**Table 1** Comparison between CO-WDEIA and HWP-WDEIA

| | CO-WDEIA | HWP-WDEIA |
|---|---|---|
| Age | School child age-old age | Adults in their 20s-60s |
| Gender | Both genders | Predominantly female |
| History of HWP-supplemented soap use | None | Essential |
| Symptoms occur while using the soap before developing WDEIA | None | Often |
| Predominant symptom of WDEIA | Urticaria (wheal) | Angioedema (especially on the eyelids) |
| Anaphylactic shock | Sometimes | Occasionally |

Abbreviations: CO-WDEIA, conventional wheat-dependent exercise-induced anaphylaxis; HWP-WDEIA, hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis.

Satisfying all of the following criteria:

1. Have used "Cha no Shizuku" soap or other products containing hydrolyzed wheat (Glupearl 19S)
2. Have had at least 1 of the following symptoms:
   2-1. Itching, eyelid edema, nasal discharge, and/or wheals within several to 30 minutes after using "Cha no Shizuku" soap or other products containing hydrolyzed wheat (Glupearl 19S)
   2-2. General symptoms such as itching, wheals, eyelid edema, nasal discharge, dyspnea, nausea, vomiting, abdominal pain, diarrhea, and decreased blood pressure within 4 hours after eating wheat products
3. Have tested positive in at least 1 of the following tests:
   3-1. Prick test using ≤0.1% Glupearl 19S solution
   3-2. Immunoassay such as dot blot, enzyme-linked immunosorbent assay (ELISA), and Western blot to identify specific IgE antibody to Glupearl 19S in the blood
   3-3. Basophil activation test using Glupearl 19S as the antigen

[Exclusion criterion]

4. Tested negative in a prick test using 0.1% Glupearl 19S solution

[Suspected cases]

Satisfying Criteria 1 and 2 but not 3

*Wheat allergy is strongly suspected if sensitization to wheat or gluten is shown in a specific IgE antibody test or a prick test but there is no hypersensitivity to ω-5 gliadin or milder hypersensitivity to ω-5 gliadin compared with that to wheat and gluten.

## COMPARISON BETWEEN CO-WDEIA AND HWP-WDEIA

Clinical features of CO-WDEIA and HWP-WDEIA observed in our hospital are summarized in Table 1. CO-WDEIA occurred more often in expanded age-group. HWP-WDEIA developed more commonly in women who use soap as cosmetics. In HWP-WDEIA, history of HWP-supplemented soap use is essential, and symptoms that occur while using the soap before the development of WDEIA often appear. A predominant symptom of CO-WDEIA is the development wheals on the entire body, whereas that of HWP-WDEIA is angioedema on the eyelids.

Anaphylactic shock sometimes developed in CO-WDEIA patients and occasionally developed in HWP-WDEIA patients.

**Table 2** Positivity rate of ω-5 gliadin-specific IgE and high molecular weight glutenin-specific IgE measurement in CO-WDEIA and HWP-WDEIA patients

| CAP-FEIA | CO-WDEIA (%, $n$ = 54) | HWP-WDEIA (%, $n$ = 30) |
|---|---|---|
| Wheat | 31.4 | 70.0 |
| Gluten | 37.0 | 76.6 |
| ω-5 gliadin | 79.6 | 6.6 |
| High molecular weight-glutenin | 18.5 | 16.6 |
| ω-5 gliadin and/or high molecular weight-glutenin | 94.4 | 16.6 |

Abbreviations: CAP-FEIA, fluorescent enzyme immunoassay combined with the CAP system; CO-WDEIA, conventional wheat-dependent exercise-induced anaphylaxis; HWP-WDEIA, hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis.

Measurement of gluten-specific IgE as well as wheat-specific IgE is possible in the diagnosis of WDEIA using the CAP-FEIA; however, more than 60% of patients with definite CO-WDEIA are considered negative by these tests. Recently, recombinant food allergens, which are consistent in quality, have been produced and applied for the diagnosis of many food allergies. Measurement of IgE that is specific to ω-5 gliadin and high molecular weight-glutenin is highly useful in diagnosing CO-WDEIA when compared with the routine diagnostic CAP-FEIA for wheat and gluten.[26] As shown in Table 2, in our hospital, 79.6% of the patients with CO-WDEIA have IgE that reacted to recombinant ω-5 gliadin. Additionally, 94.4% of the CO-WDEIA patients were positive according to the combined recombinant ω-5 gliadin-specific IgE test and the recombinant high molecular weight-glutenin-specific IgE test, whereas gluten- and

wheat-specific IgE tests positively recognized only 31.4% and 37.0% of these patients, respectively. In contrast, the positive rate of ω-5 gliadin-specific IgE was only 6.6% in HWP-WDEIA patients, whereas gluten- and wheat-specific IgE tests positively recognized 70.0% and 76.6% of these patients, respectively.

## PERCUTANEOUS SENSITIZATION TO FOOD ALLERGENS

The importance of percutaneous sensitization in the development of food allergies has been well recognized in the case of latex allergies in which fruits and vegetables are the causative agents.[27] Direct percutaneous sensitization by peanuts has recently been suggested by Fox *et al.*[28] who identified a dose-response relationship between environmental (non-oral) peanut exposure and the development of peanut allergies. HWP should now be considered a possible allergen for percutaneous sensitization, because they are currently used globally as ingredients of cosmetic products, and HWP cross-reacts to the wheat allergen in foods and may cause life-threatening anaphylaxis once sensitized. Several cases of contact urticaria due to HWP in cosmetics have been described.[12,29-32] These findings indicate percutaneous and/or rhinoconjunctival penetration of HWP in patients. Some of these patients have developed generalized allergic symptoms upon ingestion of wheat products. Some of our HWP-WDEIA patients also experienced contact urticaria by using Glupearl 19S-supplemented soap and later developed generalized symptoms upon ingestion of wheat products. Another group of patients directly developed generalized symptoms upon ingestion of wheat products. Hydrophilic moieties of allergens might be necessary for percutaneous and/or rhinoconjunctival sensitization, as Glupearl 19S was hydrolyzed under acidic conditions and was highly allergenic. This process yields new terminal amino- and carboxyl-charged groups. Matsuo *et al.* reported that serum IgE of patients with wheat protein contact dermatitis reacted to water-soluble proteins rather than water-insoluble proteins.[33] They identified 3 water-soluble proteins, peroxidase, purple acid phosphatase, and wheat 27-kDa allergen, as candidate allergens for wheat protein contact dermatitis. These results suggest that glycan moieties in these proteins are involved in IgE binding.

When considering sensitization by the percutaneous and/or rhinoconjunctival route, a penetration of the molecule through the epithelia must occur. Large molecules such as proteins are generally not expected to cross the skin barrier, unless the skin has been damaged.[34] However, a few patients with HWP-WDEIA had atopic dermatitis, a condition arising from impaired function of the skin barrier. In addition to creation of new terminal amino- and carboxyl-charged groups due to the hydrolysis of peptide bonds, soap containing HWP might facilitate the penetration of HWP into the epidermis, because soap contains surfactants that may cause destruction of skin barriers. It has been proposed by Lack that antigen exposure through inflamed skin might be involved in the establishment of allergy and tolerance.[35] He reported in his review that allergic sensitization to food could occur through low-dose cutaneous sensitization and that early consumption of food protein induced oral tolerance. He argued that low-dose exposure to environmental foods (on tabletops, hands, and dust) penetrates the skin barrier and is taken up by Langerhan's cells. This leads to T helper type 2 ($T_H2$) responses and IgE production by B cells. In contrast, early high-dose oral consumption induces tolerance, and T helper type 1 ($T_H1$) and regulatory T-cell responses occur in the gut-associated lymphoid tissue. The timing and balance of cutaneous and oral exposure determines whether a child has allergy or tolerance (dual-allergen-exposure hypothesis). Leduc *et al.* have suggested that acidic hydrolysis induces a conformational change in HWP and produces a conversion of a glutamine residue to glutamic acid and a conversion of an asparagine residue to aspartic acid.[36] As a result, new epitopes that differ from the epitopes of natural wheat proteins might be produced. It is conceivable that humans do not have sufficient tolerance to HWP, which are not natural proteins. Thus, humans appear to be easily sensitized to HWP once HWP penetrates into the skin or mucosa. As wheat proteins contain repetitive amino acid structures highly rich in glutamine and proline, it is likely that IgE produced against HWP cross-reacts to natural wheat proteins. In fact, preincubation of sera with HWP clearly revealed a decrease in the binding of IgE to natural wheat proteins (Fig. 5).

IgE produced against HWP in HWP-WDEIA patients do not react to ω-5 gliadin but instead to other undetermined protein components with specific epitopes. The patients with HWP-WDEIA had no or decreased levels of ω-5 gliadin-specific IgE.

HWPs are prepared either from insolubilized total flour proteins or more generally from gluten alone. The main modification of gluten is hydrolysis performed to overcome its insolubility. HWP, which includes large polypeptide aggregates, has a greater ability to induce sensitization than the HWP that is digested to lower molecular weight polypeptides.[37] This finding is supported by the previous observation by Palosuo *et al.*[38] that artificial polymerization of ω-5 gliadin increases its direct reactivity with IgE in an immunosorbent assay and in patients using SPT. They also hypothesized that large polymers have better IgE-bridging capacities.

## CONCLUSIONS

We experienced an outbreak of wheat allergy with systemic symptoms, which may be due to percutaneous and/or a rhinoconjunctival sensitization following

the use of cosmetics. Such an event was almost completely unknown by healthcare professionals until recently. Thus, there is limited information regarding the clinical course. The serum IgE levels of many HWP-WDEIA patients against wheat and gluten decreased after the cessation of HWP-containing soap usage. In some patients, serum IgE against these antigens had disappeared. A remission case of HWP-WDEIA has been reported[39] and we also experienced some patients who had remission of WDEIA-symptoms (unpublished observation). However, many patients with HWP-WDEIA have not recovered from WDEIA-related symptoms. Large-scale studies are needed to clarify the prognosis of HWP-WDEIA patients.

