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Antigen-Induced Expression of CD203c on Basophils Predicts IgE-mediated Wheat Allergy

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ABSTRACT

Background: For *in vitro* diagnosis of wheat allergy, specific IgE to wheat is known to be a poor predictive marker. Oral food challenge, the gold standard for the diagnosis, is accompanied by a risk of severe induced reactions. Reliable *in vitro* tests are needed to be developed for safe indication for oral challenge.

Objective: We examined the utility of a basophil activation marker, CD203c, for the diagnosis of IgE-mediated wheat allergy.

Methods: Fifty-eight children with suspected wheat allergy with positive CAP-FEIA to wheat were enrolled. On 70 occasions, the clinical distinction between patients with wheat allergy (WA) and patients tolerant to wheat (TW) was made by means of an oral food challenge test or recent history of immediate allergic reactions or tolerance after ingestion of wheat. Twelve replicate evaluations were performed in 9 patients over more than a 6-month interval. Thirty two patients on 43 occasions were diagnosed with WA and 27 were confirmed to be TW. One patient had both diagnoses 18 months apart. Peripheral blood was incubated with fractionated wheat extracts, purified native omega-5 gliadin (nOG5) and recombinant omega-5 gliadin (rOG5). Expression of CD203c on basophils was then analyzed by flow cytometry using a commercial kit.

Results: All wheat proteins induced concentration-dependent enhancement of CD203c expression in WA, but did not in TW. The analysis of receiver operating characteristics (ROC) showed that nOG5-induced CD203c^{high}% values provided the best power for discriminating between WA and TW, with a sensitivity of 85.0% and specificity of 77.0% at the cut-off level of 14.4%. AUC for CD203c with nOG5 were significantly higher than that for conventional CAP-FEIA, 0.89 and 0.73, respectively ($p < 0.01$).

Conclusions: Measurement of nOG-induced enhancement of CD203c on basophils is useful for the diagnosis of immediate wheat allergy in children.

KEY WORDS

basophil activation test, CD203c, omega-5 gliadin, wheat allergy

INTRODUCTION

Food allergy affects 5–10% of children under 6 years of age and 1–2% in the older population in Japan¹ and the prevalence appears to be increasing. Wheat is the third common allergen in those under 20 years of age, accounting for about 10% of all food-induced immediate reactions in Japan.¹ Wheat causes a variety

of IgE-mediated symptoms including baker's asthma by inhalation of wheat flour, allergic reactions including skin, respiratory, gastrointestinal symptoms, and shock by ingestion of wheat containing food,² and wheat-dependent, exercise-induced anaphylaxis (WDEIA).³ Immediate hypersensitivity reactions following ingestion of wheat is common in young children and WDEIA has been increasingly recognized

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Table 1 Demographic data of the subjects

	Number of patients	Number of diagnostic evaluations	Gender (F/M)	Age in months (Mean \pm SD)	Total IgE (Geometric mean; 95%CI)
WA	32*	43	15/28	33.4 \pm 26.2	414; 266 – 643
TA	27*	27	20/7	30.8 \pm 20.5	738; 389 – 1398

WA, wheat allergy; TA, tolerant to wheat; CI, confidence interval.

*One patient was evaluated twice. He was WA at the first evaluation and TW at the second evaluation.

in all ages.⁴

Diagnosis of wheat allergy is, however, not simple. Oral food challenge tests are the gold standard for the diagnosis but are accompanied by a risk of severe induced reactions and are impractical in busy practice settings. A reliable *in vitro* test to predict the diagnosis is thus necessary. Although the usefulness of measurements of serum specific IgE has been demonstrated for the diagnosis of egg, cow's milk, and peanut allergy, measurement of specific IgE to wheat has been shown to be much less reliable.^{5,6} A possible reason for poor predictability of wheat-specific IgE is that water-soluble wheat extracts used for the specific IgE assays may not contain major allergenic epitopes in wheat protein.

