International Archives of Allergy and Immunology

Clinical Allergy – Research Article

Int Arch Allergy Immunol 2023;184:767–775
DOI: 10.1159/000530026

Received: June 24, 2022
Accepted: March 2, 2023
Published online: April 18, 2023

Soy Gly m 8 sIgE Has Limited Value in the Diagnosis of Soy Allergy in Peanut Ara h 2-Sensitized Adults

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Keywords
Gly m 8 · Soy allergy diagnosis · 2S Albumin · Ara h 2 · Peanut sensitization · Basophil activation

Abstract
Introduction: Recently, specific IgE (sIgE) sensitization against Gly m 8 (soy 2S albumin) has been described as a good diagnostic marker for soy allergy (SA). The aim of this study was to evaluate the diagnostic value of Gly m 8 by determining the sensitization profiles based on the homologues soy allergens Bet v 1, Ara h 1, Ara h 2, and Ara h 3. ***Methods:*** Thirty soy-allergic adults were included; sIgE to total soy extract, Gly m 8, Gly m 4, Gly m 5, Gly m 6, Bet v 1, Ara h 1, Ara h 2, and Ara h 3 were determined. Sensitization patterns were analyzed and determined. The clinical relevance of sIgE of Gly m 8 sensitization was measured by assessing its capacity to degranulate basophils in Gly m8-sensitized patients by an indirect basophil activation test (iBAT). ***Results:*** Based on the sIgE patterns of sensitization, two groups of SA patients were identified: (i) peanut-associated SA group (all patients were sensitized to one or more of the peanut compounds) and (ii) non-peanut/PR-10-associated SA group (22 patients were sensitized to Gly m 4 and Bet v 1 but not to any of the peanut compounds). A high and significant correlation between total soy extract and Gly m 6 ($R^2 = 0.97$), Gly m 5 ($R^2 = 0.85$), and Gly m 8 ($R^2 = 0.78$) was observed. A nonsignificant correlation was observed between the levels of sIgE of Gly m 8 versus Ara h2. The iBAT results showed that Gly m 8 did not induce basophil degranulation in any of the peanut-associated patients, indicating that the Gly m8 sensitizations were not clinically relevant. ***Conclusions:*** Gly m 8 was not a major allergen in the selected soy-allergic population. The iBAT results indicated that Gly m 8 was not able to induce basophil degranulation in sIgE Gly m 8-sensitized soy-allergic patients. Thus, Gly m 8 would have no added value in the diagnosis of SA in the present study population.

Introduction

In Europe, prevalence of self-reported soy allergy (SA) is approximately 1.5%, dropping to 0.3% when diagnosed with an oral food challenge (OFC) [1]. This variation is

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not uncommon in food allergy diagnosis, which currently relies on a skin prick test (SPT) and specific IgE (sIgE) levels [1–3]. However, an SPT and sIgE with soy extract while specific (87.3% and 93.8%, respectively) are not sensitive enough (44.4% and 33.3%, respectively) for diagnosing IgE-mediated SA [4]. Nonetheless, an OFC is not frequently performed since it is time consuming, costly, and carries inherent health risks [5–7].

Presently, most studies describe sIgE to Gly m 5 and Gly m 6 as reliable diagnostic markers, and both have shown to contribute to a more severe form of SA [8–10]. Both have shown a higher sensitivity than soy extract, 63% for Gly m 5 and 68% for Gly m 6 but a lower specificity and 73% for both allergens [11]. Gly m 4, known as a pathogenesis-related protein (PR-10), due to cross-reactivity with the birch pollen allergen Bet v 1, has been identified as the most common allergen in SA patients with birch pollinosis with a sensitivity of 81% and a specificity of 78% [12]. Additionally, high levels of sIgE to Gly m 4 have been reported in patients with anaphylactic reactions to soy drinks [13, 14]. Recently, interest is growing in Gly m 8 (2S albumin) as a diagnostic marker of SA; however, its value as a diagnostic marker in SA has not been settled. Previous studies reported that Gly m 8 had the best accuracy in diagnosing SA when compared to Gly m 4, Gly m 5, and Gly m 6 but performed equally when compared to SPT and sIgE of soy extract [11, 15]. Furthermore, Ebisawa et al. [16] reported Gly m 8 as the best-known predictor of severe SA in children. However, Lin et al. [17] reported that 2S albumins were major allergens in SA patients.

Peanut allergy has been suggested to be the most common coexisting allergy in SA patients [18–22]. It was reported that of 133 SA children, 103 were allergic to peanuts (88%) [18]. Conversely, in a study on peanut-sensitized adults, 87% were identified to be sensitized to soy [19]. Because of the homologous proteins in peanuts and soy, it is not uncommon to encounter patients that have sIgE antibodies to these two food-based allergens without necessarily being clinically reactive to both [20]. It has been described that peanut allergens Ara h 1, Ara h 2, and Ara h 3 show similarities to soy allergens Gly m 5, Gly m 8, and Gly m 6, respectively [21, 22]. Nonetheless, the influence of cross-reactive allergens on the IgE profile to soy allergens with respect to peanuts has been poorly considered when studying SA [21], with few studies reporting the influence of peanut allergy on soy allergen sensitization profiles and thus on the clinical relevance of these sensitizations when SA is suspected [23–25]. Cross-reactivity between Gly m 8 and Ara h 2 has been reported by several studies [20–24].

