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Non-IgE mediated food allergy

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Non-IgE-mediated food allergies (FA) are highly prevalent within food allergic patients, notably in the first years of life. The most prevalent non-IgE FA are mainly induced by cow's milk and soya, but many other foods can be involved. Non-IgE FA encompass a wide range of immune-related disorders that differ in prevalence, clinical manifestation, and pathophysiology. Although some *in vivo* models have been developed for their study, further investigations are needed to fully delineate the pathogenic mechanisms involved.

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Introduction

Food allergies (FA) correspond to 'an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food' [1]. FA can be either IgE- or non-IgE-mediated, both resulting from barrier dysfunction and immune dysregulation. Although prospective cohort studies demonstrated that almost 50% of allergic infants endure non-IgE FA [2,3], they are often misdiagnosed and less well studied than IgE-mediated FA.

The most prevalent non-IgE FA are eosinophilic esophagitis (EoE), food-protein induced enterocolitis syndrome (FPIES), proctocolitis (FPIAP) and entheropathy (FPE). In the present review, we will mainly focus on EoE for which clinical data and animal models are the more abundant. Celiac disease, a prevalent adverse immune reaction triggered by gluten, has been already largely described and then will not be considered here.

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Eosinophilic esophagitis (EoE)

Prevalence, clinical manifestations, and trigger foods

EoE is considered a non-IgE FA based on immunological findings and clinical evidences such as the inefficiency of anti-IgE therapy or the increased frequency of EoE observed after oral immunotherapy for IgE FA [4]. EoE prevalence increased in the past years, reaching 0.05–0.1% of the general population in the US [5].

EoE is a chronic disease characterized by esophageal dysfunction and eosinophilic inflammation of the esophagus [6]. In symptomatic patients, EoE is diagnosed after an esophageal biopsy showing at least 15 intraepithelial eosinophils per high-power field after an 8–12 weeks course of proton pump inhibitor to rule out a gastro-esophageal reflux disease-related eosinophilia [6].

Patients with EoE are highly atopic, with elevated rates of allergic rhinitis, asthma, eczema, or even IgE FA [6,7]. In infants and children, symptoms include feeding difficulty, nausea, vomiting, and failure to thrive. School-aged children often suffer abdominal pain and frequently vomiting, whereas adolescents and adults usually have dysphagia or food impactions [6,8,9].

Although previous results suggest the role of aeroallergens in EoE, a recent meta-analysis study failed to show seasonal

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recrudescence of EoE [10]. Conversely, marked improvements of clinical and histologic symptoms upon strict amino-acid based diet, and recurrence of symptoms upon food challenge, evidenced the role of foods as triggers of EoE [11], the most commonly involved being cow's milk (CM), wheat, soy, and egg [12]. Amino acid-based elemental diet, six food elimination diet (based on the avoidance of the 6 most common triggering foods), and allergy-test result-directed food eliminations diets are effective in inducing clinical and histologic remission [13]. Swallowed topical corticosteroids demonstrated clinical and histologic improvements, but clinical symptoms recurred upon discontinuation and response varied between patients [14].

Mechanisms

EoE may result from an alteration of the oesophageal epithelial barrier induced by intrinsic dysfunction or environmental aggressions, such as gastric acid, microbiota changes and/or xenobiotic. This may lead to an increased oesophageal permeability, and activation of innate immune response. Together with exposure to food antigens, these events could initiate (local) food-specific Th2 cell activation. Recent reviews give new insights into the pathogenesis of EoE [15–17] and the more recent findings in humans are highlighted below and summarized in Fig. 1.

Alteration of epithelial integrity and activation of innate immunity

Oesophageal biopsies from patients with EoE showed a decreased expression of molecules essential for epithelial integrity, such as desmoglein-1 (DSG-1) [18]. Downstream, both thymic stromal lymphopoietin (TSLP) and IL-33, two epithelial cell-derived cytokines that may promote Th2 type response after an epithelial aggression through the activation of innate lymphoid cells 2 (ILC2) and/or basophils, are overexpressed in oesophageal biopsies from EoE patients [19,20]. Accordingly, the percentage of ILC2 and basophil responses are higher in the oesophageal mucosa of patients with active EoE than in those with inactive EoE or control subjects [19,21]. Upstream from TSLP/IL-33, TNF-related apoptosis-inducing ligand (TRAIL), which has been shown to induce TSLP production *in vitro*, is upregulated in the oesophageal mucosa of patients with EoE [22]. In addition, the eosinophil chemoattractant eotaxin-3 (CCL26) is overexpressed in oesophageal epithelial cells from patients with EoE as a result from single-nucleotide polymorphism mutation or epigenetic modification [23,24]. The numbers of invariant natural killer T cells (iNKTs) and mast cells are also increased in the oesophageal mucosa of patients with EoE compared with healthy controls [25,26]. Likewise, the expression of some histamine receptors is increased in epithelial eosinophils of patients with EoE [27].

