Immune system and microbiome in the esophagus: implications for understanding inflammatory diseases

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Introduction

The esophagus, besides the transport function of food, is an organ critical for mucosal immunity. There is an increasing awareness that antigens and metabolites derived from microorganisms and the diet are in contact with the esophagus’ mucosal immune system. Food proteins are digested and proteolyzed by the stomach’s low pH and proteases secreted by the pancreas after they have passed the esophagus. These findings have led to skyrocketing numbers of studies investigating immune responses in the small and large intestine. However, the esophagus remains a somewhat understudied immune organ, although it is easily accessible by endoscopy without specific preparations and allows biopsy sampling more straightforward than other parts of the human intestine.

Eosinophilic esophagitis (EoE) can clinically present with food impaction or dysphagia histologically characterized by an eosinophil-predominant inflammation (> 15 eosinophils per high power field) [1]. Worldwide, EoE has an increasing incidence and prevalence, with currently an incidence of 4.4–7.4 per 100 000 individuals per year and a prevalence of 43 per 100 000 individuals [2]. It has been shown that due to an impaired esophageal epithelial barrier, dietary antigens, structural components of microbes, and bacterial microorganisms penetrate the esophageal mucosa. The recognition of these dietary antigens or bacterial metabolites by esophageal epithelial cells and underlying immune cells leads to an inflammation characterized by increased expression of Th2 cytokines, such as thymic stromal lymphopoietin (TSLP), IL-5, and IL-13 [3–7]. However, the cellular composition of the mucosal immune system in the esophagus is primarily unknown.

Moreover, studies have recently appreciated that the esophagus has its distinct microbiome of predominantly

Abbreviations
DC, dendritic cells; EAC, esophageal adenocarcinoma; EoE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease; ILC, innate lymphoid cells; LC, Langerhans cells; PPI, proton pump inhibitors.
gram-positive bacteria dominated by *Streptococcus* [8–10]. The microbiome’s dynamic changes depending on the ingestion of meals, nocturnal rhythms, and diseases are beyond this review’s scope. However, they have been anticipated as a prerequisite for changes in immune cells’ cellular composition in health and disease.

With few exceptions, most reviews discussing the mucosal immune system’s structure focus on the small intestine and colon. Considering that the esophagus’ immune-mediated diseases have an increasing incidence and prevalence, in this review, we discuss the esophagus’s immune system’s organization and point out the specifics of the esophagus’ immune system.

### The anatomic structure of the esophagus

The esophagus is a continuous fibromuscular tube composed of the epithelium, lamina propria, submucosa, muscle layers, connective tissues, and the adventitia. It passes from the hypopharynx behind the trachea and heart through the mediastinum and diaphragm into the stomach. The esophagus is approximately 20–27 cm long, and its explicit function is the transportation of the alimentary bolus from the mouth into the stomach. However, the esophagus is not only a transit organ and also has a critical function in the mucosal immune response. At least three layers of squamous cells, the stratified squamous epithelium, line the esophagus’ luminal side. The esophagus and the oral cavity represent the upper gastrointestinal tract (GIT) and belong, together with the vaginal cavity, to the type 2 mucosal surfaces lined by stratified squamous epithelium, primarily serving as a physical protective barrier. The mucosal surface of the esophagus lacks mucosa-associated lymphoid tissues, the polymeric immunoglobulin receptor, goblet cells, and Paneth cells in contrast to type 1 mucosal surfaces (e.g., lower intestine, lungs, and uterus) lined by a single layer columnar epithelium. As a consequence, the esophagus lacks a thick mucus layer and IgA. However, one study investigating the presence of immunoglobulins in the esophagus of HIV-infected individuals has described IgA as the predominant immunoglobulin in the esophagus [11]. Nevertheless, it remains unclear whether, in immunodeficient individuals, IgA is secreted across the epithelium in the esophageal lumen or derived from IgA-containing saliva.

It is somewhat surprising that, in contrast to the colon, the esophagus lacks a thick mucus layer, despite the presence of hydrogen ions (H⁺) and bile acids from the stomach and duodenum. Esophageal peristalsis and gravity in an upright position clear 95% of refluxed acid, but ~ 5% of the refluxed stomach contents remain in the esophagus [12]. The esophagus has to neutralize hydrogen ions and bile acids, facilitated by bicarbonate, antimicrobial peptides, and lactoferrin containing saliva forming a soluble mucus capable of lubrication to protect the epithelium [13,14]. The unprotected esophageal epithelium is susceptible to contact with food contents, hydrogen ions, and bile acids. After entry of luminal content through the disrupted epithelial barrier into the esophagus, immune cells beneath the epithelium initiate a specific defense mechanism to protect the esophagus.

### The architecture of the squamous epithelium as a first defense line

The esophageal squamous epithelium consists of three distinctive layers: the stratum corneum (superficial layer), stratum spinosum, and stratum germinativum (basal cell layer) [15]. Each layer plays its part in maintaining epithelial integrity to protect the underlying tissue from environmental products and prevent the development of esophageal pathologies. The glycocalyx covers the esophageal epithelium’s apical membrane providing a robust physical barrier that shields the esophagus from damage. In the stratum corneum, filaggrin connects with intermediate keratin filaments creating a lipid-protein matrix that forms an impenetrable epithelial barrier and an intercellular glycocalyx [16]. Moreover, intercellular junctional complexes consisting of tight and adherens junctions intertwine individual epithelial cells of the stratum spinosum, limiting the flux of molecules, ions, and acid into the intercellular space, thereby protecting the epithelium from damage. Tight junctions interconnecting cell membranes and regulating paracellular ion-permeability are at the apical side of the epithelium. Proteins such as claudin, occludin, zonulin, and junctional adhesion molecules span over the intercellular space and connect with neighboring cells’ cytoskeleton [15]. Dysfunctional tight junctions lead to an impaired epithelial barrier facilitating electrolyte and fluid loss and increasing susceptibility to atopic diseases (e.g., atopic dermatitis, asthma, and EoE). Malfunction of the tight junction proteins (e.g., claudin and occludin) can occur in these diseases [5,17–19]. Another crucial functional structure represents the adherens junction consisting of the transmembrane protein E-cadherin and the intracellular catenins and vinculin, which ensure attachment to actin filaments. Adherens junctions are responsible for stabilizing cell-cell adhesion, regulating the actin cytoskeleton, and mediating intracellular signaling and
transcription regulation [20]. The apical junction complex's most basal structure is the desmosomes that consist of two transmembrane proteins, desmocollin, and desmoglein. Desmosomes support the adherens junctions in cell-cell adhesion [21]. The junctional complex formation is calcium-dependent [22], as paracellular permeability increases, and transepithelial resistance decreases after the experimental removal of calcium in cell cultures. Consequently, esophageal stem cells isolated from biopsies have to be cultured in a calcium-rich medium to differentiate and generate esophageal organoids [23].

When noxious agents overcome pre-epithelial and epithelial defenses, postepithelial defense mechanisms are in place to prevent tissue destruction. Paracellular glycoconjugates with buffer capacity [24] and the mucosal blood supply, which not only provides oxygen, nutrients, and bicarbonate ions but also removes metabolic byproducts, such as hydrogen ions, lactic acid, and CO₂, have protective functions for the esophageal epithelium [14,25,26]. The epithelium can renew and repair wounds after damage. Restitution is a quick repair in minutes to hours by migrating adjacent viable cells to replace dead cells and close wounds [27]. The second form of epithelial repair replaces dead cells with newly generated viable cells by mitosis, a more protracted process of days to weeks [28]. Furthermore, immune cells located beneath the epithelium serve as an additional firewall to remove incoming antigens and pathogens after a barrier breach has occurred.

