Allergology International. 2012;61:431-437
DOI: 10.2332/allergolint.11-OA-0407

ORIGINAL ARTICLE

JSA Best Presentation Award 2010

Evaluation of the Luciferase Assay-Based *In Vitro* Elicitation Test for Serum IgE

Ryosuke Nakamura[1], Ayano Ishiwatari[1,2], Masakazu Higuchi[1], Yoshimi Uchida[1], Rika Nakamura[1], Hiroshi Kawakami[2], Atsuo Urisu[3] and Reiko Teshima[1]

ABSTRACT

Background: An *in vitro* elicitation test employing human high-affinity IgE receptor-expressing rat mast cell lines appears to be a useful method for measuring mast cell activation using a patient's IgE and an allergen; however, such cell lines are sensitive to human complements in the serum. We have recently developed a new luciferase-reporting mast cell line (RS-ATL8) to detect IgE crosslinking-induced luciferase expression (EXiLE) with relatively low quantities of serum IgE.

Methods: A total of 30 patients suspected of having egg white (EW) allergy were subjected to an oral food challenge (OFC) test; then, the performances of EW-specific serum IgE (CAP-FEIA), EW-induced degranulation, and EXiLE responses in RS-ATL8 cells were compared using receiver-operating characteristic (ROC) curve analysis. The patients' sera were diluted to 1 : 100, which causes no cytotoxicity when sensitizing the RS-ATL8 cells for the degranulation and EXiLE tests.

Results: The area under the ROC curves was highest in the EXiLE test (0.977), followed by CAP-FEIA (0.926) and degranulation (0.810). At an optimal cutoff range (1.648-1.876) calculated from the ROC curve of the EXiLE test, sensitivity and specificity were 0.944 and 0.917, respectively. A 95% positive predictive value was given at a cutoff level of 2.054 (fold increase in luciferase expression) by logistic regression analysis.

Conclusions: In contrast to *in vivo* tests, the EXiLE test appears to be a useful tool in diagnosing patients suspected of having IgE-dependent EW allergy without the risk of severe systemic reactions.

KEY WORDS

allergen-specific IgE, allergy diagnosis, egg white allergy, luciferase, mast cells

INTRODUCTION

Oral food challenge (OFC) is a gold standard for food allergy diagnosis, though it is accompanied by the risk of severe allergic reactions, including anaphylaxis.[1,2] Another *in vivo* test, the skin prick test, is also known to have a slight risk of anaphylaxis.[3] *In vitro* tests, for example CAP-FEIA, often measure clinically irrelevant IgE binding to allergens, such as cross-reactive carbohydrate determinants,[4,5] since their detection sensitivity is very high. As a tool to fill the gap between *in vivo* and *in vitro* allergy tests, the basophil activation test (BAT) was recently established.[6,7] However, the BAT requires fresh basophils in the patient's blood, which cannot be preserved for long periods.

To determine whether binding between IgE and allergens would lead to mast cell activation, human high-affinity IgE receptor (FcεRI)-expressing rat mast cell lines ("humanized" RBL cell lines) have been developed by several groups, including ours.[8-11] An *in vitro* elicitation test using such humanized RBL cell lines would seem to be useful in investigating the properties of IgE and/or allergens on the basis of

[1]Division of Novel Foods and Immunochemistry, National Institute of Health Sciences, [2]Department of Food Science and Nutrition, Kyoritsu Women's University, Tokyo and [3]Department of Pediatrics, Fujita Health University, The Second Teaching Hospital, Aichi, Japan.
Conflict of interest: No potential conflict of interest was disclosed.
Correspondence: Ryosuke Nakamura, Division of Novel Foods and Immunochemistry, National Institute of Health Sciences, 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan.
Email: ryosnak@nihs.go.jp
Received 2 December 2011. Accepted for publication 21 February 2012.

