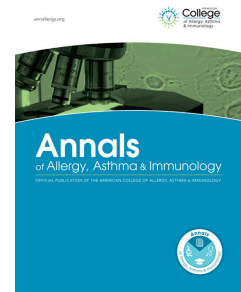


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Gastrointestinal Immunopathology of Food Protein-induced Enterocolitis Syndrome and Other Non-IgE-mediated Food Allergic Diseases

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Keywords: food protein-induced enterocolitis; food protein-induced allergic proctocolitis; food protein-induced enteropathy; eosinophilic esophagitis; atopic march; gut microbiome; serotonin; intestinal barrier.

Abbreviations:

CD — Crohn's disease

EoE — eosinophilic esophagitis

FPE — food protein-induced enteropathy

FPIAP — food protein-induced proctocolitis

FPIES — food protein-induced enterocolitis syndrome

IBD — inflammatory bowel disease

IgE — immunoglobulin E

IL — interleukin

ILC — innate lymphoid cell

iNKT — invariant natural killer T cells

MAIT — mucosal-associated invariant T cells

OFC — oral food challenge

STAT3 — signal transducer and activator of transcription 3

TGF- β — transforming growth factor-beta

Th2 — T helper 2

Th17 — T helper 17

TSLP — thymic stromal lymphopoietin

Treg — regulatory T cell

UC — ulcerative colitis

Word Count: 3655

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Table Number:2

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Objective: To provide a concise summary of current literature about gastrointestinal immunopathology of food protein-induced enterocolitis syndrome (FPIES) and other non-immunoglobulin E (IgE)-mediated food allergic diseases.

Data Sources: Data were extracted from PubMed, Medline, and Science Direct databases.

Study Selections: Original articles, review articles, and guidelines published in the recent five years in peer-reviewed journals were summarized first. The original articles cited were reviewed and relevant results were extracted.

Results: Patients with FPIES and non-IgE-mediated food allergic diseases developed vomiting, diarrhea, and food aversion expelled food allergen from their body. Not only T helper type 2 (Th2) immunity, Th1, Th17, innate immunity, and epithelial mucosal barrier defect were also important for the pathogenesis. Eosinophils, widely identified in the biopsy samples, were a key player or late-recruited cells for tissue repairs in those diseases. Intestinal dysbiosis and their metabolites stimulated enterochromaffin cells or enteroendocrine cells to produce serotonin, interfering with intestinal motility and subsequently affecting brain function. FPIES and non-IgE-mediated food allergic diseases were likely part of the atopic march. Allergic inflammation in intestinal mucosa might result in subsequent inflammation in the airway mucosa, indicating the theory of “one mucosa, one disease”.

Conclusion: The immune responses of FPIES and non-IgE-mediated food allergic diseases were not limited in the gastrointestinal tracts, but also trigger wider inflammatory response beyond the gastrointestinal tract. More research will be required for the systemic effect and intestinal microbiome of those diseases.

1 Introduction

2 Our immune system has evolved to protect us from various external threats, whether
3 infectious (bacteria, viruses, and parasites), or other noxious substances (venoms, toxins).
4 Allergy is defined as the consequence of a mistargeted and/or exaggerated immune
5 response, often to intrinsically harmless or even beneficial substances, such as foods.¹
6 Based on the nature of the immune response, allergies are categorized into
7 immunoglobulin E (IgE)-mediated, non-IgE-mediated, and mixed type.² This review will
8 focus on the gastrointestinal immunopathology of food protein-induced enterocolitis
9 syndrome (FPIES), food protein-induced proctocolitis (FPIAP), and food protein-induced
10 enteropathy (FPE). We will also draw some comparisons with eosinophilic esophagitis
11 (EoE), which is regarded as a mixed type food allergic disease,³ and has been extensively
12 and thoroughly reviewed elsewhere.⁴⁻⁶

13 The predominant symptoms of non-IgE-mediated food allergic diseases are
14 gastrointestinal, including vomiting, diarrhea, and dysphagia, which from a teleological
15 perspective is consistent with the body attempting to eliminate allergens from the
16 gastrointestinal tract.¹ Patients with acute FPIES develop delayed onset repetitive vomiting
17 and sometimes diarrhea after food ingestion, and patients with chronic FPIES, FPIAP, and
18 FPE typically present with diarrhea and/or bloody stools.⁷ EoE patients, in contrast, present
19 predominantly with abdominal pain, nausea, and vomiting in early childhood, often
20 progressing to dysphagia by adolescence and adulthood.⁸ Some patients become sensitive
21 to the tastes and smells of allergic foods, expressing their aversion and avoiding oral
22 intake.^{1,9}

Because of its increasing prevalence,^{4, 10} non-IgE-mediated food allergy has become a growing research field. In the first part of this review, we will summarize the gastrointestinal pathology and immune mechanism of each specific disease. In the following section, we will review the association of the important enteric nervous system neurotransmitter, serotonin, and the role of the microbiome in regulating it, and the pathophysiology of the non-IgE-mediated food allergy. We will hypothesize a possible mechanism of serotonin and the enteric nerve system for FPIES, in particular. We will review the association and possible function of eosinophils in non-IgE-mediated food allergy. Lastly, we will discuss the association between non-IgE-mediated food allergic diseases and IgE-mediated diseases, and suggest future research directions.

