

ORIGINAL ARTICLE

Clinical Allergy

Evaluation of the basophil activation test and skin prick testing for the diagnosis of sesame food allergy

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Summary

Background: The prevalence of sesame food allergy (SFA) has increased over recent years, with the potential of anaphylactic reactions upon exposure. Oral food challenge (OFC) remains the diagnostic standard, yet its implementation may be risky. Commercial skin prick tests (SPT) have a low sensitivity. Investigation of alternate diagnostic methods is warranted.

Objective: To evaluate the utility of SPT and the basophil activation test (BAT) for SFA diagnosis.

Methods: Eighty-two patients with suspected SFA completed an open OFC to sesame or reported a recent confirmed reaction. Patients were administered skin prick tests (SPT) with commercial sesame seed extract (CSSE) and a high protein concentration sesame extract (HPSE) (100 mg/mL protein). Whole blood from 80 patients was stimulated with sesame seed extract (40–10 000 ng/mL protein) for BAT), assessing CD63 and CD203c as activation markers.

Results: Sixty patients (73%) had IgE-mediated reactions to sesame, and 22 (27%) did not react. Receiver operating characteristic (ROC) curve analysis demonstrated an area under the curve (AUC) of 0.87 for HPSE-SPT and 0.66 for CSSE-SPT. At 1000 ng/mL of sesame protein, induction of CD63 and CD203c was weakly but significantly associated with OFC eliciting dose by rank (Spearman's $\rho = -.42$ ($P < .01$) and $-.35$ ($P < .05$) for CD63 and CD203c, respectively). By ROC analysis, the AUC was 0.86 for CD63 and was 0.81 for CD203c sesame-induced basophil expression. Using HPSE-SPT as a first test to definitively diagnose ($n = 24$) or rule-out ($n = 5$) SFA and BAT as a second test to diagnose the remainder results in the correct classification of 73 of 80 (91%) patients, leaving one false negative and 4 false positive patients. Two BAT non-responders remain unclassified by this algorithm.

Conclusions & Clinical Relevance: While prospective cohort validation is necessary, joint utilization of BAT and SPT with HPSE extract may obviate the need for OFC in most SFA patients.

KEYWORDS

basophil activation test, oral food challenge, sesame food allergy

1 | INTRODUCTION

The identified incidence of sesame seed food allergy (SFA) is increasing world-wide and contributes to significant morbidity. It is estimated that SFA affects 0.1%-0.2% of the population, in areas where the food is available (for review, see Dalal¹). It is presently a growing concern in the United States with the now widespread practice of adherence to a "Mediterranean diet." Sesame is used in the preparation of various foods including breads, crackers, granola type bars, sauces (Tahini) and confectionaries (Halva). Of some concern, many foods may contain hidden sources of sesame, deriving from the seed or oil. The latter contains the oleosins (Ses i 4 and Ses i 5), implicated in anaphylactic reactions after sesame seed exposure in susceptible individuals.²

Unlike allergens such as milk and egg, tolerance to sesame seed develops in only 20%-30% of patients.^{3,4} While only a few cases of mortality to sesame seed in allergic patients have been reported, the occurrence of anaphylaxis to sesame is not uncommon⁵ highlighting the need to properly identify patients with SFA. As with other foods, to definitively diagnose SFA an oral food challenge (OFC) has been the standard. However, due to the challenges in its implementation, there are circumstances in which an OFC is not practical or advisable.⁶ Furthermore, other diagnostic tests could prove useful in predicting the OFC outcome which might inform regarding its pace and dosing. Regarding alternative methods for diagnosis, a convincing history of an immediate reaction along with a positive skin prick test (SPT) was found to have a positive predictive value in 14 of 16 (88%) of cases³ Independent of an recent immediate reaction, however, neither SPT nor specific IgE (slgE) were proven to have a dependable positive predictive value (PPV).^{3,7-10} In addition, the source of the sesame allergen used for testing may influence the results. The reported negative predictive value of SPT, for example, was low with commercial extract and high with raw sesame extract.¹¹ Yet despite the equivocal results that are often provided by SPT, it remains a popular allergy diagnostic test, due in part, to its non-invasiveness, and its relative ease of performance. These qualities would encourage the development of strategies and methods to improve the diagnostic performance of SPT.

The basophil activation test (BAT) has been used for diagnosis for a number of allergenic foods, including cow's milk,¹²⁻¹⁴ egg^{13,15} and peanut.^{15,16} Several investigators have described the usage of BAT as related to SFA,¹⁷⁻¹⁹ but none have investigated its utility as a diagnostic tool. Here, we report on the first large-scale evaluation of the usage of BAT and SPT as complementary diagnostic tools for SFA, in a patient population with suspected allergy confirmed or disproven by OFC or recent reaction.

2 | METHODS

2.1 | Patients

This study was performed at the Institute of Allergy, Immunology and Pediatric Pulmonology of the Assaf-Harofeh Medical Center,

(Beer Yaakov, Israel) between April 2013 and February 2017). Patients (n = 84) were referred to the centre for a SFA diagnosis based on prior clinical histories of reaction to sesame seed or evidence of sesame seed sensitization based on prior SPT or sesame-specific IgE tests. Two patients, one of whom did not complete the challenge, and one with an equivocal challenge result, were excluded. SPTs were performed on the day of sesame allergy evaluation. BAT was performed on 80 of the 82 remaining patients. For 58 of them, BAT was performed on the same day or prior to OFC. For 15 patients the OFC preceded BAT, and therefore diagnosis was reconfirmed by a sesame allergic reaction, or ingestion of sesame for non-allergic patients, within a year of basophil testing. Seven other patients were not challenged, as they had sesame allergic reactions within a year prior to BAT. Asthmatics were evaluated by spirometry prior to challenge and were included if clinically stable. Patients were off of short-acting antihistamines for at least 72 hours prior to examination and 1 week for longer acting antihistamines. The research was approved by the Helsinki Review Board of the Assaf Harofeh Medical Center and all patients or their providers signed written consent.

2.2 | Preparation of allergenic extracts

A low fat, high protein (46%) sesame flour (Sukrin) was dissolved in water to a concentration of 100 mg/mL of protein. This high protein sesame extract (HPSE) preparation was used for oral food challenges for doses <240 mg of protein, as well as for the standardized skin prick tests, as described below. For the BAT assay, whole sesame seeds were ground and pestled in water and centrifuged. The protein concentration of the supernatant (containing protein and lipid components) was quantified (DC Protein Assay, Bio-Rad, Berkeley, CA, USA) and 1 mg/mL stocks were stored at -20°C.

2.3 | Oral food challenges

OFC were performed as graded open challenges of increasing increments every 15 minutes following each initial dose of 0.3 mg, 3 mg, 15 mg, and then 30-minute intervals following each subsequent dose of 30, 60, and 240 mg. Following the 480 mg dose, the final 2 doses of 1000 and 4000 mg were spaced at intervals of 45 and 60 minutes respectively, with a 90 minute observation period following the final dose. (Sesame OFC protocol can be found in Table S1 in the online repository). Tahini paste (240 mg protein/g) was used for doses ≥240 mg sesame seed protein. OFC was evaluated according to the proposed modified systemic allergic reaction (SAR) grading system.²⁰ A positive OFC was defined by the presence within 2 hours after administration of the dose of either visible skin or mucosal changes, such as urticaria, rhinitis, wheezing, abdominal symptoms such as persistent or severe abdominal pain or vomiting or a significant decline in blood pressure values in comparison to baseline measurements. The OFC was continued in patients complaining of oral pruritus or transient abdominal discomfort, in the absence of any other symptoms.

