ORIGINAL ARTICLE

Diagnostic challenges in soy allergies: utility of the basophil activation test to distinguish allergy from sensitization to Gly m 4

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ABSTRACT

Background. Soy allergy represents a diagnostic challenge, particularly when mediated by Gly m 4, a PR-10 protein known for its cross-reactivity with birch pollen allergens. Traditional diagnostic methods, including skin prick tests (SPTs) and specific IgE assays, often lack sensitivity or specificity, especially for Gly m 4-mediated allergies. **Methods.** In this study, the basophil activation test (BAT) was evaluated as a tool to distinguish true allergy from mere sensitization to Gly m 4. **Results.** A total of four patients sensitized to Gly m 4 and Bet v 1 (PR-10 of soy and birch) were included in this study. Two patients were confirmed allergic to soy based on positive BAT results with Gly m 4 and soy total extract, correlating with clinical symptoms of allergy. Conversely, two other patients were determined to be sensitized but clinically tolerant, as BAT results were negative, consistent with their symptom-free status during oral food challenges. **Conclusions.** The study highlights the limitations of traditional diagnostic methods, which often yielded false-negative or inconclusive results, and underscores the BAT's ability to provide functional evidence of allergen reactivity. We demonstrate the utility of BAT in identifying clinically relevant Gly m 4-mediated soy allergies. By enabling precise differentiation between allergy and sensitization, the BAT emerges as a valuable diagnostic tool, complementing molecular allergen-specific IgE assays and offering a safer and more specific alternative to oral food challenges

Key words
BAT; soy total extract; molecular allergen; tolerant; allergic; cross-sensitization; anaphylactic reaction; PR-10.

Impact statement

The basophil activation test reliably distinguishes true soy allergy from Gly m 4 sensitization, offering a functional, non-invasive alternative to oral food challenges in complex diagnostic cases.

INTRODUCTION

Soybeans play a crucial role as a global food source, offering both nutritional and health benefits (1). They are found in diverse culinary forms, from sprouts and immature beans, such as edamame, to products derived from mature beans, such as soy flour, tofu, and soymilk. Additionally, certain soy products have undergone fermentation, such as tempeh, miso, nattô, and soy sauce (2). Soy allergy is the most common legume allergy after peanut (3). Symptoms range in severity and may or may not be mediated by immunoglobulin E (IgE) (4). As soy consumption increases, there is a potential risk of more frequent allergic reactions. Although severe and fatal reactions are rare, it is crucial to detect and address allergies promptly. Food allergies to soy, particularly those involving the allergen Gly m 4 (a pathogenesis-related protein (PR-10)), which has been identified as the most common allergen in soy patients with birch pollen allergy (5), present significant diagnostic challenges. It is most important to differentiate between simple sensitization and true allergy.

Traditional diagnosis of soy allergy relies on skin tests and the assay of specific IgE (sIgE) to whole soy extract. However, these methods have limited sensitivity, especially in cases mediated by Gly m 4, where sufficient extraction of this allergen is challenging. This can result in false negatives or uncertain diagnoses (6–8). Component-resolved diagnosis (CRD) can overcome this hurdle by using unit sIgE assays against molecular allergens such as Gly m 4 (soy PR-10). Nevertheless, cross-reactivity between birch pollen allergens (Bet v 1) and Gly m 4 further complicates diagnosis (9), as it may point to an authentic soy allergy with a risk of anaphylactic reaction, or instead to sensitization without clinical symptoms of soy allergy. Diagnostic methods that differentiate sensitization from true allergy are therefore essential.

The basophil activation test (BAT) has emerged as a valuable tool to overcome these limitations. Several studies have shown that the basophil activation test is a useful diagnostic tool for peanut allergy (10–14). However, few studies have investigated the utility of BAT in soy allergy. One such study recently suggested that the CD203c-BAT with soymilk protein extract, in combination with the assay of sIgE to Gly m 4, could be useful for identifying patients with soymilk allergy (15). Our team had also recently described using BAT for a first confirmation of the involvement of PR-10/Gly m 4 in an anaphylactic

reaction after ingestion of soymilk in a 27-year-old female patient (5). However, the ability of BAT with soybean extract to discriminate between patients truly allergic to soybean from those sensitized but tolerant to it had not yet been assessed. In this study, we hypothesized that BAT using molecular soy allergens including Gly m 4 would both remedy the poor sensitivity of the sIgE assay and offer greater specificity.

