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Real-Life Study for the Diagnosis of House Dust Mite Allergy – The Value of Recombinant Allergen-Based IgE Serology

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Key Words

Allergen components · Allergic rhinitis · Der p 1 · Der p 2 · Der p 23 · *Dermatophagoides pteronyssinus* · House dust mites · Major allergens

Abstract

Background: *Dermatophagoides pteronyssinus* is one of the most important perennial allergen sources worldwide. Molecular diagnostics using the commercially available major allergens (Der p 1 and Der p 2) in combination with Der p 10 do not detect house dust mite (HDM) sensitization in a number of cases when used alone. The objective was to evaluate the IgE reactivity profiles of these patients using an experimental immunoassay biochip. ***Methods:*** Sera of HDM-allergic patients (positive skin prick test, CAP class ≥1 for allergen extract, and positive intranasal provocation) were tested for IgE antibodies against Der p 1, Der p 2, and Der p 10 by ImmunoCAP fluorescence enzyme immunoassay. Negatively tested sera were examined by an experimental chip containing 13 microarrayed HDM allergens. ***Results:*** Of 97 patients tested, 16 showed negative results to Der p 1, Der p 2, and Der p 10. MeDALL chip evaluation revealed 5 patients monosensitized to Der p 23, and 11 patients were negative for all HDM MeDALL chip components. Seven sera were available for further testing, and 3 of them showed IgE reactivity to dot-blotted nDer p 1, and 2 reacted with high-molecular weight components (>100 kDa) in nitrocellulose-blotted HDM extract when tested with ^{125}I-labeled anti-IgE in a RAST-based assay. The HDM extract-specific IgE levels of the 11 patients were <3.9 kU/l. ***Conclusions:*** Recombinant allergen-based IgE serology is of great value when conventional IgE diagnostics fails. Der p 23 is an important HDM allergen, especially when major allergens are negative. Therefore, it would be desirable to have Der p 23 commercially available. Further research concerning the prevalence and clinical significance of different HDM allergens is needed.

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Introduction

House dust mites (HDM), especially *Dermatophagoides pteronyssinus*, are the most important perennial allergen sources in central Europe and cause allergic rhinitis as well as allergic asthma [1]. So far, more than 20 differ-

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ent allergens of *D. pteronyssinus* have been described, with a strong variation in prevalence rates for the major allergens Der p 1 and Der p 2 in different countries [2–5]. The third currently commercially available allergen component Der p 10 also showed varying prevalence rates being responsible for a part of the cross-reactions to arthropods and mollusks [5, 6].

Molecular allergy diagnostics has become an important tool in daily allergological routine in allergy centers but also in smaller facilities within the last decade. The knowledge about major and minor allergen components and their prevalence opened new possibilities in allergy diagnostics and therapy. This powerful tool can often explain the sometimes frustrating outcomes of immunotherapy in the past and helps to choose the right treatment option (symptomatic vs. immunotherapy) for the individual patient. In comparison to immunotherapy against grass or birch pollen, immunotherapy against HDM shows considerably lower success rates [7, 8]. One reason for this reduced therapeutic success might be the fact that most commercially available therapeutic agents for immunotherapy of HDM allergy are standardized to the major allergens Der p 1, Der p 2, and Der f 1 [9, 10], thereby not matching for patients sensitized to other components. Up to now, more than 20 allergen components of *D. pteronyssinus* with a varying prevalence and importance for therapy have been described. Weghofer et al. [11] showed a high allergenic activity of Der p 23. This new allergen component is localized in the peritrophic matrix lining the midgut of *D. pteronyssinus* and also the surface of fecal pellets. Due to its association with mite feces, it becomes airborne and respirable, which might be a reason for its impact on the development of HDM allergy.

The aim of the current study was to investigate retrospectively the value of recombinant allergen-based IgE serology using an experimental allergen chip (MeDALL chip) in HDM-allergic patients tested negative for the commercially available HDM components Der p 1, Der p 2, and Der p 10 [12]. The results may identify components which can improve diagnostics and therapy of HDM allergy in the future.