**Acid-induced esophagitis**

When the esophagus fails to clear the reflux of gastric content and bile acids, gastroesophageal reflux disease (GERD) can develop. In the general population, 10–30% of adults suffer from heartburn, retrosternal pain, and regurgitation [29]. Obesity, hiatal hernia, delayed gastric emptying, and chronic pulmonary disease can increase thoracoabdominal pressure gradients and predispose to GERD [30–35]. GERD complications include erosive esophagitis, peptic strictures, Barrett's esophagus with the replacement of the esophageal squamous epithelium by intestinal columnar epithelia with sequential development of dysplasia, and esophageal carcinoma [29,36–38]. Despite symptomatic reflux, up to 70% of GERD individuals do not develop esophageal erosions [39]. Microscopically, dilated intercellular spaces are also present in nonerosive reflux disease [40,41]. It has been suggested that chronic acid exposure induces epithelial cells to secrete pro-inflammatory cytokines resulting in a mucosal immune cell infiltrate dominated by neutrophils and eosinophils, causing erosions [42]. This hypothesis gives a potential explanation as why macroscopic changes are absent in nonerosive reflux characterized by potentially lower acid exposure, mostly preserved esophageal clearance, and the absence of a pro-inflammatory cytokine signature [42–44]. These studies received further support by data that observed significant immune cell infiltration only in erosive reflux disease, despite the observation that erosive and nonerosive reflux disease both presented with microscopic changes, such as dilated intercellular spaces [38,45]. The pro-inflammatory cytokine pattern in erosive reflux disease includes the neutrophil chemoattractants IL-1β and IL-8, and IL-6 and chemokines, such as RANTES, MCP-1, MIP1-a, platelet-activating factor, and the eotaxins [46–48]. Although neutrophils dominate the immune cell infiltrate in GERD [49], eosinophils accumulate in up to 50% of GERD posing a clinical challenge for the distinction from EoE [49]. Since proton pump inhibitors (PPIs) have proven to be an effective treatment for GERD without overt side effects, immunological research in the esophagus has shifted from GERD to EoE, an immune-mediated disease induced by food allergens and with a treatment spectrum of limited efficacy.

**Immune cells as critical firewalls in the esophagus**

A unique microbiome, mainly gram-positive bacteria, colonizes the esophagus, formerly thought to be only transiently populated by swallowed bacteria from the oral cavity. When these potentially harmful microorganisms and metabolites pass the squamous epithelium, the immune system beneath the epithelium has to fight these threats and defend the host. Only a few immune cells are present in the esophagus of healthy individuals, but increase during infection or inflammation. It needs to be pointed out that most studies have so far investigated the distribution of immune cells in the esophagus, but functional studies describing immune cells in esophageal diseases are scarce. Comparable to the skin, CD1⁴⁺ Langerhans cells (LCs) are present in the esophageal epithelium. As in the skin, LCs in the esophageal epithelium are in close association with lymphocytes in the suprabasal layer [50]. Access to esophageal tissue is only possible by taking biopsies from the esophagus during invasive esophagogastroscope, in contrast to noninvasive biopsy collection from the skin and the oral cavity. Studies by Novak et al. have investigated mucosal dendritic cells (DCs) in the oral cavity [51,52]. These studies allow some assumptions on esophageal DCs' characteristics...
because of the oral cavity’s proximity to the esophagus. Furthermore, the DCs in the oral cavity reside beneath the squamous epithelium like in the esophagus [53]. In contrast to the small and large intestine, the esophagus lacks organized secondary lymphoid tissues with follicle-associated epithelium, where antigens are sampled and presented to T cells. In the esophagus, DCs phagocytose antigens directly from the lumen [54]. It has been suggested that DCs induce a tolerogenic state by presenting antigens to T cells in the epithelium, the lamina propria, or after migration to regional lymph nodes [53,55]. During inflammation, blood-borne DCs infiltrate, in addition to resident LCs, the esophageal epithelium, take up antigens, and migrate to the mediastinal lymph nodes to induce an immune response [55,56]. Comparable to the small intestine, tolerance-inducing DCs stay in an immature state and do not express CCR7, which binds CCL19 and CCL21, two cytokines responsible for the homing of immune cells to draining lymph nodes, whereas infiltrating DCs fully differentiate and begin to express CCR7 [55]. Higher expression of T-cell inhibitory surface molecules (e.g., B7-H) in tissue-resident mucosal DCs compared to epidermal DCs reflects the specific esophageal tissue environment [51].

After antigen presentation by DCs to T cells in the regional lymph nodes, the effector T cells circulate back to the esophagus. Under noninflamed conditions, only a few T cells are present in the esophageal epithelium, comprising mainly CD8+ T cells like the intraepithelial lymphocyte compartment in the small intestine [57]. During inflammation, the T cell numbers increase and can form dense clusters of CD4+ and CD8+ T cells reminding of isolated lymphoid follicles in suprabasal regions of the esophagus [53]. Although T cells are widely present in the gut and lung, T cells’ characterization in the esophagus is lacking. It is of interest to determine the distribution of IFN-γ-producing Th1, IL-13-producing Th2, and IL-17A-producing Th17 cells in the esophagus of healthy individuals and to compare them to T cells in the inflamed esophagus. There have also been innate lymphoid cells (ILCs) described in the esophagus. Doherty et al. have reported the presence of ILC2s in the human esophagus that increased in numbers in EoE and correlated with the degree of mucosal eosinophilia. The stimulation with IL-2, IL-33, and TSLP, three cytokines with increased expression in EoE, expanded the numbers of ILC2s in vitro [58]. To this end, we did not find any studies describing the presence of type 1 and type 3 ILCs in the esophagus.

Taken together, immune cells in the esophagus serve as critical firewalls in the esophagus. However, there is a great need to better phenotype and functionally describe the distribution of immune cells in the esophagus and how these cells’ composition changes in esophageal diseases.

The esophageal microbiome

In recent years, it became apparent that the microbiome is essential for the proper functioning of the GIT, the immune system, defense against pathogens, metabolism, and energy regulation. Approximately 10% of all metabolites in the peripheral blood stem from the microbiota [59]. Our microbiota provides roughly 10% of our daily ingested calories due to fermentation [60]. The microbiota also protects from infectious esophagitis, such as Candida esophagitis with the Candida albicans serotype, the most common Candida species causing esophagitis [61]. The microbiome interferes with the immune system, and its critical relevance for the digestive system and metabolism is one of the most popular current research topics. While the small intestine and colon microbiota are at the center of research, the esophageal microbiome has been widely neglected. It has been assumed that the esophageal microbiome does not exist and only reflects a transient bacterial mix of swallowed oral and refluxed gastric commensals. Methodical limitations further hampered the esophageal microbiome research to acquire samples noninvasively and without oropharyngeal cross-contamination [62].

Nonetheless, recent studies revealed an independent resident microbiome in the esophagus [63–65]. Indeed, several phyla found in the oral cavity and lungs are also present in the esophagus (e.g., Firmicutes, Fusobacteria), suggesting that swallowed oral commensals influence the formation of the resident esophageal microbiota (Table 1). The absence of several oral microbiota strains in the esophagus indicates that not all bacteria present in the oral cavity can colonize and survive in the esophagus [63–67]. Moreover, the esophageal mucosa is populated with a unique microbiota as some Firmicutes members, including Clostridium, Eubacterium, Megaplasma, Mogibacterium, and Morrella populate only the esophagus and not the oral cavity [8–10]. Approximately 140 bacterial species have been identified by 16s rRNA-sequencing in the esophagus that constitutes the esophageal microbiome, with the most common bacteria belonging to six different phyla (70% Firmicutes, 20% Bacteroidetes, 4% Actinobacteria, 2% Proteobacteria, 2% Fusobacteria, and 1% T67) [63–65]. The dominant genera characterizing the esophageal core microbiome are Streptococcus, Prevotella, Veillonella, and Fusobacterium [8–10].
esophageal core microbiome’s composition presents with variations along the esophagus but persists between gender and age groups [62,64,65,68].

