to thrive, regurgitation and vomiting in children – and histologically by intense eosinophilic infiltration [5].

An eosinophilic infiltration of ≥ 15 eosinophils per high-power field (HPF) along with symptoms of

oesophageal dysfunction points to suspected EoE. However, eosinophils are not the only inflammatory cells infiltrating the oesophageal mucosa and numerous other cell types such as lymphocytes, dendritic cells, mast cells, basophils and invariant natural killer T cells (iNKTs) can also be detected [6–16]. The epithelium in this disease is marked by a hyperplasia of epithelial cells and the lamina propria by profound remodelling; concurrently, oesophageal motor functions are altered [5].

The concomitance of EoE with other allergic diseases highlighted in epidemiologic studies, the ability of dietary interventions to resolve the inflammation, and the results of preclinical studies all indicate an allergic component to the disease. Knowledge about the disease is rapidly expanding, resulting in new recommendations [17], identification of new disease subsets [proton pump inhibitor

(PPI)-responsive oesophageal eosinophilia (PPI-REE) and EoE-like disease], better understanding of the epithelial changes (hyperplasia, intercellular space dilatation and barrier defects) [18, 19], identification of complex molecular networks (IL-13, caspase-14, NGF and IgG4) [19–23] and the proposition of new risk factors (genetic polymorphism versus epigenetic and environmental factors) [24]. The aim of this review was to integrate the recent advances in the understanding of EoE.

EoE definition and new subsets of the disease

Differential diagnoses need to be considered as other conditions, not only EoE, can lead to oesophageal eosinophilia: gastroesophageal reflux disease (GERD), adverse drug reactions, Crohn's disease of the oesophagus, parasitic infection, allergic vasculitis and oesophageal leiomyomatosis [25]. One of the most common diseases that leads to oesophageal eosinophilia is GERD, although inflammation is usually less severe and often limited to the distal oesophagus. Of note, despite the fact that EoE patients usually exhibit normal pH monitoring, this diagnostic measure is not specific as EoE and GERD can coexist and EoE can impair the function of the lower oesophageal sphincter leading to secondary reflux. With the aim of differentiating EoE from GERD, the number of infiltrating eosinophils indicating the presence of EoE was set at 15 eosinophils per HPF for patients under PPI therapy [5]. In 2006, Ngo *et al.* [26] first described patients with high-grade oesophageal eosinophilia responding to PPI therapy. Therefore, the dichotomy between GERD and EoE has been challenged by Spechler *et al.* [27]. A common aetiology for PPI-REE and EoE is highlighted by common cellular and molecular features. Interestingly, the response to PPI therapy in PPI-REE patients resembles the response of EoE patients treated with glucocorticoids [28]. The findings of these studies were rapidly confirmed by others [17, 28–34], and PPI-REE is now considered as a subtype of EoE [17].

Although eosinophilic infiltration of oesophageal tissue is a hallmark of EoE [35], individuals within EoE families have recently been identified who suffer from oesophageal dysfunction in the absence of obvious eosinophilic inflammation. As with their family members suffering from EoE, these atypical patients responded to topical anti-inflammatory treatment. Moreover, increased lymphocytic infiltration and dysregulated gene expression

suggested a pathogenicity similar to that of EoE. This clinical condition has therefore been termed 'EoE-like disease'. This attenuated inflammatory phenotype of the disease provides a warning against conventional EoE diagnosis based on eosinophil count and highlights the need for further investigation of this new subset of patients [36]. In EoE, the granule proteins have cytotoxic effects on the epithelium and mediate cell death or cell damage, suggesting that eosinophils contribute to disease pathogenesis [37]. Clearly, the identification of patients with EoE-like disease challenges the view that eosinophils are critical for EoE pathogenesis.

The epithelium: from barrier defect to Th2 inflammation

As noted above, the coexistence of EoE and other allergic diseases in epidemiologic studies, the fact that dietary intervention can alleviate symptoms and findings from preclinical studies have all highlighted the existence of a Th2 component [38] to the disease, which was also shown directly using EoE oesophageal tissues [16]. The oesophageal epithelium is in direct contact with ingested food, which is a frequent trigger of EoE. However, it is still not known whether such food contact is the primary cause as defects in the oesophageal epithelium have also been identified. Results of a recent study suggest that these defects are associated with the expression of thymic stromal lymphopoietin (TSLP), IL-25, IL-32 and IL-33 by epithelial cells together with extracellular eosinophil trap formation [18] (Fig. 1).

The oesophageal epithelium is altered in association with the disease. For instance, an intense basal zone hyperplasia and papillary elongation are observed in biopsies from EoE patients. Some groups have tried to identify the molecular events responsible for these epithelial changes, and the expression levels of numerous genes have been shown to be dysregulated using transcriptomics or RNA sequencing. Long noncoding RNAs (lncRNAs) such as B-Raf-activated nonprotein coding RNA (BANCR) have been associated with this epithelial hyperplasia. BANCR is induced by IL-13 in oesophageal epithelial cells and is overexpressed in oesophageal biopsies from EoE patients [39]. Silencing of this lncRNA allowed the identification of other genes involved in epithelial hyperplasia such as caspase-14 (CASP14) [39]. The resistin-like molecule alpha (RELM-alpha), also known as FIZZ1, is a Th2-induced gene which is upregulated

