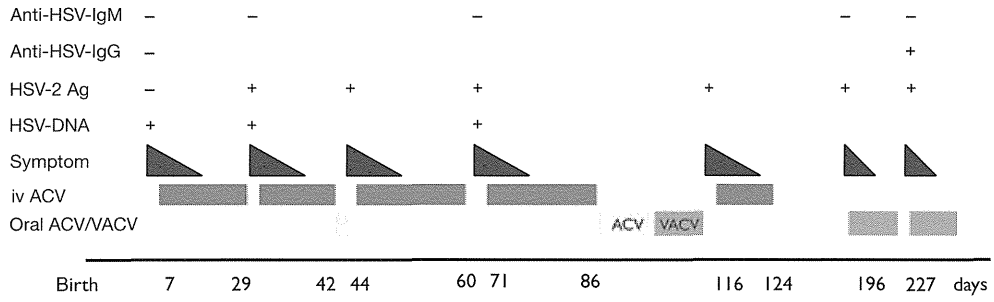

Figure 2. Clinical course of the case. The immediate recurrence after termination of each ACV course should be noted. ACV, acyclovir; Ag, antigen; HSV, Herpes simplex virus; Ig, immunoglobulin; VACV, valaciclovir.

chemotherapy improved the situation, however, there is recurrence in 46% of cases,[6] sometimes in a severer form.[4,5] HSV-2 has a tendency to show recurrence more often than HSV-1.[4] Our case had only a very limited skin lesion which resembled common recurrent herpes. The initial negative results made the diagnosis difficult and we employed a dose of 10 mg/kg per day of ACV for observation because HSV infection was not established and the lesion was very limited with neither neurological symptoms nor lab data abnormalities. But it immediately recurred after termination of i.v. ACV. Some of the recent reviews recommend 20 mg/kg per day of ACV for 14 days even for localized neonatal HSV and the same dose for 21 days for systemic disease for confirmed cases.[7] Although systematic review does not yet conclude the preference of higher doses,[8] there may have been less recurrence with a higher dose ACV at initial treatment in our case. Kimberlin *et al.*[1] reported the results of a clinical trial of 6 months of oral ACV suppression therapy on 23 neonates with SEM disease and found no recurrence. In our case, oral ACV was not effective for preventing recurrence at day 42. There is not yet a clear standard for how long suppressive antiviral chemotherapy should be maintained, however, when oral ACV fails, infusion should be employed. Prevention of NH at delivery is not yet successful, as a clinical trial of ACV versus placebo for pregnant women with a history of GH found that shedding of HSV within 7 days of delivery and the number of subjects with clinical HSV lesions at delivery did not differ between the two groups.[9] Therefore, earlier diagnosis is the most important issue and sensitive tests such as PCR and HSV Ag detection and adequate antiviral therapy are required to prevent the development of CNS disease.

Shinichi IMAFUKU,[1] Eiji OHTA,[2] Kazuko YOSHIMURA,[2] Shinichiro YASUMOTO,[3] Shinichi HIROSE,[2] Juichiro NAKAYAMA[1]
Departments of [1]Dermatology and [2]Pediatrics, Faculty of Medicine, Fukuoka University, and [3]Department of Dermatology, Faculty of Medicine, Kurume University, Fukuoka, Japan

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Novel allergen from the freshwater clam and the related allergy

Dear Editor,

Incidence of mollusc allergy reportedly varies between 0.15% and 0.4%.[1,2] Symptoms are heterogeneous among the affected ranging from mild rashes and hives to life-threatening anaphylactic shock. Tropomyosin is frequently the culprit panallergen in those cross-reactivity studies of molluscs and crustaceans.[3] When

Correspondence: Tao Wang, M.D., Ph.D., Department of Nephrology, The 4th Hospital of Hebei Medical University, Shijiazhuang 050000, China. Email: nephrology2009@hotmail.com

isolated allergy to molluscs or a single species occurs, however, tropomyosin does not seem to have a relevant role.[4,5] In these cases, mollusc-specific allergens or even species-specific ones may be involved. *Corbicula japonica*, a freshwater bivalve mollusc, is widely consumed in Japan mainly as an ingredient of "miso" soup. In this report, we present two cases of food allergy to the freshwater clam, in which the sensitization to shrimp tropomyosin was not detected.

Patient 1 was a 69-year-old woman who was referred to our hospital because of erythema of the upper torso and arms accompanied by itching 3 h after the ingestion of freshwater clam soup. No systemic symptom was noted. The patient had experienced the same symptoms 3 years prior after eating clams. There was no episode after eating shrimp, squid and octopus, or foodstuffs devoid of crustaceans and molluscs. No history of fish allergy was registered. Her sister had a history of solar urticaria and her aunt of asthma.

Patient 2 was a 64-year-old man who was rushed to our hospital by ambulance because of face wheal and erythema simultaneously with dyspnea 20 min after ingestion of freshwater clam soup. On arrival, the patient's blood pressure was 116/68 mmHg, heart rate was 125 b.p.m., SpO_2 was 96%, and the erythema extended over the whole body. He suffered from irritable bowel syndrome in 1996 and possible food-associated erythema of the back in the prior year. Unfortunately, the allergens could not be identified.

For both patients there was neither evidence of infection nor use of medication prior to the onset of the disease, and the symptoms were successfully resolved with anti-allergy treatment.

Laboratory investigation showed an elevated concentration of serum immunoglobulin (Ig)E. Serum-specific IgE antibodies, determined by using ImmunoCAP (Phadia, Uppsala, Sweden), are summarized in Table 1. The results of 2-D electrophoresis and IgE immunoblot is shown in Figure 1(a,c). The main spots were noted for the isoelectric point (IE) 4.5 and 32-kD IgE-binding constituent, which were stained not with the Coomassie Brilliant Blue (CBB) and silver stain liquid, but with the Ruby gel stain liquid (data not shown). When using serum of the healthy control against protein from the shrimp and freshwater clam, very faint bands were barely visible at 37 and 32 kD, respectively (Fig. 1b, lane 3). The impact of boiling on antigenicity is depicted in Figure 1(c) using sera of patients. In

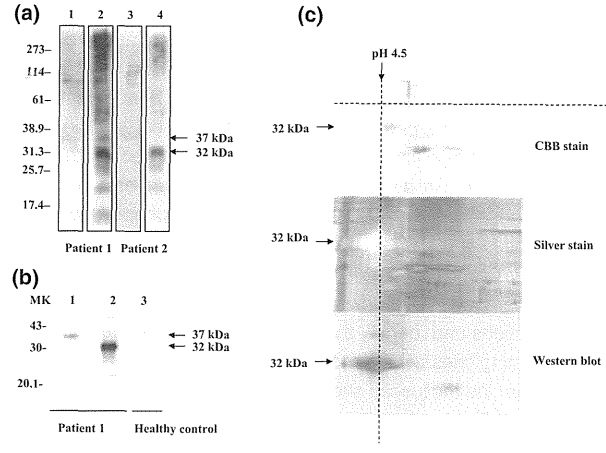

Figure 1. Analyses of shrimp and clam extracts by SDS-PAGE and IgE immunoblotting. (a) Lanes in odd and even number denotes boiled shrimp and boiled freshwater clam, respectively; lane 1 and 2, patient 1; lane 3 and 4, patient 2. (b) Lane 1 indicates raw freshwater clam, lane 2 and 3 indicate boiled freshwater clam. (c) Two dimensional gel electrophoresis of the fresh water clam extracts. Gel was stained by the CBB stain liquid, or silver stain liquid, or analyzed by IgE immunoblotting. The main spot indicates a 32 kD IgE-binding protein with an IE of 4.5, which was not stained by the CBB stain liquid and silver stain liquid.

Table 1. Radioallergosorbent test with CAP system data (Ua/mL)

Foodstuff	Patient 1	Patient 2
Egg white	<0.35	–
Milk	<0.35	–
Wheat	<0.35	–
Gluten	<0.35	<0.35
Soybean	<0.35	<0.35
Shrimp	<0.35	<0.35
Shrimp tropomyosin	<0.35	–
Squid	<0.35	<0.35
Octopus	–	<0.35
Crab	<0.35	<0.35
Asari (short necked clam)	1.65	1.98
Scallop	1.72	3.28
Total immunoglobulin E	478.9 IU/L	880.8 IU/L

comparing raw (lane 1) against boiled (lane 2) freshwater clam, IgE reactivity was dramatically enhanced at 32 kD and attenuated at 37 kD.

Herein, we report two cases of freshwater clam allergy. Both patients had a clear history of taking freshwater clam, but developed different clinical manifestations. We speculate that the difference may be due to the variations in their degree of sensitization, the dose of exposure to the offending food, and/or the sensitivity of receptors in their various tissues to the specific mediators.[6]

Tropomyosin is known to be the most frequent causative stuff in the seafood allergy and the resultant cross-reactivity is invariably found.[7] Indeed, the allergens in oyster (Cra g 1), abalone (Hal m 1) and squid (Tod p 1) have been identified as tropomyosin.[8] However, in our study, the serological and clinical cross-reactivity was not observed between freshwater clam and shrimp. The patients tolerated all crustaceans and cephalopod molluscs, and the sensitization to shrimp was not detected by immunoblotting assay as well as ImmunoCAP. Using 2-D gel electrophoresis, we found that the prominent allergen in the extract of freshwater clam corresponded to a protein with a molecular weight (MW) of approximately 32 kD and an IE of 4.5, different from those of the shrimp tropomyosin (MW 38 kD, IE 5.9). The tropomyosins of crustacean species, in fact, share only 56–68% amino acid sequence identities with those of molluscan shellfish species.[8] Sometimes, just the minor differences in the structures of tropomyosin between various molluscan and crustacean shellfish species could account for the noted differences in the immunoreactive features. Alternatively, there was evidence for the existence of both common (thus, cross-reactive) and species-specific epitopes (European Food Safety Authority, EFSA, 2006). The diversity in epitopes may likely explain the lack of uniform

allergic cross-reactivity that is observed clinically. A third possibility is that unique allergens, other than the tropomyosin, are involved in some species, such as the myosin heavy chain, hemocyanin, arginine kinase and amylase.[8] Consistently, Leung *et al.*[9] identified a major allergen in the crab *Charybdis feriatus*, which was similar to the shrimp tropomyosin but with a different MW. Moreover, the main allergen of King Broderip clam was not tropomyosin as well.[3] Whether these specific reactions are different epitope-dependent IgE responses or merely the reflection of the first shellfish exposure is currently unknown. These presently unanswered issues hence warrant more clinical studies to better define the frequency of cross-reactions and identify the underlying allergens. Nevertheless, all the existing lines of evidence suggested that there is a specific antigen from the molluscan shellfish.