Since peanut and soy share many homologous proteins, it is not uncommon to encounter positive IgE antibody tests to both of these foods in individuals who are clinically reactive to one of these allergens.

The present study aimed to evaluate the value of Gly m 8 sensitization in the diagnosis of SA considering sIgE to the major cross-reactive allergens of soy (peanut components Ara h 1, Ara h 2, Ara h 3 plus birch component Bet v 1) and by analyzing the individual clinical allergic reactivity to Gly m 8 and Ara h 2 (2S albumins) with an indirect basophil activation test (iBAT) since this functional assay mimics an in vivo allergic reaction and thus determining the clinical relevance of positive Gly m 8 sIgE [26–28].

Materials and Methods

Study Population

The study was approved by the Medical Ethical Review Committee CMO Regio Arnhem-Nijmegen, The Netherlands. A total of 30 adult patients visiting the Outpatient Allergy Clinic in Rijnstate Hospital Arnhem were selected based solely on a SA diagnosis determined by clinical evaluation in combination with sIgE measurements. All patients signed an informed consent and filled a questionnaire regarding allergy symptoms, severity of symptoms, and the specific soy products consumed upon the onset of symptoms as well as the presence of coexisting allergies and related symptoms. Before blood collection, patients were requested to stop the use of oral antihistamines for 3 days and steroid medications for 10 days. An OFC was not performed since a high number of patients enrolled in this study had a clinical history of anaphylactic shock (AS) (64%).

Determination of sIgE

sIgE to total soy extract, native Gly m 8 (nGly m 8), recombinant Gly m 4 (rGly m 4), nGly m 5, nGly m 6, rBet v 1, rAra h 1, rAra h 2, and rAra h 3 were determined using the ImmunoCAP250 with allergen caps (nGly m8 was a gift by Thermo Fischer, Uppsala, Sweden) according to the manufacturer's instructions. Patients with sIgE of ≥0.35 kU/L were considered as IgE-sensitized.

Indirect Basophil Activation Test

A 4-mL aliquot of fresh EDTA-anticoagulated blood from 8 adult nonallergic healthy blood donors with the blood group O was centrifuged for 10 min at 2,200 *g* at room temperature within 24 h of collection. Buffy coats were collected and combined and then washed with physiological salt and resuspended in a total volume of 2 mL (leucocyte count between 12.5–15 × 10^9/L). The resuspended buffy coat was centrifuged for 5 min at 1,000 *g* and 11°C after which 2 mL of cold stripping buffer (0.15 M H4NaO5P and 0.005 M KCl, pH 3.55) was added to the buffy coat, and the centrifuge protocol was repeated. After the stripping procedure, the buffy coat was washed with basophil stimulation buffer which contained calcium, heparin, and IL-3 (Bühlmann, Basel, Switzerland). A 500-μL aliquot of buffy coat was incubated with 130 μL

of undiluted serum from the tested patient for 16 h at 37°C. A BAT was performed with the resensitized donor basophils which were separately stimulated with nAra h2 (Bühlmann Laboratories AG, Schönenbuch, Switzerland) or rGly m8 (Abcam plc, Cambridge, UK).

The iBAT was performed using a Flow2-CAST kit (Bühlmann) according to the manufacturer's instructions [29]. Basophil activation was determined by the CD63 expression level of 500 basophils measured using flow cytometry (FACS Canto II; BD Biosciences, San Jose, USA). The stimulation of basophils using an anti-FcRI high-affinity IgE receptor antibody was used as a positive control (a <20% difference between the positive and negative control values indicates a putative nonresponder).

Dose-response curves were performed using nAra h 2 or rGly m 8 using a final concentration range of 0.00125–18 ng/mL for Ara h 2 and 100–2,000 ng/mL for Gly m 8 to reach a plateau phase in the dose-response curve for most patients. Basophil reactivity was expressed as the %CD63+ basophils upon stimulation with the allergen adjusted for the negative control [28–30]. Presently, the cutoffs for BAT positivity are not clearly established, and the ones defined in one population are not necessarily directly transferable to another one [31]. However, the manufacturer suggests a range from 6% to 15% [29]. Thus, for the present study, the cutoff value was set at 15% CD63+ cells for Gly m 8 and 10% CD63+ cells for Ara h 2. Dose-response curves were fitted in GraphPad Prism (version 8.0.2, GraphPad Software, San Diego, USA) using a three-parameter logistic curve fit (hill slope 1).