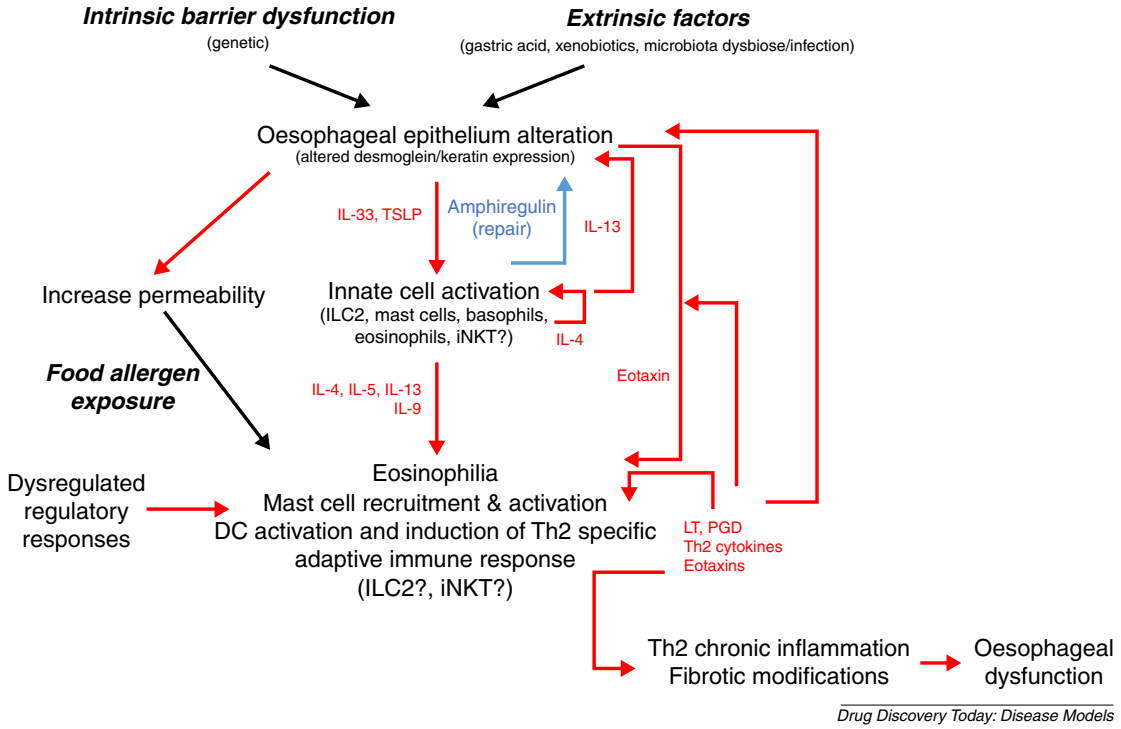

Figure 1. Pathogenesis of EoE. EoE may initially result from an alteration of the oesophageal epithelial barrier induced by intrinsic dysfunction or environmental aggressions. This may lead to an increased oesophageal permeability and activation of innate immune response. Together with exposure to food antigens, these events could initiate (local) food-specific Th2 cell activation leading to chronic inflammation, fibrotic modifications and oesophageal dysfunction.

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Evidence for a Th2 adaptive response

EoE is characterized by an increased expression of Th2 cytokines in oesophageal biopsies and blood; these cytokines may not only enhance activation and survival of eosinophils but also increase/maintain inflammation and induce fibrotic modifications [28–30]. EoE patients are more likely to present polymorphisms in gene encoding IL-13 [31] and epithelial cells from biopsies have shown an overexpression of IL-13 receptor (IL13R) and a high production of IL-13. In response to IL-13, cultured epithelial cells produce eotaxin-3 and reduce their expression of DSG-1 [18,32]. Polymorphisms in the IL-5 gene have also been associated with EoE, as well as increased circulating IL-5+ T cells and esophageal levels of the receptor IL-5R [28,31,33]. Thus, a high secretion of IL-5, potentiated by eotaxin-3, may increase the number of eosinophils in the esophagus. However, IL-5 blockage reduced only partially the recruitment and activation of eosinophils in patients with EoE [34], suggesting that other pathways may be involved in EoE pathogenesis. In that connection, an immune dysregulation may be present in EoE, with regulatory T cells (Treg) being increased in children and decreased in adults with EoE [35,36]. Other cytokines such as IL-15, IL-18 or TGF-β1 are also overexpressed in patients with EoE [37–39].

In vivo *models*

The complex interplay between epithelial barrier, innate and adaptive cells, and mediators within the esophageal mucosa is difficult to reproduce in animal models. However, various models of EoE have been proposed, mainly in mice (Table 1). Although most of these models could not reproduce EoE as observed in humans (i.e. eosinophilic inflammation restricted to the esophagus independently of IgE production, improvement of experimentally-induced EoE by local anti-inflammatory therapy or after removal of the stimuli, food impaction), they recreate some of the observations in humans and give further insights in the putative pathogenesis of EoE. These models also provide interesting results which lead to the development of potential new therapies.

