

Ara h 2 basophil activation test does not predict clinical reactivity to peanut



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Clinical Implications

- Basophil activation test has emerged as a powerful tool to predict clinical reactivity to allergens. However, as illustrated here for peanut allergy, caution should be taken because basophil stimulation with global extract and molecular allergens may lead to opposite results.

TO THE EDITOR:

Peanut allergy (PA) concerns up to 2% of children in France. Until recently, its management consisted solely in strict avoidance of peanut. Such a diet is particularly restrictive for children and their family, due to a ubiquitous presence of peanut (or small trace amounts of peanut) in our daily environment and in our alimentation. Hence, unintended exposure to peanut in patients with PA leads regularly to life-threatening anaphylactic reactions. Recently, several groups have reported the possibility of reacquisition of tolerance to peanut in allergic patients, thanks to specific oral or sublingual immunotherapy (SIT) protocols.^{1,2} Oral food challenge (OFC) is the gold standard for predicting the threshold of clinical reactivity (to assess whether a patient is likely to react or not to trace amounts of peanut), or the efficacy of SIT protocols. However, these provocation tests are not riskless for the patients, and are time consuming. There is thus a need for a safe and reliable biological marker mimicking the clinical allergic reaction. The detection of allergen specific IgE (sIgE) has been evaluated in this context, and has been revolutionized by the development of detection assays based on molecular allergens instead of the traditional whole global extract.³ Detection of molecular allergen sIgE could provide higher accuracy in diagnosing PA and thus could improve the clinical relevance. Particularly, the level of sIgE Ara h 2 was reported to be discriminative in identifying patients with PA from patients sensitized to whole peanut extract but not clinically reactive to peanut.⁴ Recently, several reports have demonstrated the performance of CD63 basophil activation test (BAT) (to whole extract peanut) to predict this sensitivity.^{5,6} More precisely, its parameter CDsens (defined as $1/LC_{50} \times 100$, where LC_{50} is the lowest concentration of allergen giving 50% of the maximum activation of basophil⁷) was the better reflect of the lowest amount of peanut necessary to induce an allergic reaction.

In line with these first reports, we have extensively evaluated BAT in predicting clinical threshold reactivity to peanut during a randomized, double-blinded, placebo-controlled SIT protocol (whose methods have been described elsewhere⁸). We focus here only on the build-up phase of this study. Briefly, all patients screened to enter the protocol experienced a clinical reaction within 1 hour after the ingestion of peanut, presented positive skin prick test to peanut (3 mm greater than control), and displayed serum sIgE to whole extract peanut (f13) over 12 kUI/L and to Ara h 2 over 5.8 kUI/L (ImmunoCAP, Phadia 250, Phadia ThermoFischer, Uppsala, Sweden). The inclusion step included a double-blinded OFC (DBOFC) and a BAT to whole extract peanut and Ara h 2. BAT were performed using Flow-CAST assay to measure CD63, and we added the CD203c Reagent Set (Bühlmann Laboratories, Schönenbuch, Switzerland). Tests were performed as the manufacturer's recommendations, within 4 hours after sampling. Allergens (Bühlmann Laboratories) were used pure (25 ng/mL for f13 and 4.4 ng/mL for Ara h 2), and diluted to 1/10, 1/100, 1/1,000, 1/10,000. At this inclusion step, 70 patients were screened: 26 patients were tolerant to 2 g of peanut and they were thus not included in the remainder of the study, whereas 44 react to less than 2 g of peanut and thus enter the protocol. The f13 and Ara h 2 sIgE of these 2 groups were not significantly different, as shown in Figure E1, available in this article's Online Repository at www.jaci-inpractice.org. The build-up phase (24 weeks) consists in oral escalating immunotherapy up to a maximum of daily level of 2 g of peanut. Desensitization was then assessed by a second DBOFC to 2 g cumulated of peanut. Tolerance to 2 g of peanut was achieved in 16 of the 18 patients treated with peanut, and 1 of the 9 patients treated with placebo ($P < .001$) (J. L. Fauquert, MD, unpublished data, 2017).

Our results first confirm that CDsens of whole peanut BAT is strongly linked to the eliciting dose of peanut, either using CD63 (Figure 1, A) or CD203c (Figure 1, B). We report these data both along the first DBOFC before the inclusion (between reactors and nonreactors using the cutoff of 2 g of peanut), and along the longitudinal comparison of the first DBOFC (before SIT) and second one (after SIT). We thus demonstrate the ability of BAT to monitor SIT protocols, and one can position BAT as a safe and noninvasive tool helping the decision of realizing an OFC.