2.4 | Standardized skin prick tests

Skin prick tests to commercial sesame seed protein (1:10 w/v, ALK-Abello, Port Washington, NY, USA), and to sesame extract prepared from a low fat, high protein (46%) sesame flour (Sukrin) were performed. The sesame flour-derived extract was dissolved in water to a concentration of 100 mg/mL of protein and frozen at -20°C . Fresh aliquots were thawed weekly and stored at 4°C for daily use. SPTs were performed on the volar surface of the forearm with single peak lancets (Heinz Herenz, Hamburg, Germany). The wheal and flare sizes were measured after 15 minutes, as previously described.²¹ Histamine 1 mg/mL (ALK-Abello) was used as the positive control.

2.5 | Basophil activation test

Whole blood was drawn from patients using a winged infusion set and syringe and placed into Vacutainer tubes. The blood was then diluted 1:1 in RPMI 1640 (Biological Industries, Beit HaEmek, Israel), containing 1 ng/mL of recombinant human IL3 (R&D Systems, Minneapolis, MN, USA), sesame seed protein supernatant at concentrations ranging from 40–10 000 ng/mL and the fluorochrome-conjugated antibodies, fluorescein isothiocyanate-anti-human CD123 (clone 6H6), R-Phycoerythrin-Cy7 anti-human HLA-DR (clone LN3) and R-Phycoerythrin anti-human CD203c (clone NP4D6), all purchased from Thermo Fisher (Waltham, MA, USA). Allophycocyanin anti-human CD63, (clone H5C6) was purchased from BioLegend (San Diego, CA, USA). Baseline stimulation tubes were set up in the presence of IL3, but without the addition of sesame protein. Positive control stimulations were with 1 $\mu\text{g/mL}$ anti-human IgE (rabbit polyclonal affinity purified, Bethyl Laboratories, Montgomery, TX, USA), 0.5 $\mu\text{g/mL}$ purified mouse monoclonal anti-human Fc ϵ Receptor (clone AER-37 (CRA1), Thermo Fisher) or 1 $\mu\text{mol/L}$ N-Formylmethionine-leucyl-phenylalanine (fMLP, Calbiochem, San Diego, CA, USA). All tubes were incubated for 15–20 minutes at 37°C . Afterwards, the stimulation reactions were stopped with addition of 2 mmol/L EDTA on ice for 10 minutes. Erythrocytes were lysed (Thermo Fisher) in the presence of 2 mmol/L EDTA at room temperature. Cells were centrifuged ($500\times g$, 4°C , 8 minutes), and resuspended in FACS buffer (Phosphate-buffered saline with 1% Bovine Serum Albumin and 2 mmol/L EDTA). Cells were acquired on a flow cytometer (Beckman FC-500, or Navios) and basophils identified as CD123⁺ and HLA-DR⁺ cells. The induced expression of CD63 and CD203c was quantitated in approximately 300 identified basophil cells per sample. Analysis was performed using Flowing Software 2.5.1 (Turku Centre for Biotechnology, Finland), by an individual blinded to patient allergic status. IL3 was added as a basophil survival factor; in initial experimentation, no consistent IL3-dependent influence in allergen-specific basophil reactivity was noted (Table S2). As such, the values provided are the percentages of basophils expressing CD63 or CD203c in the presence of stimulation above the levels of patient basophil expression of these markers in RPMI+ IL3 alone.

Three patients whose basophils did not respond to either the anti-human Fc ϵ Receptor or anti-human IgE stimulations (<10%

increase in CD63 expression compared to incubation with RPMI 1640+ IL3 alone), were considered non-responders¹⁵ and excluded from BAT analysis for generation of the ROC curve.

2.6 | Statistics

Group differences were assessed using the WINPEPI portal²² by two-tailed Mann-Whitney U analysis for ordinal variables or by chi-square test for categorical variables. Correlation was assessed by Spearman's rho test using the VassarStats website (<http://www.vassarstats.net>). Results yielding *P*-values < .05 were deemed significant. Receiver operating characteristic (ROC) analysis²³ was used to evaluate the use of BAT and SPT for distinguishing between OFC-positive and negative patient populations. The true positive (sensitivity) and false positive (1-specificity) rates were determined for each test value, and the resulting curve was plotted using MedRoc 2.0 software (<https://stenstat.com/MedRoc/MedRoc.htm>), which was also used to determine the area under the curve (AUC) and its 95% confidence interval. Test critical values corresponding to the points closest to the upper left-hand corner of each ROC curve²⁴ were considered the optimal critical values.

3 | RESULTS

3.1 | Patient population

Eighty-two patients were analysed. The study population included 51 males and 31 females. Sixty patients were children (under 18 years old) and 22 were adults. Forty-two (53%) of the patients were asthmatic, and 35 (43%) reported other food allergies. Sixty (73%) of the patients were considered SFA, either by a positive OFC (*n* = 53) or by reaction to sesame occurring within the prior 12 months (*n* = 7), and 22 (27%) did not react after challenge. No significant differences were observed between the patients with SFA and the non-allergic patients, with regard to age at diagnosis, gender, other food allergies, or prevalence of asthma (Table S3). The median eliciting dose (ED) in the OFC+ patients was 180 mg (range: 3–4000 mg). Symptoms associated with a positive OFC were cutaneous (29%, 15/52), eye (23%, 12/52), gastrointestinal (65%, 34/52), and respiratory tract (upper and lower) (46%, 24/52). No patients had non-responding asthma or upper airway obstruction. Overall, 25% (13/52) of patients experienced grade 1 reactions, 19% (10/52) had grade 2 reactions, and 56% (29/52) had grade 3 reactions during OFC; no patients experienced reactions grade 4 or higher.²⁰ Table S4 details the eliciting doses, reactions, and treatments in patients with positive challenges.

3.2 | Utility of skin prick tests for SFA diagnosis

We examined the utility of 2 preparations for SPT to discriminate between the allergic and non-allergic patients. Using a commercial sesame seed extract (CSSE), the median wheal generated was 5 mm

(Interquartile range (IQR): 3-7 mm) for OFC+ patients, compared to 3 mm (IQR: 2-4.5 mm) for the non-allergic group ($P < .05$) (Figure 1A). For the standardized high protein sesame extract (HPSE) the median wheal generated was 12 mm (IQR: 10-15.25 mm) for the patients with SFA compared to 6 mm (IQR: 3.75-9.25 mm) for the non-allergic group ($P < .001$) (Figure 1B). ROC analysis yielded an AUC of 0.66 (95% confidence interval: 0.52-0.80) for the CSSE, while for the HPSE, an AUC of 0.87 (95% confidence interval: 0.78-0.96) was obtained (Figure 1C). Notably, as shown by the ROC curve for HPSE-SPT, a specificity of up to 0.3 (ie false positive rate of 0.7) retained 100% sensitivity, and a sensitivity of up to 0.43 retained 100% specificity. This resulted in a wheal of ≤ 4 mm identifying 6 of the 20 non-allergic patients tested by HPSE-SPT with 100% negative predictive value (NPV), and a wheal of ≥ 13 mm identifying 24 of the 56 SFA patients tested by HPSE-SPT with 100% positive predictive value (PPV). These results indicate the superior performance of the HPSE over the CSSE to distinguish allergic from non-allergic patients by SPT, particularly at low or high test values, respectively.