Airway exposure to aeroallergen/cytokine

Initial studies showed that repeated intranasal (i.n.) administrations of aeroallergens such as *Aspergillus fumigatus* in mice induced eosinophilia in the lungs, esophagus and blood, but not in the stomach or small intestine [40]. Eosinophils (≈25–35 eosinophils/mm^2) were predominant in the lamina propria and the submucosa of the esophagus, and about 50% of eosinophils were undergoing cell death. Esophageal eosinophilia (≈6 eosinophils/mm^2) was also induced in mice sensitized to ovalbumin (Ova) through the intraperitoneal (i.p.) route and then repeatedly i.n. exposed. Conversely, esophageal eosinophilia was neither induced by intra-gastric (i.g.) or oral administration of *A. fumigatus*, nor by i.n. inoculation in non-anesthetized mice (i.e. in mice able to swallow). The critical role of IL-5 and the partial role of eotaxin in the induction of esophageal eosinophilia were then demonstrated using this model. As IL-5 has been related to lung eosinophilia, whereas eotaxin is critical for basal homing of eosinophils in the gastrointestinal tract (stomach and small intestine), the authors proposed a causal link between respiratory and esophageal hypersensitivity, which is debatable when considering human data [10,11,34]. The same group reproduced *A. fumigatus*-induced-eosinophil influx in esophagus (and lung), and epithelial cell hyperplasia after intratracheal delivery of IL-13, following a protocol known to induce experimental asthma [41]. The esophageal eosinophilia was dose-dependent and involved STAT5, IL-5, and partially eotaxin-1, implicating Th2 cells in EoE pathogenesis, although systemic Th2 response was not observed.

CD4+, CD8+ and B cells influx was further evidenced in this *A. fumigatus* model [42]. Interestingly, B cells or antigen-specific antibodies were not involved in the recruitment of eosinophils in the esophagus, whereas a critical role for adaptive T-cell immunity was demonstrated. However, CD4+ T cells dependency was less important in the esophagus than in the lung, and CD8+ T cells were dispensable. This study also showed that intra-tracheal (i.t.) administration was as efficient as i.n. application for inducing esophageal (and lung) eosinophilia (50–66 eosinophils/mm^2 counted after nine administrations in both cases), thus questioning the importance of topical exposure to esophagus initially stated by these authors in [40]. For both, i.n. and i.t. exposure, at least six doses were needed to induce significant eosinophilia in esophagus, whereas lung eosinophilia was induced after 4–5 applications. Unfortunately, in most of these studies, both the gender and the strain of mice were not specified. A comparison of the results obtained in BALB/c (Th2 biased) versus C57BL/6 (Th1 biased) strains would be very informative.

Recently, the roles of TRAIL and TRAIL-induced TSLP production were evidenced in the initial phase of *A. fumigatus*-induced airway and esophageal eosinophilia, in relation to EoE clinical data [22]. Esophageal eosinophilia (≈40 eo- eosinophils/mm^2) was associated with increased mast cell number, but not CD4+ T cells. This study evidenced the complex spatial and temporal expression pattern of various factors (such as TRAIL, TSLP, eotaxin, IL-5, IL-13 and TGF-β). *A. fumigatus*-induced eosinophilia in the esophagus, but not in the respiratory tract, was further shown to be dependent on IL-15 produced by macrophages/dendritic cells, suggesting that different mechanisms may be involved in esophageal and airway eosinophilia [37]. *In vitro*, IL-15 primes CD4+ T cells for Th2 cytokines production via STAT5 activation, and induces eotaxin production by esophageal epithelial cells [37].

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Table 1. Main animal models for EoE: experimental procedures and main outcomes. i.n.: intra-nasal, i.p.: intra-peritoneal, i.g.: intra-gastric, i.t.: intra-tracheal, NP: not provided