Table 1 Profiles of the egg white allergic subjects

OFC of boiled egg	*n*	Sex (F/M)	Age (y) (Median; Range)	Total IgE (IU/ml) (Geometric mean; 25-75%)	EW-specific IgE (U_A/ml) (Geometric mean; 25-75%)
Positive	18	8/10	7.5; 3-14	849; 536.5-1221.5	16.5; 7.26-33.8
Negative	12	3/9	10; 5-36	1685; 667.9-5244.0	1.04; 0.34-2.39

OFC, oral food challenge; EW, egg white.

mast cell activation using serum specimens. However, since these rat-derived cell lines are sensitive to human complements, spontaneous mediator release from these cells in the presence of human sera is not negligible.

Recently, we have established a new humanized RBL cell line, named RS-ATL8, by integrating nuclear factor of activated T cells (NF-AT)-responsive firefly luciferase reporter gene into human FcεRI-expressing RBL-SX38 cells to measure IgE crosslinking-induced luciferase expression (EXiLE).[12] In that report, we showed that by taking a twofold increase in luciferase expression as a cutoff level, EXiLE tests for egg white (EW)-allergic subjects correlated very well to both OFC and EW-specific IgE in the serum. Thus far, we have investigated 30 EW-allergic subjects (18 were OFC positive and 12 were negative). In the present study, receiver-operating characteristic (ROC) curve analysis[13,14] was performed on the EXiLE test for EW in addition to the EW-specific IgE test (CAP-FEIA) and EW-induced degranulation test using RS-ATL8 cells to compare these serum IgE tests and to calculate optimal cutoff levels.

METHODS

CELLS AND REAGENTS

A human FcεRI-expressing rat mast cell line (RS-ATL8) was maintained in minimal essential medium (MEM; Invitrogen, Rockville, MD, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS; Nichirei Biosciences, Tokyo, Japan), penicillin/streptomycin (Invitrogen), 1.2 mg/ml geneticin (Invitrogen), and 200 μg/ml hygromycin (Invitrogen), as described previously.[12] This cell line has been stably integrated with the NF-AT-responsive firefly luciferase reporter gene. Egg white extract (EWE; 100 mg of freeze-dried EW powder per 1 ml in stock solution) prepared for scratch diagnostic tests was purchased from Torii Pharmaceutical (Tokyo, Japan) for the *in vitro* elicitation tests.

SUBJECTS

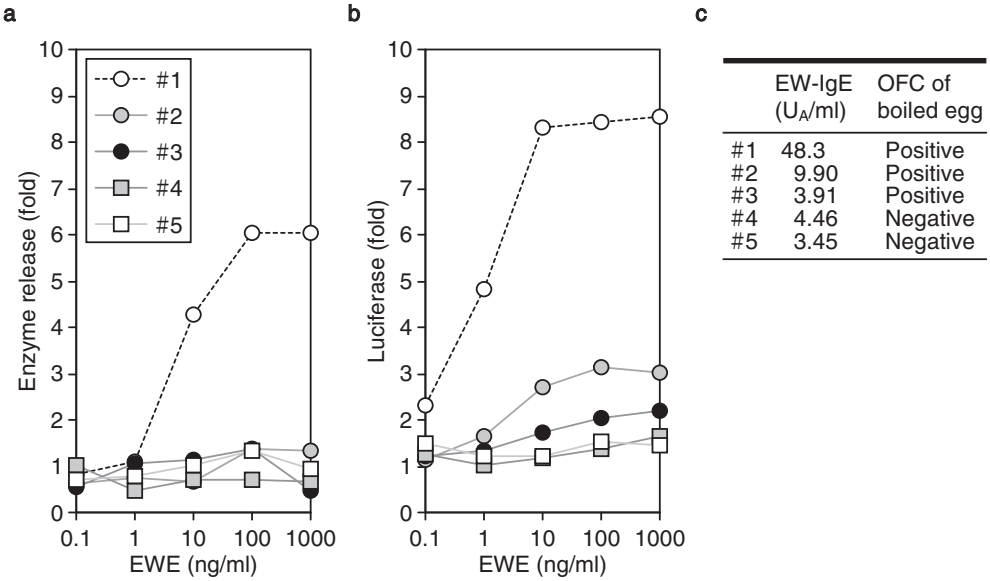
Patients with suspected egg allergy referred to a hospital for investigation were enrolled in the study (Table 1), as described previously.[12] Most of the patients had atopic dermatitis and asthma. Informed consent was obtained from patients, their parents, or both to collect and investigate serum samples further. The study was approved by the institutional review board of the National Institute of Health Sciences and Ethics Committee of Fujita Health University School of Medicine. All sera were collected before the food challenge to measure total and allergen-specific IgE levels using the CAP-FEIA system (Phadia, Uppsala, Sweden). Every patient was subjected to double-blind, placebo-controlled food challenges with boiled egg in a sequential dose-escalation manner; the patient was judged positive if he or she was unable to eat the equivalent of a whole egg due to an immediate reaction (within 2 hours), as described previously.[15] All sera were stored at -80℃.

