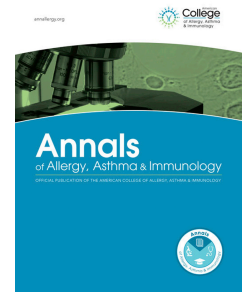


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Advances in Understanding Immune Mechanisms of Food Protein Induced Enterocolitis Syndrome

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Advances in Understanding Immune Mechanisms of Food Protein Induced Enterocolitis Syndrome

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FPIES: Food protein induced enterocolitis syndrome

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Objective: This review provides an overview of our current understanding of mechanisms of food protein-induced enterocolitis syndrome (FPIES).

Data Sources: To capture recent articles published since our last comprehensive review on the pathophysiology of FPIES, we performed a literature search through PubMed.gov, using the search terms FPIES and food protein induced enterocolitis syndrome, for 2016-current.

Study Selections: Studies in English, containing biomarker or immune data were reviewed and summarized.

Results: Studies of peripheral blood fail to demonstrate evidence of antigen-specific humoral or cellular immunity underlying clinical reactivity to foods in FPIES. However, growing evidence suggests a robust systemic innate immune activation occurring during FPIES reactions, and activation of neuroendocrine pathways.

Conclusion: FPIES reactions are associated with a marked activation of innate immune and neuroendocrine pathways, however the mechanism underlying specific recognition of foods remains elusive.

1 Introduction

Food protein induced enterocolitis syndrome (FPIES) is a food allergy of infancy that results in severe repetitive vomiting, lethargy, and pallor within hours (1-4 h) of food ingestion¹. A subset also develop diarrhea with a more delayed onset. Food allergy is defined as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food”². This is an umbrella term that includes IgE-mediated food allergies, eosinophilic gastrointestinal disorders, FPIES, and food protein-induced proctocolitis. The delay in symptom onset is not the only thing that sets FPIES apart from IgE-mediated food allergies. FPIES does not affect the skin or the respiratory tract. As a comparison, allergic reactions to red meat caused by IgE-sensitization to the sugar moiety galactose-alpha-1,3-galactose (α -gal) can be delayed in onset similar to FPIES, but do affect the skin causing hives and have a typical IgE-mediated symptom profile including abdominal cramping, hives, and anaphylaxis³. The spectrum of symptoms in FPIES is unique, specific to exposure to the triggering food, and reproducible on exposure to that food.

The majority outgrow their FPIES within the first years of life, but some will retain reactivity into adolescence and even adulthood⁴⁻⁷. The most common food triggers of FPIES are the cereals oat and rice as well as cow’s milk and soy, reflecting the foods introduced in early life. However, a wide range of foods can trigger FPIES. Adult-onset FPIES has also been described, most commonly to fish and shellfish, with individuals exposed to and tolerating the food prior to onset⁸. As will be discussed in detail in the following sections, FPIES is categorized as a non-IgE-mediated food allergy; however there is little evidence available to definitively categorize it as another form of hypersensitivity reaction. The lack of evidence for abnormal recognition of

foods by either antibodies or T cells leaves the mechanism of food specificity of this disease a compelling mystery.