We happened to observe an interesting phenomenon while processing the gel stain. The main antigen was not chromatic by both the CBB and silver stain liquid, however, the band was clearly visible by the Ruby gel stain. On the other hand, our data implied the configuration change of the specific antigen after boiling. The main band of the raw and boiled extracts was respectively detected at an MW of 37 and 32 kD, with the former similar to the shrimp tropomyosin.[4] Although the antigen protein was not thermally stable, the antigen specificity was maintained. The 32-kD allergens may play an important role, as only the boiled freshwater clam was eaten, in the ensuing anaphylactic symptoms. Concurrently, our study converged on the structure of the specific allergen.

In short, our results indicate that protein(s) involved in the mollusc allergy seem to be a specific and heat unstable allergen. Sensitization to molluscan shellfish was supported by both *in vivo* and *in vitro* studies. The mechanisms remain unknown and further studies are necessary. We speculated that the cross-reaction might be multifactorial in origin: the existence of the tropomyosin allergy, the homology in the amino acid sequences of the tropomyosins and possible involvement of other specific allergen(s).

Yan ZHANG,[1] Tao WANG,[2] Shunqiang GAO,[1] Eishin MORITA[3]

[1]*Departments of Dermatology and* [2]*Nephrology, Hebei Medical University, Fourth Hospital, Shijiazhuang, China, and* [3]*Department of Dermatology, Shimane University School of Medicine, Izumo, Shimane, Japan*

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Retrospective study of 24 patients with large or small plaque parapsoriasis treated with ultraviolet B therapy

Dear Editor,

More than a 100 years ago, parapsoriasis en plaque was characterized by Brocq as a chronic inflammation of the skin with an unknown etiology which is resistant to treatment. The possibility of malignant change in this condition has been documented,[1–3] but the effect of phototherapy in preventing malignant change has not been fully investigated.

A retrospective study was performed to evaluate the clinical outcomes of patients with parapsoriasis en plaques who consulted the Osaka Red Cross Hospital between April 1996 and December 2005. The study protocol was approved by the hospital review board. Osaka Red Cross Hospital is a referral medical center and patients with an advanced stage of disease were included in this series. If the maximum size of the lesion was 6 cm or less, the lesions were categorized as small plaque parapsoriasis (SPP). If the size of the lesion was more than 6 cm, the lesions were categorized as large plaque parapsoriasis (LPP).[2] None of the patients had axillary or inguinal lymphadenopathy. The laboratory results of all patients were unremarkable.

The mean light intensity of the broadband ultraviolet (UV)-B was 0.46 mW/cm^2 and narrowband UV-B was 5.0 mW/cm^2 as measured by the integrated light-detecting instrument (X96 Irradiance Meter; Gigahertz-Optik, Newburyport, MA, USA). UV-B therapy was conducted once a week. If all of the lesions disappeared, UV-B therapy was conducted once or twice a month thereafter or discontinued. The first exposure was 50% of the predetermined minimal

Correspondence: Rie Arai, M.D., M.P.H., Department of Dermatology, Osaka Saiseikai Izuo Hospital, 3-4-5 Kitamura, Taisho-ku, Osaka 551-0032, Japan. Email: arai88@gaia.eonet.ne.jp

laboratory abnormalities, subsequently improved, and clopidogrel and aspirin were restarted separately to prevent stent thrombosis. His symptoms did not recur, and corticosteroid was gradually tapered over the next 2 months.

One month later, after remission of DIHS/DRESS syndrome, oral provocation tests for CBZ and cilostazol were performed at 1-month intervals with informed consent. Results were positive for both, as both agents induced a fever, exfoliative skin rash and eosinophilia (Fig. 2). Human leukocyte antigen (HLA) typing was performed to determine the HLA type related to CBZ hypersensitivity, and HLA-A*1101 and 3101, HLA-B*4801 and 5101, and HLA-Cw*0801 and 1402 were detected. At the time of this report, the patient remains symptom free and his routine laboratory findings are normal.

The risk of anticonvulsant hypersensitivity syndrome (AHS) among CBZ users has been estimated to be 1.0–4.1/10 000. CBZ can show cross-reactivity with other aromatic anticonvulsants at a rate as high as 80%, but it is also known to exhibit atypical cross-reactivity with structurally dissimilar agents, such as, sodium valproate and β-lactams.[1,2]

Furthermore, a strong association has been reported between HLA and CBZ-induced drug hypersensitivity. In particular, HLA typing results suggest that HLA-B*1301, 4801 and 5101 is a genetic marker for CBZ-induced DRESS syndrome.[3] In the described case, HLA typing results were compatible with CBZ-induced DIHS/DRESS syndrome. Cilostazol is a quinolinone derivative that inhibits type-3 phosphodiesterase, structurally unrelated to clopidogrel and CBZ. No previous report has described any clopidogrel cross-reaction with cilostazol. Nevertheless, it might be unexplained cross-reactivity between cilostazol and CBZ. The detection of HHV-6 reactivation has been a diagnostic marker for DIHS/DRESS syndrome and HHV-6 reactivation is observed in more than 60% of DIHS/DRESS syndrome cases.[4] Our case showed a negative result for HHV-6 DNA by PCR and exact association with HHV-6 was not confirmed. HHV-6 reactivation was not detected, probably due to sampling at an inappropriate time or a mild clinical course of DIHS/DRESS syndrome.

Based on oral provocation test results and his medical progression, we believe that DIHS/DRESS syndrome was more likely induced by CBZ which probably played a major contributor, but had undergone polysensitivity also to cilostazol. This is the first case report issued to identify cilostazol as a causative agent of DIHS/DRESS syndrome, and to find that cilostazol and CBZ may act in concert to aggravate DIHS/DRESS syndrome. Clinicians should be aware that cilostazol is a potential cause of DIHS/DRESS syndrome.

Sung-Yoon KANG,[1,2] Ju-Young KIM,[1,2] Mi-Yeong KIM,[1,2] Suh-Young LEE,[1,2] Min-Hye KIM,[1,2] Tae-Wan KIM,[1,2] Heung-Woo PARK,[1,2] Sang-Heon CHO,[1,2] Kyung-Up MIN[1,2]

[1]*Department of Internal Medicine, Seoul National University College of Medicine, and* [2]*Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul, Korea*

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Case of allergy due to hydrolyzed wheat proteins in commercial boiled pork

Dear Editor,

Wheat is one of the most important allergens in adult food allergy,[1] but immediate hypersensitivity to hydrolyzed wheat protein (HWP) in food products has been rarely reported.[2–4] We report here a case of immunoglobulin E (IgE)-mediated food allergy due to HWP in a commercial food product.

A 30-year-old woman experienced tingling in her throat and tongue, prominent swelling of bilateral eyelids and numbness of fingers approximately 30 min after ingesting boiled pork with added HWP in a vacuum packing, boiled spinach, miso soup and a grilled alfonsino. She was prescribed an antihistamine by a clinician. However, the next day, she was referred to our clinic for examination because the swelling of her eyelids remained (Fig. 1). Her past history included atopic dermatitis in infancy. Also, she had previously experienced contact urticaria due to a cream, although she could not remember whether the cream contained HWP.

No specific IgE antibodies (ImmunoCAP) were detected for wheat, gluten, ω-5 gliadin, soybean or pork. Skin prick tests (SPTs) were performed according to standard procedures.[5] The elicited response 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. SPTs with commercial extracts, such as those of wheat and bread (Torii Pharmaceutical, Tokyo, Japan), were negative. In

Correspondence: Junko Shinoda, M.D., Department of Dermatology, Yokohama City University Hospital, 3-9 Fukuura, Kanazawa-ku, Kanagawa 236-0004, Japan. Email: shino.jun@live.jp

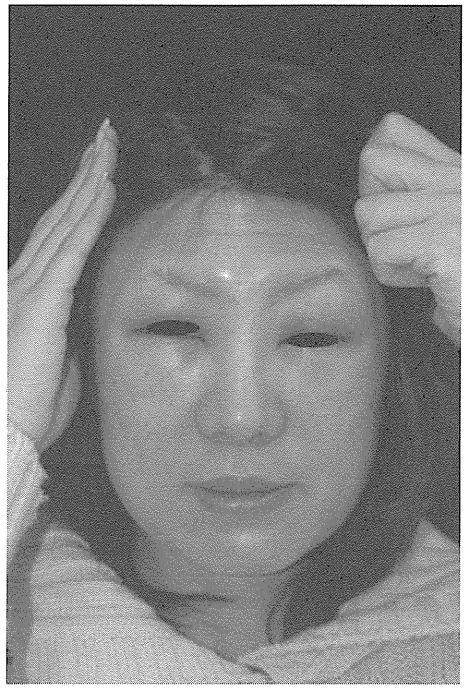

Figure 1. Bilateral eyelids were swollen at the first visit.

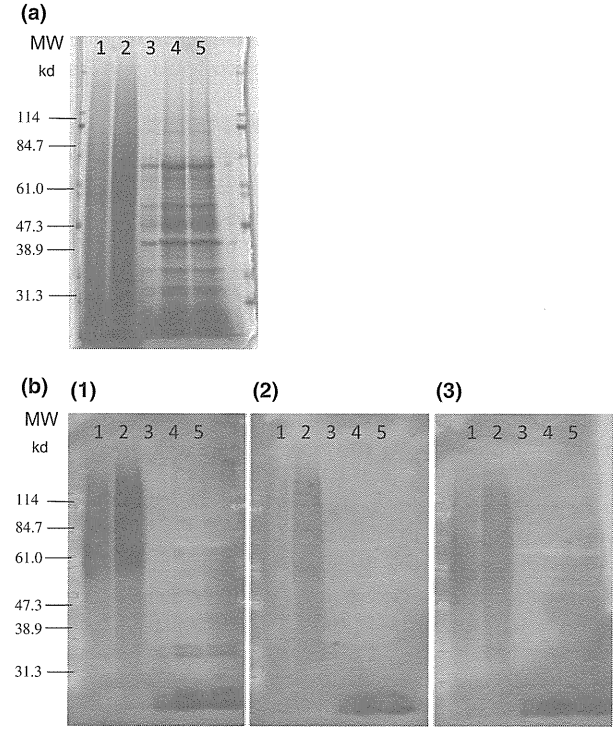

Figure 2. Sodium dodecylsulfate polyacrylamide gel electrophoresis and blotting analyses of hydrolyzed wheat protein (HWP) and wheat protein. (a) Gel stained with Coomassie blue. (b) Immunoglobulin E immunoblotting with the patient's serum. MW, molecular marker weight. Lane 1, HWP 50 μg. Lane 2, HWP 100 μg. Lane 3, wheat water-soluble protein fraction 10 μg. Lane 4, wheat water-soluble protein fraction 50 μg. Lane 5, wheat water-soluble protein fraction 75 μg.

addition, among the ingredients of the meal that induced the episode, the SPT was positive only for the boiled pork in vacuum packing but negative for others by the prick-prick method. Furthermore, the result of SPT with HWP, which was provided by the company producing the boiled pork, at 1 mg/mL was positive, whereas SPT with other constituents was negative. The SPT with HWP was negative in five control subjects. The patient was diagnosed with allergy to HWP and instructed to avoid foods and other products containing HWP.

