

Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance

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Background: Hen's egg allergy is among the most common food allergies in childhood and predicts later development of allergic disease. The optimal efficacy and mechanism(s) of egg allergen immunotherapy are poorly understood.

Objective: To enhance immunologic and clinical outcomes of egg oral immunotherapy (OIT) using a conditionally increased dosing strategy.

Methods: In an open-label clinical trial of egg OIT, egg-allergic children ingested daily doses of egg protein that were gradually increased based on the egg white (EW) IgE level. Skin prick test reactivity and EW- and ovomucoid-specific cellular and humoral responses were measured longitudinally. To confirm clinical tolerance, patients underwent double-blinded, placebo-controlled food challenges 1 month after completing the dosing protocol.

Results: Children aged 3 to 13 years with characteristics of clinical egg allergy were enrolled. All 6 patients who completed the entire protocol developed clinical tolerance to egg during the study. The median wheal diameter on EW skin prick testing decreased from 10 to 2.5 mm during OIT ($P = .03$). Both EW and ovomucoid IgE levels significantly decreased during the study (median EW IgE level: from 18.8 kU/L at baseline to 3.9 kU/L, $P = .03$), and corresponding IgG4 levels increased (median EW IgG4 level: from 0.65 mg/L at baseline to 86.15 mg/L, $P = .03$). Transient increases were seen in egg-induced interleukin 10 ($P = .06$) and transforming growth factor β ($P = .18$) levels, and the ratio of $T_H2:T_H1$ cytokine production was decreased ($P = .25$).

Conclusions: Egg OIT is associated with tolerance acquisition in children with persistent egg allergy. Individualized dosing regimens may be necessary to achieve a full therapeutic effect in some patients.

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INTRODUCTION

Food allergy is common and increasing in prevalence among US children.¹ Despite increased awareness, food allergy remains the most common cause of anaphylaxis in US emergency departments.² Because no treatment is currently available, food-allergic patients must maintain strict dietary

allergen avoidance and be prepared to treat reactions caused by accidental exposures. As a result, the quality of life of food-allergic patients and their families is significantly impaired.³ A disease-modifying intervention is greatly needed.

Hen's egg is an important food allergen, affecting approximately 1% to 2% of US children,⁴ and the prevalence is significantly higher in children with atopic dermatitis (AD).⁵ In young children, egg sensitization is a known risk factor for later progression to allergic respiratory disease.⁶ Most egg allergens are susceptible to heat and gastric digestion, and perhaps as a result, egg-allergic children are commonly expected to outgrow it in early life.⁷ However, recent studies⁸ have reported persistence into the second decade of life. These patients tend to be distinguished by more severe clinical reactions and a robust IgE response, especially to the linear epitopes of the major allergen ovomucoid, which is resistant to digestion.^{8,9} These data suggest that multiple egg allergy phenotypes may exist, which may have important therapeutic implications.

Oral immunotherapy (OIT) is an experimental interventional strategy intended to establish oral tolerance in food-allergic patients. Most published OIT trials were nonrandomized and studied a uniform dose and duration of maintenance allergen given to all patients. For example, we previously described partial desensitization in an uncontrolled proof-of-concept egg OIT trial using a 300-mg/d maintenance dose.¹⁰

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Additional patients have been enrolled,¹¹ and herein we report our updated experience in these previously unreported patients. We hypothesized that further dose escalation would enhance OIT outcomes, and we implemented a conditional updosing strategy in which the maintenance dose is individually increased based on the patient's egg white (EW) IgE level. We show that clinical tolerance developed in all 6 patients completing this OIT protocol, along with immunologic changes that may be antigen specific. Length of treatment and conditional dosing may be important variables in OIT protocols.

METHODS

Patient Recruitment and Selection

Egg-allergic children aged 1 to 16 years were recruited as part of the same ongoing trial previously reported^{10,11} from the pediatric allergy and immunology clinics and surrounding offices at Duke University Medical Center. The Duke institutional review board granted ethics approval. Written informed consent was obtained in accordance with ethics guidelines for research in children.

Patients were included with a clinical history of reaction within 60 minutes of ingesting egg, a positive EW skin prick test (SPT) reaction, and an EW CAP-FEIA (Pharmacia Inc, Uppsala, Sweden) value greater than 7 kU/L (or >2 kU/L if <2 years old). Patients were excluded for a history of severe anaphylaxis (ie, hypotension) to egg, severe or poorly controlled asthma, or a medical condition preventing completion of a food challenge.