In this context, a BAT using major recombinant Gly m 4 was developed and used, as part of routine care, and compared to traditional BAT using soy total extract in all patients with suspected soy allergy presenting at our center. This series of four cases was studied to assess the effectiveness of BAT in differentiating sensitization profiles among patients with positive IgE to Gly m 4, comparing results with available clinical and biological data. We sought a more accurate identification of patients at risk of clinically significant allergic reactions, so contributing to improved therapeutic management.

METHODS

Study population

We collected retrospectively data of subjects both sensitized solely to Gly m 4 and Bet v 1 (PR-10 of soy and birch) at the department of Pediatric of our hospital from June 2020 to November 2020 in order to distinguish truly allergic patients from sensitized but tolerant patients. Patients sensitized to Gly m 5 and Gly m 6 or with a mixed profile were excluded. All patients underwent clinical evaluation, assay of sIgE to soy (Phadia 250) and its components, and a basophil activation test. A healthy control, non-sensitized and non-allergic to soy and birch, was included to ensure that the allergen concentrations used in the BAT did not cause basophil activation. The study was approved by local Ethics Committee (IRB00013412, "CHU de Clermont Ferrand IRB #1", IRB number 2024-CF409) with compliance to the French policy of individual data protection. According to French law, patients were informed and retained the right to oppose the use of their anonymized medical data and excess samples for research purposes.

Allergen-specific IgE assay

Levels of specific IgE to soy and its components were quantified in patient serum using the ImmunoCAP and Phadia 250 analyzer (Thermo Fisher, Uppsala, Sweden) according to the manufacturer's recommendations.

Soy basophil activation test

Flow Cast® and B-CCR® kit (Bühlmann, Switzerland) were used according to the manufacturer's instructions. Briefly, EDTA whole blood was stimulated in an IL-3 containing buffer for 15 min at 37 °C with increasing concentrations of soybean extract (Bühlmann, Switzerland). As per the manufacturer's recommendations, five concentrations of soybean extract were tested in 10-fold dilution ranging from 22.5 ng/mL to 2.25 pg/mL (traditional BAT), with addition of a high concentration of soybean total extract (67.5 ng/mL). BAT using recombinant Gly m 4, Gly m 5 and Gly m 6 soybean allergen supplied by Indoor Biotech was also developed and tested, at concentrations of 67.5 ng/mL, 45 ng/mL, 22.5 ng/mL, and 11.25 ng/mL. Monoclonal antibody recognizing the high-affinity IgE binding receptor (FcεRI) and *N*-formyl-methionyl-leucyl-phenylalanine (fmlP) were used as positive controls. Cells were stained with CD63-FITC, CD203c-PE DY647 and CCR3-PE. The mixture was incubated at 37°C for 15 minutes before erythrocyte lysis. Basophils were gated as SSC-low/CCR3+, and among these, the CD63+ and/or CD203c+ cells were termed activated basophils. Cells were acquired on an FACS-CANTO II (Becton Dickinson). At least 300 basophils were analyzed using Flowlogic software (version 7.3, Inivai Technologies, Australia). Dead cells and doublet cells were excluded by an FSC/SSC gate and an SSC-A/SSC-H gate, respectively. Basophil activation was expressed as the % CD63 positive basophils (% CD63+) or % CD203c positive basophils (% CD203c+) among SSC-low/CCR3+ cells. The cut-off value for positive basophil activation in this study was set at >15% CD63 and CD203c basophils.

Cases description

Four patients from the allergology consultations of our University Hospital, sensitized to Gly m 4 and Bet v 1 (PR-10 of soy and birch), were referred to the immunology unit for BAT to confirm their allergies. The patients' characteristics are summarized in Table I.

