Quantification of Der f 1 in houses of patients allergic to house dust mite, *Dermatophagoides farinae*, using a locally produced detection reagents

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Summary

Background: **House dust mite (HDM) allergen quantification in house dust samples before and after the allergen elimination is one means of convincing the target population about the health benefits of allergen removal from their environment.**

Objective: **To produce local reagents for quantification of Der f 1 (major allergen of *Dermatophagoides farinae*) in dust samples from houses of HDM allergic Thai patients.**

Methods: **Recombinant Der f 1 was used for immunization of a BALB/c mouse for hybridoma production. Polyclonal antibody (PAb) to whole body extract of *D. farinae* was prepared from an immunized rabbit. A sandwich ELISA (MAb-allergen-PAb) was used, in comparison with the commercialized reagents (Indoor Biotechnology, UK), to quantify Der f 1 in dust samples.**

Results: **Two hybridoma clones, Df1-1 and Df1-2, were established. Their secreted MAbs (MAbDf1-1 and MAbDf1-2, respectively) bound to the homologous antigen as well as native Der f 1 and a crude extract of *D. farinae*. Epitopes of MAbDf1-1 and MAbDf1-2 were located at amino acid residues 206NSQHYGISNYCQ217 and 283DYW---NSWD—WGDSG298 of Der f 1. MAbDf1-1 had higher affinity to Der f 1 than the MAbDf1-2. A sandwich ELISA (MAbDf1-1-allergen-PAb) and commercialized reagents (MAb1-allergen-MAb2 sandwich ELISA) were used in comparison for quantification of Der f 1 in 42 dust samples collected from bedrooms and living rooms of 21 houses of the HDM allergic patients. All of the 42 dust samples measured by both ELISAs had the Der f 1 levels higher than 2 mg per gram of fine dust which is the HDM allergy sensitizing level. In addition, Der f 1 levels in 41 samples (except 1 sample from a living room) measured by the MAbDf1-1-PAb and MAb1-MAb2 sandwich ELISAs were higher than 10 mg per g of dust which is the morbidity level of HDM allergen. The local sandwich ELISA showed a high coefficient correlation (r = 0.91) in measuring known amounts of recombinant and native Der f 1. The results indicate that the reagents produced in the present study can be used for measuring the environmental levels of HDM Der f 1. The assay can also be used for standardization of the HDM extract for monitoring patient's allergenic status or for immunotherapeutic purpose. *(Asian Pac J Allergy Immunol 2011;29:78-85)***

Key words: *House dust mite (HDM), allergen, allergy,* Dermatophagoides farinae, *Der f 1, hybridoma, monoclonal antibody, ELISA, recombinant, quantification test kit, allergic patients*

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Introduction

House dust mite (HDM) allergen avoidance has been accepted as an effective measure for HDM allergy intervention[1,2]. The goals of the allergen avoidance include prevention of HDM sensitization (primary prevention), prevention of clinical asthma in HDM-sensitive subjects (secondary prevention) and reduction of asthma severity due to HDM (tertiary intervention)[3]. Allergen avoidance can be managed by decreasing HDM allergens in the environment by

using chemical and/or physical measures[4-8]. Nevertheless, the allergen reduction tends to have low compliance in the general population, particularly allergic subjects and/or their families as most people are not aware of the allergens in their dwellings. Comprehensive instruction on how to avoid the allergens should be implemented and attempts made to persuade patients to comply. Demonstration of the actual levels of HDM allergens in dust samples collected from individual houses before and after the allergen elimination is one means of convincing the target population about the health benefits of allergen removal from their environment.

Currently HDM allergen quantification in Thailand is carried out by using imported reagents which are not only expensive but also in limated supply. Thus, in this study, a mouse monoclonal antibody (MAb) specific to recombinant Der f 1 (rDer f 1) of *Dermatophagoides farinae* which reacted readily to the native Der f 1 and rabbit polyclonal antibody to the *D. farinae* whole body extract (PAb) were produced. The MAb and PAb were used in a sandwich ELISA (MAb-allergen-PAb) for quantification of the allergen in dust samples collected from houses of *D. farinae* allergic subjects.

Methods

Animal experiments

All animal experiments in the present study were approved by Siriraj Animal Care and Use Committee (SI-ACUP) no. 008/2553.