OIT Protocol

Patients underwent an egg OIT protocol consisting of 3 phases: initial-day escalation, buildup, and maintenance. The primary objective of the study was the development of clinical tolerance, defined as the successful completion of a double-blinded, placebo-controlled food challenge (DBPCFC) after a 1-month cessation of OIT. Throughout the protocol, patients were instructed to mix the OIT dose in a vehicle food and ingest it at home daily, remaining on an otherwise egg-free diet. Patients kept a diary of any missed doses or adverse symptoms, and self-injectable epinephrine was provided. The study team was readily available at all times throughout the study, and parents were instructed to call with any concerns about illness or adverse events.

Initial-day escalation. The initial-day escalation occurred on the Duke Clinical Research Unit (DCRU). A 10-mg/mL solution of powdered EW (Michael Foods, Minnetonka, Minnesota) in distilled water was prepared for all doses less than 25 mg. For doses of at least 25 mg, powdered EW was dispensed from individual preweighed containers. All doses were mixed with a vehicle food of the patient's choice. After placement of an intravenous catheter, the escalation began at 0.1 mg. The dose was approximately doubled every 30 minutes until the highest tolerated single dose was determined (maximum, 50 mg). If the patient had a mild reaction (ie, oral pruritus), the previously tolerated dose was repeated before resuming the process. If significant symptoms developed, the

escalation was stopped and the reaction was treated. The highest tolerated single dose was used as the starting dose for the buildup phase.

Standard and conditional buildup phase. Patients returned to the DCRU for the initial buildup dose and biweekly for dose escalations. Doses were increased in 25-mg increments until 150 mg was reached, and then in 50-mg increments to 300 mg. The 300-mg dose was continued for 4 months, and the EW IgE level was measured. If the EW IgE level remained greater than 2 kU/L, the patient underwent an open oral food challenge (OFC) at DCRU to assess desensitization. The following day, the dose was increased according to the highest tolerated dose during the OFC, to a maximum of 300 mg. Patients were then continued on this dose for 4 months, and the EW IgE measurement was repeated. If the EW IgE level was greater than 2 kU/L, the dose was increased by 600 mg at the DCRU. For as long as the EW IgE level remained greater than 2 kU/L, this 4-month cycle of reassessment and 600-mg updosing continued, to a maximum of 3,600 mg/d. Patients were evaluated every 4 months during continued maintenance dosing. When the EW IgE level was less than 2 kU/L, OIT was stopped and DBPCFC was performed the following day as the first step in tolerance assessment. If successful, the DBPCFC was repeated 1 month later off of OIT.

Oral food challenges. Before all OFCs, patients were asked to restrict their use of antihistamines, β -agonists, theophylline, and montelukast. To assess desensitization after 4 months of maintenance OIT, all the patients underwent an OFC consisting of 4 doses (300, 600, 1,200, and 1,800 mg) of egg protein given every 30 minutes.

The DBPCFCs were performed once the EW IgE level was less than 2 kU/L to assess tolerance. Randomization and preparation of the challenge materials was performed by the dietitian or a laboratory representative to blind the provider and supervising investigator administering the challenge. The DBPCFC involved 2 separate challenges of 7 doses of egg or placebo given every 10 to 20 minutes in increasing amounts up to a total of 10 g. All negative challenge results were confirmed by open challenge, when approximately twice the quantity of egg was given openly at mealtime or snack time.

Egg Specific IgE, IgG, and IgG4 Levels

The EW and ovomucoid specific IgE, IgG, and IgG4 levels were measured in serum samples using the ImmunoCAP 100 instrument (Phadia AB; Pharmacia Inc) according to the manufacturer's instructions.

Analyses of Cytokine Production and Regulatory T Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from approximately 25 mL of heparinized blood using Ficoll-based density separation (LymphoH; Atlanta Biologicals, Lawrenceville, Georgia). For cytokine assays, PBMCs were distributed into 96-well flat-bottom plates at a concentration of 4×10^5 cells/well in triplicate and incubated with egg protein (200 μ g/mL), concanavalin A (40 μ g/mL) (Sigma-Aldrich Corp, St Louis, Missouri), or medium alone (RPMI

1640 with 2-mmol/L L-glutamine, 25-mmol/L HEPES buffer containing 10% human AB serum, 100 IU/mL of penicillin, and 100 mg/mL of streptomycin; Mediatech, Manassas, Virginia). Cells were cultured at 37°C in 5% humidified carbon dioxide for 48 (concanavalin A) and 96 (egg) hours. Culture supernatants were collected at each time point and were analyzed using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota).

