

In vitro Analysis of Birch-Pollen-Associated Food Allergy by Use of Recombinant Allergens in the Basophil Activation Test

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Key Words

Basophil activation test · CD63 expression ·
Flowcytometry · Food allergy · Recombinant allergens ·
Bet v 1 · Api g 1 · Dau c 1 · Mal d 1

Abstract

Background: Basophil activation is associated with the expression of CD63. In birch-pollen-associated food allergy to celery, carrot and apple, Bet v 1, Api g 1, Dau c 1 and Mal d 1 are major allergens. Recombinant allergens have not yet been used in the CD63-based basophil activation test (BAT). **Objective:** To evaluate the feasibility of using recombinant allergens in the BAT in the diagnosis of allergy to apple, carrot and celery and to compare results with routine tests, i.e. skin prick tests (SPTs) and specific IgE. **Methods:** Thirty-two patients with an oral allergy syndrome induced by apple, carrot or celery and 22 controls were studied. SPTs were performed with native foods. Specific IgE was determined by the CAP method and basophil activation by flowcytometry upon double staining with anti-IgE/anti-CD63 monoclonal antibodies after incubating with purified recombinant Bet v 1, Bet v 2, Api g 1, Dau c 1 and Mal d 1. **Results:** By the

combined use of the BAT and the CAP method, sensitization to Bet v 1 and Bet v 2 was detected in 100 and 25% of all subjects, respectively. Sensitivity of specific IgE for apple, carrot and celery was 60, 70 and 75% with corresponding specificities of 64, 86 and 82%. Sensitivity of the BAT for Mal d 1, Dau c 1 and Api g 1 was 75, 65 and 75% with corresponding specificities of 68, 100 and 77%.

Conclusions: The BAT using recombinant allergens provides a valuable new in vitro method for the detection of sensitization to foods. Although double-blind placebo-controlled food challenges remain the gold standard to confirm food allergy, the CD63-based BAT with recombinant allergens may supplement routine tests for allergy diagnosis.

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Introduction

Mast cells and basophils play a dominant role as effector cells in immediate-type hypersensitivity. Therefore, basophils are often used as target cells for in vitro assays to detect immediate-type sensitization. Activation of basophils can be quantified via the measurement of media-

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1018–2438/05/1363–0230\$22.00/0

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tors, stored or de novo synthesized and released in the extracellular medium. Furthermore, it has been reported that the surface marker CD63 is expressed with high density on activated basophils, yet only weakly on resting cells [1]. The CD63 glycoprotein is present on the membrane of cytoplasmic granules and expressed only to an extent of less than 5% on the outside membrane of resting basophils, but with high density on activated basophils after the fusion of these granules with the cytoplasmic membrane [1]. Flowcytometric detection of basophil activation by CD63 expression has been studied in diagnosing immediate-type allergy to various allergens such as natural rubber latex, foods, hymenoptera venoms, aeroallergens (cypress pollen, house dust mites) and drugs [1–5]. This new in vitro method has been commonly referred to as basophil activation test (BAT) [2] or test of basophil altered surface immunofluorescence detected by cytometry [3]. To date, the BAT has not been performed applying purified recombinant allergens in food allergy.

Birch-pollen-associated allergy to foods such as apple, carrot and celery is frequently observed in Central and Northern Europe and accounts for a great proportion of all food allergies in adults [6, 7]. In the majority of these cases, food hypersensitivity is a result of crossreactivity to Bet v 1, the major allergen of birch pollen [8]. Homologous proteins to Bet v 1 have been identified in a wide range of flowering plants [9]. Mal d 1, the major allergen from apple, was the first Bet v 1 homologous fruit allergen cloned and produced as recombinant allergen [10], followed by Api g 1 from celery [11] and Dau c 1 from carrot [12].

The aim of this study was to investigate whether a two-color flowcytometric method using anti-IgE and anti-CD63 monoclonal antibodies (mAb) to analyze human basophil activation could be applied to the diagnosis of birch-pollen-associated food allergy to apple, carrot and celery by use of the recombinant allergens Api g 1, Dau c 1, Mal d 1, Bet v 1 and Bet v 2, and to compare sensitivity and specificity with well-established tests such as skin prick tests (SPTs) and the measurement of allergen-specific serum IgE.

Material and Methods

Patients

Patients with a most suggestive history of an oral allergy syndrome (OAS) [13] induced by apple and/or carrot and/or celery and a positive SPT with birch pollen were recruited for this study. For each food, 20 patients with a positive clinical history were selected. Since most patients reported an OAS induced by more than one of the

three foods, only a total of 32 patients had to be recruited to achieve three subgroups of 20 patients with a positive history for each food. Only patients with a highly suggestive history analogous to class A of the classification by Nyfeler and Pichler [14] to evaluate the reliability of a clinical history in drug allergy were considered. All of the patients had a history of seasonal allergic rhinitis or allergic asthma. Ten subjects with no history of adverse food reaction and no seasonal allergic rhinitis, asthma or atopic dermatitis were selected as controls. These 10 controls were nonatopic as defined by the absence of any clinical history of atopic diseases. All subjects provided informed consent before enrollment in this study.