Patients and Methods

Patient data were selected from the allergy database of the Department of Otorhinolaryngology, and Head and Neck Surgery of the Ludwig Maximilians University in Munich where all relevant diagnostic results of patients are stored. The study was approved by the local ethics committee and the local data protection commissioner. All patients gave informed consent. The database was scanned for consecutive patients with a proven perennial allergy to HDM who presented at our institution between 2001 and 2010. The diagnosis of HDM allergy was based on the following selection criteria: (1) A positive skin prick test for *D. pteronyssinus*. The skin prick test (ALK-Abelló, Wedel, Germany) was read after 20 min. (2) CAP class ≥1 [≥0.35 kU_A/l; ImmunoCAP fluorescence enzyme immunoassay (FEIA); Thermo Fisher Scientific, Freiburg, Germany] for the HDM *D. pteronyssinus*. (3) Positive intranasal provocation with a standard provocation solution (*D. pteronyssinus*; ALK-Abelló) according to guideline specifications [13] (decrease in rhinomanometry >40% at 150 Pa on the allergen-challenged side or symptom score >3, or decrease in rhinomanometry >20% at 150 Pa on the allergen-challenged side in combination with a symptom score >2). The provocation solution contained 200,000 SQ units/ml or 19.6 μg/ml Der p 1 [pers. notification Prof. Dr. E. Wüstenberg, ALK-Abelló]. (4) Availability of the patient's serum at our allergy serum bank.

Nasal symptoms were semiquantitatively assessed using four categories on the day of the first visit: (1) obstruction, (2) rhinorrhea, (3) sneezing, and (4) itching. Each symptom could be answered between 0 and 3: 0 = no impairment, 1 = mild impairment, 2 = moderate impairment, 3 = severe impairment.

Fluorescence Enzyme Immunoassay

Sera of the patients which fulfilled the above-mentioned criteria were analyzed for specific IgE antibodies to nDer p 1, rDer p 2, and rDer p 10 using the FEIA method with a commercial test kit (ImmunoCAP-FEIA, Thermo Fisher Scientific). Results were stated in CAP classes as well as in concentrations (kU/l).

MeDALL Allergen Chip Analysis

Sera of patients with a negative result to nDer p 1, rDer p 2, and rDer p 10 were examined for specific IgE antibodies to cross-reactive carbohydrate determinants (CCDs) and then transferred to ThermoFisher-Phadia multiplexing, Vienna, Austria, for MeDALL chip analysis.

The MeDALL allergen chip is based on the latest allergen microarray technology for diagnosis and monitoring of IgE and IgG reactivity profiles of allergic patients which was developed within the FP 7-funded European Union project MeDALL in collaboration with the Division of Immunopathology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, and ThermoFisher-Phadia multiplexing. Beside a broad variety of different allergen groups, the chip contains a panel of 13 components from *D. pteronyssinus* (Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 11, Der p 14, Der p 15, Der p 18, Der p 21, and Der p 23) and clone 16-encoded allergen. Sera from patients fulfilling the above-mentioned criteria were examined by the chip as described [12].

Statistical Analysis

Statistical analysis was performed with SigmaStat and SigmaPlot 2000 for Windows version 6.00 (Jandel Corp., San Rafael, Calif., USA). To compare nasal symptoms, normally distributed data were tested by t test and given as means ± SD. To compare non-normally distributed data, the Mann-Whitney rank sum test was used, and results are given as medians and ranges. To examine the correlation of two variables, the Pearson product correlation coefficient was used.