Role of immunoglobulins in FPIES

FPIES is a non-IgE-mediated food allergy. IgE specific for the foods causing symptoms, measured either by serum testing or skin prick testing, is not a feature of the disease, although can be found in a subset referred to as “atypical FPIES”⁹. FPIES is associated with an increased prevalence of atopic co-morbidities¹⁰. Those with IgE to the causative food outgrow their FPIES later than those without IgE⁴, and FPIES turning into IgE-mediated food allergy has also been described¹¹. The mechanistic link between atopy, IgE, and FPIES is not understood, but could possibly relate to a lack of immune regulation. The symptoms of FPIES are distinct from those of IgE-mediated food allergy, as discussed in the introduction, suggesting a unique pathophysiology. Other antibody isotypes including IgG isotypes and IgD have been shown to trigger mast cell, basophil, neutrophil or macrophage activation in mice and humans, and may contribute to non-IgE-mediated anaphylaxis¹²⁻¹⁴. Therefore food-specific antibodies of other isotypes could potentially contribute to disease in FPIES. However, milk-specific IgG1, IgG, IgM, and IgA levels were not significantly different between children with oral food challenge-proven FPIES compared to those with a history of milk FPIES who had outgrown their allergy^{15, 16}. We have also examined levels of milk-specific IgD, with no difference between active or outgrown milk FPIES (Berin, Nowak-Wegrzyn, unpublished). Adel-Patient published findings that milk-specific IgG immunoglobulin isotypes, as well as IgM and IgA were lower in milk FPIES compared to either IgE-mediated milk allergy or peanut allergy¹⁷. They also examined IgE to 8

different milk allergens, as well as milk digested with gastrointestinal enzymes, and found no IgE reactivity to any of these allergens or digested cow's milk. These comprehensive negative studies indicate that there is no evidence for antibody recognition of the trigger food, arguing against hypersensitivity reactions Types I, II, or III as the causative basis for FPIES.

Antigen-specific T cells in FPIES

FPIES has commonly been categorized as a cell-mediated (Type IV hypersensitivity) food allergy by exclusion. It has been thought that antigen-specific T cells are activated within the gastrointestinal mucosa, causing gastrointestinal symptoms and intestinal damage¹⁸. In a rare study of the histopathology of FPIES, biopsies from children with FPIES demonstrated the presence of villous blunting and an increase in mononuclear cells expressing TNF α , supporting this hypothesis¹⁹. Most studies examining food-specific T cells in FPIES have examined peripheral blood, and used an in vitro restimulation approach, and examining proliferation, cytokine production, or activation marker upregulation hours to days after stimulation. Morita et al described T cell responses to milk proteins in peripheral blood mononuclear cells (PBMCs) cultured with 5 purified milk allergens²⁰. They compared non-IgE mediated milk allergy (including milk-triggered FPIES, proctocolitis, and enteropathy) with IgE-mediated milk allergy and controls. They found an elevation of TNF α , IL-6, and Th2 cytokine secretion (IL-3, IL-5, IL-13) compared to healthy controls. Caubet et al also examined PBMC cytokine responses to casein stimulation, comparing those with milk FPIES, IgE-mediated milk allergy, and those with outgrown IgE-mediated milk allergy¹⁶. Similar to the findings by Morita, TNF α , IL-6, and Th2 cytokines were produced in response to casein stimulation in FPIES individuals. However this

response was also observed in IgE-mediated milk allergy and outgrown IgE-mediated milk allergy, indicating that there was nothing unique about the T cell response that could explain the clinical response to milk in FPIES. Adel-Patient et al found no detectable cytokine response from milk allergen-restimulated PBMCs from FPIES, although cytokines were detected from PBMCs from IgE-mediated milk allergy¹⁷. We used an activation marker based approach (CD154 upregulation) to identify milk, soy, or rice-responsive CD4+ T cells after a short stimulation with allergen extracts²¹. We found no significant difference in the frequency or phenotype of allergen-responsive T cells in active FPIES compared to healthy controls. A criticism of this approach is that it relies on the detection of cells in peripheral blood. However, food allergen-responsive CD4+ T cells can readily be detected in this manner from peripheral blood in IgE-mediated food allergy^{22, 23}. It is possible that in FPIES, food-specific T cells do not recirculate and are present as resident memory T cells in the gastrointestinal tract.

In the context of Celiac Disease, another food-triggered non-IgE mediated disease, gluten peptide-specific T cell responses are often examined a minimum of 6 days after initiating a gluten food challenge^{24, 25}. We have examined T cell responses in blood obtained 4 hours post-challenge, but more time may be needed to allow expansion of cells from central memory CD4+ T cells, or to allow time for progeny cells of the tissue resident memory cells to leave the tissue and recirculate²⁶.

