Japanese Society of Allergology task force report on standardization of house dust mite allergen vaccines — Secondary publication

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A B S T R A C T
Background: In the 1990s, the Japanese Society of Allergology (JSA) standardized Japanese cedar pollen allergen vaccines. In the present study, the task force for house dust mite (HDM) allergen standardization of the Committee for Allergens and Immunotherapy of JSA reports the standardization of HDM allergen vaccines in Japan.

Methods: In vivo allergenic potency was determined by intradermal testing of 51 Japanese adults with positive serum specific IgE to HDM allergens. In vitro total IgE binding potency was analyzed by competition ELISA using a pooled serum, with sera obtained from 10 allergic patients. The amounts of HDM group 1 (Der 1) and group 2 major allergens in eight HDM allergen extracts were measured by sandwich ELISAs. Correlation between the in vitro total IgE binding potency and major allergen levels was analyzed.

Results: We selected a JSA reference HDM extract and determined its in vivo allergenic potency. The in vitro total IgE binding potency significantly correlated with Der 1 content, group 2 allergen content, and their combined amount, indicating that measurement of major allergen contents can be used as a surrogate in vitro assay.

Conclusions: The task force determined the in vivo allergenic potency (100,000 JAU/ml) and Der 1 content (38.5 µg/ml) of the JSA reference HDM extract, selected the measurement of Der 1 content as the surrogate in vitro assay, and decided that manufacturers can label a HDM allergen extract as having a titer of 100,000 JAU/ml if it contains 22.2–66.7 µg/ml of Der 1.

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Introduction

Allergen-specific immunotherapy has been performed in general medical practice since it was first described by Noon in 1911.^

Standardization of allergen vaccines/extracts used for therapy and diagnosis is necessary because their qualities are variable depending on production methods and manufactured lots.^

In the United States, allergen standardization is based on intradermal testing of allergic patients and the potencies of lots are determined by appropriate surrogate in vitro assays, which are based on inhibition of binding of IgE from pooled allergic sera to solid phase reference allergen extracts, or measurement of specific allergen contents in the allergen vaccines.^

In the European Union, products are standardized using manufacturers’ in-house references and labeled in manufacturer-specific units.^

In Japan, the Japanese Society of Allergology (JSA) standardized Japanese cedar pollen (JCP) allergen vaccines.^

In vivo allergenic potency of the JSA reference JCP extract was determined by intradermal testing and measurement of the content of the major allergen Cry j 1 by appropriate surrogate in vitro assay.

House dust mites (HDMS) are a major allergen source that provokes allergic rhinitis, asthma, conjunctivitis, and atopic dermatitis.^

However, standardization of HDM allergen vaccines/extracts based on intradermal testing in Japanese subjects with positive serum specific IgE to HDM allergens has not been performed. This report, produced by the task force for HDM allergen standardization of the Committee for Allergens and Immunotherapy of JSA, selected a JSA reference HDM extract, determined its in vivo allergenic potency in Japanese adults, and determined a surrogate in vitro assay that is suitable for HDM allergen standardization in Japan.

Methods

HDM allergen extracts

Each HDM extract was prepared as a mixture of equivalent volumes of extracts from two mite species, Dermatophagoides pteronyssinus (DP) and Dermatophagoides farinae (DF). United States Food and Drug Administration (FDA) reference extracts, E11-DP (10,000 AU/ml) and E10-DF (10,000 AU/ml), were acquired from the Laboratory of Immunobiochemistry, Division of Bacterial, Parasitic and Allergenic Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research (CBER), FDA. Commercial extracts for subcutaneous immunotherapy (DP: lot#B3117094 and DF: lot#F21G6279, 10,000 AU/ml) were purchased from Hollister-Stier (Spokane, WA, USA). Five extracts identified by alphabet letters (Extracts A–E) were obtained from ALK-Abell (Uppsala, Sweden) and Stallergenes SA (Antony, France). JSA chose an extract other than those described above as the JSA reference HDM extract. Measurement of group 1 allergens and in vitro relative IgE binding potency testing took place in the laboratories of Sagamihara National Hospital (Laboratory 1) and Azabu University (Laboratory 2). Measurement of the combined total content of group 2 allergens was conducted only in Laboratory 1.

