Evaluation of Basophil Activation Tests to Diagnose Tolerance to Food Allergens

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To the Editor,

An oral food challenge (OFC) is the gold standard for diagnosis of food allergy, though skin prick tests (SPT) and specific IgE (sIgE) are both used to aid in the initial diagnosis and to track sensitization, despite their poor specificity and correlation with clinical reactivity[1]. Recent studies have suggested that basophil activation tests (BATs) might have higher sensitivity and specificity when compared to SPT and sIgE[2-6].

However, BATs remain heterogeneous with regard to the assay protocol, markers of basophil activation, and criteria for positive results[7].

We performed a small, exploratory prospective study to evaluate the diagnostic potential of BATs to distinguish between food tolerance and allergy to peanut, cow's milk, and/or hen's egg. We hypothesized that the combination of basophil activation markers CD63 and CD203c would be superior to either alone. The study was approved by the Colorado Multiple Institutional Review Board.

We enrolled patients between 4 months and 17 years of age, who were undergoing evaluation for peanut, cow's milk, and/or hen's egg allergy. Patients and their parents completed a survey assessing their exposure history to peanut, cow's milk, and hen's egg. Heparinized whole blood was obtained from each patient. All samples were stimulated for 20 minutes with phosphate-buffered saline (PBS, negative control), anti-FcεR1 and anti-IgE (positive controls), 10-fold dilutions each of peanut, cow's milk, and whole hen's egg extracts (50 µg/mL to 0.5 µg/mL) and 10-fold dilutions of Ara h2 (10 µg/mL to 0.1 µg/mL). Samples were placed on ice to halt basophil activation and stained with antibodies for CD45, CD63, and CD203c. Following acquisition on a Navios flow cytometer, basophil (identified as SSC^{low}/CD45+/CD203c+, figure 1 panel a and b) expression of CD63 and CD203c was analyzed using Kaluza (Beckman Coulter). The percentage of basophils with elevated expression of CD63 and/or CD203c was quantified. Samples with no basophil response to positive controls were categorized as 'non-responders'. Flow cytometry results were compared to the clinical diagnosis in patients who (1) underwent an OFC or (2) indicated on the survey that the specific allergen is regularly consumed in their diet without symptoms (food tolerant controls). The highest allergen concentrations resulted in non-specific CD63 and/or CD203c upregulation, therefore only the two lower allergen concentrations were used to evaluate BAT performance.

Of the 21 patients undergoing evaluation for food allergy who were enrolled in our study, blood samples were obtained from 19 patients. Two of the patients (11%) were 'non-responders'. Eight OFCs were performed in our study patients and regular food tolerance based on survey results was reported to peanut, cow's milk, and hen's egg in 2, 12, and 5 patients, respectively.

Seven negative food challenges were performed in six patients. Five of the six patients had previously been diagnosed with food allergies based on a history of reaction and sensitization. One patient was diagnosed with a food allergy based on sensitization, without a history of food reaction. At the time of food challenge, five of the six patients remained sensitized to the foods. Significant basophil upregulation was not observed in all six patients.

Expression of CD63 and CD203c on activated basophils was not equal for all patients. After activation with the positive control, five of the 19 patients had a differential expression of CD203 and CD63 by 30% or more (absolute difference of percentage of basophils expressing the marker). Of these, two patients were 'non-responders' based on expression of CD63, but not CD203c. Similarly, large difference in expression of CD203 and CD63 occurred after stimulation with food allergens (Table 1).

We present below, two cases that best highlight the complementary use CD63 and CD203c and potential clinical uses of BATs.

Case 1:

A female patient presented to our clinic at 16 months of age with symptoms of immediate vomiting after each ingestion of cow's milk. Testing revealed a bidirectional SPT wheal of 4x2 mm and cow's milk sIgE of 32.3 kU_A/L and she was diagnosed with cow's milk allergy. At her follow up appointment at 31 months of age, her parents reported she was accidentally exposed to a spoon of cow's milk without a reaction. Repeat testing demonstrated cow's milk bidirectional SPT wheal of 6x4mm and sIgE of 31.4 kU_A/L. She tolerated an OFC to cow's milk. BAT upregulation of CD63 was insignificant following stimulation with the two lower concentrations of cow's milk allergen whereas upregulation of CD203c was minimal at the lowest concentration of cow's milk allergen and increased relative to baseline at the intermediate concentration (Figure 1).

Case 2:

A 9-year-old boy developed difficulty swallowing and breathing after eating a candy containing peanuts. He was treated in the emergency department for anaphylaxis and referred for follow up in our clinic. The patient had a history of peanut and hen's egg allergies. He had tolerated an OFC to peanut at 5 years of age, but never introduced it into his diet. He had not introduced egg into his diet since an allergic reaction at 2 years of age. SPT at our clinic revealed bidirectional wheals to peanut and hen's egg of 4x2mm and 4x4mm respectively. sIgE levels to peanut and egg were 4.62 kU_A/L and 2.48 kU_A/L respectively. He tolerated an OFC to peanut and hen's egg. CD203c upregulation was insignificant following stimulation with the two lower concentrations of peanut and hen's egg allergens, and Ara h2. This patient's basophils did not upregulate CD63 following stimulation with the positive controls (Figure 2), therefore, allergen-specific CD63 response could not be assessed.