LUCIFERASE ASSAY

RS-ATL8 cells (5×10^4 cells/50 μl/well) were plated onto a clear-bottom white 96-well plate (ViewPlate, Perkin Elmer, Waltham, MA, USA) and incubated for 3 h at 37℃ in a 5% CO_2 incubator. Then, the cells were sensitized with 1 : 100-diluted human serum in MEM containing 10% FCS. After overnight incubation, the cells were washed once with sterile PBS, and then stimulated for 3 h at 37°C in a 5% CO_2 incubator with 0.1-1000 ng/ml EWE diluted in MEM containing 10% FCS (50 μl/well). After stimulation, 50 μl of luciferase substrate solution containing cell lysis reagent (ONE-Glo, Promega, Tokyo, Japan) was added to the cells, and chemiluminescence was measured using an EnVision multilabel plate reader (Perkin Elmer). Luciferase expression levels were represented as the fold increase of light units compared with the background expression after subtraction of a blank control (without cells). Measurements were done in duplicate, and the average was used for analysis.

DEGRANULATION ASSAY

Degranulation of RS-ATL8 cells was measured using β-hexosaminidase release as described previously with some modifications.[8,12] RS-ATL8 cells were plated and sensitized with 1 : 100-diluted sera as described above. After overnight incubation, the cells were washed twice with 1,4-piperazine-bis (2-ethanesulfonic acid) (PIPES) buffer (140 mM NaCl, 5 mM KCl, 0.6 mM $MgCl_2$, 1.0 mM $CaCl_2$, 5.5 mM glucose, 0.1% BSA, and 10 mM PIPES, pH 7.4). After incubation for 30 min at 37℃, the supernatants were collected, and residual cells were lysed with 0.2% Triton X-100. The activity of β-hexosaminidase in the medium and within the cells was determined using a



	EW-IgE (U_A/ml)	OFC of boiled egg
#1	48.3	Positive
#2	9.90	Positive
#3	3.91	Positive
#4	4.46	Negative
#5	3.45	Negative

Fig. 1 Degranulation (**a**), and luciferase expression (EXiLE) (**b**) in RS-ATL8 cells sensitized with 1 : 100-diluted serum and stimulated with 0.1-1000 ng/ml EWE. Measurements were done for 30 subjects, and representative five (three OFC positives and two negative) subjects (**c**) are shown. Data are represented as fold increase, by taking non-stimulated control as 1.0.

fluorometric assay with 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide as a substrate (0.1 mM in 100 mM citrate, pH 4.5). The reaction was stopped with 0.25-M glycine buffer after 30-min incubation at 37℃. The plate was read on a Fluoroskan Ascent plate reader (Thermo Fisher Scientific, Waltham, MA, USA) using 380-nm excitation and 440-nm emission filters. Degranulation levels were represented as the fold increase in enzyme activity as follows:

$$R = \frac{[(R_{sup} - \text{blank}) / (R_{sup} - \text{blank} + R_{ppt} - \text{blank})]_s}{[(R_{sup} - \text{blank}) / (R_{sup} - \text{blank} + R_{ppt} - \text{blank})]_c}$$

where R is the degranulation level, R_{sup} the released enzyme activity in the supernatant, R_{ppt} the residual enzyme activity within the cell, and blank the buffer control. Subscripts *s* and *c* represent *sample* and *control* (non-stimulated background), respectively. Measurements were done in duplicate, and the average was used for analysis.