HDM-sensitized subjects

Inclusion criteria for the HDM-sensitized subjects enrolled in this study were: Japanese adults between the age of 20 and 50 years who were positive for DP or DF-specific IgE, showing ≥ 0.70 UA/ml (class 2) in ImmunoCAP assay (ThermoFisher Scientific, Uppsala, Sweden). Subjects were excluded from the study if they had (a) skin disease on the forearm that affects intradermal injection reactions; (b) used (1) external application of medication within one day of study initiation, (2) oral antihistamine, oral α- or β-adrenergic agonists, and topical corticosteroid or immunosuppressive medicine for external application to the injection site within one week of study initiation, (3) tricyclic antidepressants and phenothiazines with an antihistamine effect within 2 weeks of study initiation, (4) non-selective β-adrenergic blockers within 3 weeks of study initiation, (5) systemic immunosuppressive drugs within 30 days of study initiation, (6) specific antibodies within 90 days of study initiation; or if they were (c) pregnant or were possibly pregnant on the study day; or were under (d) HDM-specific immunotherapy; or had (e) severe bronchial asthma; (f) anaphylaxis to adrenaline; (g) concomitant systemic diseases such as cardiac, hepatic, renal, and hematologic disorders or infection that could affect the study trial; or (h) were judged by the examiners as being inappropriate for study enrollment.

Intradermal testing and collection of sera were conducted at the Department of Otorhinolaryngology—Head and Neck Surgery of Chiba University Graduate School of Medicine, Department of Otorhinolaryngology of Nippon Medical School, Graduate School of Medicine, Department of Respiratory Medicine of Saitama Medical University, and Department of Otorhinolaryngology—Head and Neck Surgery of the University of Yamanashi Interdisciplinary Graduate School of Medicine and Engineering. The ethical review committee of each institution approved the protocol of this study. Written informed consent was obtained from all patients before study enrollment and anonymity was preserved using documents and methods approved by the ethical review committees.

Intradermal testing

First, we selected a candidate HDM extract as the JSA reference HDM extract to be used for intradermal testing. The candidate extract was diluted using the 0.005% Polysorbate 80-added control solution for intradermal skin testing, “TORI-I”, which contained 0.9% (w/v) NaCl and 0.5% (w/v) phenol (Torii Pharmaceutical, Tokyo, Japan). The dilution factors were from 3^2 to 3^10 (2187–1162 × 10^5). Control solution for intradermal skin testing, “TORI-I”, was used as the negative control.

Intradermal testing was performed according to a previously described method.^

The protocol for intradermal testing was largely based on the FDA protocol except for criteria for determining the threshold concentration. Briefly, 20 μl of each diluted HDM extract was administered intradermally into the forearm from low to high concentrations using 1 ml tuberculin syringes. Fifteen minutes after the injection, the diameters of the wheal or erythema were measured based on Ishizaki’s criteria.^

The threshold dilution factor was defined as the maximum dilution factor of diluted extract that can induce a positive reaction.

With respect to the determination of in vivo allergenic potency, threshold values of all patients were represented as logarithms that have 3 as the base of the threshold dilution factors, and then an average value for them was calculated. Values, which were from 9 to 11, 11 to 13, and 13 to 15, were assigned the in vivo allergenic potencies of 1000 JAU/ml, 10,000 JAU/ml, and 100,000 JAU/ml, respectively, based on the plan used in the standardization of JCP allergen vaccines.^

With this method, the same titer could be assigned to extracts with, at the most, a 3^3-fold (9-fold) difference in allergenic potencies.

Measurement of HDM group 1 major allergens

The group 1 allergens, Der p 1 and Der f 1, in the HDM extracts were measured by a previously described method. Briefly,
sandwich ELISAs for Der p 1 or Der f 1 were performed using murine monoclonal antibodies and 92-Dp or 92-Df extract, respectively, as the standard antigen for ELISA. Previously, the 92-Dp and 92-Df extracts were prepared from HDM bodies and the contents of group 1 and group 2 allergens in 92-Dp and 92-Df were determined.\textsuperscript{18,19} For validation of the measurement obtained by this method, group 1 allergen content was also determined using other ELISA kits purchased from Indoor Biotechnologies (Charlottesville, VA, USA) and Nichinichi Pharmaceutical (Mie, Japan).