3.3 | Sesame induction of CD63 and CD203c on patient basophils in BAT assay

The basophil activation test was performed on whole blood samples obtained from the patients. Sesame seed protein-induced basophil (CD123⁺ HLA-DR⁻) surface expression of CD63 and CD203c was evaluated. The gating strategy employed for the BAT is illustrated in Figure S1A. Both positive (Figure S1B) and negative (Figure S1C) basophil responses to sesame protein extract (1000 ng/mL) are demonstrated. In order to determine the optimal sesame protein concentration to be used in the BAT assay, sesame protein dose-responses were constructed for CD63 (Figure 2A) and CD203c expression (Figure 2B) for 14 of the sesame allergic patients, at protein concentrations ranging from 40-10 000 ng/mL. For both CD63 and CD203c, the response generally increased along with higher sesame protein concentrations, with the response for most patients reaching a maximum at 400 or 1000 ng/mL. We therefore used 1000 ng/mL sesame protein as the maximum dose for subsequent BAT assays.

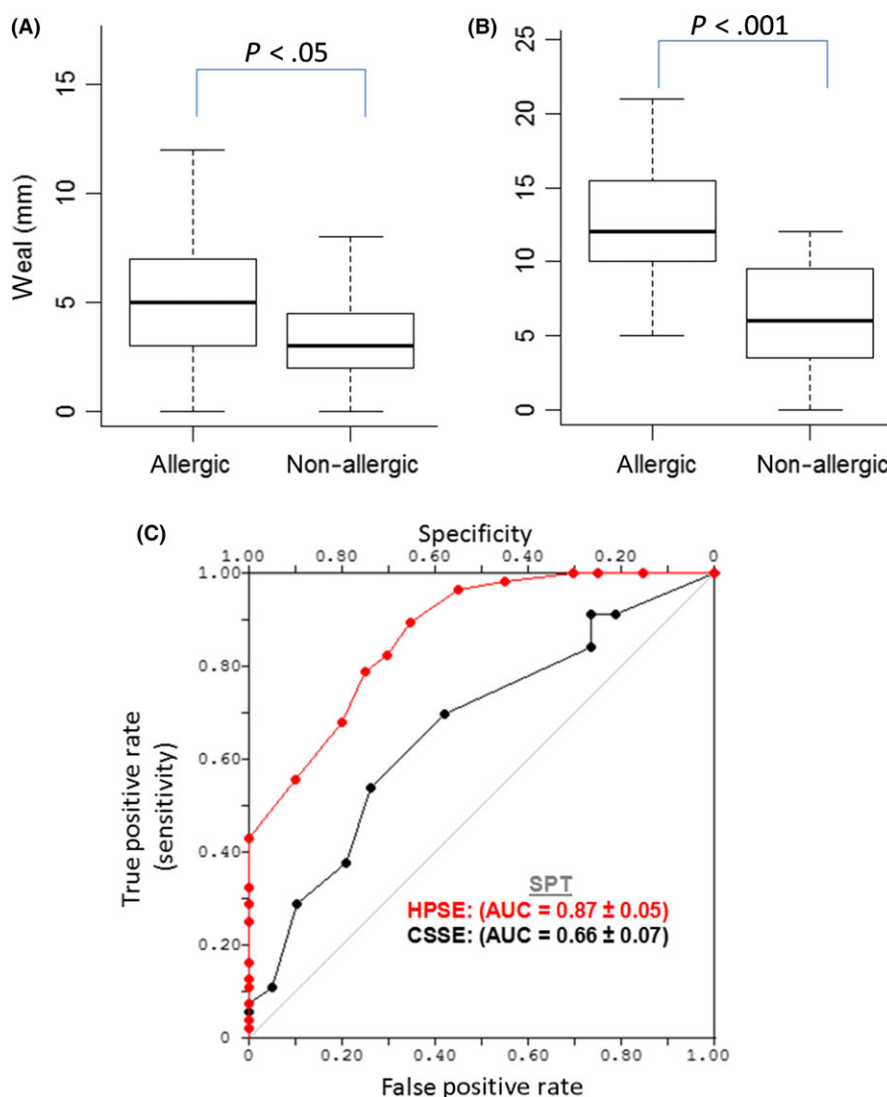


FIGURE 1 Discrimination between sesame allergic and non-allergic patients by SPT. Boxplots showing the distribution SPT results for allergic and non-allergic patients using (A) commercial sesame seed extract (CSSE) or (B) high protein sesame extract (HPSE). Boxes show the IQR with the median value as the midline, and whiskers extend 1.5 times the IQR above and below. (C) An ROC curve plotting the proportion of the true positive vs false-positive patients at SPT values. The red curve is for HPSE, and the black curve is for CSSE. Area under the curve (AUC) values are shown. Allergic patients, $n = 56$; non-allergic patients, CSSE, $n = 19$, HPSE $n = 20$

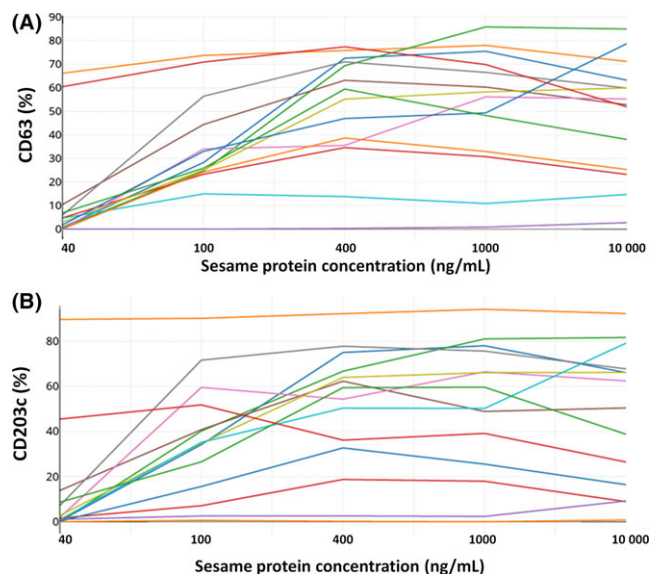


FIGURE 2 Sesame protein dose-response curves of basophil reactivity in OFC-positive patients. Each line ($n = 14$) represents the dose-response to sesame protein (40–10 000 ng/mL) of a single patient, assessed by induced (A) CD63 or (B) CD203c expression

We next compared the sesame-induced basophil response in the allergic group of patients, with that of the non-allergic group. For the allergic patient group the median percentage of basophils with sesame-induced CD63 ranged from 1.3% (IQR: 0%–6.7%) to 45.0% (IQR: 18.8%–69.8%) at 40 and 1000 ng/mL sesame protein, respectively. In the non-allergic group, the medians for these sesame concentrations ranged from 0% (IQR: 0%–0.4%) to 0.5% (IQR: 0%–6.5%) (Figure 3A). Medians were significantly different between the allergic and non-allergic groups at each dose ($P < .01$). Using CD203c, in the allergic patient group the median percentage ranged from 1.9% (IQR: 0.2%–7.9%) to 39.1% (IQR: 16.6%–60.5%), compared with the non-allergic group, in which the median ranged from 0% (IQR: 0%–0.3%) to 0.4% (IQR: 0%–2.0%) (Figure 3B). Similar to CD63, medians of CD203c at each dose were significantly different between the allergic and non-allergic groups. ($P < .001$). Noting the variability of the sesame-induced CD63 or CD203c basophil expression among the sesame allergic patients, as reflected by the broad IQR at the 1000 ng/mL dose, we reasoned that it might be reflective of sensitivity to sesame allergen, an assessment of which would be the OFC eliciting dose. At the 1000 ng/mL dose, sesame-induced CD63 ($\rho = -.42$) and CD203c ($\rho = -.35$) were both inversely associated with eliciting dose, as assessed by Spearman's rank correlation test ($P < .01$ and $P < .05$, respectively).

