

## Mechanisms of allergic diseases

Series editors: Joshua A. Boyce, MD, Fred Finkelman, MD, and William T. Shearer, MD, PhD

# Immunopathophysiology of food protein–induced enterocolitis syndrome

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There is increasing recognition of the non-IgE-mediated gastrointestinal food allergy known as food protein–induced enterocolitis syndrome (FPIES), with several recent publications summarizing the clinical experience with FPIES in the United States, the United Kingdom, Europe, and Australia. Our understanding of the mechanisms linking food exposure to typical symptoms of vomiting, hypotension, and diarrhea has lagged far behind our understanding of the immune mechanisms of IgE-mediated food allergy. The goal of this overview is to summarize and critique the current state of knowledge of the immunology of FPIES and to identify major gaps in our knowledge that need to be addressed to make significant gains in developing therapies and prevention strategies for FPIES. (*J Allergy Clin Immunol* 2015;■■■:■■■-■■■.)

**Key words:** Allergy, tolerance, vomiting, enteropathy, milk, lymphocytes, serotonin

Food allergy is an umbrella term for any immunologically mediated adverse reaction that occurs reproducibly after exposure to a given food,<sup>1</sup> including both IgE-mediated and non-IgE-mediated reactions. Within the non-IgE-mediated food allergies are the eosinophilic gastrointestinal disorders triggered by food: food protein–induced enterocolitis syndrome (FPIES) and food protein–induced proctocolitis. IgE-mediated food allergy and eosinophilic esophagitis are now well-described entities, with robust clinical and basic science research programs ongoing to understand the immune mechanisms of these disorders.<sup>2,3</sup> In contrast, our understanding of FPIES and proctocolitis falls far behind our understanding of other food-induced allergic disorders. In the past 2 years, there have been publications from 3 centers describing their extensive clinical experience with FPIES,<sup>4-6</sup> and in this issue Nowak-Węgrzyn<sup>7</sup> provides a comprehensive

### Abbreviations used

BLG:  $\beta$ -Lactoglobulin

FPIES: Food protein–induced enterocolitis syndrome

NK: Natural killer

review of the clinical features of FPIES. The purpose of this review is to assess the state of our knowledge of the immune mechanisms of FPIES.

## ACUTE VERSUS CHRONIC REACTIONS TO FOODS IN PATIENTS WITH FPIES

Reactions of profuse vomiting and lethargy occurring within 2 to 4 hours of food ingestion are commonly termed acute FPIES,<sup>8,9</sup> whereas a subacute or chronic form of FPIES has also been described in association with ongoing antigen exposure.<sup>6,10,11</sup> In the late 1960s and early 1970s, several groups described their experience with cohorts of infants presenting with a pattern of adverse reactions, primarily to milk, that they termed cow's milk intolerance, malabsorption syndrome associated with milk intolerance, cow's milk allergy, or cow's milk enteropathy.<sup>8,10,12-14</sup> These infants presented with vomiting, diarrhea, and failure to thrive. Symptoms resolved on a milk elimination diet and recurred on challenge, and infants commonly outgrew their adverse reactions to milk within the first year of life.

In many of these early studies, biopsy specimens were obtained at the time of admission during chronic milk exposure, after a milk elimination diet when symptoms resolved, and after milk challenge when symptoms recurred. These studies demonstrated marked changes in *intestinal architecture* that were triggered by milk in the diet.<sup>12,13,15</sup> These changes in intestinal architecture have led to comparisons with celiac disease; however, there is little to suggest that the pathophysiologic mechanisms are similar between the 2 diseases. In the Finnish FPIES cohorts 2 patterns based on the kinetics of symptoms after milk challenge were described: approximately half of the patients experienced an early response of vomiting and diarrhea appearing within 24 hours of milk challenge, and half had a delayed response appearing only after chronic milk ingestion.<sup>10</sup> Infants were described as progressing from an early response to a delayed response with age as they outgrew their adverse reactions to milk.<sup>15</sup> Recent descriptions of clinical experience with cohorts of FPIES (2 in the United States, 1 in the United Kingdom, 1 in Italy, and 1 in Australia) report only rare instances of delayed or chronic FPIES.<sup>4-6,16,17</sup> Much of our understanding of the gastrointestinal

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Terms in boldface and italics are defined in the glossary on page ■■■.

immune events during FPIES is derived from studies on milk enteropathy; it is not clear whether similar mechanisms might be at play in acute FPIES reactions.