Immune regulation in FPIES

The development of inappropriate immune reactivity to the first foods of infancy suggests a lack of normal oral tolerance development. Oral tolerance in experimental models is mediated

by antigen-specific Tregs²⁷. Mouse models and the LEAP study in children have indicated that IgG antibodies are also important for the development of oral tolerance to dietary antigens^{28, 29}. IgA is also induced by oral antigen exposure and is thought to contribute to oral tolerance through immune exclusion. It has been reported that individuals with milk FPIES have lower levels of IgG and IgA antibodies than various controls, leading to the hypothesis that this lack of protective antibodies is involved in pathogenesis¹⁵⁻¹⁷. However, when examining children with a history of milk FPIES who are avoiding milk and undergo a milk challenge, there is no significant difference in milk-specific antibody levels in those who react or not to challenge^{15, 16}. This suggests that it is dietary avoidance that is leading to low IgG and IgA levels in FPIES. Although children with IgE-mediated cow's milk allergy are also avoiding milk, they typically have a robust multi-isotype immunoglobulin response to milk proteins even while avoiding milk³⁰.

Immunostaining of duodenal biopsies from FPIES patients and controls demonstrated a reduced expression of TGF- β receptor II on epithelium from FPIES, which correlated with villous atrophy but was also observed in the absence of villous atrophy. Caubet et al found that IL-10 was reduced in milk-restimulated PBMCs compared to IgE-mediated milk allergy, but did not directly compare samples from milk reactive versus outgrown FPIES. Antigen-responsive Tregs have not been directly addressed in FPIES.

Innate immune activation in FPIES

Despite a lack of mechanistic insight into the food-specific immune trigger of FPIES, it is well known that FPIES reactions are associated with immune activation. An increase in circulating

neutrophils post-challenge is part of the diagnostic criteria for interpretation of FPIES food challenge results¹. Elevated TNF α and eosinophil products have been identified in fecal samples after FPIES challenge^{31, 32}. Furthermore, there is evidence for eosinophil activation in the peripheral blood during FPIES challenge³³, and eosinophils were reported to be elevated in cord blood of infants who go on to develop FPIES³⁴. Using flow cytometry, mass cytometry, and RNA sequencing of whole blood obtained before and after oral food challenge, we found that monocytes were markedly activated *in vivo* during a positive food challenge but not in those who had outgrown their FPIES²¹. Furthermore, there was evidence of pan-activation of lymphocytes, NK cells, eosinophils, and neutrophils as measured by activation marker expression. Lymphocytes were lost from the circulation after a positive food challenge, suggesting that they may have homed to tissues such as the gastrointestinal tract en masse. Mehr et al performed transcriptional profiling on whole blood obtained before and after FPIES challenges, comparing those with reactions versus no reactions³⁵. Weighted gene co-expression network analysis identified a gene module associated with FPIES reactions. This module was enriched for genes associated with granulocytes and innate immunity, and analysis of hub genes identified matrix metalloproteinase 9, IL-1 β , and STAT3 as central to the positive FPIES response. These data-driven approaches identify a systemic innate immune response during FPIES reactions. What is unclear is how the innate immune system is getting activated by foods, and how (or if) the systemic innate response triggers symptoms.

Neuroendocrine activation in FPIES

The nervous system and immune system are both sensors of the environment that provide signals for the body to respond appropriately to challenges. Together with the endocrine system, they form a comprehensive network of sensory and response mechanisms. Acute nausea and vomiting are associated with activation of the hypothalamo-pituitary-adrenal (HPA) axis and release of stress hormones, including cortisol. Cortisol is significantly elevated during FPIES reactions³⁶. Acute psychosocial stress that activates the HPA axis (and the sympathetic adreno medullary (SAM) axis) is associated with release of inflammatory cytokines, including IL-6, TNF α , and IL-1 β , that have also been implicated in FPIES. Although stress hormones are elevated in FPIES reactions, the relationship between the stress response, the immune response, and symptoms in FPIES are not understood.