Microbiota studies in the colon mainly analyzed fecal samples that may not represent the composition of upper parts of the colon or the terminal ileum. Taking biopsies from the ileum requires colon cleansing, which tremendously affects the composition of the microbiota. Similar limitations should also be considered for the esophagus as taking biopsies in the esophagus requires a fasting period of four to six hours before the examination. Novel devices for taking esophageal swabs may allow the analysis of the esophageal microbiome in future without a fasting period before the examination. We want to stress that a firm definition of a ‘healthy microbiota’ is lacking. In inflammatory bowel disease, the colon has an increased bacterial load with a simultaneous decrease of diversity, a condition termed ‘dysbiosis’ [69,70]. However, the term ‘dysbiosis’ lacks a precise definition. ‘Altered microbiota’ provides a better description of the observed changes in the microbiota composition in diseases. In the esophagus, patients with EoE or esophageal adenocarcinomas (EAC) have an altered microbiota [65,71]. In acid-induced esophagitis or Barrett’s esophagus, Yang et al. reported an enrichment in gram-negative bacteria (anaerobes and microaerophiles) together with an increase in bacterial diversity [65,72]. Other reports describe an increase in gram-negative bacteria (e.g., Veillonella, Prevotella, Campylobacter, Fusobacterium, Haemophilus, Corynebacterium, and Neisseria) in EoE or acid-induced esophagitis, further indicating an increased abundance of gram-negative bacteria in esophagitis [65,72–74].

Unresolved questions from these studies include how and which environmental factors, such as diet or drugs, influence the esophageal microbiome. For example, a low fiber diet is associated with expanding mucus degrading bacteria in the colon [75], whereas a fiber-rich diet reduces mucus degrading bacteria [76]. Simultaneously, a fiber-rich diet expands bacterial strains that degrade fibers into short-chain fatty acids (butyrate, acetate, propionate). Short-chain fatty acids have a vast range of different effects on endogenous metabolism and inflammation, such as satiety regulation [77], browning of white adipose tissue and fat accumulation [78], increased glucagon-like peptide 1, and peptide YY secretion, and intestinal gluconeogenesis [79,80]. Only limited data on the effects of diet on the esophageal microbiome exist [81]. One study in pediatric EoE patients investigating the impact of a six-food elimination diet that avoids wheat, milk, soy, nuts, eggs, and seafood/shellfish on the esophageal microbiome did not reveal any changes the microbiome. After the reintroduction of allergenic foods, Granulicatella and Campylobacter increased in EoE [65].

### Table 1. The esophagus’s core microbiome and alteration in EoE and GERD. + present; +++ highly abundant; C, Core microbiome; ↑ increased abundance; ↓ reduced abundance; N.d., not determined.

| Phylum                   | Healthy | EoEa | GERDa |
|--------------------------|---------|------|-------|
| Firmicutes               | +++ Harris et al. [74], Pei et al. [63], Fillon et al. [64] | ↓ Harris et al. [74] | ↑ Harris et al. [74], Liu et al. [73] |
| Veillonella              | C, Harris et al. [74], Fillon et al. [64] | ↓ Harris et al. [74] | ↑ Harris et al. [74] |
| Streptococcus            | C, Harris et al. [74], Fillon et al. [64], Norder Grusell et al. [66] | ↓ Harris et al. [74] | ↑ Harris et al. [74] |
| Bacteroidetes            | ++ Harris et al. [74], Pei et al. [63], Fillon et al. [64] | ++ Harris et al. [74] | ++ Liu et al. [73] |
| Prevotella               | C, Harris et al. [74], Fillon et al. [64], Norder Grusell et al. [66] | ↔ Harris et al. [74] | ↓ Harris et al. [74] |
| Actinobacteria           | + Pei et al. [63], Fillon et al. [64] | N.d. | N.d. |
| Corynebacterium          | + Benitez et al. [65] | ↑ Benitez et al. [65] | N.d. |
| Proteobacteria           | + Harris et al. [74], Pei et al. [63], Fillon et al. [64] | ↑ Harris et al. [74] | ↑ Harris et al. [74], Liu et al. [73] |
| Haemophilus              | + Fillon et al. [64], Norder Grusell et al. [66] | ↑ Harris et al. [74] | ↑ Harris et al. [74] |
| Campylobacter            | + Benitez et al. [65] | ↑ Benitez et al. [65] | N.d. |
| Neisseria                | + Fillon et al. [64], Norder Grusell et al. [66] | ↑ Harris et al. [74] | ↑ Harris et al. [74], Benitez et al. [65] |
| Fusobacteria             | + Harris et al. [74], Pei et al. [63], Fillon et al. [64] | ↓ Harris et al. [74] | ↓ Harris et al. [74], ↑ Liu et al. [73] |
| Fusobacterium            | C, Harris et al. [74], Fillon et al. [64], Norder Grusell et al. [66] | ↓ Harris et al. [74] | ↓ Harris et al. [74], ↑ Liu et al. [73] |
| TM7                     | + Pei et al. [63], Fillon et al. [64] | N.d. | ↑ Liu et al. [73] |

*aOnly nontreated EoE and GERD presented.*
Saliva
Stratum germinativum
Basement Membrane
Stratum spinosum
Stratum corneum

Blood vessel
H+, C3H6O3, CO2
O2, HCO3-, nutrients

Inflamed (EoE)

Healthy

T cells
B cells
DCs
Monocytes
Macrophages
LCs
Eosinophils
Basophils
Neutrophils

The esophageal immune system
The intake of antibiotics also tremendously influences the composition of the microbiome in the GIT. The widespread prescription of antibiotics and agricultural business use, which we are exposed to by drinking water and eating food products, was established in the 1940s [81–83]. As an unwanted side effect, antibiotic treatment may reduce the microbiome’s diversity and disrupt beneficial microbial communities. Potential pathogens may settle in the emerging niches [84]. In the colon, the increased incidence of pseudomembranous enterocolitis caused by *Clostridium difficile* toxin A or B is one of the most significant examples [85]. The intestinal microbiota’s reconstitution by fecal microbiota transplant can treat pseudomembranous enterocolitis [86–88]. In an experimental model of Barrett’s esophagus in rats, the animals’ treatment with antibiotics did not influence Barrett’s esophagus [89]. Interestingly, it has been proposed that the eradication of *Helicobacter pylori* with antibiotics inversely correlates with the development of EAC [90].

Furthermore, PPIs increase the gastric pH by inhibiting acid secretion in the stomach, which may also indirectly affect the esophageal microbiota [81,91]. PPI treatment increases the Firmicutes phylum members’ abundance and decreases the Proteobacteria phylum members’ presence in the esophagus [91]. Other widely used drugs that might affect the microbiota composition include nonsteroidal anti-inflammatory drugs and probiotics [92]. Altogether, a unique microbiome distinct from the oral cavity microbiome populates the esophagus. First studies indicate that esophagitis leads to an altered microbiome.