Gut immunity and non-IgE-mediated food allergy

As summarized in Table 1, pathogenesis of non-IgE-mediated food allergy involves not only type 2 T helper cells (Th2), but also innate, Th1, and Th17 immune responses. Activation of T cells by antigen has been demonstrated in patients with milk, soy, and rice FPIES.¹¹ However, the specificity of this response has been challenged as antigen-induced T cell activation is also observed in many normal individuals.¹² Furthermore, by analyzing antigen-activated cells following oral food challenges (OFC) of FPIES, two groups have independently demonstrated innate immune activation *ex vivo* – specifically following positive but not negative challenges.^{12, 13} By using mass cytometry analysis, Goswami *et al.*, observed that numerous innate immune cells, including monocytes, neutrophils, natural killer cells, and eosinophils were activated following positive challenges.¹³ In contrast, Mehr *et al.* found that Th17-related transcription factors and cytokines, including signal transducer and activator of transcription 3 (STAT3), transforming growth factor-beta

(TGF- β), and interleukin (IL)-6, were upregulated during an acute FPIES reaction.¹⁴ Taken together, both innate immune responses and Th17 mediated signaling pathway are likely playing a role in the pathogenesis of FPIES (Table 1), and Th2 cytokines or cells and innate immunity cells and inflammatory cytokines may also be involved in the pathophysiology of FPIAP and FPE.¹⁵

Serotonin, enteric nerve system, and non-IgE-mediated food allergy

Serotonin is an important neurotransmitter in both the central nerve system, where it mediates mood stability, and the enteric nerve system, where it regulates intestinal motility.¹⁶ More than 90% of serotonin is synthesized and stored in the enterochromaffin cells or sensory enteroendocrine cells of the intestine and can be released by mechanical pressure, nutrients, or noxious substances applied to the intestinal epithelium.^{17, 18} Serotonin subsequently activates the enteric nervous system triggering the peristalsis and secretion of the intestinal tract or, in the case of a pathological stimulus, causing vomiting and diarrhea to expulse irritants or food allergens.^{17, 18}

The receptors mediating the action of serotonin are mainly classified into 7 major groups. The 5-HT₃ receptor, distributed in the peripheral and central nerve system, is the major receptor involved both in intestinal secretion and motility and central nerve system functions.¹⁷ 5-HT₃ receptors are also expressed in immune and inflammatory cells such as monocytes, T cells, and dendritic cells.¹⁹ Therefore, serotonin is poised to be extensively involved in inflammatory and infectious intestinal diseases.^{20, 21}

Accumulating evidence supports a role for serotonin in the pathogenesis of FPIES. First, ondansetron, a highly selective 5-HT₃ antagonist most widely used for

chemotherapy-induced vomiting, has been demonstrated to be an effective treatment resolving the acute manifestation of FPIES.²²

Second, serotonin has been demonstrated to interfere with the balance between Th17 and regulatory T cells.²³ As mentioned above, Mehr *et al.*, by comparing gene expression changes before and after an oral food challenge, demonstrated that the key transcription factor and cytokines of Th17 immune response, including STAT3, TGF- β , and IL-6, were all upregulated during an FPIES acute reaction.¹⁴ In the Th17-mediated 2,4,6-trinitrobenzenesulfonic acid-induced colitis rat model, ondansetron had an anti-inflammatory effect by blocking 5-HT₃ receptor.²⁴ The imbalance between Th17 and regulatory T cells caused by serotonin participates in the pathogenesis of FPIES.

Third, serotonin is associated with eosinophils and barrier functions. Eosinophil infiltrations have been observed in the histopathological findings of FPIES and FPIAP (Table 1).^{25 26} Serotonin has also been demonstrated to be a chemotactic factor for eosinophils. Serotonin also disrupts the barrier function of mucosa.²⁴ In an organoid model, serotonin can disrupt the integrity of the esophageal barrier function.²⁷ Until now, there is still no evidence showing that high level of serotonin also disrupts the intestinal barrier function, which is worth further research. Therefore, serotonin is an important mediator for the histopathological changes in FPIES and other non-IgE-mediated food allergic diseases.

