

Variability of IgE reactivity profiles among European mite allergic patients

M. Weghofer^{*}, W. R. Thomas[†], M. Krongvist[‡], A. Mari[§], A. Purohit[¶], G. Pauli[¶], F. Horak^{*}, H. Grönlund[‡], M. van Hage[‡], R. Valenta^{*} and S. Vrtala^{*}

^{*}Medical University of Vienna, Austria, [†]University of Western Australia, Perth, Australia, [‡]Karolinska Institutet and University Hospital, Stockholm, Sweden, [§]IDI-IRCCS, Rome, Italy, and [¶]Strasbourg University Hospital, Strasbourg, France

ABSTRACT

Background House dust mites (HDM) *Dermatophagoides pteronyssinus* are a frequent indoor allergen source. Our aim was to determine the frequencies of IgE reactivity to purified HDM allergen molecules in mite allergic patients from different parts of Europe in order to establish an allergen panel for diagnosis of HDM allergy.

Materials and methods Populations of *D. pteronyssinus*-allergic patients from Austria ($n = 56$), France ($n = 55$), Italy ($n = 67$) and Sweden ($n = 65$) and storage mite allergic patients from Sweden ($n = 31$) were analysed for IgE reactivity to eight purified natural (n) and recombinant (r) *D. pteronyssinus* allergens (nDer p 1, rDer p 2, nDer p 4, rDer p 5, rDer p 7, rDer p 8, rDer p 10 and rDer p 14) in RAST-based dot blot assays.

Results Using a combination of Der p 1 and Der p 2, at least 97% of the *D. pteronyssinus*-allergic patients could be diagnosed in each of the HDM allergic populations. However, more than 50% of the patients also reacted with other allergens and significant variabilities regarding the frequencies of IgE reactivity to individual allergen molecules were found. Patients with a predominant storage mite allergy showed none or only very weak IgE reactivity to purified *D. pteronyssinus* allergens.

Conclusions Purified Der p 1 and Der p 2 are sufficient for the diagnosis of $\geq 97\%$ of *D. pteronyssinus* allergic patients in Europe, but other allergens may also play an important role for the diagnosis and treatment of HDM allergy.

Keywords Diagnosis, HDM allergy, IgE prevalence, recombinant allergens, storage mite allergy.

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Introduction

The house dust mite (HDM) *Dermatophagoides pteronyssinus* represents the most important source of mite allergy in central Europe. Sensitization to storage mites in central Europe is frequently associated with sensitivity to *D. pteronyssinus* [1] and increased prevalence rates of IgE titres to storage mites are associated with higher IgE levels to *D. pteronyssinus* [2]. Primary sensitization to storage mites has been found in rural environments [3–6], but storage mite allergic patients were also found to exhibit IgE reactivity to *D. pteronyssinus* extracts [4]. However, cross-reactivity was also observed between *D. pteronyssinus* and storage mites in studies performed with extracts [7].

D. pteronyssinus extracts used for diagnosis and therapy contain varying concentrations of Der p 1 and Der p 2 as well as non-allergenic components [8]. The contents of other important allergens (e.g. Der p 5, Der p 7 and Der p 10) in HDM extracts have so far not been evaluated in depth but some allergens (e.g. Der p

7) seem to be lacking in natural extracts. Diagnostic tests based on *D. pteronyssinus* extracts may therefore fail to identify *D. pteronyssinus* allergic patients and cannot distinguish between genuine sensitization to HDMs and storage mites.

For diagnosis and immunotherapy, the use of recombinant allergens seems to be a promising alternative to natural allergen extracts, since they represent well defined components, which can easily be characterised and quantified [7,9–12]. In order to use recombinant allergens for diagnostic or therapeutic purposes, the importance of the individual allergen molecules needs to be evaluated. So far, 23 HDM allergens have been described and it seems that the IgE binding frequency of individual allergens may show high variability at least in certain populations [7,13]. An example for a high variability in IgE reactivity profiles to *D. pteronyssinus* allergens comes from a study performed in one European (Austria) and one African (Zimbabwe) population [13].