Surprisingly, *in vitro* stimulation of basophils with Ara h 2 leads to strongly different results. Hence, no difference was observed in our study according to eliciting dose, neither at the inclusion nor during the longitudinal follow-up of patients during SIT (Figure 1, C and D). These results are unexpected in line with the interest of molecular allergens in the detection of sIgE. Our original data about Ara h 2 BAT raise thus many questions regarding the patient state of immune tolerance. Indeed, all patients are sensitized to Ara h 2, but their basophils, when stimulated *in vitro* with Ara h 2, do not reflect their tolerance state to peanut *in vivo*. One or several other factors than sIgE influence thus their clinical tolerance state to peanut. In a recent report, Santos et al⁹ have elegantly demonstrated the inhibitory role of IgG4 levels in the activation of basophils. One

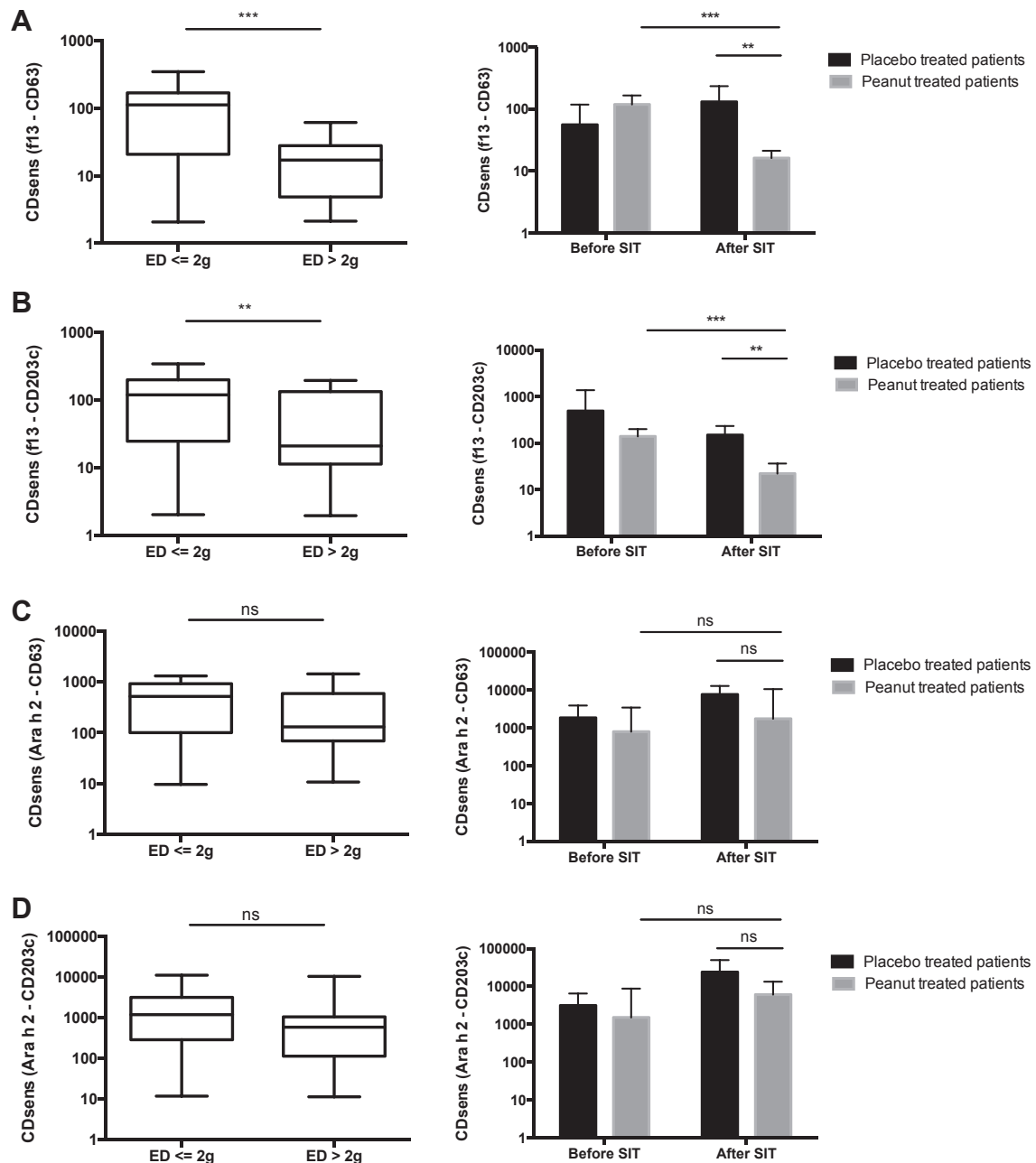


FIGURE 1. CDsens of basophil activation test to whole extract peanut (**A**, **B**), but not to its major allergen Ara h 2 (**C**, **D**), predicts threshold of clinical reactivity to peanut, using both CD63 and CD203c. ** $P < .01$ and *** $P < .001$ for the comparison between groups by using the Mann-Whitney U test (eliciting dose: “ ≤ 0.2 g of peanut” [$n = 44$] or “ > 2 g of peanut” [$n = 26$]), the paired Wilcoxon test (time effect) and the Kruskal-Wallis test (treatment effect: $n = 18$ peanut-treated and 9 placebo-treated patients). ED, Eliciting dose; SIT, specific immunotherapy.