The gut-brain axis is a bidirectional interaction between our brain and intestine, and the microbiome plays an important role in this axis.²⁸ The gut microbiota regulates the secretion of serotonin by interacting with the enterochromaffin cells in the colon.²⁹ Yano *et al.* demonstrated that metabolites including short-chain fatty acids, such as butyrate, as

well as the secondary bile acid, deoxycholate, produced by indigenous spore-forming bacteria enhanced the production of serotonin from colonic enterochromaffin cells.²⁹ Expression of tryptophan hydroxylase, a critical step of serotonin synthesis, was upregulated after germ-free mice received intrarectal infusion of deoxycholate. Yano *et al.* revealed spore-forming *Clostridium* from mice and humans had the capability to increase the production of serotonin in the colonic enterochromaffin cells in mice.²⁹ Meanwhile, Ye *et al.* demonstrated that tryptophan catabolites, indole and indole-3-carboxaldehyde, produced by the gut microbiome, activated intestinal epithelial sensory enteroendocrine cells and increased their secretion of serotonin.¹⁸ The increase of serotonin activates cholinergic enteric and vagus nerves, causing increased intestinal motility.¹⁸

In Figure 1, we illustrate the hypothesis that the interactions between gut microbiome and enterochromaffin cells/enteroendocrine cells induce serotonin production, which may then play an important role in FPIES and other non-IgE-mediated food allergies. Intestinal dysbiosis causes the overproduction of serotonin in the intestinal tract, which is similar in patients with inflammatory bowel disease or irritable bowel syndrome.³⁰ Overproduced serotonin causes dysregulation of the intestinal motility, subsequently inducing diarrhea and/or vomiting.¹⁷ Serotonin attracts eosinophils into the gastrointestinal tract, presenting as the typical histological findings of non-IgE-mediated food allergy.³¹ Serotonin also interrupts the barrier integrity of the esophagus.²⁷ Serotonin then affects the central nerve system, resulting in food aversion, nausea, and vomiting, either via stimulation of the vagus nerve, cholinergic enteric nerves, and/or delivery to the CNS via platelets where serotonin can be stored.¹⁴

Gut microbiota and non-IgE-mediated food allergy

Research about the gut microbiota alterations in non-IgE-mediated food allergic diseases is very limited. Regarding FPIES, Boyer *et al.* claimed that the microbiota from FPIES infants' stool was enriched with bacteria from the *Gammaproteobacteria* and *Porphyromonadaceae* families.³² As for FPIAP, Kumagai *et al.* enrolled 15 exclusively breastfeeding infants with blood-streaked stools. All of the infants had no evidence of diarrhea, anal fissure, stool pathogens, or other medical condition and FPIAP was diagnosed.²⁹ When compared with 15 exclusively breastfeeding age-matched healthy controls, they found that the 15 FPIAP infants had more *Klebsiella spp.*, but less *Escherichia coli* and *Bacteroides fragilis* in their stool.³³ Liu *et al.* performed fecal microbiota transplantation in 19 FPIAP infants per rectum and showed increased microbiota diversity after transplantation. After fecal microbiota transplantation, *Bacteroides*, *Escherichia*, and *Lactobacillus* genera increased in FPIAP infants, and *Clostridium*, *Veillonella*, *Streptococcus*, and *Klebsiella* genera decreased.³⁴ The FPIAP symptoms resolved in two days after fecal microbiota transplantation, and no relapse was observed. There are no reports on dysbiosis in FPE. Further gut microbiome research is required to investigate the precise mechanism of intestinal dysbiosis in FPIES and FPIAP.

Eosinophils and non-IgE-mediated food allergy

Eosinophils appear to be a common pathological feature in non-IgE-mediated food allergic diseases (Table 1). Colonic biopsies in FPIES patients showed increased eosinophil infiltration, and eosinophils were frequently identified from infants with chronic FPIES.^{26, 35} Eosinophil activation was also identified following positive OFC in FPIES patients.¹³ In FPIAP patients, the characteristic finding of the rectosigmoid biopsy was acute inflammation with 5 or more eosinophils per high-powered field.²⁵ As for FPE, even

though the predominant histological findings were villous atrophy, eosinophil granule major basic protein was significantly increased on the biopsy samples (Table 1).³⁶

Eosinophils play a diverse role in our immune system. Traditionally, eosinophils are regarded as an amplifier of type 2 immunity, playing an important role in immune defense from multicellular parasites (e.g., helminths, ticks) and in hypersensitivity reactions (such as asthma and allergic rhinitis).³⁷ ILC2 and Th2 cells produce IL-5 to recruit eosinophil into the inflammatory sites and activate the production of eosinophil.³⁸ Eosinophil counts and associated mediators were identified as severity markers and therapeutic targets in atopic diseases. One of the best examples was asthma. However, increasing evidence demonstrates that eosinophils are important for tissue remodeling and repairment after an immune response.³⁹ More research will be required to clarify the precise role of eosinophils in non-IgE-mediated food allergic diseases.

Barrier function and non-IgE-mediated food allergy

Barrier function impairment is one of the manifestations observed in non-IgE-mediated food allergies. For example, increased intestinal permeability was identified in children with non-IgE-mediated cow's milk allergy.⁴⁰ Even those cow's milk allergic infants with only cutaneous manifestations had increased intestinal permeability.⁴⁰ Before food elimination, the permeability of the mucosa from FPE patients' jejunum to horseradish peroxidase was increased in vitro. The permeability returned to normal after allergen elimination.⁴¹ The increase of intestinal permeability indicated that those infants have altered intestinal epithelial barrier function, caused presumably by the intestinal inflammation and villous atrophy secondary to the cow's milk allergy.⁴⁰ Direct evidence for barrier function change in FPIES and FPIAP is lacking.