Model	Mouse strain	Genetic modifications	Age/gender/ breeding conditions	EoE induction	Main outcome	Ref
Airway exposure to aeroallergen Aspergillus fumigatus	BALB/c C57BL/6	Wild-type Eotaxin or IL-5 deficient	8–10 week-old Males/females SPF	Repeated i.n., oral or i.g. administrations of *A. fumigatus* (3 treatments/week, for 3 weeks) I.p. sensitization to ovalbumin (alum) and repeated i.n. dosing with ova (150 μg, 7 exposures over10 days)	Esophageal (and lung) eosinophilic inflammation, extracellular granule deposition and epithelial cell hyperplasia after i.n. administration (100 μg in 50 μl) – importance of anesthesia Critical role of IL-5/partial role of eotaxin in the pathophysiological changes	[40]
	BALB/c C57BL/6	Wild-type Lymphocytes (RAG1)-, B cell (IgH6)-, T cell (Foxn1)-, CD4- or CD8α-deficient	6–8 week-old Males/females SPF	Repeated i.n. or i.t. administrations of (endotoxin-free) *A. fumigatus* in anesthetized mice	Validation of i.n. and i.t. routes for experimental airway-EoE and determination of the number of doses required Evidence of the role of adaptive T cell immunity	[42]
	BALB/c	Wild type TRAIL-deficient	8–12 week-old Male NP	Repeated i.n. administrations of *A. fumigatus* in anesthetized mice	TRAIL expression is detected as soon as 24 hours after the first administration of *A. fumigatus*. TRAIL then induced TSLP, which is sufficient to induced esophagus eosinophilia and remodeling	[22]
	BALB/c	Wild type IL-15Rα deficient	6–8 week old Males/females SPF		IL-15 produced by esophageal macrophages and dendritic cells activates CD4+ cells to produce IL-5 and IL-13 and epithelial cells to produce eotaxin, thus participates to experimental EoE but not lung eosinophilia	[37]
Intratracheal cytokine administration	BALB/c	Wild type STATS-6, eotaxin-1 or IL-5 deficient	8–12 week-old Males/females NP	Repeated i.t. administrations of various doses of IL-13, IL-4, IL-10,or IL-9	IL13 reproduce *A. fumigatus*-induced EoE and is dependent on IL-5, eotaxin-1, and STAT6	[41]
Systemic sensitization and local exposure with food antigens	BALB/c mice	Wild type Smad3 deficient	8-week old Females NP	2 i.p. sensitizations with Ova (50 μg, Alum) and repeated intra-esophageal administrations of Ova(10 mg, 3 times/ week for 4 weeks)	Development of an Ova-induced model of EoE (eosinophilia, esophagus remodeling, angiogenesis), but no info at other sites that esophagus (stomach, small intestine) TGF-β signaling is critical in esophageal remodeling	[44]
		Wild type			Role of eosinophils in inflammation but also in angiogenesis, deposition of fibronectin and basal zone hyperplasia.	[43]
	BALB/c	Wild type Lτα and CD1d deficient	6–8 week-old Males and females (mix) SPF	2 i.p. sensitizations with corn or peanut extract (200 μg, alum) and repeated i.n., oral or i.g. exposures (every other days, 100 μg)	Development of a peanut/corn induced EoE (associated with lung eosinophilia) after i.n. challenges Role of iNKTs and eotaxin 1/2 in the initiation of EoE	[45]

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Table 1 (*Continued*)

Model	Mouse strain	Genetic modifications	Age/gender/ breeding conditions	EoE induction	Main outcome	Ref
Oral sensitization and exposure to food antigen	BALB/c	Wild type	4 week-old Female Conventional breeding	Sensitization by repeated i.g. administrations of crude peanut protein extract (1 mg) with Cholera Toxin and sustained oral and i.g. exposures over 10 days	Induction of eosinophilia in esophagus (and jejunum), associated with a strong local and systemic Th2 specific immune response Pre-clinical model for testing the efficiency of epicutaneous immunotherapy, through Treg induction	[46]
Epicutaneous sensitization and i.n. or oral exposure to food antigen	BALB/c	Wild-type STAT6, IL-5, IL-13, IL-4/IL-13 deficient	4–8 week old Gender not specified SPF	Epicutaneous exposures to Ova or *A. fumigatus* using occlusive patch (2 to 3 one-week applications) and a single i.n. challenge (25 μg) on anesthetized mice	Development of esophageal (and lung) eosinophilia Essential role of IL-5 and partial role of IL-4, IL-13 and STAT6 in pathogenesis	[48]
	BALB/c C57BL/6	Wild type Igh-7-/TSLP receptor-deficient BALB/c and C57BL/6 Baso–DTR mice	8–12 week-old Males and females SPF	Epicutaneous sensitization (daily, for 14 days) to ovalbumin or crude peanut extract with TSLP-inducing agents and further sustained intra-gastric and oral exposure	Development of eosinophilic inflammation in esophagus (and stomach and small intestine), structural changes and food impactation in 30% of mice TSLP and basophil contribute to the pathogenesis of EoE No role for IgE	[19,49]

Although very informative, the *A. fumigatus* model has two main limitations. First, it could be argued that esophageal eosinophilia may be secondary to lung eosinophilia. Second, the esophageal eosinophilia observed after exposure through different routes in mice may be mechanistically distinct from that observed in humans with EoE.

Systemic sensitization combined with local delivery of food antigens

Other studies evidenced the induction of EoE in mice sensitized by the i.p. route and then repeatedly challenged by the esophageal route using food allergens such as Ova [43,44], peanut or corn [45]. I.p. sensitization and repeated esophageal exposure to a high dose of Ova led to a dramatic esophageal eosinophilia in the lamina propria (≈120 eosinophils/mm^2) [44], suggesting that previous work by Mishra and coworkers [40] used too low doses during challenges. TGF-β expression and esophagus remodeling (angiogenesis, fibronectin deposit, epithelial basal zone hyperplasia) were also evidenced, as the same as blood eosinophilia. However, although the analysis procedure and reagents were similar in both studies (anti-major basic protein (MBP) staining), the number of esophageal eosinophils in control mice highly differed (≈12 eosinophils/mm^2 vs < 1). Moreover, no information was provided on the impact of Ova treatment at other (gastrointestinal) sites. This model was used to demonstrate the role of TGF-β-Smad3 axis in the late phase of EoE, that is, fibrosis and angiogenesis [44], and the efficiency of targeting eosinophils using anti-Siglec (sialic acid-binding immunoglobulin-like lectin) antibodies as a new therapeutic approach [43].

