

LETTER TO THE EDITOR

USEFULNESS OF ATOPY PATCH TEST ON A CHILD WITH MILK PROTEIN-INDUCED ENTEROCOLITIS SYNDROME: A CASE REPORT

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We describe the case of a child affected by milk-protein induced enterocolitis, in which oral challenge with corn was performed without symptoms after a negative specific Atopy Patch Test. Food protein-induced enterocolitis syndrome (FPIES) is an uncommon non-IgE-mediated gastrointestinal food hypersensitivity of infancy, characterized by severe vomiting and diarrhea arising within 1 to 3 hours after ingestion of the causative food. Little is known about the pathophysiology of FPIES. The absence of food-specific IgE as demonstrated by negative skin prick tests suggests that the disease is not caused by an early onset IgE-mediated reaction. Atopy Patch Test has been described as sensitive and predictive in this syndrome. The hypothesis on the immunological pathogenesis has been discussed on the basis of literature data.

Food protein-induced enterocolitis syndrome (FPIES) is an uncommon non-IgE-mediated gastrointestinal food hypersensitivity of infancy, characterized by severe vomiting and diarrhea arising within 1 to 3 hours after ingestion of the causative food; symptoms typically begin in early infancy and, when severe, may lead to dehydration, hypotension and shock. Hypothermia, pallor and lethargy are other common features; laboratory tests usually show neutrophilia, thrombocytosis and metabolic acidosis (1, 2).

Although cow's milk and soy proteins are most often responsible for FPIES, typical symptoms have also been induced by egg, wheat, rice, peas, oat, peanut, tree nuts, chicken, turkey, barley, lentil, sweet potato, squash, string bean and fish (1-5).

Little is known about the pathophysiology

of FPIES. The absence of food-specific IgE, as demonstrated by negative skin prick tests and specific serum IgE, suggests that the disease is not caused by an early onset IgE-mediated reaction. Currently, oral food challenge (OFC) is the gold standard method to confirm the diagnosis of FPIES in children with suggestive symptoms, when all tests for IgE-mediated allergy resulted negative (5).

In this article, we describe the case of a child affected by milk protein-induced enterocolitis, in which Atopy Patch Test (APT) drove food introduction. We discuss potential immunological pathogenetic mechanisms underlying FPIES in pediatric age.

Case report

A 6-month-old breastfed boy was seen at the

Key words: food protein-induced enterocolitis syndrome, oral challenge, atopy patch test, pathogenetic mechanism, immunologic alterations

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Allergy and Respiratory Unit of Department of Pediatrics, University of Chieti, because of a history of gastrointestinal symptoms after the first introduction of cow's milk proteins. He had been breast-fed since birth; the first administration of a cow's milk-based formula resulted in severe vomiting, diarrhea, lethargy, drowsiness and pallor after 3 hours. A second, more severe episode occurred 2 hours after administration of a different cow's milk-based formula, and was characterized by severe vomiting, lethargy, hypotension and shock. Clinical diagnosis of FPIES was made. After the elimination diet, symptoms disappeared and the patient reached a normal weight.

Skin prick test with food extracts (Alyostal, Stallergenes) and food-specific IgE levels determination by ImmunoCAP (Phadia AB, Uppsala, Sweden; limit of the assay <0.35 KUA/L) were performed at the first evaluation, resulting negative for IgE-mediated allergy to milk, wheat, egg, cod, chicken, tomato, rice, soybeans, corn, apple, beans, peas.

Atopy patch test (APT) (CURATEST, Lohmann Rauscher International GmbH Co. Germany) was performed for milk, wheat, egg, cod, tomato, chicken, beans, peas, spinach, apple, corn. The patches were removed at 48 hours and results were read at 48 and 72 h. Reactions were classified as follows: + = erythema; ++ = erythema and papules; +++ = erythema and vesicles (6). We observed positive results (++) for milk, wheat, cod, chicken, beans and apple.

To start the weaning we followed results of a study (Fogg et al.) that showed that APT predicted the OFC results, with a negative predictive value of 100% (6). Therefore, we decided to perform in-hospital OFC with one of the negative food (corn). Before the OFC, stool examination for occult blood resulted negative. Cell blood count (CBC) and peripheral blood neutrophil count (PBNC) were in normal range for age.

A preliminary informed consent was obtained from parents and emergency medications were made available. OFC with corn cream (CC) was performed according to the guidelines suggested by Powell (7) and McDonald et al. (8). An intravenous line was inserted, and an open challenge started with 0.15 g of CC per kilogram of body weight. Since no

reaction occurred, after 4 hours 0.6 more grams of CC per kilogram of body weight were administered. CBC was repeated after 6 hours and did not show any increase in PBNC count (> 3500 cells/mm³) and no occult blood was found in stool. The patient was asymptomatic and the following day a dose of 1.25 g of CC per kilogram of body weight was administered and tolerated. Then CC was introduced into the diet at normal dose without side effects.