Skin Testing

SPTs were performed on the flexor aspect of the forearm with a standardized prick needle (ALK Scherax, Hamburg, Germany). We used histamine dihydrochloride (10 mg/ml) as a positive control and the glycerol diluent of the prick solution as a negative control. Patients and controls were skin tested with native apple (Golden Delicious), carrot and celery root by using the prick-to-prick technique [15]. In addition, all subjects were skin prick tested with birch, grass and mugwort pollen (ALK Scherax). We recorded reactions after 15 min. A skin test was considered positive if it produced a wheal with a diameter of at least 3 mm [16].

Testing for IgE

Total and specific IgE to apple, carrot, celery, Bet v 1 and Bet v 2 (purified recombinant allergens) were measured by using the CAP-FEIA system according to the recommendations of the manufacturer (Pharmacia Diagnostics, Uppsala, Sweden). Specific IgE was recorded in CAP classes 0–6 (class 0: <0.35 kU/l; class 1: 0.35–0.70 kU/l; class 2: 0.70–3.50 kU/l; class 3: 3.50–17.50 kU/l; class 4: 17.50–50.00 kU/l; class 5: 50.00–100.00 kU/l; class 6: >100 kU/l). Specific IgE exceeding 0.35 kU/l was considered positive.

Basophil Activation Test

Peripheral blood samples from all subjects were drawn into heparinized tubes. Hundred microliters of whole blood were preincubated with 20 µl of wash buffer – containing interleukin (IL)-3 at a final concentration of 4 ng/ml – at 37°C for 10 min. Hundred microliters of the cell suspension were distributed in microtiter plate wells and mixed in parallel with 100 µl of wash buffer (negative control), 100 µl of N-formyl-methionyl-leucyl-phenylalanine (fMLP; 1 µmol/l as positive control) and 100 µl of the recombinant allergens Bet v 1, Bet v 2, Api g 1, Dau c 1 and Mal d 1 (Biomay, Austria) at the following concentrations: 1 ng/ml (Bet v 1), 10 ng/ml (Bet v 2), 10 µg/ml (Api g 1) and 1 µg/ml (Dau c 1 and Mal d 1, respectively). Before this study, the BAT had been performed in a pilot study in food allergics (n = 8 per food item) with Api g 1, Dau c 1 and Mal d 1 of increasing serial dilutions with allergen concentrations ranging between 100 and 10^{−9} µg/ml. A bell-shaped dose-response curve for all three recombinant allergens was demonstrated (fig. 1a–c). Furthermore, in an additional pilot study, dose-response curves for Bet v 1 and Bet v 2 were established in 8 patients for each allergen (fig. 2). No basophil activation could be induced in controls (data not shown).

Plates were incubated for 20 min at 37°C. Degranulation was stopped by chilling on ice for 5 min. Thereafter, phycoerythrin-conjugated anti-IgE (PE anti-IgE) and fluorescein isothiocyanate (FITC)-conjugated anti-CD63 mAb were added and incubated for 20 min on ice. Finally, the heparinized whole-blood samples were lysed, fixed and washed. Plates were centrifuged (250 g, 5 min, 4°C) and ana-