There are number of IgE-binding epitopes in wheat protein. According to the method for extraction, it is composed of water/salt-soluble proteins and water/salt-insoluble proteins. The former includes albumins and globulins such as α -amylase inhibitors, peroxidase, and serpin,^{7,8} and have been considered to be major allergens in baker's asthma. The latter includes α -gliadin, ω -5 gliadin (or fast ω -gliadin), and low molecular weight glutenin and are reported to cause wheat allergy in children and WDEIA.⁹⁻¹² Recently, ω -5 gliadin-specific IgE measurement has been demonstrated to be highly predictive for the diagnosis of WDEIA.¹³ In young children with wheat allergy, IgE-binding to both salt-soluble and salt-insoluble fractions by immunoblot has also been reported.¹⁴ These observations indicate that a variety of wheat proteins are allergenic and major epitopes responsible for each type of wheat allergy are still to be determined.

Flow cytometry-based tests for basophil activation status have been described to diagnose or to confirm sensitization in allergic patients.¹⁵ CD63 can discriminate resting and allergen-activated basophils.¹⁶ Recent reports described an ectoenzyme CD203c as a more suitable basophil marker that is not only constitutively expressed on resting basophils but also up-regulated at high levels on activated basophils.¹⁷ A commercial kit for quantification of CD203c expression on basophils, Allergenicity Kit[®] (Beckmann Coulter, Fullerton, CA, USA), identifies basophils as CD3-negative and CRTH2-positive fractions from whole blood samples and measures fluorescent intensity of CD203c that is enhanced by cross-linking of surface-bound IgE molecules.

In order to establish a predictive *in vitro* test for wheat allergy in children, we measured basophil CD203c expression induced by various wheat protein fractions and evaluated the diagnostic efficiency of the reactions in patients whose allergic status to wheat had been defined by food challenge or a convincing history and compared them with that of conventional wheat-specific IgE measurement.

METHODS

SUBJECTS

Fifty-eight children who were suspected to have wheat allergy as measured by a positive wheat specific IgE antibody (CAP-FEIA, Phadia, Tokyo, Japan) were enrolled in the study. A diagnosis of wheat allergy was based on an open food challenge or a convincing history of wheat-induced immediate reactions in the previous month. Tolerance to wheat was diagnosed as a negative food challenge after ingestion of more than 50 g of udon (1.3 g of wheat protein), which is comparable to a half serving of it for toddlers, or negative symptoms with daily ingestion of wheat as a staple food. Although double-blind placebo-controlled food challenge (DBPCFC) is the gold standard for diagnosing food allergy, most of the subjects were infants or toddlers and unlikely to be influenced by suggestions. Patients who had only subjective symptoms were re-examined with single-blind placebo-controlled food challenge. Since immediate wheat allergy is often outgrown by the age of 6, serial evaluations were made in 12 patients within at least 6-month intervals and a total of 70 evaluations were performed.

Thirty-two patients were diagnosed with WA on 43 evaluations and 27 patients were diagnosed to be TW. Induced symptoms in WA occurred within 3 hours after challenge. One patient was WA on the first evaluation and became TW at the second evaluation 18 months later. Demographic data of the subjects are described in Table 1. Blood sampling for CD203c tests and IgE measurements was performed at each evaluation.

We excluded patients with negative CAP-FEIA to wheat by design since the definition of "suspected" wheat allergy can be ambiguous without objective data for IgE. However, to further test the diagnostic ability of the CD203c test, an additional 8 patients with negative wheat IgE (6 boys and 2 girls, mean

age of 35 months) who performed wheat challenge test were analyzed separately.

This study was performed with the approval of the ethics committee of the Mie National Hospital. Informed consent was obtained from the guardians of the subjects.

EXTRACTION OF WHEAT PROTEINS

Four grams of wheat flour were ultrasonicated in 40 mL of PBS on ice for 15 minutes and stirred at 4°C overnight, then the supernatant was obtained after centrifugation at 20,000 g for 15 minutes (water/salt-soluble fraction; PBS fraction). After washing with PBS three times, the sediment were then dissolved in 40 mL of 70% ethanol (EtOH) with ultrasonication on ice for 15 minutes. The supernatant was obtained after centrifugation at 20,000 g for 15 minutes (water/salt insoluble, EtOH fraction). After washing with 70% EtOH three times, the sediment was further dissolved in 40 mL of 2%Na₂CO₃/0.1N NaOH and the supernatant was obtained as an alkaline soluble fraction (alkali fraction). The fractions were dialyzed in PBS overnight and protein concentrations were determined with a densitometry, then diluted to 100 µg/ml in PBS and stored at -20°C until use.

