Published in final edited form as:

Clin Exp Allergy. 2011 October ; 41(10): 1468–1477. doi:10.1111/j.1365-2222.2011.03798.x.

Molecular characterization of Der p 10: a diagnostic marker for broad sensitization in house dust mite allergy

Y. Resch¹, M. Weghofer¹, S. Seiberler¹, F. Horak², S. Scheiblhofer³, B. Linhart¹, I. Swoboda¹, W. R. Thomas⁴, J. Thalhamer³, R. Valenta^{1,5}, and S. Vrtala^{1,6}

¹Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

²Allergy Centre Vienna West, Vienna, Austria

³Department of Molecular Biology, Division of Allergy and Immunology, University of Salzburg, Salzburg, Austria

⁴Center of Child Health Research, University of Western Australia, Telethon Institute for Child Health Research, Perth, Australia

⁵Christian Doppler Laboratory for Allergy Research, Medical University of Vienna, Vienna, Austria

⁶Christian Doppler Laboratory for the Development of Allergen Chips, Medical University of Vienna, Vienna, Austria

Summary

Background—Tropomyosins represent clinically relevant seafood allergens but the role of mite tropomyosin, Der p 10, in house dust mite (HDM) allergy has not been studied in detail.

Objective—To express and purify a recombinant Der p 10 with equivalent IgE reactivity as natural Der p 10 and to evaluate its IgE reactivity and allergenic activity in HDM-allergic patients.

Methods—rDer p 10 was expressed in *Escherichia coli*, purified and characterized by mass spectrometry and circular dichroism. It was tested for IgE reactivity in 1322 HDM-allergic patients. Detailed IgE-reactivity profiles to six HDM allergens (Der p 1, 2, 5, 7, 10, 21) were established for subgroups of Der p 10-positive and -negative patients. The allergenic activity of rDer p 10 was evaluated in basophil degranulation experiments.

Results—rDer p 10 is an α -helical protein sharing IgE epitopes with nDer p 10. It is recognized by 15.2% of HDM-allergic patients. Der p 10-negative patients were primarily sensitized to Der p 1 and/or Der p 2, whereas Der p 10-positive patients reacted to several other HDM allergens besides the major allergens (Der p 1, Der p 2) or showed a rather selective Der p 10 reactivity. The

Correspondence: Dr S. Vrtala, Christian Doppler Laboratory for the Development of Allergen Chips, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.
susanne.vrtala@meduniwien.ac.at.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

allergenic activity of Der p 10 was generally low but patients could be identified who suffered from clinically relevant HDM allergy due to Der p 10 sensitization.

Conclusion and Clinical Relevance—Der p 10 may be a diagnostic marker for HDM-allergic patients with additional sensitization to allergens other than Der p 1 and Der p 2. Such patients may require attention when allergen-specific immunotherapy is considered.

Keywords

Der p 10; house dust mite allergy; immunotherapy; recombinant allergens; tropomyosin

Introduction

House dust mites (HDMs), especially the species *Dermatophagoides pteronyssinus* and *D. farinae*, are a frequent cause of allergy world-wide, with around 20% of the population and more than 50% of allergic patients being sensitized to them [1, 2]. HDM allergens are among the most common triggers of asthma and allergic rhinitis [3, 4]. The frequency of IgE recognition, allergenic activity and clinical relevance of the major HDM allergens, Der p 1 and Der p 2, has been well investigated [5–8]. Both allergens are recognized by more than 90% of HDM-allergic patients and are considered as the major HDM allergens [5]. More than 20 other mite allergens have been identified but their importance for mite allergy has only partly been studied [9]. Among these allergens, Der p 10, a tropomyosin, has received attention because it occurs as a cross-reactive allergen in invertebrates [10] and, in particular, in seafood, where it has been described as an important allergen responsible for severe systemic anaphylaxis [11].

Although the first cDNA coding for an HDM tropomyosin Der f 10 was isolated already in 1995 [12], information regarding the prevalence of recognition and importance of HDM tropomyosins is rare or contradictory. In the initial study describing Der f 10, a prevalence of IgE recognition of 80.6% was reported, whereas for Der p 10, much lower IgE-binding frequencies (5–18%) were described [5, 13, 14]. However, in an African population, 55% of the tested mite-allergic patients showed IgE reactivity to Der p 10 [15]. Der p 10 has not been studied in large populations of HDM-allergic patients. We therefore expressed recombinant *D. pteronyssinus* tropomyosin as a biologically active recombinant non-fusion protein in *Escherichia coli* that equals its natural counterpart. We then screened sera from more than 1300 HDM-allergic patients for IgE reactivity to rDer p 10 and performed a detailed analysis of the IgE-reactivity profiles to various HDM allergens in subgroups of Der p 10-positive and Der p 10-negative patients. The allergenic activity of rDer p 10 was compared with that of clinically important and highly allergenic HDM allergens, Der p 1 and Der p 2, in basophil degranulation assays. Our results indicate that Der p 10-positive patients differ substantially from Der p 10-negative patients regarding their sensitization profiles to HDM allergens, which may have implications for specific immunotherapy treatment.