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# Higher allergenicity of high molecular weight hydrolysed wheat protein in cosmetics for percutaneous sensitization

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## Summary

**Background.** Wheat protein derivatives are used in a variety of products worldwide. Gluten is commercially used 'as is' or with modifications such as hydrolysis, which is carried out to overcome its insolubility. Several cases of contact urticaria following exposure to hydrolysed wheat protein (HWP) in cosmetics or of anaphylaxis caused by deamidated gluten in food or non-food products have been described.

**Objectives.** To evaluate the types of HWP that have higher allergenicity for percutaneous sensitization.

**Methods.** We enrolled 7 patients with wheat-dependent exercise-induced anaphylaxis who had been sensitized to HWP primarily through the percutaneous and/or the rhinoconjunctival route by using facial soap containing HWP. Reaction to wheat proteins was confirmed by IgE immunoblotting and basophil CD203c expression with six HWP variants.

**Results.** The IgE of all the patients reacted to HWPs composed of large polypeptide aggregates. High molecular weight (MW) HWPs were also found to induce significant enhancement of basophil CD203c expression.

**Conclusions.** HWPs composed of large polypeptide aggregates possibly induce sensitization to a greater degree than lower-MW HWPs. Basophil surface CD203c expression is useful for evaluating the allergenicity of HWPs.

**Key words:** cosmetics; hydrolysed wheat protein; percutaneous sensitization; wheat-dependent exercise-induced anaphylaxis.

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a special form of wheat allergy induced by wheat ingestion combined with physical exercise (1–3). Aspirin intake is another well-known trigger for allergic symptoms (4, 5). We identified wheat ω-5 gliadin as a major allergen in WDEIA patients, and found that a CAP system fluorescent enzyme immunoassay (CAP-FEIA) combined with ω-5 gliadin improves diagnostic procedures (6). A new WDEIA subtype caused by hydrolysed wheat protein (HWP) has been recently reported (7–9). WDEIA patients who used facial soap containing HWP were found to be sensitized to HWP via the percutaneous and/or the rhinoconjunctival route (7–9). Patients with this WDEIA tested positive to HWP in the prick test and had serum HWP-specific IgE. Moreover, these patients showed characteristic facial angioedema distinct from those with conventional WDEIA (CO-WDEIA); they were designated as having HWP-sensitized WDEIA (HWP-WDEIA).

In order to evaluate the allergenicity of HWP, which could be inducing percutaneous and/or rhinoconjunctival sensitization, several HWPs with different sources

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**Table 1.** Clinical features of the patients with hydrolysed wheat protein (HWP)-sensitized wheat-dependent exercise-induced anaphylaxis (HWP-WDEIA)

| Patient | Age (years)/ sex | Atopy | Use of soap with HWP-A | Contact urticaria caused by HWP | Foods | Triggers | Symptoms |
|---|---|---|---|---|---|---|---|
| 1 | 51/F | – | + | + | Spaghetti | Walking | A, U, S, NS, ND, P |
| 2 | 49/F | – | + | + | Gyoza dumplings | Table tennis | A, U, S, NS, C, D, AP, V |
| 3 | 52/F | – | + | + | Steamed bread | Jogging | A, U |
| 4 | 44/F | – | + | + | Bread, udon noodles | Tennis | A, U, AP, anaphylactic shock |
| 5 | 54/F | – | + | + | Udon noodles, gyoza dumplings | Walking | A, U, S, ND, AP, L |
| 6 | 60/F | – | + | – | Udon noodles | Walking | A, U, S, C |
| 7 | 35/F | – | + | + | Cracker | Internal use of analgesics | A, U, D |

A, angioedema; AP, abdominal pain; C, conjunctivitis; D, dyspnoea; F, female; L, lacrimation; ND, nasal discharge; NS, nasal stuffiness; P, pharyngalgia; S, sneeze; U, urticaria; V, vomiting.

were analysed with IgE immunoblotting and a basophil CD203c expression test.

## Methods

### Patients

Seven female patients with HWP-WDEIA were enrolled in this study (Table 1). All patients had used the same brand of soap containing HWP-A. Six of these patients developed contact urticaria several months later, and subsequently had WDEIA. The predominant symptom of natural wheat ingestion-induced reactions was angioedema of the eyelids. Six of the 7 patients developed anaphylaxis, and 1 patient (patient 4) also developed anaphylactic shock. Urticaria following contact with soap preceded the food ingestion-induced reactions in all cases. None of the patients had atopic diathesis.

The patients tested positive in the prick tests with wheat (5/7), bread (5/7), 0.1% soap solution supplemented with HWP-A in saline (6/7), and 1 mg/ml HWP-A in saline (7/7) (Table 2). Five healthy controls were tested, and none of them reacted to any of the allergens in the prick test. Specific IgEs for wheat and gluten were detected by CAP-FEIA (Phadia, Uppsala, Sweden; detection range, 0.35-100 $kU_A/l$) in all HWP-WDEIA patients. Challenge tests included exercise, wheat ingestion, and aspirin intake. A combination of these challenges was performed for 3 of the 7 patients, as described previously (10). Aspirin and wheat challenge induced angioedema, urticaria and/or dyspnoea in 2 of these 3 patients. Patient 2 required a combination of aspirin, wheat and exercise challenge to induce symptoms.

This study was approved by the Ethics Committee of the Shimane University Faculty of Medicine (approval Nos. 469 and 703); all participants provided written informed consent.

**Table 2.** Results of immunological studies

| Patient | Prick test | | | | Specific IgE (kUA/l) | | | Challenge test |
|---|---|---|---|---|---|---|---|---|
| | Wheat | Bread | Soap 0.1% | HWP-A, 1 mg/ml | Wheat | Gluten | ω-5 gliadin | |
| 1 | 3+ | 3+ | 3+ | 3+ | 4.70 | 4.76 | <0.34 | NT |
| 2 | 1+ | 1+ | 1+ | 2+ | 2.27 | 4.56 | <0.34 | + |
| 3 | – | – | 3+ | 2+ | 0.46 | 0.65 | <0.34 | NT |
| 4 | 2+ | 2+ | 2+ | 2+ | 9.26 | 15.8 | 1.16 | + |
| 5 | 2+ | 3+ | 3+ | 3+ | 5.17 | 7.37 | <0.34 | + |
| 6 | 2+ | 2+ | 3+ | 2+ | 0.91 | 2.16 | <0.34 | NT |
| 7 | 2+ | 2+ | 2+ | 2+ | 15.6 | 25.2 | 3.28 | NT |

HWP, hydrolysed wheat protein; NT, not tested.
*Wheat: commercial wheat flour extract* (1:20 wt/vol; Torii Pharmaceutical Co., Tokyo, Japan).
Bread: commercial bread (1:20 wt/vol; Torii Pharmaceutical Co.).
Soap 0.1%: 0.1% diluted solution of soap supplemented with HWP-F in saline.
Reactions were read at 15 min, and responses were compared with positive histamine controls (10 mg/ml): 1+, 25% of the area of the wheal induced by the positive histamine control; 2+, 50%; 3+, 100%; 4+, 200%.

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### HWP preparation

Six different HWPs (HWP-A, HWP-B, HWP-C, HWP-D, HWP-E, and HWP-F) were obtained from food or cosmetic manufacturers or directly from the producer. HWP-A and HWP-B were hydrolysed only under acidic conditions. The remaining HWPs were subjected to enzymatic and/or acidic hydrolysis.

### Preparation of wheat proteins

$\omega$-5 Gliadin was purified as described previously (11). Commercial blend wheat flour (Camellia®) was purchased from Nissin Flour Milling Inc., Kobe, Japan) for fractionation of wheat flour proteins. Wheat flour was mixed with 0.6 volumes of water with an arm kneader, and the dough was placed in water for 30 min. Water-soluble wheat proteins were extracted twice with two volumes of water with an arm kneader, and used for analysis. The residue was powdered with a tissue lyser (Qiagen, Tokyo, Japan) after lyophilization. The powdered water-insoluble wheat proteins were used for subsequent experiments.

### Western blotting

All of the sera were analysed under the same conditions. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed with 12.5% polyacrylamide gels, according to the manufacturer's specifications (Atto Corp., Tokyo, Japan). Samples were solubilized in 2% sodium dodecyl sulfate containing 6% $\beta$-mercaptoethanol for 10 min at 95°C, and electrophoresed. The separated proteins were blotted under semi-dry conditions on poly(vinylidene difluoride) (PVDF) membranes (Millipore Corp., Billerica, MA, USA) (12). For IgE immunodetection, membranes were incubated overnight with 10× diluted serum samples blocked with 5% skimmed milk in 10 mM Tris–HCl, pH 7.6, containing 150 mM NaCl and 0.1% Tween-20 (TBST). IgE fixation was detected with secondary goat anti-human IgE conjugated to horseradish peroxidase (Dako SA, Trappes, France) and the ECL-plus kit (GE Healthcare, Tokyo, Japan). Chemiluminescence was recorded on X-ray films (RX-U; Fujifilm, Tokyo, Japan) after few minutes of exposure.

### Inhibition assays

To determine cross-reactivity of wheat proteins and HWP, 100 µl of serum was incubated with serial dilutions of HWP-A (100 to 5 µg/ml) at 37°C for 2 hr with constant stirring. The serum was then diluted 1/10 in 5% skimmed milk/TBST for immunodetection of IgEs by western blotting.