Measurement of the total amount of HDM group 2 major allergens

The total amount of group 2 allergens (Der 2), Der p 2 and Der f 2, was measured using a previously described method.\textsuperscript{20,21} Briefly, a sandwich ELISA for Der 2 was performed using rabbit polyclonal antibodies and a mixture of 92-Dp and 92-Df extracts\textsuperscript{18,19} as the standard antigen for ELISA.

Sera for in vitro relative IgE binding potency testing

A total of 20 ml of blood sample was taken from 10 randomly selected patients out of 19 who had \(> 17.5\) Ua/ml (class 4) for DP- or DF-specific IgE in the ImmunoCAP assay (ThermoFisher). Blood samples were centrifuged and sera were separated and stored at \(-80^\circ\) C. A pooled serum was prepared as a mixture of equivalent volumes of the 10 sera.

In vitro relative IgE binding potency testing

Competition ELISA was used to evaluate the inhibition of allergen-specific IgE binding as described previously.\textsuperscript{22} Briefly, the plates were coated with the JSA reference HDM extract or another extract (Extract C). The pooled serum (dilution factor: 50) was mixed with an equivalent volume of each serially diluted inhibitor extract (final serum dilution factor: 100), and after incubation for 30 min at room temperature, the mixtures were added to the wells of the plates. The allergen-specific IgE binding to plate wells were detected with enzyme-conjugated anti-human IgE and a fluorogenic substrate. Relative potencies of extracts were calculated as ratios of the dilution factors of the extracts that gave half the maximum (50%) fluorescence relative to that of the JSA reference HDM extract.

Statistical analysis

Pearson correlation coefficients for the association between major allergen content and in vitro relative IgE binding potency were calculated after logarithmic transformation. \(P < 0.05\) was regarded as statistically significant.

Results

Selection of a HDM allergen extract as the JSA reference HDM extract

We selected one extract, which can be used for intradermal testing and would be approved for allergen-specific immunotherapy in Japan, as a candidate for the JSA reference HDM extract. The candidate extract showed appropriate values in terms of in vivo allergenic potency and HDM group 1 and 2 major allergen contents, as described below. Accordingly, we selected the candidate extract as the JSA reference HDM extract.
Table 1
House dust mite major allergen content in the Japanese Society of Allergology reference house dust mite extract.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Group 1 allergens</th>
<th>Group 2 allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Der p 1</td>
<td>Der f 1</td>
</tr>
<tr>
<td>Laboratory 1</td>
<td>28.2</td>
<td>14.1</td>
</tr>
<tr>
<td>Laboratory 2</td>
<td>23.2</td>
<td>11.8</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>25.6</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Der p 1, Der f 1, and Der 2 were measured by sandwich ELISA in two institutes. Der 1, combined total of Der p 1 and Der f 1; Der 2, combined total of Der p 2 and Der f 2; Laboratory 1, Sagamihara National Hospital; Laboratory 2, Azabu University; N.D., not determined.

values compared with the use of 92-Dp or 92-Df. This suggests that the small discrepancy was caused by the difference in the defined concentrations of Der p 1 and Der f 1 between 92-Dp/92-Df and UAS and was not due to the detection system, such as specificity of monoclonal antibodies. The results indicated that the ELISA system for Der p 1 and Der f 1 developed in Laboratory 1 was valid.

Major allergen contents in the HDM extracts tested

Major allergen contents in the seven HDM extracts other than the JSA reference HDM extract were measured (Table 3). Five extracts were from ALK or Stallergenes (Extract A and B) and was not due to the detection system, such as specificity of monoclonal antibodies. The results indicated that the ELISA system for Der p 1 and Der f 1 developed in Laboratory 1 was valid.

Correlation between the major allergen contents and in vitro relative IgE binding potencies

Competition ELISA was used to evaluate the inhibition of allergen-specific IgE binding to plates coated with the JSA reference HDM extract or Extract C. A pooled serum was prepared as a mixture of equivalent volumes of sera from 10 of 19 patients who had >17.5 μA/ml (class 4) for DP- or DF-specific IgE in the ImmunoCAP assay. The in vitro total IgE binding potencies of the HDM extracts relative to the JSA reference HDM extract were determined (Tables 3, 4, in vitro relative potency). Similar results were obtained using JSA reference HDM extract-coated plates and Extract C-coated plates. Similar results were obtained in the two laboratories.

