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Immunopathophysiology of Food Protein-Induced Enterocolitis Syndrome

M. Cecilia Berin, PhD

Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY

Abstract

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 US, the UK, 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 in order to make significant gains in developing therapies and prevention strategies for FPIES.

Keywords

Allergy; tolerance; vomiting; enteropathy; milk; lymphocytes; serotonin

Introduction

Food allergy is an umbrella term for any immunologically-mediated adverse reaction that occurs reproducibly after exposure to a given food ¹. This includes both IgE-mediated and non-IgE-mediated reactions to foods. 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 immune mechanisms of these disorders ^{2, 3}. In contrast, understanding of FPIES and proctocolitis fall far behind the other food allergic disorders. In the past 2 years, there have been publications from 3 centers describing their extensive clinical experience with FPIES ⁴⁻⁶, and in this issue, Dr. Nowak-Węgrzyn provides a comprehensive review of the clinical features of FPIES. The purpose of

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Address correspondence to: Cecilia Berin One Gustave L. Levy Place, Box 1198 Pediatric Allergy and Immunology Icahn School of Medicine at Mount Sinai New York, NY 10029 Tel: 212-824-8442 cecilia.berin@mssm.edu.

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this review is to assess the state of our knowledge specifically on the immune mechanisms of FPIES.

Acute versus chronic reactions to foods in FPIES

Reactions of profuse vomiting and lethargy occurring within 2-4 hours of food ingestion are commonly termed acute FPIES^{7, 8}, while a sub-acute or chronic form of FPIES has also been described in association with ongoing antigen exposure^{6, 9, 10}. In the late 1960's and early 1970's, 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^{7, 9, 11-13}. These infants presented with vomiting, diarrhea, and failure to thrive. Symptoms resolved on a milk-elimination diet, recurred upon challenge, and infants commonly outgrew their adverse reactions to milk within the first year of life. In many of these early studies, biopsies were obtained at 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^{11, 12, 14}. These change in intestinal architecture have led to comparisons with celiac disease, however there is little to suggest that the pathophysiologic mechanisms are similar between the two diseases. In the Finnish FPIES cohorts, two 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 ingestion⁹. Infants were described as progressing from an early response to a delayed response with age as they outgrew their adverse reactions to milk¹⁴. Recent descriptions of clinical experience with cohorts of food protein-induced enterocolitis syndrome (FPIES) (2 in the US, 1 in the UK, 1 in Italy and 1 in Australia) report only rare instances of delayed or chronic FPIES^{4-6, 15, 16}. Much of our understanding of the gastrointestinal immune events during FPIES is derived from studies on milk enteropathy, it is not clear if similar mechanisms may be at play in acute FPIES reactions.

Allergens inducing FPIES

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 were challenged with purified milk allergens. Freier described 6 infants with milk-induced enteropathy that improved on milk elimination diet¹³. These infants were subsequently challenged to purified milk allergens (caseins, β -lactoglobulin (BLG), α -lactoglobulin (ALA), bovine serum albumin) with the finding that BLG was the only antigen that induced symptoms in the majority of infants (5 of 6), at a dose that was present in 30 ml of milk. In a cohort described by Kuitunen, the majority of infants challenged with purified milk allergens reacted to both casein and BLG⁹. Despite the differing findings regarding the role of casein in these two 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, infants were described as reacting to BLG that had been boiled for 70 minutes¹³, 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 milk¹⁷. 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 FPIES are generally in the gram range ¹⁵, compared to IgE-mediated food allergy, which is in the microgram to milligram range ¹⁸. However, there have been case reports of children with active FPIES induced by casein hydrolysate formula ¹⁹, or reacting to antigens transmitted through breast milk ^{10, 20, 21}, 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 of FPIES. Many of the foods that trigger FPIES reactions are also IgE-mediated food allergens, including soy, fish, wheat, and egg ^{4, 5}. In adults, shellfish has been described to cause non-IgE-mediated reactions consistent with FPIES ²². However, other foods reported to trigger FPIES are not common IgE-mediated food allergens. Rice is the 3rd most common cause of FPIES in US cohorts, followed by oats ^{4, 5}. 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. While 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 one, two or multiple foods. A positive food challenge to soy reduces subsequent reactions to soy challenge, while a positive food challenge to milk does not affect a subsequent reaction to soy ²³. 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 introduction. A switch to soy formula is initially tolerated, but can result in symptoms again within approximately 2 weeks of exposure ⁷. 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 FPIES