### Basophil CD203c expression

An allergenicity kit (Beckman Coulter Inc., Brea, CA, USA) was used for quantification of basophil CD203c expression, as described previously (13). EDTA-containing whole blood samples of all patients were incubated with various concentrations of HWP-A or $\omega$-5 gliadin for 15 min after a sufficient quantity of calcium solution to override the chelating capacity of EDTA had been added. The blood samples of patients were incubated with six types of 0.1 µg/ml HWP for 15 min. Anti-IgE antibody (4 µg/ml) and phosphate-buffered saline (PBS; pH 7.4) were used as the positive and negative controls, respectively. PC7-conjugated anti-CD3, fluorescein isothiocyanate-conjugated anti-CRTH2 and phycoerythrin-conjugated anti-CD203c antibodies were added during the reaction. The samples were analysed on a FACSCan (Cell Analyzer; Becton Dickinson, Franklin Lakes, NJ, USA). Basophils were detected according to forward–side scatter characteristics and $CD3^-$ and $CRTH2^+$ expression. Upregulation of CD203c expression on basophils was determined with a threshold defined by the fluorescence of unstimulated cells (negative control), and expressed as percentage of CD203c ($CD203c^{high}$%). At least 500 basophils were analysed per assay.

### Size-exclusion chromatography (SEC)

SEC was performed with 200 µg of each HWP type loaded onto a TSK gel G2000 SWXL column (7.8 mm × 30 cm) (TOSOH Co., Tokyo, Japan) previously equilibrated with PBS at pH 7.0. A flow rate of 0.8 ml/min was used. Proteins were detected at 280 nm. Absorbance at 280 nm was expressed as absorbance unit (AU). The molecular weights (MWs) of the HWPs were estimated with a ladder of standard proteins.

### Statistical analysis

Statistical differences were determined with Student's $t$-test.

## Results

### SDS–PAGE and western blotting

Coomassie blue staining of HWP-A subjected to SDS–PAGE showed smears characteristic of random degradation and peptide rearrangement (Fig. 1a). The smears spanned most of the separation of the gel, ranging from MW 0 to MW 250 000. In contrast, wheat proteins showed characteristic bands from MW 15 000 to MW 100 000.



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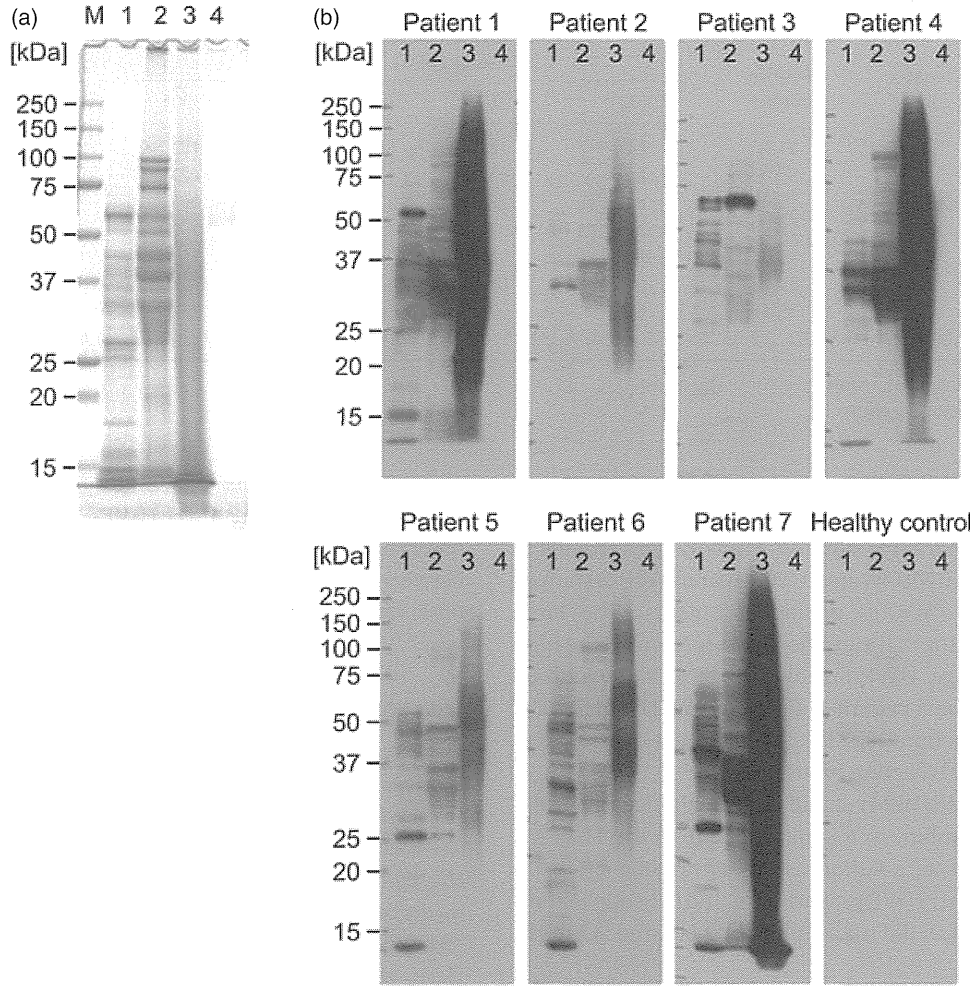


**Fig. 1.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting of wheat protein fractions and hydrolysed wheat protein (HWP)-A. (a) Gel stained with Coomassie Brilliant Blue. (b) IgE immunoblotting with the patients' sera. M, marker proteins. Lane 1: water-soluble wheat proteins. Lane 2: water-insoluble wheat proteins. Lane 3: HWP-A. Lane 4: purified ω-5 gliadin.

Western blotting showed smear patterns of IgEs of all patients reacting with HWP-A (Fig. 1b). The reaction intensity varied among the patients. The IgEs of all patients reacted with both water-soluble and water-insoluble wheat proteins, but not with ω-5 gliadin. In all patients, HWP-A inhibited the reaction of IgEs with wheat proteins in a dose-dependent manner after preincubation with the patients' sera (data not shown).

### HWP induced basophil CD203c expression

HWP-A enhanced CD203c expression in a concentration-dependent manner in the HWP-WDEIA patients (Table 3). The maximum reaction exceeded 60% at 0.1 μg/ml. No significant enhancement of CD203c was observed with purified ω-5 gliadin. Patients 4 and 7 had ω-5 gliadin-specific IgE, but the reaction to purified ω-5 gliadin was negligible. To determine whether the basophil activation was mediated by IgE, we removed the cell-surface IgE in the leukocyte mixture by using lactic acid, as described previously (14). Basophil activation was abolished by this removal of cell-surface IgE (data not shown).

### IgE and basophil activation in response to different HWPs

SDS–PAGE showed smear staining spanning the entire length of the gel for HWP-A (lane 6) and HWP-B (lane 1) (Fig. 2a). These values were considerably greater than the MWs of the native proteins, gliadins and glutenins, suggesting the presence of large polypeptide aggregates.

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**Table 3.** CD203c expression (%) on basophils induced with hydrolysed wheat protein (HWP)-A and ω-5 gliadin

| Patient | Negative control | Positive control | HWP-A (μg/ml) | | | ω-5 gliadin (μg/ml) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0.001 | 0.01 | 0.1 | 0.001 | 0.01 | 0.1 |
| 1 | 0.21 | 51.83 | ND | 18.26 | 52.07 | ND | 0.62 | 0 |
| 2 | 1.15 | 56.02 | ND | 63.13 | 63.72 | ND | 1.31 | 22.8 |
| 3 | 3.66 | 55.78 | ND | 78.31 | 60.83 | ND | 6.94 | 12.61 |
| 4 | 0 | 55.9 | 0.49 | 1.47 | 60.31 | 0.71 | 0.47 | 0.79 |
| 5 | 0.63 | 51.2 | 0.21 | 5.43 | 69.11 | 0 | 0.63 | 0.41 |
| 6 | 8.33 | 83.23 | 10.77 | 60.08 | 85.22 | 3.27 | 2.9 | 4.14 |
| 7 | 0 | 35.46 | ND | 2.6 | 61.9 | 0.67 | 0 | 1.00 |
| Mean ± SEM | 2.00 ± 1.16 | 55.63 ± 5.35* | 3.82 ± 3.47 | 32.75 ± 12.53* | 64.74 ± 3.91* | 1.16 ± 0.72 | 1.84 ± 0.92 | 5.96 ± 3.27 |

ND, not determined; SEM, standard error of the mean.
*$p < 0.05$ with negative control.

HWP-D and HWP-E showed intense staining at the lower part of the gel, with several bands. HWP-C and HWP-F showed no remarkable staining. Patients' IgEs reacted to several protein hydrolysates. HWP-A and HWP-B showed MW 15 000–250 000 or higher smears. The large proteins at the top of the gradient gel represented the high end of the range. HWP-E showed several individualized bands in 3 of the 5 patients. HWP-C, HWP-D and HWP-F showed no reaction (Fig. 2b). All patients examined had almost identical reaction patterns.

HWP-A and HWP-B significantly enhanced basophil CD203c expression, to a similar extent as the HWP-A-supplemented soap solution used by the patients (Fig. 3). In contrast, other HWP preparations and the supplement-free soap solution did not significantly enhance CD203c expression.

### SEC analysis

The SEC analysis showed dominant peaks of HWP-A and HWP-B at an elution volume corresponding to MWs of approximately 669 000 and 158 000, respectively (Fig. 4). Peaks of HWP-D, HWP-E and HWP-F were observed at elution volumes corresponding to MWs between 13 700 and 6500, and the HWP-C peak was observed at <6500.