**Eosinophilic esophagitis**

Activation of the immune system associated with an altered microbiome can induce esophageal diseases (Fig. 1). Early antibiotics and PPI in infancy, cesarean delivery, maternal fever, and preterm labor predispose to EoE [93]. EoE is a food-triggered Th2-mediated chronic inflammatory disease characterized by eosinophil infiltration (> 15 eosinophils per hpf), increased T and mast cell numbers [4], and associated with an altered microbiome [74]. EoE affects males predominantly compared to females, with a ratio of 3 : 1 [94]. In contrast to the healthy esophagus, which is devoid of eosinophils, the accumulation of eosinophils, attracted by chemokines eotaxin-1, eotaxin-3, and cytokines, such as IL-5 and IL-13, in the stratum corneum of the esophageal epithelium, characterizes EoE [95–98]. Eosinophil-derived granule proteins induce barrier breach and promote a Th2 inflammation [99–103], resulting in a sustained direct exposure of the esophageal immune system to triggering food allergens leading to transmural inflammation, smooth muscle dysfunction, basal cell hyperplasia, and consequently to fibrosis [104]. Also, mast cells infiltrate the inflamed esophageal tissue to release histamine in EoE [4,105]. An impaired epithelial barrier has been described in EoE with reduced proteins required to maintain the intact esophageal epithelial barrier, such as filaggrin and desmoglein, combined with diluted intercellular spaces between epithelial cells [5,6,106]. Interestingly, genome-wide association studies suggested genetic risk variants of genes expressed by epithelial and immune cells in EoE [107]. The identified single nucleotide polymorphisms included TSLP [108]; c11orf30, *STAT6*, and *ANKRD27* [109]; cytosolic calcium-activated cysteine proteases *CAPN14* (calpain-14) [110]; and the filament aggregating protein filaggrin [111]. The cytosolic calcium-activated cysteine proteases, including *CAPN14*, modulate integrin-cytoskeletal interactions, and *filaggrin* binds keratin intermediate filaments, reinforce a barrier dysfunction in EoE. Preclinically, experimental work with patient samples and cell lines by Azouz and colleagues indicated that the reduced expression of the serine protease inhibitor, SPINK7 leads to barrier dysfunction [112]. Further research from the same group suggested that SPINK7 restricts the activity of the serine protease kallikrein 5 (KLK5) and that *klk5*-deficient mice are protected from the development of an ovalbumin (OVA)-induced EoE mouse model [113]. It is under discussion whether a barrier defect is a prerequisite for sensitizing the esophageal immune system to EoE and requires continuous antigen exposure to drive esophageal inflammation. However, the observed barrier defect in EoE could also be secondary as a consequence of inflammation in the esophagus. One approach to solve this issue is establishing cohorts in the preclinical phase of EoE before disease onset. In individuals with a preclinical phase of EoE, researchers...
could investigate the expression of proteins involved in establishing an intact esophageal epithelial barrier.

The epithelial barrier breach further induces the expression of the cytokines TSLP, IL-25, and IL-33 that, in turn, drive a Th2-mediated inflammation [4,114]. The Th2 cytokine IL-13 increases the expression of the esophagus-specific protease CAPN14, which degrades desmoglein-1 required to form cell-cell junctions [101,115]. Furthermore, IL-13 reduces filaggrin expression in atopic dermatitis [116] and contributes to eosinophil chemotaxis by inducing eotaxin-3 expression [98,117]. Increased TSLP expression has been reported in patients with active EoE correlated with eosinophil extracellular trap formation [6]. Patients with a gain-of-function polymorphism of TSLP have increased basophil numbers in the esophagus [3]. Moreover, the IL-1 superfamily member IL-33, which is constitutively expressed in the nucleus and acts as a cytokine by binding to its receptor ST2, is expressed by the esophageal mucosa [118] and by undifferentiated epithelial cells of EoE patients [119]. Basophils express the IL-33 receptor ST2, and genetic deletion of ST2 prevents inflammation in an OVA-induced EoE mouse model [120]. Since EoE patients have increased Th2 cytokine expression, the specific targeting of cytokines with monoclonal antibodies is a promising avenue for the treatment of EoE. The human mAb Dupilumab binds the IL-4Rα receptor chain blocking both IL-4 and IL-13 signaling. In a multicenter phase II trial, Dupilumab improved dysphagia and eosinophil count at week 10 of treatment [121].

Eosinophilic esophagitis is of allergic etiology, corroborated by a high prevalence of concurrent atopic diseases and remendability by allergen avoidance. However, the frequently observed increased food antigen-specific IgE levels in EoE do not correlate with the EoE-triggering allergens [122]. Consistently, EoE patients treated with the anti-IgE antibody, omalizumab, failed in clinical case series and in prospective, randomized, double-blind, placebo-controlled studies to show a significant relieve of symptoms [123,124]. EoE is preferably associated with antigen-specific IgG4 antibodies [124], which have neutralizing properties due to their week binding affinity to IgG receptors and low complement activation [125–127]. The elevated IgG4 concentrations in active EoE decrease during dietary interventions by avoiding possible food allergens present in wheat, milk, soy, nuts, eggs, and seafood/shellfish [126]. Altogether, these data suggest that IgE does not drive the pathophysiology in EoE.

In summary, the pathophysiology of EoE is incompletely understood (Fig. 2). Since EoE presents with vomiting and feeding problems in young children and...
dysphagia and food impaction in adults [128], there is a clinical need for better treatment options. Because food antigens trigger EoE, with milk and wheat as the most prevalent food antigens [129,130], a six-food elimination diet with avoidance of wheat, milk, soy, nuts, eggs, and seafood/shellfish is an effective treatment. However, the six-food elimination diet significantly impacts life quality, limiting the compliance for this treatment for EoE [130–132]. Topical corticosteroids improve clinical symptoms and are highly efficacious for induction and maintenance therapy of EoE [133,134]. Small molecules and biologicals that specifically target checkpoints are of significant interest to further improve the treatment of EoE. In the search for targeted therapies of EoE, one has to consider that the inflammation in EoE extends eosinophils’ biology. Infiltrating T cells, B cells, and mast cells could provide specific targets for the treatment of EoE [4].

Conclusions

Increasing evidence indicates that the esophagus is a transport organ with critical importance for mucosal immunity and contributes to immune-mediated diseases. A better characterization of the esophageal immune system and its relationship with the microbiota will give insights into the development of esophageal diseases. Likely, this research will pave the way for discovering targeted therapies to improve the treatment of esophagitis. We anticipate the following research questions that may be solved before this exciting research will enter daily practice in the clinic.

1. Information on the distribution of immune cells in the healthy esophagus, GERD, and EoE is scarce. Since sampling of esophageal tissues during endoscopy is possible, systematic analysis of immune cells in esophageal biopsies with single-cell RNA sequencing and mass cytometry will help characterize the cellular composition of the esophageal immune system. These analyses are a requirement before possible targeted therapies in EoE can be explored.

2. Emerging evidence indicates that the esophagus has a core microbiome distinguishable from the oral cavity’s microbiome, the skin, the small intestine, and colon. Most assumptions on the esophageal microbiome stem from studies investigating the oral or the intestinal microbiome. There is a need to better characterize the esophageal microbiome in response to the diet, medications, and diseases, such as EoE.

With advances in this area of research, it will in future be possible to move the fascinating research on the esophageal mucosal immunity for the development of targeted therapies for EoE into the clinic. We expect that studies investigating the esophageal mucosal immune system will move into focus soon.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

TK and JHN jointly wrote the manuscript, discussed the manuscript with PH, and designed the figures.

