Systemic innate immune activation in food protein–induced enterocolitis syndrome

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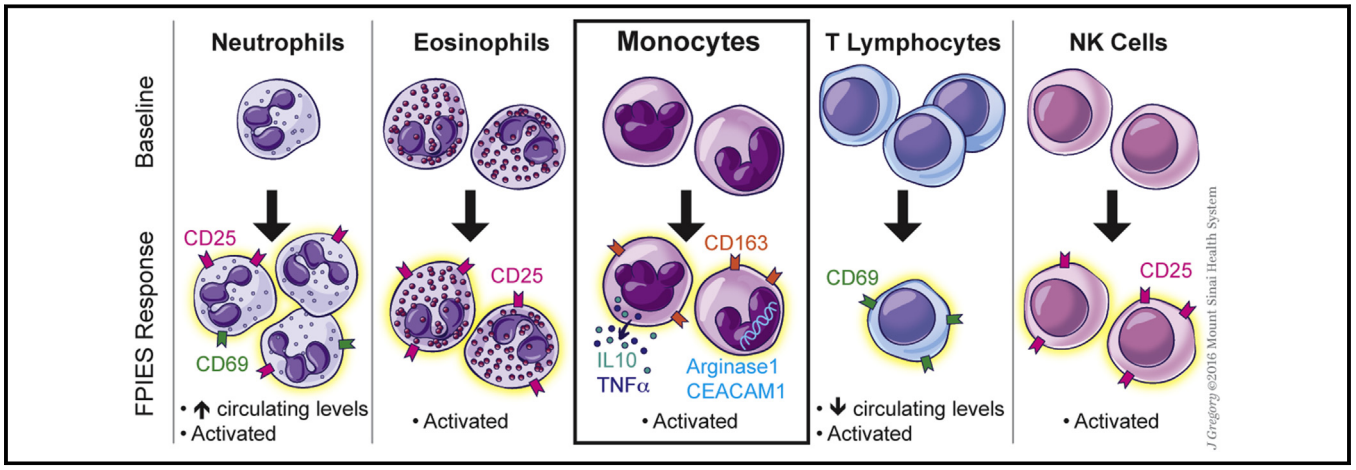
GRAPHICAL ABSTRACT



Background: Food protein–induced enterocolitis syndrome (FPIES) is a non–IgE-mediated food allergy of infancy whose pathophysiology is poorly understood.

Objectives: We set out to identify and phenotype allergen-responsive cells in peripheral blood of a cohort of subjects undergoing supervised food challenge for FPIES.

Methods: We profiled antigen-responsive cells in PBMCs by flow cytometry, and examined cells in whole blood obtained before and after challenge by CyTOF mass cytometry and RNAseq.

Results: Using a CD154-based detection approach, we observed that milk, soy, or rice-responsive T cells, and TNF-α–producing $CD154^+$ T cells, were significantly lower in those with outgrown FPIES compared with those with active FPIES. However, levels were within the normal range and were inconsistent with a role in the pathophysiology of FPIES. Profiling of whole blood by CyTOF demonstrated profound activation of cells of the innate immune system after food challenge, including monocytes, neutrophils, natural killer cells, and eosinophils. Activation was not observed in children with outgrown FPIES. We confirmed this pattern of innate immune activation in a larger cohort by RNAseq. Furthermore, we observed pan–T-cell activation and redistribution from the circulation after a positive food challenge but not in those who had outgrown their FPIES.

Conclusions: Our data demonstrate a compelling role of systemic innate immune activation in adverse reactions elicited by foods in FPIES. Further investigation is needed to identify the mechanism of antigen specificity of adverse reactions to foods in FPIES. (J Allergy Clin Immunol 2017;139:1885-96.)

Key words: *Food allergy, non-IgE, FPIES, innate, CyTOF, RNA-sequencing*

Food protein–induced enterocolitis syndrome (FPIES) is a disease of infancy characterized by profuse vomiting and lethargy beginning 2 hours after food ingestion, with a subset experiencing delayed diarrhea.[1] Although most infants outgrow FPIES by school age, a minority retain clinical reactivity into adolescence or adulthood.[2] Foods triggering FPIES are the most common foods

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Abbreviations used
FPIES: Food protein–induced enterocolitis syndrome
NK: Natural killer
OFC: Oral food challenge

introduced early into the infant's diet, including cow's milk, soy, rice, and oats, but a wide range of foods have been reported to induce FPIES symptoms. Reactions are consistent with antigen specificity, and although most individuals react to a single food, multifood reactivity also occurs.[3] There is growing awareness of FPIES as a clinical entity, highlighted by a number of publications in the last 5 years summarizing clinical experience with FPIES.[2,4-6]

FPIES is classified as a non–IgE-mediated food allergy, although allergen-specific IgE can be found in some patients with FPIES and is associated with persistent disease.[2] There are conflicting reports about levels of food-specific antibodies in FPIES or food protein–induced enteropathy.[7-9] We have examined food-specific IgG and IgA levels in subjects with milk-induced FPIES and found a relative absence of milk-specific immunoglobulins compared with tolerant controls.[10,11] In patients with food protein–induced enteropathy, a disease whose relationship to FPIES is unclear and which has not been reported in recent clinical summaries, chronic antigen exposure leads to diarrhea and vomiting and is associated with villous atrophy and T-cell infiltration.[3] PBMCs from patients with non–IgE-mediated cow's milk allergy show increased TNF-α production compared with PBMCs from patients with outgrown cow's milk allergy,[12] but it is not clear if the patients described in that cohort would fit diagnostic criteria of FPIES. Patients with FPIES have also been described to have a T_H2-skewed cytokine profile from antigen-restimulated PBMCs,[13] consistent with our recent findings.[11] It is difficult to reconcile a T_H2-skewed T-cell profile as underlying such a distinct clinical entity as FPIES. As we recently reviewed,[3] there is currently a lack of understanding of the immunologic basis of adverse reactions to foods in FPIES.