Experimental EoE was also successfully induced in mice after i.p. sensitizations and repeated i.n. or i.g. exposures with peanut extract [45]. In this study, several features of human EoE were evidenced such as the esophageal eosinophilic influx in lamina propria and within the epithelium, eosinophilic micro abscesses, extracellular MBP+ granules, eosinophil-related cytokines mRNA expression, but also mast cell accumulation and altered epithelial mucosa. I.n. challenge induced a more severe esophageal eosinophilia than i.g. challenge, and an eosinophilia in the airways, whereas i.g. challenge induced eosinophilia also in the small intestine. Conversely, oral challenge did not induce esophageal eosinophilia. However, i.n. challenged mice were apparently not anaesthetized, and then allergen could have been partially

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swallowed. Moreover, kinetic of eosinophilia in lung and esophagus was not provided, thus, the esophageal eosinophilia as a consequence of pulmonary eosinophilia cannot be ruled out – all the more para-esophagal lymph nodes, allowing trafficking of eosinophils from airway to esophagus, have a critical role in this model. Interestingly, basal influx of eosinophils was induced after systemic sensitization and was further highly increased both after i.n. and i.g. challenges, with mean values of 167–186 and 36–39 eosinophils/mm^2, respectively. This influx was far higher than that induced by i.n. exposure to *A. fumigatus*, highlighting the impact of systemic sensitization. Peanut was a more potent inducer of esophageal eosinophilia than corn, suggesting the possibility of ranking food for their potency to induce EoE. Additionally, mechanistic studies evidenced the critical role of iNKTs and eotaxin 1 and 2 in the initiation of the pathophysiology in this model, in accordance with clinical human data.

Oral exposure to food antigens

Esophago-gastro eosinophilia was observed in mice sensitized with peanut protein extract via the oral route, using cholera toxin as an adjuvant, maintained on an elimination diet for 8 weeks and then submitted to a sustained oral exposure to peanut [46]. Eotaxin and IL-13 mRNA expressions were increased as early as the second day of oral exposure, but high eosinophilic infiltration and IL-5 mRNA were only detected on day 10, that is, after combined and intense oral plus i.g. peanut protein exposures. The eosinophil influx was high (≈130 eosinophils/mm^2), although this level was not reached in another study using the same experimental procedure (≈36–70 eosinophils/mm^2) [47]. Esophageal inflammation was accompanied with jejunal lesions (necrosis, eosinophilic inflammation, villous sub-atrophy) and high systemic Th2 response (specific IgE, spleen cells specific secretion of Th2 cytokines). This model was used as a pre-clinical model for testing the efficiency of epicutaneous immunotherapy, through Treg induction.

Epicutaneous sensitization and i.n. or oral exposure to food antigen

Some groups tested the impact of cutaneous exposure on EoE development in mice. Epicutaneous exposure to Ova or *A. fumigatus* using occlusive patches induced eosinophils and mast cells influx in skin, blood eosinophilia and systemic sensitization when a further unique i.n. challenge with antigen induced esophageal (≈28–35 eosinophils/mm^2) and lung eosinophilia [48]. Esophageal eosinophilia appeared 4 hours after i.n. challenge, was totally dependent on IL-5, and partially dependent on IL-4, IL-13 and STAT6. Conversely, IL-5 deficient and wild type mice demonstrated the same levels of total IgE and specific IgG1, thus dissociating antibody response and the development of esophageal eosinophilia.

Eosinophil influx (evidenced by histology and flow cytometry), edema, inflammation, food impaction and Th2-related cytokines expression in the esophagus were also demonstrated in mice daily exposed to Ova or peanut via the cutaneous route and then submitted to sustained and combined i.g. and oral exposures to high doses of antigens [19]. The associated structural changes of esophagus were assessed using optical coherence tomography [49]. Interestingly, sensitization was achieved by exposing Ova after tape stripping or with vitamin D analog to increase TSLP production in the skin, as a model of atopic dermatitis. In this model, experimental EoE is shown to be dependent on TLSP-elicited basophils and independent on IgE. Notably, basophil depletion during cutaneous sensitization reduced eosinophilia and implication of TSLP and basophils correlated with data obtained on pediatric EoE population. Eosinophilia in stomach and small intestine and systemic Th2 cytokine responses was also evidenced, and further studies demonstrated that intestinal immediate FA is also induced in this model, which is TSLP-elicited skin basophils and IgE-dependent [50].

All these models mimicking more and more closely human EoE improved our understanding of the initial events of EoE pathogenesis. Additional studies using, for example, altered esophageal epithelium (physical/chemical/microbial stress, intrinsic dysfunction/defaults in tight junction or filaggrin mutation, among others) combined with studies in humans identifying homing and chemokine receptors, both on eosinophils and T cells, will be useful to finalize and validate EoE experimental models.

