
Case report

Rice-induced enterocolitis in an infant: T_H1/T_H2 cellular hypersensitivity and absent IgE reactivity

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Background: Although food allergy is common in children, rice allergy is unusual in Western cultures.

Objective: To report a case of T-cell-mediated rice intolerance in an 11-month-old girl.

Methods: To evaluate the intolerance to rice in this patient, a graded rice food challenge was performed. To examine the immunologic reactivity to rice, in vitro lymphoproliferative responses and cytokine synthesis of rice-stimulated peripheral blood lymphocytes (PBLs) was performed. Subsequently, skin patch testing to rice and other foods was performed.

Results: Allergy skin prick test results were negative for rice and positive for egg, milk, and soy. Specific IgE antibodies to rice, egg, peanut, wheat, walnut, codfish, milk, soybean, corn, shrimp, scallops, and clams were undetectable. Results of a single-blind rice food challenge were positive, manifested by emesis that persisted for more than an hour and required intravenous hydration. In vitro lymphoproliferation by the patient's PBLs to rice stimulation was positive. In addition, cytokine synthesis of interferon- γ , interleukin 10 (IL-10), tumor necrosis factor α , and IL-5 by the patient's rice-stimulated PBLs was elevated, indicating a T_H1/T_H2 cell response to rice. Endoscopy revealed normal esophageal, gastric, and duodenal mucosa; a biopsy specimen revealed mild esophagitis. Duodenal explant T cells were initially established by stimulation with rice and IL-2. After a 2-day rest, the lymphocytes were restimulated with rice for 7 days and revealed increased interferon- γ and IL-5 synthesis. Twenty billion colony forming units of *Lactobacillus* GG were added to the patient's diet twice daily. After 6 weeks, rice rechallenge resulted in emesis within 1 hour. Results of patch testing were positive to rice, wheat, and barley but negative to soy, which the patient tolerated on food challenge.

Conclusions: Although this patient did not demonstrate IgE antibody to rice, T_H1/T_H2 cell-mediated responses to rice were detected, and the patient experienced significant morbidity. Patch testing for gastrointestinal food allergies may be useful when the food specific IgE antibody is negative. Probiotic therapy in this patient did not ameliorate her sensitivity to rice, and food elimination remains the only reliable treatment for T_H1/T_H2-mediated food hypersensitivity.

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INTRODUCTION

Similar to other atopic diseases, the prevalence of food allergy has increased in Western countries, occurring in 6% of children younger than 3 years and in 3.5% to 4% of American adults.^{1,2} At least 50% of food hypersensitivity in children younger than 2 years is IgE mediated,³ leaving a substantial portion of food hypersensitivity that is either non-IgE mediated (cell mediated) or mixed IgE and cell mediated. The mixed disorders include allergic eosinophilic esophagitis, allergic eosinophilic gastroenteritis, and atopic dermatitis, whereas cell-mediated mechanisms are involved in food protein-induced enterocolitis, food protein-induced proctocoli-

tis, food protein-induced enteropathy syndrome, celiac disease, and food-induced pulmonary hemosiderosis.^{4–7}

Traditionally, food hypersensitivity is evaluated by history, allergy skin prick testing, and specific IgE measurement with or without food challenge. However, depending on the history, it may be important to proceed with the evaluation even if findings from specific IgE measurement and skin prick testing are negative. Beyer and colleagues⁸ demonstrated milk-specific mucosal T lymphocytes with a T_H2 cytokine profile in 60% of a milk-sensitive population, but only half of these subjects had detectable milk specific IgE antibody. Furthermore, Spergel and colleagues⁹ studied a combination of allergy skin prick testing and patch testing to identify causative foods with non-IgE-mediated reactions that had negative skin prick test results and positive patch test results.

CASE REPORT

An 11-month-old girl experienced emesis within 2 hours of ingestion of rice cereal on several occasions. She had also had similar reactions after eating barley cereal, vanilla wafers,

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and pudding. The emesis typically persisted for several hours. She did not experience any respiratory symptoms, and she typically had no skin involvement. She required intravenous hydration in the emergency department after one of the episodes. The emergency department physicians reported a few hives on her face approximately 9 hours after the ingestion of rice. The patient did not experience bloody stools or diarrhea with the emesis. The parents had eliminated all cereals, rice, soy, oat, wheat, and barley from her diet because they thought, by trial and error, that these foods caused the vomiting. The patient exhibited normal growth and development. Findings from her physical examination were normal. Informed consent to perform in vitro lymphoproliferative and cytokine synthesis studies was obtained using a Cardinal Glennon Children's Hospital consent form.