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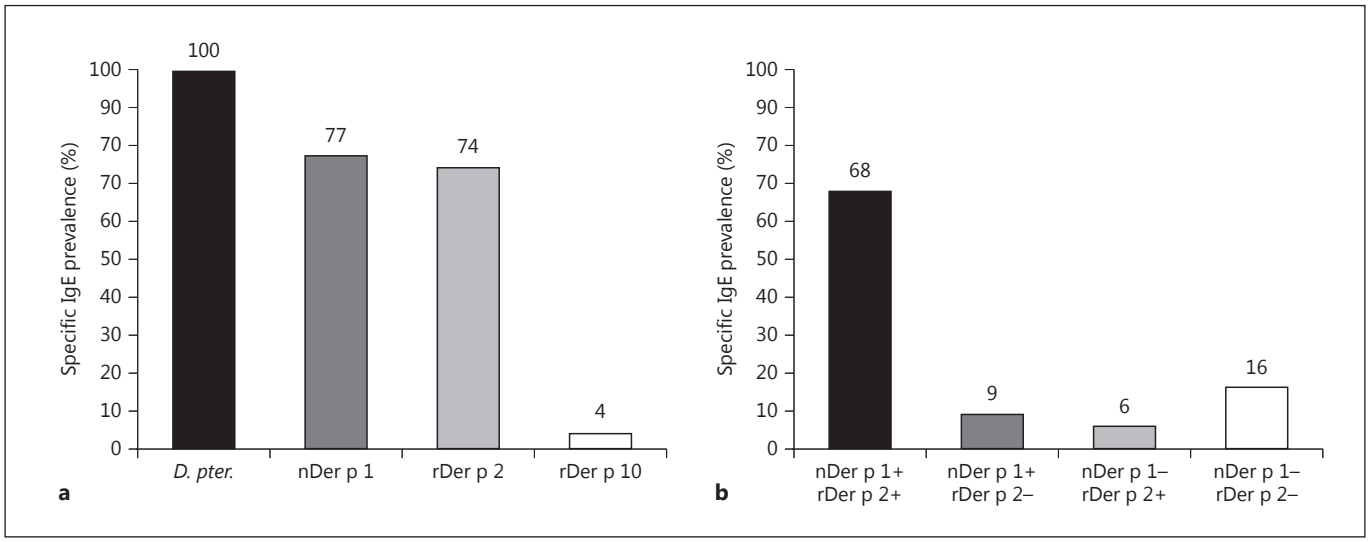

Fig. 1. a Prevalence of IgE reactivity to *D. pteronyssinus (D. pter.)*, nDer p 1, rDer p 2, and rDer p 10 in proven HDM-allergic patients in southern Bavaria (n = 97). **b** Prevalence rates for different combinations of the two major allergens Der p 1 and Der p 2. Sixteen patients negative for the two allergen components had also negative results for Der p 10. Adapted from [4], with kind permission of Springer publishing house.

Results

The database query with the above-mentioned inclusion criteria resulted in 97 patients with a proven allergy to HDM. Prevalence rates for *D. pteronyssinus*, nDer p 1, rDer p 2, and rDer p 10 were 100, 77, 74 and 4%, respectively. IgE concentrations were 15.69 ± 21.33, 10.40 ± 15.85, 17.63 ± 24.76 and 0.89 ± 0.80 kU/l (means ± SD) respectively. IgE levels to HDM extract *D. pteronyssinus* correlated well with the sum of IgE levels of Der p 1, Der p 2 and Der p 10. Pearson's correlation coefficient r = 0.851, $p < 0.05$, n = 81. Sixteen patients showed negative results to all three components (table 1; fig. 1). Within this group, gender distribution showed 9 female (56%) and 7 male (44%) patients with a mean age of 34.1 (14.7 SD) years and a range between 11 and 60 years. Nine patients (56%) showed a single sensitization to HDM, 7 patients (43%) showed additional sensitization to further aeroallergens.

Table 1. Patient demographics and study results

All patients, n (%)	97 (100)
Mean age, years (range)	30 (8–66)
Gender, n (%)	
Male	56 (58)
Female	41 (42)
Monosensitized to HDM, n (%)	30 (31)
Polysensitized, n (%)	61 (63)
No data available, n (%)	6 (6)
Nasal symptoms, means ± SD	
Obstruction	1.95±1.07
Rhinorrhea	1.41±1.00
Sneezing	1.32±0.96
Itching	1.12±0.93

Nasal symptoms were semiquantitatively assessed on the day of the first visit on a scale from 0 (no impairment) to 3 (severe impairment).