Hybridoma production. Bacterially expressed rDer f 1 in PBS (10 µg) was mixed with alum (Pierce, USA) in a ratio 1:3. The protein-adjuvant mixture (200 µl) was injected intraperitoneally into a BALB/c mouse (~6 week old). Three booster doses were given to the primed mouse at 10 day intervals. Seven days after the last booster the mouse was bled and serum antibody titer was determined against the homologous protein by indirect ELISA.[9] The mouse was then injected intravenously with 10 µg rDer f 1 in 200 µl PBS. Four days after the intravenous immunization, the mouse was bled and the serum was collected (immune serum to rDer f 1; IS). Single spleen cells of the mouse were fused with P3x-63-Ag8.653 mouse myeloma cells (~10:1) by using polyethylene glycol 4000 as a fusogen. Hybridoma clones secreting monoclonal antibodies (MAb) reactive to only rDer f 1 and crude extract of *D. farinae* were established as previously described[9].

Rabbit polyclonal antibody to a crude extract of D. farinae. Live *D. farinae* (weight ~1 g) was homogenized in 4 ml PBS using a sonicator (Model VC750, Vibra Cell™, USA) at 30% amplitude at 2.5 seconds pulse-on and 2 seconds pulse-off for 15 minutes. The preparation was centrifuged at 12,000 x *g*, 4 °C for 10 minutes and the supernatant (crude extract of *D. farinae*) was collected. A New Zealand white rabbit weighing about 1.5 kg purchased from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom province, Thailand, was immunized intramuscularly with crude extract of *D. farinae* (1 mg) mixed with alum. The rabbit was given 4 booster doses at 14 day intervals. The animal was bled on day 7 after the last booster. Total immunoglobulin was precipitated out from the antiserum by using ammonium sulfate, dialyzed against PBS, and the protein content of the PAb preparation was adjusted to 1 mg per ml.

Preparation of native Der f 1. Specific MAb to rDer f 1 derived from a hybridoma grown in a serum free medium was used to prepare affinity CNBr-Sepharose resin (5 mg per g of resin). The method described previously was followed throughout for the native Der f 1 preparation*1*[9].

SDS-PAGE and Western blot analysis

Antigen was separated in 12% SDS-PAGE[10]. The separated antigen was either stained by Coomassie Brilliant Blue G-250 dye for protein band revelation or was electroblotted onto a nitrocellulose membrane (NC). Western blot analysis was then performed. Mouse monoclonal anti-6x-His (Santa Cruz, USA) was used as the primary antibody for determining 6x-tagged rDer f 1 in lysate of *der f 1*-plasmid-transformed *E. coli*. In Western blotting for screening the antigenic specificity of MAb, the NC blotted with SDS-PAGE separated-rDer f 1, nDer f 1 or *D. farinae* crude extract was probed with the MAb; the antigen-antibody reactive band was revealed by using goat anti-mouse immunoglobulin-alkaline phosphatase (AP) conjugate and BCIP/NBT chromogenic substrate.

Determination of the antigen binding affinity of specific MAb to Der f 1

The affinity of MAb specific to Der f 1 was determined as previously described[11,12]. Briefly, each MAb (fixed amount) was mixed with

varying amounts of rDer f 1 in tubes. After a rocking (250 rpm) incubation at 37°C for 1 hour, the content of each tube (100 µl) was added to wells on an ELISA plate coated with rDer f 1 (1 µg per well) and incubated at 37°C for 1 hour. Thereafter, the content of each well was discarded and the wells were washed thoroughly with PBST. The amount of the MAb that bound to the immobilized rDer f 1 was determined by adding goat anti-mouse immunoglobulin-HRP conjugate and HRP chromogenic substrate. The OD of the content of each well was determined at an absorbance of 405 nm against blank which was the content of a well to which the MAb diluent was added to the immobilized rDer f 1 instead of the MAb. The dissociation constant (K_d) of the MAb was calculated from a Klotz plot by linear regression analysis.