References

1 Straumann A & Katzka DA (2018) Diagnosis and treatment of eosinophilic esophagitis. Gastroenterology 154, 346–359.
2 Simon D, Straumann A, Schoepfer AM & Simon HU (2017) Current concepts in eosinophilic esophagitis. Allergo J Int 26, 258–266.
3 Noti M, Wojno ED, Kim BS, Siracusa MC, Giacomin PR, Nair MG, Benitez AJ, Ruymann KR, Muir AB, Hill DA et al. (2013) Thymic stromal lymphopoietin-elicted basophil responses promote eosinophilic esophagitis. Nat Med 19, 1005–1013.
4 Straumann A, Bauer M, Fischer B, Blaser K & Simon HU (2001) Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. J Allergy Clin Immunol 108, 954–961.
5 Simon D, Page B, Vogel M, Bussmann C, Blanchard C, Straumann A & Simon HU (2018) Evidence of an abnormal epithelial barrier in active, untreated and corticosteroid-treated eosinophilic esophagitis. Allergy 73, 239–247.
6 Simon D, Radonjic-Hosli S, Straumann A, Yousefi S & Simon HU (2015) Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation. Allergy 70, 443–452.
7 Chandra mouleeeswaran PM, Shen D, Lee AJ, Benitez A, Dods K, Gambanga F, Wilkins BJ, Merves J, Noah Y, Toltzis S et al. (2016) Preferential secretion of thymic stromal lymphopoietin (TSLP) by terminally differentiated esophageal epithelial cells: relevance to eosinophilic esophagitis (EoE). PLoS One 11, e0150968.
8 Nardone G, Compare D & Rocco A (2017) A microbiota-centric view of diseases of the upper gastrointestinal tract. *Lancet Gastroenterol Hepatol* **2**, 298–312.

9 Hunt RH & Yaghoobi M (2017) The esophageal and gastric microbiome in health and disease. *Gastroenterol Clin North Am* **46**, 121–141.

10 Di Pilato V, Freschi G, Ringressi MN, Pallecchi L, Rossolini GM & Bechi P (2016) The esophageal microbiota in health and disease. *Ann N Y Acad Sci* **1381**, 21–33.

11 Rocha LP, de Melo ESAT, Gomes NC, Faria HA, Silva RB, Olegario JG, Correia RR, de Paula Antunes Teixeira V & Cavellani CL (2011) The influence of gender and of AIDS on the immunity of autopsied patients’ esophagus. *AIDS Res Hum Retroviruses* **27**, 511–518.

12 Sarosiek J & McCallum RW (2000) Mechanisms of esophageal mucosal defence. *Baillieres. Best Pract Res Clin Gastroenterol* **14**, 701–717.

13 Long JD & Orlando RC (1999) Esophageal submucosal glands: structure and function. *Am J Gastroenterol* **94**, 2818–2824.

14 Orlando RC (1998) Review article: esophageal mucosal resistance. *Aliment Pharmacol Ther* **12**, 191–197.

15 Blevins CH, Iyer PG, Vela MF & Katzka DA (2018) The esophageal epithelial barrier in health and disease. *Clin Gastroenterol Hepatol* **16**, 608–617.

16 Sandilands A, Sutherland C, Irvine AD & McLean WH (2009) Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* **122**, 1285–1294.

17 Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, Noda T, Kubo A & Tsukita S (2002) Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* **156**, 1099–1111.

18 Chen Y, Merzdorf C, Paul DL & Goodenough DA (1997) COOH terminus of occludin is required for tight junction barrier function in early Xenopus embryos. *J Cell Biol* **138**, 891–899.

19 Masterson JC, Biette KA, Hammer JA, Nguyen N, Capocelli KE, Saedi BJ, Harris RF, Fernando SD, Hosford LB, Kelly CJ et al. (2019) Epithelial HIF-1alpha/claudin-1 axis regulates barrier dysfunction in eosinophilic esophagitis. *J Clin Invest* **129**, 3224–3235.

20 Hartscock A & Nelson WJ (2008) Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochem Biophys Acta* **1778**, 660–669.

21 Orlando RC (2010) The integrity of the esophageal mucosa. Balance between offensive and defensive mechanisms. *Best Pract Res Clin Gastroenterol* **24**, 873–882.

22 Nakhoul NL, Tu CL, Brown KL, Islam MT, Hodges AG & Abdulnour-Nakhoul SM (2020) Calcium-sensing receptor deletion in the mouse esophagus alters barrier function. *Am J Physiol Gastrointest Liver Physiol* **318**, G144–G161.

23 Kijima T, Nakagawa H, Shimodosono M, Chandramouleeswaran PM, Haras T, Sahu V, Kasagi Y, Kikuchi O, Tanaka K, Giroux V et al. (2019) Three-dimensional organoids reveal therapy resistance of esophageal and oropharyngeal squamous cell carcinoma cells. *Cell Mol Gastroenterol Hepatol* **7**, 73–91.

24 Orlando RC, Lacy ER, Tobey NA & Cowart K (1992) Barriers to paracellular permeability in rabbit esophageal epithelium. *Gastroenterology* **102**, 910–923.

25 Bass BL, Schweitzer EJ, Harmon JW & Kraimer J (1984) H+ back diffusion interferes with intrinsic reactive regulation of esophageal mucosal blood flow. *Surgery* **96**, 404–413.

26 Hollwarth ME, Smith M, Kvietys PR & Granger DN (1986) Esophageal blood flow in the cat. Normal distribution and effects of acid perfusion. *Gastroenterology* **90**, 622–627.

27 Dignass AU & Podolsky DK (1993) Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor beta. *Gastroenterology* **105**, 1323–1332.

28 Tsuji H, Fuse Y, Kawamoto K, Fujino H & Kodama T (1989) Healing process of experimental esophageal ulcers induced by acetic acid in rats. *Scand J Gastroenterol Suppl* **162**, 6–10.

29 El-Serag HB, Sweet S, Winchester CC & Dent J (2014) Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* **63**, 871–880.

30 Bredenoord AJ, Pandolfino JE & Smout AJ (2013) Gastro-oesophageal reflux disease. *Lancet* **381**, 1933–1942.

31 Lee YY, Wirz AA, Whiting JG, Robertson EV, Smith D, Weir A, Kelman AW, Derakhshan MH & McColl KE (2014) Waist belt and central obesity cause partial hiatus hernia and short-segment acid reflux in asymptomatic volunteers. *Gut* **63**, 1053–1060.

32 Robertson EV, Derakhshan MH, Wirz AA, Lee YY, Seenan JP, Ballantyne SA, Hanvey SL, Kelman AW, Going JJ & McColl KE (2013) Central obesity in asymptomatic volunteers is associated with increased inrasphincteric acid reflux and lengthening of the cardiac mucosa. *Gastroenterology* **145**, 730–739.

33 El-Serag HB, Hashmi A, Garcia J, Richardson P, Alsarraj A, Fitzgerald S, Vela M, Shabib Y, Abraham NS, Velez M et al. (2014) Visceral abdominal obesity measured by CT scan is associated with an increased risk of Barrett’s oesophagus: a case-control study. *Gut* **63**, 220–229.

34 Herbella FA & Patti MG (2010) Gastroesophageal reflux disease: From pathophysiology to treatment. *World J Gastroenterol* **16**, 3745–3749.
The esophageal immune system

T. Kaymak et al.

35 Del Grande LM, Herbell FA, Bigatao AM, Abrao H, Jardim JR & Patti MG (2016) Pathophysiology of gastroesophageal reflux in patients with chronic pulmonary obstructive disease is linked to an increased transdiaphragmatic pressure gradient and not to a defective esophagogastric barrier. *J Gastrointest Surg* **20**, 104–110. discussion 110.