Native wheat ω-5 gliadin (native ω-5 gliadin; nOG5) and recombinant of ω-5 gliadin were prepared as described previously.¹⁸⁻²⁰

MEASUREMENT OF CD203c EXPRESSION ON BASOPHILS

A commercial kit (Allergenicity Kit, Beckman Coulter) was used for quantification of basophil CD203c expression as described previously.²¹ Briefly, EDTA-containing whole blood was incubated with various concentrations of the five kinds of wheat proteins at concentrations indicated for 15 minutes after addition of sufficient amounts of calcium solution to override the chelating capacity of EDTA. Anti-IgE antibody at 4 µg/ml as a positive control and PBS as a negative control were also used. PC7-conjugated anti-CD3, FITC-conjugated anti-CRTH2, and PE-conjugated anti-CD203c antibodies were also added during the reaction. The samples were analyzed on a FC500 flow cytometer (Beckman Coulter). Basophils were detected on the basis of forward side scatter characteristics and expression of negative CD3 and positive CRTH2. Up-regulation of CD203c on basophils was determined using a threshold that was defined by the fluorescence of unstimulated cells (negative control) and expressed as CD203c^{high%}. At least 500 basophils were analyzed at each assay.

WHEAT-SPECIFIC IgE

Serum levels of wheat-specific IgE were measured with a CAP-FEIA system (Phadia, Uppsala, Sweden).

STATISTICAL ANALYSIS

Differences were analyzed with the Mann-Whitney U test for unpaired samples. For multiple comparisons, two-way ANOVA followed by Dunnett's multiple comparison test was employed. The discriminative usefulness of CD203c^{high%} and CAP-FEIA was evaluated by constructing ROC curves²² where sensitivity versus 1-specificity was plotted for each possible cutoff level. For this analysis, WA was labeled as diseased compared with TW and the area under the curves (AUCs) were determined. From each ROC curve we determined the ideal cut-off levels which correspond to the closest point to the top left-hand corner and which most efficiently discriminates between the presence or absence of disease. The respective sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) were calculated. Comparison of two ROC curves was performed utilizing a method reported by Hanley *et al.*²³

RESULTS

INDUCTION OF CD203c EXPRESSION ON BASOPHILS WITH VARIOUS WHEAT PROTEINS

We first examined induced expression of CD203c on basophils with the wheat extracts and native and recombinant ω-5 gliadin at various concentrations (Fig. 1) in representative subjects of WA and TW ($n = 5$). All the fractions significantly induced the enhancement of CD203c expression in a concentration-dependent manner in WA subjects, in contrast, no significant enhancement of CD203c was observed in TA subjects. Based on the results, protein concentrations at 10 µg/ml for PBS fraction, EtOH fraction, alkali fraction, and rOG5, and 1 µg/ml for nOG5, were employed for evaluation of diagnostic usefulness. In addition, nOG5 appeared to give the highest signal to noise ratio regarding discrimination of WA and TA and we focused on the nOG5-induced reactions for further analysis.

SERUM TOTAL IgE, SPECIFIC IgE TO WHEAT, AND CD203c INDUCED BY nOG5

There was no difference in serum total IgE levels between WA and TW (Fig. 2a). Wheat-specific IgE levels by CAP-FEIA in WA were significantly higher than those in TW ($p = 0.002$) (Fig. 2b). CD203c^{high%} induced by nOG5 at 1 µg/ml in WA were again significantly higher than those in TW ($p < 0.0001$) (Fig. 2c).