Determination of the mimotope of Der f 1 specific MAb

Mimotopes of the Der f 1 specific MAb were determined as previously described[13] by using a Ph.D.-12™ Phage Display Peptide Library Kit (Promega, USA)] which consisted of M13 filamentous phages that displayed a large repertoire of 12-mer peptides on their surfaces. Briefly, wells of a micro-ELISA plate were coated individually with 1 µg of MAb from hybridoma clones and the plate was kept at 4°C overnight. After washing away the unbound protein with Tris buffered saline (TBS) containing 0.1% Tween-20 (TBST), each well was blocked with 300 µl of 0.5% BSA in TBS. After keeping the plate at 4°C for 1 hour and washing away the blocking protein with TBST, 100 µl of peptide-displaying phages (~1.5 x 10^{11} plaque forming units) were added to each MAb coated well. The phages were allowed to bind to the MAb; thereafter, unbound phages were removed by washing the wells extensively with TBST. The MAb bound phages were eluted with 0.2 M glycine-HC, pH 2.2 containing 1% BSA and propagated in K12 ER2738 *E. coli*. Three rounds of the phage bio-panning were performed. After the third round of the bio-panning, the eluted phages diluted to 10^{-2}-10^{-4} were used to transfect ER2738 *E. coli* in top agarose on an LB agar plate containing IPTG and X-gal. Isolated blue plaques were picked randomly, inoculated individually into 1 ml of LB broth and incubated at 37°C with shaking at 250 rpm for 4 hours. The bacterial cells were removed by centrifugation and phages in the supernatant were collected by precipitation with PEG/NaCl. The phage DNA was extracted by using phenol/chloroform and absolute ethanol precipitation. DNA coding for the 12-mer peptide of each phage clone was sequenced using M13-96gIII sequencing primer (New England Biolabs, UK). Amino acid sequences were deduced and subjected to multiple alignment using ClustalW2 on the EBI website[14] in order to reveal the phage mimotope peptides. MAb mimotope sequences were then aligned with the rDer f 1 peptide sequence of the present study as well as the Der f 1 sequences of the NCBI database by using ClustalW2.0.12 multiple sequence alignment for determining the MAb epitopes.

House dust collection

The experiment was approved by Siriraj Ethical Committee (no. SIEC: 096/2550). Dust samples were collected individually from 21 houses of *D. farinae* allergic patients who were positive by skin prick test to crude extract of *D. farinae*[15]. Dust sample collection was performed by the same person using the same dust collector. Each sample collection time was two minutes from an area of one square meter in bedroom and living room in each house. The course dust sample was strained through a fine strainer and 100 mg of the fine dust powder obtained was mixed with 2 ml of PBS containing 0.05% Tween-20 (PBST). The preparation was centrifuged at 5,000 x *g*, at 25°C for 10 minutes. The supernatant (dust extract) was collected and kept in small aliquots at -20°C until they were used in a sandwich ELISA for quantifying the amounts of Der f 1. This was performed in triplicate using both the locally made reagents and the commercial reagents (Indoor biotechnologies, USA).

Indirect ELISA

Indirect ELISA was used for testing specificity and determining the titer of MAb secreted by the hybridoma clones and for determining the antibody titer of the rabbit antiserum to rDer f 1. The rDer f 1 (1 µg in 100 µl of carbonate-bicarbonate buffer, pH 9.6) was immobilized in each well of a microtiter plate (Corning, USA). The plate was kept at 37 °C for 18 hours. Unbound materials were washed away by using a wash buffer (PBST). The ELISA wells were blocked by adding 200 µl of 1% bovine serum albumin (BSA) (w/v) in PBS at 37 °C for 1 hour.

After washing, hybridoma spent fluids and diluted rabbit PAb were added to appropriate wells and the plate was incubated at 37 °C for 1 hour. All wells were then washed with 100 µl of 1:5,000 goat-anti-mouse/goat anti-rabbit immunoglobulin-HRP conjugate (Southern Biotech, USA) and ABTS substrate (KPL, USA) was added sequentially. The enzyme-substrate reaction was allowed to occur at 25 °C for 20 minutes. The OD_{405nm} of the content from each well was determined against the background-- wells added with RPMI medium. The positive control was a well with rabbit PAb added to rDer f 1. A positive ELISA occurred when the content of well gave an OD_{405nm} equal to or higher than 0.05 above background.