Dot Blot

In the iBAT assay, rGly m 8 (Abcam plc, Cambridge, UK) was used. To confirm that the sIgE from patients' sera recognized rGly m 8, a dot blot was performed. Three patients were selected: patient #6, patient #8, and patient #14 as a negative control.

Results

Sensitization Profiles of the Studied Population

Sensitization Pattern against Soy Proteins

From the total study population (n = 30), sIgE to soy extract was detected in 14 patients (47%), Gly m 4 in 26 patients (87%), Gly m 5 in 7 patients (23%), Gly m 6 in 6 patients (27%), and Gly m 8 in 11 patients (33%) (Table 1). A high and significant correlation was observed between sIgE to soy extract and Gly m 5 (R^2 = 0.85, Fig. 1a), Gly m 6 (R^2 = 0.97, Fig. 1b), and Gly m 8 (R^2 = 0.78, Fig. 1c), while no significant correlation was found between soy extract sIgE and Gly m 4 (Fig. 1d), possibly because soy extract in the reagent used in the diagnostic platform contains little Gly m 4 due to the pre-treatment procedures.

Sensitization Profiles Identified: Peanut-Associated and PR-Associated SA

Based on the individual sIgE sensitization patterns, two profiles were identified:

(i) Peanut-associated SA group (26%) – represented by 8 patients, all sensitized to peanut components Ara h 1, Ara h 2, and Ara h 3 (Table 1, represented by red). All patients in this group were sensitized to Gly m 6 (100%), 87% to Gly m 5 and 75% to Gly m 8; 50% were sensitized to Gly m 4, all of whom were also sensitized to Bet v 1 (Fig. 2a).

Correlation between sIgE levels against specific soy components and their homologue allergens in peanuts and birch were analyzed. A strong positive correlation between sIgE to Gly m 6 and Ara h 3 (R^2 = 0.94, online suppl. Fig. 1A; for all online suppl. material, see www.karger.com/doi/10.1159/000530026) was observed, while nonsignificant correlations were observed between sIgE to Gly m 5 and Ara h 1, as well as Gly m 8 and Ara h 2 (online suppl. Figure 1B, C).

All 8 patients reported allergic complaints after consumption of processed soy products such as soy sauce, beansprouts, and soy flour; 4 after consumption of soy milk (50%), with 2 patients indicating they intentionally avoided consuming soy milk (online suppl. Table 1, represented by red). The most common symptom in this group was laryngeal edema (87%), followed by AS (62%). Oral allergy syndrome was described by 4 patients (50%) (online suppl. Fig. 2A).

(ii) Non-peanut/PR-associated SA group (74%) – represented by the remaining 22 patients, all sensitized to Gly m 4 and co-sensitized to Bet v 1 (Table 1, represented by gray); none were sensitized to any of the peanut components or to Gly m 5 or Gly m 6, and sIgE to Gly m 8 was detected in only 5 patients (23%) (Fig. 2b). A significant positive correlation was observed between sIgE Gly m 4 and Bet v 1 (R^2 = 0.68) (online suppl. Fig. 1D).

Most patients (77%) pointed to soy milk as the product consumed before the symptom onset, and 5 patients (23%) reported that symptoms occurred after the consumption of processed soy products in addition to soy milk (online suppl. Table 1, represented by gray). The most common reaction was AS (64%); angioedema was described by 12 patients (55%), laryngeal edema by 9 patients (41%), and oral allergy syndrome by 7 patients (32%) (online suppl. Fig. 2B).

Clinical Relevance of IgE Gly m 8 Soy Sensitization

To evaluate the clinical relevance of Gly m 8 sensitizations in the diagnosis of SA, an iBAT was performed with sera of the patients from the peanut-associated SA group (n = 8) since all patients had elevated IgE levels to the Gly m 8 (2S albumin). Moreover, to analyze the validity of this assay, an iBAT for

Table 1. Demographic data plus the sIgE profiles for soy and soy allergens Gly m 4, Gly m 5, Gly m 6, and Gly m 8 plus potential cross-allergens: Bet v 1, Ara h 1, Ara h 2, and Ara h 3