Because biopsy is not required for 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 re-challenge. 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 ^{11, 12, 14}. 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 histological finding has led to comparisons to celiac disease, but there are several important distinctions between the two 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 intraepithelial lymphocytes, including an increase in those expressing the TIA1, a marker of

cytotoxic granules²⁴. There is also an increase in CD4+ T cells in the lamina propria bearing the activation marker HLA-DR²⁵. Plasma cells producing IgM and IgA are increased in number during milk exposure, as are eosinophils^{11, 12}. Smears of fecal mucus from positive challenges reveal the presence of neutrophils, eosinophils, lymphocytes, and other unidentified mononuclear cells⁷. Fecal extracts have been studied after milk challenge in patients with milk-induced enteropathy, and are associated with an elevation of total IgM and IgA¹², and an increase in eosinophil derived neurotoxin²⁶. Eosinophils are resident cells of the small intestine, it is not clear if the measurement of EDN in the fecal extracts is due to reduced barrier function, increased eosinophil degranulation, or both. In celiac disease, villous blunting occurs due to killing of epithelial cells by cytotoxic T cells expressing natural killer receptors that kill epithelial cells expressing elevated levels of non-classical class I molecules²⁷. Gluten functions as a trigger by activating gluten-specific CD4+ T cells that recognize modified gluten peptides with high affinity, inducing the release of cytokines that facilitate 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 diseases²⁸. There is a lack of evidence for an autoimmune component in FPIES, but there is an association between FPIES and atopy^{4, 5}.

A reduction in expression of the TGF- β receptor 1 on the intestinal epithelium (but not TGF- β) has been described in the mucosa of children with FPIES compared to control children, and correlates with degree of villous atrophy²⁹. While it is interesting to speculate that this may reflect a reduced regulatory tone in the intestine of patients with FPIES, this may be secondary to altered turnover or differentiation state of the epithelial cells in the shortened villi rather than being a primary event in pathogenesis. An increased mucosal expression of TNF α by immunohistochemistry was also described by the authors²⁹. Sequential analysis of fecal samples from an infant with milk-FPIES demonstrated an elevated TNF α level in the stool during chronic milk exposure, but interestingly not in response to acute challenge³⁰.

Impact of food challenge on innate cells in circulation

In addition to local inflammatory changes in the gastrointestinal tract, food challenge in FPIES results in acute changes in circulating leukocytes, most prominently an increase in circulating neutrophils that is one of the diagnostic criteria for a positive food challenge^{7, 8}. Increase in platelets¹⁶ and decrease in eosinophils in the circulation⁴ after a positive food challenge has also been described. Circulating neutrophil levels peak at 6 h 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 may have low levels of IgE against the food, which was labeled “atypical FPIES” by Sicherer et al⁸. It has been reported that 30% of Japanese patients with symptoms consistent with FPIES have IgE against the causative food^{31, 32}. The presence of IgE to the triggering food was recently