Food protein-induced enterocolitis syndrome (FPIES), proctocolitis (FPIAP) and enteropathy (FPE): clinically relevant pathologies without animal models

The main data concerning clinical manifestations, triggered foods, biological and histologic features and supposed mechanisms for FPIES, FPIAP and FPE are gathered in Table 2. Although these pathologies are clinically relevant, pathophysiological mechanisms involved are deeply unknown. To the best of our knowledge, no animal models have been developed for FPIES, FPIAP or FPE. Human data allowing to determine the most relevant models to be used (genetic background, i.e. Th2 biased/Treg dysfunction, tight junction dysfunction or basal cytokines/chemokines secretion), the environmental factors potentially involved (factors leading to alteration of the epithelium, role of microbiota) and the key players of the immune system in initiating events and in maintaining inflammation are still lacking, which renders the development of relevant models still difficult. Moreover, some symptoms can be difficult to analyse or even to induce in 'classical' models: for example mice/rats do not vomit which renders the development of models for acute FPIES challenging.

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Table 2. FPIES, FPIAP and FPIE clinical and biological features and putative mechanisms SPT: skin prick test. CM: cow's milk

Diseases	Prevalence[a]	Clinical manifestations	Foods	Biological and histologic features	Mechanisms	References
FPIES	0.34%	**Acute form (1–3 hours after exposure)** Intermittent exposure to the offending food Repetitive vomiting, dehydration, pallor, lethargy, hypotension Delayed diarrhea No skin or respiratory involvement (≠ IgE-mediated anaphylaxis) 90% resolution at 3–5 years of age	CM, soy Single food	Variable villous blunting, colitis Intestinal inflammation (eosinophils, neutrophils, lymphocytes) Blood neutrophilia, thrombocytosis, acidosis Methemoglobinemia Positive SPT or IgE: rare	Increased duodenal mucosal expression of TNF-α/ decreased duodenal mucosal expression of TGF-β Local production of food specific IgA and IgM Immune-neuroendocrine interplay?	[51–54]
		Chronic form Regular intake of the offending food Chronic diarrhea, vomiting, failure to thrive, 90% resolution at 3–5 years of age		Hypoalbuminemia, anemia		
FPIAP	0.16%	Rectal bleeding in otherwise well children Possible mucus in stools, diarrhea, abdominal pain Resolution of most cases at 1 year of age	CM, soy, egg	Eosinophils and lymphocytes infiltration in the rectal mucosa Eosinophilia, anemia	Delayed maturation of the gastrointestinal immune system/Delayed microbiota establishment Increased mucosal expression of eotaxin-1, CXCL13	[51,53,55–57]
FPE		Protracted diarrhea, vomiting, malabsorption Abdominal distension, failure to thrive Resolution of most cases at 2–3 years of age	CM, soy	Duodenal and colic lymphonodular hyperplasia with increased intraepithelial lymphocytes Hypoprotidemia	Increased IFN-γ and IL4 expression in jejunum Increased intraepithelial cytotoxic CD8+ T cells	[51,55]

[a] Prevalence among CM-allergic infants (1%), in a prospective population-based birth-cohort study in Israel [2].

Conclusions

Elucidation of the immune mechanisms and environmental factors involved in the pathogenesis of non-IgE FA needs further investigations, in both patients (biopsies, blood samples, feces) and animal models. Animal models development using relevant mouse strains and age, clinically relevant food allergens (mainly CM and soy) and realistic route of exposure, are needed to better understand the complex mechanisms involved in epithelial barrier alteration and downstream dysregulation of the immune system. In addition, such models will be useful for the development of new preventive and therapeutic strategies, but also for the assessment of allergenicity of new foods that will soon integrate the human nutrition due to predicted shortage of proteins for human consumption.

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References

[1] Sampson HA, Aceves S, Bock SA, James J, Jones S, Lang D, et al. Food allergy: a practice parameter update – 2014. J Allergy Clin Immunol 2014;134:1016–25.
[2] Katz Y, Goldberg MR, Rajuan N, Cohen A, Leshno M. The prevalence and natural course of food protein-induced enterocolitis syndrome to cow's milk: a large-scale, prospective population-based study. J Allergy Clin Immunol 2011;127:647–53.
[3] Grimshaw KE, Bryant T, Oliver EM, Martin J, Maskell J, Kemp T, et al. Incidence and risk factors for food hypersensitivity in UK infants: results from a birth cohort study. Clin Transl Allergy 2015;6:1.
[4] Simon D, Cianferoni A, Spergel JM, Aceves S, Holbreich M, Venter C, et al. Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity. Allergy 2016;71:611–20.
[5] Dellon ES. Epidemiology of eosinophilic esophagitis. Gastroenterol Clin North Am 2014;43:201–18.
[6] Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. J Allergy Clin Immunol 2011;128:3–20.