To investigate the causative allergen in this case, IgE immunoblotting of wheat protein, including HWP, was performed by a previously described method.[5] Specific IgE for HWP with a smear pattern ranging 30–130 kDa was found in the patient's sera, whereas a band for wheat water-soluble protein fraction was detected at a molecular weight of approximately 36 kDa (Fig. 2). Additionally, in IgE immunoblotting of recombinant ω-5 gliadin and dot blot assay of purified high-molecular weight glutenin, IgE binding was not detected. IgE binding band was not detected in IgE immunoblotting and dot blotting using the serum of a healthy control.

Hydrolyzed wheat protein, which should be considered the causative allergen in this case, is produced by hydrolyzing the wheat gluten with acid and alkali. HWP is water-soluble, although gluten is not water-soluble. Furthermore, HWP has a high emulsifying property and a water retentivity, and is heat-resistant. Therefore, HWP has recently been added to various foods, such as bread, noodles, ham, cream and dressing, and cosmetics to make them soft-textured.

Several reports of food allergy due to HWP have been published.[2–4,6,7] These reports indicated that food allergy to HWP could be induced by the prior sensitization of HWP present in cosmetics and soap through skin and/or rhinoconjunctival mucosa. Lauriere *et al.*[3] reported that nine patients with contact urticaria to cosmetics containing HWP, who could eat pasta and bread without any problem, were studied. Six of them also experienced generalized urticaria or anaphylaxis to foods containing HWP. All patients had low to moderate levels of IgE specific to wheat flour or gluten. Their sensitivity to HWP and their tolerance to unmodified wheat proteins extracted from grains were confirmed using skin tests. Immunoblotting analysis of their sera showed strong IgE bindings to ω-1-2 gliadin and γ-gliadin, but not to ω-5 gliadin. These results show the role of hydrolysis on the allergenicity of wheat proteins, through both skin and digestive routes. Furthermore, in this study, it was presumed that at least part of the epitopes involved in the allergic reactions pre-existed in unmodified wheat proteins. Lauriere *et al.* suggest that aggregation of peptides carrying these epitopes and others created by hydrolysis, along with the increased solubility and the route of exposure, are possible causative factors in the allergenicity of HWP. Additionally, in another study, Lauriere *et al.*[4] reported the IgE epitopes involved in immediate hypersensitivity to HWP and conventional wheat-dependent exercise-induced anaphylaxis (WDEIA) are different.

Other reports from Japan have indicated that the development of WDEIA in patients with WDEIA due to HWP was induced by

primary skin or rhinoconjunctival sensitization to HWP in the facial soap that they used and that it accompanied sensitization to natural wheat proteins.[4,6] Fukutomi *et al.* indicated that four of five patients with HWP WDEIA showed positive results for immunoCAP against recombinant ω-5 gliadin; however, the levels in these patients were relatively low, suggesting that sensitization to other gliadins or glutenins is more important in the pathogenesis of this phenotype than that to ω-5-gliadin. In our case, cosmetics might be involved in sensitization to HWP, although the patient's information on cosmetics that induced contact urticaria was vague.

It is difficult to discriminate foods containing HWP from their appearance. Therefore, patients with allergy to HWP should be instructed to carefully avoid products containing HWP according to labeling.

Junko SHINODA,[1] Naoko INOMATA,[1]
Yuko CHINUKI,[2] Eishin MORITA,[2]
Zenro IKEZAWA[1]
[1]*Yokohama City University Hospital, Yokohama, and* [2]*Department of Dermatology, Shimane University Faculty of Medicine, Izumo, Japan*

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Drug eruption caused by sitagliptin, a dipeptidyl peptidase-IV inhibitor

Dear Editor,

Sitagliptin is a newly developed oral hypoglycemic drug, classified as a dipeptidyl peptidase (DPP)-IV inhibitor for patients with type 2 diabetes. Sitagliptin was approved by the US Food and Drug Administration and in Japan in 2006 and 2009, respectively. As for glucose regulation, incretins, including glucagons-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), play an important role.[1] These incretins were secreted from the gastrointestinal tract and act on the pancreatic β-cells to stimulate insulin release in response to the rise in glucose. It has been reported that in type 2 diabetic patients, GLP-1 levels were decreased.[2] Thus, treatment with exogenous GLP-1 improved glycemic control in type 2 diabetic patients, however incretins were rapidly inactivated via DPP-IV (CD26), a cell-surface protease. In this regard, it was impractical to use GLP-1 in a clinical setting. On the other hand, sitagliptin is an inhibitor for DPP-IV, which means that sitagliptin is an enhancer of incretins including GLP-1.

We herein report a case of sitagliptin-induced drug eruption. Given that only one case report regarding sitagliptin-induced skin reaction (sitagliptin-induced bullous pemphigoid) has been published,[3] our case is the second in the world.

A 62-year-old female patient was known to have had type 2 diabetes for 14 years and hypertension for 8 years. The patient started the treatment for type 2 diabetes with glibenclamide in 1996; however, her blood sugar and hemoglobin A1 c (HbA1c) levels were uncontrolled. Therefore, metformin hydrochloride and pioglitazone hydrochloride were added in 2002 and 2004, respectively. Glibenclamide was changed to glimepiride in 2003. In addition, the administration of trichlormethiazide and telmisartan were initiated for the treatment of hypertension in 2009 and March 2010, respectively. These five drugs were continued until her first visit in our clinic and the patient did not administrate any supplement or a single dose of medicine. To further control the elevated blood sugar, sitagliptin (50 mg/day) was started on 22 March 2011. Fifteen days after the administration of sitagliptin, red papules appeared on the abdomen and spread to the trunk and extremities. Five days after the eruption, the patient visited our clinic. The patient's general condition was good and the patient did not suffer airway constriction or edema. Red papules up to 1–2 cm were spread over the whole body except for the face, palmar and plantar. Red papules segued into slightly elevated, bright red erythema, and further erythema partially grew together (Fig. 1). These eruptions accompanied severe pruritus, resulting in

Correspondence: Youichi Ogawa, M.D., Ph.D., Department of Dermatology, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan. Email: yogawa@yamanashi.ac.jp

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ORIGINAL ARTICLE **Allergens**

Recombinant high molecular weight-glutenin subunit-specific IgE detection is useful in identifying wheat-dependent exercise-induced anaphylaxis complementary to recombinant omega-5 gliadin-specific IgE test

H. Takahashi[1], H. Matsuo[2], Y. Chinuki[1], K. Kohno[1], A. Tanaka[3], N. Maruyama[4] and E. Morita[1]

[1]Department of Dermatology, Shimane University Faculty of Medicine, Shimane, Japan, [2]Department of Pathophysiology and Therapeutics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan, [3]Scientific Affairs, Phadia K.K., Tokyo, Japan and [4]Research Institute for Food Science, Kyoto University, Uji, Kyoto, Japan

Clinical & Experimental Allergy

Summary

Background Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a special form of food allergy typically induced by exercise after ingestion of wheat products. We identified wheat omega-5 gliadin and high molecular weight-glutenin subunit (HMW-glutenin) as major allergens for WDEIA and clarified that simultaneous detection of serum IgE binding to synthetic epitope peptides of these allergens identifies more than 90% of WDEIA patients. However, the short synthetic peptides are not suitable for CAP-fluorescent enzyme-immunoassay (CAP-FEIA), which is widely utilized for detecting allergen-specific IgE.

Objective In this study, we constructed a CAP-FEIA with recombinant HMW-glutenin, and evaluated its usefulness in identifying the patients with WDEIA.

Methods Recombinant HMW-glutenin was expressed as histidine-tag protein in *E. coli* and purified by histidine-tag affinity column. Wheat, gluten, recombinant omega-5 gliadin, epitope peptide of HMW-glutenin, native and recombinant HMW-glutenin specific IgE in the sera from 48 patients with WDEIA, 16 patients with atopic dermatitis (AD) who had no immediate allergic reaction after wheat ingestion and 12 healthy controls were determined by using CAP-FEIA method.

Results In 16 AD patients without wheat allergy 12 of them (75%) had positive results for native HMW-glutenin test in contrast to epitope peptide of HMW-glutenin (12.5%) and recombinant HMW-glutenin test (12.5%). These results indicate the native HMW-glutenin test has low specificity. Sensitivity and specificity of the IgE test with recombinant HMW-glutenin were 16.7% and 92.9%. These are well compatible with results obtained by using epitope peptide of HMW-glutenin. However, sensitivity and specificity reached to 93.8% and 92.9%, when the test was combined to the test with recombinant omega-5 gliadin.

Conclusions and Clinical Relevance We demonstrated that recombinant HMW-glutenin is best for CAP-FEIA system in point of stability and specificity and confirmed that detection of specific IgE against recombinant HMW-glutenin is useful for diagnosis of WDEIA when combined with the CAP-FEIA (recombinant omega-5 gliadin) test.

Keywords diagnosis, glutenin, IgE, wheat allergy, wheat-dependent exercise induced anaphylaxis

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Correspondence:
E. Morita, Department of Dermatology, Shimane University Faculty of Medicine, Enya 89-1, Izumo 693-8501, Shimane, Japan. E-mail: emorita@med.shimane-u.ac.jp

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Introduction

Wheat-dependent exercise induced anaphylaxis (WDEIA) is a special form of food allergy typically induced by exercise after ingestion of wheat products. Detection of serum IgE in the patients with food allergy is useful for the diagnosis, however, *in vitro* tests measuring specific IgE against water/salt-soluble wheat proteins consisting albumins and globulin (wheat extract) or water/salt-insoluble wheat proteins consisting gliadin and glutenin (gluten) are not always satisfactory to identify the patients with WDEIA because of their low sensitivity and specificity [1, 2].