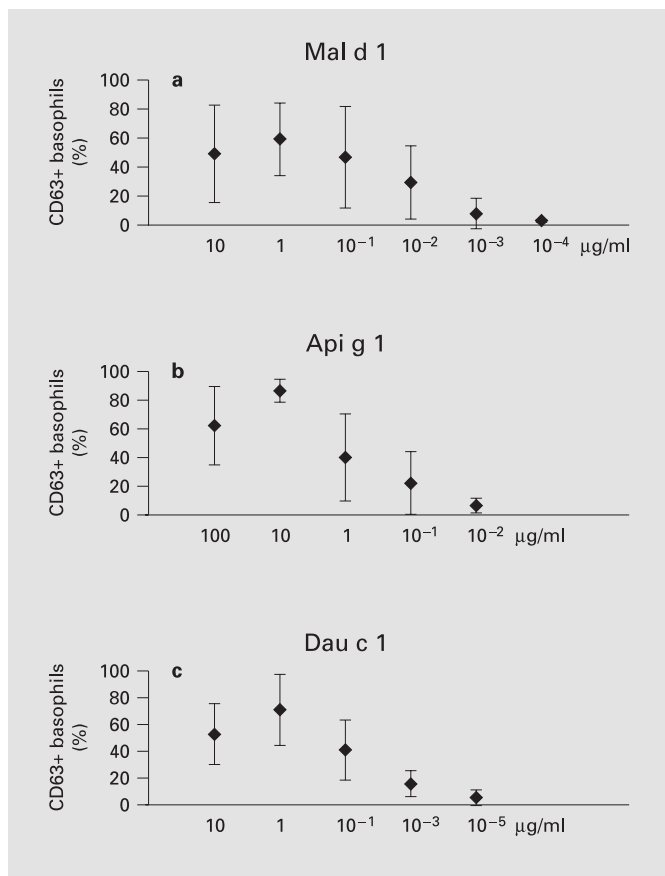


Fig. 1. Dose-response curves for CD63 expression in apple allergics (n = 8) after incubation with Mal d 1 (a), in celery allergics (n = 8) after incubation with Api g 1 (b) and in carrot allergics (n = 8) after incubation with Dau c 1 (c).

lyzed within 2 h on a FACScan flowcytometer (Becton Dickinson Immunocytometry System, San Jose, Calif., USA). IL-3, fMLP, PE anti-IgE and FITC-conjugated anti-CD63 mAb were purchased from Orpegen Pharma, Heidelberg, Germany. The basophil population was gated by the expression of PE anti-IgE. The expression of CD63 was analyzed on this gated cell population. An average of 200 IgE-expressing basophils per probe were acquired and results given as the percentage of basophils expressing CD63 according to the following formula: number of PE anti-IgE+ and FITC anti-CD63+ basophils/number of PE anti-IgE+ basophils. In a pilot study with 10 patients sensitized to Bet v 1, CD63 expression after incubating with Bet v 1 was determined with 100, 200, 400 and 500 basophils. We could demonstrate in a patient that the outcome of the BAT does not significantly vary within a range of 100–500 basophils being evaluated.

Statistics

Data are expressed as means $\pm$ SD. The comparison of the percentage of CD63+ basophils between patients and controls was performed with the Welch test. The significance level was set to 0.05.

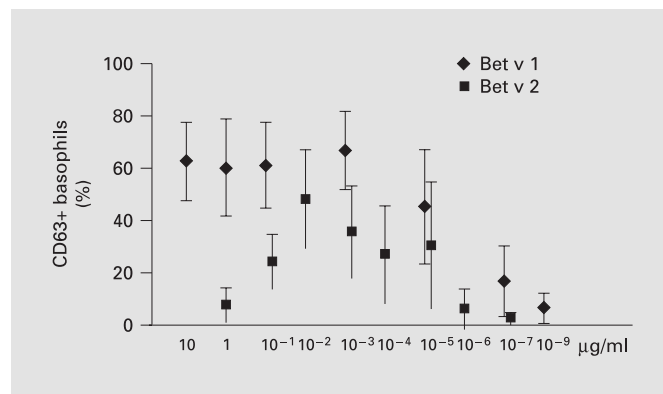


Fig. 2. Dose-response curves for CD63 expression in the BAT after incubating with Bet v 1 and Bet v 2 in sensitized patients (n = 8 for each allergen).

Because of the explorative capacity of p values, no α -adjustment was made.

It is important to note that in order to calculate sensitivity and specificity, clinical history was considered the 'golden standard'. For the study of food allergy, only patients with a highly suggestive history of an OAS were selected, since in former studies [17, 18] which included double-blind placebo-controlled food challenges to confirm food allergy a high agreement between clinical history and results of food challenges in contrast with patients showing other signs and symptoms of food allergy existed. Sensitivity and specificity were calculated according to the method of Goldman [19] for the three tests (SPT, CAP, BAT), a true-positive result (TP) was defined as a positive SPT, CAP or BAT in a patient with a positive history of an OAS; a false-positive result (FP) was defined as a positive SPT, CAP or BAT in a patient with a negative history of an OAS; a true-negative result (TN) was defined as a negative SPT, CAP or BAT in a patient with a negative history of an OAS, and a false-negative result (FN) was defined as a negative SPT, CAP or BAT in a patient with a positive history of an OAS.

$$\text{Sensitivity} = \text{TP}/(\text{TP} + \text{FN})$$

$$\text{Specificity} = \text{TN}/(\text{TN} + \text{FP})$$

Calculating specificity for each food was not only based on the 10 nonatopic controls, but also on 12 atopic subjects. In all 32 patients, SPTs with all three foods and both in vitro tests (BAT, CAP) were performed. Since only 20/32 patients had a positive clinical history for the relevant food in each subgroup, there were 12 atopic patients with a negative clinical history for this particular food item (table 1, column OAS). Hence, for each food, there was a total of 22 controls comprising 10 nonatopic (data not shown) and 12 atopic patients.