Correlation between major allergen contents and the relative IgE binding potency was analyzed. The relative IgE binding potency correlated well with each of the concentrations of Der 1 (Figs. 2, 3), Der 2, and combined total of Der 1 and Der 2 (Der 1 + Der 2) (Fig. 2). Pearson correlation coefficients were greater than 0.9 and were statistically significant.

Discussion

In the 1990s, JSA standardized the JCP allergen vaccines/extracts.11,12 The JSA standard JCP extract showed an in vivo allergen potency of 10,000 JAU/ml. The unit JAU11,12 was determined by intradermal testing, similar to the bioequivalent allergy unit (BAU) and the allergy unit (AU) defined by FDA,27–29 but criteria for the reaction threshold are different between JAU and BAU/AU, and the injection volume is 20 μl for JAU and 50 μl for BAU/AU. JSA selected measurement of Cry j 1 content as the surrogate in vitro assay for determining the potencies of other JCP extracts, with the concentration of 12.5 μg/ml of Cry j 1 corresponding to 10,000 JAU/ml. JSA decided that manufacturers can label JCP extracts as having a titer of 10,000 JAU/ml if they contain 7.3–21 μg/ml of Cry j 1 i.e., within a range that is approximately three times the lowest value, the geometric center of which is 12.5 μg/ml.11,12 In the present study, the task force selected a JSA reference HDM extract, determined its in vivo allergenic potency in JAU using the same method for JCP allergen standardization, and analyzed the correlation between the in vitro total potency determined by IgE binding inhibition ELISA and the major allergen contents in eight HDM extracts.

Through intradermal testing of 51 Japanese HDM-sensitized adults, the in vivo allergenic potency of the candidate for the JSA reference HDM extract was determined as 100,000 JAU/ml. As the candidate extract showed an appropriate in vivo allergenic potency (Fig. 1) and major allergen contents (Table 1), we decided to use it as the JSA reference HDM extract. Surrogate in vitro assay to determine the potencies of extracts from different manufacturers and lots can be based on inhibition of binding of IgE from pooled allergic sera to a reference allergen extract, or measurement of specific allergen contents in the allergen vaccines/extracts. In the United States, FDA adopted relative IgE binding potency determined by IgE binding inhibition assay for HDM and mold allergen vaccines; specific allergen contents for short ragweed pollen and cat allergen vaccines (Amb a 1 and Fel d 1, respectively); and enzymatic activity (hyaluronidase and phospholipases) for Hymenoptera venom allergen vaccines.8 It has been reported that the major allergen content correlates with the relative IgE binding potency.28–30 Among more than 20 groups of HDM allergens, group 1 and 2 allergens are considered the major allergens, although some reports showed that other HDM allergens were also important, albeit less so than the major allergens.13,14 We judged that measurement of HDM major allergens is appropriate as a surrogate assay because the relative IgE binding potency correlated well with each of the major allergen contents (Figs. 2, 3).

The in vitro measurement of major allergen content has some advantages as the surrogate assay for allergen standardization i.e., it can determine absolute and not relative values, and does not need sera in which differing individual titers are seen. Species-specific ELISAs using monoclonal antibodies to measure each HDM group 1 allergen, Der p 1 and Der f 1, with high accuracy are available commercially (see the Methods section). However, those for each group 2 allergen, Der p 2 and Der f 2, are not widely available, and the total contents of Der p 2 and Der f 2 (Der 2) determined by ELISA using polyclonal antibodies in the present study are approximate values. Therefore, we selected measurement of the total content of Der p 1 and Der f 1 (Der 1) as the surrogate in vitro assay. We determined that the in vivo allergenic potency and Der 1 content of the JSA reference HDM extract were 100,000 JAU/ml and 38.5 μg/ml, respectively. Similar to the previous JCP allergen standardization,11,12 we decided that manufacturers can label an HDM allergen...
extract as having a titer of 100,000 JAU/ml if it contains 22.2–66.7 µg/ml of Der 1 i.e., within a range that is approximately three times the lowest value, the geometric center of which is 38.5 µg/ml.