## ALLERGENS INDUCING FPIES OR MILK ENTEROPATHY

FPIES is presumed to be caused by the protein component of foods, similar to IgE-mediated food allergy. This is supported by reports in which children with FPIES or enteropathy were challenged with purified milk allergens. Freier et al<sup>14</sup> described 6 infants with milk-induced enteropathy whose symptoms improved on a milk elimination diet. These infants were subsequently challenged with purified milk allergens (*caseins*,  *$\beta$ -lactoglobulin* [BLG],  *$\alpha$ -lactoglobulin*, and BSA), with the finding that BLG was the only antigen that induced acute symptoms in the majority of infants (5/6) at a dose present in 30 mL of milk. In a cohort described by Kuitunen et al,<sup>10</sup> the majority of infants challenged with purified milk allergens reacted to both casein and BLG.

Despite the differing findings regarding the role of casein in these 2 cohorts, these findings support the concept that adverse reactions are in fact triggered by the protein fraction of milk. In the case series by Freier et al,<sup>14</sup> infants were described as reacting to BLG that had been boiled for 70 minutes, indicating that *denaturing* of the protein did not reduce its immunogenicity. In contrast, the majority of children with IgE-mediated food allergy can tolerate extensively heated milk.<sup>18</sup> This suggests that recognition by the immune system of *conformational epitopes* is not playing a role in FPIES. Threshold doses of foods causing symptoms in patients with FPIES are generally in the gram range<sup>16</sup>; in patients with IgE-mediated food allergy, these doses are in the microgram to milligram range.<sup>19</sup> However,

there have been case reports of children with active FPIES induced by casein *hydrolysate formula*<sup>20</sup> or reacting to antigens transmitted through breast milk,<sup>11,21,22</sup> showing that low-abundance antigen can infrequently trigger symptoms.

FPIES can be triggered by foods other than milk, although cow's milk is the most common cause. Many of the foods that trigger FPIES reactions are also IgE-mediated food allergens, including soy, fish, wheat, and egg.<sup>4,5</sup> In adults shellfish have been described to cause non-IgE-mediated reactions consistent with FPIES.<sup>23</sup> However, other foods reported to trigger FPIES are not common IgE-mediated food allergens. Rice is the third most common cause of FPIES in US cohorts, followed by oats.<sup>4,5</sup> Banana, sweet potato, and green peas have all been described as triggers of FPIES, as have meats, including chicken and beef. This list indicates that many common foods have the potential to induce FPIES when introduced to susceptible infants. This is in sharp contrast to celiac disease, in which pathology is triggered by a well-defined antigen (*gluten*) found in a restricted subset of foods. Although FPIES can be induced by a very broad spectrum of foods, there is selective food recognition on an individual basis, and children can be reactive to 1, 2, or multiple foods. A positive food challenge reaction to soy reduces subsequent reactions to soy challenge, whereas a positive food challenge reaction to milk does not affect a subsequent reaction to soy.<sup>24</sup> Thus there is clear specificity in the response to foods. This feature of recognition, combined with the common resolution of FPIES with age, suggests that FPIES, like IgE-mediated food allergy, is an inappropriate adaptive immune response to foods.

Furthermore, the timing of adverse reaction onset is consistent with the time required to generate an adaptive immune response. Symptoms to cow's milk typically begin within 2 weeks of

## GLOSSARY

**CASEINS,  $\beta$ -LACTOGLOBULIN,  $\alpha$ -LACTOGLOBULIN:** Caseins make up about 80% of the proteins in cow's milk.  $\beta$ -Lactoglobulin and  $\alpha$ -lactoglobulin are whey proteins.

**CONFORMATIONAL EPIOTOPE:** Nonsequential amino acid residues that become spatially juxtaposed in a folded protein form an available surface on a molecule that is recognized by an antibody.

**CYTOTOXIC GRANULES:** A principal component of cytotoxic T-lymphocyte effector function. The major components of cytotoxic granules are perforin and granzymes. Perforin disrupts target cell membranes. Granzymes are serine proteases that can cleave substrates or induce protease cascades to promote apoptosis.