MeDALL chip evaluation of these 16 sera tested positive to *D. pteronyssinus* but negative to Der p 1, Der p 2, and Der p 10 resulted in 5 patients (31%) sensitized to Der p 23. All other HDM allergens on the chip (Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, and Der p 21, and clone 16) were negative. Negative test results were also seen for the two major allergen components of *D. farinae* Der f 1 and Der f 2 in these 16 patients. Examination of specific IgE antibodies to CCDs showed negative results in 12 patients, 2 patients showed positive results (1 CAP class 2 and 1 CAP class 1). Both patients were polysensitized with high concentrations of specific IgE to birch, grass, and other plants. In 2 patients, there was no serum left for this examination. Detailed information about the 16 patients who underwent MeDALL chip evaluation can be found in table 2. In

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Table 2. Detailed evaluation of the 16 HDM-allergic patients who were negative for Der p 1, 2, and 10 by conventional IgE serology

Patient	Age, years	Obstruction	Rhinorrhea	Sneezing	Itching	SPT *D. pter.*	SPT *D. far.*	SPT histamine	sIgE *D. pter.*, kUA/l	sIgE *D. far.*, kUA/l	CCD, kUA/l	NPT rhinomanometry (decrease)	NPT symptom score	NPT evaluation	Sensibilization	MeDALL Der p 23
C.L.	**11**	**1**	**0**	**2**	**0**	**2**	**0**	**2**	**1.52**	**0.69**	**0.00**	+	–	+	**M**	+
A.S.K.	**21**	**3**	**2**	**0**	**0**	**2**	**1**	**3**	**1.44**	**0.35**	**0.00**	–	+	+	**P**	+
O.C.	53	2	2	3	3	1	1	3	0.38	0.35	0.00	+	n.a.	+	M	–
S.H.	**37**	**3**	**2**	**1**	**3**	**2**	**1**	**3**	**1.15**	**0.35**	**0.00**	+	+	+	**P**	+
D.S.	**18**	**1**	**2**	**1**	**2**	**3**	**3**	**3**	**0.37**	**0.39**	**0.35**	–	+	+	**M**	+
G.R.	60	2	1	3	2	1	1	3	0.72	0.56	0.00	–	+	+	P	–
M.B.	**27**	**n.a.**	**n.a.**	**n.a**	**n.a.**	**3**	**0**	**3**	**2.99**	**0.59**	**1.60**	**n.a.**	**n.a.**	+	**P**	+
K.L.	56	3	1	1	2	2	0	3	0.67	0.50	0.00	n.a.	n.a.	+	M	–
H.H.	27	n.a.	n.a.	n.a.	n.a.	3	1	3	3.84	3.69	0.00	+	–	+	M	–
J.K.K.	23	n.a.	n.a.	n.a.	n.a.	2	2	3	0.45	0.39	0.00	–	+	+	M	–
E.F.	27	1	0	0	0	2	2	3	1.40	1.32	0.00	–	+	+	P	–
A.K.	29	n.a.	n.a.	n.a.	n.a.	1	1	3	0.46	0.40	0.00	–	+	+	M	–
S.R.S.	38	2	2	1	1	2	2	3	0.85	0.66	0.00	+	+	+	P	–
J.T.	56	2	2	1	1	2	2	3	0.74	0.68	0.00	–	+	+	M	–
H.W.	36	3	2	2	1	1	1	3	0.75	0.86	0.00	–	+	+	M	–
S.M.	29	0	1	1	0	1	1	3	1.41	1.61	0.00	–	+	+	P	–
Mean	34.25	1.92	1.42	1.33	1.25	1.88	1.19	2.94	1.20	0.84						
SD	14.84	1.00	0.79	0.98	1.14	0.72	0.83	0.25	0.96	0.84						
Max.	60.00	3.00	2.00	3.00	3.00	3.00	3.00	3.00	3.84	3.69						
Min.	11.00	0.00	0.00	0.00	0.00	1.00	0.00	2.00	0.37	0.35						

