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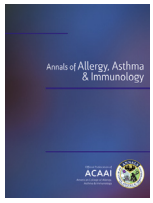

Letter

# Cytokine responses to egg protein in previously allergic children who developed tolerance naturally

Egg allergy is the second most common food allergy in childhood, with 1% to 2% of Americans affected.[1,2] Although approximately 70% of children outgrow this allergy,[3] few studies have explored the mechanism by which children naturally outgrow the allergy (natural tolerance [NT]). Most studies have compared children with allergy with those who were never allergic, have not evaluated allergen-specific responses, or have only focused on treatment inducing desensitization (oral immunotherapy). Although not previously demonstrated in food allergy, tolerance to venom has been associated with elevation of interleukin (IL)-10.[4] Our hypothesis was that peripheral blood mononuclear cells (PBMCs) stimulated with ovalbumin (OVA) in NT has increased allergen-specific IL-10 production. The objective was to determine the PBMC cytokine response to egg protein in previously allergic patients who developed NT and thus differentiate them from children with allergy.

Food allergy was defined by an allergist-immunologist as a history of IgE-mediated reaction to egg (anaphylaxis, urticaria, or significant vomiting) within 2 hours of food ingestion and positive laboratory testing (skin prick mean wheal diameter ≥4 mm larger than saline wheal and/or specific IgE [sIgE] level ≥2 kU/L). Nonallergic (control) was defined as no history of clinical reactivity to egg. NT was defined as a clinical and laboratory history of egg allergy but passing an open food challenge to 1 scrambled egg within 6 months of recruitment. The PBMCs were stimulated with or without OVA or with anti-CD3 and anti-CD28. After 48 hours, the supernatant was collected and cytokines were analyzed using multiplex assays (eMethods).

Patient characteristics, fold stimulation, and sIgE/sIgG4 ratio were compared using the Mann-Whitney or Kruskal-Wallis test. Cytokine data were logarithmically transformed, dose-dependent responses were analyzed with repeated-measures analysis of variance (ANOVA), and comparisons at specific doses were performed with ANOVA or *t* test. Data analysis was performed using SAS 9.3 (SAS Institute, Cary, North Carolina) and SPSS 14 (SPSS, Inc, Chicago, Illinois), with a 2-sided type I error rate of 5%.

Forty children (11 with NT, 20 with allergy, and 9 without allergy; median age 6 years, range 2–18 years) were recruited from a cross-sectional, case–control convenience sample (patient characteristics are listed in eTable 1, available online). Patients with NT and those with allergy were more likely to have been diagnosed with other atopy. At diagnosis, there was no difference in skin prick size or original reactions between patients with NT and those with allergy. At recruitment, there was no difference in sIgG4 among groups (median 0.45 vs 1.2 vs 1.0 kU/L, respectively, $P = .38$).

With stimulation, IL-10 was more upregulated in patients with NT and control patients using a repeated-measures approach ($P < .01$) and was the only cytokine that differentiated patients with NT from those with allergy with fold stimulation at doses of at least 10 μg/mL (Fig 1). At 100 μg/mL, the median increase was 68-fold compared with unstimulated samples.

The PBMCs from patients with NT produced significantly fewer T-helper cell type 2 cytokines (IL-4 and IL-5) than patients with allergy at baseline (Fig 1) and at every dose of OVA. Patients with NT produced similar amounts of IL-4 and slightly more IL-5 than controls. There was no dose response to OVA ($P = .41$ and .42 by repeated-measures ANOVA for IL-4 and IL-5, respectively). Patients with NT produced the most interferon-γ (IFN-γ) using repeated-measures ANOVA ($P = .02$), which was notable at doses of 1, 10, and 50 μg/mL, but was significant only in differentiating patients with NT from those with allergy with fold stimulation at 10 μg/mL (Fig 1). Other cytokines that were not significant in differentiating NT from allergy also were IL-9, IL-13, IL-6, and IL-17 (eFig1).

Median IgE/IgG4 ratio to egg white was elevated in patients with allergy vs those with NT vs those without allergy (eFig 2). The 4 patients with allergy who tolerated baked egg had decreased sIgE/sIgG4 ratios compared with other patients with allergy but had increased levels compared with patients with NT (median 5.1 vs 35.7 vs 0.4, respectively, $P = .02$ and .03). The sIgE level was similar to the sIgE/sIgG4 ratio in predicting tolerance in a receiver operating characteristic curve (area under the curve 0.97 vs 0.96).

This study is the first to address the mechanisms of food challenge-proven NT using dose-dependent cytokine responses. When comparing patients with NT with those with allergy, IFN-γ and most significantly IL-10 were the only upregulated cytokines in those with NT. Importantly, upregulation of IL-10 was antigen-specific, because the increase was seen only with OVA and did not occur with anti-CD3 and anti-CD28 stimulation. This is in contrast to research on oral immunotherapy, which is not a model of NT, that has shown no increase or transient increases in IL-10. Lack of dose-dependent T-helper cell type 2 cytokine responses to certain cytokines (IL-4 and IL-5) in patients with allergy could be secondary to high background production, obscuring the contribution of antigen-specific responses to egg (in contrast to IL-10 and IFN-γ).[5] Findings with IFN-γ have suggested that T-helper cell type 1 cytokines are involved in allergen-specific responses to food but are not clearly characteristic of NT.

