

Usability of Fag e 2 ImmunoCAP in the diagnosis of buckwheat allergy

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Abstract Currently, the detection of crude buckwheat extract-specific IgE by ImmunoCAP (f11) (Phadia AB, Uppsala, Sweden) is widely used to diagnose buckwheat allergy. However, the results of this test do not always correlate with the development of allergic symptoms. This study aimed to evaluate the diagnostic usefulness of specific IgE antibody titers for the major buckwheat allergens Fag e 1 and Fag e 2. Specific IgE antibodies were determined using the ImmunoCAP method for native Fag e 1 and Fag e 2, recombinant Fag e 1 and Fag e 2, and crude buckwheat extract (f11) in 10 buckwheat allergy patients, 14 atopic dermatitis patients, and 15 healthy subjects. All buckwheat allergy patients showed positive results for native Fag e 1- and Fag e 2-specific IgE tests and for ImmunoCAP (f11). In contrast, the rates of atopic dermatitis patients with positive results for native Fag e 1- and Fag e 2-specific IgE tests were 64.2% (9/14) and 57.1% (8/14), respectively. The sensitivities of the test using recombinant proteins were lower than those of the test

using native proteins. The area under the curve (AUC) as determined by receiver operating characteristic (ROC) curve analysis was the largest for the native Fag e 2-specific IgE test (0.967), with a sensitivity of 90% and a specificity of 89.6% (cut-off: 2.74 kUa/L). Thus, the native Fag e 2-specific IgE antibody titer obtained using the ImmunoCAP method is more reliable than the buckwheat ImmunoCAP (f11) value for predicting buckwheat allergy.

Keywords Allergen · Buckwheat · Diagnosis · Fag e 2 · IgE

Introduction

Buckwheat (*Fagopyrum esculentum*) is a popular traditional food in Asian countries, including Japan, China, and Korea. Recently, it has been widely grown and consumed in Europe [10], North America, and Canada. The prevalence of buckwheat allergy in Japan is 0.22% among school children [23] and 0.45% among adults with asthma [1, 11], and approximately 3% of these cases have reportedly experienced serious symptoms such as anaphylactic shock. The prevalence of buckwheat allergy was 4.5% in 489 patients with food anaphylaxis in France [3] and was 1% in 351 adult patients with type 1 food allergy in Italy [2]. Thus, the prevalence of buckwheat allergy is not high in clinical practice. However, ingesting, inhaling, or mucous membrane contact with only a small amount of buckwheat proteins can cause serious immediate hypersensitivity reactions such as urticaria, conjunctival hyperemia, eyelid edema, laryngeal edema, abdominal pain, vomiting, dyspnea, wheezing, and anaphylactic shock. In addition, cross-reactions between buckwheat and latex, rice, and poppy seeds have been described [13, 18, 28, 31]. Therefore, it is

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important to properly diagnose this allergy and accordingly advise patients, and thereby prevent allergic reactions.

It was suggested that an IgE-dependent allergy is involved in buckwheat allergy [25]. Currently, buckwheat ImmunoCAP (f11) (Phadia AB, Uppsala, Sweden) is widely used to detect the crude buckwheat protein-specific IgE in serum to diagnosis buckwheat allergy. However, many subjects who did not show an immediate reaction to buckwheat were judged as positive in this test [24, 30]. Atopic dermatitis patients with a high level of total IgE constitute a high proportion of patients in these cases, suggesting that the buckwheat ImmunoCAP (f11) values are weakly correlated to hypersensitivity reactions.