Patients who had a positive result on the MeDALL chip (Der p 23) are shown in bold. Nasal symptoms (obstruction, rhinorrhea, sneezing, and itching) were semiquantitatively assessed on the day of the first visit: 0 = no impairment, 1 = mild impairment, 2 = moderate impairment, 3 = severe impairment. Skin prick test (SPT): 0 = wheal <3 mm, 1 = wheal 3–4 mm, 2 = wheal 4–5 mm, 3 = wheal 5–6 mm, 4 = wheal >6 mm. Nasal provocation test (NPT): decrease in rhinomanometry >40% at 150 Pa on the allergen-challenged side or symptom score >3, or decrease in rhinomanometry >20% at 150 Pa on the allergen-challenged side and symptom score >2. *D. pter.* = *D. pteronyssinus*; *D. far.* = *D. farinae*; M = monosensitized; P = polysensitized; n.a. = not available; Max. = maximum; Min. = minimum.

the 5 Der p 23-positive patients, specific IgE levels to HDM extract (measured in kU/l) and MeDALL chip results to Der p 23 (measured in ISAC standardized units) tend to increase together with Pearson's correlation coefficient r = 0.808. Unfortunately, correlation was not significant (p = 0.084) due to the low number of patients (n = 5). The symptoms of these patients did not differ from those of the entire cohort of 97 HDM-allergic patients [obstruction median 2.00 (range 0.00–3.00) vs. 2.00 (0.00–3.00), p = 0.69; rhinorrhea 2.00 (0.00–2.00) vs. 1.00 (0.00–3.00), p = 0.96; sneezing 1.00 (0.00–3.00) vs. 1.00 (0.00–3.00), p = 0.99, and itching 1.00 (0.00–3.00) vs. 1.00 (0.00–3.00), p = 0.93] nor did Der p 23-positive in comparison to Der p 23-negative patients [obstruction 2.00 ± 1.16 (mean ± SD) vs. 1.88 ± 0.99, t(10) = 0.20, p = 0.85; rhinorrhea 2.00 (0.00–2.00) vs. 1.50 (0.00–2.00), p = 0.68; sneezing 1.00 ± 0.82 vs. 1.50 ± 1.07, t(10) = 0.82, p = 0.43, and itching: 1.25 ± 1.50 vs. 1.25 1.04, t(10) = 0.00, p = 1.00].

For the 11 sera which were tested negative for all allergen components, a correlation was calculated between the concentrations of specific IgE antibodies to the extracts of *D. pteronyssinus* and *farinae*, resulting in a correlation coefficient of 0.992, p < 0.0001.

From 7 patients, sera were available for further testing of which 3 sera showed IgE reactivity to dot-blotted nDer p 1, and 2 others reacted with high-molecular weight components (>100 kDa) in nitrocellulose-blotted HDM extract when tested with ^{125}I-labeled anti-IgE in a RAST-based assay (data not shown). The HDM extract-specific IgE levels of the 11 patients were <3.9 kUA/l (table 2).

Discussion

By means of the actually available HDM allergen components (Der p 1, Der p 2, and Der p 10), it was not possible to diagnose HDM allergy in a number of patients when used alone. Research groups from central Europe, Africa, Brazil, and Australia have published different IgE reactivity patterns in HDM-allergic patients, demonstrating that there is no single major allergen component that can be used for diagnostic purposes alone worldwide [3–5, 14–17]. Therefore, the aim of this study was to analyze

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the reactivity profile of proven HDM-allergic patients which were tested negative for the commercially available allergens Der p 1, Der p 2, and Der p 10 with an experimental allergen chip containing additional 10 HDM allergen components. Special interest was focused on Der p 23 as Weghofer et al. [11] showed in 347 patients a comparable prevalence of Der p 23 (74%) in comparison to Der p 1 and Der p 2 indicating the importance of this allergen component as a new major HDM allergen. This fact is supported by latest research from Thailand where 54% of 222 HDM-allergic patients displayed Der p 23-specific IgE responses [18]. In the current study, 5 of 16 patients showed positive results for Der p 23 whereas all other allergen components including Der p 4, which showed a high prevalence in an aboriginal community in Australia [19], were tested negative. Symptoms of Der p 23-positive patients did not differ from symptoms of patients who tested negative for this component. Der p 23 therefore may help to clarify HDM allergy in nearly one third of the cases when until now commercially available HDM component testing leads to negative results in the catchment area of our hospital in southern Germany.