All statistical analyses were performed with SAS version 8.1.

Table 1. Results of the SPTs with native apple, carrot and celery, the BAT after incubation with Bet v 1, Bet v 2, Mal d 1, Dau c 1 and Api g 1, and specific IgE (CAP class 0–6) in food allergies (n = 20 for each food) and controls (n = 12)

Pt.	Bet v 1			Bet v 2			Apple				Celery				Carrot						
	BAT		CAP	BAT		CAP	OAS	SPT	BAT	CAP	OAS	SPT	BAT	CAP	OAS	SPT	BAT	CAP			
	% CD63+	+/-		% CD63+	+/-				% CD63+	+/-			% CD63+	+/-			% CD63+	+/-			
1	77.86	+	5	1.09	-	0	+	+	95.71	+	2	-	+	61.22	+	1	-	-	1.96	-	0
2	71.82	+	3	1.24	-	0	+	+	86.57	+	0	+	+	0.54	-	0	+	+	0.53	-	0
3	27.86	+	4	31.23	+	3	+	+	5.32	-	1	+	+	17.44	+	3	+	+	33.88	+	3
4	92.63	+	4	7.16	-	0	+	+	80.36	+	2	+	+	96.52	+	0	+	+	94.12	+	0
5	42.31	+	4	40.53	+	3	-	+	0.34	-	2	+	+	66.14	+	2	+	+	12.66	+	2
6	60.12	+	3	16.75	+	2	+	+	1.92	-	3	+	+	3.18	-	3	+	+	0.91	-	3
7	90.08	+	3	31.27	+	0	+	+	4.71	-	1	+	+	88.64	+	0	+	+	9.12	-	0
8	73.87	+	2	69.25	+	2	+	+	31.55	+	0	+	+	26.68	+	0	+	+	99.71	+	0
9	64.62	+	3	1.91	-	0	-	-	2.34	-	0	+	+	87.64	+	3	-	-	2.31	-	0
10	87.12	+	5	0.96	-	0	-	-	2.45	-	0	+	+	47.62	+	2	+	+	29.53	+	2
11	82.03	+	5	2.88	-	0	+	+	93.68	+	2	+	+	63.64	+	3	+	+	88.48	+	2
12	61.52	+	4	53.77	+	3	+	+	0.45	-	3	+	+	1.99	-	3	+	+	4.17	-	3
13	58.09	+	6	1.26	-	0	+	+	58.47	+	3	-	+	41.22	+	1	+	+	1.67	-	1
14	67.89	+	4	59.33	+	0	+	+	81.25	+	3	-	+	77.15	+	3	+	+	68.41	+	3
15	71.51	+	4	4.14	+	0	+	+	92.58	+	3	+	+	80.16	+	2	+	+	4.01	-	2
16	50.54	+	4	2.14	-	0	+	+	2.18	-	2	+	+	2.11	-	1	+	+	1.65	-	2
17	88.72	+	5	7.05	-	0	-	+	87.43	+	4	+	+	92.22	+	4	+	+	85.92	+	3
18	54.47	+	4	1.71	-	0	+	+	60.81	+	0	+	+	80.55	+	2	-	-	1.97	-	0
19	64.48	+	4	7.35	-	0	+	+	72.22	+	2	-	-	6.29	-	0	-	+	7.51	-	2
20	63.13	+	6	0.67	-	0	-	+	74.13	+	2	-	-	1.83	-	0	-	-	2.34	-	1
21	46.88	+	4	1.35	-	0	+	+	77.36	+	0	-	+	66.93	+	2	-	-	1.32	-	0
22	84.89	+	3	2.63	-	0	+	+	57.69	+	0	+	+	60.71	+	0	-	-	2.55	-	0
23	45.16	+	6	1.26	-	6	-	+	75.79	+	2	+	+	47.25	+	2	+	+	19.23	+	2
24	91.62	+	6	1.54	-	0	-	+	91.79	+	3	+	+	62.65	+	1	+	+	17.08	+	1
25	60.22	+	2	2.85	-	0	+	+	52.94	+	0	+	+	62.66	+	3	-	-	6.04	-	0
26	80.42	+	3	4.03	-	0	-	-	2.16	-	0	+	+	6.62	-	0	-	-	6.05	-	0
27	88.35	+	4	0.54	-	0	-	+	83.33	+	2	-	+	2.91	-	1	-	+	0.84	-	1
28	63.39	+	3	2.62	-	0	+	+	70.86	+	0	-	-	1.44	-	0	+	+	70.86	+	0
29	87.16	+	3	3.22	-	0	-	+	86.21	+	1	-	-	2.48	-	0	-	-	0.64	-	0
30	95.81	+	4	5.26	-	0	-	-	1.46	-	0	-	-	2.94	-	0	-	-	2.64	-	0
31	90.14	+	5	7.58	-	0	+	+	78.28	+	0	-	-	7.04	-	0	+	+	55.64	+	0
32	27.71	+	6	1.11	-	0	-	+	67.48	+	5	-	-	1.23	-	0	+	+	81.06	+	4

Results

Skin Prick Testing

Results of the prick-to-prick tests with the three native foods in all 32 patients are summarized in table 1. For each subject, the relevant food (positive clinical history of OAS) is indicated in a separate column. In the 10 non-atopic controls, the foods did not elicit any positive skin tests (data not shown).