Methods

Patients' sera

One thousand and three hundred and twenty-two sera from HDM-allergic patients living in or in the surrounding area of Vienna, Austria, were randomly picked from the serum bank of an allergy clinic. For these persons, the diagnosis of HDM allergy was established based on respiratory symptoms (i.e. rhinitis and/or asthma) to *D. pteronyssinus*, positive skin prick test (SPT) reactivity to *D. pteronyssinus* [16, 17] and/or the presence of mite-specific IgE antibodies as determined by ImmunoCAP (Phadia AB, Uppsala, Sweden) or IgE reactivity to nitrocellulose-blotted *D. pteronyssinus* extract [14]. Sera from all 1322 mite-allergic patients were tested for IgE reactivity to dot-blotted Der p 10. An extensive assessment of IgE reactivities to purified HDM allergens nDer p 1, rDer p 2, rDer p 5, rDer p 10 and rDer p 21 was performed for patients from whom serum volumes 200 µL were available [i.e. 35 rDer p 10-positive (A1–A35) and 27 Der p 10-negative patients (B1–B27)]. Additional five of the Der p 10-positive sera (A36–A40) were used for ELISA and ELISA inhibitions comparing rDer p 10 and nDer p 10. Serum from a non-allergic individual was used as a control. Table 1 displays the demographic, serological and clinical characteristics of these patients.

Purified house dust mite allergens and allergen-specific rabbit antibodies

Natural Der p 1 was isolated by affinity chromatography [18]. Recombinant Der p 2 was expressed in *E. coli* with a C-terminal hexa-histidine tag using the pET17b expression system (Novagen, Madison, WI, USA) and purified by Ni-NTA agarose (Qiagen, Hilden, Germany) [19]. Recombinant Der p 5, Der p 7 and Der p 21 were expressed as non-fusion proteins in *E. coli*. rDer p 5 was purified to homogeneity by anion and cation exchange chromatography [20], rDer p 7 was purified by hydrophobic interaction chromatography [21] and hydroxyapatite chromatography and rDer p 21 by anion and cation exchange chromatography [22].

Rabbit anti-rDer p 10 antibodies were obtained by immunizing a rabbit three times with the purified protein in doses of 200 µg, using Freund's complete once and Freund's incomplete adjuvant twice (Charles River, Kisslegg, Germany).

Expression in *Escherichia coli* and purification of recombinant Der p 10

A cDNA coding for Der p 10 (database #AF016278) was obtained by reverse transcription from *D. pteronyssinus* RNA by RT-PCR using a Platinum Tag Kit (Invitrogen, Carlsbad, CA, USA) and the following primers: forward 5'-CGGGATCCCATATGGAGGCCATCAAAAAGAAAATGC-3' and reverse 5'-CGGGATCCTTAATAACCAGTAAGTTCGGC-3', containing *Bam*HI (bold letters) and *Nde*I (underlined) restriction sites (MWG, Ebersberg, Germany). The PCR product was cut with *Bam*HI and *Nde*I, gel-purified and subcloned into the *Nde*I and *Bam*HI sites of the pET17b vector (Novagen, EMD, Madison, WI, USA). Both DNA strands were sequenced (MWG).

Recombinant Der p 10 was expressed in *E. coli* BL21 (DE3) (Stratagene, Santa Clara, CA, USA) as described [20]. After lysis of cells, the protein was found in the super-natant, which was dialysed against 1 M (NH₄)₂SO₄, 50 mM Na₂HPO₄, pH 7, and 1 mM dithiothreitol (DTT) and loaded onto a Phenyl-Sepharose fast-flow column (GE Healthcare Bio-Science AB, Uppsala, Sweden). Bound proteins were eluted by a 0–100% linear gradient to 50 mM Na₂HPO₄, pH 7, 1 mM DTT. Fractions containing rDer p 10 were pooled, dialysed against 10 mM Na₂HPO₄, pH 7, 0.25 mM DTT, loaded onto a column prepacked with Bio-Gel HT Hydroxyapatite (BioRad, Hercules, CA, USA) and eluted by continuously increasing the concentration of Na₂HPO₄ to 300 mM. Fractions containing more than 90% pure rDer p 10 were pooled, dialysed against 5 mM Na₂HPO₄ pH 7 and stored at –20 °C. The purity of the protein was determined by SDS-PAGE and Coomassie blue staining [23], and its concentration was measured using the Micro BCA Assay Kit (Pierce, Rockford, IL, USA).

Preparation of an aqueous whole-body mite extract

An aliquot of 0.3 g of purified *D. pteronyssinus* whole bodies (Allergon, Vällinge, Sweden) was homogenized in 5 mL H₂O containing 1 mM benzamidine and 1 mM PMSF using an Ultra-Turrax T25 Basic disperser (IKA, Staufen, Germany). The homogenate was extracted for 2 h at 4 °C and the insoluble fraction was removed by centrifugation (20 min, 3220 g, 4 °C). The protein concentration was determined in the extract using the Micro BCA Assay Kit (Pierce). Aliquots were stored at –20 °C.

Purification of natural Der p 10

Natural Der p 10 was purified by affinity chromatography from an HDM extract using Protein G-purified rabbit anti-rDer p 10 antibodies (ImmunoPure IgG Purification Kit, Pierce). For the preparation of the affinity column, approximately 3 mg of purified rabbit anti-rDer p 10 IgG was coupled to 1 mL of a HiTrapTM NHS-activated HP column (GE Healthcare Bio-Science AB). HDM extract (4.4 mL; 28 mg protein) was diluted 1 : 1 with phosphate-buffered saline (PBS) pH 7, loaded onto the column and bound allergen was eluted using 100 mM Glycin/HCl pH 2.8. Fractions containing nDer p 10 were pooled, concentrated using centrifugal filters (Amicon Ultra, 10K, Millipore, Billerica, MA, USA) and dialysed against 5 mM Na₂HPO₄ pH 7. The purity of the protein was determined by SDS-PAGE and Coomassie blue staining and the protein concentration was determined using the Micro BCA Assay Kit (Pierce).