Another recent study demonstrated that Australian Aborigines predominantly reacted with Der p 4 but less frequently with Der p 1 and Der p 2 [14]. The IgE binding frequencies as well as the clinical relevance of the recombinant allergens need to be evaluated in order to determine the recombinant *D. pteronyssinus* allergens necessary for immunotherapy.

Our aim was to determine the prevalence of IgE reactivity to purified HDM allergen molecules (i.e. nDer p 1, rDer p 2, nDer p 4, rDer p 5, rDer p 7, rDer p 8, rDer p 10 and rDer p 14) in sera from *D. pteronyssinus* allergic patients from four European countries (Austria, France, Italy and Sweden) in order to determine the allergen panels needed for diagnostic purposes. Furthermore, we examined the IgE reactivity to purified *D. pteronyssinus* allergens in patients with a predominant storage mite allergy to study whether purified HDM allergens may discriminate HDM from storage mite allergy.

Materials and methods

Human sera

Sera from two hundred and forty-three patients with a positive case history indicative for HDM allergy and *D. pteronyssinus*-specific IgE antibodies ($\geq 0.7 \text{ kU}_A \text{ L}^{-1}$) as determined with the ImmunoCAP System (Phadia AB, Uppsala, Sweden) from four different populations in Europe (Austria: $n = 56$; France: $n = 55$; Italy: $n = 67$; Sweden: $n = 65$) were analysed in this study.

Sera from thirty-one storage mite-allergic Swedish farmers from Gotland with allergen-specific IgE or/and positive skin prick tests to at least one of the storage mites *Acarus siro*, *Glycyphagus domesticus*, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* were also studied.

Informed consent was obtained from each subject. The permission to use the sera from the different populations in an anonymous way was obtained from the Medical University of Vienna Ethics Committee.

Mite allergens

Natural Der p 1 was isolated by affinity chromatography using the monoclonal antibody 4C1 and nDer p 4 was purified by cyclodextrin affinity chromatography as described [15,16]. Recombinant Der p 2 and Der p 14 fragment (amino acids 1–260) were produced with an N-terminal His-tag using plasmid vector pET-19b (Novagen, Madison, WI, USA) [17,18], and rDer p 5, rDer p 7, rDer p 8 and rDer p 10 were produced as glutathione-S transferase (GST) fusion proteins using the pGEX vector expression system [19]. Recombinant GST protein was used as a control protein [20].

Dot blots with purified mite allergens

Natural and recombinant mite allergens (nDer p 1, rDer p 2, nDer p 4, rDer p 5, rDer p 7, rDer p 8, rDer p 10 and rDer p 14) and

recombinant GST as a control protein were dotted in $2 \mu\text{L}$ ($c = 0.25 \mu\text{g} \mu\text{L}^{-1}$) aliquots (i.e. $0.5 \mu\text{g}$ allergen/spot) onto nitrocellulose membrane strips (Schleicher & Schuell, Dassel, Germany). The strips were blocked with buffer A [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] and exposed to mite allergic patients' sera at a 1 : 5 or 1 : 10 dilution in buffer A. Bound IgE antibodies were detected as described [21]. The IgE reactivity was scored as positive by an independent investigator when there was no reactivity with sera from non-allergic patients and the buffer control in the same test. In the case of patients reacting also with GST, IgE reactivity was scored positive only if the reactivity exceeded more than twice the reactivity to GST alone in the autoradiography.

Statistical analysis

Differences of the frequency of allergen-specific IgE reactivity were assessed with multiple chi-square testing. The *P*-values were adjusted using the Shaffer procedure. A *P*-value ≤ 0.05 was considered as statistically significant.