Prick-to-prick tests with native apple, carrot and celery showed sensitivities of 100% for all foods. The corresponding specificities were 64, 91 and 77%, respectively.

SPTs with birch pollen were positive in all 32 patients, whereas grass pollen induced a positive SPT in 11/32 and mugwort in 8/32 patients. We observed no positive SPTs in the 10 nonatopic controls.

Testing for IgE

In the group of 32 subjects with a history of an OAS, mean total IgE was 227.88 kU/l (SD: 307.60 kU/l, range: 13–1391 kU/l), and in the 10 controls, mean total IgE was 91.75 kU/l (SD: 184.51 kU/l, range: 13–862 kU/l).

Table 1 summarizes the results of allergen-specific IgE determination in the 32 food allergies (CAP classes 0–6). No proof of allergen-specific IgE was found in the 10 non-atopic controls. Measurement of specific IgE against apple, carrot and celery gave a sensitivity of 60% (95% CI: 36–81%), 70% (95% CI: 46–88%) and 75% (95% CI: 46–88%), respectively; the corresponding specificities were 64% (95% CI: 41–82%), 86% (95% CI: 65–97%) and 82% (95% CI: 55–92%), respectively.

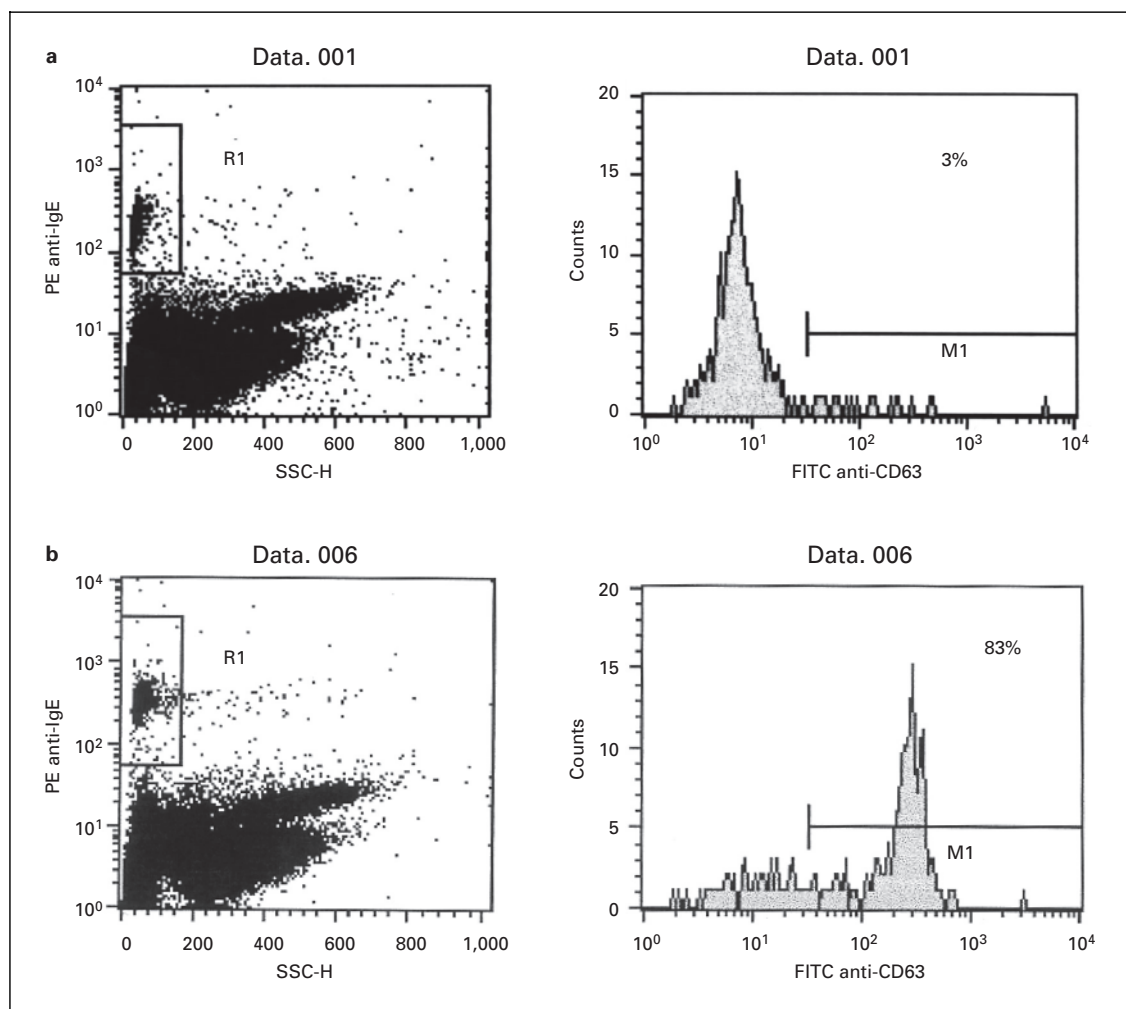


Fig. 3. CD63 expression in the BAT in an apple allergic after incubation with the negative control (**a**) and the major apple allergen Mal d 1 (**b**).

Basophil Activation Test

In the 32 patients with a history of an OAS, the mean percentage of CD63+ basophils (of the total of all IgE+ basophils) after incubating the basophils with wash buffer (negative control) was 2.7% (SD: 1.84%) as compared with an average activation of 2.99% of all basophils (SD: 1.56%) in the controls.

Incubation of the basophils with fMLP (positive control) resulted in a mean basophil activation of 30.96% (SD: 16.08%) in the food allergies and a mean basophil activation of 37.6% (SD: 16.35%) in the controls. Results of negative and positive controls in the group of allergies did not significantly differ from the outcome in the nonallergics.