can hypothesize that the quantity of serum IgG4 is sufficient to neutralize the proportion of ara h2 contained in the traditional whole global extract peanut, but is insufficient to neutralize ara h2 present in the preparation of molecular allergens. Consistent with the hypothesis of an effect mediated by IgG4, we have observed a significant increase of the Ara h 2 sIgG4/sIgE ratio during the build-up phase of our SIT protocol (J. L. Fauquert, MD, unpublished data, 2017; and Figure E2, available in this article's Online Repository at www.jaci-inpractice.org). The

removal of plasma to whole blood before the realization of BAT would avoid this issue, and this would verify this hypothesis.

In conclusion, our study confirms that whole extract peanut BAT is associated to the eliciting dose of peanut in allergic patients. This assay is a reliable, safe, noninvasive tool to assess the tolerance of a patient to a given amount of an allergen, and could thus allow the monitoring of the SIT protocol, before realization of OFC. To our knowledge, this is the first report of BAT performance with Ara h 2 for predicting clinical reactivity to

peanut along SIT protocols, and data reported here raise numerous questions concerning the use of dominant allergens in this context. Answers to these points will improve the understanding of the underlying mechanisms of immune tolerance to an allergen.

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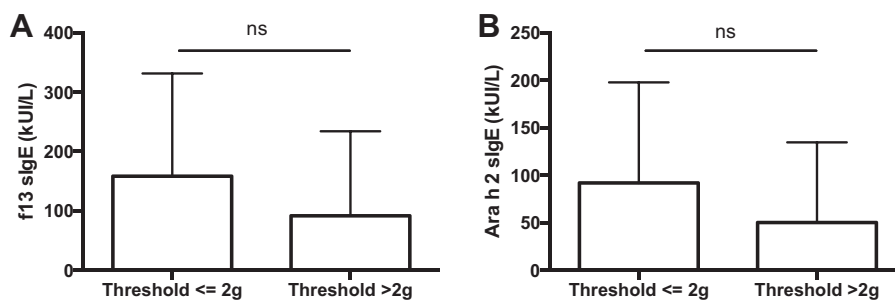


FIGURE E1. A, f13 and B, Ara h 2 specific IgE (sIgE) are not significantly different between patients who do not react to 2 g of peanut challenge and those who react to doses <2 g.

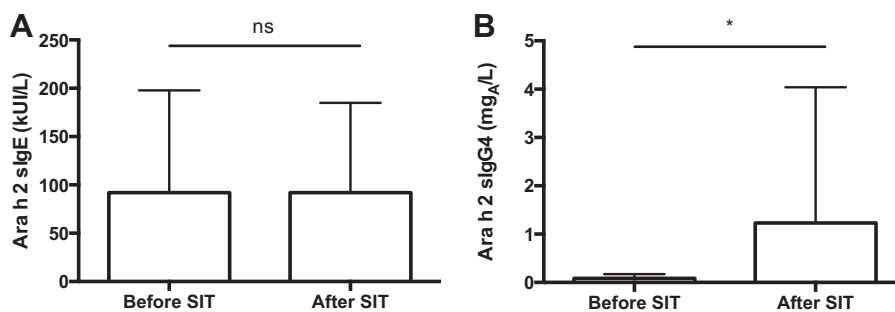


FIGURE E2. A, Ara h 2 specific IgE (sIgE) are not significantly different before and after the immunotherapy build-up phase, whereas B, Ara h 2 specific IgG4 (sIgG4) significantly increase during the immunotherapy build-up phase. * $P < .01$.