Results

IgE reactivity prevalence to *D. pteronyssinus* allergen molecules vary in different populations

Populations of *D. pteronyssinus* allergic patients from Austria, France, Italy and Sweden were analysed for IgE reactivity to dot-blotted purified nDer p 1, rDer p 2, nDer p 4, rDer p 5, rDer p 7, rDer p 8, rDer p 10 and rDer p 14. In all tested populations, Der p 1 and Der p 2 represented the most important allergens with IgE-binding frequencies ranging from 85–100% and 63–96%, respectively [Fig. 1]. The Swedish population was found to react with a significantly lower frequency to the major allergens, Der p 1 and Der p 2, than the populations of Austria, France and Italy. The frequency of IgE-reactivity to the other *D. pteronyssinus* allergens was much lower in all the tested European populations. Der p 4 was recognized by 28–45%, Der p 5 by 23–64%, Der p 7 by 17–48%, Der p 8 by 9–16%, Der p 10 by 9–18% and Der p 14 by 0–3% of the patients. The frequency of IgE reactivity to Der p 5 was significantly lower in the Swedish population compared to the other studied populations. A great variability of Der p 7 specific IgE reactivity was also observed among the four European populations (e.g. Sweden: 17%; Italy: 48%). More patients from Austria and Sweden showed IgE-reactivity to Der p 10 (18%) than patients from France and Italy (9% and 10%) [Fig. 1].

A combination of Der p 1, 2, 5 and 7 allows diagnosis of almost all *D. pteronyssinus* allergic patients in different European populations

Despite the different IgE-reactivity frequencies of the individual *D. pteronyssinus* allergens in different populations, a combination