METHODS

Allergy Skin Prick Tests

The patient underwent allergy skin prick testing to multiple foods using the Multitest II (Lincoln Diagnostics Inc, Decatur, IL). Skin prick test results were graded on a 4-point scale (1+ = 5 mm of erythema; 2+ = >5 mm of erythema; 3+ = erythema and edema [≥ 5 mm]; and 4+ = erythema, edema, and pseudopods [≥ 10 mm]) using histamine and saline as positive and negative controls, respectively. A skin test result of 2+ or greater was considered positive. In addition, food specific IgE antibodies were performed by measurement of IgE ImmunoCAPs (Pharmacia Diagnostics AB, Kalamazoo, MI).

Skin Patch Tests

The patient underwent food skin patch testing to rice, barley, wheat, soy, oat, and peanut according to the protocol described by Spergel et al.⁹ Two grams of each food product was mixed with 2 mL of isotonic sodium chloride solution. A portion of each mixture was transferred to individual Finn chambers and applied to the back. The patch tests were read at 48 and 72 hours. Peanut was applied as a negative control because the patient had not eaten or had a reaction to peanut, and rice was used as a positive control.

Food Challenge and Endoscopy

The patient participated in an open graded food challenge to rice, with 0.5, 1, 2, 4, and 8 g administered every 30 minutes in the allergy clinic under direct physician supervision. The patient was observed for 2 hours after receiving the last dose of rice. Esophagogastroduodenoscopy with multiple mucosal biopsies was performed by an experienced gastroenterologist at our facility.

Rice-Stimulated Lymphoproliferation

In vitro lymphoproliferative responses to rice were determined using methods described previously.¹⁰ The rice preparation (Greer Laboratories, Lenoir, NC) was comet natural long-grained rice, defatted, extracted at 1:10 wt/vol in 0.01 M ammonium bicarbonated, dialyzed against distilled water,

powdered, and dried. The lyophilized rice was reconstituted in RPMI media. Peripheral blood lymphocytes (PBLs) were isolated from venous blood by Ficoll-Hypaque density-gradient centrifugation and were resuspended in RPMI media supplemented with 10% fetal calf serum (FCS). Peripheral blood lymphocytes, 2.5×10^5 cells per well, were cultured in triplicate and stimulated with media and rice at 1, 5, 10, 25, and 50 $\mu\text{g/mL}$ for 7 days (37°C , 5% carbon dioxide) to determine the optimal concentration of rice to use in rice-stimulated lymphoproliferative and cytokine synthesis studies. Tritiated thymidine was added to the last 18 hours of culture, and the amount of tritiated thymidine incorporated was counted on a beta scintillation counter (Beckman Coulter Inc, Fullerton, CA). Results were calculated as net counts per minute (stimulated minus unstimulated) and as a stimulation index (stimulated/unstimulated).

Rice-Stimulated Cytokine Synthesis

For rice-specific T cells, PBLs, 1×10^6 cells/mL in RPMI containing 10% FCS, were cultured for 7 days and then stimulated with 10 $\mu\text{g/mL}$ of either medium or rice (Greer Laboratories; 37°C , 5% carbon dioxide), as previously described.¹¹ As seen in the lymphoproliferative responses to rice stimulation, at 10 $\mu\text{g/mL}$ of rice the control had little response, whereas the patient had an optimal lymphoproliferative response. At 25 and 50 $\mu\text{g/mL}$ of rice, the control had minimal lymphoproliferative responses. Thus, to avoid non-specific stimulation, 10 $\mu\text{g/mL}$ of rice was used in the rice-stimulated cytokine studies. After 7 days, the supernatants were harvested and frozen at -20°C until analysis of cytokines. Peripheral blood lymphocyte culture supernatant cytokines were analyzed for interferon- γ (IFN- γ), tumor necrosis factor α (TNF- α), interleukin 2 (IL-2), IL-4, IL-5, and IL-10 using the Cytometric Bead Array kit (BD Biosciences Pharmingen, San Diego, CA) per the manufacturer's instructions.¹¹ Transforming growth factor β (TGF- β) was analyzed by enzyme-linked immunosorbent assay using the OptEIA TGF- β kit (BD Biosciences) per the manufacturer's instructions.¹¹

Duodenal Explant Lymphocyte Cultures

Expansion of rice-stimulated duodenal lymphocytes was performed using the technique described by Beyer et al.⁸ Briefly, duodenal biopsy specimens were minced into 1-mm³ sections, cultured in 2 mL of RPMI media containing 10% FCS in 24-well plates (37°C , 5% carbon dioxide), and stimulated with 10 $\mu\text{g/mL}$ of rice and 25 U/mL of IL-2 for 7 days. After 7 days, the PBLs that had migrated out of the tissue were collected and rested for 2 days; then the PBLs, 1×10^6 cell/mL in RPMI containing 10% FCS, were restimulated with 10 $\mu\text{g/mL}$ of media or rice for 7 days as described in the previous subsection. After 7 days, the supernatants were harvested and frozen at -20°C until analysis of cytokines as described in the previous subsection.