36 Lagergren J, Bergstrom R, Lindgren A & Nyren O

37 Champion G, Richter JE, Vaezi MF, Singh S & Alexander R (1994) Duodenogastroesophageal reflux: relationship to pH and importance in Barrett’s esophagus. *Gastroenterology* **107**, 747–754.

38 Edebo A, Vieth M, Tam W, Bruno M, van Berkel AM, Stolte M, Schoeman M, Tytgat G, Dent J & Lundell L (2007) Circumferential and axial distribution of gastroesophageal mucosal damage in reflux disease. *Dis Esophagus* **20**, 232–238.

39 Fass R & Ofman JJ (2002) Gastroesophageal reflux disease—should we adopt a new conceptual framework? *Am J Gastroenterol* **97**, 1901–1909.

40 Tobey NA, Carson JL, Alkic RA & Orlando RC (1996) Dilated intercellular spaces: a morphological feature of acid reflux–damaged human esophageal epithelium. *Gastroenterology* **111**, 1200–1205.

41 Caviglia R, Ribolzi M, Maggiiano N, Gabbricielli AM, Emerenziani S, Guarino MP, Carotti S, Habib FI, Rabitti C & Cicala M (2005) Dilated intercellular spaces of esophageal epithelium in nonerosive reflux disease patients with physiological esophageal acid exposure. *Am J Gastroenterol* **100**, 543–548.

42 Souza RF, Huo X, Mittal V, Schuler CM, Carmack SW, Zhang HY, Zhang X, Yu C, Hormi-Carver K, Genta RM et al. (2009) Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. *Gastroenterology* **137**, 1776–1784.

43 Altomare A, Ma J, Guarino M, Cheng L, Rieder F, Ribolzi M, Fiocchi C, Biancani P, Harnett K & Cicala M (2012) Platelet-activating factor and distinct chemokines are elevated in mucosal biopsies of erosive compared with non-erosive reflux disease patients and controls. *Neurogastroenterol Motil* **24**, 943–e463.

44 Fass R (2007) Erosive esophagitis and nonerosive reflux disease (NERD): comparison of epidemiologic, physiologic, and therapeutic characteristics. *J Clin Gastroenterol* **41**, 131–137.

45 Isomoto H, Suenko VA, Kanazawa Y, Nishi Y, Ohtsuru A, Inoue K, Akazawa Y, Takeshima F, Omagari K, Miyazaki M et al. (2004) Enhanced expression of interleukin-8 and activation of nuclear factor kappa-B in endoscopy-negative gastroesophageal reflux disease. *Am J Gastroenterol* **99**, 589–597.

46 Fitzgerald RC, Onwuegbusi BA, Bajaj-Elliott M, Saeed IT, Burnham WR & Farthing MJ (2002) Diversity in the esophageal phenotypic response to gastro-oesophageal reflux: immunological determinants. *Gut* **50**, 451–459.

47 Ma J, Altomare A, Guarino M, Cicala M, Rieder F, Fiocchi C, Li D, Cao W, Behar J, Biancani P et al. (2012) HCl-induced and ATP-dependent upregulation of TRPV1 receptor expression and cytokine production by human esophageal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* **303**, G635–G645.

48 Monkemuller K, Wex T, Kuester D, Fry LC, Peitz U, Beyer M, Roessner A & Maifertheiner P (2009) Interleukin-1beta and interleukin-8 expression correlate with the histomorphological changes in esophageal mucosa of patients with erosive and non-erosive reflux disease. *Digestion* **79**, 186–195.

49 Brown LF, Goldman H & Antonioli DA (1984) Intraepithelial eosinophils in endoscopic biopsies of adults with reflux esophagitis. *Am J Surg Pathol* **8**, 899–905.

50 Fries PN & Griebel PJ (2011) Mucosal dendritic cell diversity in the gastrointestinal tract. *Cell Tissue Res* **433**, 33–41.

51 Allam JP, Peng WM, Appel T, Wenghofer M, Niederhagen B, Bieber T, BERGE S & Novak N (2008) Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. *J Allergy Clin Immunol* **121**, 368–374.e1.

52 Allam JP, Wurtzen PA, Reinnart M, Winter J, Vrtala S, Chen KW, Valenta R, Wenghofer M, Appel T, Gros E et al. (2010) Ph1 p5 resorption in human oral mucosa leads to dose-dependent and time-dependent allergen binding by oral mucosal Langerhans cells, attenuates their maturation, and enhances their migratory and TGF-beta1 and IL-10-producing properties. *J Allergy Clin Immunol* **126**, 638–645.e1.

53 Lucendo AJ, Navarro M, Comas C, Pascual JM, Burgos E, Santamaria L & Larrauri J (2007) 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* **31**, 598–606.

54 Novak N, Haberstok J, Bieber T & Allam JP (2008) The immune privilege of the oral mucosa. *Trends Mol Med* **14**, 191–198.

55 Novak N & Allam JP (2011) Mucosal dendritic cells in allergy and immunotherapy. *Allergy* **66** (Suppl 95), 22–24.

56 Iwasaki A (2007) Mucosal dendritic cells. *Annu Rev Immunol* **25**, 381–418.
T. Kaymak et al.

The esophageal immune system

57 Cerf-Bensussan N, Schneeberger EE & Bhan AK (1983) Immunohistologic and immunoelectron microscopic characterization of the mucosal lymphocytes of human small intestine by the use of monoclonal antibodies. *J Immunol* **130**, 2615–2622.

58 Doherty TA, Baum R, Newbury RO, Yang T, Dohil R, Aquino M, Doshi A, Walford HH, Kurten RC, Broide DH et al. (2015) Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis. *J Allergy Clin Immunol** **136**, 792–794.e3.

59 Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC & Siuzdak G (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA* **106**, 3698–3703.

60 Nieuwdorp M, Gilijamse PW, Pai N & Kaplan LM (2014) Role of the microbiome in energy regulation and metabolism. *Gastroenterology* **146**, 1525–1533.

61 Sant’Ana Pde L, Alcantara AP, Carvalho MT & Colombo AL (2002) Multicenter Brazilian study of the esophageal microbiome. *Mem Inst Oswaldo Cruz* **97**, 253–257.

62 Okereke IC, Miller AL, Hamilton CF, Booth AL, Reep GL, Andersen CL, Reynolds ST & Pyles RB (2019) Microbiota of the oropharynx and endoscope compared to the esophagus. *Sci Rep* **9**, 10201.

63 Pei Z, Bini EJ, Yang L, Zhou M, Francois F & Blaser MJ (2004) Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci USA* **101**, 4250–4255.

64 Fillon SA, Harris JK, Wagner BD, Kelly CJ, Stevens MJ, Moore W, Fang R, Schroeder S, Masterson JC, Robertson CE et al. (2012) Novel device to sample the esophageal microbiome—the esophageal string test. *PLoS One* **7**, e42938.

65 Benitez AJ, Hoffmann C, Muir AB, Dods KK, Spergel JM, Bushman FD & Wang ML (2015) Inflammation-associated microbiota in pediatric eosinophilic esophagitis. *Microbiome* **3**, 23.

66 Norder Grusell E, Dahlen G, Ruth M, Ny L, Quiting-Telles F, Ferreira MS, Alcantara AP, Carvalho MT & Colombo AL (2013) Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am J Respir Crit Care Med* **187**, 1067–1075.

67 Morris A, Beck JM, Schliss PD, Campbell TB, Crothers K, Curtis JL, Flores SC, Fontenot AP, Ghedin E, Huang L et al. (2013) Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am J Respir Crit Care Med* **187**, 1067–1075.

