ORIGINAL ARTICLE

# Is the Quantification of Antigen-Specific Basophil Activation a Useful Tool for Monitoring Oral Tolerance Induction in Children With Egg Allergy?

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■ **Abstract**

*Objectives:* To assess modifications in baseline specific IgE- and anti-IgE- and antigen-specific–mediated basophil activation in egg-allergic children. The values were compared before and after the children completed specific oral tolerance induction (SOTI) with egg.

*Patients and Methods:* We studied 28 egg-allergic children who completed SOTI with egg. The basophil activation test and specific IgE determinations with egg white, ovalbumin, and ovomucoid were performed in all 28 children.

*Results:* A decrease in antigen-specific activation with egg white, ovalbumin, and ovomucoid was observed only at the 2 lowest concentrations used (5 and 0.05 ng/mL). Baseline activation was higher in patients with multiple food allergies and in those who developed anaphylaxis during SOTI; this activation decreased in both groups after completion of SOTI. A significant decrease was also observed in specific IgE values for egg white, ovalbumin, and ovomucoid after tolerance induction.

*Conclusions:* Food tolerance induction is a specific process for each food that can be mediated by immunologic changes such as a decrease in specific IgE values and in specific and spontaneous basophil activation.

Key words: Egg allergy. Oral tolerance induction. Basophil activation test. Specific IgE. Anaphylaxis.

■ **Resumen**

*Objetivos:* Valorar los cambios en la IgE específica y en la activación de basófilos basal, mediada por Anti-IgE y antígeno específica en niños con alergia a huevo, antes y después de finalizar el proceso de inducción oral de tolerancia.

*Métodos:* Se estudiaron 28 niños con alergia a huevo que finalizaron una inducción oral de tolerancia con este alimento. En todos ellos se realizó test de activación de basófilos e IgE específica con clara de huevo, ovoalbúmina y ovomucoide.

*Resultados:* Se produjo una reducción en la activación antígeno específica con clara de huevo, ovoalbúmina y ovomucoide únicamente con las dos concentraciones más bajas (5 y 0,05 ng/ml) empleadas. La activación basal era más alta en los pacientes con alergia alimentaria múltiple y en aquellos que desarrollaron anafilaxia en el proceso de inducción de tolerancia, disminuyendo esta activación en ambos grupos tras la finalización del proceso de inducción de tolerancia. Se observó igualmente una reducción significativa en los valores de IgE específica frente a clara de huevo, ovoalbúmina y ovomucoide tras finalizar la inducción de tolerancia.

*Conclusiones:* La inducción de tolerancia con alimentos es un proceso específico para cada alimento que puede estar mediado por cambios inmunológicos como un descenso en los valores de IgE específica, así como en la activación de basófilos tanto específica como espontánea.

Palabras clave: Alergia a huevo. Inducción oral de tolerancia. Test de activación de basófilos. IgE específica. Anafilaxia.

## Introduction

Allergy to milk and/or egg is the most common form of food allergy in childhood. While most children outgrow these allergies by the age of 4 to 6 years, a significant number do not. Treatment has traditionally consisted of avoidance of milk and/or egg for many years or even a lifetime. However, new treatments, such as specific oral tolerance induction (SOTI), administered orally [1,2] or sublingually, have emerged in recent years [3,4]. The aim of SOTI is to achieve tolerance to usual doses of the allergenic food or at least tolerance of certain doses in order to prevent reactions due to the inadvertent ingestion of small quantities hidden in other foods.

Several immunological mechanisms have been identified as possibly responsible for the development of tolerance, namely, a decrease in specific IgE [5], an increase in specific IgG4 [6,7], formation of specific regulatory T cells [7,8], and modifications in basophil response [9,10]. Whatever the reason, any immunological process would have to act either directly or indirectly on the effector cells of type I reactions: basophils and mast cells. Basophils are scarce but easily accessible cells whose activation is quantifiable by the study of the expression of flow cytometric activation markers. This application is of special interest in pediatric populations. The aim of the current study was to investigate the presence of changes in basophil reactivity in children who underwent oral immunotherapy treatment for egg allergy.

## Methods

### Participants

Twenty-eight children (20 boys and 8 girls) with egg allergy participated in the study. Their mean (SD) age was 7.5 (2.3) years. Thirteen of the children had exclusive egg allergy, while the rest were allergic to eggs and other foods. Twenty children had bronchial asthma, all due to dust mite allergy, and 10 had atopic dermatitis. To be included in the study the children had to fulfil all 4 of the following diagnostic criteria: 1) a clinical history of immediate allergic reaction to egg; 2) positive in vitro specific IgE to egg white, ovalbumin, and ovomucoid; 3) positive skin tests to egg white, ovalbumin, and ovomucoid; and 4) a positive open oral challenge test with scrambled egg. This fourth criterion was not required in 6 children who had developed anaphylaxis following the ingestion of egg in the previous months.