Comparison of EoE with FPIES and other non-IgE-mediated food allergy

EoE is an immune-mediated inflammatory disease of the esophagus characterized by esophageal dysfunction and esophageal eosinophilic infiltration. EoE is regarded as a food allergic disease because food is a common trigger of EoE and food elimination is an effective treatment for EoE.^{3,42} EoE is by far the most well-studied entity in non-IgE and IgE-mediated mixed food allergies. We will make a parallel comparison of EoE with FPIES and other non-IgE-mediated food allergic diseases here.

FPIES, FPIAP, FPE, and EoE are different in several clinical aspects. The diagnosis of FPIES, FPIAP, and FPE usually begin around infancy and early childhood while the first peak of EoE diagnosis is somehow later, around 5 to 10 years in age.^{8, 35, 43, 44} FPIES, FPIAP, and FPE commonly resolve over time, but EoE does not.^{8, 35, 43, 44}

Even though eosinophils were reported to be identified in FPIES and other non-IgE-mediated food allergic diseases,^{25, 26, 36} EoE is the only entity with a specific pathological diagnostic criterion of ≥ 15 eosinophils per high power field on esophageal biopsies.⁸ Eosinophil and its associated mediator and cytokines (e.g. IL-5, IL-13, and eotaxin-3) are important for the pathogenesis of EoE,⁵ but the role of eosinophils in FPIES and other non-IgE-mediated food allergic diseases are much less well defined.

Barrier impairment is another feature of EoE. Desmoglein-1, a major component of the desmosome maintaining epithelial cell-to-cell adhesion, is downregulated in EoE patients.⁴⁵ IL-13 in EoE patients induces the loss of desmoglein-1 via calpain 14.⁴⁶ The EoE patients also have decreased expression of filaggrin, a crucial factor preserving the epithelial integrity.⁴⁷ The barrier defect of EoE, measured by electrical tissue impedance

spectroscopy during endoscopy, was correlated well with esophageal eosinophilia and the severity of EoE.⁴⁸ And as mentioned above, serotonin disrupts the integrity of the esophageal barrier function in an organoid model.²⁷ In comparison, the evidence of barrier dysfunction for FPIES or non-IgE-mediated food allergic diseases are not sufficient.

The esophageal microbiota associated with the pathogenesis of EoE is quite different from that in FPIES and other non-IgE-mediated diseases. When the bacterial DNA was extracted from the biopsied samples, *Neisseria* and *Corynebacterium* genera were enriched in patients with active EoE, and *Streptococcus* and *Atopobium* genera were more common in non-EoE controls.⁴⁹ When the bacterial DNA was extracted from the capsule of the esophageal string test, *Hemophilus* genera were predominant and the bacterial loads were higher in the untreated EoE patients.⁵⁰ Until now, no research demonstrated that the EoE microbiota change was associated with serotonin production. However, Wen *et al.* demonstrated that short-chain fatty acids produced by the esophageal microbiome bound to free fatty acid receptor 3 on pathogenic Th2 cells, inducing the production of Th2 cytokines (e.g. IL-4, IL-5, and IL-13) in EoE patients.⁵¹

One mucosa, one disease: Non-IgE-mediated food allergy as part of atopic march

We believe that increasing evidence shows that non-IgE-mediated food allergic diseases are part of the disease spectrum associated with the atopic march.⁵² Chronologically, FPIAP occurs early in infancy (2 weeks to 2 months), followed by atopic dermatitis (eczema), FPIES, IgE-mediated food allergies, and later-on EoE and asthma. To be specific, 40.4% of FPIES children had eczema, and children with FPIAP had 1.5 times more with a history of eczema when compared with healthy controls.^{9, 44} Su

205 *et al.* demonstrated 23% of FPIES patients had a past history of FPIAP.⁹ Martin *et al.*
 206 recently demonstrated that FPIAP infants, independent from its association with eczema,
 207 were more than twice as likely to develop IgE-mediated food allergy, especially to cow's
 208 milk, the most common trigger of FPIAP.⁵³ Hill *et al.* demonstrated that children with
 209 atopic dermatitis, IgE-mediated food allergy, and allergic rhinitis were at higher risk to
 210 develop EoE later in their life. EoE was considered by these authors to be a late
 211 manifestation of the atopic march.⁵⁴ The loss-of-function mutation of filaggrin is
 212 common to both atopic dermatitis and EoE – one of the possible mechanisms explaining
 213 EoE as a late manifestation of the atopic march.⁴⁷ Radano *et al.* reported that EoE
 214 patients had higher probabilities to have symptoms compatible with FPIAP before 1 year
 215 old.⁵⁵ Taking all those findings together, non-IgE-mediated food allergic diseases are
 216 intertwined with IgE-mediated diseases, as seen by the odds ratios for comorbidity
 217 summarized in Figure 2. While EoE is considered as a late manifestation, FPIAP might
 218 be the earliest presentation of the atopic march, even earlier than IgE-mediated food
 219 allergy (Figure 3).^{53, 54} We acknowledge that the precise mechanism of adult-onset FPIES,
 220 FPIES that does not remit in young childhood, and early-onset of inflammatory bowel
 221 diseases are less well understood and are likely more complicated, and their roles in the
 222 allergic march are not well defined. But these could be of great interest in future studies.