68 Yang L, Chaudhury N, Baghdadi J & Pei Z (2014) Microbiome in reflux disorders and esophageal adenocarcinoma. *Cancer J* **20**, 207–210.

69 Schirmer M, Denson L, Vlamakis H, Franzosa EA, Thomas S, Gotman NM, Rufo P, Baker SS, Sauer C, Markowitz J et al. (2018) Compositional and temporal changes in the gut microbiome of pediatric ulcerative colitis patients are linked to disease course. *Cell Host Microbe* **24**, 600–610.e4.

70 Yilmaz B, Juillerat P, Øyäs O, Ramon C, Bravo FD, Franc Y, Fournier N, Michetti P, Mueller C, Geuken M et al. (2019) Microbial network disturbances in relapsing refractory Crohn’s disease. *Nat Med* **25**, 323–336.

71 Elliott DRF, Walker AW, O’Donovan M, Parkhill J & Fitzgerald RC (2017) A non-endoscopic device to sample the oesophageal microbiota: a case-control study. *Lancet Gastroenterol Hepatol* **2**, 32–42.

72 Yang L, Lu X, Nossa CW, Francois F, Peek RM & Pei Z (2009) Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology* **137**, 588–597.

73 Liu N, Ando T, Ishiguro K, Maeda O, Watanabe O, Funasaka K, Nakamura M, Miyahara R, Ohmiya N & Goto H (2013) Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett’s esophagus. *BMC Infect Dis* **13**, 130.

74 Harris JK, Fang R, Wagner BD, Choe HN, Kelly CJ, Schroeder S, Moore W, Stevens MJ, Yeekes A, Amsden K et al. (2015) Esophageal microbiome in eosinophilic esophagitis. *PLoS One* **10**, e0128346.

75 Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A et al. (2016) A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* **167**, 1339–1353.e21.

76 Kelly CJ, Zheng L, Campbell EL, Saedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A et al. (2015) Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* **17**, 662–671.

77 Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J, Ghourab S, Hankir M, Zhang S et al. (2014) The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* **5**, 3611.

78 Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashiwara D, Hirano K, Tani T et al. (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun* **4**, 1829.

79 Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F & Gribble FM (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G–protein-coupled receptor FFAR2. *Diabetes* **61**, 364–371.

80 Larraufie P, Martin-Gallusiaux C, Lapaque N, Dore J, Gribble FM, Reimann F & Blottiere HM (2018)
SCFAs strongly stimulate PYY production in human enteroendocrine cells. Sci Rep 8, 74.

81 May M & Abrams JA (2018) Emerging insights into the esophageal microbiome. Curr Treat Options Gastroenterol 16, 72–85.

82 Wypych TP & Marsland BJ (2018) Antibiotics as instigators of microbial dysbiosis: implications for asthma and allergy. Trends Immunol 39, 697–711.

83 Lambrecht BN & Hammad H (2017) The immunology of the allergy epidemic and the hygiene hypothesis. Nat Immunol 18, 1076–1083.

84 Buffie CG, Jarchum I, Equinda M, Lipuma L, Gobourne A, Viale A, Ubeda C, Xavier J & Pamer EG (2012) Profound alterations of intestinal microbiota following a single dose of clindamycin results in sustained susceptibility to Clostridium difficile-induced colitis. Infect Immun 80, 62–73.

85 Awad MM, Johansen PA, Carter GP, Rose E & Lyras D (2014) Clostridium difficile virulence factors: Insights into an anaerobic spore-forming pathogen. Gut Microbes 5, 579–593.

86 Ekmekciu I, von Klitzing E, Fiebiger U, Escher U, May M & Abrams JA (2018) Antibiotics as instigators of microbial dysbiosis: implications for asthma and allergy. Trends Immunol 39, 697–711.

87 Islami F & Kamangar F (2008) Helicobacter pylori and esophageal cancer risk: a meta-analysis. Cancer Prev Res (Phila) 1, 329–338.

88 van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijsen JG et al. (2013) Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 368, 407–415.

89 Sawada A, Fujiwara Y, Nagami Y, Tanaka F, Yamagami H, Tanigawa T, Shiba M, Tominaga K, Watanabe T, Gobourne A, Viale A, Ubeda C, Xavier J & Pamer EG (2012) Long-term follow-up of colonicoscopy for recurrent Clostridium difficile infection. Am J Gastroenterol 107, 1079–1087.

90 van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijsen JG et al. (2013) Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 368, 407–415.

91 Rogers MAM & Aronoff DM (2016) The influence of non-steroidal anti-inflammatory drugs on the gut microbiome. Clin Microbiol Infect 22, 178e1–178e9.

92 Jensen ET, Kuhl JT, Martin LJ, Rothenberg ME & Dellon ES (2018) Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. J Allergy Clin Immunol 141, 214–222.

93 HRU P, Straumann A, Bussmann C, Heer P, Simon HU, Zwahlen M, Beglinger C, Schoepfer AM; Swiss EoE study group (2011) Escalating incidence of eosinophilic esophagitis: a 20-year prospective, population-based study in Olten County, Switzerland. J Allergy Clin Immunol 128, 1349–1350.e5.

94 Mishra A, Hogan SP, Lee JJ, Foster PS & Rothenberg ME (1999) Fundamental signals that regulate eosinophil homing to the gastrointestinal tract. J Clin Invest 103, 1719–1727.

95 Mishra A, Hogan SP, Brandt EB & Rothenberg ME (2002) IL-5 promotes eosinophil trafficking to the esophagus. J Immunol 168, 2464–2469.

96 Mishra A & Rothenberg ME (2003) Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. Gastroenterology 125, 1419–1427.

97 Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, Jameson SC, Kirby C, Konikoff MR, Collins MH et al. (2006) Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest 116, 536–547.

98 Rothenberg ME & Hogan SP (2006) The eosinophil. Annu Rev Immunol 24, 147–174.

99 Yang D, Chen Q, Su SB, Zhang P, Kurosaka K, Caspi RR, Michalek SM, Rosenberg HF, Zhang N & Oppenheim JJ (2008) Eosinophil-derived neurotoxin acts as an alarm to activate the TLR2-MyD88 signal pathway in dendritic cells and enhances Th2 immune responses. J Exp Med 205, 79–90.

100 Litosh VA, Rochman M, Rymer JK, Porollo A, Kottyan LA & Rothenberg ME (2017) Calpain-14 and its association with eosinophilic esophagitis. J Allergy Clin Immunol 139, 1762–1771.e7.

101 Masterson JC, McNamee EN, Hosford L, Capocelli KE, Ruybal J, Fillon SA, Doyle AD, Eltzschig HK, Rustgi AK, Protheroe CA et al. (2014) Local hypersensitivity reaction in transgenic mice with squamous epithelial IL-5 overexpression provides a novel model of eosinophilic esophagitis. Gut 63, 43–53.

102 Lee HM, Lee SH, Baek SK & Kim TH (2016) Decreased expression of E-cadherin and ZO-1 in the nasal mucosa of patients with allergic rhinitis: altered regulation of E-cadherin by IL-4, IL-5, and TNF-alpha. Am J Rhinol Allergy 30, 173–178.

103 Lucendo AJ (2014) Cellular and molecular immunological mechanisms in eosinophilic esophagitis: an updated overview of their clinical implications. Expert Rev Gastroenterol Hepatol 8, 669–685.
The esophageal immune system

105 Gupta SK, Fitzgerald JF, Kondratyuk T & HogenEsch H (2006) Cytokine expression in normal and inflamed esophageal mucosa: a study into the pathogenesis of allergic eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* **42**, 22–26.