Many immunoblotting studies have been conducted to investigate buckwheat allergens [17, 25, 26, 32]. Urisu et al. [25] identified proteins of 24 kDa (Fag e 1), 26 kDa, and 67–70 kDa as important allergens. Fag e 1, which is homologous to the 11S or 12S globulin β chain [26], reacted with the serum IgE of all buckwheat allergy patients, indicating that Fag e 1 is the most important allergen in the induction of the immediate hypersensitivity reaction in buckwheat allergy. Moreover, Nagata et al. [17] showed that the 58, 62, 70, and 73 kDa proteins that were identified as major buckwheat allergens were heterodimers containing a 24 kDa protein that was identical to Fag e 1. The relevance of the Fag e 1 protein to buckwheat allergy and its use in diagnosis are controversial because Fag e 1 is sensitive to the digestive enzyme pepsin, and many individuals with the Fag e 1-specific IgE antibody do not show a hypersensitivity reaction after buckwheat ingestion [19]. On the other hand, Fag e 2, which consists of 127 amino acids and has a molecular weight of 16 kDa, was identified as a major buckwheat allergen in Japanese and Korean patients with buckwheat allergy [15, 19]. Fag e 2 shares 50% homology with the 8-kDa buckwheat allergen [4] and is homologous to other known food allergens belonging to the 2S albumin family, such as Ara h 2 (34% homology) and Ara h 6 (30% homology) from peanuts, Ber e 1 (18.9% homology) from Brazil nuts, and Jug r 2 (30% homology) from English walnuts [4]. Unlike Fag e 1, Fag e 2 is resistant to pepsin digestion, suggesting that it is a major allergen responsible for allergic symptoms [24]. It was deduced that Fag e 1 and Fag e 2 do not have the N-linked oligosaccharide that is known to be a cross-reactive carbohydrate determinant in plant allergens because these proteins do not have the N-glycosylation consensus sequence Asn-X-Ser/Thr (where X is any amino acid) [8] in their primary structure [12, 34].

Thus far, recombinant proteins of these allergens (rFag e 1 and rFag e 2) have been prepared and their usefulness in diagnosing buckwheat allergy has been independently shown [7, 12, 34]. However, because the simultaneous determination of Fag e 1- and Fag e 2-specific IgEs in a

single patient has not been reported, it is unclear which allergen is more relevant in the diagnosis of buckwheat allergy. In this study, we determined the titers of IgE antibodies specific for native and recombinant Fag e 1 and Fag e 2 (nFag e 1, nFag e 2, rFag e 1, and rFag e 2) by using the ImmunoCAP method, which is widely used in clinical settings to determine specific IgE antibody titers, to examine the use of these proteins in diagnosing buckwheat allergy.

Materials and methods

Sera

The study protocol was approved by the Ethics Committee of Shimane University Faculty of Medicine (approval number: 469). The buckwheat-allergic patients (8 male and 2 female patients, age: 4–43 years) were diagnosed on the basis of a history of recurrent immediate hypersensitivity reactions immediately after ingestion of buckwheat noodles made from buckwheat flour and water and a positive buckwheat ImmunoCAP (f11) (>0.7 kUa/L) result. In patients 2 and 5, the allergic symptoms were also provoked by steamed buckwheat breads. Inhalation of buckwheat flour while making buckwheat noodles induced symptoms in patients 6 and 8. Patient 10 also showed allergic symptoms after consumption of tempura that contained a small amount of buckwheat flour as a hidden allergen. The characteristics of the patients are summarized in Table 1. This study enrolled the following individuals as controls: (1) 14 atopic dermatitis patients (10 male and 4 female patients, age: 4–43 years) with positive buckwheat ImmunoCAP (f11) (>0.7 kUa/L) who did not show immediate hypersensitivity reactions after buckwheat ingestion, and (2) 15 healthy subjects (3 male and 12 female, age: 26–52 years) with negative buckwheat ImmunoCAP (f11) (<0.7 kUa/L) without a history of buckwheat allergy. Serum total IgE and buckwheat-and horseradish peroxidase (HRP)-specific IgE levels were determined using the ImmunoCAP method [29].

Purification of native allergens

Yokota Native Soba (*Fagopyrum esculentum* Moench), a native buckwheat breed in Japan maintained at Shimane Agricultural Technology Center, was used for buckwheat allergen preparation.