In our previous studies, we identified wheat omega-5 gliadin and high molecular weight-glutenin subunit (HMW-glutenin) as major allergens for WDEIA and successfully defined IgE-binding epitopes of these allergens [3, 4]. Moreover, we clarified that simultaneous detection of serum IgE binding to synthetic peptides consisting IgE-binding epitopes of these allergens identifies more than 90% of the patients with WDEIA [4] by CAP-fluorescent enzyme-immunoassay (CAP-FEIA; ImmunoCAP, Phadia, Uppsala, Sweden) which is widely utilized for detecting allergen-specific IgE all over the world. However, the short synthetic peptides are not suitable for CAP-FEIA, because coupling of peptide on solid phase of cellulose is unstable for longtime storage than that of protein. Thus, we have constructed a recombinant omega-5 gliadin protein covering one-thirds of the molecule from C-terminus, which become soluble protein [5]. CAP-FEIA by using the recombinant omega-5 gliadin is found to have an ability to identify approximately 80% of the patients with WDEIA, resulting in similar results comparable to CAP-FEIA with the synthetic peptide [6]. The CAP-FEIA by using the recombinant omega-5 gliadin is now widely used for diagnosing wheat allergy as well as WDEIA in clinics [7, 8]. In this study, we constructed a CAP-FEIA with recombinant HMW-glutenin, and evaluated its usefulness in identifying the patients with WDEIA, especially those who showed negative omega-5 gliadin-specific IgE testing.

Methods

Subjects

Sera were obtained from 48 patients with WDEIA who had recurrent episodes of anaphylaxis and positive challenge testing with wheat ingestion combined with exercise and/or aspirin pre-treatment. Sera obtained from 16 patients with atopic dermatitis (AD) who had no immediate allergic reaction by ingestion of wheat products although they had detectable scores in CAP-FEIA (wheat), and 12 healthy subjects were used as controls. This study was approved by the ethics committee of the Shimane University Faculty of Medicine (approval No. 469).

ImmunoCAP for wheat components

The wheat 1Ax2 type HMW-glutenin (458 amino acids) expression vector, pEWHx, was kindly gifted by Utsumi [9]. To express the recombinant HMW-glutenin with histidine-tag, inverse polymerase chain reaction (PCR) was conducted using KOD Plus mutagenesis kit (TOYOBO Co. Ltd, Osaka, Japan) with pEWHx as a template. The additional DNA sequence coding 6×histidine with start codon was inserted at 5′ end of expression region of HMW-glutenin by site-directed mutagenesis with forward (5′-atgcatcatcatcatcatatggaaggtgaggcctctgagcaactaca-3′) and reverse (5′-ggtatatctccttcttaaagttaaacaaaat-3′) primers. A PCR cycle consisted of denaturation at 96°C for 20 s, annealing at 62°C for 20 s, and 68°C for 7 min. After eight cycles, template DNA was digested with DpnI. Self-ligated PCR product with T4 kinase was transformed into *E. coli* DH5α. The insertion sequence was verified by DNA sequencing with BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and Genetic Analyzer ABI PRISM 310 (Applied Biosystems). The constructed plasmid was transformed into *E. coli* Rosetta (DE3) cells. The recombinant HMW-glutenin with histidine-tag at N-terminal was purified as follows. *E. coli* Rosetta (DE3) cells harbouring the expression vector were cultured in NZ medium supplemented with glucose (12 g/L), ampicillin (100 μg/mL) and chloramphenicol (20 μg/mL) for 37°C [10]. The protein expression was induced with 1 mM IPTG at OD_{600} 0.6. After cultivation for overnight, cells were harvested by centrifugation and stored −20°C until use. The cells were disrupted by sonication in a solution (20 mM Tris-HCl, 500 mM NaCl, 5 mM imidazole at pH 7.9) at 4°C after incubation with lysozyme (100 μg/mL). Bacterial lysate was then centrifuged (20 000 g, 20 min, 4°C). The pellet was dissolved with a solution A (6 M Urea, 20 mM Tris-HCl, 500 mM NaCl, 5 mM Imidazole at pH 7.9). The supernatant was collected by centrifugation and applied to Ni-affinity chromatography column (HisTrap FF crude column 1.6 × 2.5 cm, GE Healthcare UK Ltd.). The recombinant protein was eluted with imidazole gradient using buffer A and buffer B (6 M Urea, 20 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole at pH 7.9) after washing of column with 4% of buffer B. The fractions containing recombinant HMW-glutenin were lyophilized after dialysis against 0.1% acetic acid. Protein concentration was measured with Lowry assay by using DC protein assay kit (Bio-Rad Laboratories Inc., Hercules, California, USA).

Native HMW-glutenin was partially purified from wheat flour (Kameria, Nisshin Foods Inc.) as described previously [11] and epitope peptide of HMW-glutenin (PTSPQQSGQGQQPGQGQQ) containing three IgE-binding epitopes of HMW-glutenin subunit was prepared as described previously [4].

Specific IgE against wheat, gluten, recombinant omega-5 gliadin, epitope peptide of HMW-glutenin, native and recombinant HMW-glutenin were measured with CAP-FEIA (ImmunoCAP, Phadia Uppsala Sweden), as described previously [5]. Specific IgE values of > 0.7 kUa/L were determined as positive.

Immunoblotting for HMW-glutenin

Purified HMW-glutenins were electrophoresed with 12.5% SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride membrane (PVDF; Immobilon-P, Millipore Corp., Bedford, MA, USA). The membrane was incubated with the serum from WDEIA patient. Bound IgE was visualized on RX-U Fuji medical X-ray film (FUJIFILM Co., Tokyo, Japan) by using horseradish peroxidase-conjugated goat anti-human IgE antibody (BioSource Int., Camarillo, CA, USA) and ECL plus kit (GE Healthcare).

Results

IgE-binding activity for purified HMW-glutenins

Native HMW-glutenin consists of several types with molecular size of between 70 and 100 kDa [4, 12, 13]. Among them the 1Ax2 type HMW-glutenin was expressed with histidine-tag in *E. coli.* and purified. SDS-PAGE stained with CBB showed single band with molecular size of 90 kDa for recombinant HMW-glutenin (Fig. 1).

Bindings of serum IgE from the patients with WDEIA to purified native and recombinant HMW-glutenin were confirmed by IgE immunoblotting. For native HMW-glutenin four band with molecular size of between 70 and 100 kDa corresponding to x- and y-type HMW-glutenin. The IgE from two WDEIA patients who had HMW-glutenin epitope peptide-specific IgE reacted to both recombinant and native proteins (Fig. 1).

Measurement of specific IgE against native and recombinant HMW-glutenin

We compared the specificity and sensitivity of the serum IgE tests against native and recombinant HMW-glutenin, epitope peptide of HMW-glutenin and recombinant omega-5 gliadin in 10 patients with WDEIA, 16 patients with atopic dermatitis and 12 healthy subjects as controls (Fig. 2). Four patients who had positive IgE

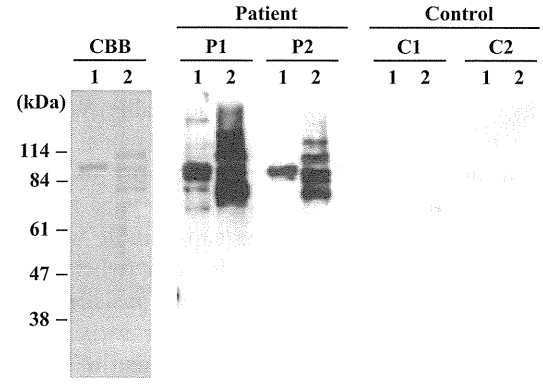

Fig. 1. IgE immunoblot analysis with recombinant HMW-glutenin and native HMW-glutenin. IgE binding to recombinant HMW-glutenin (lane 1) and native HMW-glutenin (lane 2) was determined with immunoblot by using sera of WDEIA patients (P1 and P2) who had serum IgE binding predominantly to epitope peptide of HMW-glutenin. As a negative control, sera of healthy subjects were used (C1 and C2).

value (> 0.7 kU/L) to synthetic epitope peptide of HMW-glutenin had positive IgE value to recombinant HMW-glutenin. In the test using native HMW-glutenin, in addition to these four patients, two patients showed positive result, however, 12 of 16 atopic dermatitis controls who are non-allergic to wheat showed positive results. In the 12 healthy subjects, 11 subjects were negative to native HMW-glutenin. These results indicate that the sensitivity of test by using native protein is little higher than those of the tests using recombinant protein or epitope peptide, however, the specificity of the test is much lower.

To confirm the usefulness of recombinant wheat component-specific IgE test in the diagnosis of WDEIA, we measured IgE values specific to water/salt-soluble wheat protein extract (wheat), water/salt-insoluble proteins (gluten), recombinant omega-5 gliadin, and recombinant HMW-glutenin in 48 patients with WDEIA (Table 1). In patients with WDEIA, positive subjects were 21 (43.8%), 39 (81.3%), and 8 (16.7%) for wheat and/or gluten, recombinant omega-5 gliadin, and recombinant HMW-glutenin, respectively. Six of eight WDEIA patients with positive results for recombinant HMW-glutenin-specific IgE tests had negative for recombinant omega-5 gliadin-specific IgE test. In the 16 patients with AD, positive subjects were 14 (87.5%), 0 (0%), and 2 (12.5%) for wheat and/or gluten, recombinant omega-5 gliadin and recombinant HMW-glutenin, respectively. In the 12 healthy subjects, no subjects were positive to those proteins. The sensitivity and specificity of the IgE test with recombinant omega-5 gliadin was 81.3% (39/48) and 100% (28/28). Sensitivity and specificity of the IgE test with recombinant HMW-glutenin remained 16.7% (8/48) and 92.9%

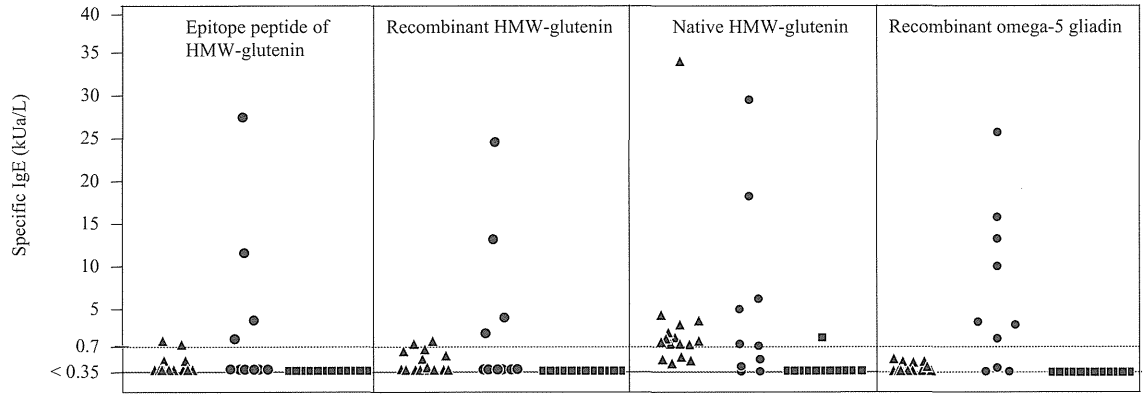

Fig. 2. Allergen-specific IgE values against epitope peptide of HMW-glutenin, recombinant HMW-glutenin, native HMW-glutenin and recombinant omega-5 gliadin. Allergen-specific IgE antibodies in sera were measured by using CAP-FEIA; ▲, AD patients (n = 16) ●, WDEIA patients (n = 10), and ■, healthy subjects (n = 12).