Sandwich ELISA

The sandwich ELISA (MAb-allergen-PAb) for quantification of Der f 1 was performed as previously described.[16] Briefly, each well of an ELISA plate (Corning, USA) was coated with 4 µg of Der f 1 specific MAb in 100 µl of carbonate-bicarbonate buffer, pH 9.6. After incubation at 37°C for 18 hours, the wells were washed and blocked with 1% BSA in PBST, 100 µl of each dust extract was added to each and they were incubated at 37°C for one hour. After incubation, all wells were washed with PBST, 100 µl of PAb (diluted 1:1,000) was added to each well and the plate was incubated at 37°C for one hour. Excess PAb was removed by washing and the color reaction was developed by using goat anti-rabbit immunoglobulin-HRP conjugate (Santa Cruz, USA) with a peroxidase substrate. The OD_{405nm} against blank (a well to which PBS was added instead of the dust sample) was determined with an ELISA reader (Multiscan *EX*; LabSystem, Helsinki, Finland). Series of different amounts of rDer f 1 and nDer f 1 were included in the same plate as allergen standards. The amount of Der f 1 in the dust sample was extrapolated from the respective standard curves constructed by plotting the amounts of Der f 1 standards against the read-out sandwich ELISA OD_{405nm}.

The amount of Der f 1 in each sample was also determined concurrently by sandwich ELISA (MAb1-allegen-MAb2) using commercial reagents (Indoor Biotechnologies, USA).

Statistical analysis

A paired *t*-test was used for comparing the amounts of Der f 1 in the dust samples determined by the sandwich ELISA using rDer f 1 and nDer f 1 as standards and the amounts of Der f 1 in dust samples detected by the commercialized reagents and the locally made reagents. The level of statistical significant difference was $p < 0.05$.

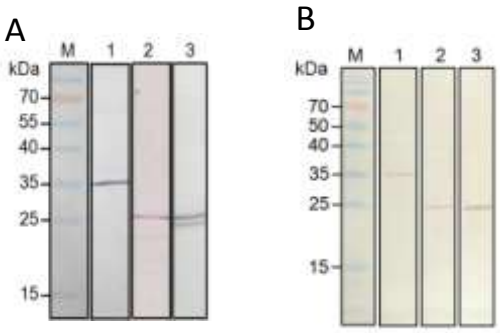

Figure 1. (A), Western blot patterns of rDer f 1 probed with anti-6x-Histidine tag (lane 1) and nDer f1 and *D. farinae* extract probed with MAbDf1-1 (lanes 2 and 3, respectively). (B), Western blot patterns of rDer f 1 probed with anti-6x-Histidine tag (lane 1) and nDer f1 and *D. farinae* extract probed with MAbDf1-2 (lanes 2 and 3, respectively). Lane M, standard protein marker. Numbers at the left of both blocks are the relative molecular masses of proteins in kDa.

Results

Spleen cells (1.3×10^8 cells) of the immune BALB/c mouse (serum antibody titer 1:51,200 against rDer f 1) were fused with the myeloma cells. Culture fluids of 35 from 75 wells (40%) which showed growing cells gave positive indirect ELISA to the homologous antigen, rDer f 1. After cloning the cells of only 6 hybridomas, which were designated clones of Df1-1 to Df1-6, retained the antibody secreting activity. The MAb of clones of Df1-3 to Df1-6 also bound to crude extracts of BL21(DE3) *E. coli* which was used as the rDer f 1 expression host. The MAb of clones Df1-1 and Df1-2 reacted to the rDer f 1 and the nDer f 1 and *D. farinae* whole body extract but they did not react to other heterologous antigens including extracts of BL21(DE3) *E. coli*, *D. farinae*, *D. pteronyssinus* and American cockroach, *Periplaneta americana*. The immunoglobulin isotype of both clones was IgG1. The antigenic specificity of the MAb of clones Df1-1 (MAbDf1-1) and Df1-2 (MAbDf1-2) against SDS-PAGE separated- rDer f 1, nDer f 1 and *D. farinae* extract by Western blot analysis are shown in Figure 1.