## Discussion

We have already shown that the *in vitro* wheat protein-induced basophil activation test for quantifying CD203c expression aids in the diagnosis of HWP-WDEIA and CO-WDEIA, as CD203c expression clearly differentiates the sensitization conditions of both WDEIA subtypes in accordance with the results obtained with the prick test, serum allergen-specific IgE, and immunoblotting (9). Here, we successfully evaluated the allergenicity of six different HWP variants by IgE immunoblotting and surface CD203c expression on basophils. We devised a new procedure for detecting the allergenicity of HWP by measuring HWP-induced enhancement of CD203c expression on basophils.

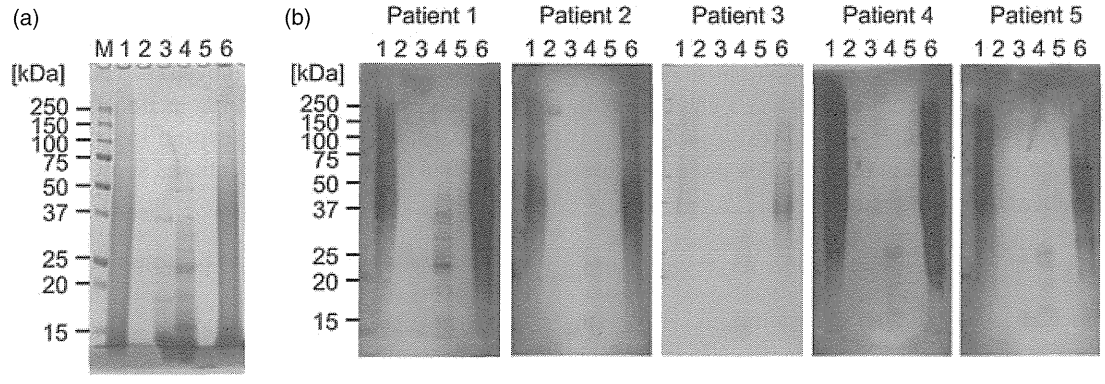


**Fig. 2.** Immunoblotting analyses. (a) Gel stained with Coomassie Brilliant Blue. (b) Immunoblotting of patients' sera. M, marker proteins. Lane 1: Hydrolysed wheat protein (HWP)-B. Lane 2: HWP-C. Lane 3: HWP-D. Lane 4: HWP-E. Lane 5: HWP-F. Lane 6: HWP-A.


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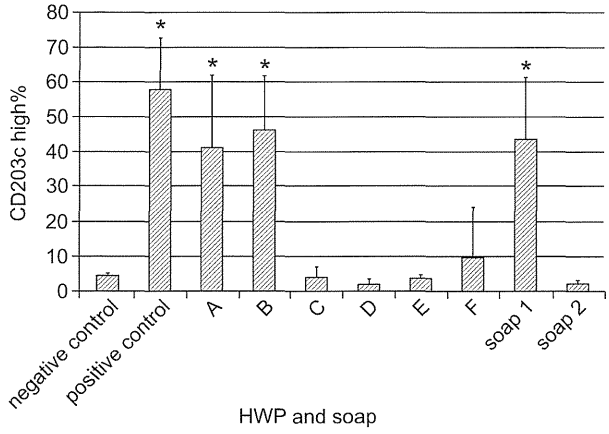


**Fig. 3.** Expression of CD203c on basophils induced with several hydrolysed wheat proteins (HWPs) and two soap solutions. (a) HWP-A. (b) HWP-B. (c) HWP-C. (d) HWP-D. (e) HWP-E. (f) HWP-F. Soap 1: soap containing HWP-A. Soap 2: soap containing no HWP. Each sample was added at 0.1 μg/ml, $^{*}p < 0.05$ as compared with negative control.

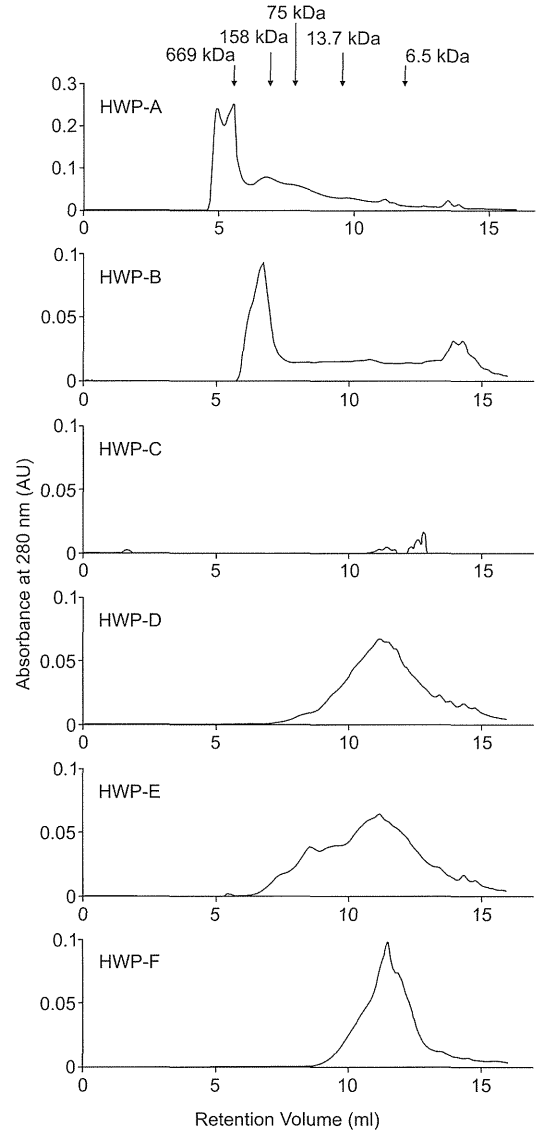


**Fig. 4.** Size-exclusion chromatography (SEC) profiles of the hydrolysed wheat proteins (HWPs).

Our results indicate that HWPs composed of large polypeptide aggregates induce sensitization to a greater degree than lower-MW HWPs, because HWP-A and HWP-B showed larger MWs than HWP-C, HWP-D, HWP-E, and HWP-F. HWPs are prepared either from insolubilized total flour proteins or gluten. In order to increase the solubility of gluten, acidic or enzymatic hydrolysis was performed. The procedure used and the degree of hydrolysis depend on the desired functionality and on the manufacturer. Large undefined aggregates of HWP-A and HWP-B produced by acid hydrolysis were observed as smears in SDS–PAGE (Fig. 2). Laurière et al. reported that IgE reacted mostly with large aggregates of HWP in patients with contact urticaria caused by cosmetics containing HWP (15). This is supported by a previous report (16) showing that artificial polymerization of ω-5 gliadin increases its direct reactivity with IgE in an immunosorbent assay and the prick test. It was also hypothesized that larger polymers have better IgE-bridging capacities.

CD203c is an ecto-enzyme belonging to a family of ectonucleotide pyrophosphatases and phosphodiesterases. It is expressed on the cell membranes of human peripheral basophils and mast cells, and cross-linking of the high-affinity IgE receptor upregulates CD203c expression on the cell membrane, indicating that the CD203c test is an excellent *in vitro* test for evaluating IgE sensitization and detecting allergens causing allergic symptoms. CD203c was previously proposed to be a useful marker in the diagnosis of paediatric allergies induced by wheat, hen eggs, and cow's milk (17, 18). In our previous study, we showed that the *in vitro* wheat protein-induced basophil activation test for quantifying CD203c expression was very useful for diagnosing the subtypes of WDEIA, namely, HWP-WDEIA and CO-WDEIA, as the determination of CD203c expression clearly differentiated the sensitization conditions of both types of WDEIA in good accordance with the clinical conditions (9). In this study, native ω-5 gliadin did not induce upregulation of CD203c expression in patients with HWP-WDEIA, even in the case of the patient who had serum ω-5 gliadin-specific IgE as determined with the CAP-FEIA system. This indicated that IgE produced against HWP in patients with HWP-WDEIA

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does not react to ω-5 gliadin, but to other undetermined protein components with specific epitopes. The present study extends the use of the CD203c test to evaluate the allergenicity of different kinds of protein such as HWP. The fact that the basophil activation test requires only small amounts of blood and allergen is another advantage of the test, because it is possible to use the test to simultaneously evaluate a series of allergens. To diagnose WDEIA, we typically perform an exercise/aspirin challenge test combined with wheat ingestion for patients who have episodes of anaphylaxis after wheat intake. However, the challenge test is unsafe for patients, because anaphylactic shock was sometimes elicited during the test. Therefore, an *in vitro* diagnostic method predicting the development of symptoms from wheat and exercise/aspirin challenge is necessary for patients with WDEIA.

All of our patients were considered to be sensitized to HWP-A via the percutaneous and/or rhinoconjunctival route, because they presented symptoms following use of the HWP-supplemented soap. The importance of percutaneous sensitization in the development of food allergies has already been well recognized in the case of latex allergies in which fruits and vegetables are the causative agents (19). Direct percutaneous sensitization by peanuts has recently been suggested by Fox et al. (20), who identified a dose–response relationship between environmental (non-oral) peanut exposure and the development of peanut allergies. It is possible that our patients have been sensitized by eating food containing HWP. However, almost all of our patients with HWP-WDEIA initially experienced contact urticaria after using HWP-A-supplemented soap, and later developed generalized symptoms upon ingestion of wheat products. In Japan, this soap was very popular, and more than 46 million soaps had been sold between March 2004 and September 2010. As a result, more than 1300 people who have used the same brand of soap have developed allergic symptoms after ingesting natural wheat proteins to date (21). Unfortunately, neither the manufacturer or the producer have ever noticed the danger of high-MW HWP, because they had little information about the protein itself. HWP has been widely used in cosmetics and foods, but only several cases of contact urticaria following exposure to HWP in cosmetics had been described before the outbreak in Japan (7–9, 15, 22–25). Among them, some patients developed generalized allergic symptoms upon ingestion of wheat products. This study provides evidence that HWP with high-MW components could selectively cause percutaneous sensitization when added as an ingredient in soaps or cosmetics. This remains to be determined with rodent sensitization tests.