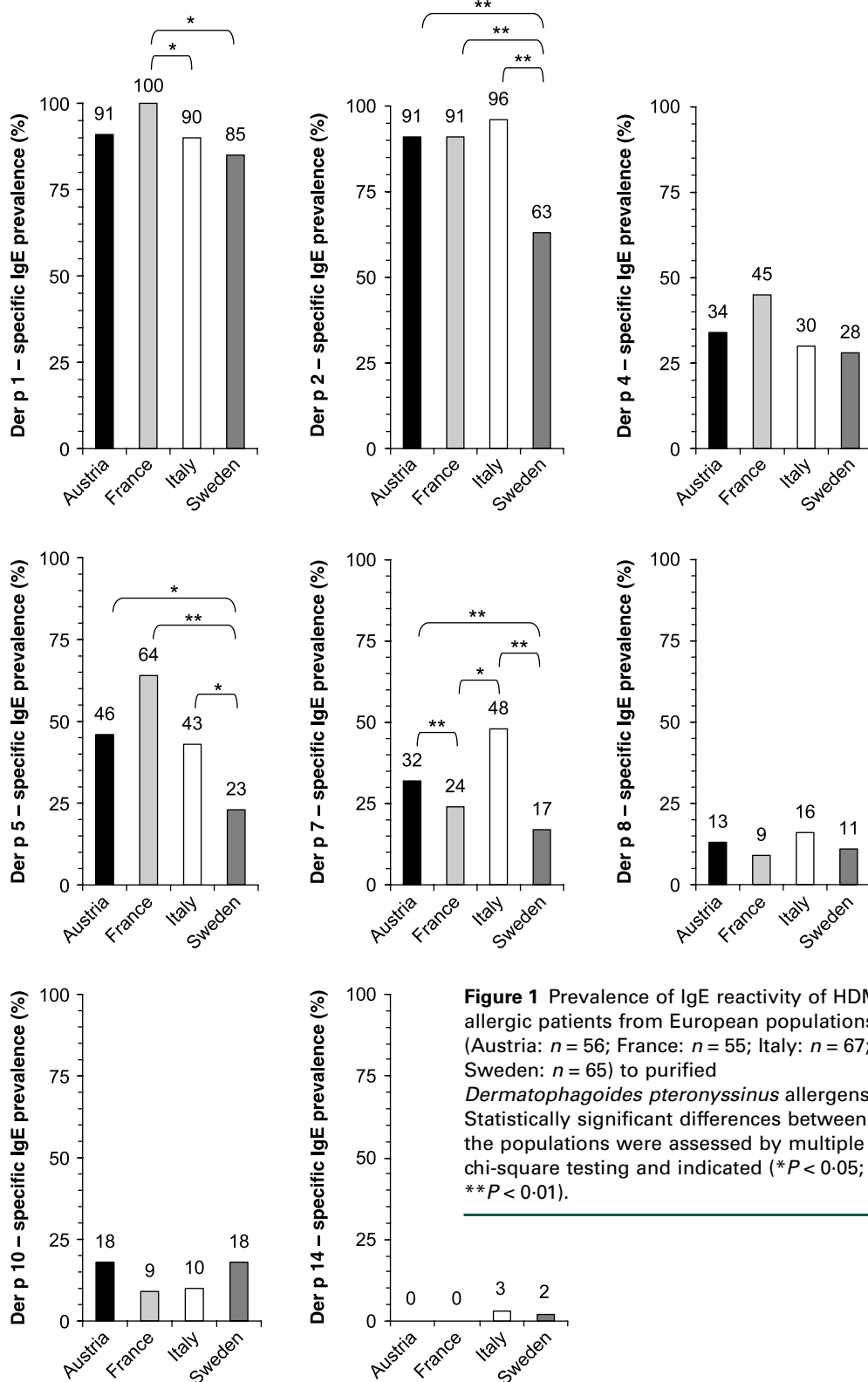


Table 1 Patients diagnosed with combinations of *D. pteronyssinus* allergens (nDer p 1, rDer p 2, 5 and 7)

Populations from	Number of patients	nDerp 1/rDer p 2 positive		nDer p 1/rDer p 2/rDer p 5/rDer p 7 positive	
		<i>n</i>	%	<i>n</i>	%
Austria	56	56	100	56	100
France	55	55	100	55	100
Italy	67	66	99	66	99
Sweden (D.p.)	65	63	97	65	100
Sweden (STM)	31	3	10	6	19

n, number of IgE-reactive sera; %, percentage of IgE-reactive sera; D.p., *Dermatophagoides pteronyssinus* allergic; STM, storage mite allergic.

Table 2 Patients diagnosed with combinations of *D. pteronyssinus* allergens (nDer p 1, rDer p 2, nDer p 4, rDer p 5, 7, 8, 10 and 14)

Populations from	Number of patients	nDer p 1/rDer p 2 positive		nDer p 1/rDer p 2/rDer p 5/rDer p 7 positive		nDer p 1/rDer p 2/nDer p 4/rDer p 5/rDer p 7 positive	
		+		+		+	
		nDer p 4/rDer p 5/rDer p 7/rDer p 8/rDer p 10/rDer p 14 negative		nDer p 4/rDer p 8/rDer p 10/rDer p 14 negative		rDer p 8/rDer p 10/rDer p 14 negative	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Austria	56	17	30	33	59	43	66
France	55	10	18	25	45	46	84
Italy	67	20	30	40	60	54	81
Sweden (D.p.)	65	27	42	39	60	51	78
Sweden (STM)	31	1	3	5	16	6	19

n, number of IgE-reactive sera; %, percentage of IgE-reactive sera; D.p., *Dermatophagoides pteronyssinus* allergic; STM, storage mite allergic.

of Der p 1 and Der p 2 was sufficient to diagnose at least 97% of *D. pteronyssinus* allergic patients in each of the tested populations (Table 1). In France and Austria, the combination of Der p 1 and Der p 2 allowed diagnosis of all patients. One of the two negative Swedish patients showed IgE-reactivity to Der p 5 and Der p 10, whereas the other patient reacted with Der p 7. The addition of the two mite allergens, Der p 5 and Der p 7, to the mixture of Der p 1 and Der p 2 increased the percentage of diagnosed patients to 100% in most of the populations (Austria, France, Sweden) (Table 1). Only one patient from Italy, who showed IgE reactivity with Der p 8 and Der p 10, could not be diagnosed with the combination of Der p 1, Der p 2, Der p 5 and Der p 7. Although most of the patients could be diagnosed with a combination of Der p 1 and Der p 2, only 18–42% of the patients were exclusively sensitized to the two major *D. pteronyssinus* allergens (Table 2). Between 45 and 60% reacted with Der p 1, 2, 5 and 7 but not with the other tested allergens. A great geographic difference was observed. Approximately 60% of the patients from Austria, Italy

and Sweden but only 45% of the French patients were exclusively sensitized to a combination of Der p 1, Der p 2, Der p 5 and Der p 7 and did not show IgE reactivity to the other tested *D. pteronyssinus* allergens (Der p 4, Der p 8, Der p 10 and Der p 14) (Table 2). Since Der p 4 is a frequently recognized mite allergen [Fig. 1], we included Der p 4 to the combination of Der p 1, Der p 2, Der p 5 and Der p 7. The addition of Der p 4 increased the percentage of diagnosed French patients from 45% to 84% but had lower effects on the diagnosis in the other populations (Table 2).