223 The concept of “one airway, one disease” was proposed by Bachert *et al.* Most
 224 asthmatic patients have concomitant allergic rhinitis, and the allergic inflammation
 225 occurring both in nasal and bronchial mucosa was indicative of the comorbidity.⁵⁶ The
 226 barrier defect, such as filaggrin in EoE and atopic dermatitis, was shared by IgE-mediated
 227 and non-IgE-mediated allergic diseases.⁴⁷ EoE triggered by aeroallergens in seasonal

allergic patients was another hint that esophageal and airway mucosal inflammation have certain interaction in those patients.⁵ Allergic cytokine profiles in the gastrointestinal mucosa were similar to that in the airway mucosa of asthma patients.⁵⁷ In a mouse model of parasitic infection, inflammatory ILC2 induced in the intestine circulated to the lung, contributing to type 2 immunity.⁵⁸ We believe that ILC2 would be a possible link between non-IgE-mediated food allergy and other IgE-mediated allergy.

Although there is no direct evidence of their involvement in FPIES, FPIAP and non-IgE mediated food allergies, Mucosal-associated invariant T cells (MAIT) could play an important role in the pathogenesis of these conditions. MAIT cells are innate-like T cells present in both blood stream and barrier tissue, including the intestine, oral, airway, skin, and female genital mucosa.⁵⁹ They serve an important role in the cross-talk between diverse environmental factors, such as microbiome, and the adaptive immune system, and are considered crucial in homeostatic function as well as inflammatory conditions. Studies of MAIT cells function in non-IgE food allergies could be fruitful.

Invariant natural killer T cells (iNKT) are a unique group of T lymphocytes that are activated by both microbial glycolipid antigens and dietary lipid from cow's milk.⁶⁰ iNKT could provide a clue in understanding the mechanisms of FPIES, as well as FPIAP and other non-IgE mediated food allergies. Furthermore, antigen-specific human Treg cells in the lungs play an important role in regulating tolerance versus allergy to minute quantities of inhaled aeroallergens.⁶¹ This not only provided insight into antigen-specific Treg cell response, but also implicated potential therapeutic strategies in the treatment and prevention of allergies. No such data exist for food allergens in the gastrointestinal tract.

With the support from research in the future, we might extend the concept of “one airway, one disease” into “one mucosa, one disease”.⁵²

The association of non-IgE-mediated food allergy with IBD, celiac disease, and EGID

Non-IgE-mediated food allergic diseases may also have some links with celiac diseases and bowel inflammatory diseases, the other 2 disease entities of intestinal mucosa inflammation with underlying immune mechanisms. Our group observed that FPIES patients had a higher prevalence of family histories with inflammatory bowel disease (IBD) (9.4% vs. 0.6%) and celiac disease (7.3% vs. 0.75%) than the general population.⁹ In a large population-based prospective epidemiological study of IBD patients, either Crohn’s disease (CD) or ulcerative colitis (UC), were at increased relative risk to have EoE (5.4 for CD and 3.5 for UC).⁶² In another case-control study, Mintz *et al.* found that patients with EoE alone, versus those with both IBD and EoE, were diagnosed earlier and were male predominant.⁶³ Finally, Thompson *et al.*, found that children and adults with celiac diseases were 16 times more likely to have eosinophilic esophagitis.⁶⁴ Although there are not yet well-characterized common mechanisms/pathways to explain the possible link among EoE, celiac disease and IBD, the interplay among innate immunity, regulatory T cell response, antigen-driven adaptive immune reactions and microbiome-derived inflammation likely plays a role in the development of these clinical conditions. Further studies are needed.

Overlapping features between chronic FPIES and FPE/Eosinophilic gastroenteritis.

Chronic FPIES is difficult to differentiate from FPE and/or eosinophilic gastritis/gastroenteritis (Table 2).⁶⁵ Chronic FPIES usually has progressive vomiting and bloody stools if the offending food is not eliminated. The predominant symptom of FPIAP is bloody stool. The typical symptoms of FPE are chronic diarrhea with malabsorption and steatorrhea, but blood in stool is rarely noted.⁴³ As for eosinophilic gastroenteritis, food intake is not acutely associated with symptoms, and eosinophil infiltration of the mucosa is necessary for the diagnosis of eosinophilic gastritis/gastroenteritis. Our group revealed that 23.2% of children with FPIES had a past history of FPIAP.⁹ Further research is needed to investigate the precise relationship between chronic FPIES, FPIAP, FPE, and eosinophilic gastroenteritis.

Conclusion

The dramatic rise in the prevalence of non-IgE-mediated food allergies over the past few decades cannot be explained by genetic factors alone.¹ Environmental and diet changes have modified the microbiome of our skin, respiratory, and intestinal mucosa. As the proposed model in this review, these changes to our intestinal microbiome may favor the increased production of serotonin, with the subsequent consequence to our gut motility and feeding behavior. The original proper immune responses to noxious substances and parasites have turned increasingly to react to the foods we need.