Please cite this article in press as: Lozano-Ojalvo D, et al. Non-IgE mediated food allergy, Drug Discov Today: Dis Model (2016), http://dx.doi.org/10.1016/j.ddmod.2016.09.003

[7] Spergel JM. An allergist's perspective to the evaluation of eosinophilic esophagitis. Best Pract Res Clin Gastroenterol 2015;29:771–81.
[8] Furuta GT, Katzka DA. Eosinophilic esophagitis. N Engl J Med 2015;373:1640–8.
[9] Cianferoni A, Spergel J. Eosinophilic esophagitis: a comprehensive review. Clin Rev Allergy Immunol 2016;50:159–74.
[10] Lucendo AJ, Arias A, Redondo-Gonzalez O, Gonzalez-Cervera J. Seasonal distribution of initial diagnosis and clinical recrudescence of eosinophilic esophagitis: a systematic review and meta-analysis. Allergy 2015;70:1640–50.
[11] Kelly KJ, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. Gastroenterology 1995;109:1503–12.
[12] Spergel JM, Brown-Whitehorn TF, Cianferoni A, Shuker M, Wang ML, Verma R, et al. Identification of causative foods in children with eosinophilic esophagitis treated with an elimination diet. J Allergy Clin Immunol 2012;130:461–7.
[13] Greenhawt M, Aceves SS, Spergel JM, Rothenberg ME. The management of eosinophilic esophagitis. J Allergy Clin Immunol Pract 2013;1:332–40.
[14] Rajan J, Newbury RO, Anilkumar A, Dohil R, Broide DH, Aceves SS. Long-term assessment of esophageal remodeling in patients with pediatric eosinophilic esophagitis treated with topical corticosteroids. J Allergy Clin Immunol 2016;137:147–56.
[15] Hill DA, Spergel JM. The immunologic mechanisms of eosinophilic esophagitis. Curr Allergy Asthma Rep 2016;16:9.
[16] Sherrill JD, Rothenberg ME. Genetic and epigenetic underpinnings of eosinophilic esophagitis. Gastroenterol Clin North Am 2014;43:269–80.
[17] Leung J, Beukema KR, Shen AH. Allergic mechanisms of eosinophilic oesophagitis. Best Pract Res Clin Gastroenterol 2015;29:709–20.
[18] Sherrill JD, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, et al. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. Mucosal Immunol 2014;7:718–29.
[19] Noti M, Wojno ED, Kim BS, Siracusa MC, Giacomin PR, Nair MG, et al. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. Nat Med 2013;19:1005–13.
[20] Judd LM, Heine RG, Menheniott TR, Buzzelli J, O'Brien-Simpson N, Pavlic D, et al. 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 2016;310:G13–25.
[21] Doherty TA, Baum R, Newbury RO, Yang T, Dohil R, Aquino M, et al. Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis. J Allergy Clin Immunol 2015;136:792–4.
[22] Collison AM, Sokulsky LA, Sherrill JD, Nightingale S, Hatchwell L, Talley NJ, et al. TNF-related apoptosis-inducing ligand (TRAIL) regulates midline-1, thymic stromal lymphopoietin, inflammation, and remodeling in experimental eosinophilic esophagitis. J Allergy Clin Immunol 2015;136:971–82.
[23] Lim E, Rothenberg ME. Demethylation of the human eotaxin-3 gene promoter leads to the elevated expression of eotaxin-3. J Immunol 2014;192:466–74.
[24] Krishnamurthy P, Sherrill JD, Parashette K, Goenka S, Rothenberg ME, Gupta S, et al. Correlation of increased PARP14 and CCL26 expression in biopsies from children with eosinophilic esophagitis. J Allergy Clin Immunol 2014;133:577–80.
[25] Jyonouchi S, Smith CL, Saretta F, Abraham V, Ruymann KR, Modayur-Chandramouleeswaran P, et al. Invariant natural killer T cells in children with eosinophilic esophagitis. Clin Exp Allergy 2014;44:58–68.
[26] Arias A, Lucendo AJ, Martinez-Fernandez P, Gonzalez-Castro AM, Fortea M, Gonzalez-Cervera J, et al. Dietary treatment modulates mast cell phenotype, density, and activity in adult eosinophilic oesophagitis. Clin Exp Allergy 2016;46:78–91.
[27] Merves J, Chandramouleeswaran PM, Benitez AJ, Muir AB, Lee AJ, Lim DM, et al. Altered esophageal histamine receptor expression in eosinophilic esophagitis (EoE): implications on disease pathogenesis. PLOS ONE 2015;10:e0114831–114840.
[28] Blanchard C, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, et al. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. J Allergy Clin Immunol 2011;127:208–17.
[29] Cheng E, Zhang X, Huo X, Yu C, Zhang Q, Wang DH, et al. Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. Gut 2013;62:824–32.
[30] Rieder F, Nonevski I, Ma J, Ouyang Z, West G, Protheroe C, et al. T-helper 2 cytokines, transforming growth factor beta1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. Gastroenterology 2014;146:1266–77.
[31] Namjou B, Marsolo K, Caroll RJ, Denny JC, Ritchie MD, Verma SS, et al. Phenome-wide association study (PheWAS) in EMR-linked pediatric cohorts, genetically links PLCL1 to speech language development and IL5-IL13 to Eosinophilic Esophagitis. Front Genet 2014;5:401–10.
[32] Blanchard C, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu TX, et al. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. J Allergy Clin Immunol 2007;120:1292–300.
[33] Kinoshita Y, Furuta K, Ishimura N, Ishihara S. Elevated plasma cytokines in Japanese patients with eosinophilic esophagitis and gastroenteritis. Digestion 2012;86:238–43.
[34] Assa'ad AH, Gupta SK, Collins MH, Thomson M, Heath AT, Smith DA, et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. Gastroenterology 2011;141:1593–604.
[35] Stuck MC, Straumann A, Simon HU. Relative lack of T regulatory cells in adult eosinophilic esophagitis - no normalization after corticosteroid therapy. Allergy 2011;66:705–7.
[36] Fuentebella J, Patel A, Nguyen T, Sanjanwala B, Berquist W, Kerner JA, et al. Increased number of regulatory T cells in children with eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2010;51:283–9.
[37] Zhu X, Wang M, Mavi P, Rayapudi M, Pandey AK, Kaul A, et al. Interleukin-15 expression is increased in human eosinophilic esophagitis and mediates pathogenesis in mice. Gastroenterology 2010;139:182–93.
[38] Niranjan R, Rajavelu P, Ventateshaiah SU, Shukla JS, Zaidi A, Mariswamy SJ, et al. Involvement of interleukin-18 in the pathogenesis of human eosinophilic esophagitis. Clin Immunol 2015;157:103–13.
[39] Frischmeyer-Guerrerio PA, Guerrerio AL, Oswald G, Chichester K, Myers L, Halushka MK, et al. TGFbeta receptor mutations impose a strong predisposition for human allergic disease. Sci Transl Med 2013;5:195ra94.
[40] Mishra A, Hogan SP, Brandt EB, Rothenberg ME. An etiological role for aeroallergens and eosinophils in experimental esophagitis. J Clin Invest 2001;107:83–90.
[41] Mishra A, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. Gastroenterology 2003;125:1419–27.
[42] Mishra A, Schlotman J, Wang M, Rothenberg ME. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. J Leukoc Biol 2007;81:916–24.
[43] Rubinstein E, Cho JY, Rosenthal P, Chao J, Miller M, Pham A, et al. Siglec-F inhibition reduces esophageal eosinophilia and angiogenesis in a mouse model of eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2011;53:409–16.
[44] Cho JY, Doshi A, Rosenthal P, Beppu A, Miller M, Aceves S, 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.
[45] 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.
[46] Mondoulet L, Dioszeghy V, Larcher T, Ligouis M, Dhelft V, Puteaux E, et al. Epicutaneous immunotherapy (EPIT) blocks the allergic esophago-gastro-enteropathy induced by sustained oral exposure to peanuts in sensitized mice. PLoS ONE 2012;7:e31967.
[47] Dioszeghy V, Mondoulet L, Dhelft V, Ligouis M, Puteaux E, Dupont C, et al. The regulatory T cells induction by epicutaneous immunotherapy is