DISCUSSION

Sicherer et al. (5) defined the criteria for "typical" FPIES as follows (1):

- 1) age less than 9 months at initial diagnosis;
- 2) elicitation of diarrhea and/or repetitive vomiting within 24 hours, caused by repeated exposure to the incriminated food without any other cause for the symptoms;
- 3) symptoms limited to the gastrointestinal tract;
- 4) removing the offending allergen should lead to rapid and complete symptom resolution within a few days;
- 5) elicitation of FPIES symptoms can be obtained with a standardized OFC.

"Atypical" FPIES occurs when subjects with clinical criteria of FPIES were over 9 months of age at diagnosis or had specific IgE to the incriminating food (5).

Caubet and Nowak-Wegrzyn described endoscopic evaluations reported by several case series that included patients who fulfilled criteria for a diagnosis of FPIES and described varied and nonspecific histological features: diffuse colitis with variable ileal involvement; mild friability to severe spontaneous hemorrhage of colonic mucosa, associated with minute ulcers; crypt abscesses in some patients; flattened villi, edema and increased numbers of lymphocytes, IgM- and IgA-containing plasma cells, eosinophils and mast cells at jejunal biopsies; friability of rectal mucosa, with ulceration and bleeding; in some cases, focal erosive gastritis and esophagitis with eosinophilia and villus atrophy (9).

The above-described features, together with the negativity of IgE-mediated reactions, suggest a cellular immune mechanism with consequent patchy

and nonspecific inflammation and damage to the digestive tract walls at the basis of FPIES. Untreated children develop tissue changes, with moderate or intense partial atrophy and moderate intraepithelial lymphocyte infiltration. Thus, immunohistochemistry shows an increase in activated CD4⁺ cells within the lamina propria of the small bowel, as well as of intraepithelial CD8⁺ cells. These tissue changes lead to protein-losing enteropathy, with secondary hypoalbuminemia, complement depletion, edema, anemia and other signs of denutrition (10). Furthermore, FPIES is characterized by the failure of the barrier and immunological function of the digestive tract (9).

The pathophysiology of FPIES is still unknown. The hypothesis that the disease is not caused by an early onset IgE-mediated reaction is supported by the absence of food-specific IgE, as demonstrated by negative skin prick tests and RAST (6).

The immune response at the basis of the tolerance to food antigens induced via the gut is known as "oral tolerance", characterized by the generation of local and systemic immunological tolerance, that may be explained by T-cell anergy, clonal deletion, and induction of regulatory T (Treg) cells. The role of CD4⁺CD25⁺Treg cells in mediating oral tolerance is supported by feeding experiments in mice and by indirect evidence as patients with enteropathy, immune dysregulation, polyendocrinopathy, X-linked syndrome, or X-linked autoimmunity-allergic dysregulation syndrome lack this T-cell subset. Similarly, Karlsson et al. hypothesized that mucosal induction of tolerance against dietary antigens was associated with the appearance of circulating CD4⁺CD25⁺ Treg cells, capable of suppressing the effector T cells; this characteristic was shown to be partly cell contact-dependent (11).

Many immunologic alterations have been described in FPIES as humoral antibody-specific responses, especially the involvement of antigen-specific T cells with proinflammatory cytokine production, and subsequent regulation of the intestinal barrier permeability (8, 9).

The food specific *in-vitro* stimulation of blood lymphocyte in infants with FPIES, correlated with the results of OFC with the same foods (soy, cow's milk, and egg white), results in the enhancement of specific lymphocyte responses to the incriminated

food proteins. This suggests that circulating lymphocytes, sensitive to the food antigens, that produce clinical symptoms are frequent in infants with this form of food protein hypersensitivity (12).

In the setting of T-cell-mediated response, high levels of proinflammatory cytokine TNF- α released by antigen-specific T cells act synergistically with IFN- γ to increase intestinal permeability, contributing to the influx of antigens into the submucosa with further activation of antigen-specific T cells (9).

TNF- α production, which alters intestinal permeability, is a feature of both gastrointestinal allergy to cow's milk and FPIES (6). Majamaa et al. found increased fecal levels of TNF- α in the stool after positive cow's milk challenge in patients with enterocolitis (13). Therefore, TNF- α is thought to play a major role in FPIES (6).

The regulatory cytokine TGF- β 1 protects the epithelial barrier of the gut from the penetration of foreign antigens, stimulating the synthesis of extracellular matrix proteins, collagen and fibronectin and enhancing the binding between cells and matrix proteins by altering the expression of integrins. It also induces T-cell suppression. Chung et al. observed that infants with FPIES have a decrease of TGF- β 1 receptor, in addition to the increase of TNF- α . It is suggested that the decreased TGF- β 1 activity against the barrier-disrupting effect of T-cell cytokines in young infants may play a role in the pathogenesis of FPIES. Older children presented increased TGF- β 1 expression after weaning, which may partially explain why FPIES is only seen in young children (9, 14, 15). Interestingly, TGF- β blockade also strongly induced IFN- γ production (16). The acute FPIES reaction seems to be associated with a Th2 skewing of the T-cell cytokine profile.