The study was approved by the local ethics committee, and all the parents provided informed consent before their children were enrolled in the study.

### In Vivo Tests

Skin prick tests were performed with commercial extracts of egg white (10 mg/mL), ovalbumin (10 mg/mL), and ovomucoid (10 mg/mL) (Laboratorio BIAL) following the recommendations of the European Academy of Allergy and Clinical Immunology [11]. The skin tests were regarded as positive if the wheal diameter was at least 3 mm. A positive control of histamine 10 mg/mL and a negative control of saline 0.9% were included.

The open food challenges were carried out 15 days before the SOTI protocol was started. The challenges were carried out with incremental doses of scrambled egg, starting with a quantity of 1/1000 of an egg and ending, 8 steps later, with the ingestion of a whole egg. All the doses were administered at 20-minute intervals in the hospital.

SOTI with egg consisted of the administration of incremental doses of raw egg white following the protocol described by Patriarca et al [12]. The egg could be administered in water or orange juice. The first dose was administered in the hospital and the subsequent doses were administered at home on a daily basis for 1 week. Every week the dose was increased, and this increased dose was administered in the hospital. Finally, tolerance to 1 raw egg was verified. Subsequently, the children had to continue consuming 1 scrambled egg at least 3 days a week in addition to any amount of other foods containing egg (cakes, omelettes, etc.).

### In Vitro Tests

#### Specific IgE

Specific IgE to egg white, ovalbumin, and ovomucoid was measured with the ImmunoCAP FEIA system (Thermo Fisher Scientific) according to the manufacturer's instructions. Values higher than 0.35 kU/L were considered positive.

#### Basophil Activation Test

For flow cytometry, peripheral blood was collected in ACD tubes. Aliquots were resuspended with 100 mcg HEPES calcium buffer containing IL-3 (10 ng/mL). Then, primed blood samples were incubated with egg white, ovalbumin, and ovomucoid antigens (Laboratórios BIAL) at 8 different concentrations (1/10 dilutions from an initial concentration of 5 mg/L to a final concentration of 0.5 ng/mL). Cells were then incubated for 20 minutes at 37ºC. A monoclonal anti-IgE receptor antibody (1 mcg/mL) (Buhlmann Laboratories) was used as a positive control and washing solution was used as a negative control. Basophils were double-labeled with anti-CD63 antibody and anti-IgE FITC-labeled antibody. After erythrocyte lysis, washing and centrifugation, the supernatants were discarded. Samples were then studied for expression and upregulation of CD63. Flow cytometric analysis of basophil activation was performed at 488 nm with a FACS-Canto flow cytometer (BD Biosciences).

The BAT result was considered positive when allergen stimulation resulted in the activation of greater than 10% of basophils and when the stimulation index (percentage of activated basophils after stimulation/percentage of activated basophils at baseline) was 2 or higher. A basophil activation of over 10% without stimulation (baseline stimulation), was considered baseline activation. An activation level of less than 15% after stimulation with anti-IgE and with allergen was considered a nonresponse, and was excluded.

The initial extraction was performed on the same day, just before SOTI was started, and the final extraction was performed after 15 days, on completion of SOTI.

## Statistical Analysis

The statistical analysis was carried out in SPSS (version 16 for Windows). Quantitative data were expressed as means and SD and qualitative data as frequencies. Means were compared with the Wilcoxon test for paired data. The $\chi^2$ test was used for the comparison of qualitative variables. In all cases the comparisons were bilateral, with a *P* value of less than .05 considered to be statistically significant.

## Results

Of the 28 patients with egg allergy initially included in the study, 27 tolerated the ingestion of a whole egg on completion of SOTI. The other patient achieved tolerance of half an egg.

In the course of SOTI, 5 patients experienced an anaphylactic episode that did not require interruption of the process. One of these children experienced a second episode after eating scrambled egg 1 week after finalization of SOTI. In this case, SOTI was interrupted as the patient subsequently failed to tolerate minimal doses of egg. Six children developed systemic reactions (disseminated urticaria-angioedema and abdominal pain) that subsided with corticosteroids and oral antihistamines. Interruption of SOTI was not necessary, and all 6 children completed the process successfully. The remaining patients did not present any systemic symptoms during SOTI and tolerated the ingestion of a raw egg and subsequently, of a scrambled egg at least 3 days a week.

In 3 of the 28 patients, it was not possible to evaluate the BAT due to nonresponse to stimulation with anti-IgE in either the pre-SOTI or post-SOTI tests. Of the valid BATs performed before SOTI, 11 (44%) showed baseline activation (>10% of basally activated basophils). Baseline activation was observed in 11 (44%) of the BATs considered valid and performed after completion of SOTI.