Thus, overall the sesame-induced basophil surface expression of CD63 and CD203c was able to discriminate between the SFA and non-allergic patient groups, and for the SFA group, was associated with challenge eliciting dose.

3.4 | Utility of BAT for SFA diagnosis

To assess the utility of BAT in SFA diagnosis, we generated receiver operating characteristic (ROC) curves plotting the true positive vs

the false positive rate of SFA diagnosis at different sesame-induced basophil expression levels of CD63 (Figure 3C) or CD203c (Figure 3D). For CD63, the AUC values ranged from 0.86 (95% CI: 0.75–0.98) using a 1000 ng/mL concentration of sesame protein, to 0.72 (95% CI: 0.60–0.85) with a 40 ng/mL concentration. For CD203c, the AUC values at the different sesame concentrations displayed less variation. An AUC value of 0.81 was obtained for both 1000 ng/mL (95% CI: 0.67–0.95) and 40 ng/mL (95% CI: 0.70–0.92), while an AUC of 0.83 was obtained for 400 ng/mL (95% CI: 0.67–0.98) as well as for 100 ng/mL (95% CI: 0.72–0.94). Further data on the ROC curves can be found in Table 1.

3.5 | Complementary usage of BAT and SPT

As described in the methods section, the sesame extract used for BAT differed from that used for the HPSE-SPT. Differences were noted in the protein composition between the extracts leading to differences in the patient IgE binding profile, as assessed by immunoblot (Figure S2 in the online repository). Furthermore, the ROC curve plot (Figure 4A) shows that HPSE-SPT had perfect sensitivity with fewer false positives and perfect specificity with fewer false negatives than BAT, while BAT had a higher AUC than HPSE-SPT at intermediate critical test points. Notably, HPSE-SPT consistently outperformed CSSE-SPT, as did BAT, aside from at very high critical points (where specificity $\geq 90\%$). This suggested that BAT and HPSE-SPT could potentially be used complementarily for SFA diagnosis. We therefore examined the joint usage of HPSE-SPT and BAT to correctly categorize SFA in the 80 patients for whom BAT was performed (Figure 4B). Given the broad clinical adoption of SPT, we placed HPSE-SPT as the first step in this scheme. The generally accepted SPT wheal cut-off of 3 mm²⁵ was used to identify 5 non-allergic patients, and a 13 mm wheal cut-off was used to unambiguously identify 24 SFA patients. BAT was implemented for the categorization of the remaining 51 patients (Figure 4B), using the optimal diagnostic points of CD63 and CD203c determined for our data at 1000 ng/mL (Table 1). Thirty-two SFA patients were positive in both tests (CD63 and CD203c, $n = 29$), or by one parameter, (CD63 alone $n = 1$; CD203c alone, $n = 2$). Four non-allergic patients were also falsely classified as positive by both CD63 and CD203c ($n = 3$) or by CD203c alone ($n = 1$). Of the patients whose BAT results for both CD63 and CD203c were below the cut-offs, 12 were not allergic and one patient was allergic. Two patients, one with SFA and one without, were BAT non-responders. Overall, joint use of HPSE-SPT and BAT correctly classified 73/80 (91%) of patients (Sensitivity = 0.97; Specificity = 0.77). 56 of the 58 sesame allergic patients could be identified, with 4 false positive patients, 2 BAT non-responders, and one false negative. The eliciting dose for the false negative patient was at 720 mg protein resulting in gastrointestinal reactions and urticaria that was treated with antihistamines. Regarding the false positive patients, while not significant, we noted that their median age was higher than that of the general patient population (14.3 vs 8.8 years).