Case 1 experienced Grade 3 (oFASS-5) anaphylaxis after drinking 200 mL of soymilk. Patient symptoms included rhinoconjunctivitis, sneezing, trunk rash, abdominal pain, and palmoplantar pruritus, but no laryngeal edema or voice modification. The patient did not seek medical attention, and no tryptase assay was performed. The symptoms resolved spontaneously after a few hours. The patient reported no prior symptoms from occasional soymilk consumption. The patient had an atopic condition with eczema, birch pollen allergic rhinitis for three years and experienced oral syndrome from Rosaceae fruits, mainly raw apple. Since this original reaction, the patient had avoided raw soybeans but consumed cooked soybeans without experiencing any new severe reaction. The patient reported several instances of mild oral reactions after ingestion of soy cream in boiled form. In 2018, his sIgE against whole soybean extract was weakly positive (0.15 kU/mL) and negative in 2020, while CRD revealed a positive anti-Gly m 4 sIgE (6.41 kUA/l in 2018 and 5.45 kUA/l in 2020), unlike those directed against Gly m 5 and Gly m 6, which were always negative. The patient had specific IgE to birch pollen (21.3 kUA/l in 2020) and Bet v 1 (25.9 kUA/l in 2018). His SPT results were negative for soybean total extract and positive for birch total extract (6 mm) (Table I). Despite the near-negativity of the sIgE assay directed against the total soy extract and the negativity of the soy SPT, an oral food challenge (OFC) was recommended to confirm the diagnosis of soy-induced anaphylaxis and to determine the reactivity threshold, but the patient declined. As a result, a BAT was proposed to assess his allergic status. Interestingly, BATs using the highest concentration of soybean extract (67.5 ng/mL) revealed basophil activation (figure 1). Also, BAT showed a strong positive reaction to Gly m 4 at 67.5 ng/mL for CD63 and up to 45 ng/mL for CD203c in soybean allergic subject #1 (Figure 1 and Table II). No degranulation was elicited using Gly m 5 and Gly m 6 allergens.

Case 2 experienced birch pollen rhinoconjunctivitis. The patient's medical background comprised atopic dermatitis, asthma, and a previously resolved food allergy to egg white, shrimp, and mustard. In view of possible cross-allergy, he sought medical advice to determine whether he could safely consume soy. He had previously consumed soy yogurts and tofu without any adverse reactions, and his recent exposure to soy in all its forms had been minimal. We conducted a molecular analysis and found that his sIgE levels were negative for soybean (<0.10 kUA/L), Gly m 5, and Gly m 6, but positive for birch pollen (10.1 kUA/L), Bet v 1 (8.05 kUA/L), and Gly m 4 (4.14 kUA/L) (Table I). The soybean total extract

SPT yielded negative results, while the birch total extract SPT was positive (4 mm), with a histamine control of 7 mm (Table I). In the BAT, no basophil activation was observed with either the soybean total extract or the specific allergens Gly m 4, Gly m 5, and Gly m 6, as indicated by the lack of reactivity on both the CD63 and CD203c markers (Figure 1 and Table II).

Case 3 was allergic to birch pollen, for which he had been desensitized. This patient had asthma and was also allergic to house dust mites, grass pollen and plantain. He presented an anaphylactic reaction associating an oral syndrome and digestive disorders (nausea, vomiting, and liquid stools), after ingesting a meal containing chocolate soymilk and on another occasion after ingesting soy yogurt. He also had an oral syndrome on eating raw apple, cherry, hazelnut, almond, carrot, and celery.

Birch extract-sIgE and Bet v 1-sIgE were positive (41.1 kUA/L and 8.39 KUA/L, respectively). Soybean total extract-sIgE were weakly positive (0.19 kUA/L), whereas specific-Gly m 4 sIgE were fully positive (13.3 kUA/L). Gly m 5- and Gly m 6-specific IgE were negative. SPT were performed with histamine at 9 mm. Results were positive for birch pollen (10 mm), inconclusive for soy commercial extract (2 mm), and negative for carrot (0 mm). No test was run for celery.

In 2020, the patient underwent two OFCs, first with raw carrots and then with soy yogurt. The ingestion of 50 g of carrot gave no reaction. New skin tests were carried out and revealed positivity for raw celery (histamine 6 mm, celery 6 mm, carrot 0 mm), and the biological report found carrot sIgE 1.19 kU/l, celery 3.3 kU/l, Api g 1.01 4.67 kU/l. The ingestion of 120 ml of soy yogurt did not cause any reaction, confirming the results of the prick test. No basophil activation was detected in BAT with the soybean total extract or the specific allergens Gly m 4, Gly m 5, and Gly m 6, as shown by the absence of reactivity on both CD63 and CD203c markers (Table II).