RESULTS

Allergy Skin Prick Testing

The patient's skin prick test results were positive for egg, milk, and soy but negative for all other foods tested, including rice. Specific IgE ImmunoCAPs were undetectable to rice, milk, soy, egg, corn, peanut, walnut, wheat, barley, codfish, and seafood.

Skin Patch Testing

The patient had erythema and vesicles at the site of rice application at 48 and 72 hours, with additional erythema and vesicles in an area outside the Finn chamber where a stray portion of rice rested. She had eczematous erythema and induration to barley and wheat and negative findings on patch testing for soy, oat, and peanut.

Food Challenge and Endoscopy

Rice food challenge resulted in protracted emesis that started within 2 hours of administration of the last dose of rice (8 g), and the patient subsequently required intravenous hydration. She did not have hypotension, wheezing, or diarrhea. The esophageal, gastric, and duodenal mucosa were normal in appearance. The esophageal biopsy sample showed mild esophagitis, with 5 to 6 eosinophils per high-power field. The gastric and duodenal biopsy results were normal.

Lymphoproliferation to Rice Stimulation

The PBLs of the patient demonstrated strong lymphoproliferative responses to rice at 1 to 50 $\mu\text{g/mL}$, with peak response at 10 $\mu\text{g/mL}$ (Fig 1). In contrast, the PBLs of the control did not demonstrate a lymphoproliferative response to rice stimulation at 10 $\mu\text{g/mL}$; subsequently, cytokine synthesis was performed using 10 $\mu\text{g/mL}$ of rice.

Cytokine Synthesis by Rice Stimulation

Rice-stimulated PBLs from the patient revealed increased IFN- γ , IL-5, TNF- α , and IL-10 synthesis, in contrast to an atopic control (allergic rhinitis; Fig 2A). However, IL-2 and TGF- β synthesis was not elevated and was comparable in the

patient and the control. Similarly, the patient's duodenal lymphocytes stimulated with rice also demonstrated elevated IFN- γ and IL-5 synthesis but not IL-10, TNF- α , and TGF- β synthesis (Fig 2B).

Treatment

Initially, the patient was treated with a strict elimination diet, eliminating egg, milk, rice, oat, wheat, barley, and soy, with her diet consisting of fresh fruits, vegetables, and meats. She underwent a trial of *Lactobacillus* GG, 20 billion CFU twice a day for 6 weeks, along with rice elimination based on the findings by Isolauri and colleagues¹²⁻¹⁴ that probiotics may prevent and treat atopic dermatitis. After 6 weeks, the patient was rechallenged with rice; however, she again experienced vomiting. After the negative soy patch test result, a soy food challenge was performed, the results of which were negative, and soy was reintroduced into the patient's diet. The parents have refused food challenges to oat, wheat, and barley.

DISCUSSION

Skin prick tests and specific IgE measurements are a good starting point for food allergy evaluation, but these tests alone must not be considered 100% predictive. In light of a positive food challenge result, it is important to continue with the food allergy investigation. This case demonstrated a T_H1/T_H2 response to rice by the patient's lymphocytes isolated from PBLs and duodenal biopsy samples in the absence of rice-specific IgE antibody.

As we explore ways to further evaluate patients for food hypersensitivity, food patch testing may come to the fore. Multiple researchers^{9,15-20} have reported using patch testing to evaluate for non-IgE-mediated food hypersensitivity, but the predictive value is not yet known, and nonspecific irritation is a common problem. Broader studies need to be performed to define the sensitivity and specificity of patch testing for routine food allergy evaluation, and this holds promise. Furthermore, measurement of T_H1/T_H2 responses from either vesicular fluid or skin biopsy samples might be informative in determining the immunologic response to foods identified by skin patch tests. In this patient, the parents were willing to submit their child to a soy food challenge after they were reassured by the negative patch test result. The soy food challenge result was negative, and the patient has since broadened her diet with the addition of soy products.