Buckwheat powder (40 g), from which fat was removed by chloroform extraction, was added to 400 ml buffer (0.038 M NaCl and 0.033 M NaHCO₃) and stirred overnight at 4°C. The mixture was centrifuged at 15,000g for 20 min. The supernatant was filtered with DISMIC-25cs

Table 1 Characteristics of buckwheat allergy patients

Patients (No.)	Age (years)	Gender	Total IgE (IU/mL)	Buckwheat-specific IgE (kUa/L)	Type of food	Immediate hypersensitivity reactions
1	27	Male	220	2.16	Noodles	Generalized urticaria, facial angioedema
2	29	Male	110	1.54	Noodles, steamed breads	Dyspnea, generalized urticaria
3	25	Male	641	8.77	Noodles	Dyspnea, laryngeal discomfort, facial angioedema, shock
4	27	Male	1,420	1.64	Noodles	Laryngeal discomfort, shock
5	32	Male	45.5	1.55	Noodles, steamed breads	Laryngeal discomfort, abdominal pain, generalized urticaria
6	4	Male	1,036	5.50	Noodles Flour (inhalation) ^a	Generalized urticaria Facial angioedema
7	29	Female	1,905	1.42	Noodles	Urticaria, exacerbation of atopic dermatitis
8	43	Male	4,950	90.9	Noodles Flour (inhalation) ^a	Dyspnea, generalized urticaria Attack of asthma
9	17	Male	4,705	2.30	Noodles	Dyspnea, generalized urticaria
10	32	Female	1,831	1.05	Noodles Tempura ^b	Dyspnea, generalized urticaria, vomiting Dyspnea, generalized urticaria, vomiting

^a The patients inhaled buckwheat flour while preparing buckwheat noodles

^b The tempura batter contained a small amount of buckwheat flour as a hidden allergen

(0.45 μ m, ADVANTEC) and used as a salt-soluble buckwheat protein. From the salt-soluble buckwheat protein, nFag e 1 was purified as a complex of a legumin-like protein α subunit by using the method described by Urisu et al. [19]. Briefly, the salt-soluble buckwheat protein was applied to an anion-exchange column (HiTrap Q HP, GE Healthcare) with 50 mM phosphate buffer (pH 7.3) as a mobile phase, and the NaCl concentration was linearly increased from 0 to 0.5 M to elute the protein.

nFag e 2 was purified on an anion-exchange column (HiTrap Q HP) and a gel-filtration column (Superdex 75 10/300 GL, GE Healthcare) according to the method described by Tanaka et al. [24].

Preparation of recombinant allergens

Twenty buckwheat seeds (*Fagopyrum esculentum* Moench) were sprouted at room temperature and the green shoots were harvested 10 days after seeding. The whole shoot, including the leaf and stem, was crushed in liquid nitrogen and total RNA was extracted using an RNeasy Plant Mini Kit (QIAGEN). cDNA was synthesized with an Omniscript Reverse Transcription Kit (QIAGEN) by using the extracted total RNA as a template. The target cDNA was amplified by polymerase chain reaction (PCR) using specific primers (Fag e 1; 5'-GGGAATCCATATGGGATTGGAGCAAGCGTTC-3', 5'-CCGCTCGAGGAAACGCTCCCTCTCCTT-3', and Fag e 2; 5'-GGGAATCCATATGAGAGATGAAGGCTTCGAT-3', 5'-CCGCTCGAGCACAAAATACCGATTTC-3'). These primers were

designed on the basis of the known genetic sequences of Fag e 1 and Fag e 2 (GenBank accession Nos. D87982 and AY966013, respectively). PCR was performed using KOD-Plus-DNA polymerase (TOYOBO) under the following conditions: 96°C for 1 min followed by 4 cycles at 96°C for 15 s, 52°C for 15 s, 68°C for 1 min, 28 cycles at 96°C for 15 s, and 68°C for 1 min, and a final hold at 68°C for 7 min. The amplified target gene was cloned into the pEU3-NII vector (TOYOBO) and transfected into the *Escherichia coli* (*E. coli*) DH5 α strain. Gene fragments cut with *Nde*I and *Xho*I restriction enzymes were incorporated into the pET21a (+) vector and transfected into the *E. coli* BL21 (DE3) strain. After a 3-h incubation at 37°C, 1 mM IPTG was added and the strain was further incubated at 28°C for 16 h to induce expression of histidine-tagged recombinant buckwheat proteins. The bacterial body was crushed by ultrasonic waves and solubilized by lysis buffer [4 M urea, 20 mM sodium phosphate, 0.5 M NaCl, and 20 mM imidazole (pH 7.4)]. The recombinant protein conjugated with a histidine tag was adsorbed in a Ni affinity column (His-Trap HP, GE Healthcare) and eluted by raising the imidazole concentration from 20 to 500 mM. The eluate was dialyzed with 0.1% acetic acid and lyophilized.