DIAGNOSTIC ABILITY OF WHEAT PROTEIN-INDUCED CD203c^{HIGH%} AND SPECIFIC IgE TO WHEAT

To evaluate the diagnostic ability of the different *in vitro* tests for the diagnosis of wheat allergy, the ROC analyses for each test were performed. The AUC for nOG5-induced CD203c^{high%} test gave the highest value, 0.89, compared with the other tests (Table 2)

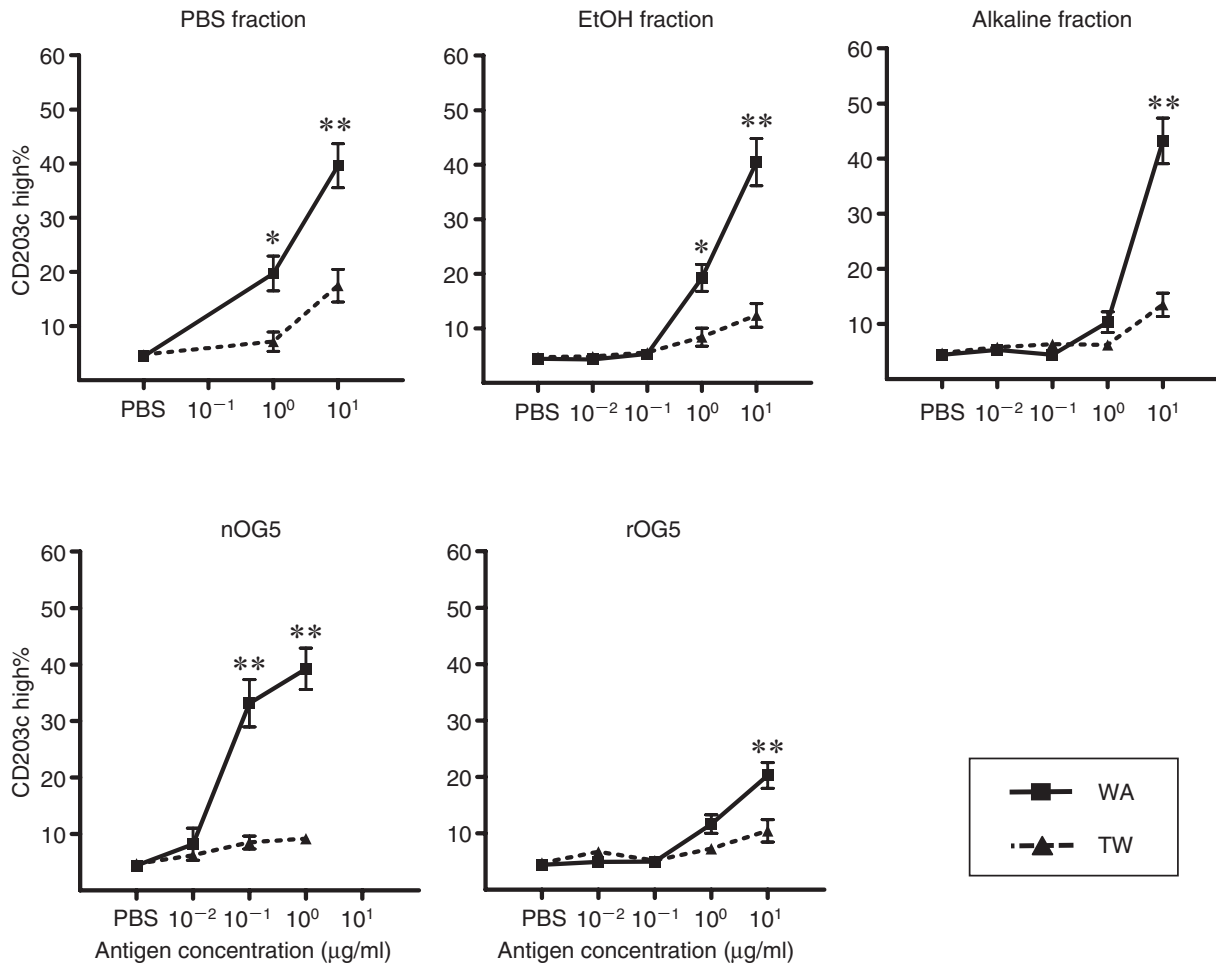


Fig. 1 Expression of CD203c on basophils induced by various wheat protein extracts and gliadins. WA, subjects with definite wheat allergy ($n = 5$); TW, subjects tolerant to wheat ($n = 5$). Data are expressed as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, Dunn's multiple comparison test.