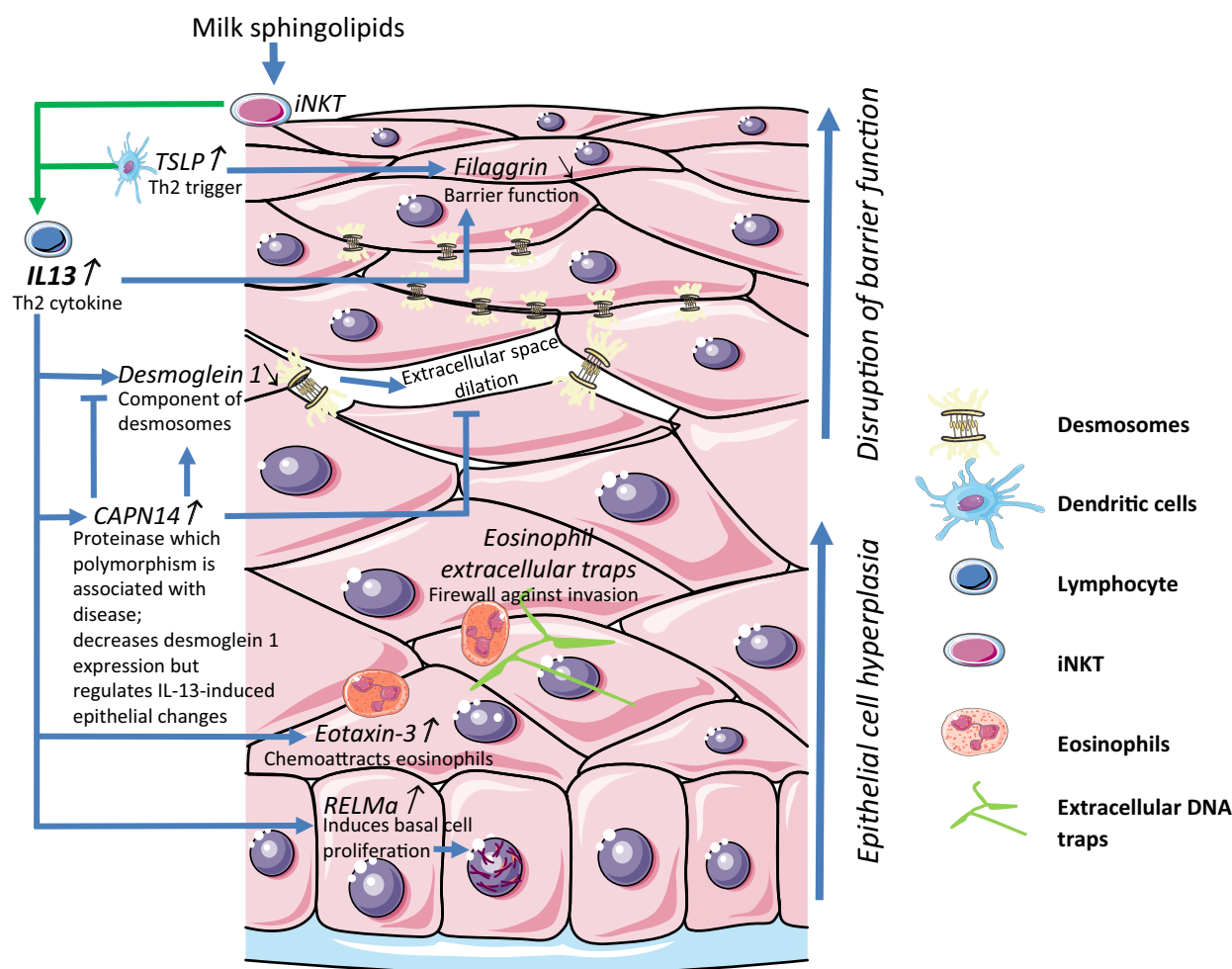


Fig. 1 From epithelial cells to Th2 inflammation, back and forth. The oesophageal mucosa undergoes histologic changes in eosinophilic esophagitis (EoE): hyperplasia of the basal layer, dilated extracellular spaces and inflammatory cell infiltration. Eosinophils infiltrate the tissue and form microabscesses and/or extracellular DNA traps. Genome-wide association studies have identified genetic variants in the region of the thymic stromal lymphopoietin (TSLP) and calpain-14 (CAPN14) genes. TSLP is a key regulator of the T helper 2 (Th2) response and contributes to interleukin-13 (IL-13) upregulation. IL-13 is highly involved in the molecular changes observed in EoE, regulating genes linked with inflammatory cell infiltration such as eotaxin-3, a chemoattractant of eosinophil infiltration. Calpain-14 downregulates desmoglein 1, but may also contribute to reducing dilated extracellular spaces. IL-13 induces genes involved in epithelial proliferation, such as RELM- α , and downregulates genes involved in epithelial cell differentiation and barrier function, such as filaggrin or desmoglein 1. The barrier function of the epithelium is altered and may allow passage of environmental factors such as milk sphingolipids which induce invariant natural killer T cells (iNKTs), potentiating the Th2 response. This figure was produced using Servier Medical Art (www.servier.com).

in EoE, compared to control patients. It has been demonstrated in knockout mice that RELM- α participates in the thickening of the basal layer of the oesophageal epithelium in experimental EoE [40]. The central role of the epithelium in disease onset is indirectly highlighted in another mouse

model of EoE. SHARPIN mice develop spontaneous EoE with histologic features resembling the disease, that is epithelial hyperplasia and eosinophilic infiltration of the oesophageal mucosa [41]. Interestingly, these animals crossed with lymphocyte-deficient mice still develop the disease with the

same severity, suggesting that lymphocytes are not required for the eosinophilic oesophageal inflammatory response in SHARPIN mice [41].