The dissociation constant (K_d) of MAbDf1-1 and MAbDf1-2 to rDer f1 was 0.31×10^{-8} M and

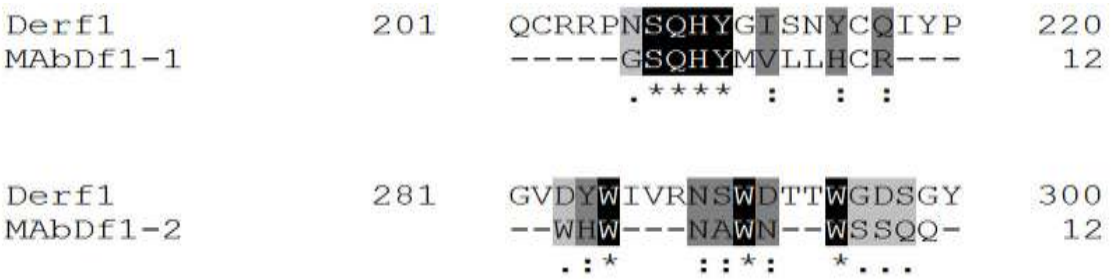

Figure 2. Alignment of mimotopes of MAbDf1-1 and MAbDf1-2 with deduced amino acids of Der f 1 (accession nos. EU095368 and EF139428) Black shading and *****, identical amino acids; dark grey shades and "**:**", conserved amino acid substitution; and light grey shades and **"."**, semi-conserved amino acid substitution.

0.18 x 10^{-8} M, respectively, while the K_d of MAbDf1-1 and MAbDf1-2 to nDer f 1 was 0.48 x 10^{-8} M and 0.22 x 10^{-8} M, respectively.

The deduced peptide sequences of the 12 mer peptides displayed on the phage clones that bound to MAbDf1-1 and MAbDf1-2 were GSQHYMVLLHCR and WHWNAWNWSSQQ, respectively. When these mimotope sequences were aligned with the deduced amino acid sequences of rDer f 1 of the database (accession nos. EU095368 and EF139428), it was found that the mimotope sequence of MAbDf1-1 matched with the amino acids 206NSQHYGISNYCQ217 of the Der f 1 while the mimotope sequence of MAbDf1-2 matched with amino acids 283DYW---NSWD—WGDSG299 (Figure 2). Thus, the peptides NSQHYGISNYCQ and DYW---NSWD—WGDSG should contain epitopes of the MAbDf1-1 and MAbDf1-2, respectively.

The lower limit of the sandwich ELISA for detecting rDer f 1 and nDer f 1 using the MAbDf1-1 as the allergen capture reagent was 8 ng/g of dust.

Levels of Der f 1 in house dust samples collected from bedrooms and living rooms of the *D. farinae* allergic patients determined by using the locally made reagents (MAbDf1-1-allergen-PAb) and rDer f 1 and nDer f 1 as standards in comparison to the measurement by using the commercial reagents are shown in Table 1. The means (X) ± SD and ranges of the amounts of Der f 1 per g of dust in bedrooms measured by MAbDf1-1 based sandwich ELISA and extrapolated from a standard curve constructed from values for varying amounts of rDer f 1 and nDer f 1 were 40.31 ± 19.83 µg (range 10.03-84.44 µg) and 33.55 ± 13.07 µg (range 15.63-60.89 µg). The levels measured by using the commercialized reagents were 29.92 ± 13.24µg (range 10.90-60.22 µg). For living room samples measured by the MAbDf1-1 based sandwich ELISA and extrapolated from standard curve constructed from values for varying amounts of rDer f 1 and nDer f 1 were 29.90 ± 10.89 µg (range 10.02-43.83 µg) and 25.35 ± 8.39 µg (range 10.22-40.17 µg). The levels measured in living room samples measured by the commercial reagents were 25.60 ± 9.86 µg (range 4.12-42.49 µg) per g of dust.