In the group of food allergies ($n = 20$ for each food), incubating the basophils with the relevant allergen gave the following results with regard to mean basophil activation: Api g 1: 49.75% (SD: 34.27%); Mal d 1: 55.24% (SD: 34.54%) and Dau c 1: 38.22% (SD: 37.02%). Figure 3 shows basophil activation induced by Mal d 1 in one subject allergic to apple. In the 22 controls for each food, mean basophil activation after incubating with Api g 1, Mal d 1 and Dau c 1 was 13.13% (SD: 24.14%), 26.94% (SD: 38%) and 2.22% (SD: 1.91%), respectively. Comparison of the mean percentage of CD63+ basophils shows significant differences between patients and controls in all three allergens. The corresponding p value of the Student's test was <0.0155 for Mal d 1, <0.0004 for Api g 1

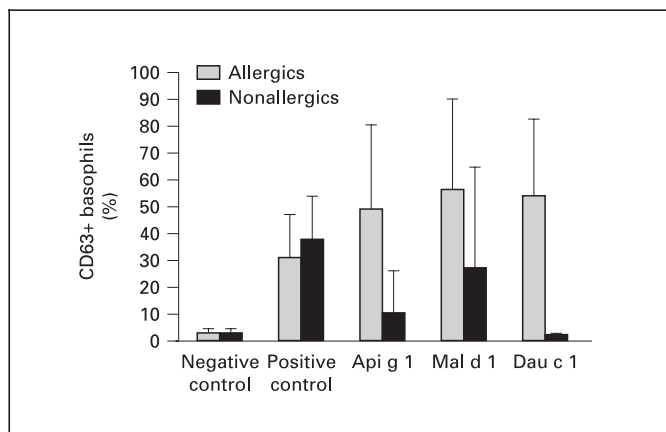


Fig. 4. Mean basophil activation in the BAT in celery, apple and carrot allergics ($n = 20$ for each food item) after incubating with Api g 1, Mal d 1 and Dau c 1 and a negative and positive test control (fMLP). In each pair of columns, the right column represents the nonallergic controls ($n = 22$ for each food).

and <0.0003 for Dau c 1. All data are summarized in figure 4.

Comparing the rate of concordance between the BAT and CAP (both tests negative or positive) in each group of allergics ($n = 20$) including controls ($n = 22$), results were as follows: in apple allergy, both tests gave the same test result in 25/42 (59.52%) subjects, in carrot allergy in 30/42 (71.43%) subjects and in celery allergy in 32/42 (76.19%) subjects. Spearman's correlation coefficients indicated a moderate positive correlation between CAP class and the percentage of CD63+ basophils for Dau c 1, Api g 1 and Mal d 1: r (Dau c 1/carrot) = 0.40, r (Api g 1/celery) = 0.58, r (Mal d 1/apple) = 0.41. Spearman's correlation coefficients for CAP class and the percentage of CD63+ basophils for Bet v 1 and Bet v 2 were 0.53 and 0.38, respectively.