For sensitization by the percutaneous and/or the rhinoconjunctival route, the molecule must penetrate the epithelium. Large molecules are not expected to cross the skin barrier unless the skin is damaged (26). Antigen exposure through inflamed skin or gastrointestinal mucosa might be involved in the establishment of allergy and tolerance (27). However, our patients did not have atopic dermatitis, which arises from impaired function of the skin barrier. In addition to the creation of new terminal amino-charged and carboxyl-charged groups, owing to the hydrolysis of peptide bonds, the presence of HWP in soap might facilitate its penetration into the epidermis, because soap contains surfactants that may destroy skin barriers.

The mechanism of IgE cross-reactivity in patients with HWP-WDEIA caused by natural wheat has not yet been clarified. Interestingly, native ω-5 gliadin did not enhance basophil CD203c expression in our patients, even in patients 4 and 7, who had serum ω-5 gliadin-specific IgE. This indicates that IgE produced against HWP-A in HWP-WDEIA patients does not react to ω-5 gliadin, but to other undetermined protein components with specific epitopes. This is in accordance with the previous observations of HWP-WDEIA patients with no or lower levels of ω-5 gliadin-specific IgE (7). It is suggested that acidic hydrolysis induces a conformational change in HWP, converting a glutamine to glutamic acid and an asparagine to aspartic acid (28), yielding new epitopes that differ from the epitopes of natural wheat proteins. As we discussed in the previous report (9), it is conceivable that humans do not have sufficient tolerance to HWPs that are not natural proteins, and are therefore easily sensitized to HWP. As wheat proteins contain repetitive amino acid structures rich in glutamine and proline, IgEs against HWP probably cross-react with natural wheat proteins. This is supported by the fact that preincubation of sera with HWP decreased binding of IgE to the natural wheat proteins.

In conclusion, HWPs composed of large polypeptide aggregates possibly induce sensitization to a greater degree than lower-MW HWPs. We devised a new procedure for detecting the allergenicity of HWP by measuring HWP-induced enhancement of surface CD203c expression on basophils. Because HWPs are used in a variety of products worldwide, physicians and industry should be aware of their allergenicity.

## Acknowledgements

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JAPANESE DERMATOLOGICAL ASSOCIATION
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# Case of rice allergy induced by epicutaneous sensitization to rice bran due to handling rice bran pickles

Dear Editor,

There have been several reports of immunoglobulin (Ig) E-mediated reactions following contact with raw rice, inhalation of vapors from boiling rice and ingestion of cooked rice.[1–3] However, rice bran allergy has never been reported. In this report, we describe the first case of occupational contact urticaria in a housewife due to the handling of rice bran in the form of rice bran pickles; this initial reaction was followed by allergic reactions after the ingestion of cooked, unpolished and unwashed rice in paella.

A 50-year-old Japanese housewife was referred to our clinic for a history of oral itch, face angioedema and cough after the ingestion of paella while travelling in Spain. She had begun pickling vegetables in rice bran paste 3 years previously. However, 6 months previously, she developed wheals and itch on the hands and forearms, while handling rice bran paste. Thereafter, she also felt oral itch after the ingestion of rice bran pickles and cooked, unpolished rice. Furthermore, wheals on the hands had developed during the washing of raw polished rice, although she regularly consumed polished rice, which was cooked after washing, without symptoms. Her past history included pollinosis due to Japanese cedar pollen but not to grass pollen.

Serum total IgE level was 652 IU/mL. The specific IgE measurements using the ImmunoCAP system (Phadia, Uppsala, Sweden) showed positive for rice, which was extracted from unpolished rice, wheat, barley and rye, whereas they were negative for ingredients of paella, such as shrimp, squid and scallop. The elicited response of skin prick tests (SPT) was considered positive when the average wheal diameter induced by the allergen was 50% of the positive control response induced by histamine chloride at 10 mg/mL. SPT with commercial extracts (Torii Pharmaceutical, Tokyo, Japan) for rice and wheat were positive. SPT with rice bran at 0.2 g/mL in saline, and unpolished and polished rice at 0.5 g/mL in saline were also positive. Additionally, the average wheal diameter (17.8 mm) induced by polished rice was diminished by washing (8.4 mm) and cooking (7.9 mm). The SPT with extracts of rice bran, unpolished and polished rice was negative in five control subjects.

In IgE immunoblotting, IgE binding at approximately 50 kDa was shown by the serum of the patient, whereas not by two controls without rice allergy (Fig. 1).

Rice bran is the layer between the inner white rice grain and the outer hull and is a by-product of the rice polishing process. Rice bran is used for foods and skin care products such as soap in not only Asian countries but also westernized nations because it is rich in vitamins and minerals. Rice bran pickles, *nukazuke*, are a traditional Japanese food. This patient first developed IgE-mediated contact urticaria due to rice bran during pickling vegetables in rice bran paste. Furthermore, continuous skin exposure to rice bran while dealing with rice bran pickles and washing rice was found to result in allergic reactions after ingestion of cooked, unpolished rice and unwashed rice, such as that found in paella.

Contact urticaria to rice while handling raw rice or throwing it during weddings has been reported in both Southern Europe and Japan.[1,2] The relevant allergens in rice causing contact urticaria have never been identified, although several allergenic components in rice have been described, including Ory s 1 (35 kDa), a 14–16-kDa member of the α-amylase/trypsin inhibitor family, a 33-kDa allergen with glyoxalase I activity, and a 9-kDa lipid transfer protein.[4,5] The molecular weight of the allergen involved in rice bran allergy in this patient was 50 kDa, indicating that the allergen involved in epicutaneous sensitization of rice bran can be different from the inhalant and food rice allergens reported. Additionally, in SPT, cooked rice induced much smaller wheals than uncooked rice, indicating that the rice bran allergens could be unstable during heating.

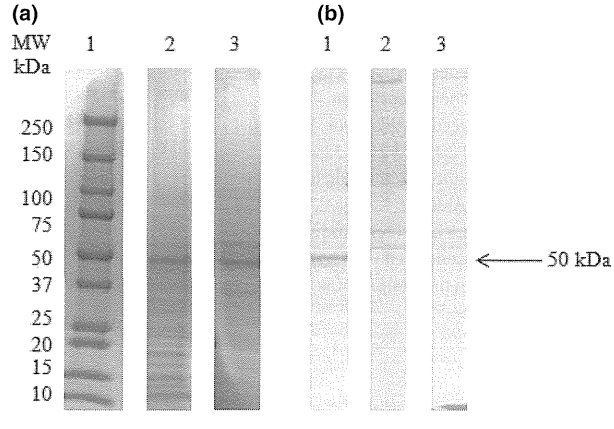


**Figure 1.** Sodium dodecylsulfate polyacrylamide gel electrophoresis and blotting analyses of rice bran. (a) Gel stained with Coomassie Blue. Lane 1, molecular weight; lane 2, rice bran under the non-reducing condition; lane 3, rice bran under the reducing condition. (b) Immunoglobulin E immunoblotting using rice bran. Lane 1, patient's serum; lanes 2 and 3, non-allergic patients.

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# Drug eruption induced by gonadotropin-releasing hormone analogs accompanying radiation-recall phenomenon

Dear Editor,

Goserelin acetate and leuprorelin acetate are gonadotropin-releasing hormone analogs (GnRHA), and are used to suppress production of the sex hormones in the treatment of breast and prostate cancer. Although several cases of granulomatous reactions at injection sites have been reported, generalized drug eruptions induced by GnRHA are rare.[1–3] Herein, we report a rare case of drug eruption induced by goserelin acetate. In addition to generalized maculopapular eruption, the patient exhibited intense erythema in the previously irradiated area, which was considered to be radiation-recall phenomenon.

A 48-year-old Japanese woman with breast cancer underwent partial mastectomy followed by postoperative radiation therapy. She also received adjuvant chemotherapy with s.c. injection of depot goserelin acetate once a month and oral tamoxifen citrate. She had taken no other drugs. After the third goserelin acetate injection, she developed pruritic eruption on her back, and was referred to our department. Multiple erythema and red papules were distributed all over the body. In addition, pronounced erythema with erosion was observed in the relatively sharply demarcated area corresponding to the previously irradiated region (Fig. 1). There was no eruption at the goserelin acetate injection site. Laboratory examination revealed no significant change. A skin biopsy specimen taken from an erythema showed small foci of spongiosis with focal vacuolar change in the basal layer, and lymphocytic infiltration around the vessels in the superficial dermis. We made a presumptive diagnosis of drug eruption, and tamoxifen acetate was discontinued. Because the eruptions were intense, she

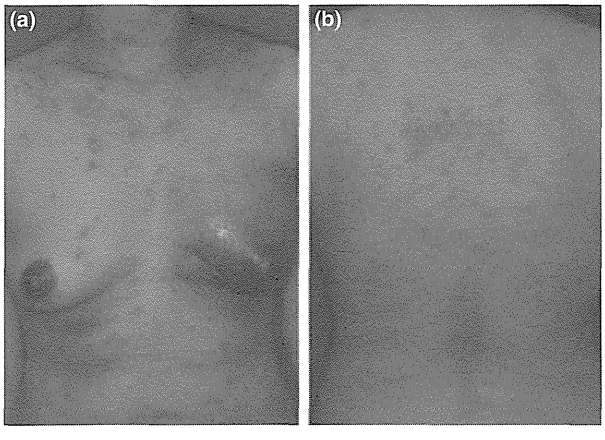


**Figure 1.** (a,b) Multiple erythema and red papules distributed all over the body. Pronounced erythema with erosion was observed in the demarcated area that corresponded to the previously irradiated region.