STATISTICAL ANALYSIS

Differences were analyzed using the Mann-Whitney U test (two-tailed) for unpaired samples. The discriminative usefulness of serum IgE testing methods was evaluated by constructing ROC curves, where sensitivity versus 1 - specificity was plotted for each possible cutoff level, and the area under the curves (AUCs) was determined. From each ROC curve, we determined the optimal cutoff range, which was selected at the point closest to the upper-left corner of the ROC curve and which most efficiently discriminated between the OFC-positive and negative subjects. The respective sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using Microsoft Excel. Fisher's exact probability and logistic regression formula were calculated using R software (ver. 2.9.2).

RESULTS

IgE CROSSLINKING-DEPENDENT DEGRANULATION AND LUCIFERASE EXPRESSION IN RS-ATL8 CELLS

RS-ATL8 cells were sensitized with 1 : 100-diluted EW-allergic patients' sera overnight, and EWE-induced degranulation and luciferase expression was measured. Five representative patients' responses are shown in Figure 1. To enable comparison, both responses are presented as the fold increase by taking the background (non-stimulated) levels as 1.0. As shown in Figure 1a, degranulation was detected with serum #1, which contained a high concentration of EW-specific IgE (48.3 U_A/ml), but no response was observed when moderate (#2, 9.90 U_A/ml) or low (#3, 3.91 U_A/ml) levels of IgE were used. On the other hand, the EXiLE test clearly detected mast cell activation caused by different levels of specific IgE (Fig. 1 b). As expected, the OFC-negative sera (#4, 5) did not induce any luciferase expression; nevertheless, serum #4 contained a higher amount of EW-specific IgE (4.46 U_A/ml) than did the OFC-positive serum #3. It should be noted that the EXiLE test was performed in a culturing medium supplemented with 10% FCS, which contains a substantial amount of non-specific proteins.

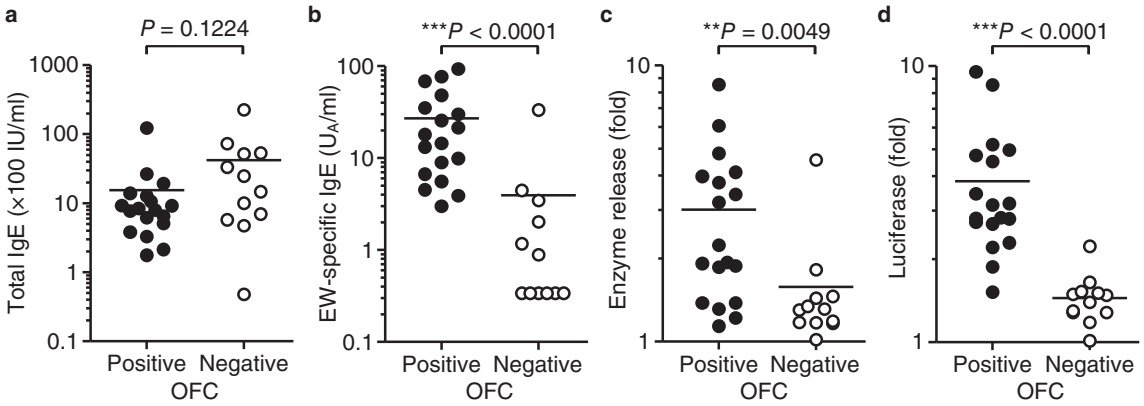


Fig. 2 Total IgE (**a**), EW-specific serum IgE (**b**), degranulation test (**c**), and EXiLE test (**d**) in the OFC positive and negative subjects. Degranulation and EXiLE tests were performed using RS-ATL8 cells sensitized with 1 : 100-diluted serum and stimulated with 0.1-1000 ng/ml EWE. The maximum responses in each serum subject within the EWE concentration range above are shown. $^{**}P < 0.01$, $^{***}P < 0.001$, Mann-Whitney U test.