Mean basophil activation (percentage of CD63+ basophils) without stimulation ('background') was 2.7% (SD: 1.8%) in the group of allergics. We set the positivity threshold ('cutoff') for a positive BAT at 10% CD63+ basophils (cutoff > mean basal activation +4 SD).

On the basis of this cutoff, sensitivity and specificity of Mal d 1 were 75% (95% CI: 51–91%) and 68% (95% CI: 45–86%), respectively. Sensitivity and specificity of Api g 1 were 75% (95% CI: 51–91%) and 77% (95% CI: 55–92%), respectively. Sensitivity and specificity of Dau c 1 were 65% (95% CI: 41–85%) and 100% (95% CI: 87–100%), respectively.

Discussion

In this study, flowcytometry was applied to study allergen-induced basophil activation in IgE-mediated food allergy using a double-staining method with anti-IgE and anti-CD63 mAb as described in the literature [1]. Patients with a pollen-associated OAS induced by apple, carrot and celery were selected. These foods were investigated, since the recruitment of food-allergic patients was facilitated due to the high prevalence of OAS in patients with seasonal respiratory allergy to birch pollen in particular [11, 20], ensuring a large study population for the measurement of sensitivity and specificity. The identification of basophils was based on the presence of IgE on the cell surface.

In Central Europe, pollen-associated food allergy to apple, carrot and celery is mostly caused by crossreactivity to the major birch pollen allergen Bet v 1 [8]. To our knowledge, in this study, recombinant allergens were applied in the BAT for the first time to study pollen-associated food hypersensitivity: Bet v 1, Bet v 2 (profilin), Api g 1 (major allergen of celery), Dau c 1 (major allergen of carrot) and Mal d 1 (major allergen of apple). Results of the BAT were compared with routine tests such as SPTs with native foods and the measurement of allergen-specific IgE. Clinical history was considered the 'golden standard' for calculating sensitivity and specificity of the three applied tests (SPT, the BAT and measurement of specific IgE).

In our study, SPT with native apple, carrot and celery ($n = 20$ for each food item) showed a sensitivity of 100% for all three foods in the patients with an OAS. High sensitivity of SPTs with fresh foods as compared with commercially available food allergen extracts is a well-known factor, particularly with regard to studying hypersensitivity to fruits and vegetables [4, 21].

Sensitivity of SPTs and determination of specific IgE by the CAP-FEIA method for carrot and celery are consistent with results of previous studies [17, 18]. In a study by Ballmer-Weber et al. [17], sensitivity of specific IgE to celery was 73% and of SPTs with crude celery 96%. Interestingly, celery allergy was confirmed by double-blind placebo-controlled food challenges in 83% of all patients with a history of an adverse reaction to this food.

In 32 patients with a suggestive history of an OAS induced by apple, carrot and celery, we determined CD63 expression in the BAT after incubating the patients' basophils with the relevant recombinant allergen Mal d 1, Dau c 1 and Api g 1 in 20 tests for each food. In addition, we performed the BAT after incubating the basophils with

Bet v 1 and Bet v 2. Basophil responses are considered to be highly heterogeneous in the same and between different individuals [22]. Therefore, different allergen concentrations covering several logs have been proposed for basophil-based tests [22]. Since recombinant allergens have not been applied in the BAT before, we established dose-response curves for each allergen (fig. 1, 2), selecting appropriate allergen concentrations. Dose-response curves showed that appropriate allergen concentrations varied considerably between the different recombinant allergens Bet v 1, Bet v 2, Api g 1, Dau c 1 and Mal d 1, covering a range between 1 ng/ml (Bet v 1) and 10 µg/ml (Api g 1). Dose-response curves were bell shaped with a plateau covering several tenfold allergen dilutions which means that within a relatively wide range of recombinant allergen concentrations the BAT remains positive. This is of great importance for practical use. If the optimal concentration is in a narrow range and varies considerably from patient to patient, it is necessary to use several allergen concentrations per test allergen. However, if on the other hand basophil activation occurs over a broad range of allergen concentrations, it is possible to use a single concentration per test. To date, the latter has only been proven for some natural protein allergens such as house dust mites, grass pollen or hymenoptera venoms [5]. However, for small molecules such as drugs, the range of basophil-activating concentrations has been found to be small [5]. In our study, the use of one single allergen concentration for each allergen facilitated the evaluation of 'cellular reactivity' of the basophils (i.e. maximal response), but not the cellular sensitivity (i.e. shift of the increasing dose-dependent activation) [22]. Therefore, in future studies applying additional recombinant allergens in the BAT besides Api g 1, Dau c 1, Mal d 1, Bet v 1 and 2, dose-response curves will remain indispensable.