Matrix-assisted laser desorption and ionization-time-of flight mass spectrometry of rDer p 10

rDer p 10 was desalted and further purified via reversed phase-HPLC (HP 1050 binary gradient; column, Nucleosil-100 RP-18 150×4 mm, 5 µm; solvent A, water, 0.1% trifluoroacetic acid (TFA); solvent B, acetonitrile, 0.1% TFA; gradient, 10% B to 90% B in 15 min; flow, 1 mL/min; detection, UV at 215 nm; injected volume, 10 µL). Laser desorption mass spectra were acquired in a linear mode with a time-of-flight Compact matrix-assisted laser desorption and ionization (MALDI) II instrument (Kratos, Manchester, UK; piCHEM, Research and Development, Graz, Austria). rDer p 10 was dissolved in 10% acetonitrile, 0.1% TFA, and α-cyano-4-hydroxycinnamic acid (dissolved in 60%

acetonitrile, 0.1% TFA) was used as a matrix. For sample preparation, a 1 : 1 mixture of protein and matrix solution was deposited onto the target and air-dried.

Circular dichroism analysis of natural and recombinant Der p 10

Far UV circular dichroism (CD) spectra of natural and recombinant Der p 10 were collected on a Jasco J-810 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) using a 1 mm path length quartz cuvette at protein concentrations of 0.1 and 0.26 mg/mL, respectively. Spectra were measured from 260 to 180 nm, with a 0.5 nm resolution at a scanning speed of 50 nm/min, and resulted from averaging of three scans. All measurements were performed in 10 mM Na₂HPO₄ pH 7. For the temperature scan, spectra of rDer p 10 were recorded by gradually increasing the temperature (5 °C steps) from 25 to 95 °C at a heating rate of 1 °C/min, and after cooling down to 25 °C again. The final spectra were baseline corrected by subtracting the corresponding buffer spectrum. Results were expressed as the mean residue ellipticity [θ] at a given wavelength. The secondary structure content of rDer p 10 was calculated using the secondary structure estimation program CDSSTR [24].

Enzyme-linked immunosorbent assay inhibition

For ELISA inhibitions, 96-well plates (Nunc Maxi-Sorp, Roskilde, Denmark) were coated with 100 µL of nDer p 10 and rDer p 10 (1 µg/mL diluted in PBS) o/n at 4 °C. Sera from eight mite-allergic patients with IgE reactivity to Der p 10 (diluted 1 : 5) were pre-incubated with 10 µg/mL of nDer p 10, rDer p 10 or, for control purposes, with bovine serum albumin (BSA) at 4 °C o/n. After blocking the plates with 1% BSA in PBS-T (PBS 0.05% Tween-20) for 2.5 h at room temperature, the pre-incubated sera were applied o/n at 4 °C. Bound IgE antibodies were detected with 1 : 2500 diluted horseradish peroxidase (HRP)-labelled goat anti-human IgE (KPL, Gaithersburg, MD, USA). The signal was developed by the addition of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (100 µL/well; Sigma-Aldrich, St Louis, MO, USA). Optical density (OD) measurements were carried out at 405 and 490 nm in an ELISA reader (Spectramax Plus, Molecular Devices, Sunnyvale, CA, USA). The percentage of IgE inhibition was calculated as follows: $100 - (OD_{\text{inhibited}}/OD_{\text{uninhibited}} \times 100)$, where OD_{inhibited} represents extinction after pre-incubation with nDer p 10 or rDer p 10 and OD_{uninhibited} represents extinction after pre-incubation with BSA.

Immunoglobulin E reactivity to purified allergens in a non-denaturing radio allergosorbent test-based dot-blot assay

Two µL aliquots of natural and recombinant HDM allergens (nDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 10 and rDer p 21) [19–22] as well as BSA as a control protein (each 0.25 µg/µL) were dotted onto nitrocellulose membranes (Schleicher & Schuell). The membranes were blocked with gold buffer [50 mM sodium phosphate pH 7.4, 0.5% (v/v) Tween-20, 0.5% (w/v) BSA and 0.05% (w/v) sodium azide], twice for 5 min and once for 30 min, and incubated with mite-allergic patients' sera and, for control purposes, with serum from a non-allergic individual, diluted 1 : 10 in gold buffer o/n at 4 °C. Bound IgE antibodies were detected with ¹²⁵I-labelled anti-human IgE antibodies (Demeditec Diagnostics, Kiel, Germany) and visualized by autoradiography [25]. A semi-quantitative analysis of dot-blot signals was achieved using the NIH ImageJ software (version 1.42; <http://rsb.info.nih.gov/>

ij/). The scanned film was analysed after conversion into an 8-bit greyscale image, background correction and inversion of the image. Thus, pixel values ranged from 0 (black) to 255 (white). After outlining the individual dots using the elliptical tool, the integrated density was calculated as a product of 'area' value (area of selection in square pixels) and the 'mean grey value' (sum of the grey values of all the pixels in the selection divided by the number of pixels). An example of the dot's integrated density is shown for patient A35 (see supporting information Fig. S1). Three independent measurements were performed and averaged for each dot. The individual dots were grouped into three categories (weak, middle and strong) according to their 'integrated density' values and marked white, grey and black in supporting information Table S1.