and was significantly higher than wheat-CAP-FEIA ($p < 0.001$) (Fig. 3). With the cut-off values obtained from the ROC analyses, sensitivity, specificity, PPV, and NPV were calculated. The nOG5 CD203c test showed the highest sensitivity and specificity among the tests, 85.0% and 77.2% respectively. Conventional wheat-CAP-FEIA gave comparable sensitivity and PPV, but lower specificity and NPV.

To further confirm the utility of the CD203c test, 8 patients with negative wheat-CAP-FEIA were also analyzed. By the food challenge test, 4 patients were confirmed to be WA and nOG5-induced CD203c^{high%} in 3 of them were above the cut-off level (positive). Although one patient with WA showed a negative nOG5 CD203c, significant enhancement of CD203c expression was observed with the alkaline fraction. All 3 patients with TA were negative in nOG-induced upregulation of CD203c. One patient was tolerant to udon but showed immediate symptoms after ingestion of bread. CD203c upregulation in the patient was negative with nOG5 but positive with PBS and alkaline

fractions.

DISCUSSION

In the present study, we demonstrated that wheat protein-induced basophil activation test quantifying CD203c expression had efficient diagnostic ability for immediate wheat allergy in children. Since patients with wheat allergy may respond to a variety of epitopes contained in wheat, we tested different kinds of wheat extracts and purified ω -5 gliadins (native and recombinant forms) for the CD203c test and found that native ω -5 gliadin (nOG5) gave the best sensitivity and specificity for the diagnosis, 85.0% and 77.2%, respectively. The AUC received from ROC analysis for the nOG5 CD203c test was significantly higher than that for the conventional wheat-specific IgE test.

There are numerous wheat allergens which cause immediate reactions including skin, respiratory, and systemic symptoms after wheat ingestion. A study using a number of purified protein fractions in immunoblotting and RAST demonstrated that water/

