

Letter to the Editor

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 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 μ g/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 (AllergenCity 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 al⁹ 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 and proline, IgE produced against HWPs probably cross-reacts with natural wheat proteins. In fact, preincubation of sera with

TABLE I. Clinical features and results of immunologic studies

Patient no.	Age (y/sex)	Food	Triggers	Symptoms	SPT				Specific IgE (kU _A /L)				CT
					Wheat	Bread	Soap 0.1%	HWP-A 0.1%	Wheat	Gluten	ω-5	Gliadin	
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.

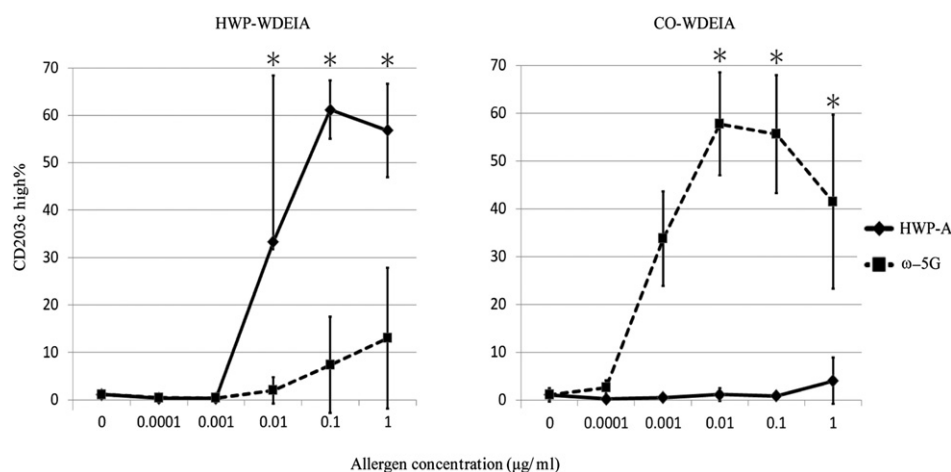


FIG 1. Expression of CD203c on basophils induced by HWP-A and ω -5 gliadin (ω -5G). Mean levels of CD203c expression in 5 patients with HWP-WDEIA and 5 patients with CO-WDEIA are presented. Data are expressed as means $\pm$ SEMs. * P < .01 when comparing HWP-A and ω -5 gliadin, as determined by using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

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.

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