Dilated extracellular spaces between the oesophageal epithelial cells have been identified in EoE patients [42–44]. It has recently been reported that these intercellular spaces correlate with endoscopic mucosal impedance measurements of ion flux [42]. Indeed, both a mucosal impedance of less than 2300 Ω and the presence of dilated extracellular spaces can predict actively inflamed disease with 90% sensitivity and 91% specificity and 89% sensitivity and 82% specificity, respectively. These extracellular spaces may be a consequence of altered desmosomes. Desmoglein 1, an essential constituent of desmosomes, has been shown to be involved in the reduced barrier function observed in EoE oesophageal epithelium. Additionally, IL-13 is also responsible for the downregulation of desmoglein 1 mRNA [19] along with other genes involved in the skin barrier function [45] (Fig. 1). The inflammation observed in atopic dermatitis resembles in many ways EoE and the genes involved in the epidermal differentiation complex are largely downregulated in both diseases, along with numerous genes known to be dysregulated by Th2 cytokines [45].

IL-13 transcriptional targets in EoE

IL-13 is a key player in the oesophageal inflammation (Fig. 1). Many transcriptional targets of IL-13 have been shown to be involved in EoE pathogenesis, and complex gene interaction networks have been identified. In a recent clinical trial, oesophageal eosinophilia was significantly decreased by anti-IL-13 antibody treatment [46]. However, when the outcome was defined as a 75% decrease in peak eosinophil numbers, a nonsignificant decrease in the response in the antibody-treated group (12.5%) was observed as compared to the placebo group (40%). These initial results were thus less encouraging than expected as IL-13 is known to induce dysregulation of numerous genes in oesophageal epithelium. A further clinical trial testing the efficacy of another anti-IL-13 antibody is ongoing, and results seem more promising.

Many molecules have been implicated in eosinophil accumulation. Most are directly or indirectly associated with the presence of IL-13. For instance, the expression of *eotaxin-3*, an eosinophil chemoattractant and a transcriptional target of IL-13, is

highly dysregulated in EoE patients (Fig. 1). Produced by epithelial cells, its expression levels in biopsies correlate directly with eosinophil numbers [8]. Numerous genes are indirectly linked to oesophageal eosinophilia, such as the neurotrophic tyrosine kinase receptor type 1 (*NTRK1*). *NTRK1* upregulation in EoE patients increases the responsiveness to its ligand NGF and, together with IL-13, allows a synergistic dysregulation of genes such as *eotaxin-3* and *serpin B4*, highlighting the complex gene interaction network required for the disease phenotype [20]. Other recent data suggest that prostaglandin D₂, derived from activated mast cells, participates in the chemoattraction of eosinophils in the oesophageal epithelium [47], pointing to the possibility that CRT2 antagonism is indeed a promising new strategy for the treatment of EoE [48].

Food antigens: is there a place for IgE and IgG4 in the disease?

The role of IgE in EoE is puzzling. IgE is definitely associated with EoE, but not all EoE patients have high levels of either total or specific IgEs [16]. It is still unclear whether IgE plays an active role in the disease pathogenesis or whether it is only an associated marker [49]. Intensive efforts have been made by the medical community to determine whether specific IgEs are causative in the pathogenesis of EoE. IgE switching has been identified in the oesophageal mucosa of EoE patients, suggesting possible local rather than systemic IgE production in the disease [50]. Animal models have shown that IgE production is not necessary for oesophageal eosinophilia [51]. Interestingly, targeted food avoidance and trials testing the effect of omalizumab (antibody against IgE) seem to be less promising than might be expected for an allergic disease and the suggestion of Th2 involvement. On the other hand, specific IgG4, an antibody isotype known to be upregulated by IL-4, is increased by 20-fold in oesophageal biopsies from adult EoE patients [23]. IgG4 is a Th2-induced isotype and is considered as a key marker for desensitization in immunotherapy. Recently, oral immunotherapy (OIT) has been shown to induce EoE in about 2.7% of the patients undergoing this treatment [52]. Although the exact role of IgG4 in EoE is still unclear, these data suggest that (i) production of IgG4 is associated with EoE, and (ii) sustained exposure to an allergen whilst undergoing OIT can induce eosinophilic inflammation in the oesophagus. Moreover, although IgE was not found to be predictive of EoE, the utility of IgG4 for predicting the disease has recently been assessed with

positive results. Intraepithelial IgG4 deposits have been detected in EoE patients, allowing differentiation of the disease from GERD with a sensitivity of 88% and a specificity of 100% [53].

Milk has been identified as one of the main triggers for EoE symptoms. It has recently been demonstrated that the milk sphingolipids induce iNKTs to produce Th2 cytokines [11] (Fig. 1). iNKTs are overrepresented in EoE patients [11, 14, 15, 54] and are known to promote oesophageal eosinophilic inflammation in experimental EoE [15].

The role of milk in EoE was also highlighted by the findings of a recent study which suggested that breastfeeding practices might be associated with the risk of developing the disease [24]. It has also been shown that EoE is associated with season of birth and penicillin allergy [55]. Moreover, the use of antibiotics in early infancy seems to lead to a sixfold increase in the risk for developing EoE [56], pointing towards a role for the microbiota. In addition, interest in environmental factors has recently increased as they challenge the dogma that EoE is a disease with a predominant of genetic heritability.