Storage mite allergic patients exhibit almost no IgE reactivity to purified *D. pteronyssinus* allergens

Sera from patients with predominant storage mite allergy from Sweden (sera 1–31) were tested for IgE reactivity to the panel of purified *D. pteronyssinus* allergens (nDer p 1, rDer p 2, nDer p 4, rDer p 5, rDer p 7, rDer p 8, rDer p 10 and rDer p 14) [Fig. 2]. Only seven of the 31 sera exhibited very low IgE reactivity to *D. pteronyssinus* allergens (Der p 1, 2, 4, 7 and 10). Four of these

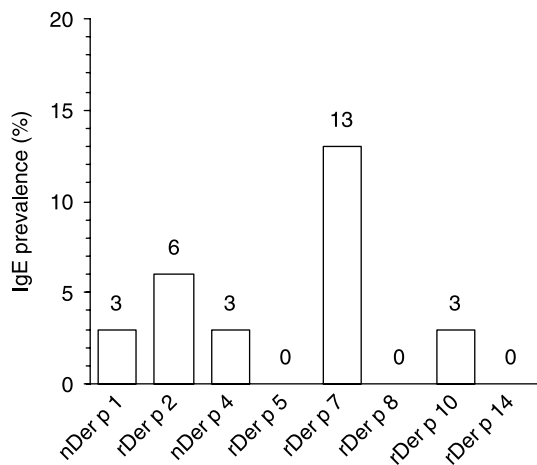


Figure 2 Prevalence of IgE reactivity of storage mite allergic patients (31 Swedish patients) to purified *Dermatophagoides pteronyssinus* allergen molecules.

seven sera reacted with Der p 7 (sera 1, 10, 17 and 22; 13%), two sera with Der p 2 (sera 9 and 22; 6%), one serum with Der p 1 and Der p 4 (serum 28; 3%) and one serum with Der p 10 only (serum 23; 3%) (Table 1 and Table 2).

Discussion

Allergen extracts from HDMs are frequently of poor quality. The use of purified recombinant and/or natural allergens for diagnostic and therapeutic purposes may therefore be considered as an alternative, or even an improvement over the traditional allergen extracts. In order to determine the importance of the individual HDM allergens for diagnosis and therapy, we determined the molecular IgE reactivity profiles to purified *D. pteronyssinus* allergens in sera from mite allergic patients from different parts of Europe (Austria, France, Italy and Sweden). The determination of the IgE reactivity prevalence to the individual allergen molecules in the different populations is an important first step in deciding which of the purified allergens are necessary for diagnosis of mite allergy and may be included in a vaccine cocktail.

Our results demonstrate that the two major allergens, Der p 1 and Der p 2, appear to be sufficient for the diagnosis of more than 97% of *D. pteronyssinus* allergic patients in each of the tested European populations and thus are in agreement with a survey carried out in an Austrian population [13]. With the addition of Der p 5 and Der p 7, the percentage of diagnosed patients could be increased to nearly 100%. Only one out of 243 patients could not be diagnosed with a combination of Der p 1, 2, 5 and 7, but reacted with Der p 8 and Der p 10. This patient also showed IgE reactivity to cockroaches and crustaceans, which may have been the primary sensitizing allergen sources. In fact, Der p 8 and Der

p 10 are cross-reactive allergens. Der p 8 belongs to the glutathione S-transferase (GST) superfamily [22], and Der p 10 (mite tropomyosin) represents a cross-reactive allergen also found in insects (cockroaches), crustaceans (e.g. shrimp, lobster, crab), molluscs (e.g. squid) and helminths [23–25].