Case 4 had severe eczema in childhood. Given his origin, soy had been incriminated to explain his eczema, but the patient did not remember consuming any. In this context during an allergologic workup, various food skin tests were carried out. Soybean SPT and soybean total extract-sIgE were found positive and an avoidance diet was recommended. He had never shown an anaphylactic reaction. In 2020, he consulted to review these allergies, while eliminating soy in all its forms from his diet. Soybean and birch SPT came back positive. sIgE assay (Table I) showed an allergic profile to birch pollen (birch pollen total extract- and Bet v 1-sIgE > 100 kUA/L) and a sensitization profile to soybean (0.2 kUA/L)

with positive Gly m 4-sIgE (74.3 kUA/L) and Mal d 1 of apple was also positive (95.3 kU/AL), demonstrating a PR-10 profile.

In October 2020, the OFC involving the ingestion of 87.5 g of cooked soybean and 5 ml of soy sauce (cooked soy) did not produce any local or general reaction. However, given the clinical history, the positive results from the soy prick-test, and specific IgE assays, a BAT was subsequently proposed to further evaluate the patient's reactivity to the soy allergen. CD63-BAT with soybean total extract was negative even at the highest allergen concentration tested (67.5 ng/mL) but a weak activation of basophils was observed with the analysis of the expression of CD203c (Figure 1 and Table II). BAT to Gly m 4 solution was positive up to the concentration of 22.5 ng/mL and 45 ng/mL based on the expression of CD203c and CD63, respectively, evidencing a strong reactivity of the patient (Table II). No degranulation was observed with Gly m 5 and Gly m 6 (Table II).

DISCUSSION

Although the prevalence of soy allergy in Europe is low, increasing consumption of soy flour-containing industrial products and vegan diets raises concerns about rising soy allergies. Soy CRD-specific IgE tests identify soy allergy patients with higher sensitivity and predictive positive value than soybean whole extract sIgE tests (9). However, the specificity of this molecular allergen-sIgE test is not always satisfactory. Hence the need to develop a functional test, easier to perform, cheaper, and safer than an OFC, with better specificity and predictive positive value for soy allergy diagnosis. In this series of four cases, we demonstrate the added value of BAT in patients sensitized to Gly m 4, showing that BAT with soy total extract or Gly m 4 triggers basophil degranulation only in allergic patients, enabling discrimination between sensitized but tolerant and sensitized and allergic patients.

Case #1 highlights the need for an optimized BAT with a higher concentration of soy total extract to accurately detect soy allergies. Initially, when testing with the manufacturer-recommended concentration of 22.5 ng/mL of the total soy extract, no basophil activation was observed, even though the patient with positive Gly m 4-sIgE and negative Gly m 5- and Gly m 6-sIgE had experienced a severe anaphylactic reaction following soymilk ingestion. This raised concerns about the concentration of Gly

m 4 in the total extract. When the concentration was increased to 67.5 ng/mL, basophil activation was detected, confirming the patient's allergy. This result underscores the importance of adjusting allergen concentrations in the BAT to improve its sensitivity, particularly for allergens like Gly m 4, which may be present in small quantities in commercial soy extracts (8). Optimizing allergen concentrations ensures that the BAT can detect clinically relevant allergies that might otherwise go unnoticed with standard testing protocols.

In our cohort of four patients, we focused on isolated soy PR-10 monosensitization (focusing only on soy). Only two patients (Cases #1 and #4) were finally considered allergic to soy. These findings are consistent with the observation made by Fukutomi et al. where only half of pollen-sensitized cases with PR-10 sensitization were allergic to soy (7), showing the need for another confirmatory test for etiological purposes. In Case #1, the positive anti-Gly m 4-sIgE combined with the clinical anaphylactic reaction after soymilk intake were informative, but the negativity of the soy prick test was disconcerting. It is known that the SPT against whole natural extract of soy lacks sensitivity when the soy allergy is mediated by the PR-10 family (6). This stems from the difficulty met in extracting the Gly m 4 allergen from whole extracts and their low content of this allergen (8). To be sure of the involvement of this legume, the BAT to whole soy total extract advantageously replaced the OFC refused by the patient and enabled us to classify the patient as allergic. Also, by demonstrating the *ex vivo* degranulation of basophils in contact with Gly m 4, BAT to Gly m 4 provided valuable mechanistic evidence that the anti-Gly m 4 sIgE previously measured by the serum unitary assays have a functional activity and clinical relevance in this anaphylactic reaction, even in a patient with negative IgE and BAT results for Gly m 5 and Gly m 6. In Case #4, neither anaphylactic reaction to soy nor positive OFC to raw soy (not carried out to avoid any risk for the patient) were evidenced in a subject naïve to soy and under preventive avoidance for a long time, but a significant very high positivity of Gly m 4-sIgE and positive soybean SPT were observed. In this patient, we observed basophil degranulation with soy total extract and Gly m 4, which made it possible to firmly conclude on a soybean allergy mediated by Gly m 4, even though the patient's food allergy was not evidenced by the oral provocation test with cooked soy sauce. This could be explained by the small amount of Gly m 4 present in the soy sauce as in other cooked