For flow cytometry, PBMCs (4×10^6 cells/well) were cultured in 24-well plates under the same stimulation conditions. After 6 days, cells were collected and stained with fluorescent monoclonal antibodies: anti-CD3-PerCP, CD4-fluorescein isothiocyanate, and CD25-phycoerythrin (BD Biosciences, San Jose California). Isotype controls were included for each condition. Three-color events were detected using a FACSCalibur flow cytometer (Beckman-Coulter, Brea, California). At least 10,000 events were acquired for each experimental condition, and data were analyzed using FlowJo (Tree Star Inc, Ashland, Oregon).

Statistical Analysis

Means, medians, and variances were tabulated for all the data and evaluated for normality. Pairwise SPT, antibody, and cytokine data were analyzed using the nonparametric Wilcoxon signed rank test. Simple linear regression compared trends in milk, peanut, and egg specific IgE levels across time. All the analyses were performed using Prism for Windows (Graphpad, La Jolla, California). A $P < .05$ was considered significant.

RESULTS

Patients

Eight patients (5 boys and 3 girls) were enrolled in the study (Table 1). The median age was 5 years (age range, 3–13 years). Seven patients (88%) had comorbid atopic disease,

primarily AD and other food allergies. For all patients at baseline, the median and mean, respectively, for EW SPT reactions were 9 and 9.3 mm (range, 5–14 mm); for EW IgE levels, 12.5 and 25 kU/L (range, 0.7–127 kU/L); and for ovomucoid IgE levels, 8.6 and 25.3 kU/L (range, 0.4–110 kU/L).

Patient 1 was voluntarily withdrawn from the study for the worsening of unrelated preexisting behavioral symptoms. Patient 3 built up to 300 mg and received this maintenance dose for 4 months but was withdrawn from the study before challenge owing to poor AD control and nonadherence to topical AD therapy. Six patients (75%) successfully completed the protocol and are discussed further herein.

Safety

Five of six patients (83%) developed allergic symptoms during the initial escalation day, before ingestion of 50 mg. In 3 patients, these symptoms were dose limiting. Four patients (50%) developed abdominal pain, which was accompanied by vomiting and diarrhea in 2. Two patients developed urticaria, which resolved with diphenhydramine administration. During the observation period after administration of the 50-mg dose, 1 patient with asthma developed nasal congestion, sneezing, and wheezing, which resolved with albuterol use.

During the buildup phase, 3 patients developed transient oral pruritus at doses less than 100 mg, none of which required dose adjustment or treatment. Eight days after building up to 250 mg, patient 8 unexpectedly developed swelling of her left eye and face within 15 minutes of receiving the OIT dose, which resolved with diphenhydramine administration.

There were no adverse events associated with maintenance dosing. No patients required unscheduled physician evaluation or epinephrine use during the trial. Patient 8 reported an accidental allergen ingestion while taking 1,800 mg when she

Table 1. Characteristics of the Study Population

Patient No./sex/age, y ^a	Comorbidities	Baseline SPT reaction, mm	Baseline IgE level, kU/L	Length of OIT treatment	Highest maintenance dose, mg
1/M/13	Probable EE	9	6.03	26 d (withdrew) ^b	NA
2/M/6	None	8	0.7	18 mo	300
3/M/3	AD	9	11.2	182 d (withdrew) ^c	NA
4/F/6	AD, AR, asthma, FA (wheat, PN)	14	127	50 mo	2,400
5/F/4	AD, AR, FA (milk, PN)	5	23.2	50 mo	2,400
6/M/6	AD, asthma, FA (PN)	10	13.8	38 mo	3,600
7/M/4	AD, AR, asthma, FA (PN)	5.5	3.7	19 mo	1,200
8/F/4	AD, AR, FA (shrimp, PN)	14	14.4	28 mo	2,400
Mean (SD)	NA	9.3 (3.4)	25 (41.8)	33.8 (14.5) mo	2,050 (1,145)
Median	NA	9	12.5	33 mo	2,400

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; EE, eosinophilic esophagitis; FA, food allergy; NA, not applicable; OIT, oral immunotherapy; PN, peanut; SPT, skin prick test.