The correlation coefficient (r) of Der f 1 amounts in dust samples measured by the MAbDf1-1 based-sandwich ELISA using rDer f 1 and nDer f 1 as standards were 0.91 (Figure. 3A). Nevertheless, the r values of Der f 1 in dust

Table 1. Levels of Der f 1 in house dust samples collected from bedrooms and living rooms of *D. farinae* allergic patients determined by using the locally made reagents (MAbDF1-1-allergen-PAb) using rDer f 1 and nDer f 1 as standards in comparison to the measurement using the commercial reagents

Parameter	Bed room (µg/g of dust)			Living room (µg/g of dust)		
	In house (rDer f 1 as std.)	In house (nDer f 1 as std.)	Commercial reagents	In house (rDer f 1 as std.)	In house (nDer f 1 as std.)	Commercial reagents
Range	10.03-84.44	15.63-60.89	10.90-60.22	10.02-43.82	10.22-40.17	4.12-42.49
Mean	40.31	33.55	29.92	29.90	25.35	25.60
SD	19.83	13.07	13.24	10.89	8.39	9.86

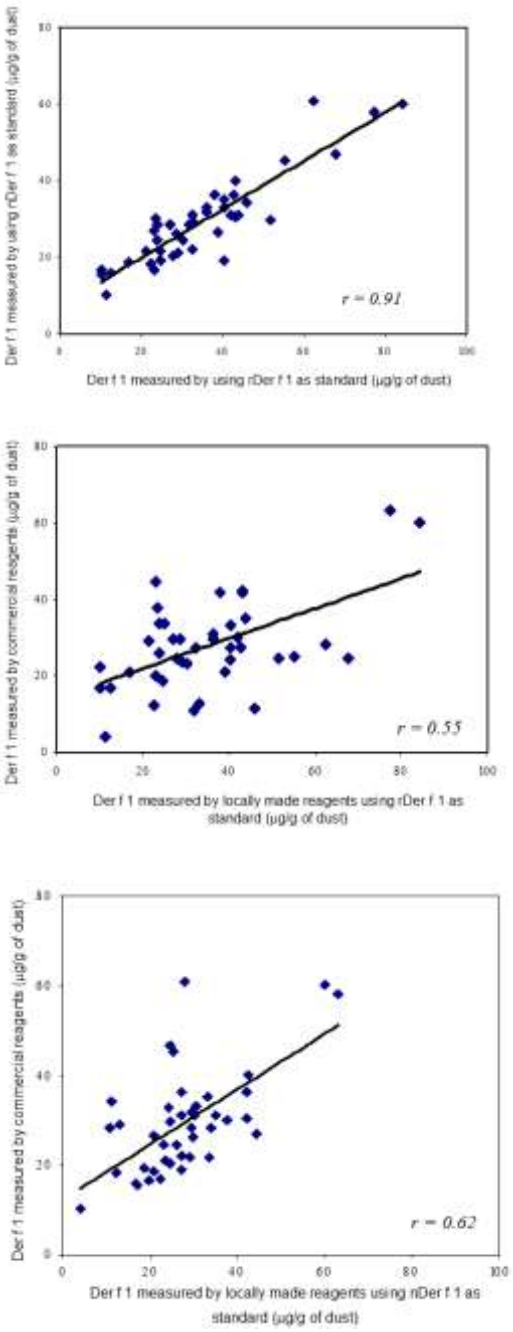

Figure 3. Correlation coefficient (*r*) of Der f 1 amounts in dust samples measured by: A, the MAbDf1-1 based-sandwich ELISA using rDer f 1 and nDer f 1 as standards (*r* = 0.91); B, the MAbDf1-1 based- and the commercial reagent based-ELISAs using rDer f 1 as standard (*r* = 0.55); and C, the MAbDf1-1 based- and commercial reagent based-ELISAs using the nDer f 1 as standard (*r* = 0.62).

samples quantified by the MAbDf1-1 based-using rDer f 1 as standard and commercial reagents based-ELISA was 0.55 (Figure. 3B), while the *r* values using the nDer f 1 as standard compared with the commercial reagents based-ELISA was 0.62 (Figure. 3C).

All 42 dust samples from the 21 houses measured by using the MAbDf1-1-based sandwich ELISA and the rDer f 1 as well as the nDer f 1 as standards contained >10 µg per g of dust, while the samples measured by commercialized reagent revealed that 41 of 42 samples contained Der f 1 >10 µg per g of dust and one sample from a living room contained 4.12 µg Der f 1 per g of dust.