described as a marker of persistent FPIES by Caubet et al ⁴. Furthermore, patients with resolved milk-FPIES have been reported to develop IgE-mediated reactions to milk ⁴. It has been speculated that there may 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 reported a dramatic elevation in IgE+ cells in the lamina propria of infants with milk-induced enteropathy, and this elevated number of IgE+ cells was not reduced by elimination diet or increased by allergen challenge ³³. This elevation in IgE was not replicated by Savilahti et al, who did not find IgE+ cells in the jejunum of infants with milk enteropathy, but did report an increase in IgM+ and IgA+ cells after milk challenge ¹². A decrease in intact mast cells (suggesting degranulation) was reported after milk challenge in infants with milk enteropathy ³⁴, although this was also observed in celiac disease where mast cells are not thought to be important contributors to pathology. Thus this mast cell degranulation may be a non-specific feature of intestinal inflammation. Nonetheless, the link between food-specific IgE and 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 cells in the lamina propria, an increase in IgM and IgA in fecal extracts, and an increase in cow's milk-specific antibodies in the serum after allergen challenge ¹². These changes were not observed in those that did not develop symptoms. As IgM and IgA are primarily neutralizing mucosal antibodies, this increase may be a response to a breakdown of the epithelial barrier associated with allergen-induced inflammation and be functioning as an immunologic barrier to prevent the influx of antigens from the gastrointestinal lumen. McDonald et al studied food-specific immunoglobulin levels in a cohort of 18 children undergoing food challenge for FPIES ³⁵. Milk, egg, and soy-specific IgA antibodies were elevated in infants who reacted to a food challenge compared to those who passed a food challenge. Egg and soy-specific IgG antibodies were also elevated in those who reacted compared to those who did not. In contrast with these reports of elevated immunoglobulin levels, a significant suppression of milk-specific immunoglobulins has been observed in patients with FPIES. Shek et al observed that patients with milk-induced FPIES had significantly lower milk-specific IgG4 levels than healthy controls, patients with IgE-mediated milk allergy, or patients with allergic eosinophilic gastroenteritis ³⁶. Konstantinou et al examined casein-specific IgA responses in patients with milk-FPIES, outgrown milk-FPIES, non-milk-FPIES, and IgE-mediated milk allergy ³⁷. Although casein-specific IgA was significantly suppressed in milk-FPIES compared to both controls and non-milk-FPIES, there was no difference between those with active versus outgrown milk-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 immunoglobulins.

Cellular Immunity in 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 ³⁸, although Hoffman et al found that proliferation responses to milk were not significantly different in

patients with milk-induced FPIES compared to controls³⁹. The same study showed a significant increase in lymphocyte proliferation in patients with IgE-mediated milk allergy. Heyman et al published an oft-cited study showing that PBMCs from children with milk enteropathy produced more TNF α than controls or those with outgrown milk enteropathy when stimulated with milk proteins⁴⁰. Although the increase in TNF α was relatively modest (less than 2-fold difference between those with active versus outgrown milk enteropathy after 5 days of milk re-stimulation), treatment of intestinal epithelial monolayers with supernatants from milk-restimulated PBMCs showed a dramatic impact 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 α , although neutralization experiments with anti-TNF α 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 α 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 α in disease manifestations⁴¹. Subjects with IgE-mediated and eosinophilic gastroenteritis also have allergen-specific lymphocytes producing TNF α ⁴². It is difficult to understand how a modest increase in TNF α -producing cells could lead to such unique and severe symptoms in FPIES, although a systemic release of TNF α would be consistent with shock-like symptoms. A systemic increase in circulating TNF α during acute FPIES reactions has not been described. Morita et al 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)⁴³. Patients were compared to control subjects and those with IgE-mediated allergy to milk. Those with non-IgE-mediated gastrointestinal allergies to milk produced significantly more TNF α , IL-2, IL-3, IL-5, IL-6, IL-10 and IL-13 compared to control subjects, while IFN- γ and IL-17 were not increased. Proliferative responses were noted to α -, β -, and κ -casein and β -lactoglobulin, with minimal proliferative responses to α -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 may contribute to the unique spectrum of symptoms observed in FPIES. It is possible that differences in homing receptor expression may explain difference 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 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 that there may be a regulatory defect in FPIES. The role of Tregs has not yet been addressed in FPIES, although it has been reported that resolution of non-IgE-mediated milk allergy is associated with an expansion of milk-specific Tregs detectable after milk challenge⁴⁴.