Our results thus indicate that a diagnostic test consisting of Der p 1, Der p 2, Der p 5 and Der p 7 seems to be sufficient for the diagnosis of a genuine HDM sensitization in Europe and, according to another study, even in Australia [14].

Sensitization to storage mites is often found in *D. pteronyssinus* allergic patients and it may be difficult to differentiate between a genuine sensitization to HDM or to storage mites. Several studies have been performed to examine the cross-reactivity between storage mites and HDMs [2,26,27]. Some cross-reactivity could be observed but IgE reactivity to both mite types seems more likely to be the result of a co-sensitization. This assumption is confirmed by our finding that storage mite allergic patients reacted only very weakly and only with few of the purified *D. pteronyssinus* allergens. It is therefore possible that the diagnostic cocktail of Der p 1, Der p 2, Der p 5 and Der p 7 can also be used to discriminate between sensitization to HDMs and storage mites.

We observed significant differences regarding IgE reactivity to certain allergens in the tested populations. For example, significantly less Swedish patients reacted with rDer p 2 compared to patients from Austria, France and Italy. Significantly more Italian patients reacted with Der p 7 while Der p 5 was most frequently detected by French patients.

The observed differences cannot be attributed to different selection criteria, since all sera were selected randomly from groups of patients with specific IgE-levels $> 0.7 \text{ kU}_A \text{ L}^{-1}$ and clinical symptoms to HDMs.

One possible explanation for the differences between the Swedish patients and the other European populations may be that in Sweden mite allergen antibody levels are very low which is consistent with a low level or absence of detectable mite allergens in homes and fewer people are sensitised to HDMs [28,29]. However the fact that the IgE reactivity frequency to certain mite allergens was similar or even higher in the Swedish population (e.g. Der p 10) compared to Austria, France and Italy suggests that differences of *D. pteronyssinus*-specific IgE levels alone are not responsible for the variability in the IgE reactivity profiles.

Our finding that there is a considerable variability of IgE recognition frequencies to individual HDM allergens may have important implications for the selection of mite allergens to be included into a therapeutic vaccine for HDM allergy. Only 18–42% of our patients reacted exclusively with Der p 1 and/or Der p 2, whereas the majority of patients (i.e. $\geq 50\%$) reacted also with other *D. pteronyssinus* allergens. In the case that the other *D. pteronyssinus* allergens are responsible for clinical symptoms, a mix of Der p 1 and Der p 2 alone will not be sufficient for effective specific immunotherapy. Based on the frequency of IgE-recognition, Der

p 5, Der p 7 and Der p 4 are possible candidate molecules for a vaccine cocktail whereas less than 20% of the patients reacted with Der p 8, Der p 10 and Der p 14. As the next steps towards the selection of a vaccine cocktail, IgE recognition prevalences need to be established for the remaining HDM allergens (e.g. Der p 3, Der p 6, Der p 9, Der p 11, Der p 15, Der p 18, Der p 20 and Der p 21) [9,30].

However, serum IgE levels and IgE reactivity frequencies cannot predict clinical relevance [31,32]. The final definition of an optimal vaccine cocktail for HDM allergy will therefore require the clinical evaluation of the frequently recognized allergens. For this purpose, the allergenic activity of the individual mite allergens should be determined *in vivo* by provocation testing (e.g. by skin prick testing of different populations). As recently demonstrated for grass pollen allergens, those molecules with weak or no allergenic activity may be excluded from the vaccination cocktail because they will not induce relevant symptoms in patients [33]. After selection of the most frequently recognized and clinically relevant allergens a therapeutic vaccine cocktail can be established and then needs to be tested in immunotherapy studies in humans for efficacy.

In conclusion we determined a minimal panel of purified HDM allergens which allows us to diagnose genuine sensitization to HDMs. Our study may also be considered as a first step towards the selection of allergens to be included in a therapeutic vaccine for HDM allergy.