We validated the measurement of Der p 1 and Der f 1 (Table 2). The amounts of group 1 and group 2 allergens in 92-Dp and 92-Df extracts were determined previously in Laboratory 118, 19. The measurement of the concentrations of purified group 1 and group 2 allergens previously used as the standard antigens for the ELISA that determine the major allergen contents in 92-Dp and 92-Df were based on absorbance at 280 nm. UAS is composed of eight allergens previously used as the standard antigens for the ELISA that measures the inhibition of allergen-specific IgE binding to plates coated with the JSA reference house dust mite extract (JSA coating) or Extract C (Extract C coating).

Table 3
House dust mite major allergen contents and in vitro total IgE binding potencies of the eight house dust mite extracts determined in Laboratory 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>In vitro relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Der p 1</td>
<td>Der f 1</td>
</tr>
<tr>
<td>JSA</td>
<td>28.2</td>
<td>14.1</td>
</tr>
<tr>
<td>CBER/FDA</td>
<td>16.7</td>
<td>16.3</td>
</tr>
<tr>
<td>Hollister-Stier</td>
<td>10.1</td>
<td>2.54</td>
</tr>
<tr>
<td>Extract A</td>
<td>80.0</td>
<td>54.2</td>
</tr>
<tr>
<td>Extract B</td>
<td>2.69</td>
<td>0.98</td>
</tr>
<tr>
<td>Extract C</td>
<td>3.59</td>
<td>25.0</td>
</tr>
<tr>
<td>Extract D</td>
<td>0.27</td>
<td>5.45</td>
</tr>
</tbody>
</table>

Der 1, combined total of Der p 1 and Der f 1; Der 2, combined total of Der p 2 and Der f 2; JSA, JSA reference house dust mite extract.

1 In vitro total IgE binding potency relative to the Japanese Society of Allergology (JSA) reference house dust mite extract was determined on the basis of the results of a competition ELISA that measures the inhibition of allergen-specific IgE binding to plates coated with the JSA reference house dust mite extract (JSA coating) or Extract C (Extract C coating).

Figure 2. Correlation between house dust mite major allergen contents and in vitro total IgE binding potencies of the eight house dust mite extracts determined in Laboratory 2. The values in Table 3 were used for the analysis. In vitro total IgE binding potency relative to the Japanese Society of Allergology (JSA) reference house dust mite extract was determined based on the results of the competition ELISA that measured the inhibition of allergen-specific IgE binding to plates coated with the JSA reference house dust mite extract (A) or Extract C (B). r: Pearson correlation coefficient. *p < 0.01.
the HDM extract useful as the standard antigen for ELISAs that measure the amounts of Der p 1 and Der f 1.

Acknowledgments

The authors thank CBER/FDA for supplying FDA reference HDM extracts and FDA reference HDM-allergic serum; ALK-Abello AS and Stallergenes SA for supplying HDM allergen extracts; and Torii Pharmaceutical Co., Ltd. and Shionogi & Co., Ltd. for their communication with ALK-Abello AS and Stallergenes SA, respectively.

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Conflict of interest
YO received lecture fees from Torii, Shionogi, Kyowa Kirin, MSD, GlaxoSmithKline (GSK), and Kyorin, and research funding from the Japanese Rhinologic Society and GSK. KO received lecture fees from Torii, GSK, MSD, Mitsubishi Tanabe, Ono, and Kyowa Kirin, and research funding from GSK. KN received lecture fees from MSD and AstraZeneca. KM received lecture fees from GSK, MSD, and Kyorin, and research funding from the Ministry of Health, Labour, and Welfare of Japan.

Authors’ contributions
TT: study design and interpretation of the in vitro study, and drafting and revision of the manuscript; YO, KO, MN, and KM: study design, data acquisition, analysis and interpretation of intradermal testing, and drafting and revision of the manuscript; MS, YF, and HY: study design, data acquisition, analysis and interpretation of the in vitro study, and drafting and revision of the manuscript; AS: data acquisition and analysis of the in vitro study.

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