^a The mean (SD) patient age was 5.8 (3.2) years (median age, 5 years).

^b Stress exacerbated by a comorbid psychological disorder.

^c Concern over worsening AD complicated by nonadherence to standard therapy.

accidentally ate a sibling's sandwich containing mayonnaise, but she did not develop symptoms.

OFC Outcomes

The median time to reach the 300-mg maintenance dose was 174 days (interquartile range [IQR], 125–177 days). Four months after reaching maintenance, all 5 patients with EW IgE levels greater than 2 kU/L passed an OFC to 3,900 mg of total egg protein and were considered desensitized. The EW IgE level was undetectable in patient 2, and he continued taking 300 mg for 18 months without undergoing the initial desensitization challenge. After a median length of treatment of 33 months with a median maintenance dose of 2,400 mg, all 6 patients passed the first DBPCFC. After decreasing early on, the EW IgE levels in patients 4, 5, and 6 plateaued after years of maintenance. As a result, parents and investigators agreed to challenge these patients at EW IgE levels of 9.94, 4.83, and 3.95 kU/L, respectively. All these patients subsequently passed the second DBPCFC.

EW-Specific SPT and IgE and IgG4 Levels

The median EW SPT wheal diameter and the IgE level decreased from baseline values of 10 mm and 18.8 kU/L to 2.5 mm and 3.9 kU/L, respectively, at the time of the tolerance challenge ($P = .03$ for both) (Figs 1 and 2A). The median EW IgG4 level increased from 0.65 mg/L at baseline to 86.15 mg/L ($P = .03$) (Fig 2B). The EW IgG4 level dropped at 6 and 12 months after OIT was discontinued, but this was not statistically significant. Similar changes were seen across time in ovomucoid IgE and IgG4 levels (Fig 2C and D).

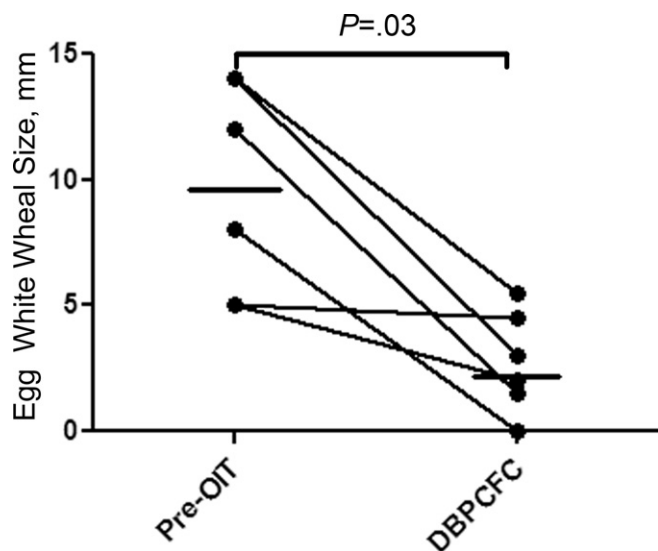


Figure 1. Egg oral immunotherapy (OIT) is associated with suppression of IgE responses in vivo. Titrated skin prick testing was performed at the indicated time points using a commercially available egg white extract. The short horizontal lines are medians. DBPCFC indicates double-blinded, placebo-controlled food challenge.

The impact of egg OIT on IgE levels may be allergen specific. We examined the IgE responses to other foods in 2 patients (patients 4 and 5) with comorbid peanut allergy and peanut plus milk allergy, respectively. Only EW specific IgE levels decreased during egg OIT, whereas peanut and milk specific IgE levels remained unchanged (Fig 3).

Cytokines

Median transforming growth factor β levels were 44 pg/mL (IQR, 22–237 pg/mL) at baseline and 291 pg/mL (IQR, 175–463 pg/mL) at 6 months ($P = .18$) (Fig 4A). The ratio of IL-13 to interferon γ was 65.7 at baseline and 5.1 at 18 months ($P = .25$) (Fig 4B). Interleukin 10 (IL-10) was generally undetectable at baseline (median, 0 pg/mL; IQR, 0–38.5 pg/mL), and at 12 months the median level was 172 pg/mL (IQR, 73.75–267 pg/mL) ($P = .06$) (Fig 4C).