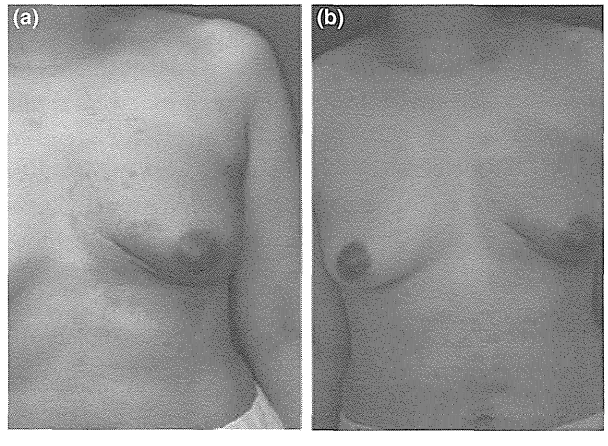


**Figure 2.** (a) Erythematous lesion flared up on her left breast. (b) Maculopapular eruptions gradually developed all over the body. The erythema on the left breast extended into the whole irradiated region and resolved with pigmentation.

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Conflict of interest: none declared.


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# Allergic Contact Dermatitis from Carmine in Cosmetic Blush

*Kayoko Suzuki, Keiko Hirokawa, Akiko Yagami, and Kayoko Matsunaga*

**Although there are many reported cases of immediate allergy after ingestion of foods containing cochineal, there are few reports of allergic contact dermatitis from carmine. We present a rare case of allergic contact dermatitis due to carmine. A 52-year-old female presented with an itchy erythema on her cheeks at the site where blush had been applied. Patch-tested with her cosmetics, she showed a positive reaction to the blush (30% in petrolatum) and to 0.2% (but not 0.1%) carmine in petrolatum. In this case, the optimum patch-test concentration of carmine was 0.2% in petrolatum.**

CARMINE is a natural red dye and is the aluminum lake of the pigment from cochineal (Fig 1). Carmine is obtained from the dried female cochineal insect *Dactylopius coccus* (or *Coccus cacti*). Carmine is an ancient textile and ornament dye and has also been widely used in foods, drinks, drugs, and cosmetics. Although immediate allergy due to carmine has been reported, reports of allergic contact dermatitis caused by carmine are rare. Here we describe a case of contact dermatitis caused by carmine in a cosmetic blush.

## Case Report

A 52-year-old female presented with a 6-month history of itchy erythema on her cheeks. Two years earlier, she had exhibited the same symptoms, which improved after she stopped using her cosmetics. She had no history of atopic dermatitis or allergic rhinitis. On examination, a scaly erythema was seen on her cheeks where blush had been applied (Fig 2). Patch tests with her personal cosmetics and 17 cosmetic allergens (not containing carmine) were applied with Finn Chambers (EPITEST Ltd Oy, Hyryla, Finland) on Scanpor (Actavis Norway AS). Readings were made on day 2 (D2) and day 4 (D4) according to International Contact Dermatitis Research Group guidelines. The patient showed a positive reaction only to the blush (30% in petrolatum). Additional patch testing was performed with the ingredients of the blush provided by the cosmetics supplier. The patient reacted to the 1% petrolatum mixture of carmine and talc but did not react to talc alone. Results of patch tests with the same 1% petrolatum mixture of carmine and talc on four control subjects were negative, which suggested that the causative allergen was carmine. As the cosmetics company was unable to provide carmine, we patch-tested with carmine sold by Kanto Kagaku (Tokyo, Japan) at concentrations of 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% in petrolatum. The patient showed positive reactions to carmine at concentrations of 0.2% and greater. We concluded that the causative allergen was carmine, and the minimum positive concentration of carmine in this case was 0.2% in petrolatum (Table 1).

## Discussion

There have been only three reports of allergic contact dermatitis from carmine after the initial cases described by Sarkany and colleagues in 1961.[1–4] Immediate allergic reaction to carmine was first reported in 1979 as occupational asthma,[5] and a case of anaphylaxis after a patient drank a Campari Orange containing cochineal extract was reported in 1994. Since then, many cases of immediate allergy after the ingestion of food containing cochineal have been reported.

The many reported cases of immunoglobulin E–mediated hypersensitivity due to carmine in drinks or foods are likely caused by exposure to mucous membranes. It appears that carmine in the form of a lake with aluminum oxide rarely induces delayed-type hypersensitivity owing to its inability to penetrate the epidermal barrier; carmine is thus considered to be a rare sensitizer of delayed-type

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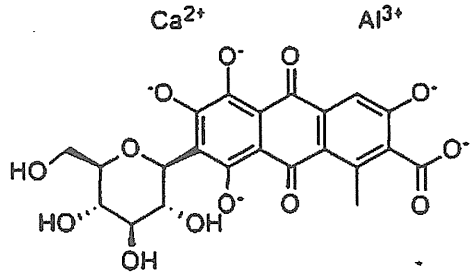


Color : red
Stability : Stable. Incompatible with strong oxidizing agents

Molecular formula: C22H20O13
Molecular weight; 492.39

CAS number: 1390-65-4
Color index: 75470

**Figure 1.** Chemical structure of carmine. (CAS = Chemical Abstracts Service.)

hypersensitivity. Although the major allergenic protein in cochineal that causes immediate allergy has been identified as a 38 kD protein that may be a phospholipase or related enzyme,[6] the causative allergen of the allergic contact dermatitis remains unidentified. Because carmine is a natural pigment from cochineal extract, it contains several proteins as well as the main pigment.

The carmine used in the patch test was 42% pure. Endo and colleagues performed patch tests on their two patients with three kinds of carmine.[3] Although one patient had positive reactions to all three carmines, the other reacted to only two. It was suspected that an impurity in the carmine

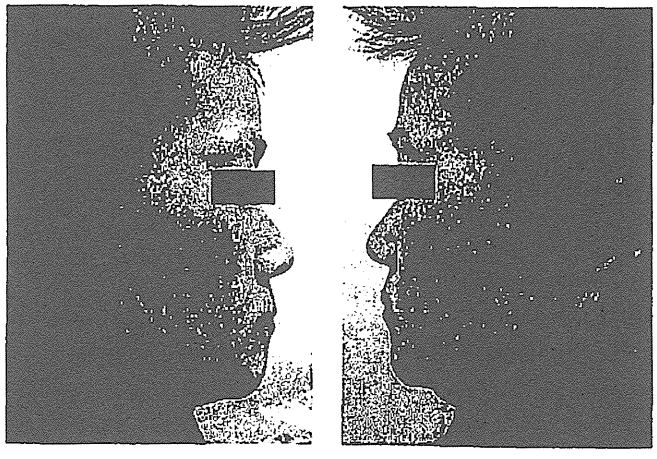

**Figure 2.** Photographs of the patient at presentation. Note the clinical appearance of scaly erythema on the cheeks, where blush had been applied.

**Table 1.** Positive Patch-Test Reactions

| | | *Reactions* | |
|---|---|---|---|
| *Material* | *Conc* | *D2** | *D4** |
| Blush | 30.0% pet | +? | + |
| Carmine | 0.5% pet | +? | + |
| Carmine | 0.4% pet | +? | + |
| Carmine | 0.3% pet | +? | + |
| Carmine | 0.2% pet | +? | + |
| Carmine | 0.1% pet | − | +? |

Conc = concentration; D = day; pet = in petrolatum; +? = doubtful.
*According to International Contact Dermatitis Research Group recommendations.

reagent was responsible for causing the delayed-type hypersensitivity.

With respect to the patch-test concentration of carmine, positive reactions were documented by Sarkany and colleagues[1] and Yamamoto and colleagues,[2] who used carmine 0.1% in liquid paraffin and in petrolatum, respectively. Our patient had a doubtful reaction to carmine 0.1% in petrolatum and a + reaction to carmine 0.2% in petrolatum. From these findings, it appears that the optimum patch-test concentration of carmine is 0.2% in petrolatum. Shaw reported positive patch-test reactions and antecubital repeated open application test results with carmine 2.5% in petrolatum.[4]

Antecubital repeated open application testing seems to be useful for identifying the causative agents of contact dermatitis.