Patient	Age/sex	ImmunoCAP IgE (kU/L)								
		soya bean	nGly m 4	nGly m 5	nGly m 6	nGly m 8	rBet v 1	rAra h 1	rAra h 2	rAra h 3
1	26/F	2.66	4.84	1.67	2.74	0.38	13.5	19.0	92.90	10.7
2	23/F	9.59	<0.35	3.58	12.3	<0.35	<0.35	81.1	>100	28.3
3	32/M	2.69	<0.35	2.75	1.78	0.97	<0.35	19.5	36.00	2.93
4	55/M	1.63	0.81	0.36	0.86	<0.35	14.5	<0.35	1.64	<0.35
5	40/F	1.96	20.4	<0.35	1.32	0.41	45.0	3.84	5.71	1.38
6	21/F	19.5	<0.35	9.75	19.7	3.76	<0.35	68.4	>100	71
7	34/F	3.35	<0.35	2.39	2.88	0.47	<0.35	63.4	71.5	12.8
8	25/F	19.8	2.51	4.86	25.5	5.59	>100	63.3	>100	67.3
9	56/F	<0.35	1.24	<0.35	<0.35	<0.35	8.62	<0.35	<0.35	<0.35
10	32/F	2.23	2.00	<0.35	<0.35	0.60	26.1	<0.35	<0.35	<0.35
11	43/F	<0.35	8.28	<0.35	<0.35	<0.35	28.5	<0.35	<0.35	<0.35
12	26/F	0.93	28.0	<0.35	<0.35	<0.35	64.8	<0.35	<0.35	<0.35
13	65/F	<0.35	1.84	<0.35	<0.35	<0.35	9.88	<0.35	<0.35	<0.35
14	46/F	<0.35	4.98	<0.35	<0.35	<0.35	12.8	<0.35	<0.35	<0.35
15	51/F	<0.35	7.2	<0.35	<0.35	<0.35	14.7	<0.35	<0.35	<0.35
16	36/M	<0.35	1.43	<0.35	<0.35	<0.35	9.55	<0.35	<0.35	<0.35
17	68/F	0.83	8.67	<0.35	<0.35	0.66	45.5	<0.35	<0.35	<0.35
18	50/F	0.36	5.16	<0.35	<0.35	<0.35	15.6	<0.35	<0.35	<0.35
19	21/M	0.58	11.3	<0.35	<0.35	<0.35	78.1	<0.35	<0.35	<0.35
20	52/F	<0.35	0.63	<0.35	<0.35	<0.35	2.84	<0.35	<0.35	<0.35
21	69/F	<0.35	10.8	<0.35	<0.35	<0.35	25.4	<0.35	<0.35	<0.35
22	67/M	<0.35	8.12	<0.35	<0.35	1.00	37.0	<0.35	<0.35	<0.35
23	60/F	<0.35	0.95	<0.35	<0.35	<0.35	8.25	<0.35	<0.35	<0.35
24	52/F	<0.35	5.78	<0.35	<0.35	<0.35	36.4	<0.35	<0.35	<0.35
25	58/F	<0.35	0.99	<0.35	<0.35	<0.35	4.93	<0.35	<0.35	<0.35
26	58/F	<0.35	11.8	<0.35	<0.35	<0.35	45.1	<0.35	<0.35	<0.35
27	46/M	<0.35	1.05	<0.35	<0.35	<0.35	4.59	<0.35	<0.35	<0.35
28	54/M	1.97	3.27	<0.35	<0.35	0.50	19.4	<0.35	<0.35	<0.35
29	26/F	<0.35	4.34	<0.35	<0.35	<0.35	16.3	<0.35	<0.35	<0.35
30	20/F	<0.35	37.8	<0.35	<0.35	0.51	69.5	<0.35	<0.35	<0.35

Peanut-associated SA group. Non-peanut/PR-associated SA group.

Ara h 2 was performed in this group as well. The reliability of the iBAT Gly m 8 results are strengthened by the fact that the iBAT Ara h 2 outcomes correspond with the recorded medical history.

For Gly m 8, there was no increase in basophil activation, with an average of %CD63 activation of 3.68 at an allergen concentration of 300 ng/mL, rising to 5.5 when the allergen concentration was 1,000 ng/mL (Fig. 3a). Thus, Gly m 8 did not induce basophil degranulation in the iBAT assay. For Ara h 2 ($n = 8$), dose-response curves reached positive IBAT results in 7 patients (Fig. 3b). Patient #4 had a negative iBAT to Ara h 2, which corresponded to the absence of a recorded allergic reaction to peanuts or other type of nuts. The seven positive iBAT results also correlate well to the clinical history of the patients, with all describing a clear medical history of peanut allergy that started at an early age and a recorded medical episode of AS after peanut consumption.

Dot Blot

Following preincubation of patient's sera with raw peanut extract to block binding of cross-reactive IgG antibodies, a positive signal was observed for patients #6 and #8 (Fig. 4a), thereby confirming that rGly m 8 is recognized by sIgE. The binding for patient #6 was much lower when compared to the binding shown for patient #8 (as show in Fig. 4b), possibly because the total IgE binding is mostly based on Gly m 8 plus the cross-reactive peanut allergens Gly m 4 and Bet v 1; while patient #6 shows IgE partially from Gly m 8 and the cross-reactive peanut allergens but not from Gly m 4 nor Bet v 1. The negative control, patient #14, showed insignificant binding to rGly