This work was presented at the Pediatric Academic Societies meeting in April 2011, the American College of Allergy & Immunology meeting in October 2011, and the American Academy of Allergy, Asthma & Immunology meeting in March 2012.

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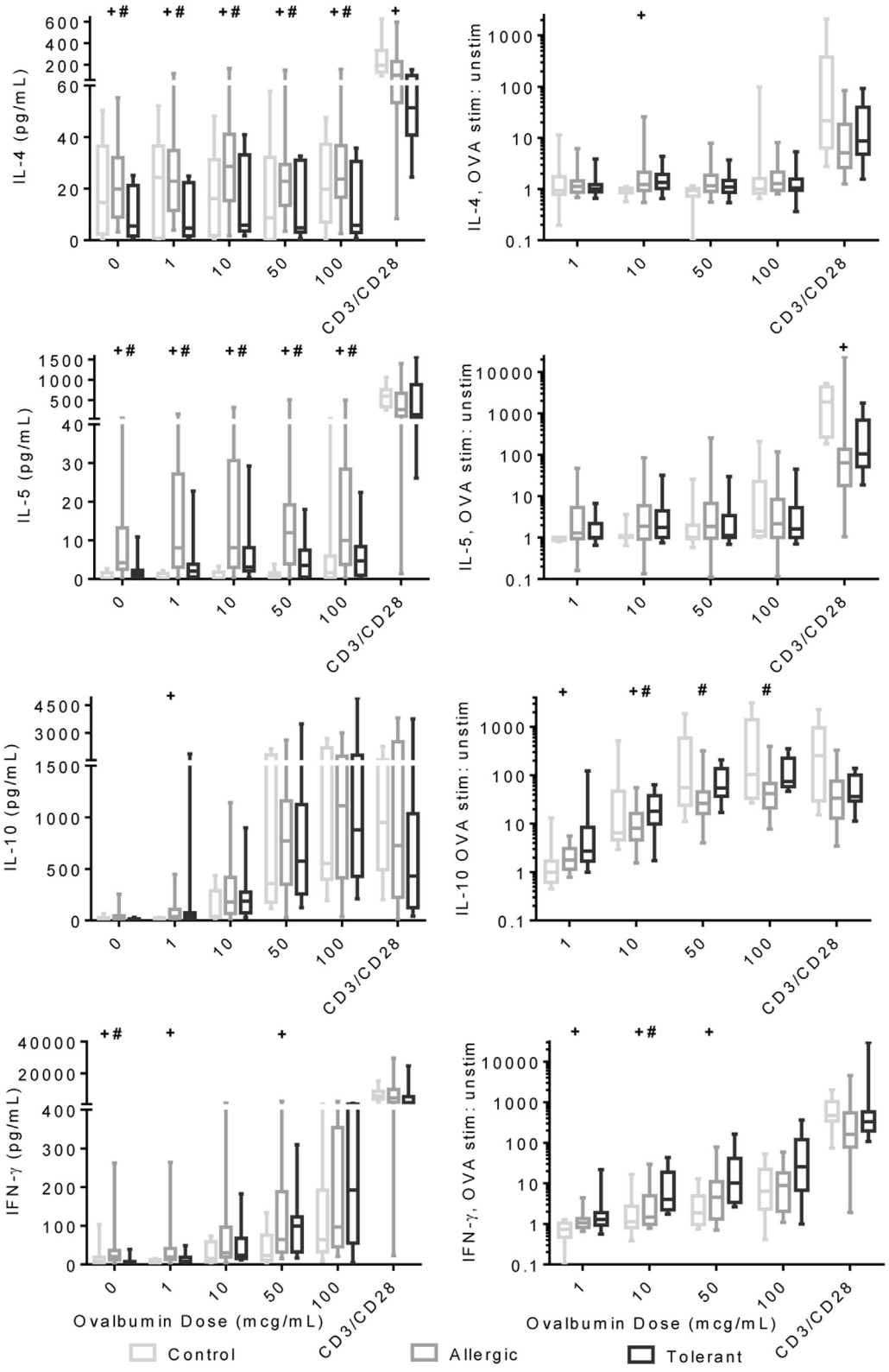


**Figure 1.** Cytokine responses to ovalbumin (OVA) in patients without allergy (control), with allergy, and with natural tolerance. Boxplots depict cytokine responses to OVA and anti-CD3 and anti-CD28 (0.2 mg/mL) after 48-hour culture of peripheral blood mononuclear cells for interleukin (IL)-4, IL-5, IL-10, and interferon-γ (IFN-γ) (*left*) and upregulation after stimulation compared with unstimulated samples (*right*). Boxplots display median values (*horizontal lines within bars*), lower and upper quartiles (*areas within bars below and above lines, respectively*), and minimum and maximum values (*whiskers*). Within-dose comparisons: $^{+}P < .05$ among all 3 groups, $^{\#}P < .05$ between allergic and tolerant groups.

It is unlikely IL-10 is the only immunomodulatory cytokine involved in NT. IL-6 showed OVA responsiveness but did not differ between patients with NT and those with allergy but was important in the response of those without allergy. This suggests a role of IL-6 in food allergen nonresponsiveness but not necessarily in the acquisition of NT. These data and those from other mechanistic