soybeans, because Gly m 4 is partially destroyed by cooking (16). BAT to soybean molecular allergens thus provides important evidence supporting the medical relevance of the sensitization measured against Gly m 4 and enabled us here to conclude without a raw soy OFC.

In the remaining cases (Cases #2 and #3), patients were ultimately identified as Gly m 4-sensitized but clinically tolerant. Both cases exhibited positive Gly m 4-specific IgE yet displayed negative results in soy prick tests. However, due to the poor specificity of these tests, diagnosis was challenging. The BAT provided significant assistance in reaching the final diagnosis, as both cases showed negative BAT results with the soy total extract and Gly m 4 protein. Additionally, Case #3 had a negative raw soy OFC. The concordance of negative results in BAT and OFC led us to conclude on a simple sensitization to soy, with the absence of clinical relevance for anti-Gly m 4 IgE assays. Since IgE cross-linking is essential to elicit allergic reactions, the low specificity of positive Gly m 4-sIgE may be partly due to occurrence of sIgE with no ability to be cross-linked on mast cells/basophils. The anaphylactic reaction observed in Case #3 with positive Gly m 4-sIgE subsequent to an initial birch pollinosis, combined with the negativity of SPT, OFC and BAT, raises questions about cofactors or the involvement of other unidentified allergens.

The soy or molecular allergen-based BAT emerges as a promising diagnostic tool, effectively discerning true allergy from sensitization. BAT triggered basophil degranulation exclusively in allergic patients using soy total extract and Gly m 4, so differentiating between sensitized but tolerant and sensitized and allergic individuals. This approach overcomes the limitation of biological cross-reactivity in species-specific IgE tests, improving diagnostic accuracy and patient management. In this context, the exploration of specific soy allergens, particularly Gly m 4, Gly m 5, and Gly m 6, becomes crucial. Our results evidence that at least some anaphylactic reactions in soy patients who are sensitized to these components are linked to BAT reactivity against Gly m 4. However, the roles of Gly m 5 and Gly m 6 in this process are less clear. Further studies involving larger patient cohorts are necessary to validate the utility of BAT with these molecular allergens in differentiating sensitization profiles (PR-10 vs. storage proteins) among soy-allergic patients. Investigating whether BAT reactivity directed against Gly m 4 rather than Gly m 5 or Gly m 6 correlates with clinical symptoms will be essential in refining

diagnostic strategies and understanding the immunological mechanisms and cross-reactivity underlying soy allergy.

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Contribution

BB: Conceptualization, Data curation, Formal Analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. BE: Supervision, Writing – review & editing. EM: Methodology, Writing – review & editing. ET: Writing – review & editing. JC: Writing – review & editing. MG : Writing – review & editing. MR: Data curation, Writing – original draft.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table I. Patient characteristics and laboratory findings in screened patients