**DENATURING:** Modifying the molecular structure of a protein, especially by using heat, acid, alkali, or UV radiation, so as to destroy or diminish some of the original properties.

**GLUTEN:** A water-insoluble storage protein moiety of certain cereal grains, including wheat, barley, and rye. Storage proteins (gluten) in wheat are composed of gliadin and glutenin.

**HOMING RECEPTOR:** Adhesion molecules expressed on lymphocytes. Gut-homing T cells express the  $\alpha 4\beta 7$  integrin, which binds to mucosal addressin cell adhesion molecule 1 on gut endothelial cells, and CCR9, a chemokine receptor that binds to the chemokine CCL25 expressed by intestinal epithelial cells. A subset of T cells (intraepithelial lymphocytes) express an integrin (CD103) that allows binding to E-cadherin on the intestinal epithelium.

**HYDROLYSATE FORMULA:** A formula that has been exposed to either enzymatic or chemical treatments designed to reduce proteins to small

peptides. The goal of hydrolysis is to alter the functional properties of ingredients. Hydrolysis reduces IgE reactivity but might not eliminate it completely.

**INTESTINAL ARCHITECTURE:** In general, there are 5 layers to the small intestinal wall: mucosa, submucosa, circular muscularis, longitudinal muscularis, and serosa. The mucosal surface contains finger-like projections called villi, and the epithelial cells lining the mucosa contain microvilli, which enhance the absorptive surface of the intestine. In addition to absorptive columnar epithelial cells, secretory epithelial cells, including goblet cells, Paneth cells, and enterochromaffin cells, are found within the epithelial layer. The lamina propria is the layer beneath the epithelium and contains connective tissue, lymphocytes, plasma cells, macrophages, dendritic cells, mast cells, and eosinophils.

**INVARIANT NATURAL KILLER (NK) T CELLS:** A subset of lymphocytes that express surface molecules characteristic of both NK and T cells. NKT cells recognize self-lipids and foreign lipids bound to CD1, a class I MHC-like molecule. They are capable of rapidly secreting cytokines after stimulation. The T-cell receptor  $\alpha$  chains in invariant NKT cells have limited diversity and are characterized by a unique V $\alpha$ 24-J $\alpha$ 18 rearrangement.

**TGF- $\beta$ :** A regulatory cytokine produced by stromal and hematopoietic cells. TGF- $\beta$  inhibits proliferation of T and B cells, inhibits activation of macrophages, induces IgA production by B cells through isotype switching, and increases collagen synthesis from fibroblasts to promote tissue repair. Regulatory T-cell production of TGF- $\beta$  can block T<sub>H</sub>1 and T<sub>H</sub>2 development in CD4<sup>+</sup> T cells.

introduction. A switch to soy formula is initially tolerated but can result in symptoms again within approximately 2 weeks of exposure.<sup>8</sup> Although it is reasonable to assume that these adverse reactions are immune mediated, it has been difficult to identify a causative immune mechanism of FPIES that is consistent with the nature of the symptoms.

## GASTROINTESTINAL INFLAMMATION IN PATIENTS WITH FPIES OR ENTEROPATHY

Because biopsy is not required for the diagnosis or management of FPIES, our information about immune events in the gastrointestinal mucosa during FPIES reactions is limited primarily to early reports of chronic allergen exposure and rechallenge. A consistent finding of these reports is shortening of the villi of the jejunum (partial villous atrophy) while consuming milk, which is the basis of the malabsorption.<sup>12,13,15</sup> Maintenance of a milk avoidance diet results in normalization of the intestinal architecture, and milk challenge results in a recurrence of partial villous atrophy. This histologic finding has led to comparisons with celiac disease, but there are several important distinctions between the 2 diseases that suggest unique mechanisms of pathophysiology.