(26/28), however, the values reached to 93.8% and 92.9%, when the test was combined to the test with recombinant omega-5 gliadin.

Discussion

In this study, we established a stable CAP-FEIA detecting specific IgE for recombinant HMW-glutenin and demonstrated that a combination of CAP-FEIA (recombinant HMW-glutenin) and CAP-FEIA (recombinant omega-5 gliadin) provides a highly useful tool for identifying patients with WDEIA, although positive rate of CAP-FEIA (recombinant HMW-glutenin) is not high in the patients with WDEIA. This observation is well compatible with our previous results obtained by using CAP-FEIA (HMW-glutenin epitope peptide) [2]. On the other hand, four patients with IgE having above cut-off value in epitope peptide had positive IgE value with recombinant HMW-glutenin, but those values were not parallel (Fig. 2). In two cases, the specific IgE value to recombinant HMW-glutenin was increased from the value with epitope peptide test and they were decreased in other two cases. This may be explained by differences in content of epitope sequence that recognized with specific IgE individually between epitope peptide and recombinant HMW-glutenin. Our results are consistent with the previous findings that recombinant food proteins such as recombinant Ara h1, Gly m3 and Mal d1 are highly useful to identify the patients with several food allergies [14–17].

We have also demonstrated that the 75% (12/16) of AD patients who had never experienced wheat allergic reaction had positive to the native HMW-glutenin specific IgE test, although 12.5% (2/16) of AD patients had specific IgE to recombinant HMW-glutenin (Table 1). This indicates that recombinant HMW-glutenin test is more specific than the test used native HMW-glutenin. The non-specific reactions with AD patients may be explained by the contaminating water-soluble proteins in the native HMW-glutenin preparation because the IgE value was well correlated to the IgE value of CAP-FEIA (wheat), which was constructed with water-soluble fraction of wheat proteins.

Six of eight WDEIA who had HMW-glutenin specific IgE were negative to omega-5 gliadin. This supports the results of our previous study that additional HMW-glutenin-specific IgE test increases diagnostic accuracy of WDEIA [4]. Five of eight (62.5%) WDEIA patients positive for the CAP-FEIA (recombinant HMW-glutenin) were under 20 years old suggesting that young people

Table 1. Positive rate of allergen-specific IgE tests (CAP-FEIA) with wheat proteins

CAP-FEIA	WDEIA (n = 48) Positive (%)	AD (n = 16) Positive (%)	Healthy (n = 12) Positive (%)	Sensitivity (%)	Specificity (%)
Wheat	35.4	87.5	0	35.4	50.0
Gluten	37.5	18.8	0	37.5	89.3
Wheat and/or Gluten	43.8	87.5	0	43.8	50.0
rO5G	81.3	0.0	0	81.3	100
rHMW	16.7	12.5	0	16.7	92.9
rO5G and/or rHMW	93.8	12.5	0	93.8	92.9

rO5G, Recombinant omega-5 gliadin; nHMW, Native high molecular weight gulutenin; rHMW, Recombinant high molecular weight gulutenin.

are easily sensitized by HMW-glutenin than adult. It remains to be elucidated whether or not the CAP-FEIA (recombinant HMW-glutenin) test is useful in diagnosis of wheat allergy in paediatric patients. The CAP-FEIA (recombinant omega-5 gliadin) test was reported to be useful to determine clinical reactivity in wheat-sensitized children who do not need exercise to develop allergic symptoms, because the presence of serum specific IgE against omega-5 gliadin was related to the reaction to wheat challenge outcome in wheat-sensitized children [18]. Moreover, it has recently been reported that detection of IgE to omega-5 gliadin can be a useful tool in monitoring wheat allergy in the assessment of tolerance development in children with wheat allergy [19]. However, approximately 80% of the wheat-sensitized children were positive on the CAP-FEIA (recombinant omega-5 gliadin) test. It is likely that the CAP-FEIA (recombinant HMW-glutenin) test may cover the wheat-sensitized children who were negative to the CAP-FEIA (recombinant omega-5 gliadin) test.

Several gliadins in wheat, such as alpha-gliadin, beta-gliadin, omega-1,2 gliadin, were considered to be allergens in WDEIA [20–23] suggesting that additional studies are required to improve diagnosis of WDEIA by using these high purity proteins in addition to a combination of recombinant omega-5 gliadin and recombinant HMW-glutenin. In fact, three patients had neither specific IgE to recombinant omega-5 gliadin nor recombinant HMW-glutenin in this study.

In conclusion, we have demonstrated that CAP-FEIA (recombinant HMW-glutenin) is the best among the epitope peptide and native protein in point of stability and specificity. It was confirmed that detection of specific IgE against recombinant HMW-glutenin is useful for diagnosis of WDEIA when combined with the CAP-FEIA (recombinant omega-5 gliadin) test.

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Conflict of interest: There is no conflict of interest for any of the authors in this study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Allergen-specific IgE values of 48 patients with WDEIA.

Table S2. Allergen-specific IgE values of 16 patients with AD who had no immediate allergic reaction after ingestion of wheat products.

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Policies promoting use of fewer variations of precautionary statements and use only when the risk of contamination is unavoidable should be promoted. Furthermore, all subjects with food allergies, particularly those who are not members of allergy advocacy groups, must be made aware of the importance of meticulous avoidance of the offending allergen.

Moshe Ben-Shoshan, MD, MSc[a]*
Shashank Sheth, MD[b]*
Daniel Harrington, PhD[c]
Lianne Soller, MSc[d]
Joe Fragapane, BSc[d]
Lawrence Joseph, PhD[d,e]
Yvan St Pierre, MA[d]
Sebastien La Vieille, MD[f]
Susan Elliott, PhD[g]
Susan Waserman, MD[h]
Reza Alizadehfar, MD[a]
Laurie Harada, BA[i]
Mary Allen[j]
Marilyn H. Allen[i]
Ann E. Clarke, MD, MSc[b,d]

From [a]the Division of Pediatric Allergy and Clinical Immunology, Department of Pediatrics, and the Divisions of [b]Allergy and Clinical Immunology and [d]Clinical Epidemiology, Department of Medicine, McGill University Health Center, Montreal, Quebec, Canada; [c]the School of Geography and Earth Sciences and [h]the Division of Clinical Immunology/Allergy, Department of Medicine, McMaster University, Hamilton, Ontario, Canada; [e]the Departments of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada; [f]Food Directorate, Health Canada, Ottawa, Ontario, Canada; [g]the Faculty of Applied Health Sciences, University of Waterloo, Waterloo, Ontario, Canada; [i]Anaphylaxis Canada, Toronto, Ontario, Canada; and [j]the Allergy/Asthma Information Association, Montreal, Quebec, Canada. E-mail: daliamoshebs@gmail.com.

*These authors contributed equally to this work.

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CD203c expression–based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis

To the Editor:

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a special form of wheat allergy induced by the combination of wheat ingestion and physical exercise. We previously identified wheat ω-5 gliadin, a component of the water/salt-insoluble protein (gluten), as a major allergen in patients with WDEIA.[1] Recently, increased incidence of a new WDEIA subtype caused by hydrolyzed wheat protein (HWP) has been observed.[2,3] We have encountered several patients with WDEIA who were sensitized to HWP primarily through percutaneous routes, rhinoconjunctival routes, or both by using HWP-containing facial soaps.[3] Patients with this type of WDEIA showed HWP-positive results in a skin prick test (SPT) and had serum HWP-specific IgE. In addition, these patients had characteristic features of facial angioedema distinct from those seen in patients with conventional WDEIA (CO-WDEIA). Thus they were designated as having WDEIA sensitized by HWP (HWP-WDEIA). We examined the sera of several patients with HWP-WDEIA using the CAP-FEIA (Phadia, Uppsala, Sweden; detection range, 0.35-100 kU_A/L) and found that these patients have no or only low levels of ω-5 gliadin–specific IgE.

To establish a predictive *in vitro* test for differentiating these 2 subtypes of WDEIA (HWP-WDEIA and CO-WDEIA), we measured basophil CD203c expression induced by different types of wheat proteins and evaluated the diagnostic efficiency of the reactions in the patients. CD203c is an ectoenzyme belonging to a family of ectonucleotide pyrophosphatases and phosphodiesterases. It is expressed on the cell membrane of human peripheral basophils and mast cells, and cross-linking of the high-affinity IgE receptor upregulates CD203c expression on the cell membrane.

Ten patients with WDEIA were enrolled in this study: 5 with HWP-WDEIA and 5 with CO-WDEIA. The clinical features and the results of immunologic studies of these patients are summarized in Table I. All of the patients with HWP-WDEIA had been using the same brand of soap, which included HWP-A (Katayama Chemical, Osaka, Japan). None of the patients with CO-WDEIA had a prior history of using soap or other cosmetic products supplemented with HWP. Sensitization to wheat proteins was determined by means of SPT responses, specific IgE levels (CAP-FEIA), and challenge test results with wheat combined with exercise/aspirin.[4] None of the patients had atopic diathesis.