The BAT showed reductions in activation values after completion of SOTI for the 2 lowest concentrations (5 ng/mL and 0.05 ng/mL) of egg white, ovalbumin, and ovomucoid, and for the 0.05-µg/mL concentration of ovalbumin (Table). No significant differences were observed for the higher concentrations of allergens on comparing pre- and post-SOTI data (Table). Baseline activation was significantly lower for post-SOTI values compared with pre-SOTI values (*P*<.05). Pre-SOTI baseline activation was more common in patients with egg allergy and other food allergies (60% vs 15%). This group of children with multiple food allergies also had higher mean (SD) baseline activation values than children with egg allergy only (35.7% [30%] vs 10% [10.6%], *P*<.05). Nevertheless, on completion of SOTI, baseline activation diminished in both groups (13% [9.7%] vs 5.8% [5.3%]), but the differences remained significant (*P*<.05). There were no significant differences in activation levels after stimulation with anti-IgE before and after SOTI (data not shown).

Likewise, 4 of the 5 patients who developed anaphylaxis during SOTI presented baseline activation, while the fifth presented values very close to baseline activation (9.2%). Mean baseline activation values were clearly higher in the 5 patients who developed anaphylaxis during SOTI than in

**Table.** Specific Basophil Activation Test With Egg Proteins Before and After Egg-Specific Oral tolerance Induction[a]

| | EW, mg/mL | | | EW, µg/mL | | | EW, ng/mL | | OVA, mg/mL | | | OVA, µg/mL | | | OVA, ng/mL | | OM, mg/mL | | | OM, µg/mL | | | OM ng/mL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 0.5 | 0.05 | 5 | 0.5 | 0.05 | 5 | 0.5 | 5 | 0.5 | 0.05 | 5 | 0.5 | 0.05 | 5 | 0.5 | 5 | 0.5 | 0.05 | 5 | 0.5 | 0.05 | 5 | 0.5 |
| PRE-SOTI | 23.3 (22.6) | 39.2 (25.7) | 36.4 (27.2) | 40.3 (27.8) | 48.8 (26) | 48.1 (24.4) | 33.1 (22.4)[b] | 22.5 (20.7)[b] | 31.3 (26.1) | 36 (27.7) | 40.7 (25.9) | 41.8 (27.1) | 46.4 (27.2) | 49.7 (26.2)[b] | 32.6 (22.2)[b] | 29.1 (26.1)[b] | 21.1 (23.1) | 35.7 (28.1) | 37.5 (23.4) | 38.9 (27.5) | 37.2 (25.9) | 41.6 (23.8) | 38 (23.3)[b] | 26.5 (21.5)[b] |
| POST-SOTI | 25.7 (30.8) | 37.4 (30.7) | 35.4 (29.5) | 31.8 (26.4) | 33.6 (28.5) | 29.7 (30.1) | 14 (17.7)[b] | 10.9 (15.8)[b] | 30.2 (33.3) | 32.1 (29) | 32 (29.5) | 34.8 (27.5) | 37.8 (28.5) | 31.6 (30.7)[b] | 13.9 (16.6)[b] | 11.7 (12.9)[b] | 28.9 (32.8) | 27.8 (26.9) | 24.3 (25.9) | 31.2 (29.9) | 28.7 (28.2) | 30.2 (29.5) | 22.8 (26.9)[b] | 14 (20.1)[b] |

Abbreviations: EW, egg white; OVA, ovoalbumin; OM, ovomucoid.
[a]Values expressed as mean (SD).
[b]Values with significant differences before and after induction tolerance.

those who did not (53.8% [30.3%] vs 17.1% [20.3%], *P*<.05). On completion of SOTI, baseline activation levels was very similar in the groups with and without anaphylaxis during SOTI (10.1% [8.8%] vs 8.6% [9.5%]). We found no significant differences between the 2 groups with regard to specific antigen activation of basophils.

Specific IgE values dropped significantly on completion of SOTI with respect to pre-SOTI values for the 3 egg allergens, as follows: egg white, 11.1 (13.5) kU/L vs 4.7 [(5.1) kU/L; ovalbumin, 8.1 (7.5) vs 2.6 (2.6) kU/L; and ovomucoid, 10.9 (18.2) vs 3.4 (3.4) kU/L (*P*<.05 for all comparisons). The patients who experienced an anaphylactic episode during SOTI had higher specific IgE levels before starting SOTI for the 3 egg allergens: egg white, 29.5 (24.7) vs 8 (8.4) kU/L; ovalbumin, 18.9 (7.6) vs 6.2 (6.2) kU/L; and ovomucoid, 38.4 (37.8) vs 5.7 (5.8) kU/L (*P*<.05 for all comparisons). Nevertheless, these significant differences were no longer observed on completion of SOTI: egg white, 6.3 (3.6) vs 4.5 (5.5) kU/L; ovalbumin, 4.2 (3.8) vs 2.3 (2.5) kU/L; and ovomucoid, 6 (2.6) vs 3.1 (3.5) kU/L.