N-Terminal amino acid sequence analysis

The target protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a PVDF membrane (Immobilon-PSQ,

Millipore), and the band was clipped. The N-terminal amino acid sequences of purified proteins were determined by the Edman degradation method using PPSQ-10 (SHI-MADZU, Kyoto, Japan).

Construction of the ImmunoCAP and ImmunoCAP inhibition tests

To determine allergen-specific IgE for native and recombinant buckwheat proteins in sera, the ImmunoCAP method was used as previously described [29]. Solid-phase carrier cellulose was activated by cyanogen bromide and buckwheat proteins were applied for immobilization of the carrier cellulose. The allergen-specific IgE titer was determined using an anti-human IgE monoclonal antibody labeled with β -galactosidase secondary antibody.

The ImmunoCAP inhibition test was performed by the method described previously [33]. Fag e 1 and Fag e 2 were prepared with 0.1 M phosphate buffer to obtain 0.1, 1, 10, and 100 μ g/ml solutions. Crude buckwheat protein (f11) solution (1.1 mg/ml) (Phadia) was diluted with 0.1 M phosphate buffer to obtain 1 $\times$, 10 $\times$, 100 $\times$, and 1,000 $\times$ diluted solutions. The diluted buckwheat protein solutions (30 μ l) were added to 45 μ l of each patient's serum and incubated at room temperature for 2 h. Crude buckwheat protein (f11)-specific IgE antibody titer was determined using the ImmunoCAP method. Phosphate buffer (0.1 M) was used as a blank.

Statistical analysis

The correlation coefficient was obtained using a simple regression analysis (Excel software). Receiver operating characteristic (ROC) curves were prepared using data from patients with buckwheat allergy, atopic dermatitis, and healthy controls. The analysis was performed using a commercially available software program (SPSS, version 17.0).

Results

Determination of purified allergen-specific IgE antibody titer by ImmunoCAP

We confirmed that purified buckwheat allergens to be target proteins by using N-terminal amino acid sequence data. SDS-PAGE analysis showed single bands for nFag e 2, rFag e 2, and rFag e 1. In contrast, several bands were observed from 35–45 kDa in addition to the 24 kDa band for nFag e 1 (Fig. 1).

Specific IgE antibody titers to the native and recombinant buckwheat allergens were determined by ImmunoCAP in the sera derived from 10 patients with buckwheat allergy, 14 atopic dermatitis patients without buckwheat allergy, and 15 healthy subjects (Fig. 2a). With a cut-off value of 0.35 kUa/L, the nFag e 2-specific IgE test results were positive in all buckwheat allergy patients (100%), 8 of 14 atopic dermatitis patients (57.1%), and 1 of 15 healthy subjects (6.7%). The nFag e 1-specific IgE test result was positive for all buckwheat patients (100%), 9 of 14 atopic dermatitis patients (64.2%), and 1 of 15 healthy subjects (6.7%). The rFag e 2-specific IgE test result was positive for 5 of 10 buckwheat allergy patients (50%), 5 of 14 atopic dermatitis patients (35.7%), and none of the 15 healthy subjects. The rFag e 1-specific IgE test result was positive for 1 of 10 buckwheat-allergic patients (10%), 3 of 14 atopic dermatitis patients (21.4%), and none of the 15 healthy subjects. HRP-specific IgEs were also measured to evaluate the cross-reactive carbohydrate determinant-specific IgE in the patients with buckwheat allergy and atopic dermatitis. HRP-specific IgE was detected in 2 of 10 patients with buckwheat allergy and 7 of 13 tested patients with atopic dermatitis.