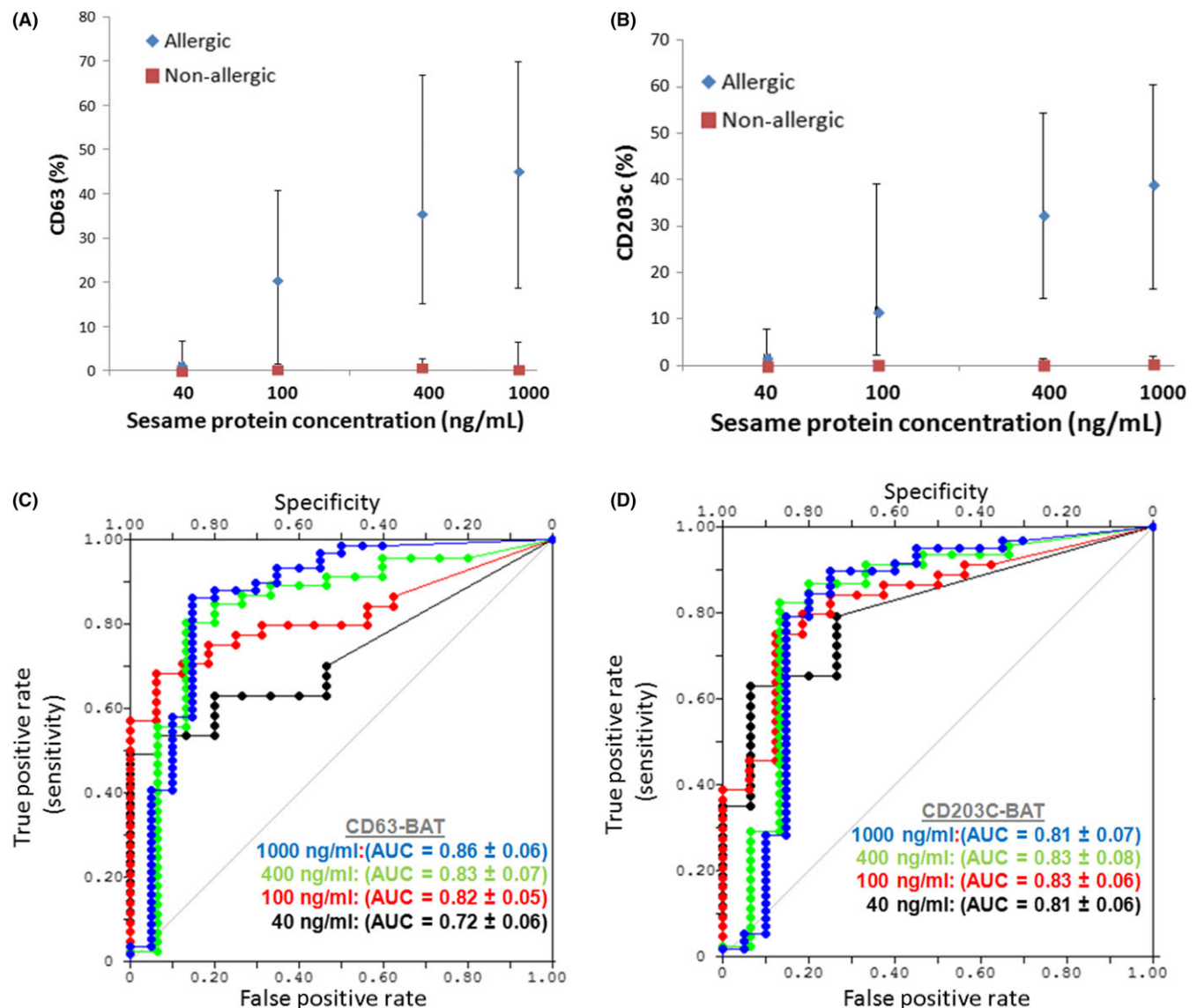


FIGURE 3 Discrimination between sesame allergic and non-allergic patients by BAT. A, B, Median sesame protein induction of (A) CD63 or (B) CD203c on the basophils of SFA patients (blue diamonds) and non-allergic patients (red boxes) at doses ranging from 40 to 1000 ng/mL is shown. The extended bars denote the interquartile ranges. C, D, ROC curves plotting the proportion of the true positive vs false positive patients for induction values of (C) CD63 or (B) CD203c. The curve for each dose of sesame protein is depicted by a different colour. AUC values are shown. For the SFA-positive group, $n = 43, 44, 45, 57$ patients for the 40, 100, 400, 1000 ng/mL doses, respectively. For the non-allergic group, $n = 15, 16, 15, 20$ patients for the 40, 100, 400, 1000 ng/mL doses, respectively

4 | DISCUSSION

The serious health effects of sesame allergy and its incidence necessitate the development and implementation of accurate diagnostic tests. While the oral food challenge remains the definitive diagnostic assessment of food allergy, there can be complications in its implementation, as well as considerable investments in cost and effort. As such, it is important to evaluate other diagnostic methods, particularly for cases in which an OFC is not feasible.

Regarding other methods for diagnosis of SFA, SPT,^{7,9,10,17} detection of specific serum IgE against sesame protein^{8-10,17,26} and recently, specific component testing against a recombinant Sesi 1 (rSesi i1)

allergen²⁷ have been reported in the literature. Two of these studies^{7,10} reported critical values generating >95% specificity for commercial SPT, yet with sensitivities reaching <50%. In terms of specific serum IgE, >95% specificity was obtained with sensitivities <30%,⁸⁻¹⁰ while the study investigating serum reactivity to rSesi1 yielded specificity and sensitivity of 85%.²⁷ SPT remains a critical diagnostic tool, in that it is a relatively straightforward inexpensive technique, with a quick readout. Our analysis therefore began with SPT, testing 2 formulations, and finding that a high protein extract which could be reproducibly generated was more accurate than commercial extract in detecting SFA. Our findings are in accordance with a previous small study, which compared these types of SPT on 6 patients.¹¹

TABLE 1 ROC analysis of the BAT performed at different doses of sesame protein

	Dose	AUC	Optimal critical value ^a	Sensitivity	Specificity	Accuracy	95% PPV critical value	95% NPV critical value
BAT test								
CD63	1000 ng/mL	0.86 ± 0.06	10.9%	0.86	0.85	0.86	56.0%	–
	400	0.83 ± 0.07	13.8%	0.80	0.87	0.82	13.8%	–
	100	0.82 ± 0.05	1.8%	0.75	0.81	0.77	5.8%	–
	40	0.72 ± 0.06	0.6%	0.63	0.80	0.67	1.3%	–
CD203c	1000 ng/mL	0.81 ± 0.07	5.2%	0.84	0.80	0.83	–	–
	400	0.83 ± 0.08	4.9%	0.82	0.87	0.83	4.9%	–
	100	0.83 ± 0.06	1.4%	0.80	0.81	0.80	15.6%	–
	40	0.81 ± 0.06	0.1%	0.79	0.73	0.78	0.9%	–
SPT test								
CSSE	1:10 w/v	0.66 ± 0.07	4	0.70	0.58	0.67	–	–
HPSE	100 mg/mL	0.87 ± 0.05	10	0.79	0.75	0.78	13	5

^aAs determined by the upper left-hand point on the ROC curve.

Over the last 20 years, the use of patient basophils has become appreciated in testing for food allergy. Several methods have been developed for typing human basophils and for determining their reactivity,²⁸ most reports however, have focused on the surface expression of CD63²⁹ or CD203c¹² which is associated with basophil degranulation in response to stimulation through the FcεR1a receptor. Prior to this report, there have been several reports concerning the usage of BAT as related to SFA.^{17–19} To our knowledge, however, this is the first large-scale report evaluating the usage of BAT as a diagnostic tool for SFA in a patient population with suspected allergy confirmed or disproven by OFC or recent reaction.

Our metric for assessing basophil reactivity was the percentage of basophils expressing CD63 or CD203c in response to sesame seed extract supernatant, at a level greater than the maximal expression of these markers by basophils in media containing only IL3. Under this scheme, both CD63 and CD203c percentage expression levels were able to discriminate between sesame allergic and non-allergic patient groups. This discrimination was observed for all of the sesame protein concentrations used in our BAT assay. Additionally, for the sesame allergic patients, the values for both CD63 and CD203c induction at the higher protein concentrations (400 and 1000 ng/mL) showed a weak but significant inverse correlation with OFC eliciting doses, possibly reflecting the association of basophil reactivity with allergic sensitivity to sesame. The optimal cut-off values for CD63 and CD203c at these concentrations were in line with previously-reported BAT cut-offs.³⁰ Despite its overall accuracy, as a single test the BAT did not yield sufficient sensitivity and specificity for clinical use. However, given the broad ability for BAT to discriminate between sesame allergic and non-allergic patients, we reasoned that BAT might be utilizable in the context of other diagnostic methods.

We examined the joint-diagnostic utility of HPSE-SPT and BAT. HPSE-SPT performed well at its extreme test values, identifying 24 of 60 sesame allergic patients, and 6 of 22 sesame non-allergic

patients without identifying any false positive or false negative patients, respectively. Considering these results, and that skin testing is already well-adopted into clinical use, it seemed reasonable to place the HPSE-SPT as the first test in our testing scheme. As HPSE-SPT did not perform as well for the remaining patients, we investigated whether BAT might be useful for their classification, including patients with HPSE-SPT >3 and <13 mm. Using the optimal test cut-off values generated by ROC analysis for CD63 and CD203c, BAT identified 32 of the remaining 34 sesame allergic patients. Notably, both CD63 and CD203c were required for this identification, as 3 patients were positive for one but not the other marker. Twelve of the remaining 17 non-allergic patients were also identified correctly, with BAT results below the optimal cut-off values for both CD63 and CD203c. Our results therefore suggest that a sesame extract with high protein content could be used for SPT as a first step to diagnose SFA and subsequently BAT to classify the remaining patients. We did not investigate the underlying driver behind this complementation. The explanation may be partially due to the intrinsic differences between the 2 tests. SPT primarily demonstrates mast cell allergen reactivity, while BAT tests the allergen reactivity of circulating basophils. However, however it might also be reasoned that the differences in the protein extraction methods used for each test plays a role. The defatting process of the HPSE removes the fat-soluble proteins that might have been present in the seed. Indeed, given the relevance of oleosins to SFA,^{2,31} the relatively positive performance by the HPSE in our hands is of interest. One possibility is that in general, oleosin-reactive patients may also react to other sesame proteins whose presence is enhanced in the HPSE, especially considering its higher protein content compared to standard, commercial sesame extracts. As we demonstrate (Figure S2), some proteins in the HPSE are not present in the BAT sesame extract. At the same time, the use of HPSE would be inadvisable for performing BAT, due to the risk of residual particulate matter affecting flow cytometry fluidics. Additionally, reliable SFA