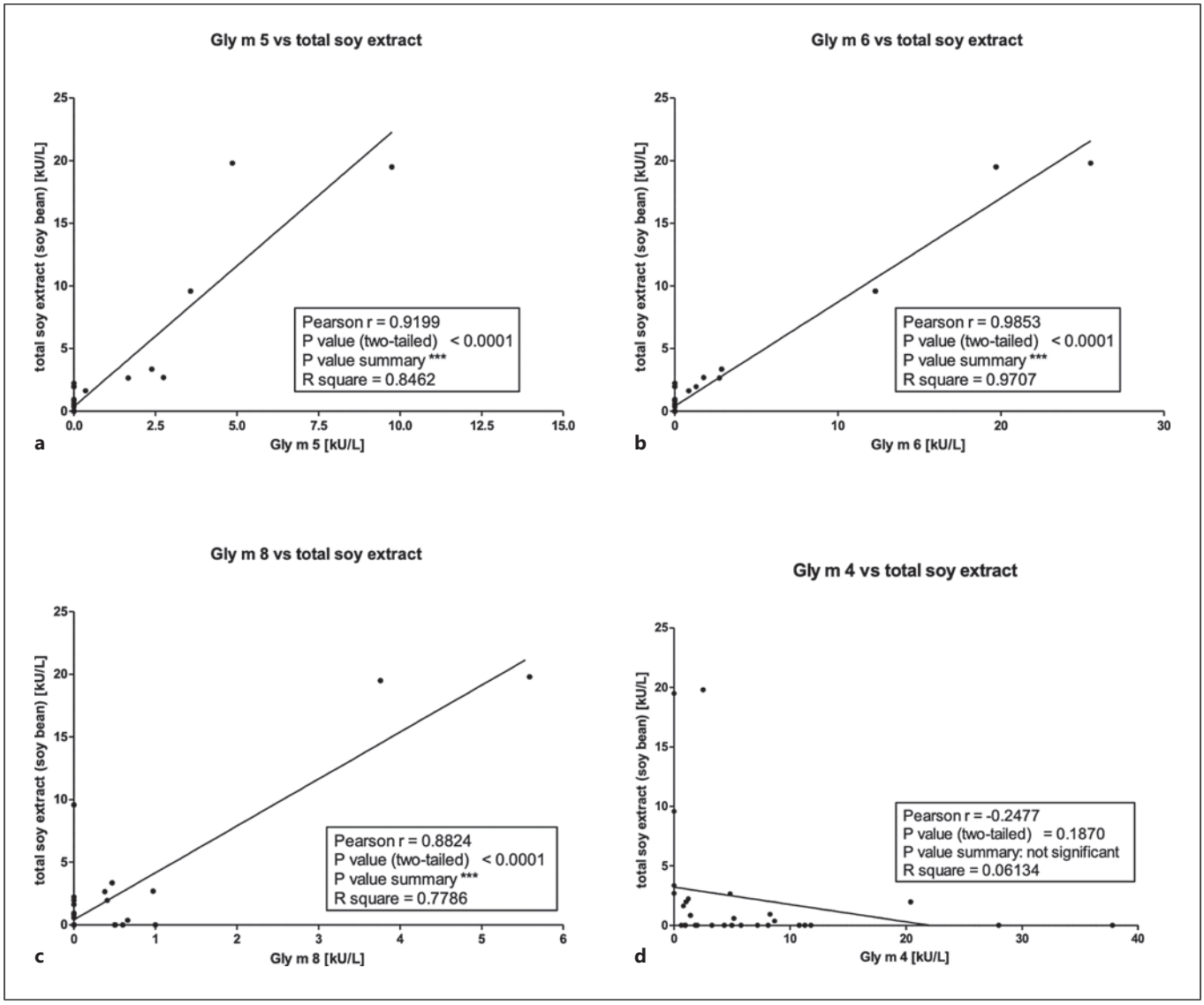

Fig. 1. Correlation between total soy extract sIgE levels and soy allergen markers (**a**) Gly m 5 vs total soy extract; (**b**) Gly m 6 vs total soy extract; (**c**) Gly m 8 vs total soy extract; (**d**) Gly m 4 vs total soy extract.

m 8 in the dot blot, showing IgE binding to Gly m 4 and partially from cross-reactive Bet v 1 (Fig. 4a, b).

Discussion

Currently, the diagnostic capacity of Gly m 8 remains unclear, and the present study analyzed the diagnostic value of Gly m 8 within an SA population by determining the sensitization profiles based on the homologues soy allergens Bet v 1, Ara h 1, Ara h 2, and Ara h 3 [16, 18]. Moreover, since sensitization does not equal allergy, in contrast to previous studies, the clinical relevance of sIgE Gly m 8 sensitization was analyzed using the iBAT.