Patient	**HC**	**1**	**2**	**3**	**4**
Demographic and clinical characteristics					
Sex	F	F	F	M	M
Age (y)	28	27	26	11	25
Clinically reactive to soybean or suspected soy allergy	No	Yes (Grade III)	No	Yes	No (naïve subject)
Soy eviction	No	Yes (for raw soy)	No (but minimal exposure)	Yes	Yes
Oral syndrome mediated by an allergen of the PR-10 family	No	Yes	No	Yes	No
Atopic history					
Asthma	No	No	Yes	Yes	No
Rhino-conjunctivitis	No	Yes	Yes	Yes	No
Eczema	No	Yes	Yes	No	Yes
Specific IgE (KUA/L)					
Birch pollen (total extract)	<0.1	**21.3**	**10.1**	**41.1**	**>100**
Bet v 1 (PR10)	<0.1	**25.9**	**8.1**	**8.4**	**>100**
Bet v 2 (Profilin)	NT	<0.1	NT	NT	NT
Mal d 1 (PR-10)	NT	**3.2**	**2.6**	**49.1**	**95.3**
Mal d 3 (LTP)	NT	<0.1	<0.1	NT	NT
Celery	NT	NT	NT	**3.3**	NT
Api g 1.01	NT	NT	NT	**4.7**	NT
Peanut	NT	NT	NT	NT	NT
Ara h 1	NT	NT	NT	<0.1	0.14
Ara h 3	NT	NT	NT	<0.1	0.13
Ara h 2	NT	NT	NT	<0.1	NT
Ara h 6	NT	NT	NT	NT	NT
Ara h 8	NT	NT	NT	NT	NT
Soybean (total extract)	<0.1	0.2	<0.1	**0.2**	**0.7**
Gly m 4 (PR10)	<0.1	**6.4**	**4.1**	**13.3**	**74.3**
Gly m 5 (globulin 7S)	<0.1	<0.1	<0.1	<0.1	<0.1
Gly m 6 (globulin 11S)	<0.1	<0.1	<0.1	<0.1	<0.1
Skin prick test (mm)					
Histamine	NT	3	7	9	10
NaCl 0.9%	NT	0	0	0	0
Soybean commercial extract	NT	0	0	2	4
Soy milk	NT	NT	NT	NT	NT
Soy sauce	NT	NT	NT	NT	NT

Flour soy	NT	NT	NT	NT	NT
Birch pollen	NT	6	4	10	6
OFC					
Raw soy	NT	NT (patient refusal)	NT	Negative	NT
Cooked soy	NT	NT (patient refusal)	NT	NT	Negative

Specific IgE antibodies against the PR-10 of interest and the major soy allergens were assayed by fluorescence enzyme immuno-assay (FEIA) (Immunocap, ThermofisherScientific) in the patients studied and in a negative control. HC, healthy control ; NT, not tested.

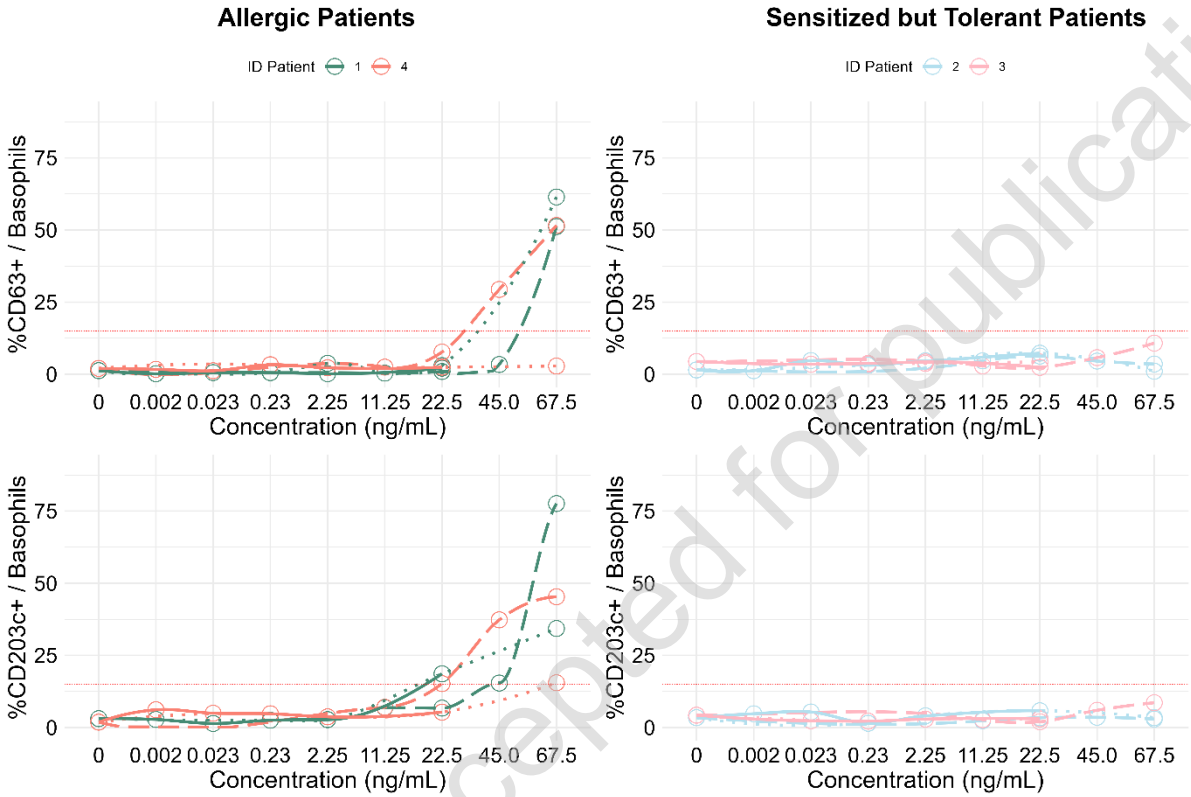
Table II. BAT of mono-sensitized, truly allergic or sensitized but tolerant to soybean patients