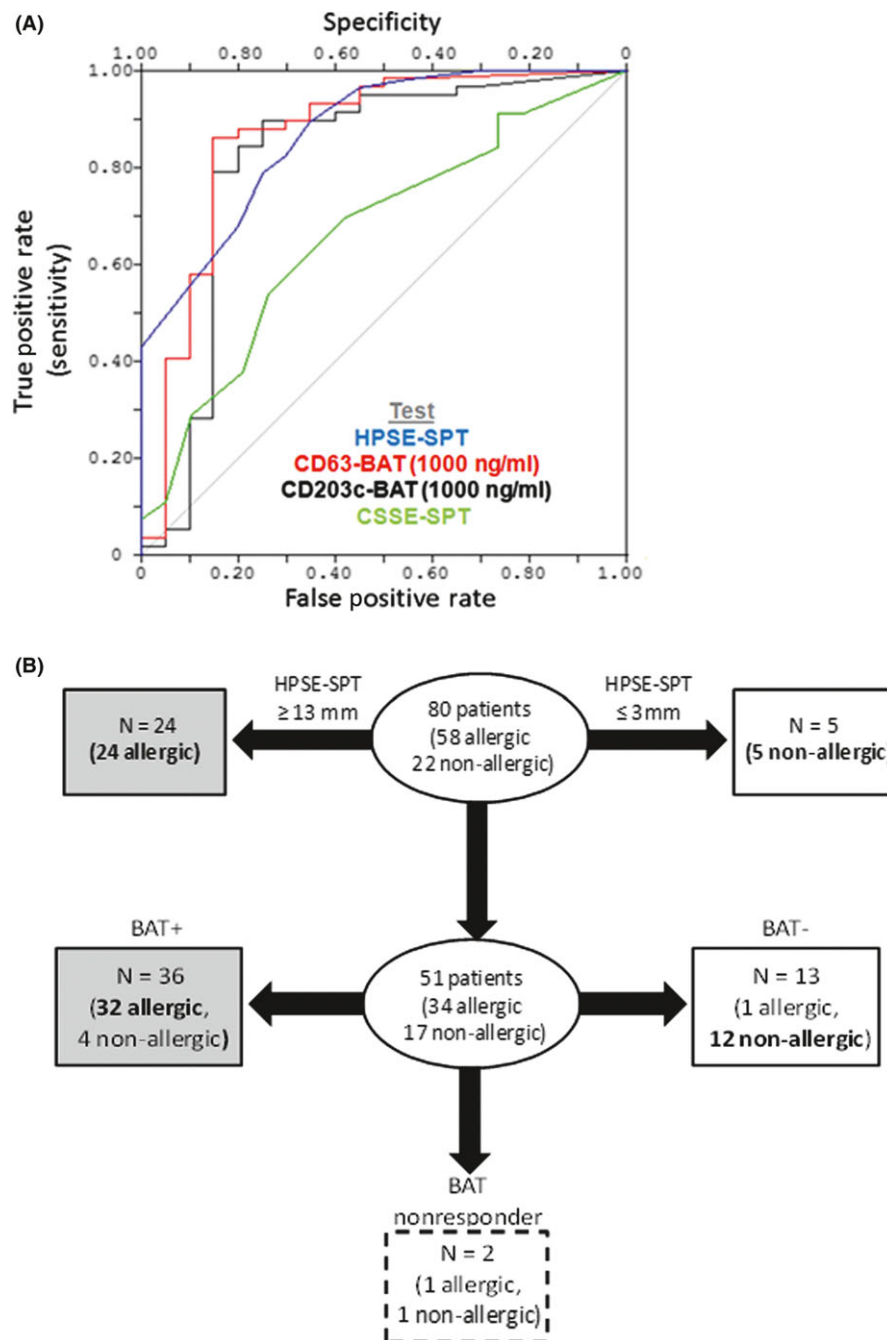


FIGURE 4 Joint utilization of HPSE-SPT and BAT in SFA diagnosis. A, The ROC curves for BAT and SPT diagnostic tests presented previously are plotted on the same axes. B, Schematic diagram depicting a strategy for the diagnosis of SFA, with sequential utilization of HPSE-SPT and BAT. HPSE-SPT is administered initially to rule-in (weal ≥ 13 mm) or rule-out SFA (weal ≤ 3 mm) SFA. For patients with equivocal HPSE-SPT results, BAT is performed using optimal test cut-off values ($\geq 10.9\%$ to classify as CD63 + and $\geq 5.2\%$ to classify as CD203c+). A positive test for either CD63 or CD203c would be considered BAT+. The HPSE-SPT, BAT and allergic state of the patients in this study were utilized in a simulation of the strategy. Grey boxes denote results in which the majority of patients are allergic, and white boxes denote results in which the majority of patients are non-allergic. Two patients with equivocal HPSE-SPT were BAT non-responders. Two patients for whom BAT was not performed are not depicted

diagnosis would likely require a more full representation of sesame proteins in extracts. The protein composition of the sesame extract supernatant used for the BAT assay, which was not completely defatted might have been useful for diagnosing other patients, including those with oleosin reactivity.³²

In terms of clinical utility of this method, a prospective trial would be required to validate our findings. The usefulness of this approach may well depend as to whether the usage would be to identify allergic patients (as a "rule-in" test) or in addition to classify suspected SFA patients as non-allergic ("rule-out" SFA). Used

exclusively to identify allergic patients, the results of our study would suggest that nearly all allergic patients could be identified without the need for an OFC, with an approximately 10% false positive rate. The remaining patients would require an OFC, most of whom would likely test negative. Although OFC could largely be obviated if the approach were used as a rule-out test, caution would be warranted due to the risk of false negatives.

Several limitations remain in our findings. There may be room to optimize the performance of BAT in the assessment of SFA. As of yet there are no definitive guidelines regarding proper BAT procedure. Reports vary regarding the formulation of stimulation media, proper basophil identification markers, and outcome metrics.³⁰ It is possible that modifications to the performance of BAT might yield test improvements. Furthermore, additional testing doses may improve the sensitivity of BAT over the use of only a single testing concentration. Multiple doses would facilitate the generation of other BAT metrics such as complete BAT dose-response curves, such as would be necessary to obtain EC₅₀ values which might facilitate the assessment of sensitivity.³³ As a practical matter, however, the feasibility of testing multiple concentrations on patients with multiple allergies can be limited by factors such as the blood volume obtained. A further limitation is that the determination of optimum cut-off values for diagnostic use is complex and dependent on factors such as cohort composition, and methodology.³⁰ Furthermore, the decision to adopt a particular critical value may differ among clinical settings³⁴ and may be dependent for example on the requirement for a 100% negative predictive value, such as in the diagnosis of allergy. Future work testing or adapting the diagnostic algorithm described here will require consideration of these aspects. Finally, while we have shown that the joint diagnostic utility of HPSE-SPT and BAT is quite robust, it might be worth considering whether the model could be strengthened through the addition of other diagnostic methods such as specific component testing of Ses i 1, particularly due to its specificity, which could be useful to lower the incidence of false positive results.