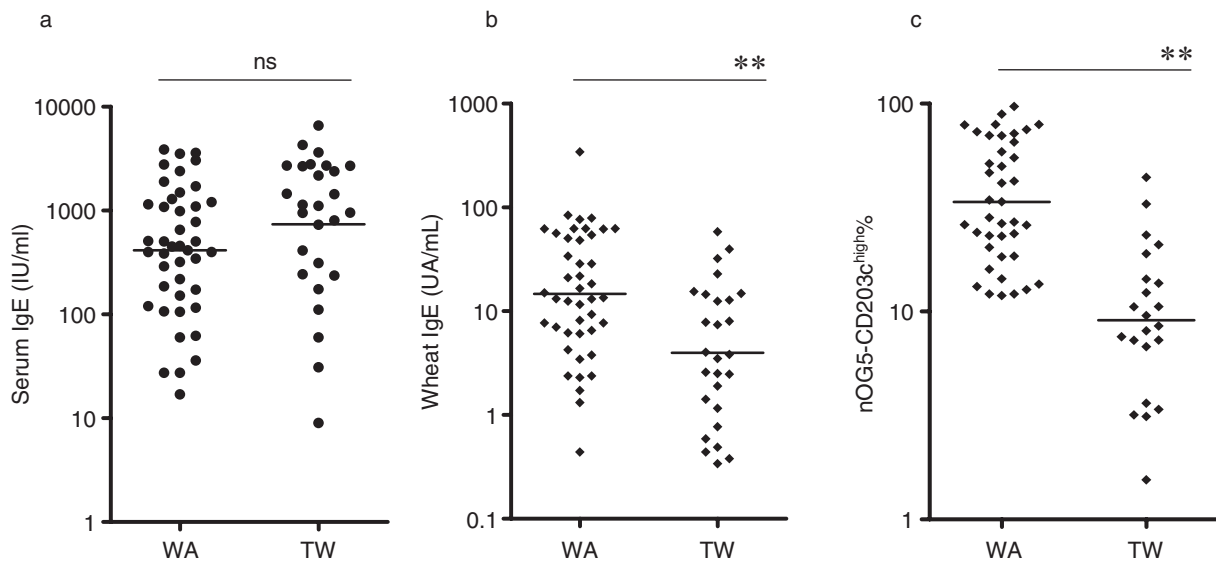


Fig. 2 Total IgE (a) , wheat-specific IgE (b) , and nOG5-induced CD203c expression on basophils (c) in subjects with wheat allergy (WA) and tolerance to wheat (TW) . Concentration of nOG5 used was 1 µg/ml. ***P* < 0.01, Mann-Whitney U test.

Table 2 Diagnostic abilities of *in vitro* tests for wheat allergy

	IgE (CAP-FEIA)		CD203c ^{high%}			
	Wheat	PBS Fraction	EtOH fraction	Alkaline fraction	nOG5	rOG5
AUC	0.73	0.77	0.81	0.84	0.89	0.74
<i>P</i> value	0.002	0.0002	< 0.0001	< 0.0001	< 0.0001	0.0017
Cut off	4.1 UA/mL	11.1%	8.1%	11.7%	14.4%	7.9%
Sensitivity	81.4	86.1	83.3	83.7	85.0	82.5
Specificity	55.6	57.7	69.2	66.7	77.2	63.4
PPV	74.5	77.1	81.4	80.0	86.8	80.5
NPV	65.2	71.4	69.2	72.0	70.8	66.7

AUC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value.

salt insoluble gliadins and glutenins as well as water/salt soluble albumins and globulins were IgE-binding allergens in patients with wheat allergy.²⁴ Another study employing confirmed wheat-allergic patients with DBPCFCs in Italy stated that lipid transfer protein in the albumin/globulin fraction and low molecular glutenins were major allergens in these patients.²⁵ Recently, IgE antibodies to ω-5 gliadin have been reported to be present in sera from wheat-induced anaphylaxis²⁶ and WDEIA¹³. These findings are in agreement with our present study showing that ω-5 gliadin-induced basophil activation most efficiently predicted wheat allergy in young children. In addition, the fact that water/salt soluble and alkali soluble fractions, which contain allergen proteins other than gliadins, also induced significant basophil activation indicates that many epitopes are involved in immediate wheat allergy.

Although we found that the nOG5 CD203c test

showed high sensitivity and specificity, the rOG5 CD203c test had lesser discriminating power for wheat allergy with AUCs of 0.89 and 0.74, respectively. In addition, a higher concentration of the recombinant form of the protein in inducing CD203c expression were needed, compared to the native form (Fig. 1), indicating lesser IgE binding ability of rOG5 in this group of patients. A possible explanation for the discordance may be the difference in allergenic epitopes contained in the proteins. Several IgE-binding epitopes in WDEIA have been identified and sequenced.⁹ The recombinant protein used in the present study was the C-terminal half of the omega-5 gliadin protein²⁰ and was confirmed to contain the identified 11 IgE epitope sequences related to WDEIA. However, it may not contain some of the major allergenic epitopes in wheat allergy in young children. There is a possibility that distinct epitopes are involved in WDEIA and immediate wheat allergy. In

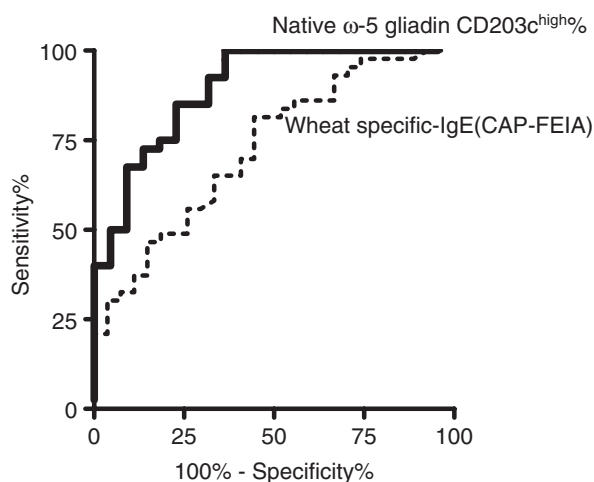


Fig. 3 ROC curves for native ω -5 gliadin-induced CD203c expression test and wheat specific IgE test. Horizontal bars indicate geometric means.