Rat basophil leukaemia cell mediator release assay

The rat basophil leukaemia (RBL) cell-release assay was performed as described previously by Schulmeister et al. [26]. Briefly, RBL cells (clone RBL-703/21) [27] transfected with human FcεRI were sensitized with mite-allergic patients' serum IgE in a final dilution of 1 : 30 o/n at 37 °C. Degranulation of the cells was induced by adding nDer p 1, rDer p 2 and rDer p 10 (100, 10, 1 and 0.1 ng/mL) or buffer as a control and assessed by measuring the β-hexosaminidase release. Results are reported as the percentage of total β-hexosaminidase release, where values obtained with buffer instead of allergen were subtracted.

Statistical analysis

Statistically significant differences between Der p 10-positive and -negative patients were assessed using the Mann–Whitney *U*-test as well as the multiple χ^2 testing. Differences were considered statistically significant if the *P*-value was lower than 0.05.

Results

Expression and purification of folded rDer p 10, purification of nDer p 10

The cDNA coding for Der p 10, which was obtained by RT-PCR from *D. pteronyssinus* RNA, was completely identical to the earlier described nucleotide sequence of Der p 10 (AF016278) and resulted in two amino acid exchanges compared with the deduced amino acid sequence published by Asturias (Y149906) [13]. The cDNA coding for the Der p 10 protein was subcloned into expression plasmid pET17b and was expressed as a soluble non-fusion protein in *E. coli* BL21 (DE3). Between 3 and 7 mg of recombinant protein/L of bacterial culture was purified by hydrophobic interaction and ion exchange chromatography to homogeneity (Fig. 1a) Natural Der p 10, purified by affinity chromatography from an aqueous *D. pteronyssinus* extract, yielded a single band at 36 kDa in SDS-PAGE (Fig. 1a).

Comparative biochemical analysis of natural and recombinant Der p 10

In SDS-PAGE (Fig. 1a) both natural and recombinant Der p 10 migrated at a molecular mass (i.e. 36 kDa) that was greater than that calculated from the amino acid sequence (32.972 kDa) [13]. However, MALDI-time-of-flight (MALDI-Tof) mass spectrometry of rDer p 10 confirmed the calculated mass (32.939 kDa) (Fig. 1b), which corresponded to previous studies showing that tropomyosins migrate at higher molecular masses in SDS-PAGE than predicted from their sequence [12, 13, 28, 29]. The far UVCD spectra of

purified natural and recombinant Der p 10 recorded at 25 °C showed almost identical curves with two minima at 209 and 221.5 nm and a large maximum at 191 nm, which is characteristic for proteins with predominantly α -helical structure (Fig. 2a). Calculation of the secondary structure using the program CDSSTR with the reference data set SP175 resulted in 90%, 1%, 4%, 5% (nDer p 10) and 89%, 1%, 4% and 4% (rDer p 10) of α -helix, β -sheets, turns and random-coils, respectively. A melting temperature, defined as the midpoint of transition, of 44 °C was determined for rDer p 10 at 222 nm (data not shown). Total unfolding of rDer p 10 occurred at 50 °C. However, even after heating to 95 °C, the protein almost completely regained its fold after cooling to 25 °C (Fig. 2b).

rDer p 10 contains the immunoglobulin E epitopes of nDer p 10 and reacts with immunoglobulin E from 15% of house dust mite-allergic patients

To investigate whether rDer p 10 contains the IgE epitopes of the natural Der p 10, ELISA assays and ELISA inhibition experiments were performed. nDer p 10 and rDer p 10 were tested for their IgE-binding capacity with sera from eight HDM-allergic patients in an ELISA assay (Fig. 3), which showed that the sera reacted equally well to natural and recombinant Der p 10 (correlation coefficient: 0.978). In ELISA inhibition experiments, the same sera were pre-incubated with purified nDer p 10 or rDer p 10 and then allowed to bind to solid phase-bound nDer p 10. The results showed that natural and recombinant Der p 10 inhibited patients' IgE binding to nDer p 10 in a comparable manner (82% and 78%), indicating that rDer p 10 contains the relevant IgE epitopes of the natural allergen (Table 2).

Der p 10 was then used to search for specific IgE antibodies in 1322 sera from *D. pteronyssinus*-allergic patients. These patients suffered from respiratory symptoms to HDM (i.e. rhinitis, conjunctivitis, cough, dyspnoea and asthma). We found that 201 (15.2%) of the patients had specific IgE antibodies to rDer p 10 (data not shown).

rDer p 10-reactive patients are also frequently sensitized to house dust mite allergens other than Der p 1 and Der p 2

A detailed analysis of the IgE-reactivity profiles to other HDM allergens was performed in 35 of the 201 Der p 10-positive sera where sufficient serum was available. For comparison, 27 randomly picked sera from the Der p 10-negative patients were analysed. Table 1 summarizes the demographic, serological and clinical data of these patients. Der p 10-positive patients showed significantly higher total IgE levels (median: 546.5 kU/L) and RAST classes (median: 5) than the Der p 10-negative group [total IgE: median: 160 kU/L ($P < 0.000$); RAST: median: 3 ($P = 0.018$)]. Figure 4 and Table S1 show the frequency and intensities of IgE reactivity to nDer p 1, rDer p 2, rDer p 5, rDer p 7 and rDer p 21 in the two groups.