Heritability of EoE

Familial cases of EoE [57] and sibling recurrence risk ratio have led to the suspicion of a strong genetic inheritance for this disease. Consequently, for the last 10 years, efforts have focused on identifying genetic polymorphisms associated with EoE, using either candidate gene approaches or genome-wide association studies. The latter have identified genetic variants associated with EoE disease: rs3806932 in *TSLP* gene locus, [58], 2p23 spanning *CAPN14*, 8p23 in the intron *XKR6* and 15q13 between *LOC283710* and *KLF13* [59], 1p13 in *SLC25A24*, 5q23 near *SEMA6A*, 10p12 in *MIR467S*, 11q13.5 containing *c11orf30*, 11q14 in the intron of *CCDC81*, 12q13.3 spanning *STAT6*, 19q12.11 spanning *ANKRD27*, and 21q22 in *HSF2BP* [59, 60]. The plurality of these genetic markers is testimony to the polygenic aetiology of the disease, but also to the missing heritability. Indeed, a recent twin cohort study, comparing the risk of developing EoE amongst the general population and amongst siblings, monozygotic twins and dizygotic twins, has shown that genetics can explain only 14% of the disease inheritance. The contribution of environment, and more specifically the

familial household, was previously underestimated and can explain 81% of the disease phenotypic variance [24]. Currently, however, the effort to understand the environmental causes of EoE is at an early stage.

Remodelling, fibrosis and pain

The inflamed oesophagus is subjected to intense remodelling possibly explaining at least some oesophageal dysfunction in adult EoE patients [61]. Remodelling is secondary to tissue damage, but collagen deposition in the lamina propria and intraepithelial inflammation may co-occur [62, 63]. TGF- β 1 is highly expressed in the oesophageal epithelial layer of EoE patients and is believed to be involved in remodelling processes [37, 61, 64, 65]. Recently, additional mechanistic insights have been obtained. For instance, Smad 3 knockout mice which lack a key molecule within the TGF- β 1 signalling pathway exhibit reduced oesophageal eosinophilia [66]. Moreover, TGF- β 1 induces expression of periostin, a protein that facilitates the passage of eosinophils from the blood stream into the oesophageal tissue. TGF- β 1 is also an inducer of matrix metalloproteinases such as MMP-2, which is known to be involved in fibrosis. It has also recently been shown that expression of another matrix metalloproteinase, MMP-14, is associated with the extent of fibrosis in the oesophagus [67]. Additionally, phosphorylated phospholamban, a transcriptional target of TGF- β 1, is overexpressed in EoE and modulates smooth muscle cell contractility [68]. Finally, transient receptor potential vanilloid type 1 (TRPV1) is involved in the acid responsiveness of the vagal afferent neurons and may contribute to retrosternal pain and the heartburn symptoms frequently described by EoE patients [69]. In both adults and children, oesophageal inflammation may lead to oesophageal remodelling and associated symptoms. In adults, the delay in EoE diagnosis may contribute to oesophageal strictures as a consequence of an established untreated inflammation [70]. In children, a study has shown that 42% of patients with lamina propria fibrosis have dysphagia [71]; however, these remodelling features are reversible with appropriate treatment, whether it is dietary intervention or topical steroids.

The quest for specific and sensitive markers

EoE symptoms and the characteristics of EoE histology are not sufficiently specific, thus making