fact, we recently observed profound CD203c expression by rOG5 and less expression by nOG5 in young adults with WDEIA (data not shown), which is the opposite reaction profile we often observed in children with immediate allergy to wheat. Both immediate wheat allergy and WDEIA are caused by IgE antibodies to wheat proteins, however, there are a number of differences between the two ailments in terms of age,⁴ symptoms, and pathogenic mechanisms.³ These findings warrant further studies to identify major epitopes for these similar but distinct diseases.

There are limitations to the present study, which are noteworthy. We did not compare diagnostic abilities of the CD203c test and specific IgE quantification by using identical antigens. Although not presented in the results, we measured CAP-FEIA titer to rOG in a limited number of the subjects. The AUC received from ROC analysis for rOG-specific IgE was 0.78, which was comparable to the value in the corresponding CD203c test. For nOG-specific IgE, we could not perform an ELISA since a large amount of antigens, about 100-fold than those for the basophil activation test, are necessary to establish a regular ELISA and we did not have an ample amount of the antigen to assay a large number of samples. An additional study to solve the problem may be necessary. The fact that the basophil activation test needs only a small amount of antigens, however, can be an advantage of the test since it is possible to utilize the test for rare antigens.

The CD203c assay system employs whole blood during incubation with wheat allergens, which allows not only surface-bound IgE on basophils but possibly serum factors or other circulating cells to affect activation status of basophils. We recently found that induced expression of CD203c by Japanese cedar pollen (JCP) extract significantly decreased after rush immunotherapy (RIT) in patients with JCP pollinosis

without decrease in specific IgE levels to JCP.²¹ In passive sensitization experiments, the patients' sera obtained both before and after RIT showed essentially similar sensitizing capacity for basophils. In contrast, basophil degranulation in response to the pollen extract was effectively suppressed by addition of post-RIT serum samples, indicating the presence of blocking antibody in the serum²⁷ and that the CD203c test reflected not only the presence of IgE antibodies but serum factors such as blocking IgG antibodies. Even though IgE is the key molecule in food allergy, it is not a single factor to determine severity of the disease or development of tolerance. In this sense, the CD203c test may have an advantage over specific IgE measurements since it may possibly detect factors modifying the food allergy.

In conclusion, the findings in this study suggest that measurement of basophil CD203c expression by ω -5 gliadin is highly useful in predicting a positive food challenge in children who are suspected of wheat allergy with positive CAP-FEIA to wheat. This test may help to determine a safe indication for the challenge.

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REFERENCES

1. Mukoyama T, Nishima S, Arita M *et al*. Guidelines for diagnosis and management of pediatric food allergy in Japan. *Allergol Int* 2007;**56**:349-61.
2. Pourpak Z, Mansouri M, Mesdaghi M, Kazemnejad A, Farhoudi A. Wheat allergy: Clinical and laboratory findings. *Int Arch Allergy Immunol* 2004;**133**:168-73.
3. Palosuo K. Update on wheat hypersensitivity. *Curr Opin Allergy Clin Immunol* 2003;**3**:205-9.
4. Fujii H, Kambe N, Fujisawa A, Kohno K, Morita E, Miyachi Y. Food-dependent exercise-induced anaphylaxis induced by low dose aspirin therapy. *Allergol Int* 2008;**57**: 97-8.
5. Sampson HA. Utility of food-specific ige concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;**107**:891-6.
6. Komata T, Soderstrom L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum ige concentrations to challenge outcomes for egg and milk varies by patient age. *J Allergy Clin Immunol* 2007;**119**:1272-4.
7. Amano M, Ogawa H, Kojima K *et al*. Identification of the major allergens in wheat flour responsible for baker's asthma. *Biochem J* 1998;**330** (Pt 3):1229-34.
8. Sander I, Flagge A, Merget R, Halder TM, Meyer HE, Baur X. Identification of wheat flour allergens by means of 2-dimensional immunoblotting. *J Allergy Clin Immunol*