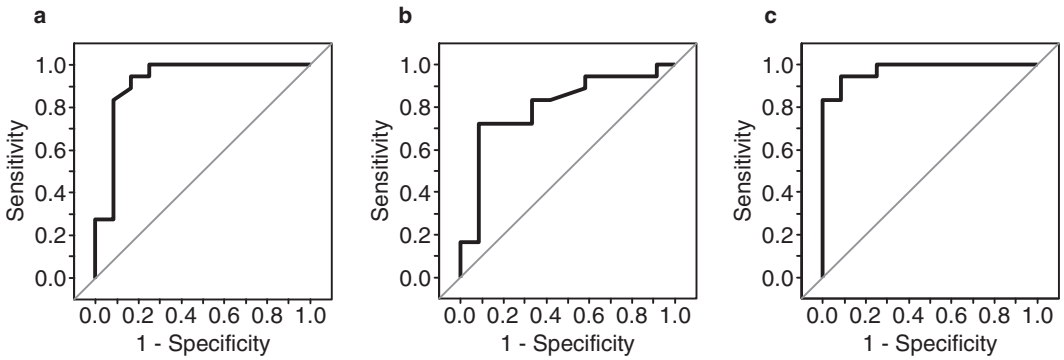


Fig. 3 Area under the ROC curves of EW-specific serum IgE (**a**), degranulation test (**b**), and EXiLE test (**c**). Conditions are similar to those of Figure 1.

DIAGNOSTIC ABILITIES OF TOTAL IgE, SPECIFIC IgE, DEGRANULATION, AND EXiLE

A total of 30 patients suspected of having EW allergy were subjected to an OFC test with boiled egg, and 18 were found to be positive and 12 negative (Table 1). EW-specific IgE was detected in every OFC-positive subject, i.e., IgE-independent food allergy subjects were not included in this study. There was no difference in the total IgE levels in the sera between OFC-positive and negative patients (Fig. 2a). On the other hand, EW-specific serum IgE were significantly higher ($P < 0.0001$) in OFC-positive than negative subjects (Fig. 2b). Both the degranulation (Fig. 2c) and EXiLE (Fig. 2d) tests were also able to distinguish between positive and negative subjects with a significant difference ($P = 0.0049$ and <0.0001, respectively).

EVALUATION OF SPECIFIC IgE, DEGRANULATION AND EXiLE TESTS

To evaluate the diagnostic ability of the different *in vitro* tests in diagnosing EW allergy, ROC curve analyses were performed for each test (Fig. 3).[13,14] Since RS-ATL8 cells were stimulated with 0.1-1000 ng/ml EWE in each test, five distinct ROC curves could be drawn for each concentration in the degranulation and EXiLE tests. However, the maximum response in each subject's serum within the above EWE concentration range was considered to be representative of the overall response (e.g., the maximum EXiLE response of #1 in Figure 1b was 8.559 at 1000 ng/ml). As summarized in Table 2, the AUC of the ROC curve for the EWE-induced EXiLE test gave the highest value (0.977), followed by the EW-specific IgE (0.926) and EWE-induced degranulation tests (0.810). With the optimal cutoff range obtained from the ROC curve analyses, sensitivity, specificity, PPV, NPV, and Fisher's exact probability were calculated. Both specificity (0.944) and sensitivity (0.917) were highest in the EXiLE tests; the CAP-FEIA and degranulation tests gave comparable sensitivity and specificity, though the specificity (0.833) and sensitivity (0.722), respectively, were lower. PPV (0.944) and NPV (0.917) were also highest in the EXiLE tests;