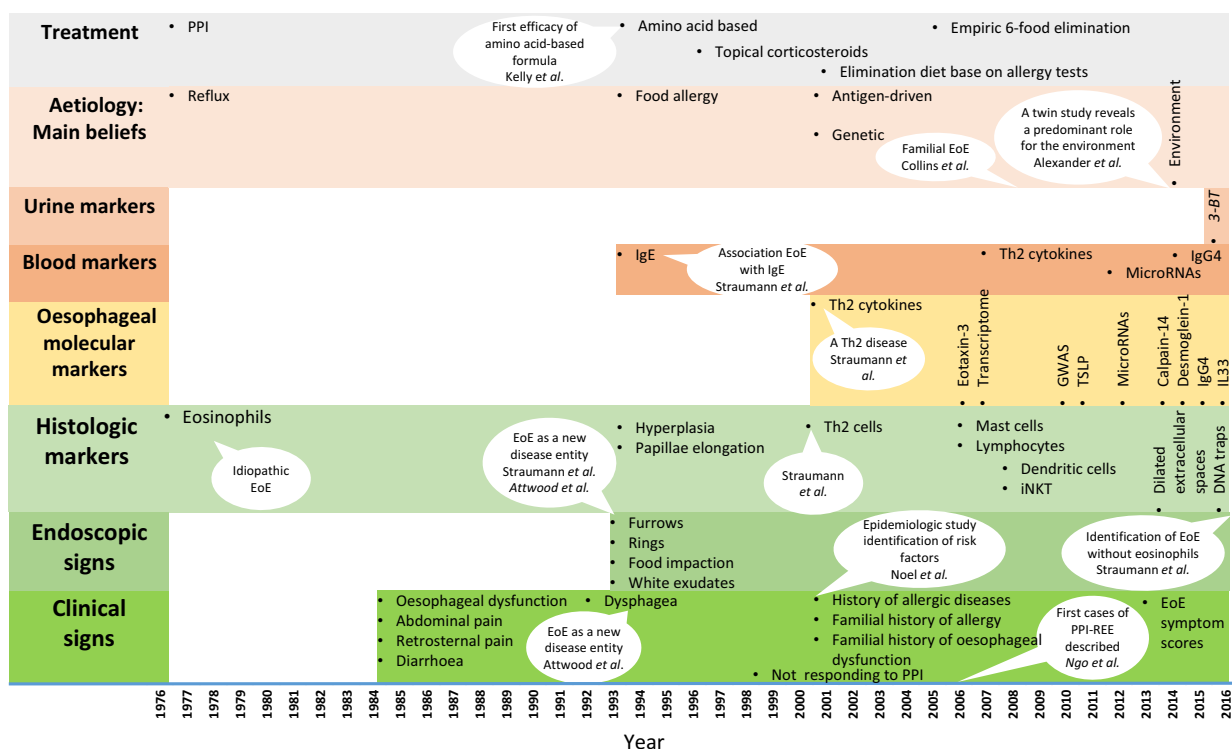


Fig. 2 The markers of eosinophilic esophagitis (EoE) disease: a 40-year journey. In the last 40 years, extensive research has led to a better understanding of EoE. Characteristics of the disease, such as clinical symptoms, endoscopic phenotypes, histologic hallmarks, as well as molecular and blood markers, have been identified. These findings have led to understanding the disease and have resulted in development of therapies. Although the infiltration of eosinophils is observed in most cases and remains a key diagnostic criterion, the role of eosinophil infiltration in the pathogenesis is unclear. Similarly, the role of IgE antibodies in EoE pathogenesis remains uncertain. 3-BT, 3-bromotyrosine; Th2, T helper 2; TSLP, thymic stromal lymphopoietin; GWAS, genome-wide association study; iNKT, invariant natural killer T cell; PPI, proton pump inhibitor; PPI-REE, PPI-responsive oesophageal eosinophilia.

diagnosis difficult. Many research groups have focused attention on methods for disease scoring, identification of cellular and molecular biomarkers and development of noninvasive diagnostic tools (Fig. 2). Yet numerous limitations still reduce the utility and applicability of current disease scores, biomarkers and diagnostic tools. Blood markers seem to be the most convenient, as they are minimally invasive (only requiring blood collection). In allergic diseases, specific IgE levels often help in establishing the diagnosis (Fig. 2). In EoE, however, specific IgE levels are often misleading. Therefore, other markers have been investigated. For instance, peripheral blood mononuclear cells of EoE patients have shown some differences in Th2 cytokine expression compared to control subjects without EoE [72] and some studies have shown that blood eosinophils of EoE patients differently

express surface markers [73]. Additional studies have focused on panels of different cytokines in the blood, but other allergic diseases also strongly influence expression of Th2 markers, decreasing the specificity of the test. So far, this approach has been shown to be less sensitive than oesophageal markers [74].

The eosinophil count in the epithelial layer of the oesophagus is central for EoE diagnosis. However, as discussed above, the emergence of the new EoE subsets PPI-REE and EoE-like disease question even the utility of this marker. In addition to tissue eosinophil numbers, a panel of 94 EoE transcripts has been developed to differentiate EoE from non-EoE controls with a sensitivity of 92% and a specificity of 96%. However, this EoE score has limitations in cases with an eosinophil count

ranging from 6 to 14 per HPF [75]. The approach of immunohistochemistry and a panel of epithelial cell-derived genes may be able to complement the results from the EoE 94-transcript panel [76]. Cellular and molecular markers, as well as panels of markers for the oesophagus seem to be useful in terms of specificity and sensitivity, but still require endoscopy and biopsies, thus remaining invasive.

To differentiate between EoE and non-EoE controls, genetic markers have also been investigated (Fig. 2). This approach could potentially be very relevant because EoE in remission would also be recognized along with EoE. Genome-wide association studies have identified several single-nucleotide variants associated with EoE. The two genes with the most significant association were *TSLP* and *CAPN14* [59, 60]. Calpain-14 is a calcium-activated cysteine protease, and *CAPN14* is an IL-13-induced gene recently identified in two genome-wide association studies (Fig. 1). *CAPN14* mRNA expression is upregulated in active EoE. The proteolytic activity of calpain-14 can impair oesophageal barrier function in air-liquid interphase oesophageal epithelial cell culture, as a result of its role in desmoglein-1 gene expression. Calpain-14 may also have regulatory functions on IL-13-induced epithelial structural changes (such as epithelial dilated intracellular spaces, Fig. 1), and is thus relevant to allergic disease and tissue cell infiltration [77]. Polymorphism in *TSLP* is associated with EoE susceptibility [58]. As a master regulator of Th2 inflammation, *TSLP* has been considered as a marker of the disease. Its involvement in EoE pathogenesis is likely. Unfortunately, however, both *TSLP* polymorphism and expression are not sensitive enough by themselves and cannot be used as EoE biomarkers [78]. Therefore, a combination of several genetic markers may lead to better specificity and sensitivity, but such an approach is still limited by numerous technical factors (Fig. 2).

MicroRNAs are stable small RNA sequences that can be considered as blood biomarkers. MiR-146a, miR-146b, miR-223, miR-203 and miR-21 have been found to be dysregulated in oesophageal biopsies, but also in the blood of EoE patients compared to non-EoE control subjects [79–83]. These data need to be confirmed in larger cohorts and assessed for specificity and sensitivity.

Very recently, using a mass spectrometry-based method, Cunnion *et al.* [84] have reported increased levels of urinary 3-bromotyrosine as a

surrogate marker for eosinophil degranulation in EoE disease. Although yet to be confirmed, this approach may lead to the development of noninvasive biomarkers (Fig. 2).

In the absence of reliable noninvasive biomarkers, assessment of disease symptoms using a validated patient-reported outcome (PRO) instrument and determination of histologic alterations in EoE [85] with a scoring system [86] are certainly important developments (Fig. 2). In other diseases, these PRO scores have allowed better recognition and diagnosis. Several groups have initiated research in this area [86].