- 2001;**107**:907-13.
9. Matsuo H, Morita E, Tatham AS *et al.* Identification of the ige-binding epitope in omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Biol Chem* 2004;**279**:12135-40.
 10. Sandiford CP, Tatham AS, Fido R *et al.* Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. *Clin Exp Allergy* 1997;**27**:1120-9.
 11. Tanabe S, Arai S, Yanagihara Y, Mita H, Takahashi K, Watanabe M. A major wheat allergen has a gln-gln-gln-pro-motif identified as an ige-binding epitope. *Biochem Biophys Res Commun* 1996;**219**:290-3.
 12. Maruyama N, Ichise K, Katsube T *et al.* Identification of major wheat allergens by means of the escherichia coli expression system. *Eur J Biochem* 1998;**255**:739-45.
 13. Matsuo H, Dahlstrom J, Tanaka A *et al.* Sensitivity and specificity of recombinant omega-5 gliadin-specific ige measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergy* 2008;**63**:233-6.
 14. Takizawa T, Arakawa H, Tokuyama K, Morikawa A. Identification of allergen fractions of wheat flour responsible for anaphylactic reactions to wheat products in infants and young children. *Int Arch Allergy Immunol* 2001;**125**:51-6.
 15. de Weck AL, Sanz ML, Gamboa PM *et al.* Diagnostic tests based on human basophils: More potentials and perspectives than pitfalls. *Int Arch Allergy Immunol* 2008;**146**:177-89.
 16. Sturm GJ, Bohm E, Trummer M, Weiglhofer I, Heine-mann A, Aberer W. The cd63 basophil activation test in hymenoptera venom allergy: A prospective study. *Allergy* 2004;**59**:1110-7.
 17. Buhning HJ, Streble A, Valent P. The basophil-specific ectoenzyme e-npp3 (cd203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol* 2004;**133**:317-29.
 18. Rumbo M, Chirido FG, Giorgieri SA, Fossati CA, Anon MC. Preparative fractionation of gliadins by electrophoresis at ph 3.1 (a-page). *J Agric Food Chem* 1999;**47**:3243-7.
 19. Matsuo H, Kohno K, Niihara H, Morita E. Specific ige de-termination to epitope peptides of omega-5 gliadin and high molecular weight glutenin subunit is a useful tool for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Immunol* 2005;**175**:8116-22.
 20. Matsuo H, Kohno K, Morita E. Molecular cloning, recombinant expression and ige-binding epitope of omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *FEBS J* 2005;**272**:4431-8.
 21. Nagao M, Hiraguchi Y, Hosoki K *et al.* Allergen-induced basophil cd203c expression as a biomarker for rush immunotherapy in patients with Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2008;**146** (Suppl 1):47-53.
 22. Zweig MH, Campbell G. Receiver-operating characteristic (roc) plots: A fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;**39**:561-77.
 23. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983;**148**:839-43.
 24. Battais F, Pineau F, Popineau Y *et al.* Food allergy to wheat: Identification of immunoglobulin e and immunoglobulin g-binding proteins with sequential extracts and purified proteins from wheat flour. *Clin Exp Allergy* 2003;**33**:962-70.
 25. Pastorello EA, Farioli L, Conti A *et al.* Wheat ige-mediated food allergy in european patients: Alpha-amylase inhibitors, lipid transfer proteins and low-molecular-weight glutenins. Allergenic molecules recognized by double-blind, placebo-controlled food challenge. *Int Arch Allergy Immunol* 2007;**144**:10-22.
 26. Daengsuwan T, Palosuo K, Phankingthongkum S *et al.* Ige antibodies to omega-5 gliadin in children with wheat-induced anaphylaxis. *Allergy* 2005;**60**:506-9.
 27. Kawakami A, Koketsu R, Suzukawa M *et al.* Blocking antibody is generated in allergic rhinitis patients during specific immunotherapy using standardized Japanese cedar pollen extract. *Int Arch Allergy Immunol* 2008;**146** (Suppl 1):54-60.