A strong correlation was found between the nFag e 1- and nFag e 2-specific IgE titers in buckwheat allergy patients but not in atopic dermatitis patients (Fig. 2b). In contrast, no correlations were observed between buckwheat (f11)-specific IgE and nFag e 1 or nFag e 2-specific IgE titers in patients with either buckwheat allergy or atopic dermatitis (data not shown).

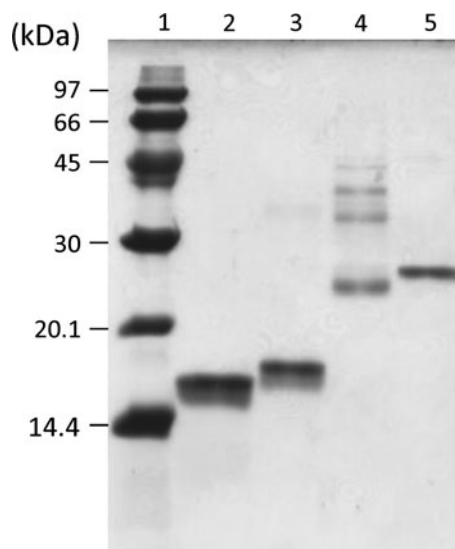
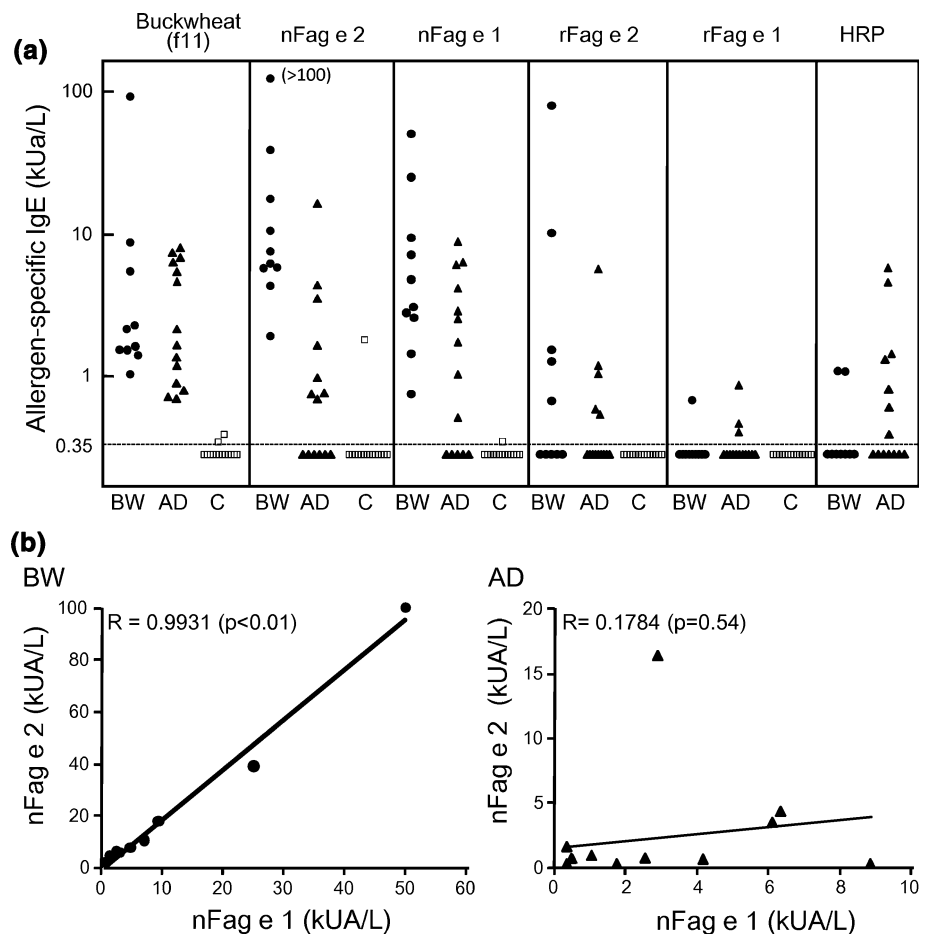