In addition to villous blunting, a number of inflammatory changes have been documented in the gastrointestinal tract after food challenge. Milk exposure leads to increased numbers of intraepithelial lymphocytes, including an increase in those expressing TIA1, a marker of *cytotoxic granules*.<sup>25</sup> There is also an increase in numbers of CD4<sup>+</sup> T cells in the lamina propria bearing the activation marker HLA-DR.<sup>26</sup> Plasma cells producing IgM and IgA are increased in number during milk exposure, as are eosinophils.<sup>12,13</sup> Smears of fecal mucus from positive challenge results reveal the presence of neutrophils, eosinophils, lymphocytes, and other unidentified mononuclear cells.<sup>8</sup> Fecal extracts have been studied after milk challenge in patients with milk-induced enteropathy and are associated with an increase in total IgM and IgA levels<sup>13</sup> and an increase in eosinophil-derived neurotoxin levels.<sup>27</sup> Eosinophils are resident cells of the small intestine. It is not clear whether measurement of eosinophil-derived neurotoxin in the fecal extracts is due to reduced barrier function, increased eosinophil degranulation, or both. In patients with celiac disease, villous blunting occurs because of killing of epithelial cells by cytotoxic T cells expressing natural killer (NK) receptors that kill epithelial cells expressing increased levels of nonclassical class I molecules.<sup>28</sup> Gluten functions as a trigger by activating gluten-specific CD4<sup>+</sup> T cells that recognize modified gluten peptides with high affinity, inducing the release of cytokines that facilitate the cytotoxic activity of intraepithelial lymphocytes and upregulate stress molecules on epithelial cells. Thus celiac disease is thought to be a food antigen-triggered autoimmunity, and genome-wide association studies have identified multiple susceptibility genes common to celiac disease and other autoimmune diseases.<sup>29</sup> There is a lack of evidence for an autoimmune component in FPIES, but there is an association between FPIES and atopy.<sup>4,5</sup>

A reduction in the expression of *TGF-β* receptor 1 on the intestinal epithelium (but not TGF-β) has been described in the mucosa of children with FPIES compared with that seen in control children and correlates with the degree of villous atrophy.<sup>30</sup> Although it is interesting to speculate that this might reflect a reduced regulatory tone in the intestines of patients with FPIES,

this might be secondary to altered turnover or differentiation state of the epithelial cells in the shortened villi rather than being a primary event in pathogenesis. Increased mucosal expression of TNF-α detected by using immunohistochemistry was also described by the authors.<sup>30</sup> Sequential analysis of fecal samples from an infant with milk-induced FPIES demonstrated an increased TNF-α level in the stool during chronic milk exposure but, interestingly, not in response to acute challenge.<sup>31</sup>

## EFFECT OF FOOD CHALLENGE ON INNATE CELLS IN CIRCULATION

In addition to local inflammatory changes in the gastrointestinal tract, food challenge in patients with FPIES results in acute changes in circulating leukocytes, most prominently an increase in circulating neutrophil numbers, which is one of the diagnostic criteria for a positive food challenge result.<sup>8,9</sup> An increase in platelet numbers<sup>17</sup> and a decrease in eosinophil numbers in the circulation<sup>4</sup> after a positive food challenge result have also been described. Circulating neutrophil levels peak at 6 hours after challenge, which is after symptom onset, but the role of these innate cell changes in the pathophysiology of FPIES remains unclear.

## HUMORAL IMMUNE PROFILE OF FPIES

Although FPIES is a non-IgE-mediated food allergy, many patients with FPIES have low IgE levels against the food, which was labeled “atypical FPIES” by Sicherer et al.<sup>9</sup> It has been reported that 30% of Japanese patients with symptoms consistent with FPIES have IgE against the causative food.<sup>32,33</sup> The presence of IgE to the triggering food was recently described as a marker of persistent FPIES by Caubet et al.<sup>4</sup> Furthermore, patients with resolved milk-induced FPIES have been reported to have IgE-mediated reactions to milk.<sup>4</sup> It has been speculated that there might be local production of IgE in the intestinal mucosa that does not reach the serum but participates in reactions to the food. Rosekrans et al.<sup>34</sup> reported a dramatic increase in numbers of IgE<sup>+</sup> cells in the lamina propria of infants with milk-induced enteropathy, and this increase was not reduced by an elimination diet or increased by allergen challenge. This increase in IgE levels was not replicated by Savilahti,<sup>13</sup> who did not find IgE<sup>+</sup> cells in the jejunum of infants with milk enteropathy but did report an increase in IgM<sup>+</sup> and IgA<sup>+</sup> cells after milk challenge. A decrease in intact mast cell numbers (suggesting degranulation) was reported after milk challenge in infants with milk enteropathy,<sup>35</sup> although this was also observed in patients with celiac disease, in whom mast cells are not thought to be important contributors to pathology. Thus this mast cell degranulation might be a nonspecific feature of intestinal inflammation. Nonetheless, the link between food-specific IgE and the persistence of FPIES is an important factor that requires further investigation.