Table 2 Summary of the comparison of three *in vitro* IgE tests

Test		AUC	*P*-value	Optimal cutoff range †	Sens	Spec	PPV	NPV	Fisher's exact probability
CAP-FEIA (EW)		0.926	<0.0001	3.46-3.91 (U_A/mL)	0.944	0.833	0.895	0.909	1.548×10^{-5}
Degranulation				(fold increase)					
EWE (ng/ml)	*0.1*	0.616	0.2900	1.016-1.117	0.944	0.583	0.773	0.875	0.07172
	1	0.764	0.01586	0.839-0.897	0.833	0.583	0.750	0.700	0.04504
	10	0.782	0.009842	1.271-1.399	0.667	0.833	0.857	0.625	0.01061
	100	0.838	0.002010	1.187-1.219	0.889	0.750	0.842	0.818	0.001221
	1000	0.708	0.05684	1.437-1.489	0.667	0.917	0.923	0.647	0.002397
Maximum		0.810	0.004582	1.821-1.860	0.722	0.917	0.929	0.688	7.964×10^{-4}
EXiLE				(fold increase)					
EWE (ng/ml)	*0.1*	0.713	0.05155	1.298-1.332	0.611	0.750	0.786	0.563	0.07172
	1	0.940	<0.0001	1.217-1.294	1.000	0.917	0.947	1.000	2.197×10^{-7}
	10	0.972	<0.0001	1.338-1.537	0.944	0.917	0.944	0.917	2.509×10^{-6}
	100	0.977	<0.0001	1.526-1.765	0.944	0.917	0.944	0.917	2.509×10^{-6}
	1000	0.958	<0.0001	1.648-1.866	0.944	0.917	0.944	0.917	2.509×10^{-6}
Maximum		0.977	<0.0001	1.648-1.876	0.944	0.917	0.944	0.917	2.509×10^{-6}

AUC, area under the curve; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value.

† Cutoff levels closest to the upper left corner in the ROC curve optimizing both sensitivity and specificity. Cutoff values between the range shown here will give the same prediction results. Sens, Spec, PPV, NPV, and Fisher's exact probability at the optimal cutoff levels are shown.

they were moderate in the CAP-FEIA test (0.895 and 0.909, respectively). In the degranulation test, PPV was very high (0.929), but NPV was relatively low (0.688).

DISCUSSION

It is well known that antigen-specific IgE in the serum has good diagnostic usability in distinguishing OFC-negative and positive subjects with EW allergies.[16-18] We found that the optimal cutoff range of EW-specific IgE determined from ROC curve analysis of CAP-FEIA was 3.46-3.91 U_A/ml (Table 2), which is slightly higher than the 2.82 U_A/mL been reported by Ando *et al.* using ROC curve analysis in 108 subjects.[15] This small inconsistency may have arisen from the difference in populations and their sizes between that study and the present one.

Conventional degranulation tests based on β-hexosaminidase release have been widely used to measure the activation of RBL cell lines[19-21] because of the convenience of such tests compared with other methods, such as ^{3}H-labeled serotonin release.[9,10] However, in measurements of humanized RBL cell lines, human complements in the serum are cytotoxic to the rat-derived cells, such that high "spontaneous release" is observed in the β-hexosaminidase assay, resulting in a high background with the assay. Figure 1a illustrates that degranulation can be detected with a relatively high concentration of EW-specific IgE-containing serum (#1, 48.3 U_A/ml), but not with moderate (#2, 9.90 U_A/ml) or low (#3, 3.91 U_A/ml) levels of IgE. Regarding OFC-negative subject #4, neither degranulation nor the EXiLE test detected mast cell activation, although the EW-specific serum IgE was 4.46 U_A/ml, which is higher than the optimal cutoff range of CAP-FEIA (Table 2). Considering the high specificity (0.944) of these tests, it is unsurprising that clinically irrelevant IgE did not cause mast cell activation, resulting in negative in these tests. In addition, this result is consistent with that of our previous report, which described how clinically irrelevant IgE bondable to ovomucoid in an outgrown subject could not activate RS-ATL8 cells.[22] To avoid cytotoxicity due to human complements, serum specimens had to be diluted to 1 : 100 in the present study. However, this dilution would seem to be too low to sensitize RS-ATL8 cells effectively and to detect a marked degranulation response, which may have led to the low sensitivity (0.722) of this test (Table 2). However, even under these conditions, the specificity (0.917) and PPV (0.929) were higher than with the CAP-FEIA test (0.833 and 0.895, respectively) at each optimal cutoff level.