Gaps in the understanding of the immune mechanisms of FPIES

The possibility that antigen-specific cytokine-producing T lymphocytes may produce changes in intestinal architecture observed during chronic FPIES is consistent with our knowledge of mucosal immunology. Landmark studies by MacDonald and Spencer showed that polyclonal activation of T cells in human fetal gut explants led to crypt hyperplasia and villous atrophy⁴⁵, and mouse studies have confirmed that polyclonal T cell activation *in vivo* can result in intestinal damage and diarrhea through TNF α -dependent mechanisms⁴⁶. 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 may not be feasible as biopsies 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 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 et al, milk challenge administered by enema resulted in diarrhea and weight loss, while drinking the milk induced vomiting, pallor, and diarrhea in the same infant¹³. Thus the chronic and acute manifestations of FPIES may 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-HT₃ receptor antagonist ondansetron effectively suppresses vomiting triggered by FPIES challenge^{47, 48}. The question remains how a chemosensor cell such as an enterochromaffin cell in the gut could recognize specific foods, or what the nature of a possible neuroendocrine-immune communication leading to acute symptoms of FPIES could be.

In addition to these fundamental questions about how immune activation can lead to symptoms observed in FPIES (summarized in Figure 1), there are several areas that should be considered. Is processing of the allergen required in order to trigger symptoms? For example, deamidation of gluten peptides is an essential step in celiac disease pathogenesis leading to high-affinity TCR binding, and it is possible that we have not yet observed a distinct T cell phenotype in 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 IgE-mediated food allergy and eosinophilic esophagitis^{49, 50}, a similar recognition may be at work in FPIES. NK cells residing in the liver have been shown to acquire features of antigen-specific memory in mice⁵¹. There is unfortunately a lack of a suitable animal model for FPIES. Mice and rats do not vomit, and it is not clear if 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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ABBREVIATIONS

ALA	α -lactoglobulin
BLG	β -lactoglobulin
FPIES	Food protein induced enterocolitis syndrome
NK cell	Natural killer cell
NKT cell	Natural killer T cell
PBMC	peripheral blood mononuclear cells
TNFα	Tumor necrosis factor alpha
TGF-β	Transforming growth factor-beta

Immunopathophysiology of Food Protein-Induced Enterocolitis Syndrome

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.
Caseins, β-lactoglobulin, α-lactoglobulin	Caseins make up about 80% of the proteins in cow's milk. β -lactoglobulin and α -lactoglobulin are whey proteins.
Denaturing	Modifying the molecular structure of a protein, especially by heat, acid, alkali, or ultraviolet radiation so as to destroy or diminish some of the original properties.
Conformational epitope	Amino acid residues not in a sequence that become spatially juxtaposed in a folded protein form an available surface on a molecule that is recognized by an antibody.
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 may not eliminate it completely.
Cytotoxic granules	A principal component of cytotoxic T lymphocyte (CTL) 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.
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.
TGF-β	A regulatory cytokine produced by stromal and hematopoietic cells. TGF- β inhibits proliferation of T and B cells, inhibits activation of macrophages, induces IgA production by B cells via isotype switching, and increases collagen synthesis from fibroblasts to promote tissue repair. Treg production of TGF- β can block T _H 1 and T _H 2 development in CD4 ⁺ T cells
Homing receptor	Adhesion molecules expressed on lymphocytes. Gut-homing T cells express the α 4 β 7 integrin, which binds to MAdCAM-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.
Invariant NKT cells	A subset of lymphocytes that express surface molecules characteristic of both NK and T cells. NKT cells recognize self and foreign lipids bound to CD1, a class I MHC-like molecule. They are capable of rapidly secreting cytokines following stimulation. The TCR α chains in invariant NKT cells have limited diversity and are characterized by a unique V α 24-J α 18 rearrangement.

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Figure 1. Immune mechanisms of FPIES in comparison to IgE-mediated food allergy
 The general sequence of immune events from first exposure to re-exposure leading to symptoms in IgE-mediated food allergy is understood. In contrast, while 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 (uncertainty indicated by red question marks). To date, only serotonin has been clearly implicated in symptoms of FPIES, although it is not clear if serotonin is involved as a peripheral trigger of reactions or is restricted to central control of the vomiting reflex.