Comparing the frequencies of IgE reactivity to the indicated HDM allergens in the Der p 10-positive and negative group, we observed striking differences regarding the reactivity profiles. More than 50% of the Der p 10-positive sera reacted with at least five of the tested HDM allergens (Fig. 4, supporting information Table S1). Eleven patients showed IgE reactivity to all six tested HDM allergens (31.4%) and eight reacted with five allergens (22.9%). The frequencies of IgE reactivities of rDer p 5 ($P < 0.000$), rDer p 7 ($P = 0.004$) and

rDer p 21 ($P = 0.001$) were approximately four- to fivefold higher in the Der p 10-positive compared with the Der p 10-negative patients (Fig. 4). By contrast, the majority of the Der p 10-negative sera (77.8%) were characterized by a sensitization to only one to two HDM allergens (supporting information Table S1). Interestingly, five patients showed IgE reactivity only to Der p 10 but to none of the other tested HDM allergens (supporting information Table S1). IgE inhibition studies performed with sera from patients A11, A27, A28, A33 and A35 indicated that Der p 10 and not shrimp tropomyosin is the primary sensitizing agent (data not shown).

In contrast to total IgE levels, RAST classes and IgE-reactivity frequencies, no significant difference was found regarding age and sex distribution, SPT results as well as clinical symptoms between Der p 10-positive and negative patients.

Der p 10 generally has low allergenic activity but can induce strong basophil degranulation in certain patients

In order to study the allergenic activity of recombinant Der p 10, mediator release assays with an RBL cell line that had been transfected with the human FCεRI receptor were performed. RBL cells, loaded with serum IgE from the 35 Der p 10-positive patients, were exposed to different concentrations of nDer p 1, rDer p 2 and rDer p 10. The mediator release at the allergen concentration (i.e. 100 ng/mL) yielding the strongest basophil degranulation is displayed in Fig. 5 and supporting information Table S1. In the majority of the patients, rDer p 10 induced weak or no basophil degranulation. Relevant rDer p 10-induced degranulation was, however, obtained with four sera, which were derived from patients who also showed strong IgE reactivity to Der p 10 (Fig. 4, supporting information Table S1). A relevant mediator release was observed for 19 (54.3%) and 17 (48.6%) sera with nDer p 1 and rDer p 2, respectively, indicating that these allergens exhibit much higher allergenic activity than Der p 10. However, Der p 10 may be a clinically relevant HDM allergen for certain patients. For example, patients A11 and A33 had not reported any seafood allergy but showed respiratory symptoms to HDM. These patients had shown IgE antibody reactivities only to Der p 10 but not to any of the other tested HDM allergens.

Discussion

Tropomyosins are important food allergens that can cause severe anaphylactic reactions in sensitized patients [30]. They show extensive cross-reactivity with mite tropomyosins but their relevance for HDM allergy has not been studied in detail. In order to investigate the importance of tropomyosin in HDM allergy, we expressed and purified rDer p 10, the tropomyosin from the mite *D. pteronyssinus* as folded protein and purified nDer p 10 from an HDM extract. rDer p 10 equalled the natural one in terms of IgE reactivity and secondary structure. The reported prevalences of IgE recognition of mite tropomyosins in HDM patients showed a large variability from 5% to 80% [12, 13]. In a population of more than 1300 HDM-allergic patients, we found that Der p 10 is recognized by 15.2% patients.

When we performed an in-depth analysis of sera from Der p 10-positive and -negative patients regarding IgE reactivity to five other HDM allergens (nDer p 1, rDer p 2, rDer p 5, rDer p 7 and rDer p 21), striking differences of IgE-reactivity profiles between these two

groups were noted. The majority of the Der p 10-positive patients were polysensitized to most of the tested allergens. Certain patients showed strong and exclusive IgE reactivity to Der p 10. By contrast, the majority of Der p 10-negative patients reacted only with Der p 1 and/or Der p 2 but not with the other HDM allergens. In addition, the Der p 10-positive group showed significantly higher total IgE as well as mite-specific IgE antibody levels than the Der p 10-negative group. Similar associations between the number of IgE sensitizations and specific IgE levels could also be observed in grass pollen allergy [31, 32].

Our finding may have important clinical implications for HDM-specific immunotherapy. In fact, at present, HDM extracts are only tested for the presence of Der p 1 and Der p 2 so that no information is available regarding the contents of allergens other than Der p 1 and Der p 2 in HDM extracts [33]. Furthermore, it has been demonstrated that allergens other than Der p 1 and Der p 2 are missing in most of the commercially available allergen extracts [34] (A. Casset et al., unpublished data). It is very likely that patients from the Der p 10-positive group who are also sensitized to other clinically highly relevant allergens such as Der p 5, Der p 7 or Der p 21 may not benefit so well from HDM-specific immunotherapy with extracts containing only Der p 1 and Der p 2.

Our *in vitro* results suggest that the clinical relevance of Der p 10 among HDM-allergic patients may be rather low compared with patients with food allergy to tropomyosin. In fact, only a relatively few HDM-allergic patients were sensitized to Der p 10. Their IgE reactivities were rather weak and also the allergenic activity of Der p 10 was very low compared with that of Der p 1 and Der p 2. For a few sera, the antibody levels and biological activity were not associated (i.e. A2, A7, A29), but this phenomenon has already been described for several allergens including Der p 1 and Der p 2 [7, 35, 36]. Several factors such as the number and geometry of IgE epitopes as well as affinity/avidity of IgE binding may influence the mediator release activity.

For a few Der p 10-sensitized patients (i.e. two of the 35 Der p 10-positive patients in our study, A11, A33), Der p 10 appeared to be the most important HDM allergen, exhibited high allergenic activity in the basophil degranulation assay, and caused clinically relevant sensitization to HDM. These two patients showed a monosensitization to the mite tropomyosin (patients A11 and A33). Inhibition experiments were performed, which indicated that mite was the original sensitizing agent for these patients, who also did not report allergy to shrimps (data not shown).