Conclusion

Despite the application of state-of-the-art techniques, research in FPIES has not achieved a mechanistic explanation for how specific foods trigger a set of symptoms unique from other food allergies. We are developing a better understanding of the neuro-endocrine-immune events that occur during FPIES reactions, which is summarized in **Figure 1**. However, we lack two key areas of understanding: how food triggers a specific response, and how/if the immune response is linked to the major symptom of vomiting. A somewhat similar phenomenon is associated with Staphylococcal enterotoxins (SE) that can induce vomiting in addition to systemic inflammation. Staphylococcal enterotoxin A (SEA) binds to mast cells in the submucosa through a yet-unidentified receptor, leading to serotonin release which binds to 5-HT₃ receptors on vagal afferents, which then leads to activation of the brainstem emetic loci to

initiate the vomiting reflex³⁷. Systemic inflammation (toxic shock) is mediated through a distinct mechanism related to its ability to cross-link MHC II/TCR as a superantigen. Chemotherapy drugs³⁸ and rotavirus infection³⁹ have been shown to activate enterochromaffin cells in the intestine, leading to serotonin release from these cells, activation of vagal afferents, and initiation of the vomiting reflex. Mast cells and enterochromaffin cells are the main non-neuronal sources of serotonin in the gastrointestinal tract. The serotonin antagonist ondansetron has been shown to effectively suppress FPIES reactions, providing evidence for a role for serotonin in the vomiting reaction in FPIES^{40, 41}. If we hypothesize that FPIES vomiting is initiated by enterochromaffin cells or mast cells, as shown in **Figure 1**, we do not yet have a proposed mechanism by which foods can activate these cells in an antigen-specific manner. We also do not understand if the 3 systems activated in FPIES (the innate immune system, the nervous system, and the neuroendocrine systems) are independent or interconnected, as highlighted in **Figure 1**.

A key roadblock to progress in the field of FPIES research is the lack of access to gastrointestinal tissue. As endoscopy and biopsy is not required for clinical care of FPIES, it has been difficult to obtain these specimens, particularly in the young children affected by FPIES. The development of innovative non-invasive methods to identify cells in the gastrointestinal tract activated by food exposure is necessary to understand the true mechanism underlying FPIES reactions.

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Figure Legend

Figure 1: Pathophysiology of FPIES.

Ingestion of the specific triggering food in FPIES leads to activation of 3 systems: the neural pathway leading to vomiting, the innate immune system, and the neuroendocrine system. The mechanism of recognition of foods, and the involvement of the immune system in symptoms, remain poorly understood. Figure created with Biorender.