The basic immune responses of most non-IgE-mediated food allergic diseases are still not clearly understood. Even though eosinophils are widely identified in those diseases, their role as a key player or as a late responder to the exaggerated immune response requires further research. FPIES, FPIAP, and other non-IgE-mediated food allergic diseases behave as antigen-specific diseases. Even though MAIT cells, iNKT, or

antigen-specific Treg are potential candidates, the primary sensor(s) of allergen is(are) still unknown. More research about the immunopathogenesis of non-IgE-mediated food allergy is urgently needed.

Finally, non-IgE mediated food allergy likely shares disease mechanisms with other atopic and mucosal inflammatory conditions. The original atopic march refers to the natural history of allergic disease, initially presented as atopic dermatitis, followed by asthma and allergic rhinitis. “One airway, one disease” suggests the close association of allergic rhinitis and asthma. However, increasing evidence suggests that the trivial immune change of the gut also contributes substantially to respiratory allergic diseases. EoE, an allergic inflammatory disease limited to the esophagus, has been suggested as a late manifestation of the atopic march. We hypothesize that allergic inflammation within the mucosa of either the respiratory tract or esopaphiogastrointestinal tract is mechanistically related. The overlap of common diseases and the additional associations of these disorders with celiac disease and IBD further suggest that common pathogenic mechanisms of mucosal inflammation likely exist. The common genetic or epigenetic background is another possible link between those diseases. Gut microbiome association and mucosa innate immune responses are likely at the center of future research in understanding the mechanisms of FPIES, FPIAP, and other non-IgE mediated food allergies. Insight into the mechanisms of these disease entities will help with developing strategies to prevent allergic inflammation of all the mucosa, rather than treat them as separated diseases.

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Table 1. Pathological and Immunological Characteristics of Non-IgE-mediated Food Allergic Diseases

	FPIES	FPIAP	FPE	EoE
Pathological findings	<u>Upper endoscopic findings:</u> Gastric edema, erythema, and mucosal friability, and erosion in vomiting and failure-to-thrive infants. <u>Colonoscopic findings:</u> Decreased vascularity, friability, ulceration, and occasional crypt abscess in those with rectal bleeding	<u>Colonoscopic findings:</u> Focal mucosal erythema, friability, ulceration, lymphoid nodular hyperplasia, ecchymosis.	<u>Endoscopic findings:</u> Villous atrophy and crypt hyperplasia on duodenal and jejunal biopsy	<u>Endoscopic findings:</u> Decreased vasculature, white plaques, linear furrows, stricture, ring formation. <u>Pathological findings:</u> Esophageal biopsy ≥ 15 eosinophils/hpf
Cytokine milieu	<u>From mucosal biopsy:</u> $\uparrow$ TNF-α $\downarrow$ TGF-β receptor <u>From serum:</u> $\uparrow$ IL-10 $\uparrow$ IP-10 (CXCL-10) $\downarrow$ Latent TGF-β produced by PBMCs in response to food protein	<u>From colonic biopsy:</u> $\uparrow$ CXCL13 (B cell attractant) $\uparrow$ CCL11 (eotaxin-1) $\uparrow$ IL-6 <u>From sera:</u> $\downarrow$ Interferon-γ /IL-4 ratio	<u>From jejunal biopsy:</u> $\uparrow$ IFN-γ and IL-4 in jejunal biopsy <u>From duodenal biopsy:</u> $\uparrow$ eosinophil granule major basic proteins	$\uparrow$ IL-4 $\uparrow$ IL-5 $\uparrow$ IL-13 $\uparrow$ CCL26 (eotaxin-3)
Cellular changes	$\uparrow$ Eosinophils in intestinal biopsies, blood, and stool $\uparrow$ Lymphocytes, plasma cells, polymorphonuclear leukocytes in those with rectal bleeding.	<u>From colonic biopsies:</u> $\uparrow$ CD3+ cells $\uparrow$ Eosinophils (≥ 5 eosinophils/hpf), Neutrophil, lymphocytes. <u>From sera:</u>	<u>From jejunal biopsy:</u> $\uparrow$ Lymphocytes, plasma cells, eosinophils, activated CD4+ cells,	$\uparrow$ Eosinophils $\uparrow$ Mast cells $\uparrow$ Th2 cells $\uparrow$ iNKT $\uparrow$ ILC2

	After a positive OFC, innate immune cells were activated. Innate immune gene and Th17-related gene were upregulated.	↑ Eosinophils ↓ T regulatory cells ↑ Ratio of naïve to memory T cells CD4+ cells shifted toward Th2	<u>From duodenal biopsy</u> : ↑ Cytotoxic lymphocytes ↑ IL-13 secreting lymphocytes	
Immunoglobulin	↓ Food-specific levels of IgG, IgG4, and IgA in peripheral blood • 2-12% patients have coexisting IgE-mediated food allergy or sensitization.	↑ IgA-bearing cell in mucosal biopsies No evidence for role of IgE	From serum: ↑ Food-specific IgA From jejunal biopsy: ↑ IgA-and IgM-containing cells No association with IgE	↑ IgG4, as well as other subtypes of IgG ↑ IgA ↑ IgM

Adapted and modified from Feuille et al. Curr Allergy Asthma Rep (2015)15:50.