It is possible that the low frequency of sensitization and the low IgE reactivity and allergenic activity of Der p 10 may be due to the fact that the allergen is not well represented in the respirable mite faeces compared with other HDM allergens (e.g. Der p 1, Der p 2, Der p 5, Der p 7 and Der p 21) [37, 38] but only occurs in the possibly less immunogenic mite body particles [39]. In contrast to HDM, tropomyosins are present in high concentrations in seafood (e.g. for shrimp approximately 20% of the dry weight [40]), where they also represent major and clinically relevant allergens with IgE-reactivity frequencies of more than 80% [41].

Although our *in vitro* results indicate that the clinical relevance of Der p 10 for respiratory allergy to HDM is rather low, provocation tests may be required to further determine the clinical relevance of Der p 10 as a respiratory allergen in sensitized patients.

However, IgE reactivity to Der p 10 allows the identification of a subset of HDM-allergic patients with a broad sensitization to HDM allergens other than Der p 1 and Der p 2. This may have important implications for HDM-specific immunotherapy, as Der p 10-sensitized patients may not be suitable for immunotherapy with most of the currently available HDM extracts.

Clinical relevance

Diagnostic testing with Der p 10 may identify patients with broad sensitization to HDM allergens other than Der p 1 and Der p 2 who may not benefit so well from immunotherapy with HDM extracts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was funded by Grants F1803 and F1815 of the Austrian Science Fund, the Christian Doppler Research Association and Phadia, Uppsala, Sweden.

References

1. Bousquet PJ, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. *Allergy*. 2007; 62:301–9. [PubMed: 17298348]
2. Boulet LP, Turcotte H, Laprise C, et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy*. 1997; 27:52–9. [PubMed: 9117881]
3. Sunyer J, Jarvis D, Pekkanen J, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol*. 2004; 114:1033–9. [PubMed: 15536406]
4. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J*. 2004; 24:758–64. [PubMed: 15516669]
5. Weghofer M, Thomas WR, Kronqvist M, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest*. 2008; 38:959–65. [PubMed: 19021722]
6. Hales BJ, Martin AC, Pearce LJ, et al. IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol*. 2006; 118:361–7. [PubMed: 16890759]
7. Witteman AM, Stapel SO, Perdok GJ, et al. The relationship between RAST and skin test results in patients with asthma or rhinitis: a quantitative study with purified major allergens. *J Allergy Clin Immunol*. 1996; 97:16–25. [PubMed: 8568133]
8. Alvarez MJ, Olaguibel JM, Acero S, Garcia BE, Tabar AI, Urbiola E. Effect of current exposure to Der p 1 on asthma symptoms, airway inflammation, and bronchial hyperresponsiveness in mite-allergic asthmatics. *Allergy*. 2000; 55:185–90. [PubMed: 10726735]
9. Thomas WR, Heinrich TK, Smith WA, Hales BJ. Pyroglyphid house dust mite allergens. *Protein Pept Lett*. 2007; 14:943–53. [PubMed: 18220991]
10. Reese G, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol*. 1999; 119:247–58. [PubMed: 10474029]

11. Lopata AL, Lehrer SB. New insights into seafood allergy. *Curr Opin Allergy Clin Immunol*. 2009; 9:270–7. [PubMed: 19398906]
12. Aki T, Kodama T, Fujikawa A, et al. Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, *Dermatophagoides farinae*. *J Allergy Clin Immunol*. 1995; 96:74–83. [PubMed: 7622766]
13. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez A, Martinez J, Palacios R. Sequencing and high level expression in *Escherichia coli* of the tropomyosin allergen (Der p 10) from *Dermatophagoides pteronyssinus*. *Biochim Biophys Acta*. 1998; 1397:27–30. [PubMed: 9545522]
14. Pittner G, Vrtala S, Thomas WR, et al. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy*. 2004; 34:597–603. [PubMed: 15080813]
15. Westritschnig K, Sibanda E, Thomas W, et al. Analysis of the sensitization profile towards allergens in central Africa. *Clin Exp Allergy*. 2003; 33:22–7. [PubMed: 12534545]
16. Dreborg S, Holgersson M, Nilsson G, Zetterstrom O. Dose response relationship of allergen, histamine, and histamine releasers in skin prick test and precision of the skin prick test method. *Allergy*. 1987; 42:117–25. [PubMed: 2438960]
17. Dreborg S. Skin testing. The safety of skin tests and the information obtained from using different methods and concentrations of allergen. *Allergy*. 1993; 48:473–5. [PubMed: 8238806]
18. Hales BJ, Shen HD, Thomas WR. Cross-reactivity of T-cell responses to *Dermatophagoides pteronyssinus* and *D. farinae*. Studies with group 1 and 7 allergens. *Clin Exp Allergy*. 2000; 30:927–33. [PubMed: 10848914]
19. Chen KW, Fuchs G, Sonneck K, et al. Reduction of the in vivo allergenicity of Der p 2, the major house-dust mite allergen, by genetic engineering. *Mol Immunol*. 2008; 45:2486–98. [PubMed: 18295887]
20. Weghofer M, Grote M, Dall'Antonia Y, et al. Characterization of folded recombinant Der p 5, a potential diagnostic marker allergen for house dust mite allergy. *Int Arch Allergy Immunol*. 2008; 147:101–9. [PubMed: 18520154]
21. Reese G, Schick Tanz S, Lauer I, et al. Structural, immunological and functional properties of natural recombinant Pen a 1, the major allergen of Brown Shrimp, *Penaeus aztecus*. *Clin Exp Allergy*. 2006; 36:517–24. [PubMed: 16630158]
22. Weghofer M, Dall'Antonia Y, Grote M, et al. Characterization of Der p 21, a new important allergen derived from the gut of house dust mites. *Allergy*. 2008; 63:758–67. [PubMed: 18445190]
23. Fling SP, Gregerson DS. Peptide and protein molecular weight determination by electrophoresis using a high-molarity tris buffer system without urea. *Anal Biochem*. 1986; 155:83–8. [PubMed: 3454661]
24. Whitmore L, Wallace BA. DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucl Acids Res*. 2004; 32:W668–73. [PubMed: 15215473]
25. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med*. 1992; 175:377–85. [PubMed: 1370681]
26. Schulmeister U, Hochwallner H, Swoboda I, et al. Cloning, expression, and mapping of allergenic determinants of alphaS1-casein, a major cow's milk allergen. *J Immunol*. 2009; 182:7019–29. [PubMed: 19454699]
27. Ladics GS, van Bilsen JH, Brouwer HM, Vogel L, Vieths S, Knippels LM. Assessment of three human FcepsilonRI-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. [PubMed: 18534732]
28. Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Rao PV. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *J Immunol*. 1993; 151:5354–63. [PubMed: 7693809]
29. Jeong KY, Lee J, Lee IY, Ree HI, Hong CS, Yong TS. Allergenicity of recombinant Bla g 7, German cockroach tropomyosin. *Allergy*. 2003; 58:1059–63. [PubMed: 14510726]
30. Taylor, SL.; Bush, RK. Allergy by ingestion of seafoods. In: Tu, AT., editor. *Marine toxins and venoms*. Dekker; New York: 1988. p. 149-83.