Regulatory T Cells

We attempted to quantify egg-specific T cells that might have regulatory properties. These early studies used a flow cytometric approach to gate on CD4⁺CD25^{hi} populations after egg stimulation in vitro. All the patients had detectable populations, but we could not detect a change across time (data not shown).

DISCUSSION

Egg allergy is one of the most common allergic disorders of childhood, especially in children with AD, and it predicts later allergic disease. We previously reported¹⁰ interim clinical results from a pilot egg OIT trial that used a maintenance dose of 300 mg. After 24 months, 4 of 7 patients (57%) were considered to be desensitized based on increased OFC thresholds; 2 of these 4 patients tolerated egg after stopping OIT. Patient enrollment continued at both sites,¹¹ and based on these findings, we revised the protocol. Herein we report the impact of individualized dosing on the clinical and immunologic outcomes of different patients. This trial, which has been ongoing for nearly 60 months, is the longest of its kind reported and offers important insight about OIT protocols. Although preliminary data from OIT trials are promising, critical questions remain unanswered, such as the appropriate dose and length of treatment.¹²

Most OIT studies, including our previous study,¹⁰ have treated all patients with the same dose and for the same often relatively short duration. In contrast, the patients in this study underwent conditional stepwise increases in an attempt to reciprocally drive EW IgE levels below 2 kU/L before assessing tolerance. This was not possible in 3 patients in whom progressive dose increases had no further impact. Yet, in contrast to our previous study,¹⁰ all 6 patients who completed the protocol passed DBPCFCs during egg OIT and again 1 month after stopping treatment, indicating clinical tolerance to egg. All these patients have incorporated egg into their diets without difficulty. The higher doses and longer treatment courses delivered in a monitored setting by experienced investigators were safe and well tolerated. These results suggest that patients may derive additional benefit from

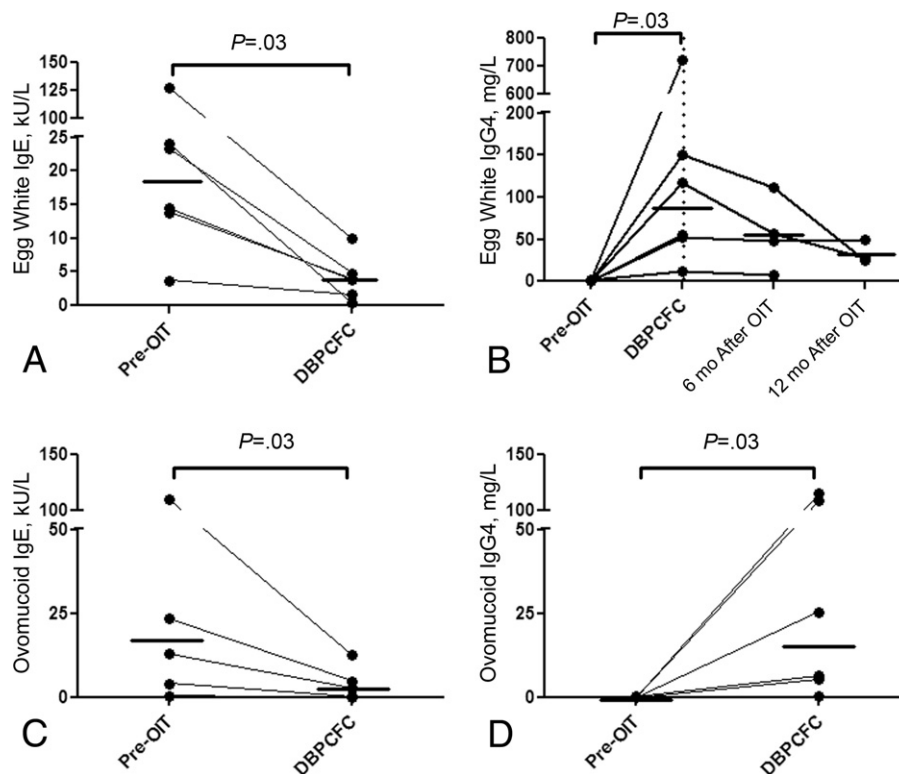


Figure 2. Production of egg white and ovomucoid specific IgE and IgG4 is decreased and increased, respectively, during egg oral immunotherapy (OIT). Serum samples were collected at the indicated time points, and IgE and IgG4 levels specific for egg white (A and B, respectively) and ovomucoid (C and D, respectively) were measured using CAP-FEIA. The short horizontal lines are medians; dotted vertical line, end of treatment. DBPCFC indicates double-blinded, placebo-controlled food challenge.