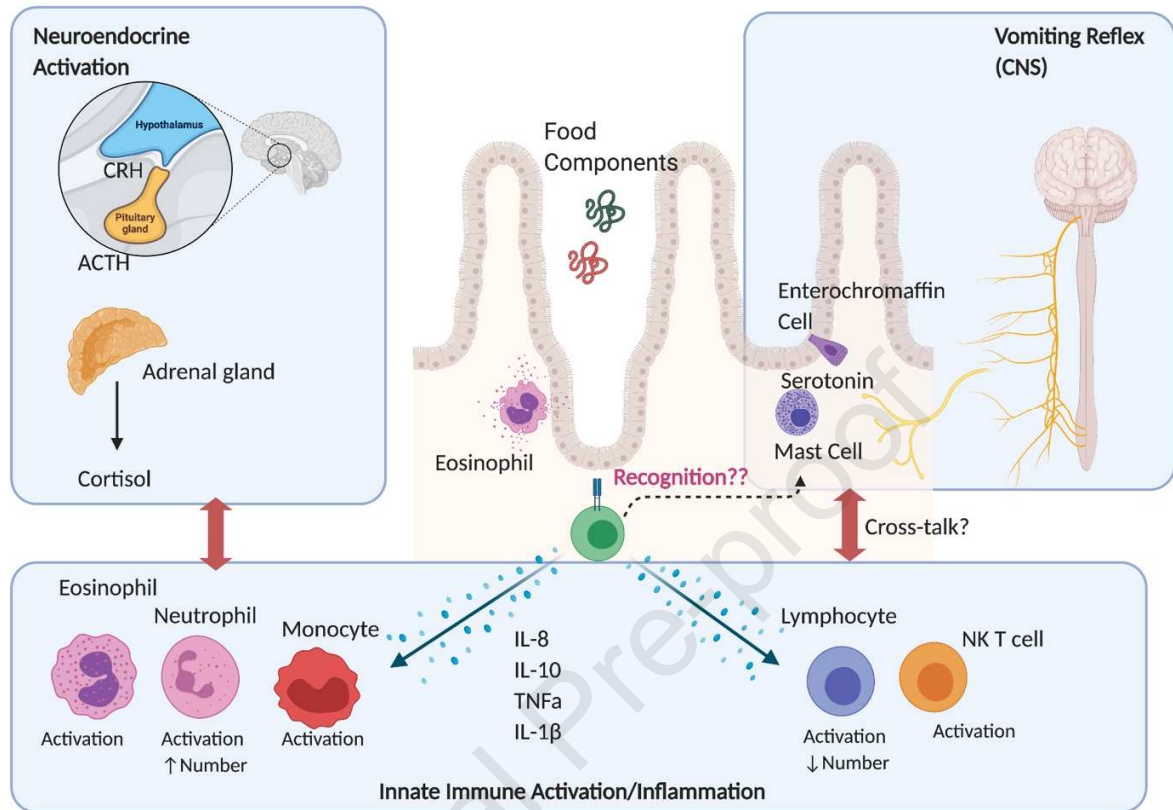


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Key Messages

- Careful studies examining antibody isotypes and food components have failed to identify any food-specific humoral immune response underlying FPIES.
- Although there is conflicting data on the detection of food-responsive T cells in peripheral blood in FPIES, no studies have been able to identify a phenotype of food-responsive T cell that is unique from control groups and could explain the clinical response in FPIES.
- Despite the unclear mechanism of food recognition, there is clear evidence for a systemic innate immune activation affecting monocytes, neutrophils, and eosinophils in FPIES.
- The link between innate inflammation and activation of neural pathways that lead to vomiting remains unclear.

1 Introduction

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different milk allergens, as well as milk digested with gastrointestinal enzymes, and found no IgE reactivity to any of these allergens or digested cow's milk. These comprehensive negative studies indicate that there is no evidence for antibody recognition of the trigger food, arguing against hypersensitivity reactions Types I, II, or III as the causative basis for FPIES.

Antigen-specific T cells in FPIES

FPIES has commonly been categorized as a cell-mediated (Type IV hypersensitivity) food allergy by exclusion. It has been thought that antigen-specific T cells are activated within the gastrointestinal mucosa, causing gastrointestinal symptoms and intestinal damage¹⁸. In a rare study of the histopathology of FPIES, biopsies from children with FPIES demonstrated the presence of villous blunting and an increase in mononuclear cells expressing TNF α , supporting this hypothesis¹⁹. Most studies examining food-specific T cells in FPIES have examined peripheral blood, and used an in vitro restimulation approach, and examining proliferation, cytokine production, or activation marker upregulation hours to days after stimulation. Morita et al described T cell responses to milk proteins in peripheral blood mononuclear cells (PBMCs) cultured with 5 purified milk allergens²⁰. They compared non-IgE mediated milk allergy (including milk-triggered FPIES, proctocolitis, and enteropathy) with IgE-mediated milk allergy and controls. They found an elevation of TNF α , IL-6, and Th2 cytokine secretion (IL-3, IL-5, IL-13) compared to healthy controls. Caubet et al also examined PBMC cytokine responses to casein stimulation, comparing those with milk FPIES, IgE-mediated milk allergy, and those with outgrown IgE-mediated milk allergy¹⁶. Similar to the findings by Morita, TNF α , IL-6, and Th2 cytokines were produced in response to casein stimulation in FPIES individuals. However this