Early descriptions of cow's milk-associated malabsorption and villous blunting identified an increase in IgM and IgA plasma cell numbers in the lamina propria, an increase in IgM and IgA levels in fecal extracts, and an increase in cow's milk-specific antibody levels in serum after allergen challenge.<sup>13</sup> These changes were not observed in those without symptoms. Because IgM and IgA are primarily neutralizing mucosal antibodies, this increase might be a response to a breakdown of the epithelial barrier associated with allergen-induced inflammation and function as an immunologic barrier to prevent the influx of antigens from the

gastrointestinal lumen. McDonald et al<sup>36</sup> studied food-specific immunoglobulin levels in a cohort of 18 children undergoing food challenge for FPIES. Levels of milk-, egg-, and soy-specific IgA antibodies were increased in infants who reacted to a food challenge compared with those who passed a food challenge. Egg- and soy-specific IgG antibody levels were also increased in those who reacted compared with those who did not.

In contrast with these reports of increased immunoglobulin levels, a significant suppression of milk-specific immunoglobulins has been observed in patients with FPIES. Shek et al<sup>37</sup> observed that patients with milk-induced FPIES had significantly lower milk-specific IgG<sub>4</sub> levels than healthy control subjects, patients with IgE-mediated milk allergy, or patients with allergic eosinophilic gastroenteritis. Konstantinou et al<sup>38</sup> examined casein-specific IgA responses in patients with milk-induced FPIES, outgrown milk-induced FPIES, non-milk-induced FPIES, and IgE-mediated milk allergy. Although casein-specific IgA level were significantly suppressed in patients with milk-induced FPIES compared with those in both control subjects and patients with non-milk-induced FPIES, there was no difference between those with active versus outgrown milk-induced FPIES. This lack of consistent association of food-specific antibody levels and clinical symptoms indicates that food-specific antibodies are unlikely to play a key role in the pathogenesis of FPIES unless there is a qualitative rather than a quantitative difference in food-specific immunoglobulin levels.

## CELLULAR IMMUNITY IN PATIENTS WITH FPIES

FPIES is often described as a cell-mediated response to food proteins. Food-specific proliferation of lymphocytes has been documented in patients with FPIES,<sup>39</sup> although Hoffman et al<sup>40</sup> found that proliferation responses to milk were not significantly different in patients with milk-induced FPIES compared with those in control subjects. The same study showed a significant increase in lymphocyte proliferation in patients with IgE-mediated milk allergy. Heyman et al<sup>41</sup> published an oft-cited study showing that PBMCs from children with milk enteropathy produced more TNF- $\alpha$  than those from control subjects or those with outgrown milk enteropathy when stimulated with milk proteins. Although the increase in TNF- $\alpha$  levels was relatively modest (<2-fold difference between those with active vs outgrown milk enteropathy after 5 days of milk restimulation), treatment of intestinal epithelial monolayers with supernatants from milk-restimulated PBMCs showed a dramatic effect on epithelial permeability to macromolecules. Supernatants from PBMCs of patients with active milk enteropathy, but not outgrown milk enteropathy, induced a significant increase in flux of the tracer horseradish peroxidase across epithelial monolayers. This was proposed to be due to TNF- $\alpha$ , although neutralization experiments with anti-TNF- $\alpha$  antibodies showed quite modest effects. The unique strengths of this study included the use of a functional readout (disruption of epithelial barrier) of lymphocyte activation and a comparison of those with active versus outgrown milk allergy. A different kinetic pattern of TNF- $\alpha$  production was also observed by the same authors in children with different manifestations of milk allergy (gastrointestinal versus cutaneous symptoms), again supporting a role of TNF- $\alpha$  in disease manifestations.<sup>42</sup> Subjects with IgE-mediated and eosinophilic gastroenteritis also have allergen-specific lymphocytes producing TNF- $\alpha$ .<sup>43</sup> It is difficult to understand how a modest increase in

TNF- $\alpha$ -producing cell numbers could lead to such unique and severe symptoms in patients with FPIES, although a systemic release of TNF- $\alpha$  would be consistent with shock-like symptoms. A systemic increase in circulating TNF- $\alpha$  levels during acute FPIES reactions has not been described.