In the present study, we have shown that the diagnostic performance of the EXiLE test was the highest in the AUC (0.977), sensitivity (0.944), specificity (0.917), PPV (0.944), NPV (0.917), and Fisher's exact probability (2.509×10^{-6}) among the three *in vitro* IgE tests at each optimal cutoff level (Table 2). Although the highest sensitivity, PPV, and NPV were

obtained when the cells were stimulated with 1 ng/ml of EWE, the optimal cutoff range was very narrow (1.217-1.294). Therefore, the maximum response within 0.1-1000 ng/ml EWE in each subject appears to be a more reliable and practical indicator for the EXiLE test. The optimal cutoff range of maximum responses calculated here was 1.648-1.876, which is slightly lower than the 2.0 that was taken as the cutoff level in our previous study.[12] When the PPVs were plotted against the luciferase-expression levels (in terms of fold increase) in the present study, the logistic regression curve can be drawn using the following formula:

$y = 1/[1 + \exp(2.7157 - 2.7553x)]$

In this formula, 95% PPV is given at a cutoff level of 2.054, which may be used as the level with a 95% predicted probability of EW allergy.[16-18] Meanwhile, 95% specificity, which is independent of disease prevalence and thus may be considered another useful benchmark,[15] is given at a cutoff level of 1.962 from another regression curve (formula is not shown). Accordingly, in practice, a twofold increase in luciferase expression in the EXiLE test seems to be a reasonable cutoff level for predicting OFC positives.

The BAT based on histamine release[6] or CD203c expression[7] is another possible *in vitro* method for diagnosing food allergies without the risk of anaphylaxis. When performed in the presence of patient serum, BAT may detect serum-derived factors that modify the allergic responses, such as neutralizing antibodies.[23] The EXiLE test is also capable of being performed in the presence of human serum (i.e., without washing cells before stimulation); this results in reduced responses, which may be caused by neutralizing antibodies (unpublished results). In such a testing situation, however, the maximum concentration of human serum is 1% because of cytotoxicity[12]; with the EXiLE system, this would make it difficult to fully elucidate the effects of serum factors on allergic responses. Nevertheless, the BAT requires a whole-blood specimen, which cannot be preserved for long periods, and in this context BAT should more correctly be classified as an *ex vivo* test rather than an *in vitro* test such as CAP-FEIA. Conversely, the EXiLE test is a bona fide *in vitro* test, consisting just of the serum specimen and the cultured mast cell line and allowing reproducible results to be obtained. Thus, the EXiLE test is expected to be suitable not only for diagnosis, but also retrospective epidemiological studies. Additionally, our preliminary results suggest that the EXiLE test is so robust that most anti-allergics, such as disodium cromoglycate and ketotifen fumarate, do not affect the results at least until a concentration of 1 μg/ml, which is much higher than the clinical concentration in plasma (data not shown). Accordingly, unlike with the BAT, it would not appear necessary to discontinue administering such anti-allergics before EXiLE testing, which would be beneficial to the patients.

In conclusion, a luciferase assay-based *in vitro* elicitation test, which measures IgE crosslinking-induced mast cell activation as an indicator of clinically meaningful serum IgE, would be useful in diagnosing patients who are suspected of having IgE-dependent EW allergy without the risk of severe systemic reactions.

ACKNOWLEDGEMENTS

This study was supported by Grants-in-Aid from the Food Safety Commission, Japan (No.0708) and by Grants-in-Aid for Challenging Exploratory Research from the Japan Society of the Promotion of Science (22659027). We are grateful for receiving the 7th JSA Best Presentation Award for the present study.