Table 2. Comparison of chronic FPIES, FPE, and eosinophilic gastrointestinal disease

	Chronic FPIES	FPE	Eosinophilic gastrointestinal disease
Symptoms			
Vomiting	Intermittent but progressive	Intermittent	Intermittent
Diarrhea	Moderate	Severe	Moderate
Bloody stool	Occasional	Rare	Sometimes
Failure to thrive	In severe form	Moderate	Occasional
Laboratory findings			
Anemia	Moderate	Moderate	Mild-to-moderate
Hypoalbuminemia	In severe form	Moderate	Mild-to-severe
Methemoglobinemia	Occasional in severe form	No	No
Peripheral blood eosinophilia	No	No	Present
Stool occult blood	Frequently	No	Sometimes
Malabsorption/Stool reducing substances	No	Yes	No
Food intake association	Yes	Yes	No
Diagnosis	Vomiting and diarrhea subsided after triggering food elimination. Acute FPIES symptoms (delayed vomiting with lethargy and pallor) developed after triggering food reintroduction.	Patients with malnutrition and malabsorption with villous atrophy and crypt hyperplasia in biopsied samples.	Biopsy demonstrated increased eosinophils in the samples.

Central Nervous System:

- Food aversion
- Nausea
- Vomiting

Spore-Forming Bacteria

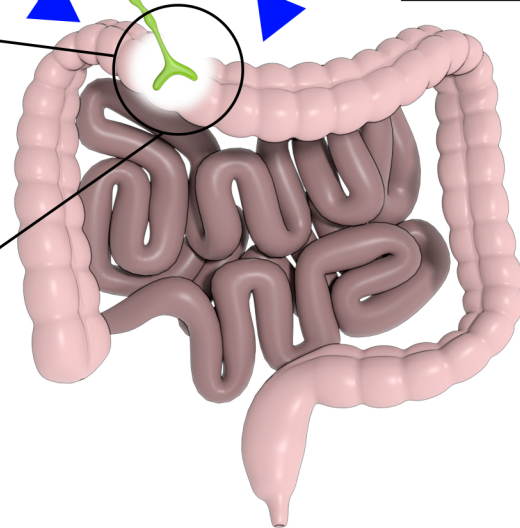
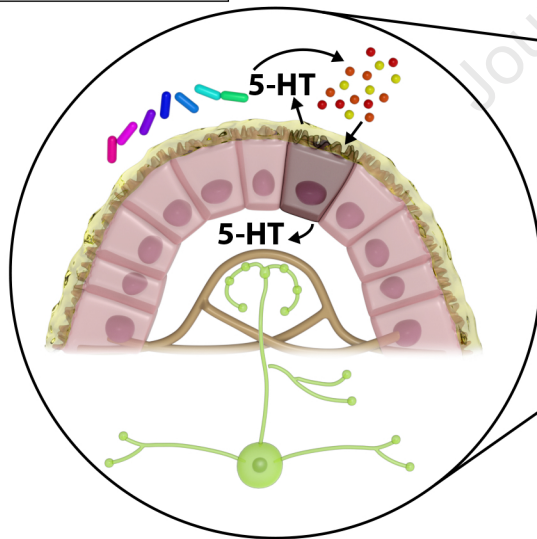
↑ Increase serotonin

SEROTONIN

Vagus
Nerve

Enteric Nervous System:

- Peristalsis
- Secretion of intestinal glands



		Primary Diagnosis					
		FPIAP	AD	IgE-FA	Asthma	AR	EoE
Secondary Diagnosis	IgE-FA	2.3	2.5	–	–	–	–
	Asthma	–	1.5	1.5	–	–	–
	AR	–	1.9	1.7	1.7	–	2.5
	EoE	5.0	3.2	9.7	1.9	2.8	–