Morita et al<sup>44</sup> recently published a comprehensive analysis of T-cell cytokine responses in 65 children with non-IgE-mediated milk allergies (comprised of a mix of patients with symptoms consistent with FPIES, food protein-induced enteropathy, and food protein-induced proctocolitis). Patients were compared with control subjects and those with IgE-mediated allergy to milk. Those with non-IgE-mediated gastrointestinal allergies to milk produced significantly more TNF- $\alpha$ , IL-2, IL-3, IL-5, IL-6, IL-10, and IL-13 compared with control subjects, whereas IFN- $\gamma$  and IL-17 levels were not increased. Proliferative responses were noted to  $\alpha$ -,  $\beta$ -, and  $\kappa$ -casein and BLG, with minimal proliferative responses to  $\alpha$ -lactalbumin. There was overlap between PBMC responses in those with IgE-mediated food allergy, which again begs the question of how this T-cell response might contribute to the unique spectrum of symptoms observed in patients with FPIES. It is possible that differences in *homing receptor* expression might explain differences in allergic manifestations by directing these cells to the gastrointestinal mucosa. It would be of interest to determine how these T-cell responses compare in those with active versus outgrown reactivity to identify which, if any, of these T-cell responses correlate with clinical reactivity. Is this lymphocyte profile causative in patients with FPIES or related to the association of FPIES with atopy?

The observation that FPIES commonly develops to the first food introduced into the diet or that adverse reactions can develop to such a wide range of foods that are not typically allergenic suggests there might be a regulatory defect in patients with FPIES. The role of regulatory T cells has not yet been addressed in patients with FPIES, although it has been reported that resolution of non-IgE-mediated milk allergy is associated with an expansion of milk-specific regulatory T cells detectable after milk challenge.<sup>45</sup>

## GAPS IN THE UNDERSTANDING OF THE IMMUNE MECHANISMS OF FPIES

The possibility that antigen-specific cytokine-producing T lymphocytes can produce the intestinal architecture changes observed during chronic FPIES is consistent with our knowledge of mucosal immunology. Landmark studies by MacDonald and Spencer<sup>46</sup> showed that polyclonal activation of T cells in human fetal gut explants led to crypt hyperplasia and villous atrophy, and mouse studies have confirmed that polyclonal T-cell activation *in vivo* can result in intestinal damage and diarrhea through TNF- $\alpha$ -dependent mechanisms.<sup>47</sup> However, the presence and phenotype of food-specific T cells in the intestinal mucosa of patients with FPIES needs to be demonstrated, as has been done in patients with celiac disease. Access to gut tissues in this patient group might not be feasible because biopsy specimens are not required for clinical care. Alternatively, detection of allergen-specific T cells in peripheral blood with a phenotype that can explain the unique gastrointestinal manifestations of disease observed in patients with FPIES needs to be demonstrated. There is currently a lack of any solid data to support the hypothesis that FPIES is mediated by allergen-specific T cells.

There is even less evidence available to explain the immune basis of acute FPIES reactions. In a case series described by Freier