Wheat fractionation and purification of ω-5 gliadin were performed according to a previously described method.[5] SDS-PAGE and IgE immunoblotting were also performed as described previously.[5] All IgE from patients with HWP-WDEIA reacted with HWP-A, and the molecular size ranged from 15 to 250 kDa. The IgE of all patients with HWP-WDEIA also reacted to both water-soluble and water-insoluble wheat proteins but not to ω-5 gliadin. The IgE of the patients with CO-WDEIA did not react to HWP-A but reacted to

TABLE I. Clinical features and results of immunologic studies

Patient no.	Age (y/sex)	Food	Triggers	Symptoms	SPT				Specific IgE (kU_A/L)			
					Wheat	Bread	Soap 0.1%	HWP-A 0.1%	Wheat	Gluten	-5 Gliadin	CT
HWP-WDEIA												
1	51/F	Spaghetti	Walking	A, U, S, NS, ND, P	3+	3+	3+	3+	4.70	4.76	<0.34	NT
2	49/F	Gyoza dumplings	Table tennis	A, U, S, NS, C, D, AP, V	1+	1+	1+	2+	2.27	4.56	<0.34	+
3	52/F	Steamed bread	Jogging	A, U	−	−	3+	2+	0.46	0.65	<0.34	NT
4	44/F	Bread, Udon noodles	Tennis	A, U, AP, anaphylaxis	2+	2+	2+	2+	9.26	15.8	1.16	+
5	54/F	Udon noodles, Gyoza dumplings	Walking	A, U, S, ND, AP, L	2+	3+	3+	3+	5.17	7.37	<0.34	+
CO-WDEIA												
6	55/F	Pan-fried noodles, Chinese noodles, bread, spaghetti	Working	U, anaphylaxis	2+	2+	−	1+	<0.34	<0.34	2.14	+
7	41/F	Bread, cake, pudding	Working	U, D, anaphylaxis	3+	2+	−	−	0.76	0.90	14.1	+
8	73/M	Bread, Chinese noodles	Driving, internal use of analgesics	U, anaphylaxis	2+	2+	−	1+	<0.34	<0.34	2.36	+
9	39/F	Bread, cookie	Tennis	U, P	3+	3+	−	−	<0.34	1.90	3.82	+
10	60/M	Fried chicken, Chinese noodles, curry and rice	Football, jogging, internal use of analgesics	U, C, anaphylaxis	2+	2+	−	1+	1.82	5.39	11.0	+

Wheat was a commercial wheat flour extract (1:20 wt/vol; Torii Pharmaceutical Co, Tokyo, Japan). Bread was commercial bread (1:20 wt/vol, Torii Pharmaceutical Co). Soap 0.1% was 0.1% diluted solution of soap supplemented with HWP-A in saline. SPT responses were read at 15 minutes, and responses were compared with those after positive histamine controls (10 mg/mL): *1+*, 25%; *2+*, 50%; *3+*, 100%; and *4+*, 200% of the area of the wheal induced by the positive histamine control. The SPT responses were negative for all 5 nonallergic control subjects.

A, Angioedema; *AP*, abdominal pain; *C*, conjunctivitis; *CT*, challenge test; *D*, dyspnea; *L*, lacrimation; *ND*, nasal discharge; *NS*, nasal stuffiness; *NT*, not tested; *P*, pharyngalgia; *S*, sneeze; *U*, urticaria; *V*, vomiting.

water-insoluble wheat proteins and ω-5 gliadin. One hundred microliters of serum was incubated with HWP-A and serially diluted from 100 to 5 μg/mL at 37°C for 2 hours during constant stirring to perform immunoblotting inhibition assays for determining specific binding of IgEs to HWP-A on a polyvinylidene difluoride membrane. The serum was then diluted 1:10 in 5% skim milk/Tris-buffered saline with Tween 20 for immunodetection of IgEs by means of Western blotting. The reaction of the IgE with water-soluble and water-insoluble wheat proteins was also inhibited by HWP-A in a dose-dependent manner (data not shown).

A commercial kit (Allergenicity Kit; Beckman Coulter, Fullerton, Calif) was used for quantifying basophil CD203c expression, as described previously.[6] HWP-A was found to enhance CD203c expression in a concentration-dependent manner in the patients with HWP-WDEIA (Fig 1). No significant enhancement of CD203c was observed with purified ω-5 gliadin. Interestingly, native ω-5 gliadin did not enhance CD203c expression in patients with HWP-WDEIA, including patient 4, who had serum ω-5 gliadin–specific IgE, as determined by using the CAP-FEIA. This indicates that IgE produced against HWP in patients with HWP-WDEIA does not react to ω-5 gliadin but to other undetermined protein components with specific epitopes. Instead, purified ω-5 gliadin enhanced CD203c expression in a concentration-dependent manner in the patients with CO-WDEIA (Fig 1). No significant enhancement of CD203c was observed in the presence of HWP. To study whether the basophil activation was mediated by IgE, we removed the cell-surface IgE in the leukocyte mixture with lactic acid treatment, as described previously.[7] We found that basophil activation was abolished by this removal of cell-surface IgE (data not shown).

In the present study we showed that the *in vitro* wheat protein–induced basophil activation test for quantifying CD203c expression is highly useful for diagnosing the subtypes of WDEIA: HWP-WDEIA and CO-WDEIA. Specifically, the determination of CD203c expression clearly differentiated the sensitization conditions of both types of WDEIA, which is consistent with SPT responses, determination of serum allergen-specific IgE levels, and results of immunoblotting. CD203c was previously proposed to be a useful marker in the diagnosis of wheat-induced pediatric allergies.[8] The present study extends the use of the CD203c test to the determination of major allergens in adult patients with wheat allergies. The fact that the basophil activation test requires only small amounts of blood and allergen is an additional advantage of the test because it can be used to simultaneously identify a series of allergens.

The mechanism of IgE cross-reactivity to natural wheat in patients with HWP-WDEIA remains unclear. Leduc et al[9] have suggested that acidic hydrolysis induces a conformational change in HWP and converts a glutamine residue to glutamic acid and an asparagine residue to aspartic acid. Thus new epitopes that differ from the epitopes of natural wheat proteins might be produced. A tolerance to wheat proteins can develop essentially during the infantile stage through recognition of wheat allergen epitopes. It is conceivable that human subjects do not have sufficient tolerance to HWPs that are not natural proteins. Thus human subjects appear to be sensitized easily to HWPs. Because wheat proteins contain repetitive amino acid structures highly rich in glutamine

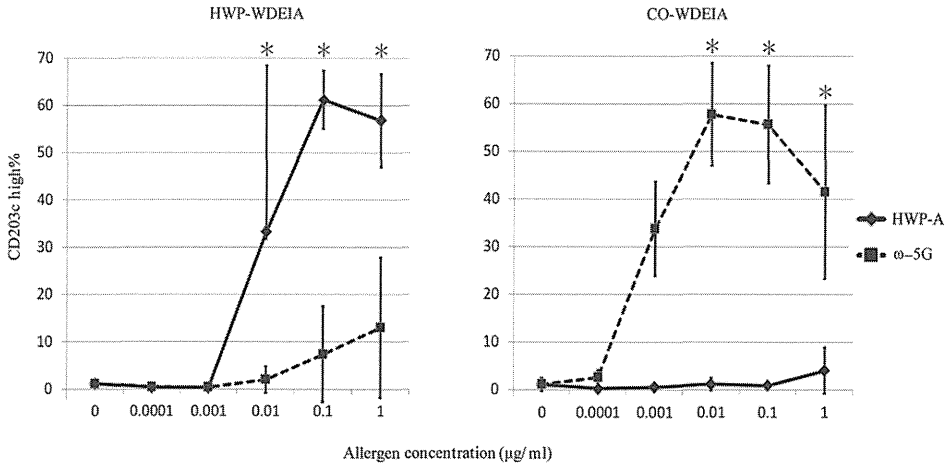

FIG 1. Expression of CD203c on basophils induced by HWP-A and ω-5 gliadin *(ω-5G)*. Mean levels of CD203c expression in 5 patients with HWP-WDEIA and 5 patients with CO-WDEIA are presented. Data are expressed as means ± SEMs. $*P < .01$ when comparing HMP-A and ω-5 gliadin, as determined by using the Student *t* test.

and proline, IgE produced against HWPs probably cross-reacts with natural wheat proteins. In fact, preincubation of sera with HWP-A clearly revealed a decreased binding of IgE to natural wheat proteins.

In conclusion, measurement of basophil CD203c expression induced by various preparations of wheat proteins is highly useful in predicting causative allergens in patients with WDEIA. Furthermore, the basophil activation test based on the expression of CD203c might help determine causative allergens for a wide variety of food allergies.

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.

Yuko Chinuki, MD[a]
Sakae Kaneko, MD, PhD[a]
Itaru Dekio, MD, PhD[a]
Hitoshi Takahashi, PhD[a]
Reiko Tokuda, MD, PhD[b]
Mizuho Nagao, MD, PhD[b]
Takao Fujisawa, MD, PhD[b]
Eishin Morita, MD, PhD[a]

From [a]the Department of Dermatology, Shimane University Faculty of Medicine, Izumo, Japan, and [b]the Institute for Clinical Research, Mie National Hospital, Mie, Japan. E-mail: ychinuki@med.shimane-u.ac.jp.

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High sensitivity of CAP-FEIA rVes v 5 and rVes v 1 for diagnosis of *Vespula* venom allergy

To the Editor:

Ves v 5 (antigen 5) is a 23-kDa protein from *Vespula* venom, and it is recognized as the most potent allergen in venoms of the *Vespidae* family.[1] There is a high sequence similarity of Ves v 5 within species of the same genus, such as yellow jacket, that is, *Vespula* (>95%); however, when it is compared with other genera such as *Dolichovespula* or *Polistes*, the sequence identity is much lower (about 60%).[2] Another potential *Vespula* allergen is a 37-kDa phospholipase A1, known as Ves v 1.[1] Neither Ves v 5 nor Ves v 1 is found in honeybee venom. Ves v 5 and Ves v 1 recombinant allergen components, both expressed in insect cells, became available in 2010 and 2011, respectively, for analyses on the ImmunoCAP solid-phase IgE assay (CAP-FEIA; Phadia, Uppsala, Sweden).

Very recently we demonstrated that the current CAP-FEIA recombinant major honeybee venom allergen rApi m 1 (i208) has a limited clinical usefulness for the detection of honeybee venom allergy because of its low diagnostic sensitivity, which

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REVIEW ARTICLE

Review Series: Advances in Consensus, Pathogenesis and Treatment of Urticaria and Angioedema

Wheat-Dependent Exercise-Induced Anaphylaxis Sensitized with Hydrolyzed Wheat Protein in Soap

Yuko Chinuki[1] and Eishin Morita[1]

ABSTRACT

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a specific form of wheat allergy typically induced by exercise after ingestion of wheat products. Wheat ω-5 gliadin is a major allergen associated with conventional WDEIA, and detection of serum immunoglobulin E (IgE) specific to recombinant ω-5 gliadin is a reliable method for its diagnosis. Recently, an increased incidence of a new subtype of WDEIA, which is likely to be sensitized via a percutaneous and/or rhinoconjunctival route to hydrolyzed wheat protein (HWP), has been observed. All of the patients with this new subtype had used the same brand of soap, which contained HWP. Approximately half of these patients developed contact allergy several months later and subsequently developed WDEIA. In each of these patients, contact allergy with soap exposure preceded food ingestion-induced reactions. Other patients directly developed generalized symptoms upon ingestion of wheat products. The predominant observed symptom of the new WDEIA subtype was angioedema of the eyelids; a number of patients developed anaphylaxis. This new subtype of WDEIA has little serum ω-5 gliadin-specific serum IgE.