## Acknowledgments

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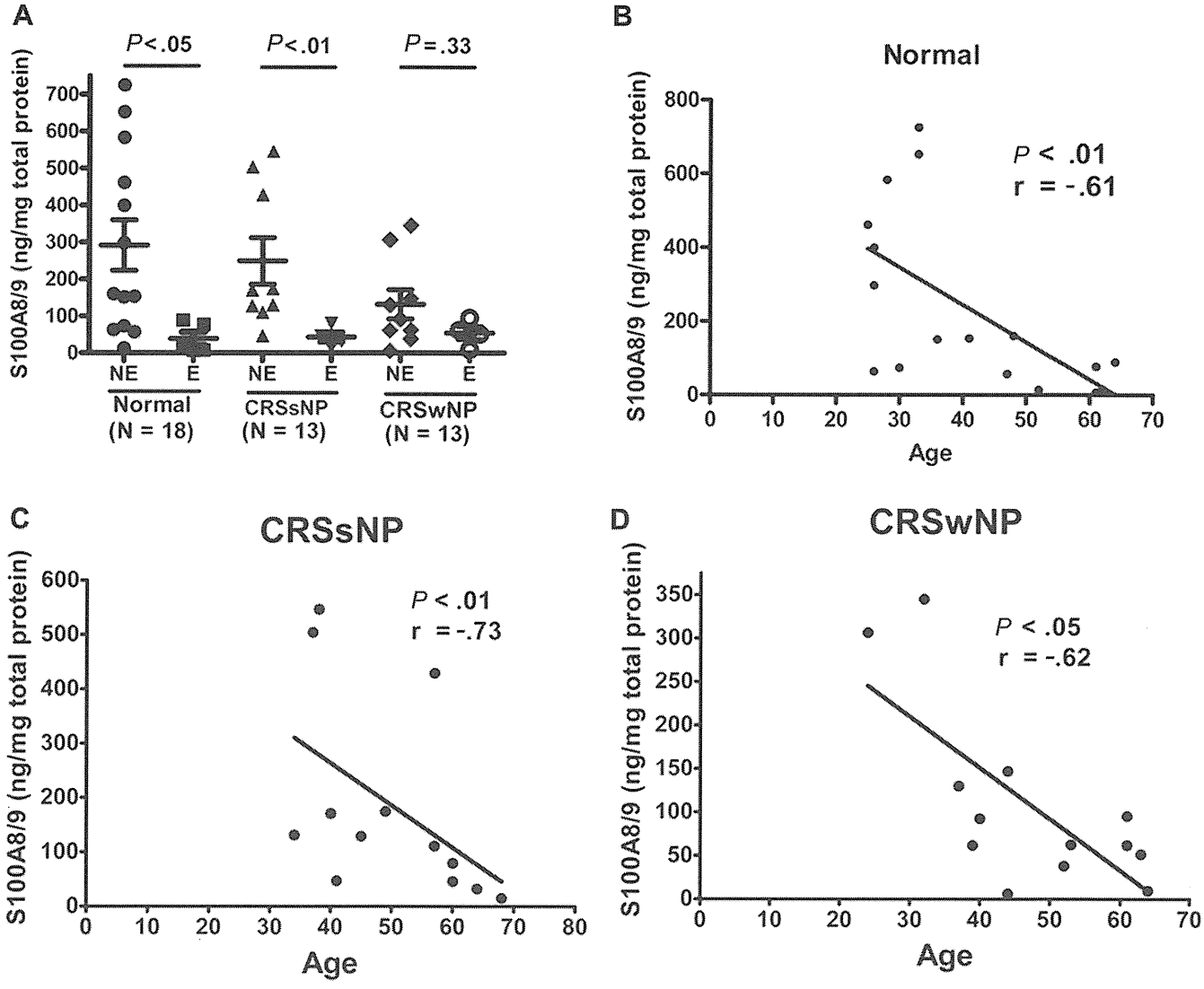


**FIG 2.** Age-related distribution of the human S100A8/A9 protein levels in nasal lavage fluids from normal subjects and from patients with CRS as determined by ELISA. **A,** In normal controls and patients with CRSsNP, S100A8/9 levels in elderly subjects were significantly lower compared with those in nonelderly subjects. **B-D,** Levels of S100A8/A9 were significantly diminished with increasing age in normal controls and patients with both subtypes of CRS. *E,* Elderly; *NE,* nonelderly.

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## Clinical relevance of IgE to recombinant Gly m 4 in the diagnosis of adult soybean allergy

*To the Editor:*

Soybean is one of the most important foods causing food allergy in childhood.[1] In addition, studies have shown that allergy to soybean can be caused by IgE cross-reactivity between Bet v 1, a major allergen of birch pollen, and its homologue pathogenesis-related class 10 protein in soybean, Gly m 4.[2,3] However, the clinical relevance of measuring the level of IgE antibody (IgE-ab) to recombinant Gly m 4 (rGly m 4) is still

**TABLE I.** Demographics and frequencies of sensitization to allergens in study subjects

| | Soybean-allergic patients (n = 21) | Alder-pollen–sensitized control patients (n = 93) | *P* value |
|---|---|---|---|
| Sex (female), n (%) | 17 (81) | 57 (61) | .128 |
| Age (y), mean ± SD | 41.4 ± 16.1 | 44.5 ± 16.6 | .438 |
| Comorbidity, n (%) | | | |
| Allergic rhinitis | 21 (100) | 93 (100) | 1.000 |
| Asthma | 3 (14) | 31 (33) | .449 |
| Reported food allergy to soybean, n (%) | 21 (100) | 0 (0) | <.001 |
| Reported OAS due to any other plant foods,* n (%) | 18 (86) | 17 (18) | <.001 |
| Sensitization determined by CAP-FEIA (≥0.35 kUA/L), n (%) | | | |
| Alder pollen | 21 (100) | 93 (100) | 1.000 |
| Birch pollen | 21 (100) | 93 (100) | 1.000 |
| rBet v 1 | 21 (100) | 66 (71) | .003 |
| Soybean | 10 (48) | 30 (32) | .211 |
| rGly m 4 | 21 (100) | 53 (57) | <.001 |
| nGly m 5 | 1 (5) | ND | |
| nGly m 6 | 1 (5) | ND | |

*ND*, Not done; *OAS*, oral allergy syndrome.
*Defined as reporting OAS due to any plant foods other than soybean in the structured questionnaire.

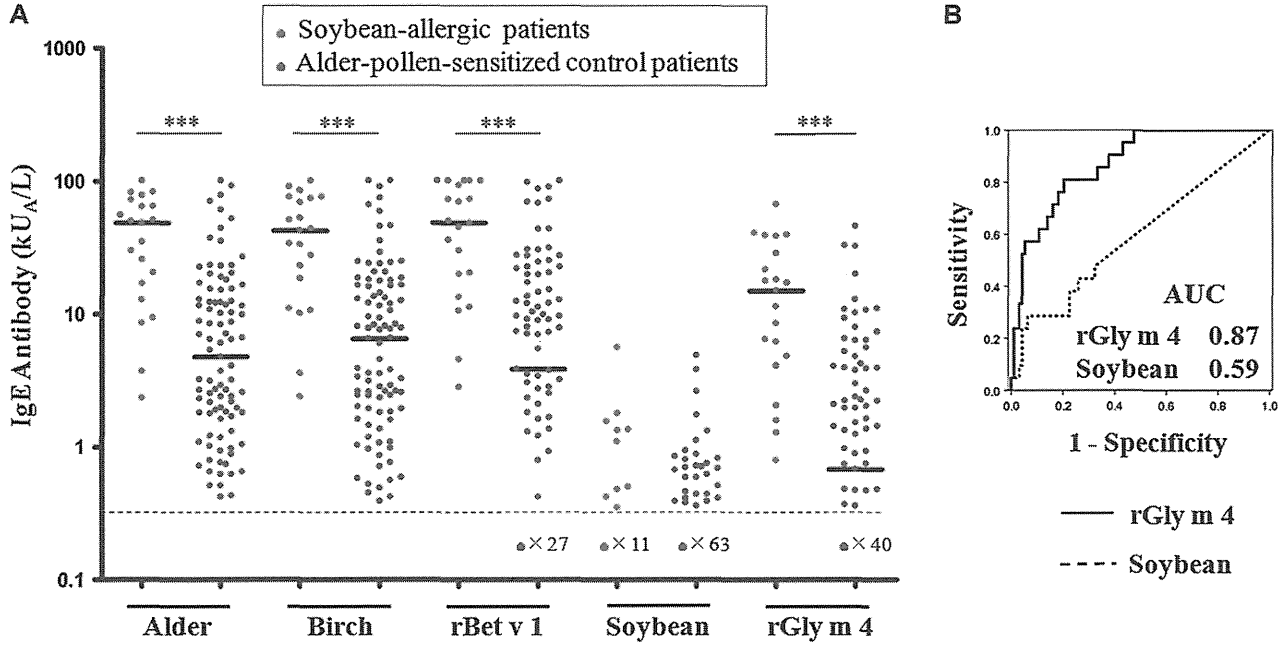


**FIG 1. A,** Levels of IgE-ab to alder pollen, birch pollen, recombinant Bet v 1 (rBet v1), soybean, and rGly m 4 in sera from soybean-allergic patients and alder-pollen–sensitized control patients. **B,** Receiver-operating characteristic curve for levels of IgE-ab to soybean and rGly m 4 in the diagnosis of soybean allergy. The number of negative test results in each set of measurement is indicated below the dotted cutoff line (0.35 kUA/L). ***$P < .001$. *AUC*, Area under the curve.

debated, considering that the concentration of Gly m 4 in soybean extract is quite low and that a considerable proportion of the patients allergic to birch pollen have IgE-ab to Gly m 4 without reporting any symptoms to soybean.[2,4] Sensitization to the soybean storage proteins Gly m 5 and Gly m 6 have been shown to contribute to more severe soybean allergy.[1,5] Data from studies on soybean allergy outside Europe, particularly in Asian populations, are still limited.

Japan has one of the largest soybean-consuming populations in the world and tofu, miso, and natto are common soybean foods.[6] Soybean allergy is the fourth most common cause of food allergy in Japan among infants but, although reported, has been thought until recently to be rare in adults.[7,8]

The aim of this study was to elucidate the characteristics of soybean allergy among adults and the impact of sensitization to Gly m 4 on soybean allergy and to analyze the diagnostic efficiency of measuring the level of IgE-ab to Gly m 4 in patients with soybean allergy compared with Betulaceae-pollen–sensitized patients without soybean allergy.

We recruited 21 consecutive adult patients with soybean allergy who visited our clinic during 2009. Soybean allergy was diagnosed on the basis of a convincing case history and results of positive skin

prick test to soybean extract (extract named "edamame," Torii, Tokyo, Japan) and/or soymilk (Luckme Yakult, Tokyo, Japan). The control group consisted of 93 alder-pollen–sensitized allergic rhinitis patients without soybean allergy recruited from the outpatient clinic during the same study period. Details of the criteria for both patient groups are shown in this article's Online Repository at www.jacionline.org.