In summary, we have demonstrated the joint use of BAT and HPSE-SPT for the diagnosis of sesame allergy. While validation of our findings requires a prospective trial, this study sets the stage for the accurate diagnosis of sesame food allergy, with minimal reliance on oral food challenges.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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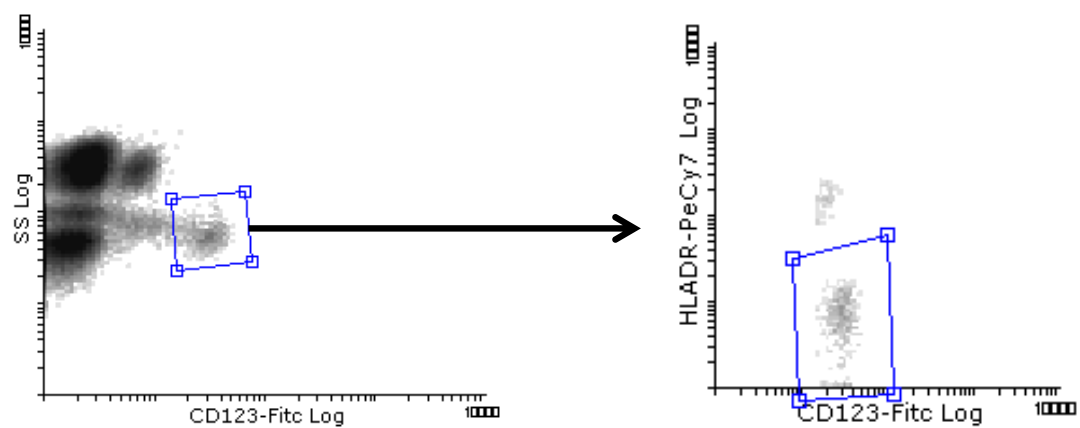
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SUPPORTING INFORMATION

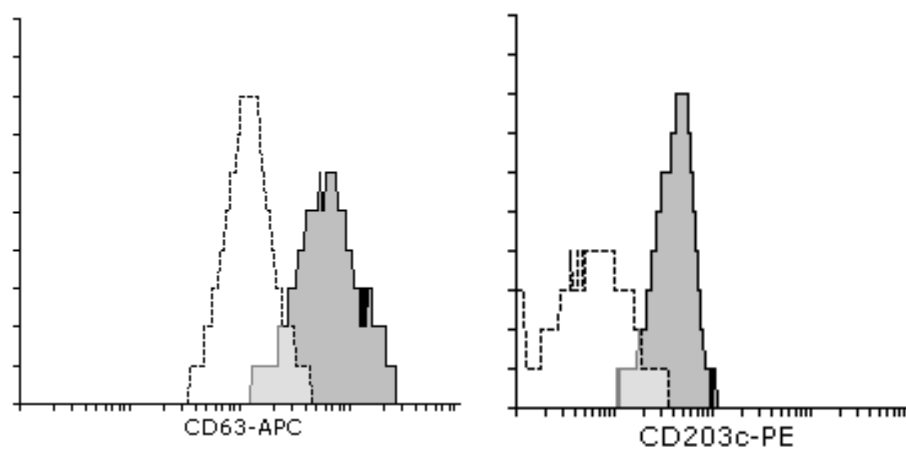
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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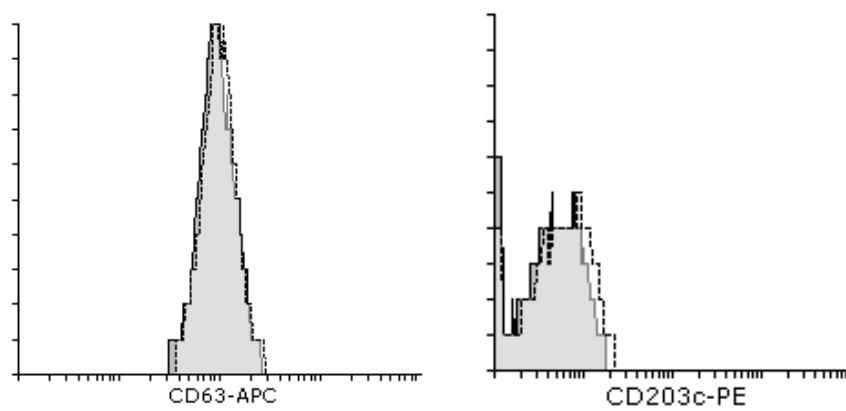
Figure S1



A

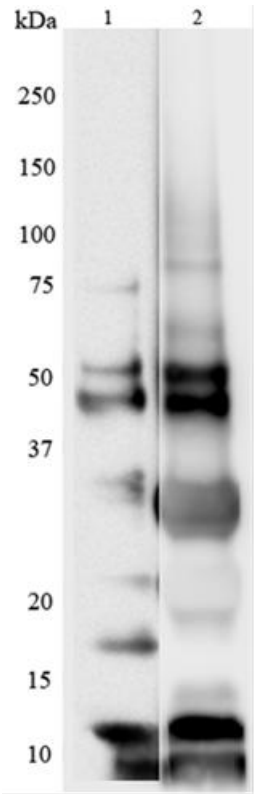


B



C

Figure S2



Supplemental Methods

SDS-PAGE analysis and IgE-immunoblot

Thirty micrograms of sesame extract used for BAT, and the high protein extract used for SPT were run on reducing SDS-PAGE 4-20% polyacrylamide gels (Bio-Rad) at constant voltage. Proteins were transferred to PVDF membranes (MilliporeSigma, Burlington, MA) for immunoblotting with IgE from patients with SFA. For the latter, membranes were cut into strips corresponding to lanes from the gel, blocked with 5% Bovine Serum Albumin in 0.1% Tween PBS, and incubated overnight at 4⁰ C with patient plasma (1:7 dilution). Membrane strips were washed and probed with horseradish peroxidase conjugated mouse anti human IgE Fc (clone B3102E8, Southern Biotech, Birmingham, AL) in the presence of mouse gamma globulin (Jackson ImmunoResearch, West Grove, PA). Electrochemiluminescent substrate was added (ThermoFisher). Images were acquired on a MicroChemi digital gel camera (DNR Bio-Imaging Systems, Israel).

Table S1. Sesame Oral Food Challenge Protocol

Dose	Interval (min.)	Sesame protein (mg)*	Tahini (g)*
1	0	0.3	-
2	15	3	-
3	15	15	-
4	15	30	-
5	30	60	-
6	30	240	1
7	30	480	2
8	45	1000	4.2
9	60	4000	16.8
Observation	90		
Total (min.)	330		

*Intermediate doses of sesame were occasionally administered to patients with indeterminant allergic reactions, pursuant to the judgment of the attending physician.