Cross-reacting group 1 and group 2 allergens from *D. pteronyssinus* and *farinae* are well known [20]. In all the 16 patients, Der f 1 and Der f 2 as well as Der p 1 and Der p 2 showed negative results in immunoassay biochip testing in spite of positive results for allergen extracts of *D. pteronyssinus* and *farinae* by FEIA. In patients tested negative for all HDM components, concentrations of IgE antibodies against both allergen extracts correlated well, indicating a cross-reacting allergen component which is not covered by the MeDALL chip so far. Chan et al. [21] were able to identify a new allergen component of *D. farinae* (Der f 24), an ubiquinol-cytochrome c reductase binding protein originating from *Enterobacter* species inhabiting the mite gut. The authors highlight the fact that the internal HDM body is host to more than 100 bacterial species, with a predominance of *Enterobacter* species, and that allergen components from their microbiome can be of importance for HDM allergenicity and immunotherapy mechanisms. Although not proven yet, the results of Chan et al. [21] might explain our findings for group 1 and 2 allergens from *D. pteronyssinus* and *farinae* via reactions to bacterial allergen components which can be found in both allergen extracts.

CCDs from plants and invertebrates are able to induce IgE production with cross-reacting properties [22–24]. CCD sensitization is normally considered clinically irrelevant due to a poor activity in vivo but can serve as a disturbing factor in specific IgE assays especially in patients allergic to plants and in patients with a *Hymenoptera* venom allergy [25, 26]. Only 2 of 15 patients in the current study showed positive results for CCDs indicating that the good correlation between the allergen extracts of *D. pteronyssinus* and *farinae* is not caused by these cross-reacting antibodies.

Subcutaneously or sublingually applied allergen-specific immunotherapy represents the only disease-modifying and allergen-specific approach with long-lasting effects. In contrast, symptomatic therapy is only able to decrease symptoms like nasal obstruction, rhinorrhea and itching but cannot influence the allergic immune response itself. From this perspective, immunotherapy is superior to symptomatic therapy. In our study, 11 patients (11%) showed negative results for the previous HDM major allergens Der p 1 and Der p 2 but additionally showed negative results for further 10 HDM allergens when using the experimental MeDALL chip. Most companies in the market of HDM immunotherapy standardize their formulations to Der p 1, Der p 2, or Der f 1 [9, 10]. Analysis of HDM extracts from different manufacturers revealed varying allergen compositions and contents when examined for several *D. pteronyssinus* allergen components (Der p 1, 2, 5, 7, 10, and 21) by Casset et al. [27]. Der p 1 and Der p 2 could be detected in all extracts, but high variations in concentrations were seen, whereas other components, e.g. Der p 21, were completely absent in many formulations. Consequently, patients with a proven allergy due to one of these two major allergen components should benefit from therapy. But what should be done with patients not sensitized to theses major components, showing sensitization to Der p 23 or the HDM extract only? Are these patients suitable for immunotherapy with the actually available therapeutic solutions? These questions should be addressed in the future to improve clinical results of immunotherapy and to reduce treatment failure. Eleven of the 97 patients (11%) in our study showed a reactivity profile with none of 13 HDM allergen components in commercially available test platforms. As a consequence of these findings, molecular diagnostics for major allergen components should be mandatory before immunotherapy in HDM-allergic patients. For diagnostic purposes, measurement of specific antibodies to HDM extracts is a safe and routine procedure. To optimize therapy outcome and to improve reporting and comparability of clinical trials, it would be desirable to have more information about the content of major and minor allergen components in therapeutic solutions used in daily routine as well as in clinical trials.

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Der p 23 is a new important HDM allergen which can help to clarify perennial allergic symptoms when the known major HDM allergen components Der p 1 and Der p 2 as well as Der p 10 were negative. Further research concerning prevalence and clinical relevance of Der p 23 is needed to improve diagnostics and therapy of house dust mite allergy in the future. It is desirable to have Der p 23 commercially available for research purposes as well as in clinical routine.

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