The IgE-ab levels to alder pollen, birch pollen, soybean extracts, recombinant Bet v 1 (rBet v 1), rGly m 4, native Gly m 5 (nGly m 5), and native Gly m 6 (nGly m 6) were determined by using the commercially available ImmunoCAP system (Phadia, Uppsala, Sweden). IgE-ab level of 0.35 $kU_A/L$ or more was regarded as positive. IgE-ab levels to nGly m 5 and nGly m 6 were measured in the soybean-allergic group only.

Statistical testing was performed by using the Fisher exact test for the categorical variables and Mann-Whitney *U* test for the continuous variables. The receiver-operating characteristic curves were plotted for IgE-ab levels to rGly m 4 and soybean extract separately and the area under the receiver-operating characteristic curve was calculated and the Student *t* test was conducted for comparison (SPSS ver. 19.0, IBM, Tokyo, Japan). A *P* value of <.05 was considered significant.

The most frequent symptom among the soybean-allergic patients (see Table E1 in this article's Online Repository at www.jacionline.org) was oral allergy syndrome (17 of 21, 81%), followed by cough/dyspnea (10 of 21, 48%) and abdominal pain/diarrhea (8 of 21, 38%). Soymilk was the most provoking allergen in relation to exposure. Fifteen (71%) of the 21 patients reported reaction to any of the moderately heated soy products such as tofu and boiled edamame, and none to the fermented soy products such as natto, miso, and soy sauce (see Table E2 in this article's Online Repository at www.jacionline.org).

The frequency of sensitization to soybean in soybean-allergic patients was relatively low (48%), whereas all the soybean-allergic patients were sensitized to rGly m 4 (Table I). Only one patient was sensitized to the soybean storage proteins nGly m 5 and nGly m 6.

IgE-ab levels to rGly m 4, as well as to alder pollen, birch pollen, and rBet v 1, were significantly higher in the soybean-allergic group than in the control group (median levels of IgE-ab to rGly m 4, 15.0 vs 0.68 $kU_A/L$; alder, 48.4 vs 4.77; birch, 42.4 vs 6.49; rBet v 1, 48.4 vs 3.85 $kU_A/L$; Fig 1, *A*). The receiver-operating characteristic curve showed that the area under the curve for IgE-ab levels to rGly m 4 was 0.87 (Fig 1, *B*), which was significantly higher than that for soybean (0.59; $P < .01$). Applying a 4.0-$kU_A/L$ cutoff level, the diagnostic efficiency of rGly m 4 reached 81% and 78%, respectively, in terms of sensitivity and specificity.

This study revealed the characteristics of adult soybean allergy and the impact of sensitization to Gly m 4 in a Japanese population. In Japan, alder pollen (alder is part of the Betulaceae family) is of minor clinical importance in terms of respiratory sensitization, compared with Japanese cedar pollen. However, although sensitization to alder pollen was not an inclusion criterion for the soybean-allergic patient group, all the patients were sensitized to alder pollen and rGly m 4. Moreover, IgE-ab levels to rGly m 4 were markedly higher in the soybean-allergic patients than in the alder-pollen–sensitized control patients. These findings highlight the clinical impact of respiratory sensitization to pollen-derived pathogenesis-related class 10 protein on the development of adult soybean allergy.

The most frequent symptom in the soybean-allergic patients was oral allergy syndrome. Soymilk was the allergen that most prevalently induced symptoms in those who had been exposed, whereas none of the soybean-allergic patients reacted to fermented soybean products. This finding was in agreement with that of Mittag et al.[2] The explanation is that Gly m 4 is somewhat heat-labile but also apparently susceptible to degradation by fermentation.

This study was carried out in a population with high soybean consumption. Indeed, a study in Japan has shown that all the pediatric patients with soybean allergy were sensitized to Gly m 5 and Gly m 6, and sensitization to these allergens contributes to more severe soybean allergy.[5] However, only 1 of the 21 adult patients in our study was sensitized to Gly m 5 and Gly m 6, suggesting that primary sensitization to soybean (ie, the oral route) was relatively rare. Therefore, we consider that sensitization to Gly m 4 is of more importance for adult patients with soybean allergy. Moreover, it is important to highlight the finding that some of these Gly m 4–mediated soybean-allergic adult patients experienced severe allergic symptoms including anaphylaxis.

One possible limitation of this study is related to the diagnosis of soybean allergy. Soybean allergy was diagnosed on the basis of a convincing case history and positive results of skin prick test. A double-blind placebo-control food challenge was not performed. However, regarding oral symptoms, subjective symptoms are relatively reliable because they occur immediately after the ingestion of specific foods. Recently, Skypala et al[9] have shown the validity of self-completed structured questionnaire, namely, subjective allergic symptoms, as the basis of the diagnosis of oral allergy syndrome.

We conclude that the discrimination between patients with pollen-related soybean allergy and Betulaceae-pollen–sensitized patients was effectively achieved in this Japanese adult population with a low prevalence of Betulaceae pollen allergy and high soybean consumption, by measuring the IgE-ab level to rGly m 4. A high level of IgE-ab to rGly m 4 was associated with adult soybean allergy.

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## IgE against bed bug (*Cimex lectularius*) allergens is common among adults bitten by bed bugs

*To the Editor:*

*Discuss this article on the JACI Journal Club blog: www.jaci-online.blogspot.com.*

*Cimex lectularius*, the bed bug, is found worldwide and likely has been sharing human dwellings dating back to prehistoric times. With increased use of pesticides after World War II, the reports of bed bugs in developed countries decreased significantly.[1] Recently, *C lectularius* has made a resurgence, including in New York City (NYC) where housing violations for bed bugs rose from 82 to 4084 between 2005 and 2009.[2] The potential health implications of this increase in domestic exposure are not known; however, there is some evidence that exposed individuals can make a type 1 allergic response to *C lectularius* bites (see Table E1 in this article's Online Repository at www.jacionline.org).[3] In the 1990s, researchers in Egypt reported skin test positivity to crude *C lectularius* extract among asthmatic patients.[4] Another case report identified *C lectularius* nitrophorin protein (cNP), a nitric oxide–carrying protein found in the injected saliva, that elicited an IgE antibody response in 1 patient.[5-7] The uniqueness of the predicted cNP sequence[8] suggests that IgE to cNP would indicate a hypersensitivity response that is specific to *C lectularius*. Aside from the single case described above,[5] the development of IgE antibodies to cNP among individuals bitten by bed bugs has not been reported. Our goal was to develop assays for measuring IgE antibodies against both the *C lectularius* extract and the cNP protein in order to determine the prevalence of sensitization to bed bug allergens among adults with reported bed bug bites.

Thirty NYC residents who reported being bitten by bed bugs were recruited through Web advertisement (www.craigslist.org), fliers, and physician referral. Entry criteria for the study included report of having been bitten by a bed bug resulting in an itchy raised bump within the past year. Qualifying consenting participants donated serum and were queried about bed bug exposure and respiratory and allergic symptoms (wheezing, coughing, pruritic rash, itchy eyes, or runny nose) at the time of being bitten. Columbia University's Institution Review Board approved this study.

The *C lectularius* extract was prepared from 160 mg of dried *C lectularius* (generously donated by Louis Sorkin, Museum of Natural History and George Keeney, Ohio State University) by freezing it in liquid nitrogen and then grinding it to a powder by using mortar and pestle. The powder was incubated for 2 hours in 600 μL of PBS with Tween 20 at 30°C. After 10 minutes of centrifugation, the supernatant was dialyzed against PBS to remove Tween 20. Recombinant cNP (prepared from *Escherichia coli* as described previously)[7] was dialyzed in PBS and diluted to 1.7 μg/mL. Both recombinant cNP and the *C lectularius* extracts were biotinylated (separately) by using EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, Ill) in a 10 mM solution of the Sulfo-NHS-LC-Biotin reagent. The biotinylated cNP and *C lectularius* extracts were bound separately to streptavidin ImmunoCAPs as described previously (Phadia, Portage, Mich).[9] Serum from the subjects was then incubated separately with *C lectularius* and cNP ImmunoCAPs for 30 minutes at 37°C. IgE concentrations were measured by using standard ImmunoCAP methods.[9] Optimum *C lectularius* and cNP coating dilutions were determined to be 67 and 264 μg/mL, respectively, on the basis of maximal IgE binding experiments with serum samples identified as positive in our initial screening. IgE levels against cockroach and dust mite, 2 major allergens in NYC, and the total IgE level were also measured to assess seroatopy. Inhibition of IgE binding to *C lectualrius* experiments were conducted with dust mite and cockroach allergen extracts (see the Methods section in this article's Online Repository at www.jacionline.org).

Seventeen (57%) subjects had detectable IgE levels (≥0.1 IU/mL) against the *C lectularius* extract (Fig 1). Of these 17, 9

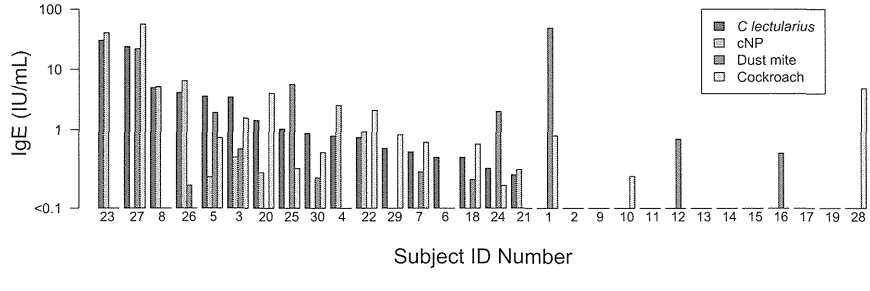


**FIG 1.** Concentrations of IgE antibodies against *C lectularius* extract, *C lectularius* nitrophorin recombinant protein, *Dermataphagoides farinae*, and German cockroach among the 30 adults reporting bed bug bites in the past year. Absence of a bar indicates that the subject had undetectable IgE levels (ie, <0.1 IU/mL).