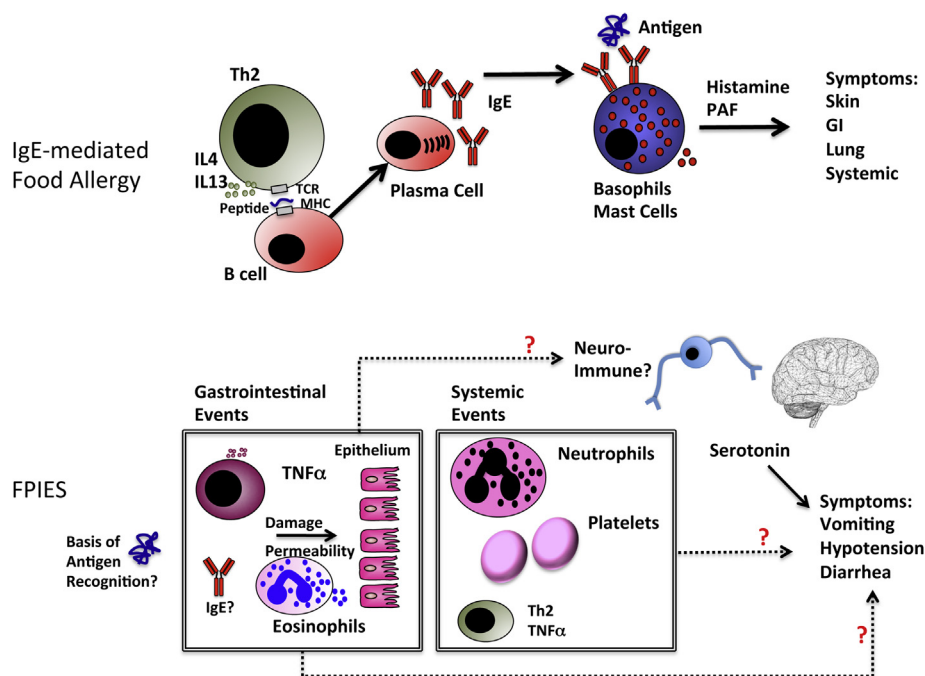


**FIG 1.** Immune mechanisms of FPIES in comparison with IgE-mediated food allergy. The general sequence of immune events from first exposure to re-exposure leading to symptoms in patients with IgE-mediated food allergy is understood. In contrast, although there have been many observations about gastrointestinal and systemic immune events associated with FPIES as depicted, there is no clear understanding of how these immune events relate to symptoms of vomiting, hypotension, and diarrhea. Red question marks indicate uncertainty. To date, only serotonin has been clearly implicated in symptoms of FPIES, although it is not clear whether serotonin is involved as a peripheral trigger of reactions or is restricted to central control of the vomiting reflex. *GI*, Gastrointestinal; *PAF*, platelet-activating factor; *TCR*, T-cell receptor.

et al,<sup>14</sup> milk challenge administered by enema resulted in diarrhea and weight loss, whereas drinking the milk induced vomiting, pallor, and diarrhea in the same infant. Thus the chronic and acute manifestations of FPIES can be triggered at different sites along the gastrointestinal tract. Vomiting is triggered by chemosensors or mechanosensors in the upper gastrointestinal tract. For example, enterochromaffin cells of the gastrointestinal tract release serotonin that can activate the vagus nerve and trigger the vomiting reflex. Treatment of patients with the serotonin 5-HT<sub>3</sub> receptor antagonist ondansetron effectively suppresses vomiting triggered by FPIES challenge.<sup>48,49</sup> Questions remain. How could a chemosensor cell, such as an enterochromaffin cell, in the gut recognize specific foods? What is the nature of a possible neuroendocrine-immune communication leading to acute symptoms of FPIES?

In addition to these fundamental questions about how immune activation can lead to symptoms observed in patients with FPIES (Fig 1), there are several areas that should be considered. Is processing of the allergen required to trigger symptoms? For example, deamidation of gluten peptides is an essential step in celiac disease pathogenesis, leading to high-affinity T-cell receptor (TCR) binding, and it is possible that we have not yet observed a distinct T-cell phenotype in patients with FPIES because the allergens need to be modified by the digestive tract to be pathogenic. The role of innate cells in antigen recognition should be considered. For example, *invariant NKT cells* are activated by milk sphingolipids in patients with IgE-mediated food allergy and eosinophilic esophagitis<sup>50,51</sup>; a similar recognition might be at work in patients with FPIES. NK cells residing in the liver have

been shown to acquire features of antigen-specific memory in mice.<sup>52</sup> Unfortunately, there is a lack of a suitable animal model for FPIES. Mice and rats do not vomit, and it is not clear whether models relying on chronic antigen exposure to generate villous atrophy will provide answers to the pathogenesis of acute FPIES. In the absence of a robust animal model, we need to look to the patients to answer these fundamental questions about the elusive immune mechanisms underlying non-IgE-mediated reactions to foods.

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