Discussion

Incidence of HDM allergy has increased and become an important non-infectious ailment that places a big burden on the healthcare systems of various countries worldwide including Thailand[17,18,19]. One of the reasons for the increase of the incidence of allergy might be changes in people's life styles. There has been more urbanization, with people moving to big cities where air-conditioning is used in preference to fresh air ventilation in workplaces and dwellings, conditions which favor the growth of HDM fauna. HDM allergen avoidance is one of the most effective intervention measures for preventing naïve individuals from being sensitized and as an intervention for those that have been sensitized to prevent allergic morbidity. Allergen quantification reagents are needed for measuring the levels of HDM allergens, especially the major allergenic components. However, such reagents not only have limitedly availablity from only one or two commercial sources but are also expensive, which have prevented the HDM allergen quantification from being practiced widely. For this reason, in the present study, locally made sandwich ELISA reagents for measurement of the major HDM allergen, Der f 1, levels were produced.

Two hybridoma clones secreting IgG1 MAb (MAbDf1-1 and MAbDf1-2) that reacted specifically to Der f 1, both recombinant and native, as well as crude extract of *D. farinae* were

established using conventional hybridoma technology. The MAb epitopes were indirectly identified by first identifying the phage mimotopes in order to obtain the mimotope sequences and secondly the mimotope sequences were aligned with the Der f 1 peptide for epitope determination. Epitopes of the MAbDf1-1 and MAbDf1-2 are located at amino acid residues 206-217 and 283-299 of Der f 1 molecule, respectively. Currently, it is not known how important these residues are for the functional activity of the native Der f 1. Nevertheless, the MAb that is specific for these Der f 1 peptides can be used as a biological tool for *in situ* localization of the protein or for standardization of the Der f 1 B cell epitopes in the HDM extract which is important and useful for patient screening and designing an immunotherapeutic reagent.

Although the two MAb bound with relatively high affinity to Der f 1, the MAbDf1-1 was selected for use in the sandwich ELISA for quantification of Der f 1 in dust samples simply because 1) the hybridoma Df1-1 grew better and at the stationary phase of growth the cells produced a higher indirect ELISA antibody titer than the clone Df1-2 and 2) the MAbDf1-1 has a slightly higher affinity to the target antigen (K_d = 0.31 x 10^{-8} M to rDe f 1) than the MAbDf1-2 (K_d = 0.18 x 10^{-8} M to rDer f 1).

Rabbit immune serum against the rDer f 1 produced in the present study reacted readily and equally with the homologous antigen and the native Der f 1 when tested in the indirect ELISA using the two proteins as antigens, implying that most epitopes, linear and conformational, of the native Der f 1 were conserved in the recombinant counterpart. Moreover the coefficient correlation (*r*) of Der f 1 amounts in dust samples measured by the MAbDf1-1 based-sandwich ELISA using rDer f 1 and nDer f 1 as standards was 0.91, indicating that the recombinant HDM allergen, which can be produced economically and adequately without HDM culture and extraction, can be used as an alternative standard of the native Der f 1 in the assay which would reduce the overall cost of the quantification reagents. Details of the production of the bacterially expressed Der f 1 which resembles the native HDM Der f 1 will be published elsewhere.

The relatively low *r* values between the locally made sandwich ELISA and the commercial reagents in quantification of the Der f 1 allergens in the dust samples reflects the different reagents from the two sources. The sandwich ELISA of the present study used MAbDf1-1 as an allergen capture reagent and the rabbit PAb as the detection reagent, while the commercial test kit used MAb1 and MAb2 as the capture and detection reagents, respectively. Moreover, different antibody affinity and abundance of the target epitopes might also contribute to the low *r* values between the two assays.

There are threshold levels of HDM allergen for sensitization of the naïve individuals (2 µg per g of dust) and disease manifestation/aggravation in the sensitized subjects (10 µg per g of dust)[17,20]. Almost all of the dust samples collected from homes of HDM allergic Thai subjects, when measured by using the locally made and commercial reagents, revealed >10 µg per gram of dust indicating that the household's inhabitants were at high risk of clinical manifestations, which might be as serious as hospitalization for or death from asthma.