31. Moverare R, Petays T, Vartiainen E, Haahtela T. IgE reactivity pattern to timothy and birch pollen allergens in Finnish and Russian Karelia. *Int Arch Allergy Immunol.* 2005; 136:33–8. [PubMed: 15591811]
32. Moverare R, Kosunen TU, Haahtela T. Change in the pattern of IgE reactivity to timothy grass and birch pollen allergens over a 20-year period. *J Invest Allergol Clin Immunol.* 2006; 16:274–8.
33. Meyer CH, Bond JF, Chen MS, Kasaian MT. Comparison of the levels of the major allergens Der p I and Der p II in standardized extracts of the house dust mite, *Dermatophagoides pteronyssinus*. *Clin Exp Allergy.* 1994; 24:1041–8. [PubMed: 7874602]
34. Brunetto B, Tinghino R, Braschi MC, Antonicelli L, Pini C, Iacovacci P. Characterization and comparison of commercially available mite extracts for in vivo diagnosis. *Allergy.* 2010; 65:184–90. [PubMed: 19796217]
35. Niederberger V, Stubner P, Spitzauer S, et al. Skin test results but not serology reflect immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. *J Invest Dermatol.* 2001; 117:848–51. [PubMed: 11676821]
36. Purohit A, Laffer S, Metz-Favre C, et al. Poor association between allergen-specific serum immunoglobulin E levels, skin sensitivity and basophil degranulation: a study with recombinant birch pollen allergen Bet v 1 and an immunoglobulin E detection system measuring immunoglobulin E capable of binding to Fc epsilon RI. *Clin Exp Allergy.* 2005; 35:186–92. [PubMed: 15725190]
37. Tovey ER, Chapman MD, Platts-Mills TA. Mite faeces are a major source of house dust allergens. *Nature.* 1981; 289:592–3. [PubMed: 7464922]
38. Park GM, Lee SM, Lee IY, et al. Localization of a major allergen, Der p 2, in the gut and faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy.* 2000; 30:1293–7. [PubMed: 10971476]
39. Gattuso JR, Hales BJ, Thomas WR, Bi XZ, Chew FT, Tovey ER. Localization of recently discovered allergens in *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol.* 2005; 115:S91.
40. Lehrer SB, Ayuso R, Reese G. Current understanding of food allergens. *Ann N Y Acad Sci.* 2002; 964:69–85. [PubMed: 12023195]
41. Daul CB, Slattey M, Reese G, Lehrer SB. Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *Int Arch Allergy Immunol.* 1994; 105:49–55. [PubMed: 7916224]

Fig. 1. Biochemical characterization of rDer p 10. (a) Comparison of natural and recombinant Der p 10 by Coomassie Blue-stained SDS-PAGE. Lane M, molecular weight marker; lane 1, 1.5 μ g of purified nDer p 10; lane 2, 0.5 μ g of purified rDer p 10. (b) Matrix-assisted laser desorption and ionization-time-of-flight (MALDI-ToF) analysis of rDer p 10. The x-axis shows the mass/charge ratio. Signal intensities as percentage of the most abundant signal (y-axis).

Fig. 2. Circular dichroism (CD) analysis of natural and recombinant Der p 10. The molecular ellipticities $[\theta]$ (y-axes) at different wavelengths (190–250 nm, x-axis) are displayed for nDer p 10 and rDer p 10 (a). (b) CD spectra of rDer p 10 at different temperatures (25–95 °C) and refolding after heating to 95 °C and cooling down to 25 °C.

Fig. 3.