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sustained and mediates long-term protection from eosinophilic disorders in peanut-sensitized mice. Clin Exp Allergy 2014;44:867–81.
[48] Akei HS, Mishra A, Blanchard C, Rothenberg ME. Epicutaneous antigen exposure primes for experimental eosinophilic esophagitis in mice. Gastroenterology 2005;129:985–94.
[49] Alex A, Noti M, Wojno ED, Artis D, Zhou C. Characterization of eosinophilic esophagitis murine models using optical coherence tomography. Biomed Opt Express 2014;5:609–20.
[50] Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. J Allergy Clin Immunol 2014;133:1390–9.
[51] Nowak-Wegrzyn A, Katz Y, Mehr SS, Koletzko S. Non-IgE-mediated gastrointestinal food allergy. J Allergy Clin Immunol 2015;135:1114–24.
[52] Berin MC. Immunopathophysiology of food protein-induced enterocolitis syndrome. J Allergy Clin Immunol 2015;135:1108–13.
[53] Nowak-Wegrzyn A. Food protein-induced enterocolitis syndrome and allergic proctocolitis. Allergy Asthma Proc 2015;36:172–84.
[54] Chung HL, Hwang JB, Park JJ, Kim SG. Expression of transforming growth factor beta1, transforming growth factor type I and II receptors, and TNF-alpha in the mucosa of the small intestine in infants with food protein-induced enterocolitis syndrome. J Allergy Clin Immunol 2002;109:150–4.
[55] Feuille E, Nowak-Wegrzyn A. Food protein-induced enterocolitis syndrome, allergic proctocolitis, and enteropathy. Curr Allergy Asthma Rep 2015;15:50–60.
[56] Ohtsuka Y, Jimbo K, Inage E, Mori M, Yamakawa Y, Aoyagi Y, et al. Microarray analysis of mucosal biopsy specimens in neonates with rectal bleeding: is it really an allergic disease? J Allergy Clin Immunol 2012;129:1676–8.
[57] Kumagai H, Maisawa S, Tanaka M, Takahashi M, Takasago Y, Nishijima A, et al. Intestinal microbiota and secretory immunoglobulin A in feces of exclusively breast-fed infants with blood-streaked stools. Microbiol Immunol 2012;56:657–63.

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