The results of the present study showed two different sensitization profiles in the selected soy-allergic population based on homologues soy allergens present in peanuts and birch: peanut-associated (26%) and non-peanut/PR-10-associated (74%) SA groups. These sensitization patterns are in line with an SA phenotype described by Savage et al. [18] possibly related to either birch pollen cross-reactivity or persistent peanut allergy. In the non-peanut/PR-10-associated SA group, sIgE against Gly m 4 and Bet v 1 was identified in all patients,

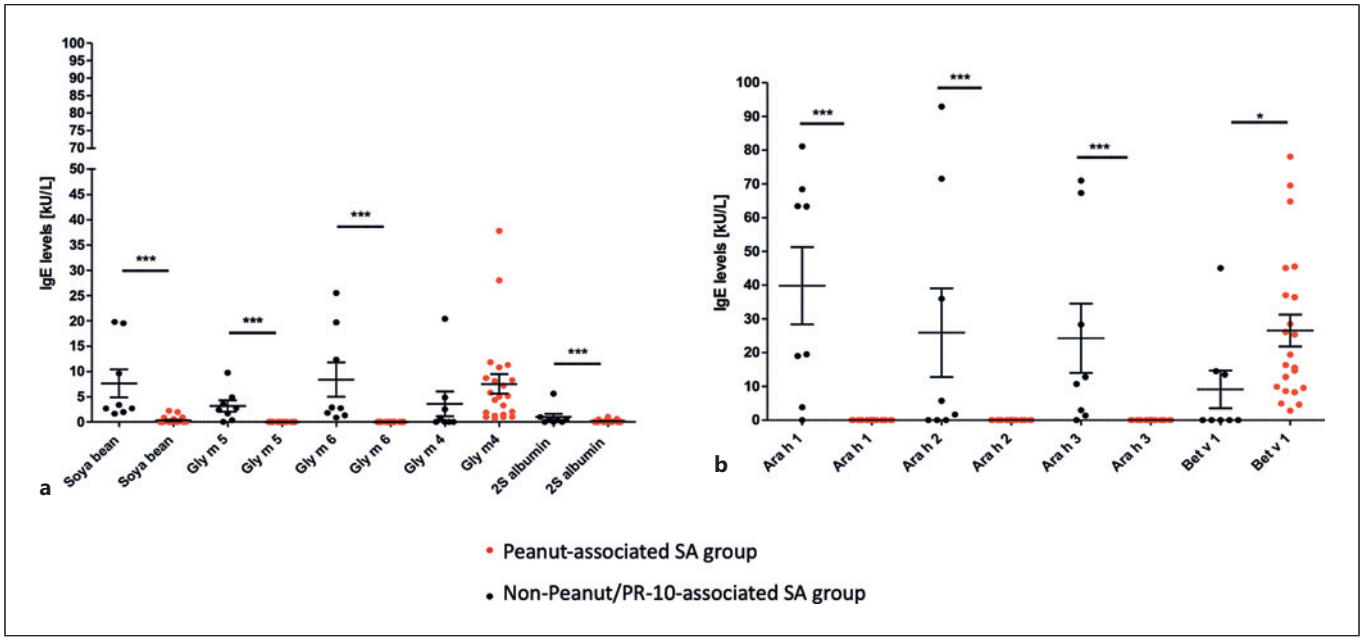

Fig. 2. sIgE distribution in the two sensitization profiles observed in the studied population (n = 30); black – peanut-associated SA; red – non-peanut/PR-10-associated SA. **a** sIgE levels against soy compounds. **b** Peanut compounds (Ara h 1, Ara h 2, and Ara h 3) and birch pollen compound (Bet v 1).

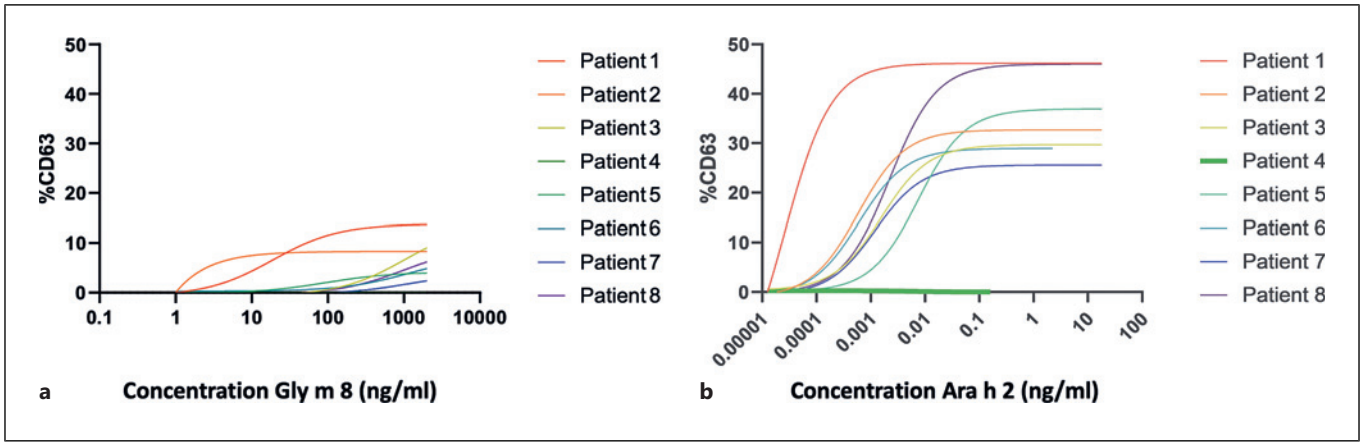

Fig. 3. Nonlinear fitted curves for CD63% basophil activation (%) for patients in the peanut-associated SA group. **a** Different (final) concentrations of rGly m 8. **b** Different (final) concentrations of nAra h 2.