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Address

Division of Immunopathology, Department of Pathophysiology, Centre for Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria (M. Weghofer, R. Valenta, S. Vrtala), Centre for Child Health Research, University of Western Australia, Telethon Institute of Child Health Research, Perth, Australia (W. R. Thomas), Clinical Immunology and Allergy Unit, Department of Medicine, Karolinska Institute and University Hospital, Stockholm, Sweden (M. Kronqvist, H. Grönlund, M. van Hage), Centre for Clinical and Experimental Allergology, IDI-IRCCS, Rome, Italy (A. Mari), Division of Asthma and Allergology, Department of Chest Disease, Strasbourg University Hospital, Strasbourg, France (A. Purohit, G. Pauli), Department of Otorhinolaryngology, Medical University of Vienna, Vienna, Austria (F. Horak), Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology, Centre for Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria (R. Valenta).

Correspondence to: Susanne Vrtala, PhD, Division of Immunopathology, Department of Pathophysiology, Centre for Physiology and Pathophysiology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria.
Tel.: +43 140400 5132; fax: +43 140400 5130;
e-mail: susanne.vrtala@meduniwien.ac.at

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References

- Musken H, Fernandez-Caldas E, Maranon F, Franz JT, Masuch G, Bergmann KC. In vivo and in vitro sensitization to domestic mites in German urban and rural allergic patients. *J Investig Allergol Clin Immunol* 2002;12:177–81.
- van der Heide S, Niemeijer NR, Hovenga H, de Monchy JG, Dubois AE, Kauffman HF. Prevalence of sensitization to the storage mites *Acarus siro*, *Tyrophagus putrescentiae*, and *Lepidoglyphus destructor* in allergic patients with different degrees of sensitization to the house-dust mite *Dermatophagoides pteronyssinus*. *Allergy* 1998;53:426–30.
- Kronqvist M, Johansson E, Pershagen G, Johansson SG, van Hage-Hamsten M. Increasing prevalence of asthma over 12 years among dairy farmers on Gotland, Sweden: storage mites remain dominant allergens. *Clin Exp Allergy* 1999;29:35–41.
- van Hage-Hamsten M, Johansson SG, Hoglund S, Tull P, Wiren A, Zetterstrom O. Storage mite allergy is common in a farming population. *Clin Allergy* 1985;15:555–64.
- Tee RD, Gordon DJ, van Hage-Hamsten M, Gordon S, Nunn AJ, Johansson SG *et al.* Comparison of allergic responses to dust mites in U.K. bakery workers and Swedish farmers. *Clin Exp Allergy* 1992;22:233–9.
- Kronqvist M, Johansson E, Pershagen G, Johansson SG, van Hage-Hamsten M. Risk factors associated with asthma and rhinoconjunctivitis among Swedish farmers. *Allergy* 1999;54:1142–9.
- Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F *et al.* Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 2004;34:597–603.
- 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.
- Thomas WR, Heinrich TK, Smith W-A, Hales BJ. Pyroglyphid house dust mite allergens. *Protein Pept Lett* 2007;14:943–53.
- Valenta R. The future of antigen-specific immunotherapy of allergy. *Nat Rev Immunol* 2002;2:446–53.
- Vieths S, Scheurer S, Reindl J, Lüttkopf D, Wangorsch A, Kästner M *et al.* Optimized allergen extracts and recombinant allergens in diagnostic applications. *Allergy* 2001;56(Suppl. 67):78–82.
- Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004;59:243–67.
- Westritschnig K, Sibanda E, Thomas W, Auer H, Aspöck H, Pittner G *et al.* Analysis of the sensitization profile towards allergens in central Africa. *Clin Exp Allergy* 2003;33:22–7.
- Hales BJ, Laing IA, Pearce LJ, Hazell LA, Mills KL, Chua KY *et al.* Distinctive immunoglobulin E anti-house dust allergen-binding specificities in a tropical Australian Aboriginal community. *Clin Exp Allergy* 2007;37:1357–63.