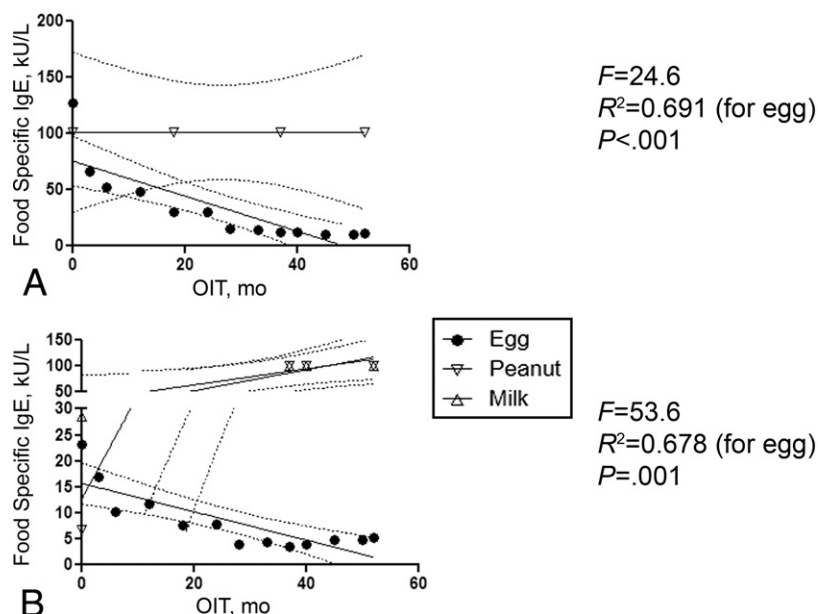


Figure 3. The reduction in IgE production during egg oral immunotherapy (OIT) is allergen specific. Serum samples were collected from 2 patients, and IgE levels specific for egg and peanut in patient 4 (A) and for egg, peanut, and milk in patient 5 (B) were measured using CAP-FEIA across time. Values greater than 100 kU/L were transformed to 101 kU/L. The solid lines represent regression lines; dashed lines, 95% confidence intervals for slope variables.