IL-10 seems to play an important role in regulating the Th1 and Th2 response, in particular at the gut level (17, 18); its increased expression is associated with the ingestion of the same food when tolerance is reached. On the contrary, IL-4 has been reported as inflammatory cytokines involved in the gut immune response by inhibiting the induction of Foxp3 and then the generation of inducible regulatory T cells (19). In a recent study, Mori and colleagues described with documented FPIES to rice an increase in T-cell IL-4 expression and a decrease in IFN- γ in an 8-month-old child, after a positive

challenge with rice; the authors observed an increase in T-cell IL-10 expression after rice challenge 6 months later after a negative challenge, when the child had acquired tolerance to rice. They concluded that a Th2 activation, associated with high IL-4 levels, may be involved in the pathophysiology of FPIES, while T-cell-derived IL-10 may play a role in the immunotolerance acquisition by regulating the Th2 responses (20).

Humoral immune response may also be involved in the pathophysiology of FPIES, with an increase of specific IgA and IgG and a decrease in specific IgG4 antibodies (8, 9, 21).

Shek et al., determining IgE, IgA, IgG1 and IgG4 antibody levels and lymphocyte proliferative responses to the major cow's milk allergens in patients with IgE- and non-IgE-mediated cow's milk allergy (CMA), observed elevation of milk protein-specific IgA in patients with FPIES, but they did not find elevated IgG1 or IgG4 antibodies compared with controls. In fact, patients with FPIES produced almost no milk protein-specific IgG4 that could have a protective role in competing with other subclasses that could activate complement. This relative lack of IgG4 may be involved in the pathogenesis of FPIES (21).

Eosinophil accumulation in the gastrointestinal tract is a frequent feature of many gastrointestinal disorders: IgE-mediated food hypersensitivity, eosinophilic gastroenteropathies, food-induced proctocolitis, gastroesophageal reflux inflammatory and bowel diseases. Eosinophils, originating from hematopoietic stem cells in the bone marrow, are effector cells with an immunoregulatory function through cytokine release and presentation of antigens; their migration to the gut is regulated by granulocyte-macrophage colony-stimulating factor, IL-3 and IL-5. The presence of clusters of eosinophils in intestinal biopsies from infants with FPIES suggests that FPIES and eosinophilic gastroenteritis might represent a continuum of eosinophilic gastrointestinal disorders, with similar immunopathogenic mechanism (9, 22).

Positive patch test, a useful tool in food allergic T-cell-mediated disorders, such as atopic dermatitis, supports the role of T cells in FPIES. However, there are important differences between skin T cells that express cutaneous leukocyte antigen,

and gastrointestinal T cells that are primarily $\gamma\delta$ T cells and express different homing adhesion molecules, making the detection of gastrointestinal T lymphocytes in the skin unlikely (9, 23, 24).

Currently, the only way to make diagnosis of FPIES is through a careful history and an OFC carried out only on patients with doubtful and mild symptoms, as it is considered a high-risk procedure in FPIES. The study of Fogg et al. suggests that, if the APT is negative for the suspected food, an OFC can be immediately performed as there is a strong likelihood that the patient is either not sensitive to the food or has outgrown his sensitivity; while, if the APT is positive, the OFC will be delayed at least for 1 year after the last known reaction. Indeed, in 16 cases of FPIES confirmed by OFC, the authors observed a positive APT to the suspected food (however, the APT was positive in five cases with negative OFC), while patients with a negative APT had a negative OFC to the suspected food (6).

Recently, Järvinen et al. (25) performed APT on children with FPIES before OFC to monitor tolerance development; however, they found an APT sensitivity of 11.8%, specificity of 85.7%, positive predictive value of 40%, and negative predictive value of 54.5% vs sensitivity of 100%, specificity of 71%, positive predictive value of 75% and negative predictive value of 100% by Fogg et al. (6). This discrepancy may be attributable to the fact that Järvinen et al. considered only palpable infiltration as a positive reaction, the median age in their study was older (3.3 years; range, 1.5–16.8 years) and the median time since the most recent FPIES episode was longer (24.5 months, range, 14.5–79 months) before the OFC, while Fogg's (6) patients represent a group of children with more "active" disease. Probably, the negative APT is a result of a reduction in activation capacity of responsible T cells over time, while there is not the same reduction in the gut mucosa during a positive OFC (25).

According to diagnostic algorithm of Fogg and colleagues (6), we carried out an OFC with corn on a patients with negative APT for corn. Our little patient tolerated the OFC with corn and now he eats and tolerates corn cream and has a normal weight gain.

Therefore, further studies are needed to determine the usefulness of APT in patients with FPIES, because,

although FPIES prognosis is usually good and most children develop tolerance to the incriminated food by 3 years of age without long-term sequelae (1, 2), a safety food introduction before 3 years is needed in these high-risk patients.

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