Conclusions

The incidence of EoE is still underestimated and its diagnosis still requires upper endoscopy. Specific IgE antibodies and eosinophil activation may contribute to the pathogenesis in subgroups of patients, but neither seems to be definitive. The complexities of the disease aetiology and molecular pathogenesis are starting to be unravelled and new noninvasive diagnostic tools are being developed. Ongoing and future studies focusing on associated environmental factors and epigenetics will improve the understanding of the disease onset and thus possibly help to develop strategies for preventing the development of EoE in individuals at risk.

Funding

This review was supported in part by the Nestlé Research Center and Nestlé Health Science (C.B.). A.S., A.Sch. and H.-U.S. are supported by the Swiss National Science Foundation (32003B_160115 and 310030_166473).

Conflict of interest statement

C.B. is employed by Nestec Ltd. A. Sch. has consultancy contracts with Falk, Receptos and Regeneron. A.S. has consultancy contracts with Actelion, Falk, Genentech-Roche, Nutricia, Receptos and Regeneron. The remaining authors have no potential conflict of interest.

References

- 1 Dobbins JW, Sheahan DG, Behar J. Eosinophilic gastroenteritis with esophageal involvement. *Gastroenterology* 1977; **72**: 1312–6.

- 2 Landres RT, Kuster GG, Strum WB. Eosinophilic esophagitis in a patient with vigorous achalasia. *Gastroenterology* 1978; **74**: 1298–301.
- 3 Attwood SE, Smyrk TC, DeMeester TR, Jones JB. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. *Dig Dis Sci* 1993; **38**: 109–16.
- 4 Straumann A, Spichtin HP, Bernoulli R, Loosli J, Vogtlin J. Idiopathic eosinophilic esophagitis: a frequently overlooked disease with typical clinical aspects and discrete endoscopic findings. *Schweiz Med Wochenschr* 1994; **124**: 1419–29.
- 5 Liacouras CA, Furuta GT, Hirano I *et al.* Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011; **128**: 3–20.
- 6 Abonia JP, Blanchard C, Butz BB *et al.* Involvement of mast cells in eosinophilic esophagitis. *J Allergy Clin Immunol* 2010; **126**: 140–9.
- 7 Aceves SS, Chen D, Newbury RO, Dohil R, Bastian JF, Broide DH. Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF-beta1, and increase esophageal smooth muscle contraction. *J Allergy Clin Immunol* 2010; **126**: 1198–204.
- 8 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**: 536–47.
- 9 Dellon ES, Chen X, Miller CR *et al.* Tryptase staining of mast cells may differentiate eosinophilic esophagitis from gastroesophageal reflux disease. *Am J Gastroenterol* 2011; **106**: 264–71.
- 10 Iwakura N, Fujiwara Y, Tanaka F *et al.* Basophil infiltration in eosinophilic oesophagitis and proton pump inhibitor-responsive oesophageal eosinophilia. *Aliment Pharmacol Ther* 2015; **41**: 776–84.
- 11 Jyonouchi S, Smith CL, Saretta F *et al.* Invariant natural killer T cells in children with eosinophilic esophagitis. *Clin Exp Allergy* 2014; **44**: 58–68.
- 12 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**: 598–606.
- 13 Niranjana R, Rajavelu P, Ventateshaiah SU *et al.* Involvement of interleukin-18 in the pathogenesis of human eosinophilic esophagitis. *Clin Immunol* 2015; **157**: 103–13.
- 14 Rajavelu P, Rayapudi M, Moffitt M, Mishra A, Mishra A. 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**: G645–54.
- 15 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 Immunology* 2014; **3**: e9.
- 16 Straumann A, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J Allergy Clin Immunol* 2001; **108**: 954–61.
- 17 Molina-Infante J, Bredenoord AJ, Cheng E *et al.* Proton pump inhibitor-responsive oesophageal eosinophilia: an entity challenging current diagnostic criteria for eosinophilic oesophagitis. *Gut* 2016; **65**: 524–31.
- 18 Simon D, Radonjic-Hosli S, Straumann A, Yousefi S, Simon HU. Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation. *Allergy* 2015; **70**: 443–52.
- 19 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**: 718–29.
- 20 Rochman M, Kartashov AV, Caldwell JM *et al.* Neurotrophic tyrosine kinase receptor 1 is a direct transcriptional and epigenetic target of IL-13 involved in allergic inflammation. *Mucosal Immunol* 2015; **8**: 785–98.
- 21 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**: 1292–300.
- 22 Philpott H, Royce S, Nandurkar S, Thien F, Gibson P. IgG and EoE: too soon for a paradigm shift away from IgE. *Gastroenterology* 2015; **148**: 453–4.
- 23 Clayton F, Fang JC, Gleich GJ *et al.* Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* 2014; **147**: 602–9.
- 24 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**: 1084–92.
- 25 Furuta GT, Katzka DA. Eosinophilic esophagitis. *N Engl J Med* 2015; **373**: 1640–8.
- 26 Ngo P, Furuta GT, Antonioli DA, Fox VL. Eosinophils in the esophagus - peptic or allergic eosinophilic esophagitis? Case series of three patients with esophageal eosinophilia. *Am J Gastroenterol* 2006; **101**: 1666–70.
- 27 Spechler SJ, Genta RM, Souza RF. Thoughts on the complex relationship between gastroesophageal reflux disease and eosinophilic esophagitis. *Am J Gastroenterol* 2007; **102**: 1301–6.
- 28 Molina-Infante J, Katzka DA. Proton-pump inhibitor-responsive esophageal eosinophilia. *Curr Opin Gastroenterol* 2014; **30**: 428–33.
- 29 Dellon ES, Speck O, Woodward K *et al.* Clinical and endoscopic characteristics do not reliably differentiate PPI-responsive esophageal eosinophilia and eosinophilic esophagitis in patients undergoing upper endoscopy: a prospective cohort study. *Am J Gastroenterol* 2013; **108**: 1854–60.
- 30 Dellon ES, Speck O, Woodward K *et al.* Markers of eosinophilic inflammation for diagnosis of eosinophilic esophagitis and proton pump inhibitor-responsive esophageal eosinophilia: a prospective study. *Clin Gastroenterol Hepatol* 2014; **12**: 2015–22.
- 31 Eluri S, Dellon ES. Proton pump inhibitor-responsive oesophageal eosinophilia and eosinophilic oesophagitis: more similarities than differences. *Curr Opin Gastroenterol* 2015; **31**: 309–15.
- 32 Molina-Infante J, Ferrando-Lamana L, Mateos-Rodriguez JM, Perez-Gallardo B, Prieto-Bermejo AB. Overlap of reflux and eosinophilic esophagitis in two patients requiring different therapies: a review of the literature. *World J Gastroenterol* 2008; **14**: 1463–6.
- 33 Molina-Infante J, Rodriguez-Sanchez J, Martinek J *et al.* Long-term loss of response in proton pump inhibitor-responsive esophageal eosinophilia is uncommon and influenced by CYP2C19 genotype and rhinoconjunctivitis. *Am J Gastroenterol* 2015; **110**: 1567–75.
- 34 Park JY, Zhang X, Nguyen N, Souza RF, Spechler SJ, Cheng E. Proton pump inhibitors decrease eotaxin-3 expression in