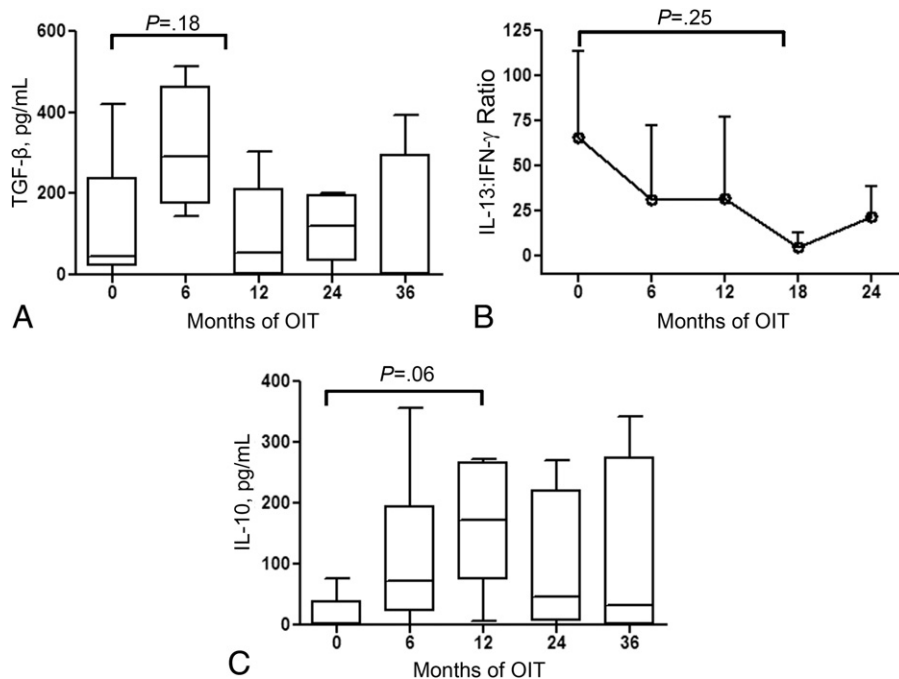


Figure 4. Transient changes in regulatory and proallergic cytokine production occur during egg oral immunotherapy (OIT). Peripheral blood mononuclear cells were collected at the indicated time points and were stimulated with egg allergen for 96 hours. Supernatants were collected, frozen, and later analyzed for the production of transforming growth factor β (TGF- β) (A), interleukin 13 (IL-13) to interferon γ (IFN- γ) ratio (B), and IL-10 (C) using standard enzyme-linked immunosorbent assay. In parts A and C, the horizontal line in the middle of each box indicates the median, and the top and bottom borders of the box mark the 75th and 25th percentiles, respectively. The whiskers above and below the box mark the 90th and 10th percentiles, respectively. In part B, open circles represent means and error bars represent standard deviation.

OIT with longer treatment or higher doses. These findings are consistent with those of Narisety et al,¹³ who enrolled partially desensitized children in an extended open-label dose escalation protocol after a short double-blinded milk OIT study. In these children, prolonged higher-dose treatment induced new immunologic changes and enhanced the desensitization effect.

Changes in antibody production observed in OIT patients were consistent with other forms of allergen immunotherapy. The longitudinal trends in EW IgE and IgG4 levels were inversely related to each other. The EW IgG4 level was undetectable in the present study at baseline and increased across time before falling in 2 clinically tolerant patients once OIT was discontinued. We demonstrate for the first time, to our knowledge, that OIT's effect on IgE levels may be allergen specific because peanut and milk IgE levels did not change in 2 patients cosensitized to these foods. Although a small sample size prevents definitive conclusions, these observations provide interesting trends for future study. Long-term data from controlled studies, including detailed dietary histories and follow-up immunologic analyses, are necessary to better understand OIT-induced antibody changes.

Trends in egg-induced cytokine production suggested transient production of the immunoregulatory cytokines IL-10 and transforming growth factor β and a shift from T_H2 to T_H1 ,

but these data were not statistically significant. However, they are similar to reports from allergen immunotherapy trials and are internally consistent because IgG4 production has been correlated with IL-10 levels in this setting. We hypothesized that OIT would be associated with regulatory T-cell development. All the patients expressed an egg-specific $CD4^+CD25^{hi}$ population, but we could not identify changes across time, presumably because we did not use a specific marker (ie, FOXP3). No previous studies of egg allergy treatment have reported egg-specific cytokine production or the identification of egg-specific regulatory T cells.^{4,14–16} A more mechanistic study in a larger cohort is needed.

The small sample size and lack of a control group are limitations of this study because many children will naturally outgrow egg allergy. However, these patients were atopic, older, and had a history of convincing IgE-mediated symptoms after egg ingestion and average EW IgE levels exceeding established thresholds for predictive clinical reactivity. Similar characteristics predicted persistent egg allergy in a study of more than 850 children aged 4 to 6 years in which the median EW IgE level in persistent patients was 7.6 kU/L compared with 1.8 kU/L in those who went on to develop tolerance.⁸ Using the strictest definition of clinical tolerance, these authors calculated that 12% of patients naturally developed tolerance by age 6 years and 37% by age 10 years, for

a 25% acquisition rate across a 4-year period. By comparison, baseline median values for age and EW IgE level in the present study were 5 years and 12.5 kU/L, respectively, and the ovomucoid specific IgE level, a known predictor of persistent allergy,⁹ was also elevated; and all 6 patients developed tolerance at a median of 33 months. Taken together, these data suggest that these patients were at risk for persistent egg allergy, and although they may have naturally outgrown it, the use of egg OIT with a progressive dosing strategy is likely to be faster and more effective than waiting for tolerance to develop naturally. Even if the present patients ultimately developed natural tolerance, OIT offers the substantial protection of clinical desensitization. A large, multicenter, double-blinded, placebo-controlled egg OIT trial is under way that should have sufficient statistical power to address efficacy.

In summary, we report herein our 5-year experience with an original study of egg OIT. Using an innovative, IgE-dependent dosing strategy safely induces favorable immunologic changes and may facilitate clinical egg tolerance. Such an individualized approach may be necessary to achieve a full therapeutic response in some patients.

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