Diagnosis of FPIES is based on clinical history and response to food elimination. Patients with a history of FPIES undergo a supervised food challenge when there is reason to believe that the patients may have outgrown their reactivity, usually 12 to 24 months since their last reaction. In our center, food challenges for FPIES are performed in the clinical research center, with full resuscitation facilities for rapid intravenous fluid repletion. We collected specimens from patients undergoing a food challenge for FPIES. Blood specimens were collected immediately before the challenge, and again 4 to 6 hours after the challenge. We found evidence for a profound systemic innate immune activation associated with FPIES reactions, in the absence of an abnormal or pathogenic antigen-specific T-cell response. These data point to a critical role of the innate immune system in mediating adverse reactions to foods in FPIES.

METHODS

Study population

The research protocol was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board. Written informed consent was obtained before enrollment. Patients aged 1 to 21 years previously diagnosed with FPIES underwent an oral food challenge (OFC) in the inpatient clinical research unit to evaluate for resolution. Table I presents the clinical characteristics of the study subjects. A peripheral intravenous line was inserted before the OFC. During the OFC, the challenge food was administered in 3 equal portions over 30 minutes. The OFC was considered positive on the basis of diagnostic criteria defined by Powell[14]: emesis and/or diarrhea, and an increase in blood polymorphonuclear leukocyte count (>3500 cells/mm^3 peaking at 6 hours). Following a negative OFC result, children were observed for 4 hours, whereas following a positive OFC result they were treated (2 of 14 treated with steroids, the remainder with intravenous fluid with or without Zantac) and observed until stable, usually discharged within 6 hours. Blood samples were obtained immediately before the OFC as well as 4 hours after a negative OFC result and 6 hours after a positive OFC result. There were sex differences between groups, with positive challenges being overwhelmingly male, whereas negative challenges were gender balanced. Healthy adult (non–age-matched) controls who were non–food-allergic by self-report were recruited to provide a reference of a healthy CD154 response to foods.

TABLE I. Subjects' characteristics

Characteristic	FPIES	Outgrown
N	14	16
Age (y), median (range)	7.5 (1.3-21)	4.6 (1-21)
Sex	13 M/1 F	7 M/9 F
Foods used for challenge	Milk (4) Rice (4) Soy (3) Oat (1) Banana (1) Beef (1)	Milk (7) Rice (3) Soy (1) Egg (2) Wheat (1) Salmon (1) Bean (1)
Food-specific IgE	1 of 14	0 of 16

F, Female; *M*, male.

Cell isolation and culture

Blood was obtained in 10 mL heparinized vacutainer tubes. PBMCs were isolated, and cultured in AimV with 5% Human AB serum. A total of 4×10^6 cells in 1 mL were plated in 24-well plates. Cells were stimulated for 6 or 18 hours with milk antigens (a mix of 50 μg/mL each of α, β, and κ caseins) (Sigma Aldrich, St Louis, Mo), or soy or rice extract prepared from flour at 100 μg/mL. Extracts were cleaned of endotoxin using DetoxiGel columns (ThermoFisher, Rockford, Ill) and verified by Pierce LAL Endotoxin quantification kit (ThermoFisher) before use.

Four hours before harvest, Brefeldin A (BD Biosciences, San Jose, Calif) was added to cells. Cells were harvested, stained with fixable live/dead stain, followed by surface markers (CD3-APC-Cy7 [eBioscience, San Diego, Calif], CD4-Brilliant Violet 405 [Biolegend, San Diego, Calif], CXCR5-PerCP-Cy5.5 [BD Biosciences], CCR6-PE-Cy7 [BD Biosciences], and CCR9-FITC [BD Biosciences]). Cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, Pa), followed by permeabilization with Permeabilization Buffer (eBioscience), and intracellular staining (CD154-PE [eBioscience], TNFα-AlexaFluor700, IL-13-v450, IL-10-PE-CF594, and IL-9-AlexaFluor647). Cells were acquired on a BD LSRFortessa, and analysis was performed on FlowJo Software (TreeStar, Ashland, Ore). In some studies, CD14-PE-Cy7 was used to identify monocytes.

Mass cytometry analysis (CyTOF)

Sample preparation. Whole blood samples were treated with BD PhosFlow Lyse/Fix Buffer (BD Biosciences) before freezing the sample in 10% dimethyl sulfoxide/PBS at −80°C. Thawed samples were first barcoded with Cell-ID 20-Plex Pd Barcoding Kit (Fluidigm, San Francisco, Calif), according to the manufacturer's protocol. All the antibodies used in this study were either purchased preconjugated from Fluidigm or were conjugated