	Concentration ng/mL	HC		Case 1		Case 2		Case 3		Case 4	
Allergen		CD 63 %	CD 203 %	CD 63 %	CD 203 %	CD 63 %	CD 203 %	CD 63 %	CD 203 %	CD 63 %	CD 203 %
C -		2.64	2.84	1.26	3.06	1.51	3.40	4.44	4.25	2.04	1.84
Fc□RI		87.6	79.6	70.7	74.3	90.2	79.1	85.4	35.9	91.2	55.2
fmlP		45.4	60	37.5	58.3	16.5	38.1	28.5	16.9	30.5	29.3
F14 (total extract)	67.5	3.01	3.41	**61.5***	**34.3***	1.06*	3.40*	NT	NT	2.85	**15.5**
	22.5	4.02	3.62	2.11	**18.6**	7.3	5.79	2.38	2.98	2.45	5.31
	2,25	2.51	5.23	0.19	2.72	4.67	4.03	4.26	2.91	2.25	3.69
	0,225	3.42	3.42	0.58	2.51	3.11	1.45	3.77	2.18	3.31	4.76
Gly m 4	67.5	2.85	2.03	**51.2**	**77.6**	3.56	2.93	10.8	8.57	**51.6**	**45.3**
	45	1.32	1.64	3.38	**15.4**	4.56	3.53	5.77	5.96	**29.4**	**37.3**
	22.5	3.4	4.08	0.96	6.7	6.32	3.37	2.38	1.98	7.69	**15.2**
	11.25	3.36	2.68	0.38	6.9	4.65	2.33	2.9	2.7	2.47	7
Gly m 5	67.5	2.88	3.19	0.19	2.33	3.33	1.88	4.31	4.31	2.66	3.68
	45	4.32	4.65	0.39	2.91	5.01	2.3	1.58	2.17	2.64	3.66
	22.5	2.69	2.36	0.78	2.73	3.21	1.28	3.15	3.35	1.82	2.23
	11.25	3.6	3.6	0.4	3.78	4.54	2.89	3.85	3.45	1.41	2.83
Gly m 6	67.5	2.99	4.98	0.59	2.55	5.22	3.34	4.15	3.56	1.43	4.07
	45	2.37	3.32	0.78	2.72	5.21	2.71	3.53	3.14	1.62	4.65
	22.5	4.43	3.45	0.8	2.79	6.42	4.14	2.19	4.37	2.04	3.89
	11.25	3.86	1.5	0.84	4.63	5.07	2.33	3.52	2.93	1.42	2.83

This table shows the percentage of basophil activation (according to CD63 and CD203 markers) that were restimulated by the total soybean extract and molecular allergen Gly m 4, Gly m 5 and Gly m 6 at several concentrations, for 15 min at 37°C. The BAT is considered positive when one of the percentages of CD63 or CD203c exceeds 15% (written in bold). *The concentration of 67.5 ng/mL was tested independently in a second round of BAT for Cases # 1 and # 2 after the initial tests at concentrations of 22.5, 2.25, and 0.225 ng/mL showed no basophil activation,

even in highly allergic patients. Although the 67.5 ng/mL concentration was tested separately, all data are presented together in a single table for a comprehensive view.
HC: healthy control, NT: not tested.

Figure 1: Activation of basophils as a function of allergen concentrations



The positivity threshold (basophil activation >15%) is represented by the dotted red line. BAT protocols are indicated as follows: F14 traditional BAT (solid line), F14 high concentration BAT (dotted line), Gly m 4 BAT (dashed line)