IgE-binding capacity of nDer p 10 and rDer p 10. ELISA-plate-bound nDer p 10 and rDer p 10 were incubated with sera from eight house dust mite (HDM)-allergic patients (A11, A27, A35–A40) and the buffer control (NC). Bound IgE antibodies were detected with an horseradish peroxidase (HRP)-conjugated anti-human IgE antiserum. The optical densities (OD) corresponding to the amount of bound antibodies are displayed on the y-axis.

Fig. 4.

Frequencies (percent of reactive sera) of IgE recognition (y-axis) of house dust mite (HDM) allergens (x-axis) are shown for HDM-allergic patients with (grey bars) or without (white bars) IgE antibodies to rDer p 10. Statistically significant differences between the two groups are indicated (* $P < 0.05$).

Fig. 5.

Allergenic activity of nDer p 1, rDer p 2 and rDer p 10. Rat basophil leukaemia (RBL) cells were loaded with IgE from Der p 10-sensitized patients and degranulation was induced with nDer p 1, rDer p 2 or rDer p 10 (x-axis). The intensities of basophil degranulation are shown as percentages of total β-hexosaminidase release (y-axis) as a scatter plot. Median mediator release values and the cut-off (5% mediator release) are indicated by a horizontal black and dashed line, respectively. Statistically significant differences are indicated (* $P < 0.05$).

Table 1

Characterization of mite-allergic patients

Patient	Sex	Age	Total IgE (kU/L)	Der p sIgE (RAST class)	Der p SPT	Mite-related symptoms
A1	F	18	155	3	+++	D, As
A2	M	8	309	4	+++	R, C, Co, D
A3	M	42	526	4	+++	R, C
A4	M	30	252	4	+++	R, C, D
A5	F	12	3205	6	+++	R, D
A6	M	44	369	ND	+++	R, C
A7	M	7	781	5	+++	R, Co, As
A8	M	16	1107	4	+++	R, C
A9	M	11	1081	6	+++	R, C, As
A10	F	31	213	3	+++	R, C
A11	M	14	493	4	+++	R, D, As
A12	M	27	283	3	+++	R, D
A13	M	11	624	6	+++	R
A14	F	13	801	5	+++	R, Co
A15	F	7	521	6	+++	R
A16	F	35	205	4	+++	R, C
A17	M	9	567	5	+++	R
A18	M	9	>2000	6	+++	R
A19	M	16	438	5	+++	R, C
A20	M	10	1132	4	+++	R, C
A21	M	20	319	5	+++	R, C
A22	M	NK	3333	6	+++	R, C
A23	M	16	970	5	+++	R, C, D
A24	M	45	183	1	+	R, C
A25	F	48	1008	6	+++	R, C, As
A26	M	16	1210	ND	+++	R
A27	F	31	604	3	+++	R, C
A28	F	71	615	2	+++	R, C
A29	F	30	292	4	+	R
A30	F	43	226	2	+++	R, C
A31	F	21	320	3	+++	R, C, S
A32	M	26	65	1	+++	R
A33	M	17	131	4	+++	D, As
A34	M	8	579	6	+++	R, D
A35	F	13	1039	6	+++	R, C, As
B1	F	8	111	4	ND	As
B2	F	9	433	5	ND	R, C
B3	F	20	21	2	+++	D, As
B4	M	10	971	6	+++	R, C, D, As

Patient	Sex	Age	Total IgE (kU/L)	Der p sIgE (RAST class)	Der p SPT	Mite-related symptoms
B5	F	13	69	3	+++	R, D, As
B6	M	14	47	3	+++	R
B7	M	27	140	ND	+++	R, As
B8	M	11	145	5	+++	R, Co
B9	M	39	160	3	+++	R
B10	M	33	127	2	–	R
B11	M	6	134	4	+++	R, C, D
B12	M	26	266	3	+	R, C, Co, As
B13	M	17	178	2	–	R, C
B14	F	23	305	4	+++	R, C
B15	F	27	212	2	+++	R, C
B16	F	27	122	4	+++	R, C, D
B17	F	32	921	4	+++	R, C
B18	M	10	223	4	+++	R, C, As
B19	F	25	1043	4	+++	R, C
B20	M	22	72	3	+++	R, C
B21	F	32	1332	3	+++	R, C, D, As
B22	F	17	218	5	+++	R, C, Co, D
B23	M	12	65	3	+++	R, C
B24	F	34	27	2	+++	R, Co, D
B25	F	26	288	3	–	R, C, D
B26	F	43	35	2	+++	R, C, Co
B27	F	9	232	5	+++	R

A1–A35, Der p 10-positive patients; B1–B27, Der p 10-negative patients; F, female; M, male; ND, not done; NK, not known; SPT, skin prick test; –, no reaction; +, weak reaction; +++, strong reaction; As, asthma; C, conjunctivitis; Co, cough; D, dyspnoea; R, rhinitis; S, sinusitis.

Table 2

Inhibition of patients' IgE binding to solid phase-bound nDer p 10 by nDer p 10 or rDer p 10 in the fluid phase

Patient	nDer p 10				
	+BSA	+nDer p 10		+rDer p 10	
	OD values	OD values	% Inhibition	OD values	% Inhibition
A11	0.660	0.100	85	0.088	87
A27	0.441	0.110	75	0.142	68
A35	0.831	0.110	87	0.309	63
A36	0.519	0.062	88	0.070	87
A37	0.240	0.059	76	0.059	76
A38	2.237	0.262	88	0.153	93
A39	1.802	0.366	80	0.228	87
A40	0.782	0.170	78	0.264	66
Mean	0.939	0.155	82	0.164	78