Fig. 1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of purified buckwheat allergens. Lane 1 molecular weight marker, lane 2 nFag e 2, lane 3 rFag e 2, lane 4 nFag e 1, lane 5 rFag e 1

Fig. 2 Serum levels of IgE specific to buckwheat-related allergens and horseradish peroxidase (HRP) (a) and the correlation between nFag e 1 and nFag e 2-specific IgE titers (b). *BW* patients with buckwheat allergy ($n = 10$), *AD* patients with atopic dermatitis ($n = 14$ and 13 for buckwheat-related allergens and HRP, respectively), *C* healthy controls ($n = 15$). The antigen-specific IgE values less than 0.35 kUa/L are depicted as 0.35



Comparison of sensitivity and specificity among buckwheat (f11), nFag e 1, and nFag e 2 ImmunoCAP tests

ROC analysis was conducted with buckwheat (f11), nFag e 1, and nFag e 2 ImmunoCAP values, and the area under the curve (AUC) of buckwheat (f11), nFag e 1, and nFag e 2 was determined to be 0.823, 0.890, and 0.967, respectively. This finding showed that the nFag e 2 ImmunoCAP test had the highest precision (Fig. 3).

The optimal cut-off value was determined as the point of intersection of the 2 lines when sensitivity and specificity were shown on the y axis and the cut-off value was shown on the x axis (data not shown). The sensitivities and specificities of these ImmunoCAP tests with buckwheat allergens at the optimal cut-off values were as follows: 90% (9/10) and 89.6% (26/29) for nFag e 2 with a cut-off value of 2.74 kUa/L, 80% (8/10) and 79.3% (23/29) for nFag e 1 with a cut-off value of 2.15 kUa/L, and 80% (8/10) and 72.4% (21/29) for buckwheat (f11) with a cut-off value of 1.48 kUa/L. On the other hand, the sensitivities of recombinant buckwheat allergens were low even if the

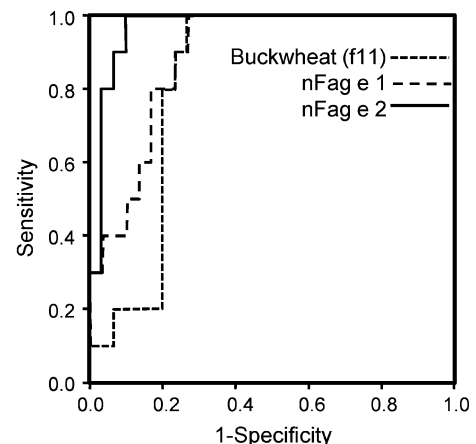


Fig. 3 Receiver operating characteristics (ROC) curves of buckwheat (f11), nFag e 1, and nFag e 2 ImmunoCAP tests

cut-off value was set at 0.35 kUa/L: 50% (5/10) for rFag e 2 and 10% (1/10) for rFag e 1. In contrast to the low sensitivities, the specificities were relatively high: 83% (24/29) for rFag e 2 and 89.6% (26/29) for rFag e 1 (Fig. 2a).

ImmunoCAP inhibition test with purified native and recombinant buckwheat allergens

An IgE-binding inhibition test for buckwheat ImmunoCAP (f11) was performed with Fag e 1 and Fag e 2 because there was a clear difference in the reactivity of the native and recombinant allergens in the ImmunoCAP test (Fig. 2a). These results are shown in Fig. 4. A dose-dependent inhibition was observed when sera from patients 1 and 5 (Table 1) were preincubated with buckwheat extract (f11), with an almost 100% inhibition of IgE binding at a buckwheat (f11) concentration of 100 µg/ml. Preincubation with nFag e 2 elicited a lesser but significant dose-dependent inhibition, reaching 80.5% of IgE binding in patient 1 and 81.5% in patient 5 at a concentration of 100 µg/ml. rFag e 2 showed less inhibition than did nFag e 2 in both the patients, even at a concentration of 100 µg/ml. Preincubation with nFag e 1 elicited less inhibitory effect than did preincubation with nFag e 2, reaching 46.9% of IgE binding in patient 1 and 63.0% in patient 5 at a concentration of 100 µg/ml. rFag e 1 did not inhibit binding of IgE to buckwheat (f11).