reinforcing the well-known and relevant cross-reactivity between Gly m 4 and Bet v 1 [21, 25]. It has been suggested that SA individuals with pollen-food syndrome could possibly be sensitized to other legumes, particularly peanuts [21, 25, 32]. This association was observed in the present study in 4 patients who were sIgE-positive to Gly m 4 and Bet v 1 as well as one or more of the peanut allergen components (Ara h 1, Ara h 2, and/or Ara h 3). This association is mainly because many food allergies are acquired due to cross-reactivity between aeroallergens and food allergens, mainly pollen; thus pollen-allergic patients can suffer from many different plant food allergens, including peanuts [33, 34].

In the total study population (n = 30), sIgE against Gly m 8 was only found in about a third of the patients (33%). In the

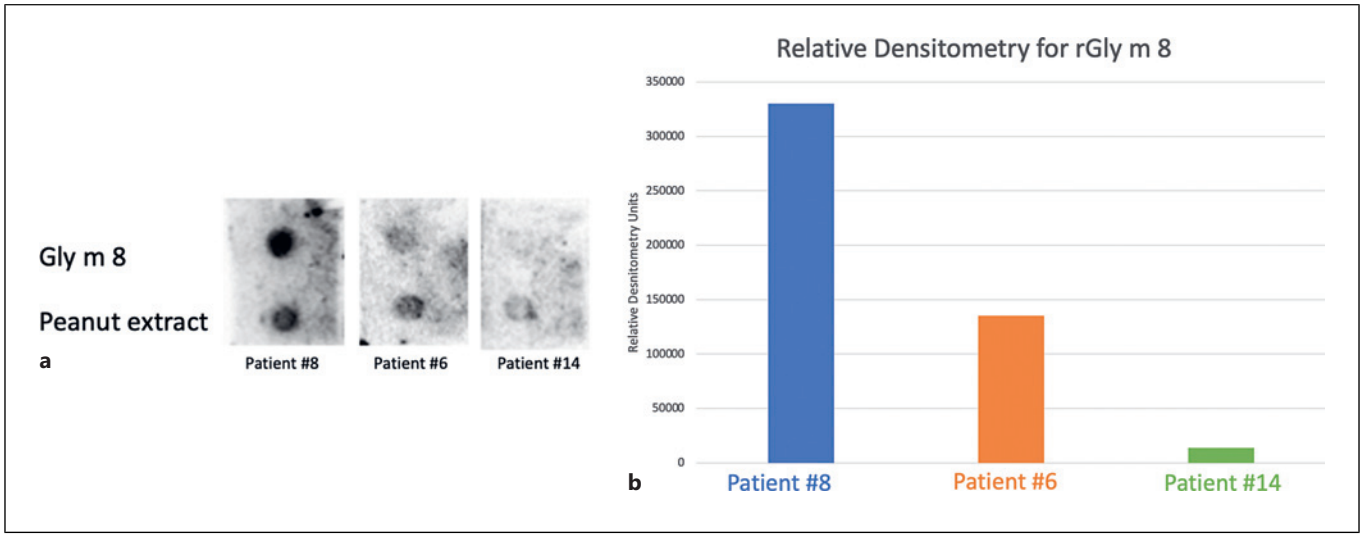

Fig. 4. Dot blot results. **a** Dot blot images for rGly m 8 and peanut extract for patients #6, #8, and #14. **b** Relative densitometry histogram for rGly m 8 for patients #6, #8, and #14 (relative densitometry values are corrected for the dot blot background reading).