KEY WORDS

angioedema, hydrolyzed wheat protein, percutaneous sensitization, wheat-dependent exercise-induced anaphylaxis, ω-5 gliadin

INTRODUCTION

Wheat protein derivatives are widely used in the composition of products worldwide. Industry uses gluten either with or without modifications. The main modification is hydrolysis, which is used to overcome its insolubility. Hydrolysis is performed either in acid conditions or increasingly with the use of enzymes. The procedure that is chosen and the degree of hydrolysis depend on the desired functionality and the manufacturer. Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a distinct form of wheat allergy induced by the combination of wheat ingestion and physical exercise.[1,2] Aspirin intake is another well-known trigger for allergic symptoms.[3] We identified wheat ω-5 gliadin, a component of water/salt-insoluble protein (gluten), as a major allergen in patients with WDEIA.[1,4-7] When recombinant ω-5 gliadin was used in a fluorescent enzyme immunoassay combined with the CAP system (CAP-FEIA; Phadia, Uppsala, Sweden; detection range, 0.35-100 kUA/L), approximately 80% of patients with WDEIA tested positive.

Recently, increased incidence of a new subtype of WDEIA caused by hydrolyzed wheat protein (HWP) has been observed.[8-10] Patients with this new subtype were likely to be sensitized to HWP primarily through percutaneous and/or rhino-conjunctival routes by using HWP (Glupearl 19S)-supplemented soap (Cha no shizuku). In Japan, this soap was very popular and more than 46 million soaps had been sold from March 2004 to September 2010. More than 1300 individuals who had used the soap developed allergic symptoms after ingesting natural wheat pro-

[1]Department of Dermatology, Shimane University Faculty of Medicine, Shimane, Japan.
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Correspondence: Yuko Chinuki, MD, Department of Dermatology, Shimane University Faculty of Medicine, 89–1 Enya-cho, Izumo, Shimane 693–8501, Japan.
Email: ychinuki@med.shimane-u.ac.jp
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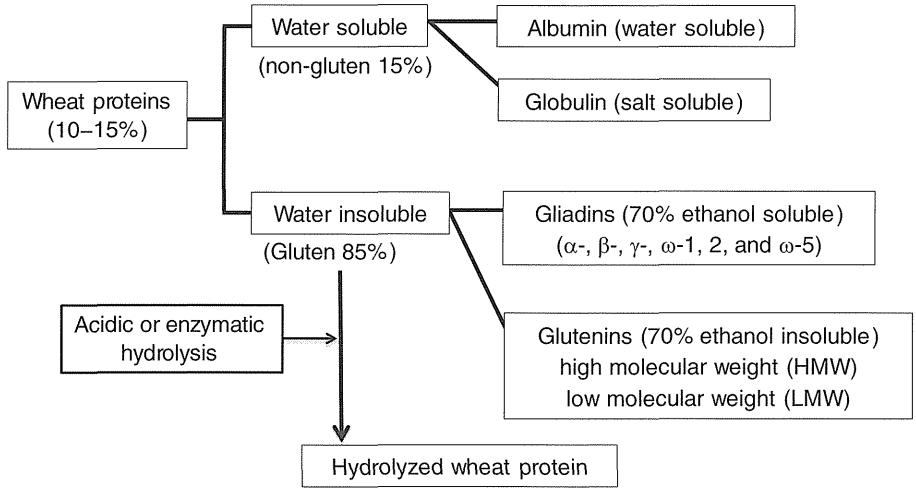

Fig. 1 Components of wheat protein.

teins to date.[11] Patients with this new WDEIA subtype tested positive for the HWP allergy by skin prick test (SPT) and had serum HWP-specific immunoglobulin E (IgE). In addition, the patients had characteristic features of facial angioedema distinct from the patients with conventional WDEIA (CO-WDEIA); they were designated as having WDEIA sensitized by HWP (HWP-WDEIA). We examined the sera of several patients with HWP-WDEIA by CAP-FEIA and found that these patients have no or low levels of ω-5 gliadin-specific IgE.

COMPONENTS OF WHEAT PROTEIN AND HWP[12]

Wheat flour contains 10-15% w/w protein. The wheat proteins are fractionated according to their solubility characteristics: albumins and globulins are soluble in salt solutions, while gluten proteins are precipitated by salt (Fig. 1). Among the gluten proteins, gliadins are soluble in 70% ethanol, while low and high molecular weight glutenin subunits are not. Wheat protein derivatives are used in a variety of products worldwide. HWPs are prepared either from insolubilized total flour proteins or more generally from gluten only. In order to increase the solubility of gluten, acidic or enzymatic hydrolysis is performed. Glupearl 19S, which was added to the soap (Cha no Shizuku), was produced by acid hydrolysis.

CASE REPORT[9]

We present our first patient who was given a diagnosis of HWP-WDEIA. The patient appeared to be sensitized to Glupearl 19S by using Glupearl 19S-supplemented soap.

A 49-year-old woman was referred to our clinic complaining of eyelid edema while working after ingesting bread. She had experienced similar episodes 3 times while working and 4 times while walking during the past 11 months. Her wheat, gluten, and ω-5 gliadin antigen-specific serum IgE levels were 1.35, 1.78, and >0.34 kUA/L (CAP-FEIA, Phadia), respectively, whereas SPTs with wheat and bread were negative (Torii allergen extracts for scratch, Torii Pharmaceutical Co., Ltd., Tokyo, Japan). WDEIA was diagnosed based on a positive challenge test with the combination of wheat (120 g) and aspirin (500 mg). Eyelid edema induced by the challenge test is shown in Figure 2. No symptoms were observed with either the wheat challenge or aspirin intake alone.

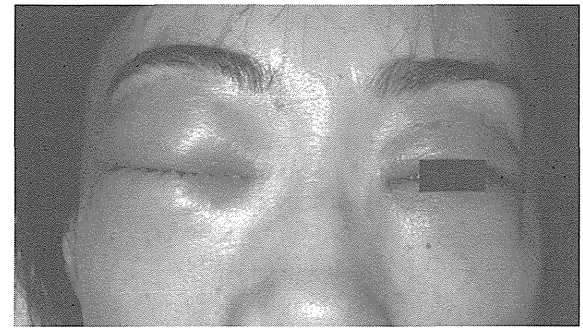

Fig. 2 Eyelid edema induced by challenge test with a combination of wheat and aspirin.

A precise medical history revealed that the patient had used Glupearl 19S-supplemented soap for 1 year, and she had noticed facial wheals and nasal discharge occasionally while washing her face with the soap. She was not atopic. She was confirmed to have no other disease by blood and physical examinations. SPT showed a positive reaction to 0.1% soap solution and 0.01% Glupearl 19S solution. A face wash challenge test with the soap induced facial wheals. Sensitization to HWP was also confirmed by Western blotting. Coomassie blue staining of Glupearl 19S using sodium dodecyl sulfate polyacrylamide gel electro-

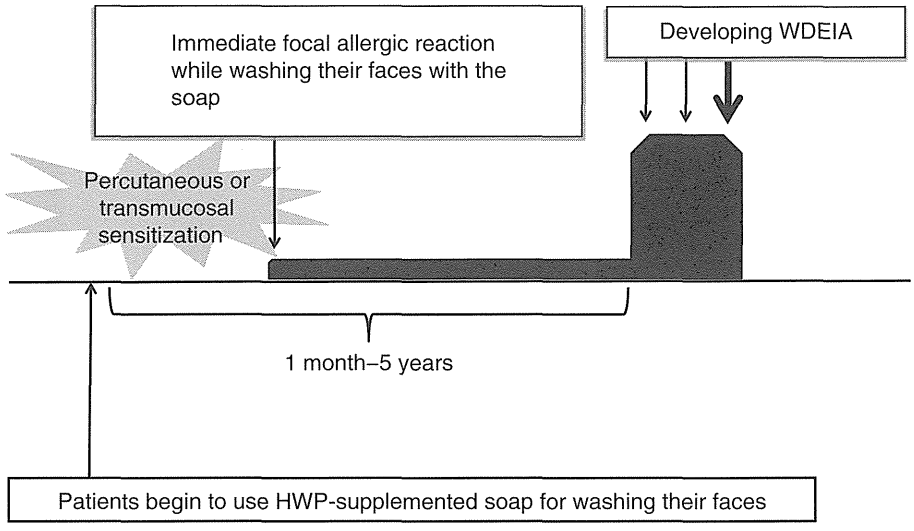

Fig. 3 Presumable time course of wheat-dependent exercise-induced anaphylaxis (WDEIA) patients sensitized to hydrolyzed wheat protein (HWP).

phoresis (SDS-PAGE) showed smears (ranging from 0-250 kDa) characteristic of random degradation and peptide rearrangement. The serum IgE of the patient reacted with the smear proteins ranging from 15-250 kDa, indicating that the IgE was specific to Glupearl 19S. In addition, the IgE of the patient reacted with both water-soluble and water-insoluble wheat proteins but not with ω-5 gliadin.

CLINICAL FEATURES OF THE PATIENTS WITH HWP-WDEIA

Almost all of the patients with HWP-WDEIA had used the same brand of soap (Cha no Shizuku), which included HWP (Glupearl 19S). Several patients developed contact urticaria on their faces several months later, and subsequently developed WDEIA. We summarized a presumable time course of patients with HWP-WDEIA (Fig. 3). After starting to use HWP-supplemented soap, patients were likely to be sensitized to HWP through the percutaneous and/or rhino-conjunctival route within 1 month to 5 years. Several patients had an immediate focal allergic reaction while washing their faces with the soap. Patients had systemic allergic symptoms when they were exposed to wheat products and subsequently exercised. In each of these patients, contact urticaria with soap preceded the food ingestion-induced reactions. The other patients directly developed generalized symptoms upon ingestion of wheat products. In some cases, allergies were triggered by mild exercise such as walking and bathing. The predominant observed symptom in all of the patients was angioedema of the eyelids; most of the patients developed anaphylaxis, and some of them developed anaphylactic shock. Several patients had pollinosis, and a few patients had atopic dermatitis.

IMMUNOLOGICAL STUDIES OF HWP-WDEIA PATIENTS

In our hospital, approximately half of the patients with HWP-WDEIA tested positive in the SPT to wheat and bread allergens. Almost all of the patients tested positive by SPT to 0.1% solution of soap supplemented with HWP in saline and 0.01% HWP solution diluted with saline. None of the patients with CO-WDEIA had positive reactions to diluted soap and HWP solution. None of the healthy subjects reacted to SPT with any of these allergens. Wheat protein-specific IgE was detected by CAP-FEIA. Wheat- and gluten-specific IgE were detected in almost all of the patients with HWP-WDEIA. A few patients with HWP-WDEIA had ω-5 gliadin-specific IgE. However, the level of IgE to ω-5 gliadin was significantly lower in patients with HWP-WDEIA than in those with CO-WDEIA. Challenge tests including exercise, wheat ingestion, aspirin intake, and/or a combination of these tests were performed for some patients.[13] In almost all of the patients, a combination of aspirin/exercise and wheat challenge induced allergic symptoms such as angioedema, urticaria, and dyspnea.