## Discussion

Food-allergic individuals are continuously at risk of accidental exposure that can induce allergic reactions, including life-threatening anaphylaxis, and this fear can have a notable impact on their quality of life. Food oral immunotherapy is a new therapeutic approach that can induce tolerance of or desensitization to different foods such as egg, milk, and peanuts. In egg-allergic patients, oral immunotherapy is associated with a median success rate of over 80% [13].

In a previous study, significant decreases in basophil activation were recorded for SOTI with concentrations of 500, 50, and 5 ng/mL of egg white, ovalbumin, and ovomucoid [10]. In our patients, however, these decreases were observed at lower concentrations. This could be due to the different tolerance induction protocols used (raw vs scrambled egg), to the later extraction of post-SOTI samples in the other study (1-4 months after completion of SOTI), or to the presence of higher specific IgE values in our series: egg white 11.2 kU/L vs 2.98 kU/L; ovalbumin 8.3 kU/L vs 2.33 kU/L, and ovomucoid 10.8 kU/L vs 2.53 kU/L. In another study involving patients with similar egg-specific IgE levels to those in our series (10.3 kU/L), the decrease in activation of antigen-specific basophils occurred at similar concentrations to those observed in our series of patients (0.01 and 0.1 ug/mL egg white) [14]. This observation suggests that it is important to analyze basophil response to a wide range of concentrations of the food involved, as this response can be influenced by specific circulating IgE levels and surface-bound IgE on the basophils, and/or by changes in the number of IgE receptors on the basophil surface. The most suitable concentrations for children with more serious symptoms or with higher specific IgE levels are probably lower. Allergen response in the activation of basophils appears to typically follow a bell-shaped distribution [15], and accordingly, high concentrations would nullify the activation of basophils, as has already been described, for example, with the release of mediators [15], and as was seen in our study. This would seem to indicate the need to use a wide range of antigen concentrations in studies of basophil kinetics after SOTI in patients with food allergies.

The decrease in specific antigen activation observed in our study may have various explanations, including a decrease in specific IgE levels, which occurred for all allergens studied in our patients, and other immunological phenomena that occur during SOTI, such as an increase in specific IgG4, which can compete with IgE for binding to the antigen, the formation of antigen-antibody complexes with sufficient avidity to interact with the inhibitory receptors FcgammaRIIb [16], or mechanisms of desensitization or downregulation that occur in basophils after IgE-mediated reactions [17,18].

We observed that the decrease in basophil activation was not only antigen-specific, but was also manifested in spontaneous (baseline) activation, without allergen stimulation, as this also decreased after SOTI. Furthermore, the decrease occurred more frequently in patients with allergy to several foods, and it may have been due either to a decrease in the releasability of basophils in these patients [19] or to the occurrence of immunological responses that impede the activation of basophils following stimulation with low concentrations of allergen, or to the fact that they are low-affinity bindings that can occur in vitro, but not in vivo. This theory could perhaps explain the findings published years ago on the high release of plasma histamine and the increase in the expression of basophil activation markers observed in patients with food allergy with chronic exposure to foods [20,21].

Recently, Gernez et al [22] and Ford et al [23], respectively, observed greater spontaneous basophil activation in nut-allergic patients and patients with more severe clinical milk reactivity. Their findings are supported by ours, as 80% of the children with anaphylaxis in our study had high baseline activation, both quantitatively and percentagewise, and furthermore, these levels were significantly higher than those observed in children who tolerated SOTI without anaphylaxis. After SOTI, this increase in baseline activation decreased, regardless of tolerance of the induction process. A similar observation was reported in the study by Wanich et al [9], in which children who regularly consumed and tolerated heated milk presented a reduction in baseline basophil activation values and stimulation with anti-IgE. This decrease in anti-IgE–induced activation was not reproduced in our sample.

In our series, we also observed a decrease in specific IgE levels after the finalization of SOTI with egg white, ovalbumin, and ovomucoid. This supports previous findings [24-27] and has also been described in desensitization protocols with other foods such as milk [28-30], peanuts [7], and wheat [31].

In short, SOTI produces a reduction in basophil activation after allergen stimulation as well as in baseline basophil activation (ie, activation without antigen). These facts seem to be the result of the action of different mechanisms that can cause tolerance or desensitization in these individuals in terms of the behavior of the effector cell (in this case the basophil), and which are expressed in numerous ways, such as a reduction in the size of skin test wheals, a reduction in specific IgE figures, production of regulatory T cells, and a decrease in circulating IL-3.

In our opinion, the BAT constitutes a useful tool for the in vitro monitoring of children who food allergy who undergo SOTI.

*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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