106 Sherrill JD, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, Kemme KA, Costello MS, Mingler MK, Blanchard C *et al.* (2014) Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. *Mucosal Immunol* **7**, 718–729.

107 Kottyan LC, Trimarchi MP, Lu X, Caldwell JM, Maddox A, Parameswaran S, Lape M, D’Mello RJ, Bonfield M, Ballaban A *et al.* (2021) Replication and meta-analyses nominate numerous eosinophilic esophagitis risk genes. *J Allergy Clin Immunol* **147**, 255–266.

108 Rothenberg ME, Spergel JM, Sherrill JD, Annaiah K, Martin LJ, Cianferoni A, Gober L, Kim C, Gillesner J, Frackelton E *et al.* (2010) Common variants at 5q22 associate with pediatric eosinophilic esophagitis. *Nat Genet* **42**, 289–291.

109 Steiman PM, Wang ML, Cianferoni A, Aceves S, Gonsalves N, Nadeau K, Bredenoord AJ, Furuta GT, Spergel JM & Hakonarson H (2014) GWAS identifies four novel eosinophilic esophagitis loci. *Nat Commun* **5**.

110 Kottyan LC, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, Weirauch MT, Vaughn S, Lazar S, Rupert AM *et al.* (2014) Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet* **46**, 895–900.

111 Blanchard C, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, Buckmeier BK, Jameson SC, Greenberg A, Kaul A *et al.* (2010) Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. *J Immunol* **184**, 4033–4041.

112 Azouz NP, Ynga-Durand MA, Caldwell JM, Jain A, Rochman M, Fischew DM, Ray LM, Bedard MC, Mingler MK, Forney C *et al.* (2018) The antiprotease SPINK7 serves as an inhibitory checkpoint for esophageal epithelial inflammatory responses. *Sci Transl Med* **10**, eaap9736–eaap9750.

113 Azouz NP, Klingler AM, Pathe R, Besse JA, Baruch-Morgenstern NB, Ballaban AY, Osswald GA, Brusilovsky M, Habel JE, Caldwell JM *et al.* (2020) Functional role of kallikrein 5 and proteinase-activated receptor 2 in eosinophilic esophagitis. *Sci Transl Med* **12**, eaaz7773–eaaz7787.

114 Kitajima M, Lee HC, Nakayama T & Ziegler SF (2011) TSLP enhances the function of helper type 2 cells. *Eur J Immunol* **41**, 1862–1871.

115 Davis BP, Stucke EM, Khorki ME, Litosh VA, Rymer JK, Rochman M, Travers J, Kottyan LC & Rothenberg ME (2016) Eosinophilic esophagitis-linked culprit 14 is an IL-13-induced protease that mediates esophageal epithelial barrier impairment. *JCI Insight* **1**, e86355.

116 O’Regan GM, Sandilands A, McLean WH & Irvine AD (2008) Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* **122**, 689–693.

117 Blanchard C, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu TX, Collins MH, Putnam PE, Wells SI & Rothenberg ME (2007) IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol* **120**, 1292–1300.

118 Judd LM, Heine RG, Menheniott TR, Buzzelli J, O’Brien-Simpson N, Pavlic D, O’Connor L, Al Gazali K, Hamilton O, Scorr M *et al.* (2016) Elevated IL-33 expression is associated with pediatric eosinophilic esophagitis, and exogenous IL-33 promotes eosinophilic esophagitis development in mice. *Am J Physiol Gastrointest Liver Physiol* **310**, G13–25.

119 Travers J, Rochman M, Caldwell JM, Besse JA, Miracle CE & Rothenberg ME (2017) IL-33 is induced in undifferentiated, non-dividing esophageal epithelial cells in eosinophilic esophagitis. *Sci Rep* **7**, 17563.

120 Venturelli N, Lexamond WS, Ohsaki A, Nurko S, Karasuyama H, Fiebiger E & Oyoshi MK (2016) Allergic skin sensitization promotes eosinophilic esophagitis through the IL-33-basophil axis in mice. *J Allergy Clin Immunol* **138**, 1367–1380.e5.

121 Hirano I, Dellon ES, Hamilton JD, Collins MH, Peterson K, Chehade M, Schoepfer AM, Safroneeva E, Rothenberg ME, Falk GW *et al.* (2020) Efficacy of dupilumab in a phase 2 randomized trial of adults with active eosinophilic esophagitis. *Gastroenterology* **158**, 111–122.e10.

122 Straumann A, Aceves SS, Blanchard C, Collins MH, Furuta GT, Hirano I, Schoepfer AM, Simon D & Simon HU (2012) Pediatric and adult eosinophilic esophagitis: similarities and differences. *Allergy* **67**, 477–490.

123 Rocha R, Vitor AB, Trindade E, Lima R, Tavares M, Lopes J & Dias JA (2011) Omalizumab in the treatment of eosinophilic esophagitis and food allergy. *Eur J Pediatr* **170**, 1471–1474.

124 Clayton F, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, Lowchik A, Chen X, Emerson L, Cox K *et al.* (2014) Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* **147**, 602–609.

125 Davies AM & Sutton BJ (2015) Human IgG4: a structural perspective. *Immunol Rev* **268**, 139–159.
126 Wright BL, Kulis M, Guo R, Orgel KA, Wolf WA, Burks AW, Vickery BP & Dellon ES (2016) Food-specific IgG4 is associated with eosinophilic esophagitis. J Allergy Clin Immunol 138, 1190–1192.e3.

127 Mahajan VS, Mattoo H, Deshpande V, Pillai SS & Stone JH (2014) IgG4-related disease. Annu Rev Pathol 9, 315–347.

128 O’Shea KM, Aceves SS, Dellon ES, Gupta SK, Spergel JM, Furuta GT & Rothenberg ME (2018) Pathophysiology of eosinophilic esophagitis. Gastroenterology 154, 333–345.

129 Spergel JM, Brown-Whitehorn TF, Cianferoni A, Shuker M, Wang ML, Verma R & Liacouras CA (2012) Identification of causative foods in children with eosinophilic esophagitis treated with an elimination diet. J Allergy Clin Immunol 130, 461–467.e5.

130 Gonsalves N, Yang GY, Doerfler B, Ritz S, Ditto AM & Hirano I (2012) Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. Gastroenterology 142, 1451–1459.e1; quiz e14–5.

131 Markowitz JE, Spergel JM, Ruchelli E & Liacouras CA (2003) Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. Am J Gastroenterol 98, 777–782.

132 Warners MJ, Vlieg-Boerstra BJ, Verheij J, van Rhijn BD, Van Ampting MT, Harthoorn LF, de Jonge WJ, Smout AJ & Bredenoord AJ (2017) Elemental diet decreases inflammation and improves symptoms in adult eosinophilic oesophagitis patients. Aliment Pharmacol Ther 45, 777–787.

133 Lucendo AJ, Miehlke S, Schlag C, Vieth M, von Arnim U, Molina-Infante J, Hartmann D, Bredenoord AJ, de Los C, Rios C et al. (2019) Efficacy of budesonide orodispersible tablets as induction therapy for eosinophilic esophagitis in a randomized placebo-controlled trial. Gastroenterology 157, 74–86.e15.

134 Straumann A, Lucendo AJ, Miehlke S, Vieth M, Schlag C, Biedermann L, Vaquero CS, de Los C, Rios C, Schmoecker C et al. (2020) Budesonide orodispersible tablets maintain remission in a randomized, placebo-controlled trial of patients with eosinophilic esophagitis. Gastroenterology 159, 1672–1685.e5.