response was also observed in IgE-mediated milk allergy and outgrown IgE-mediated milk allergy, indicating that there was nothing unique about the T cell response that could explain the clinical response to milk in FPIES. Adel-Patient et al found no detectable cytokine response from milk allergen-restimulated PBMCs from FPIES, although cytokines were detected from PBMCs from IgE-mediated milk allergy¹⁷. We used an activation marker based approach (CD154 upregulation) to identify milk, soy, or rice-responsive CD4+ T cells after a short stimulation with allergen extracts²¹. We found no significant difference in the frequency or phenotype of allergen-responsive T cells in active FPIES compared to healthy controls. A criticism of this approach is that it relies on the detection of cells in peripheral blood. However, food allergen-responsive CD4+ T cells can readily be detected in this manner from peripheral blood in IgE-mediated food allergy^{22, 23}. It is possible that in FPIES, food-specific T cells do not recirculate and are present as resident memory T cells in the gastrointestinal tract.

In the context of Celiac Disease, another food-triggered non-IgE mediated disease, gluten peptide-specific T cell responses are often examined a minimum of 6 days after initiating a gluten food challenge^{24, 25}. We have examined T cell responses in blood obtained 4 hours post-challenge, but more time may be needed to allow expansion of cells from central memory CD4+ T cells, or to allow time for progeny cells of the tissue resident memory cells to leave the tissue and recirculate²⁶.

Immune regulation in FPIES

The development of inappropriate immune reactivity to the first foods of infancy suggests a lack of normal oral tolerance development. Oral tolerance in experimental models is mediated

by antigen-specific Tregs²⁷. Mouse models and the LEAP study in children have indicated that IgG antibodies are also important for the development of oral tolerance to dietary antigens^{28, 29}. IgA is also induced by oral antigen exposure and is thought to contribute to oral tolerance through immune exclusion. It has been reported that individuals with milk FPIES have lower levels of IgG and IgA antibodies than various controls, leading to the hypothesis that this lack of protective antibodies is involved in pathogenesis¹⁵⁻¹⁷. However, when examining children with a history of milk FPIES who are avoiding milk and undergo a milk challenge, there is no significant difference in milk-specific antibody levels in those who react or not to challenge^{15, 16}. This suggests that it is dietary avoidance that is leading to low IgG and IgA levels in FPIES. Although children with IgE-mediated cow's milk allergy are also avoiding milk, they typically have a robust multi-isotype immunoglobulin response to milk proteins even while avoiding milk³⁰.

Immunostaining of duodenal biopsies from FPIES patients and controls demonstrated a reduced expression of TGF- β receptor II on epithelium from FPIES, which correlated with villous atrophy but was also observed in the absence of villous atrophy. Caubet et al found that IL-10 was reduced in milk-restimulated PBMCs compared to IgE-mediated milk allergy, but did not directly compare samples from milk reactive versus outgrown FPIES. Antigen-responsive Tregs have not been directly addressed in FPIES.

Innate immune activation in FPIES

Despite a lack of mechanistic insight into the food-specific immune trigger of FPIES, it is well known that FPIES reactions are associated with immune activation. An increase in circulating