- the proximal esophagus of children with esophageal eosinophilia. *PLoS One* 2014; **9**: e101391.
- 35 Muller S, Puhl S, Vieth M, Stolte M. Analysis of symptoms and endoscopic findings in 117 patients with histological diagnoses of eosinophilic esophagitis. *Endoscopy* 2007; **39**: 339–44.
 - 36 Straumann A, Blanchard C, Radonjic-Hoesli S *et al.* A new eosinophilic esophagitis (EoE)-like disease without tissue eosinophilia found in EoE families. *Allergy* 2016; **71**: 889–900.
 - 37 Straumann A, Conus S, Degen L *et al.* Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. *Gastroenterology* 2010; **139**: 1526–37.
 - 38 Lee JJ, Blanchard C, Masterson JC *et al.* Animal models of human pathology. In: Lee J, Rosenberg H, eds. *Eosinophils in Health and Disease*. San Diego (CA), USA: Elsevier Inc., 2013; 537–75.
 - 39 Sherrill JD, Kiran KC, Blanchard C *et al.* Analysis and expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. *Genes Immun* 2014; **15**: 361–9.
 - 40 Mavi P, Niranjana R, Dutt P *et al.* Allergen-induced resistin-like molecule- α promotes esophageal epithelial cell hyperplasia in eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2014; **307**: G499–507.
 - 41 Chien SJ, Silva KA, Kennedy VE, HogenEsch H, Sundberg JP. The pathogenesis of chronic eosinophilic esophagitis in SHARPIN-deficient mice. *Exp Mol Pathol* 2015; **99**: 460–7.
 - 42 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**: 1242–8.
 - 43 Ravelli A, Villanacci V, Cadei M, Fuoti M, Gennati G, Salemm M. Dilated intercellular spaces in eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2014; **59**: 589–93.
 - 44 Rodrigo S, Abboud G, Oh D *et al.* High intraepithelial eosinophil counts in esophageal squamous epithelium are not specific for eosinophilic esophagitis in adults. *Am J Gastroenterol* 2008; **103**: 435–42.
 - 45 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**: 4033–41.
 - 46 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**: 500–7.
 - 47 Zhang S, Wu X, Yu S. Prostaglandin D2 receptor D-type prostanoid receptor 2 mediates eosinophil trafficking into the esophagus. *Dis Esophagus* 2014; **27**: 601–6.
 - 48 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**: 375–85.
 - 49 Simon D, Cianferoni A, Spergel JM *et al.* Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity. *Allergy* 2016; **71**: 611–20.
 - 50 Vicario M, Blanchard C, Stringer KF *et al.* Local B cells and IgE production in the oesophageal mucosa in eosinophilic esophagitis. *Gut* 2010; **59**: 12–20.
 - 51 Noti M, Wojno ED, Kim BS *et al.* Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. *Nat Med* 2013; **19**: 1005–13.
 - 52 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**: 624–9.
 - 53 Zukerberg L, Mahadevan K, Selig M, Deshpande V. Oesophageal intrasquamous IgG4 deposits: an adjunctive marker to distinguish eosinophilic oesophagitis from reflux oesophagitis. *Histopathology* 2016; **68**: 968–76.
 - 54 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**: 646–57.
 - 55 Lee YJ, Redd M, Bayman L, Frederickson N, Valestin J, Schey R. Comparison of clinical features in patients with eosinophilic esophagitis living in an urban and rural environment. *Dis Esophagus* 2015; **28**: 19–24.
 - 56 Jensen ET, Kappelman MD, Kim H, Ringel-Kulka T, Dellon ES. Early life exposures as risk factors for pediatric eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2013; **57**: 67–71.
 - 57 Collins MH, Blanchard C, Abonia JP *et al.* Clinical, pathologic, and molecular characterization of familial eosinophilic esophagitis compared with sporadic cases. *Clin Gastroenterol Hepatol* 2008; **6**: 621–9.
 - 58 Rothenberg ME, Spergel JM, Sherrill JD *et al.* Common variants at 5q22 associate with pediatric eosinophilic esophagitis. *Nat Genet* 2010; **42**: 289–91.
 - 59 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**: 895–900.
 - 60 Sleiman PM, Wang ML, Cianferoni A *et al.* GWAS identifies four novel eosinophilic esophagitis loci. *Nat Commun* 2014; **5**: 5593.
 - 61 Rieder F, Nonevski I, Ma J *et al.* T-helper 2 cytokines, transforming growth factor β 1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. *Gastroenterology* 2014; **146**: 1266–77.
 - 62 Simon D, Aeberhard C, Erdemoglu Y, Simon HU. Th17 cells and tissue remodeling in atopic and contact dermatitis. *Allergy* 2014; **69**: 125–31.
 - 63 Straumann A, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU. Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. *Gastroenterology* 2003; **125**: 1660–9.
 - 64 Straumann A, Bussmann C, Conus S, Beglinger C, Simon HU. Anti-TNF- α (infliximab) therapy for severe adult eosinophilic esophagitis. *J Allergy Clin Immunol* 2008; **122**: 425–7.
 - 65 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**: 400–9.
 - 66 Cho JY, Doshi A, Rosenthal P *et al.* Smad3-deficient mice have reduced esophageal fibrosis and angiogenesis in a model of egg-induced eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2014; **59**: 10–6.
 - 67 Beppu L, Yang T, Luk M *et al.* MMPs-2 and -14 are elevated in eosinophilic esophagitis and reduced following topical corticosteroid therapy. *J Pediatr Gastroenterol Nutr* 2015; **61**: 194–9.
 - 68 Beppu LY, Anilkumar AA, Newbury RO, Dohil R, Broide DH, Aceves SS. TGF- β 1-induced phospholamban expression