Our two cases highlight the potential benefit of multiple basophil activation markers. For case 1, CD63 was a more accurate marker of tolerance to milk allergen whereas case 2 was a 'non-responder' based on absence of CD63 upregulation, however, analysis of CD203c upregulation in this patient enabled interpretation of BAT results.

Our results add to the growing body of literature, suggestive of the clinical utility of BATs in understanding tolerance to food allergens. In both cases, the BATs correctly predicted tolerance to foods despite a history of food allergy and persistent sensitization. While previous reports on BATs have concentrated on the potential to avoid OFCs in patients with BATs above an established threshold, our cases suggest a different purview on the context of use of BATs– to identify patients with history of food allergies who may have developed tolerance, despite persistent elevations in sIgE and SPT [2,8,9]. These patients may therefore benefit from OFCs, and BATs may be a confirmatory test that indicates the OFC has a stronger likelihood of success

Our case series is limited by the small number of patients who underwent OFCs and will benefit from ongoing study to better determine the clinical utility of BAT in the aforementioned context. However, close examination of these two cases effectively highlights (1) the potential benefit of evaluating multiple basophil markers and (2) the potential to identify sensitized but food tolerant patients with BAT, as a tool that helps select for OFC candidates more likely to have successful outcomes.

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Figure Legends:

Figure 1: Case 1 Flow Cytometry Results for Milk Allergen

A and B: Gating strategy to identify basophil population (green).

C and D: Expression of CD63 and CD203c when stimulated with two concentrations of milk allergen and negative (PBS) and positive (αFcεR1) controls

Figure 2: Case 2 Flow Cytometry Results for Peanut, Ara h2, and Egg Allergen

Expression of CD63 and CD203c when stimulated with two concentrations of allergen and negative (PBS) and positive (αFcεR1) controls

A and D: Peanut allergen

B and E: Ara h2 allergen

C and F: Egg allergen

Table 1: Diagnostic Test Results

BAT performed with 100 µl of the reported allergen concentration added to 100 µl of whole blood

OFC: Oral food challenge

+: Based on combination of history, sIgE and SPT. Patients who had an equivocal diagnosis of food allergy (e.g. did not complete a recommend food challenge) were included as allergic in this table

NR: Non-responder

ND: Not done

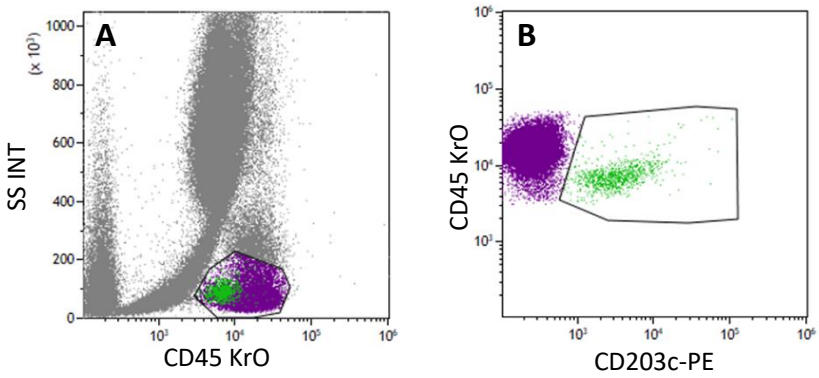
++passed an oral food challenge to baked milk

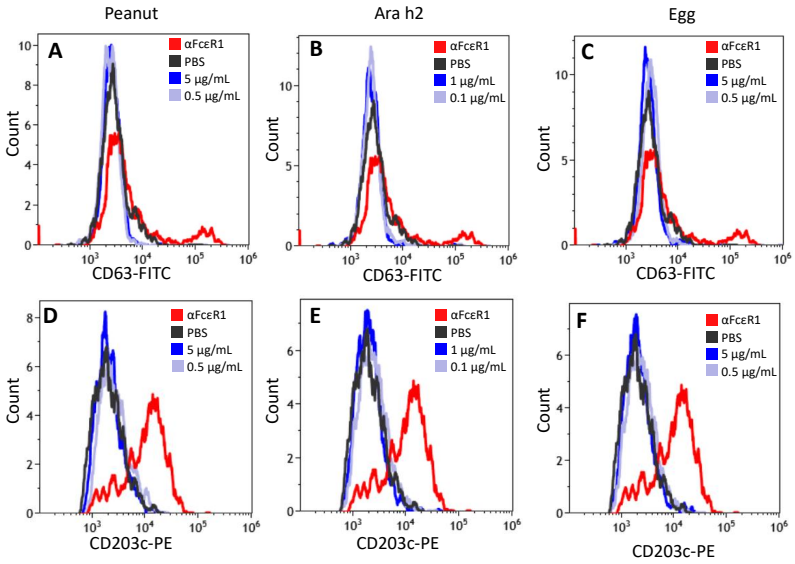
a: regular consumption without symptoms

b: infrequent consumption, but without symptoms

c: avoidance of food because of previous reaction

d: avoidance of food without prior exposure





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