neutrophils post-challenge is part of the diagnostic criteria for interpretation of FPIES food challenge results¹. Elevated TNF α and eosinophil products have been identified in fecal samples after FPIES challenge^{31, 32}. Furthermore, there is evidence for eosinophil activation in the peripheral blood during FPIES challenge³³, and eosinophils were reported to be elevated in cord blood of infants who go on to develop FPIES³⁴. Using flow cytometry, mass cytometry, and RNA sequencing of whole blood obtained before and after oral food challenge, we found that monocytes were markedly activated *in vivo* during a positive food challenge but not in those who had outgrown their FPIES²¹. Furthermore, there was evidence of pan-activation of lymphocytes, NK cells, eosinophils, and neutrophils as measured by activation marker expression. Lymphocytes were lost from the circulation after a positive food challenge, suggesting that they may have homed to tissues such as the gastrointestinal tract en masse. Mehr et al performed transcriptional profiling on whole blood obtained before and after FPIES challenges, comparing those with reactions versus no reactions³⁵. Weighted gene co-expression network analysis identified a gene module associated with FPIES reactions. This module was enriched for genes associated with granulocytes and innate immunity, and analysis of hub genes identified matrix metalloproteinase 9, IL-1 β , and STAT3 as central to the positive FPIES response. These data-driven approaches identify a systemic innate immune response during FPIES reactions. What is unclear is how the innate immune system is getting activated by foods, and how (or if) the systemic innate response triggers symptoms.

Neuroendocrine activation in FPIES

The nervous system and immune system are both sensors of the environment that provide signals for the body to respond appropriately to challenges. Together with the endocrine system, they form a comprehensive network of sensory and response mechanisms. Acute nausea and vomiting are associated with activation of the hypothalamo-pituitary-adrenal (HPA) axis and release of stress hormones, including cortisol. Cortisol is significantly elevated during FPIES reactions³⁶. Acute psychosocial stress that activates the HPA axis (and the sympathetic adreno medullary (SAM) axis) is associated with release of inflammatory cytokines, including IL-6, TNF α , and IL-1 β , that have also been implicated in FPIES. Although stress hormones are elevated in FPIES reactions, the relationship between the stress response, the immune response, and symptoms in FPIES are not understood.

CONCLUSION

Despite the application of state-of-the-art techniques, research in FPIES has not achieved a mechanistic explanation for how specific foods trigger a set of symptoms unique from other food allergies. We are developing a better understanding of the neuro-endocrine-immune events that occur during FPIES reactions, which is summarized in **Figure 1**. However, we lack two key areas of understanding: how food triggers a specific response, and how/if the immune response is linked to the major symptom of vomiting. A somewhat similar phenomenon is associated with Staphylococcal enterotoxins (SE) that can induce vomiting in addition to systemic inflammation. Staphylococcal enterotoxin A (SEA) binds to mast cells in the submucosa through a yet-unidentified receptor, leading to serotonin release which binds to 5-HT₃ receptors on vagal afferents, which then leads to activation of the brainstem emetic loci to

initiate the vomiting reflex³⁷. Systemic inflammation (toxic shock) is mediated through a distinct mechanism related to its ability to cross-link MHC II/TCR as a superantigen. Chemotherapy drugs³⁸ and rotavirus infection³⁹ have been shown to activate enterochromaffin cells in the intestine, leading to serotonin release from these cells, activation of vagal afferents, and initiation of the vomiting reflex. Mast cells and enterochromaffin cells are the main non-neuronal sources of serotonin in the gastrointestinal tract. The serotonin antagonist ondansetron has been shown to effectively suppress FPIES reactions, providing evidence for a role for serotonin in the vomiting reaction in FPIES^{40, 41}. If we hypothesize that FPIES vomiting is initiated by enterochromaffin cells or mast cells, as shown in **Figure 1**, we do not yet have a proposed mechanism by which foods can activate these cells in an antigen-specific manner. We also do not understand if the 3 systems activated in FPIES (the innate immune system, the nervous system, and the neuroendocrine systems) are independent or interconnected, as highlighted in **Figure 1**.

A key roadblock to progress in the field of FPIES research is the lack of access to gastrointestinal tissue. As endoscopy and biopsy is not required for clinical care of FPIES, it has been difficult to obtain these specimens, particularly in the young children affected by FPIES. The development of innovative non-invasive methods to identify cells in the gastrointestinal tract activated by food exposure is necessary to understand the true mechanism underlying FPIES reactions.