REFERENCES

1. Urisu A, Ebisawa M, Mukoyama T, Morikawa A, Kondo N; Japanese Society of Allergology. Japanese guideline for food allergy. *Allergol Int* 2011;**60**:221-36.
2. Mukoyama T, Nishima S, Arita M *et al*, and Food Allergy Committee, Japanese Society of Pediatric Allergy and Clinical Immunology. Guidelines for diagnosis and management of pediatric food allergy in Japan. *Allergol Int* 2007;**56**:349-61.
3. Codreanu F, Moneret-Vautrin DA, Morisset M *et al*. The risk of systemic reactions to skin prick-tests using food allergens: CICBAA data and literature review. *Eur Ann Allergy Clin Immunol* 2006;**38**:52-4.
4. van Ree R, Aalberse RC. Specific IgE without clinical allergy. *J Allergy Clin Immunol* 1999;**103**:1000-1.
5. Mari A, Iacovacci P, Afferni C *et al*. Specific IgE to cross-reactive carbohydrate determinants strongly affect the *in vitro* diagnosis of allergic diseases. *J Allergy Clin Immunol* 1999;**103**:1005-11.
6. Nishi H, Nishimura S, Higashiura M *et al*. A new method for histamine release from purified peripheral blood basophils using monoclonal antibody-coated magnetic beads. *J Immunol Methods* 2000;**240**:39-46.
7. Bühring 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.
8. Takagi K, Nakamura R, Teshima R, Sawada J. Application of human FcεRI α-chain-transfected RBL-2H3 cells for estimation of active serum IgE. *Biol Pharm Bull* 2003;**26**: 252-5.
9. Wiegand TW, Williams PB, Dreskin SC, Jouvin MH, Kinet JP, Tasset D. High-affinity oligonucleotide ligands to human IgE inhibit binding to Fcε receptor I. *J Immunol* 1996;**157**:221-30.
10. Dibbern DA Jr, Palmer GW, Williams PB, Bock SA, Dreskin SC. RBL cells expressing human FcεRI are a sensitive tool for exploring functional IgE-allergen interactions: studies with sera from peanut-sensitive patients. *J Immunol Methods* 2003;**274**:37-45.
11. Vogel L, Lüttkopf D, Hatahet L, Haustein D, Vieths S. Development of a functional *in vitro* assay as a novel tool for the standardization of allergen extracts in the human system. *Allergy* 2005;**60**:1021-8.
12. Nakamura R, Uchida Y, Higuchi M *et al*. A convenient and sensitive allergy test: IgE crosslinking-induced lu-

ciferase expression in cultured mast cells. *Allergy* 2010; **65**:1266-73.
13. Akobeng AK. Understanding diagnostic tests 3: receiver operating characteristic curves. *Acta Pædiatrica* 2007;**96**: 644-7.
14. Tokuda R, Nagao M, Hiraguchi Y *et al*. Antigen-induced expression of CD203c on basophils predicts IgE-mediated wheat allergy. *Allergol Int* 2009;**58**:193-9.
15. Ando H, Movérare R, Kondo Y *et al*. Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. *J Allergy Clin Immunol* 2008;**122**:583-8.
16. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;**100**:444-51.
17. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;**107**:891-6.
18. Komata T, Söderström 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.
19. Gavériaux C, Renard P, Cammisuli S, Loor F. A comparison of five different methods for the detection of TNP specific mouse IgE: ELISA, ELISA on cells, rosetting, granule enzyme release assay and passive cutaneous anaphylaxis. *J Immunol Methods* 1986;**93**:107-14.
20. Aketani S, Teshima R, Umezawa Y, Sawada J. Correlation between cytosolic calcium concentration and degranulation in RBL-2H3 cells in the presence of various concentrations of antigen-specific IgEs. *Immunol Lett* 2001;**75**: 185-9.
21. Ladics GS, van Bilsen JH, Brouwer HM, Vogel L, Vieths S, Knippels LM. Assessment of three human FcεRI-transfected RBL cell-lines for identifying IgE induced degranulation utilizing peanut-allergic patient sera and peanut protein extract. *Regul Toxicol Pharmacol* 2008;**51**:288-94.
22. Teshima R, Nakamura R. [The method of the prediction for food-protein allergenicity]. *[J Food Hyg Safety Sci]* 2011;**52**:1-9 (in Japanese).
23. Wanich N, Nowak-Wegrzyn A, Sampson HA, Shreffler WG. Allergen-specific basophil suppression associated with clinical tolerance in patients with milk allergy. *J Allergy Clin Immunol* 2009;**123**:789-94.