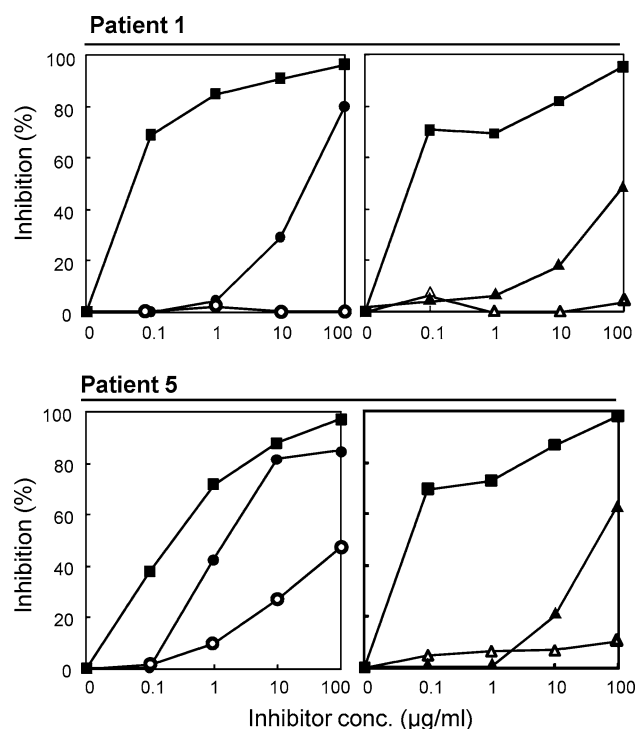


Fig. 4 Buckwheat ImmunoCAP (f11) inhibition test with Fag e 1 and Fag e 2 in patients with buckwheat allergy. Sera from buckwheat-allergic patient 1 or 5 (Table 1) was incubated with native or recombinant allergens, and the specific IgE antibodies to buckwheat (f11) were measured. Inhibition to buckwheat ImmunoCAP (f11) was observed with buckwheat (f11) (closed squares), nFag e 2 (closed circles), rFag e 2 (open circles), nFag e 1 (closed triangles), and rFag e 1 (open triangles)

Discussion

Using the ImmunoCAP with purified native and recombinant buckwheat allergens, we observed the best diagnostic precision with the nFag e 2 ImmunoCAP test with a sensitivity of 90%, a specificity of 89.6%, and a cut-off value of 2.74 kUa/L. These values were higher than those of the commercially available buckwheat ImmunoCAP (f11) test, which showed a sensitivity of 80% and a specificity of 72.4% in this study. The nFag e 1 ImmunoCAP test also had a higher AUC than did buckwheat ImmunoCAP (f11) (Fig. 3), but the sensitivity and the specificity of the nFag e 1 ImmunoCAP were comparable to those of buckwheat ImmunoCAP (f11), indicating that the nFag e 1 ImmunoCAP does not have an advantage over the buckwheat ImmunoCAP (f11). These observations are consistent with the results of previous studies showing that the Fag e 2 allergen is more responsible for allergic symptoms than is Fag e 1 [4, 19, 24].