non-peanut/PR-associated SA group, a low number of patients (23%) had a positive sIgE against Gly m 8 compared to the peanut-associated SA group where most of the patients (75%) had a positive sIgE against Gly m 8. This prevalence of Gly m 8 sensitization differs from previous studies that have evaluated the accuracy of sIgE to Gly m 8 for the diagnosis of SA [11, 15]. However, previous studies had a high prevalence of peanut co-sensitization in their study population, ranging from 57% to 89% [11, 15], which differs from the present study population, 23%. This concomitant high prevalence of peanut allergy could have influenced the diagnostic value of Gly m 8, possibly due to cross-reactivity to Ara h 2 [11]. Thus, sIgE to Gly m 8 appears to not be a very frequent soy marker when the soy-allergic population is not highly sensitized to peanuts. The cross-reactivity between Gly m 8 and Ara h 2, both δ-conglutin proteins, has been previously described [20–24]. However, data regarding the clinical implications of their cross-reactivity are lacking [35]. 2S albumins, such as Ara h 2 and Ara h 6, have been described as the most important allergens of peanuts, and their structure is likely to contribute to the cross-reactivity between peanuts and other foods, such as soy [36]. Clinically relevant cross-reactivity of 2S albumins of different species has been reported as uncommon [37], probably due to the fact that sequence homology between peanuts and soy 2S albumins is too low, i.e., approximately 40% [23, 38], while a sequence homology of greater than 70% is associated with a clinically relevant cross-reactivity [39]. The homology of Gly m 8 to Ara h 2 is higher than to Ara h 6; thus, a clinically relevant cross-reactivity between Gly m 8 and Ara h 6 is not expected [20, 21, 40].

The clinical relevance of sIgE Gly m 8 sensitization can only be confirmed with a functional assay. To our knowledge, this is the first time the iBAT has been used to assess the clinical relevance of sIgE Gly m 8. The observed results showed that sIgE sensitization to Gly m 8 was not clinically relevant in all studied patients, compared to Ara h 2 which showed positive iBAT results with adequate correlation with clinical symptoms. Therefore, studies in which sIgE sensitization profiles are correlated with the clinical outcome would benefit from the inclusion of in vitro functional assays, such as the iBAT. The results of the present study show the reliability and possible future uses for this type of assay in SA diagnosis, even though the iBAT for Gly m 8 needs to be validated in larger populations.

The discrepancy between the present study and previous reports regarding the diagnostic value of Gly m 8 may be due to criteria for population selection, geographical reasons, and/or different 2S albumin preparations. In the present study population, selection appears to be similar to previous studies, namely, based on an SA diagnosis [11, 12, 15, 16]. However, the main difference of the present study population is that the rate of peanut co-sensitization is considerably lower, i.e., 23% versus 89% [15]. There is no obvious reason for this co-sensitization difference in study populations with the exception that there is an age discrepancy with Kattan et al. [15] since the study was performed in a pediatric outpatient setting.

Therefore, it is possible that the high prevalence of peanut co-sensitization might have previously overestimated the diagnostic value of Gly m 8. On the other hand, Lin et al. [17] concluded that soy 2S albumins were not major allergens in their study population, which results in line with the present study. Additionally, the selected study population of Lin et al. [17] reported a 37% peanut co-sensitization, similar to the rate in the present study. Even though regional variations have been reported both for SA as well as for cross-sensitization and clinical-reactivity patterns for different legumes, these differences would not justify the different results since the studied population is mainly European, including different European regions [11, 17]. These described differences might be reminiscent of variability in diet, including cross-reactive pollen-fruit allergies that are common in Europe, together with a changing food allergy pattern in Asia [41]. The allergen sources, the handling of these preparations, and the main products derivatives consumed could all contribute to the difference in results.

A dot blot experiment was carried out to test the IgE binding ability of serum of patients, reflecting the different profiles to the recombinant Gly m8 allergen. The dot blot results confirmed that sIgE from the sera of the selected patients was recognized by the utilized form of rGly m 8 in the iBAT. Moreover, recombinant allergen preparations have been evaluated and showed results that are highly specific plus avoided false positives by elimination of cross-reactive allergens [42]. Therefore, even though this is the first time the iBAT has been used with rGly m 8, the CD63-based iBAT has been validated for the use of recombinant allergens for the detection of sensitization to foods before [43].

In conclusion, the results of the present study show that Gly m 8 was not clinically relevant, as demonstrated by the negative iBAT results despite sIgE sensitization. In line with other studies, 2S albumins from soybean, Gly m 8, was not a major allergen in the selected soy-allergic population. Hence, Gly m 8 would have no added value in the diagnosis of SA in the present study population. The present study highlights why it is crucial to analyze co-sensitization patterns in allergic populations and verify the clinical relevance of sensitization with the use of functional assays such as the BAT to avoid over- or underestimating the diagnostic value of sIgE sensitizations.

Statement of Ethics

This study protocol was reviewed and approved by the CMO Regio Arnhem-Nijmegen with the registration number 2013/235 and the NL NR.: 44,545.091.13. Written informed consent was obtained from the patient for publication of the details of their medical case and when applicable any accompanying images. All the participants in the study have given their written informed consent.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Funding Sources

This research received no external funding.

Author Contributions

All the authors contributed to the design and implementation of the research. D.B., L.H., and A.B. performed the measurements, processed the experimental data, performed the analysis, and designed the figures, under the supervision of A.J., H.S., and J.R.K. M.T., D.B., and L.H. interpreted the results. D.B. drafted the manuscript. All the authors discussed the results and commented on the manuscript.

Data Availability Statement

All the data generated or analyzed during this study are included in this article and/or its supplementary material files. Further inquiries can be directed to the corresponding author.

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