- alters esophageal smooth muscle cell contraction in patients with eosinophilic esophagitis. *J Allergy Clin Immunol* 2014; **134**: 1100–7.
- 69 Hu Y, Liu Z, Yu X, Pasricha PJ, Udem BJ, Yu S. Increased acid responsiveness in vagal sensory neurons in a guinea pig model of eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2014; **307**: G149–57.
 - 70 Schoepfer AM, Safroneeva E, Busmann C *et al.* Delay in diagnosis of eosinophilic esophagitis increases risk for stricture formation in a time-dependent manner. *Gastroenterology* 2013; **145**: 1230–6.
 - 71 Chehade M, Sampson HA, Morotti RA, Magid MS. Esophageal subepithelial fibrosis in children with eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2007; **45**: 319–28.
 - 72 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**: 22–31.
 - 73 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**: 594–604.
 - 74 Blanchard C, Stucke EM, Rodriguez-Jimenez B *et al.* A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin Immunol* 2011; **127**: 208–17.
 - 75 Wen T, Stucke EM, Grotjan TM *et al.* Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. *Gastroenterology* 2013; **145**: 1289–99.
 - 76 Matoso A, Mukkada VA, Lu S *et al.* Expression microarray analysis identifies novel epithelial-derived protein markers in eosinophilic esophagitis. *Mod Pathol* 2013; **26**: 665–76.
 - 77 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**: e86355.
 - 78 Sherrill JD, Gao PS, Stucke EM *et al.* Variants of thymic stromal lymphopoietin and its receptor associate with eosinophilic esophagitis. *J Allergy Clin Immunol* 2010; **126**: 160–5.
 - 79 Lu S, Mukkada VA, Mangray S *et al.* MicroRNA profiling in mucosal biopsies of eosinophilic esophagitis patients pre and post treatment with steroids and relationship with mRNA targets. *PLoS One* 2012; **7**: e40676.
 - 80 Lu TX, Sherrill JD, Wen T *et al.* MicroRNA signature in patients with eosinophilic esophagitis, reversibility with glucocorticoids, and assessment as disease biomarkers. *J Allergy Clin Immunol* 2012; **129**: 1064–75.
 - 81 Lu TX, Lim EJ, Wen T *et al.* MiR-375 is downregulated in epithelial cells after IL-13 stimulation and regulates an IL-13-induced epithelial transcriptome. *Mucosal Immunol* 2012; **5**: 388–96.
 - 82 Sawant DV, Yao W, Wright Z *et al.* Serum MicroRNA-21 as a Biomarker for Allergic Inflammatory Disease in Children. *Microna* 2015; **4**: 36–40.
 - 83 Zahm AM, Menard-Katcher C, Benitez AJ *et al.* Pediatric eosinophilic esophagitis is associated with changes in esophageal microRNAs. *Am J Physiol Gastrointest Liver Physiol* 2014; **307**: G803–12.
 - 84 Cunnion KM, Willis LK, Minto HB *et al.* Eosinophil quantitated urine kinetic: a novel assay for assessment of eosinophilic esophagitis. *Ann Allergy Asthma Immunol* 2016; **116**: 435–9.
 - 85 Collins MH, Martin LJ, Alexander ES *et al.* Newly developed and validated eosinophilic esophagitis histology scoring system and evidence that it outperforms peak eosinophil count for disease diagnosis and monitoring. *Dis Esophagus* 2016; doi: 10.1111/dote.12470. [Epub ahead of print].
 - 86 Schoepfer A, Safroneeva E, Straumann A. How to measure disease activity in eosinophilic esophagitis. *Dis Esophagus* 2015; doi: 10.1111/dote.12391. [Epub ahead of print].

Correspondence: Hans-Uwe Simon, Institute of Pharmacology, University of Bern, Inselspital, INO-F, CH-3010 Bern, Switzerland.
(fax: +41-31-632-4992; e-mail: hus@pki.unibe.ch).■