In this particular patient, the food protein-induced enteropathies must be included in the differential diagnosis, but the patient did not demonstrate gastrointestinal bleeding, hypotension, diarrhea, or failure to thrive. Celiac disease was ruled out by the normal duodenal biopsy findings. The prevalence of allergic eosinophilic esophagitis and allergic eosinophilic gastroenteritis has increased in Western cultures and must be considered in this case as well. This patient does not meet the diagnostic criteria for eosinophilic esophagitis, with only 5 to 6 eosinophils per high-power field; however, the study was performed while the patient was on a strict elimination diet.⁶ She demonstrated a T_H1/T_H2 cytokine profile

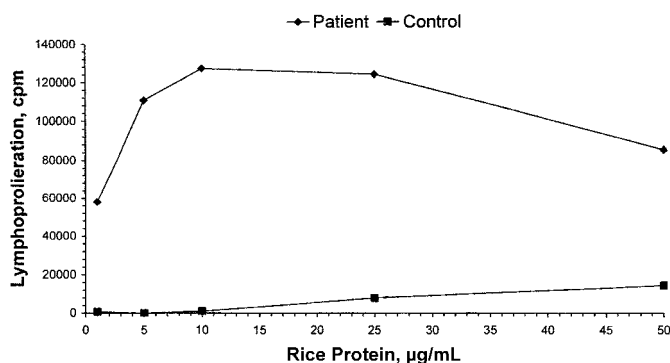


Figure 1. Lymphocyte proliferative response to rice in a patient with rice allergy and a control. The patient had positive lymphoproliferative responses to rice stimulation at 1 to 50 $\mu\text{g/mL}$, whereas the control demonstrated only slight responses at 25 and 50 $\mu\text{g/mL}$.

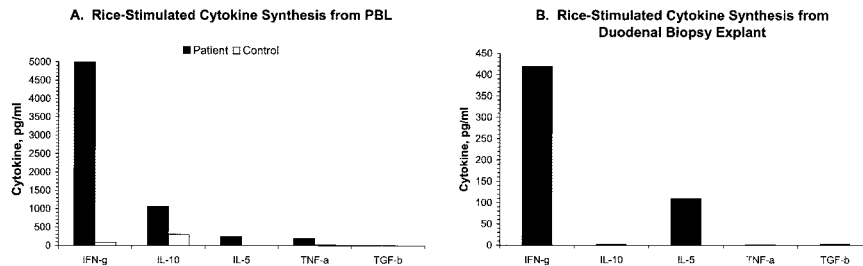


Figure 2. A, Peripheral blood lymphocytes (PBLs), 1×10^6 , were stimulated for 7 days with $10 \mu\text{g/mL}$ of rice, and supernatant was analyzed for cytokine synthesis. The patient's PBLs, when stimulated with rice, demonstrated significant interferon- γ (IFN- γ), interleukin 5 (IL-5), IL-10, and tumor necrosis factor α (TNF- α) synthesis but not transforming growth factor β (TGF- β) and IL-2 (not shown) synthesis compared with a control. B, The duodenal biopsy explant was initially stimulated with rice, $10 \mu\text{g/mL}$, and IL-2, 25 IU/mL, for 7 days, rested, and then stimulated with rice, $10 \mu\text{g/mL}$, for 7 days. Rice-stimulated duodenal lymphocytes from the patient demonstrated increased IFN- γ and IL-5 synthesis but not IL-10, TNF- α , and TGF- β synthesis.

from her peripheral blood and tissue. Allergic eosinophilic gastroenteritis studies have shown increased levels of T_H2 cells in the peripheral blood and the intestinal mucosa,^{8,21} which were also present in this patient. Other investigators have demonstrated that the role of TNF- α in milk-induced enterocolitis increased either on gastrointestinal epithelial cells²² or from milk-stimulated PBL synthesis of TNF- α .²³ The present study also demonstrated that TNF- α synthesis was increased in this patient. In addition, IFN- γ , IL-5, and T_H1 and T_H2 cytokine synthesis was also elevated.

Gut flora plays an important role in the developing mucosal immune responses in the intestinal mucosa. Although probiotics have proven useful in the prevention and treatment of atopic dermatitis, similar benefits have not yet been shown in the prevention and treatment of food allergy.^{4,12–14} Further studies need to be performed to evaluate their role in the prevention and treatment of gastrointestinal food allergies, but it seems feasible that enhancing the gut microbiota with a product such as *Lactobacillus* GG could play a role in shifting the immune response to food allergens encountered by the gastrointestinal mucosa to one of tolerance. However, as in our patient, if the food-induced enterocolitis is a mixed T_H1 and T_H2 immunologic response, then *Lactobacillus* may not be helpful.

This case report emphasizes the developing areas of diagnosis and treatment of food hypersensitivities. Further studies that define the sensitivity and specificity of food patch testing and studies that broaden our understanding of the benefits of probiotics are likely to change the clinical practice of allergists when they evaluate patients with food hypersensitivities. It is becoming more difficult to accept negative skin test results and undetectable specific IgE levels as the end of a food allergy evaluation.

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