Table S2. Sesame -induced CD63 and CD203c, in the presence or absence of exogenous IL3

CD63+ (%)		CD203c		Sesame Allergic (Y/N)
IL3-*	IL3+**	IL3-*	IL3+**	
67.5	78.0	93.7	94.2	Y
51.3	69.8	10.3	39.1	Y
33.8	10.9	8.5	0.0	Y
36.6	33.3	69.7	60.1	N
0.8	0.0	0.7	0.2	N
63.9	58.2	62.3	66.1	Y

Values are the percentages of basophils expressing CD63 or CD203c in the presence of 1000 ng/mL sesame above the levels of patient basophil expression of these markers in basal media (*RPMI or **RPMI + IL3) for six patients

Table S3. Demographic comparison of allergic versus nonallergic groups

Parameter	Allergic (N=60)	Nonallergic (N=22)	P-value
Age, years* (median (IQR))	9.0 (5.9-15.0)	9.0 (5.3-18.5)	NS
Gender (male)	63.3 %	59.1 %	NS
Other food allergies ¹	79.3%	65.0%	NS
Asthma ³	61.4 %	38.9 %	NS

* At diagnosis

¹ N=58 (Allergic); N=20 (Nonallergic)

² N=57 (Allergic); N=18 (Nonallergic)

NS, Nonsignificant

Table S4. Sesame Oral Food Challenge Reactions

#	Age (mo)	Gender	ED (mg)	Perioral	Skin	UR	LR	Eyes	GI	CV	Grade 1	Grade 2	Grade 3	UR-stridor or LR-severe	AH	BD	Epi*
1	156	F	15	N	N	N	N	N	Y	N	N	N	Y	N	Y	N	Y
2	324	M	15	N	N	N	N	Y	Y	N	N	Y	N	N	N	N	N
3	227	F	30	N	N	N	N	N	Y	N	N	N	Y	N	N	N	Y
4	231	M	30	N	N	Y	N	N	N	N	Y	N	N	N	Y	N	N
5	255	M	30	N	N	N	N	Y	Y	N	N	N	Y	N	Y	N	Y
6	142	F	30	Y	N	N	N	N	Y	N	Y	N	N	N	N	N	N
7	230	F	30	N	N	N	N	N	Y	N	N	N	Y	N	Y	N	Y
8	151	M	30	N	Y	Y	N	N	N	N	N	Y	N	N	Y	N	Y
9	89	M	120	N	N	N	N	N	Y	N	Y	N	N	N	N	N	N
10	75	M	120	N	N	N	N	N	Y	N	Y	N	N	N	N	N	N
11	108	F	120	N	N	Y	N	N	N	N	Y	N	N	N	Y	Y	N
12	155	F	240	N	N	N	N	N	Y	N	N	N	Y	N	N	N	Y
13	61	M	240	N	Y	N	N	Y	Y	N	N	N	Y	N	Y	N	Y
14	67	F	240	N	N	N	N	N	Y	N	N	N	Y	N	Y	N	Y
15	133	M	240	N	N	N	N	N	Y	N	N	N	Y	N	N	N	N
16	61	F	240	N	Y	Y	N	N	N	N	N	Y	N	N	Y	N	Y
17	154	M	300	N	N	N	N	N	Y	N	N	N	Y	N	Y	N	N
18	93	M	300	Y	N	N	N	N	Y	N	N	N	Y	N	Y	N	N
19	82	F	480	N	Y	N	N	N	N	N	Y	N	N	N	Y	N	N
20	77	M	3840	N	Y	Y	N	Y	N	N	N	Y	N	N	Y	N	N
21	7	M	480	N	Y	N	N	N	N	N	Y	N	N	N	Y	N	N
22	79	M	1000	N	Y	Y	N	N	N	N	N	Y	N	N	Y	N	N
23	244	M	1000	N	Y	Y	N	Y	Y	N	N	N	Y	N	Y	N	Y
24	162	F	1440	N	Y	N	N	N	N	N	Y	N	N	N	Y	N	N
25	52	F	4000	N	N	Y	N	Y	N	N	Y	N	N	N	Y	N	N
26	58	M	4000	N	Y	N	N	N	Y	N	N	N	Y	N	Y	N	N
27	94	M	15	N	N	Y	N	N	Y	N	N	N	Y	N	Y	Y	N
28**		M															
29	147	F	60	N	Y	N	N	N	Y	N	N	Y	N	N	Y	N	N
30	80	M	240	N	N	N	N	N	Y	N	N	N	Y	N	Y	N	N
31	488	M	4000	N	N	Y	N	N	N	N	N	Y	N	N	Y	N	N
32	72	M	60	N	Y	N	N	N	Y	N	N	N	Y	N	Y	N	N
33	140	M	480	Y	Y	Y	Y	N	N	N	N	N	Y	N	Y	Y	N
34	225	M	480	N	N	N	N	Y	Y	N	N	Y	N	N	N	N	N
35	58	M	30	N	N	Y	N	N	Y	N	N	N	Y	N	Y	N	N
36	106	M	120	N	N	Y	N	N	Y	N	N	N	Y	N	Y	Y	N
37	53	M	720	N	Y	N	N	N	Y	N	N	N	Y	N	Y	N	N
38	58	M	120	N	N	Y	N	N	N	N	Y	N	N	N	Y	N	N
39	120	F	480	N	N	Y	N	N	N	N	Y	N	N	N	Y	N	N
40	69	F	120	N	N	Y	N	N	Y	N	N	N	Y	N	Y	N	N
41	69	F	1000	N	Y	N	N	Y	N	N	N	Y	N	N	Y	N	Y
42	179	M	60	N	N	N	N	Y	Y	N	N	N	Y	N	Y	N	N
43	248	M	2880	N	N	N	N	Y	Y	N	N	N	Y	N	Y	N	Y
44	233	M	3	N	N	N	Y	N	N	N	N	N	Y	N	Y	Y	N
45	34	M	3	N	N	Y	N	N	N	N	Y	N	N	N	Y	N	N
46	136	F	400	N	N	Y	N	N	Y	N	N	N	Y	N	Y	Y	N
47	54	M	120	N	N	Y	N	N	Y	N	N	N	Y	N	Y	N	N
48	89	F	120	N	N	Y	N	N	Y	N	N	N	Y	N	Y	N	N
49	37	M	63	N	N	N	N	Y	Y	N	N	Y	N	N	Y	N	N
50	111	F	30	N	N	Y	N	N	N	N	Y	N	N	N	Y	N	N
51	337	M	240	N	N	Y	N	Y	Y	N	N	N	Y	N	Y	N	N
52	222	F	240	N	N	N	Y	N	Y	N	N	N	Y	N	Y	Y	N
53	71	M	15	N	N	N	N	N	Y	N	N	N	Y	N	N	N	N

UR=Upper Respiratory, LR= Lower Respiratory, GI=Gastrointestinal,
CV=Cardiovascular,AH=Antihistamine, BD=Bronchodilator, Epi=Epinephrine

* Epinephrine administered for anaphylactic allergic reactions involving respiratory
compromise, reduced BP or persistent GI complaints

**OFC for patient 28 was performed at another medical center.

Figure legends-Supplementary material

Figure S1. Flow cytometry gating strategy for basophils and basophil reactivity after stimulation. (A) Cells were gated on the side-scatter low, CD123⁺, HLA-DR⁻ population. Induction of CD63 and CD203c expression with 1000ng/ml sesame protein (solid line) over IL3 alone (hatched line) in a sesame allergic (B) or nonallergic, sensitized (C) individual.

Figure S2. Immunoblot of sesame allergic patient IgE against sesame extracts. 30 µg of protein from sesame extracts were run by SDS-PAGE on a 4-20% polyacrylamide gel. Proteins were transferred to a PVDF membrane for immunoblotting with IgE from a patient with SFA. Lane 1-blot against sesame extract used for BAT (10 minute exposure), lane 2-blot against HPSE (2 minute exposure).