- 15 Hales BJ, Shen H, Thomas WR. Cytokine responses to Der p 1 and Der p 7: house dust mite allergens with different IgE-binding activities. *Clin Exp Allergy* 2000;**30**:934–43.
- 16 Lake FR, Ward LD, Simpson RJ, Thompson PJ, Stewart GA. House dust mite-derived amylase: allergenicity and physicochemical characterization. *J Allergy Clin Immunol* 1991;**87**:1035–42.
- 17 Hales BJ, Hazell LA, Smith W, Thomas WR. Genetic variation of Der p 2 allergens: effects on T cell responses and immunoglobulin E binding. *Clin Exp Allergy* 2002;**32**:1461–7.
- 18 Epton MJ, Dilworth RJ, Smith W, Hart BJ, Thomas WR. High-molecular-weight allergens of the house dust mite: an apolipoprotein-like cDNA has sequence identity with the major M-177 allergen and the IgE-binding peptide fragments Mag1 and Mag3. *Int Arch Allergy Appl Immunol* 1999;**120**:185–91.
- 19 Lynch NR, Thomas WR, Garcia NM, Di Prisco MC, Puccio FA, L'Opez RI *et al*. Biological activity of recombinant Der p 2, Der p 5 and Der p 7 allergens of the house-dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol* 1997;**114**:59–67.
- 20 Weghofer M, Thomas WR, Pittner G, Horak F, Valenta R, Vrtala S. Comparison of purified *Dermatophagoides pteronyssinus* allergens and extract by two-dimensional immunoblotting and quantitative immunoglobulin E inhibitions. *Clin Exp Allergy* 2005;**35**:1384–91.
- 21 Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P *et al*. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;**175**:377–85.
- 22 Arruda LK, Vailes LD, Platts-Mills TA, Hayden ML, Chapman MD. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (*Blattella germanica*). *J Biol Chem* 1997;**272**:20907–12.
- 23 Reese G, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol* 1999;**119**:247–58.
- 24 Arruda LK, Santos AB. Immunologic responses to common antigens in helminthic infections and allergic disease. *Curr Opin Allergy Clin Immunol* 2005;**5**:399–402.
- 25 Asturias JA, Eraso E, Moneo I, Martinez A. Is tropomyosin an allergen in Anisakis? *Allergy* 2000;**55**:898–9.
- 26 van Hage-Hamsten M, Johansson SG, Johansson E, Wiren A. Lack of allergenic cross-reactivity between storage mites and *Dermatophagoides pteronyssinus*. *Clin Allergy* 1987;**17**:23–31.
- 27 Gafvelin G, Johansson E, Lundin A, Smith AM, Chapman MD, Benjamin DC *et al*. Cross-reactivity studies of a new group 2 allergen from the dust mite *Glycyphagus domesticus*, Gly d 2, and group 2 allergens from *Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae* with recombinant allergens. *J Allergy Clin Immunol* 2001;**107**:511–8.
- 28 Erwin EA, Ronmark E, Wickens K, Perzanowski MS, Barry D, Lundback B *et al*. Contribution of dust mite and cat specific IgE to total IgE: relevance to asthma prevalence. *J Allergy Clin Immunol* 2007;**119**:359–65.
- 29 Schram-Bijkerk D, Doekes G, Boeve M, Douwes J, Riedler J, Ublagger E *et al*. Nonlinear relations between house dust mite allergen levels and mite sensitization in farm and nonfarm children. *Allergy* 2006;**61**:640–7.
- 30 Weghofer M, Dall'Antonia Y, Grote M, Stocklinger A, Kneidinger M, Balic N *et al*. Characterization of Der p 21, a new important allergen derived from the gut of house dust mites. *Allergy* 2008;**63**:758–67.
- 31 Heiss S, Mahler V, Steiner R, Spitzauer S, Schweiger C, Kraft D *et al*. Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol* 1999;**113**:830–7.
- 32 Niederberger V, Stübner P, Spitzauer S, Kraft D, Valenta R, Ehrenberger K *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.
- 33 Westritschnig K, Horak F, Swoboda I, Balic N, Spitzauer S, Kundi M *et al*. Different allergenic activity of grass pollen allergens revealed by skin testing. *Eur J Clin Invest* 2008;**38**:260–7.