Our findings also support the concept of component-resolved diagnosis that uses purified individual allergens instead of total protein extracts in allergen-specific IgE measurement [9, 22]. The advantage of component-resolved diagnosis using recombinant allergens [5, 6, 27] or IgE-binding epitope peptides [15, 16] was also reported. Choi et al. [4] reported that IgE-specific antibodies for Fag e 2 were detected in 14 of 18 (77.8%) patients with buckwheat allergy by using recombinant protein expressed in *E. coli*. Therefore, although we expressed recombinant Fag e 2 in the same way as did Choi et al., the ImmunoCAP test for rFag e 2 showed a low sensitivity compared with nFag e 2 (Fig. 2a). This difference in allergenicity is also supported by the results of the ImmunoCAP inhibition test using these 2 preparations. Although the nFag e 2 preparations inhibited binding of IgE to buckwheat extract (f11) in patients 1 and 5 (Fig. 4), the rFag e 2 protein showed a much lower or no activity in the inhibition assay. Similar low activities were observed for Fag e 1. The low allergenicity of recombinant preparations in this study may be attributable to differences in the three-dimensional structures of the recombinant and native proteins. Satoh et al. [21] showed that mutation of each of the 10 cysteine residues of rFag e 2 to serines resulted in a remarkably decreased ability of the mutant molecules to bind to the patients' IgE, indicating that the conformation of Fag e 2 plays an important role in its allergenicity. Our purification method after denaturing of Fag e 2 with urea was different from Choi's method and might have consequently changed the intramolecular disulfide bonds in the protein. Thus, we speculate that the epitope structure might be destroyed or hidden inside the protein structure. However, we did not compare the structure between native and recombinant buckwheat allergens in the present study.

Buckwheat extract (f11) contains a variety of allergenic and non-allergenic components and may therefore result in non-specific IgE binding, but nFag e 2 can specifically determine the IgE relevant to clinical symptoms. Our six atopic dermatitis patients who showed a significant decrease in IgE reactivity with the Fag e 2 ImmunoCAP compared with the buckwheat (f11) ImmunoCAP (Fig. 2a) presumably have IgE that reacts to proteins unrelated to Fag e 2. The binding of IgE to cross-reacting carbohydrate determinants reportedly affects in vitro diagnostic tests in cases where in vitro test results do not match clinical history or where in vitro test results are positive for numerous allergens [14]. Many allergens are glycoproteins containing carbohydrate moieties called glycans, which have some common structures among various carbohydrate chains. Therefore, a patient that produced IgE reacting to these common glycan structures would show a positive reaction in the allergen-specific IgE tests without showing allergic symptoms relevant to these allergens. In fact, 7 of 15 patients with atopic dermatitis had IgE antibodies specific to HRP that included cross-reactive carbohydrate determinants. Moreover, the buckwheat (f11) ImmunoCAP inhibition experiments with HRP showed that the IgE antibodies from 2 patients with atopic dermatitis cross-reacted with HRP and buckwheat proteins (f11) (data not shown). This may partly explain the discrepancy in the data of our atopic dermatitis patients that showed positive buckwheat ImmunoCAP (f11) test results, but no clinical symptoms associated with buckwheat ingestion.

The ImmunoCAP inhibition test results imply that cross-reactivity exists between Fag e 1 and Fag e 2 because binding of IgE to buckwheat extract (f11) was inhibited up to 80% with nFag e 2-treatment and up to 60% with nFag e 1-treatment. In fact, we found a strong association between the nFag e 1- and nFag e 2-specific IgE titers in buckwheat allergy patients but not in atopic dermatitis patients (Fig. 2b). Fag e 2 is known to be a 2S albumin and Fag e 1 is the beta subunit of a 13S globulin. Although whole amino acid sequence homology between these two categories of proteins is not observed, the amino acid sequence RDEKE in Fag e 1 is homologous to EGVRDLKE, which was recently identified as the IgE-binding epitope in Fag e 2 [20]. Therefore, the nFag e 2-specific IgE antibodies from the buckwheat allergy patients may cross-react with nFag e 1. In contrast, we propose that the IgE-binding epitopes of Fag e 2 in atopic dermatitis are different from those in buckwheat allergy, and that the nFag e 2-specific IgE antibodies from patients with atopic dermatitis do not cross-react with nFag e 1. This may cause difficulty in clarifying the causative allergens of buckwheat. The present study did not identify the IgE-binding epitopes of Fag e 2, which would improve our understanding of the cross-reactivity between Fag e 1 and Fag e 2.

In conclusion, we provided evidence that the allergen-specific IgE test with nFag e 2 may help in an accurate diagnosis of buckwheat allergy, although this should be confirmed by studies in a large number of patients and controls.

Conflict of interest The authors declare that they have no conflict of interest.

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