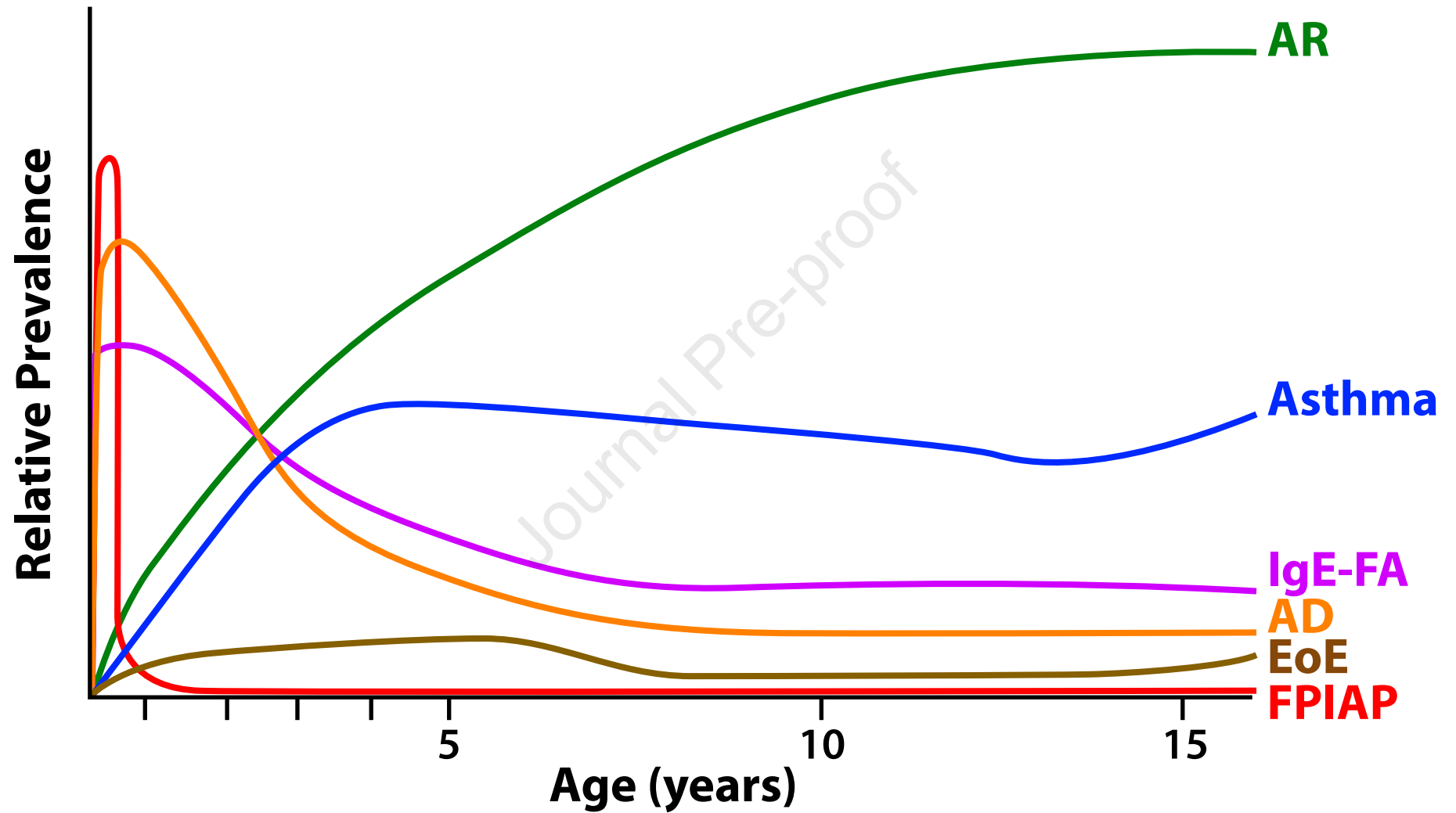


Figure 1. Serotonin might be the key mediator participating in the FPIES and non-IgE-mediated food allergic diseases. Intestinal dysbiosis increased the production of serotonin in the enterochromaffin cells or sensory enteroendocrine cells. And then, serotonin affects the peristalsis in the intestine and induced nausea, vomiting, and food aversion in the central nerve system. FPIES: Food protein-induced enterocolitis syndrome; IgE: immunoglobulin E.

Figure 2. The hazard ratio matrix demonstrated the effect of primary allergic diagnosis on the likelihood of secondary allergic diagnosis in children. AD: Atopic dermatitis; IgE-FA: immunoglobulin E-mediated food allergy; EoE: Eosinophilic esophagitis; AR: Allergic rhinitis; FPIAP: Food protein-induced allergic proctocolitis. The hazard ratio presented in this figure were adapted from Martin *et al.* 2020^{44,53}, Hill *et al.* 2018⁵⁴, and Radano *et al.* 2014⁵⁵.

Figure 3. The proposed new atopic march including IgE-mediated atopic disease and non-IgE-mediated food allergy. Food protein-induced allergic proctocolitis (FPIAP) is the earliest manifestation in the atopic march, and eosinophilic esophagitis (EoE) is the late manifestation in the march. AR: Allergic rhinitis; AD: Atopic dermatitis.

Key Messages:

- Non-IgE-mediated food allergic diseases are part of atopic march, indicating “one mucosa, one disease”.
- Not only Th2 immune responses, Th1, Th17, innate immunity, and epithelial mucosal barrier defect are important for immunopathogenesis.
- Serotonin, a key mediator of the gut-brain axis, plays a role in the pathogenesis of FPIES and other non-IgE-mediated food allergic diseases.
- Eosinophils, widely identified in FPIES and non-IgE-mediated food allergic disease, are key amplifiers of type 2 immune responses or late-recruited cells for repair, which require further researches.
- Cells sensing food allergen and further intestinal microbiome researches are required.