SDS-PAGE AND WESTERN BLOTTING[9,10]

To detect HWP-specific IgE in HWP-WDEIA patients, we performed SDS-PAGE and Western blotting. Coomassie blue staining of Glupearl 19S electrophoresed using SDS-PAGE showed smears characteristic of random degradation and rearrangement of peptides (Fig. 4). These smears spanned most of the gel, ranging from 0-250 kDa. In contrast, SDS-PAGE of wheat proteins showed characteristic bands mainly existing in the area of 25-100 kDa.

Representative blots are shown in Figure 4. The

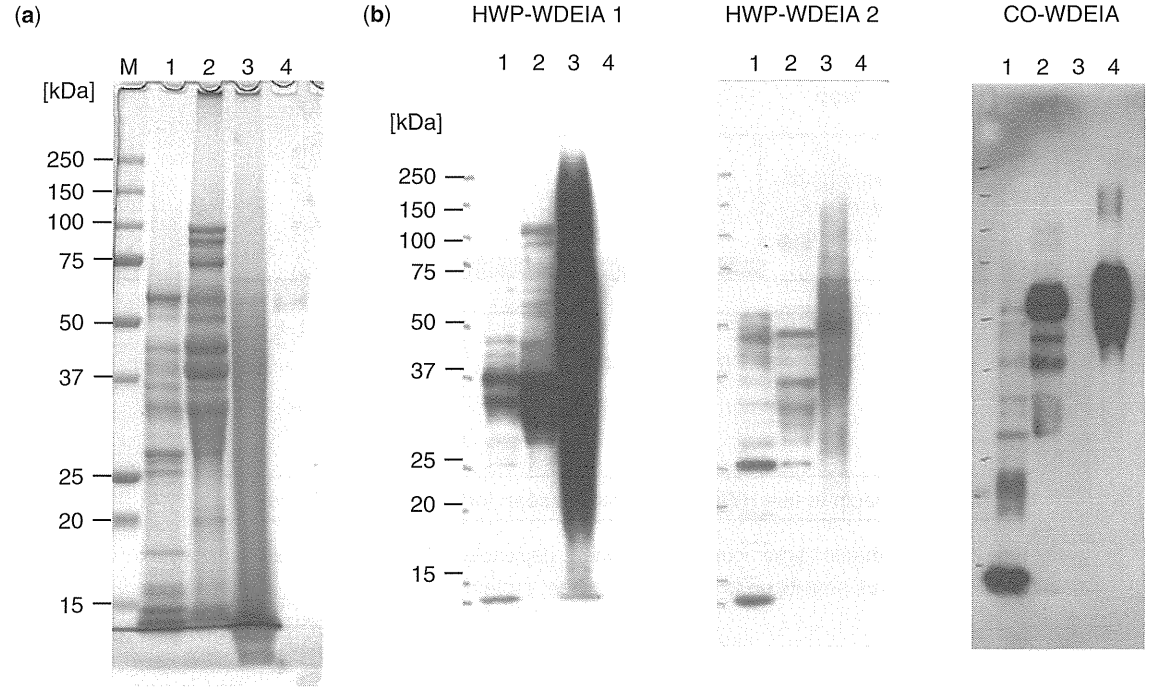

Fig. 4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analyses of wheat protein fractions and hydrolyzed wheat protein (HWP [Glupearl 19S]). (**a**) Gel stained with Coomassie Brilliant Blue; (**b**) Immunoglobulin E (IgE) immunoblotting with the patients' sera (2 HWP-wheat-dependent exercise-induced anaphylaxis [WDEIA] patients and 1 conventional [CO]-WDEIA-patient). M, marker proteins; Lane 1, salt-soluble wheat proteins; Lane 2, salt-insoluble wheat proteins; Lane 3, Glupearl 19S; Lane 4, purified ω-5 gliadin.

IgE of all of the HWP-WDEIA patients reacted to Glupearl 19S with a smear pattern. The intensity of the reaction varied among the patients. The IgE of all of the patients also reacted with both water-soluble and water-insoluble wheat proteins, whereas they did not react with ω-5 gliadin. The IgE of the CO-WDEIA patients did not react with HWP, but reacted with water-soluble and water-insoluble wheat proteins, and ω-5 gliadin.

To perform immunoblotting inhibition assays for determining cross-reactivity of wheat proteins and Glupearl 19S, 100 μL of serum was previously incubated with Glupearl 19S serially diluted from 100 to 5 μg/mL. The reaction of the IgE to water-soluble and water-insoluble wheat proteins was inhibited by Glupearl 19S in a dose-dependent manner when the sera of HWP-WDEIA patients were preincubated with a series of Glupearl 19S concentrations (Fig. 5).

INDUCTION OF BASOPHILIC CD203c EXPRESSION BY GLUPEARL 19S AND ω-5 GLIADIN

Flow cytometry-based tests of basophil activation status have been used to diagnose or confirm sensitization in allergic patients.[14-25] CD203c is an ectoenzyme that belongs to a family of ectonucleotide pyrophosphatases and phosphodiesterases. It is expressed on the cell membrane of human peripheral basophils and mast cells, and cross-linking of the high-affinity IgE receptor upregulates membrane CD203c expression.

Glupearl 19S enhanced CD203c expression of basophils in a concentration-dependent manner in all of the HWP-WDEIA patients.[10] In these patients, no significant enhancement of CD203c was observed with purified ω-5 gliadin. In contrast, purified ω-5 gliadin induces enhancement of CD203c expression in a concentration-dependent manner in CO-WDEIA patients, whereas no significant enhancement of CD203c is observed in the presence of Glupearl 19S. A representative reaction is shown in Figure 6.

INFORMATION FROM THE JAPANESE SOCIETY OF ALLERGOLOGY[11]

Information concerning the wheat allergy associated with the use of soap bars containing Glupearl 19S provided in the Rheumatism & Allergy Information Center website includes "FAQs (for general consumers)," the "Definition of the disorder and diagnostic guidelines (for healthcare professionals)," and a "List of institutions offering treatment for wheat allergy associated with the use of 'Cha no Shizuku' soap." The "Diagnostic criteria for immediate wheat allergy to the hydrolyzed wheat (Glupearl 19S) contained in

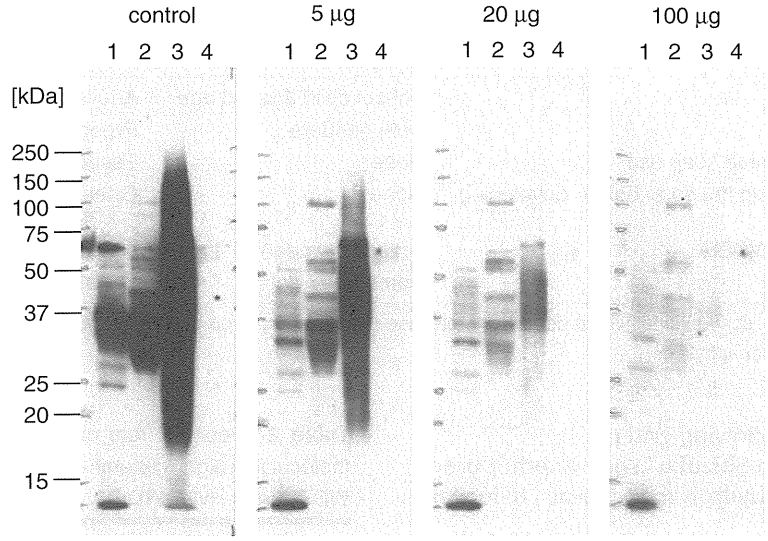

Fig. 5 Immunoblot inhibition assay for determining cross-reactivity of wheat proteins and Glupearl 19S. Lane 1, salt-soluble wheat proteins; Lane 2, salt-insoluble wheat proteins; Lane 3, Glupearl 19S; Lane 4, purified ω-5 gliadin. Electrophoresed membranes were blotted against patient sera (hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis [HWP-WDEIA]) without Glupearl 19S (control) or with increasing amounts of Glupearl 19S (5 μg, 20 μg, and 100 μg).

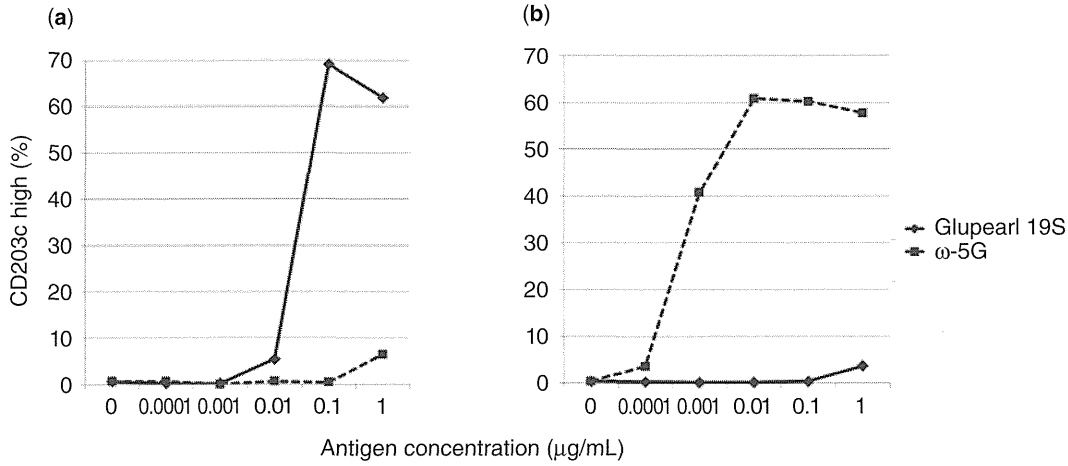

Fig. 6 Expression of CD203c on basophils induced by Glupearl 19S and ω-5 gliadin (ω-5G). (**a**) Results from a 44-year-old woman with hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis (HWP-WDEIA). Serum allergen-specific IgE to wheat, gluten, and ω-5G was 9.26 kUA/L, 15.8 kUA/L, and 1.16 kUA/L, respectively. (**b**) Results from a 39-year-old woman with conventional wheat-dependent exercise-induced anaphylaxis (CO-WDEIA). Serum allergen-specific IgE to wheat, gluten, and ω-5G was <0.34 kUA/L, 1.90 kUA/L, and 3.82 kUA/L, respectively.

'Cha no Shizuku' soap and some other products" have been published by the Special Committee for the Safety of Protein Hydrolysate in Cosmetics of the Japanese Society of Allergology (see below).

Diagnostic criteria for immediate wheat allergy to the hydrolyzed wheat (Glupearl 19S) contained in "Cha no Shizuku" soap and some other products (Prepared by the Special Committee for the Safety of Protein Hydrolysate in Cosmetics on October 11, 2011)

[Definitive diagnosis]