In conclusion, the overall results indicate that the locally made MAbDf1-1 and the rabbit PAb will be useful and cost effective reagents for assessing the level of the major HDM allergen for exposure intervention as well as for epidemiological work

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References

1. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house dust- mite allergen (Der p 1) and the development of asthma in children. New Eng J Med. 1990; 323: 502-7.
2. Ehnert B, Lau-Schadendorf S, Weber A, Buettner P, Schou C, Wahn U. Reducing domestic exposure to dust mite allergen reduces bronchial hyperreactivity in sensitive children with asthma. J Allergy Clin Immunol. 1992; 90: 135-8.
3. Boner A, Pescollderungg L, Silverman M. The role of house dust mite elimination in the management of childhood asthma: an unresolved issue. Allergy 2002; 57: 23-31.
4. Dietemann A, Bessot JC, Hoyet C, Ott M, Verot A, Pauli G. A double-blind, placebo controlled trial of solidified benzyl benzoate applied in dwellings of asthmatic patients sensitive to mites: clinical efficacy and effect on mite allergens. J Allergy Clin Immunol. 1993; 91: 738-46.
5. Woodfolk JA, Hayden ML, Miller JD, Rose G, Chapman MD, Platts-Mills TA. Chemical treatment of carpets to reduce allergen: a detailed

study of the effects of tannic acid on indoor allergens. J Allergy Clin Immunol. 1994; 94: 19-26.
6. Lee IS. Effect of bedding control on amount of house dust mite allergens, asthma symptom, and peak expiratory flow rate. Yonsei Med J. 2003; 44: 313–322.
7. Moon JS, Choi SO. Environmental controls in reducing house dust mites and nasal symptoms in patients with allergic rhinitis. Yonsei Med. 1999; 40: 238-43.
8. Vojta PJ, Randels SP, Stout J, Muilenberg M, Burge HA, Lynn H, et al. Effects of physical interventions on house dust mite allergen levels in carpet, bed, upholstery dust in low-income, urban homes. Env Health Perspect. 2001; 109: 815-9.
9. Sookrung N, Diraphat P, Chaicumpa W, Tongtawe P, Sakolvaree Y, Tapchaisri P,et al. Cockroach allergen detection and cockroach allergens of allergic Thai patients. Asian Pac J Allergy Immunol. 2003; 21: 1-9.
10. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227: 680-5.
11. Friguet B, Chaffotte AF, Djavadi-Ohaniance L, Goldberg ME. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay. J Immunol Methods. 1985; 77: 305-19.
12. Thanongsaksrikul J, Srimanote P, Maneewatch S, Choowongkomon K, Tapchaisri P, Makino S, et al. A V_HH that neutralizes the zinc metalloprotease activity of botulinum neurotoxin type A. J Biol Chem. 2010; 285: 9657-66.
13. Kulkeaw K, Sakolvaree Y, Srimanote P, Tongtawe P, Maneewatch S, Sookrung N, et al. Human monoclonal ScFv neutralize lethal Thai cobra, *Naja kaouthia*, neurotoxin. J Proteomics. 2009; 72: 270-82.
14. http://ebi.ac.uk/Tools/clustal w2/. Accessed on Dec 2010.
15. Tungtrongchitr A, Sookrung N, Indrawattan N, Sae-Lim J, Pooduang S, Phonrat B, Wanachiwanawin D, Chaicumpa W. Seasonal levels of the major American cockroach allergen Per a 9 (arginine kinase) in Bangkok and their relevant for disease severity. Asian Pac J Allergy Immunol. 2009; 27: 1-7.
16. Sookrung N, Chaicumpa W, Tungtrongchitr A, Vichyanond P, Bunnag C, Ramasoota P, et al. *Periplaneta americana* arginine kinase as a major cockroach allergen among Thai patients with major cockroach allergy. Env Health Perspeck. 2006; 114: 875-80.
17. Platts-Mills TAE, de Weck AL. Dust mite allergens and asthma – a worldwide problem. J Allergy Clin Immunol. 1989; 83: 416-27.
18. Pumhirun P, Towiwat P and Mahakit P. Aeroallergen sensitivity of Thai patients with allergic rhinitis. Asian Pac J Allergy Immunol. 1997; 15:183-5.
19. Thomas WR. Geography of house dust mite allergens. Asian Pac J Allergy Immunol. 2010; 28: 211-24.
20. Platts-Mills TAE, Thomas WR, Alberse RC, Vervloet D, Chapman MD. Dust mite allergens and asthma: report of the second international work-shop. J Allergy Clin Immunol. 1992; 89: 1046-60.