As a positive control, anti-IgE would be most appropriate for allergen-mediated activation of basophils, since the same pathway after crosslinking of FcεRI receptors on basophils is triggered. However, due to the staining procedure with anti-IgE, another secretagogue has been chosen as positive control: fMLP activates basophils through IgE-independent mechanisms [24]. As a consequence, nonreleasing basophils with a defect in signaling IgE-mediated stimuli, occurring in 15–20% of the general population [25], will not be picked up by the fMLP. However, IL-3 coinubation is able to restore FcεRI-associated protein tyrosine kinase (Syk) expression and IgE-mediated basophil activation [26], decreasing the rate of nonresponders to approximately 5%. As a consequence, positive SPTs and detectable IgE concentrations to the food allergens,

but a negative outcome using CD63 expression, might be explained by this phenomenon – nonresponding basophils with regard to IgE-mediated stimuli – even if the fMLP control is positive. Mean basophil activation induced by fMLP was lower than the average basophil activation after incubation with the relevant allergens in the food allergics, probably due to reduced potency of this secretagogue as compared with the allergens.

An increase in CD63 expression exceeding more than 10% of the total (IgE+) basophil population (cutoff) was considered to be a positive BAT. On the basis of this positivity threshold, 43 out of 60 tests were considered positive with the relevant food allergen. On the other hand, in a total of 66 controls (22 controls for each of the three foods), 12 tests were also positive. Since most of these controls also had a positive SPT with the same food or proof of allergen-specific IgE, the BAT was classified 'false positive' with regard to a lacking history of food allergy. However, in these cases, a positive BAT correctly identified sensitization. These data suggest that the BAT, such as SPT or allergen-specific IgE, does not provide definitive information about the clinical relevance of sensitization.

'False-positive' test results (i.e. clinically nonrelevant sensitization) due to multiple crossreactive allergens are a well-known problem, in particular in pollen-associated food allergy [27]. In celery allergy, IgE crossreactivity is due to at least three distinct crossreacting allergens [28]. Besides Bet v 1 and profilin, particularly carbohydrate structures (crossreactive carbohydrate determinants) on glycoproteins are also involved in pollen-vegetable food crossreactivity. However, their clinical relevance is still a matter of debate [29]. Crossreactive carbohydrate determinants are particularly relevant with regard to false-positive IgE serology [30].

Our results confirm that the detection of basophil activation by double staining with anti-CD63 and anti-IgE and the application of recombinant allergens is a new method feasible in the diagnosis of IgE-mediated immediate-type allergy which widens the spectrum of in vitro diagnostics. These results are consistent with the outcome of several previous studies which also evaluated basophil activation by applying flowcytometry and different cell surface markers, in particular CD63 [3–5]. Flowcytometric detection of basophil activation by CD63 expression has been studied to detect sensitization in immediate-type allergy to various allergens such as natural rubber latex, foods, hymenoptera venoms, aeroallergens (cypress pollen, house dust mites), betalactam antibiotics and muscle relaxants [3–5]. However, in these studies, only commer-

cially available allergen extracts and no recombinant allergens were applied.

In our study, sensitivity of the BAT applying the relevant recombinant allergen Mal d 1, Dau c 1 and Api g 1 in the group of apple, carrot and celery allergics was 75, 65 and 75% with corresponding specificities of 68, 100 and 77%, respectively. Previous studies showed that IgE reactivity to Api g 1 is observed in 59–80% of celery allergics [28, 31, 32]. In apple allergy, IgE reactivity to Mal d 1 is seen in 60–70% of all allergics [33], and in carrot allergy, there is proof of allergen-specific IgE to Dau c 1 in 85% of all subjects [18]. Therefore, in this study, sensitivity of the three allergens Api g 1, Dau c 1 and Mal d 1 ranging between 65 and 70% reflects a rather high diagnostic yield of the BAT. The BAT was positive with Bet v 1 in all 32 food allergics, which is not surprising, since selection was based on clinical history of an OAS and a positive SPT with birch pollen. A positive BAT with Bet v 2 occurred in 7/32 patients.

Interestingly, the determination of allergen-specific IgE for apple and carrot by the well-established CAP-FEIA method was less sensitive than the BAT in detecting sensitization in the group of apple and carrot allergics. In our study applying the BAT in conjunction with the measurement of allergen-specific IgE, combined sensitivity of in vitro diagnosis in apple allergy was 100% (sensitivity of specific IgE: 60%), in carrot allergy 90% (sensitivity of specific IgE: 70%) and in celery allergy 95% (sensitivity of specific IgE: 75%).

Our results suggest that the application of recombinant allergens in the BAT is feasible in detecting IgE-mediated food hypersensitivity. When used to evaluate patients with a strong history of pollen-associated food allergy, the BAT reaches the sensitivity of in vivo testing of native foods.

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