

Evaluation of Specific IgE to the Recombinant Group 2 Mite Allergens Lep d 2 and Tyr p 2 in the Pharmacia CAP System

Eva Johansson Tove Lisa Jenny Eriksson Susanna Olsson
Marianne Kronqvist Paul Whitley S.G.O. Johansson Guro Gafvelin
Marianne van Hage-Hamsten

Department of Laboratory Medicine, Division of Clinical Immunology, Karolinska Institute and Hospital, Stockholm, Sweden

Key Words

CAP system · Group 2 allergens · *Lepidoglyphus destructor* · Recombinant allergens · Lep d 2 · Tyr p 2 · *Tyrophagus putrescentiae*

Abstract

Background: Several recombinant allergens have been shown to be potentially useful for diagnosis of IgE-mediated allergy, but only a few recombinant allergens are at present commercially available in serological assays for detection of specific IgE. The aim of this study was to evaluate the IgE binding to the recombinant major dust mite allergens rLep d 2 and rTyr p 2 and compare it with the IgE binding to the commercial mite extracts *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* in the Pharmacia RAST CAP System. **Methods:** The recombinant allergens rLep d 2 and rTyr p 2 were immobilised on ImmunoCAPs, and sera from 461 Swedish farmers who are frequently exposed to mites were analysed for specific IgE antibodies. Immunoblotting was performed to evaluate discrepancies between the results obtained with the recombinant and the commercial CAP assays. **Results:** The IgE values of each recombinant assay significantly correlated with the IgE values of the corresponding commercial CAP assay. The sensitivity of the rLep d 2 assay was 73.3% and that of the rTyr p 2 assay,

60.5% of that provided by the commercial *L. destructor* and *T. putrescentiae* assays. Two subjects out of 416, who tested negative in the commercial *L. destructor* assay, were positive to rLep d 2. The corresponding figures for rTyr p 2 and the *T. putrescentiae* extract were 5/418. The possibility that these subjects were sensitised to *L. destructor* and *T. putrescentiae* could not be excluded. **Conclusions:** The data suggest that it may be possible to use rLep d 2 and rTyr p 2 on ImmunoCAPs to detect and quantify IgE antibodies to these, the major allergens of *L. destructor* and *T. putrescentiae*. It appears likely that the addition of just a few more recombinant *L. destructor* and *T. putrescentiae* allergens in the CAP assay will be sufficient for in vitro diagnosis of IgE mediated allergy to *L. destructor* and *T. putrescentiae*.

Introduction

Allergy to the dust mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* is not only common among individuals living in rural environments, e.g. farmers [1–4], but has also been shown to affect city dwellers [5, 6]. Immunoblotting studies have revealed at least one major allergen in each species, Lep d 2 and Tyr p 2, respectively [7, 8], and these have been cloned and expressed as recombinant

allergens [9–11]. Both allergens belong to the group 2 mite allergens, which have also been found in *Dermatophagoides pteronyssinus*, *D. farinae* and *Euroglyphus maynei* [12].

A serological diagnostic test, usually RAST (radio allergosorbent test) [13], is a valuable tool to help clinicians identify the allergens of importance in allergic disease. A great disadvantage with many in vitro systems for the determination of specific IgE, is that they are based on allergen extracts from non-purified biological sources, which makes standardisation difficult and batch-to-batch variation likely. This problem can be overcome by using recombinant allergens, as they can be produced in a pure and defined form and in practically unlimited amounts [14]. However, the recombinant allergens that have been produced do not all bind IgE antibodies to the same extent as their natural counterparts [15–18], making it necessary to evaluate the IgE binding capacity of each recombinant allergen before it is used in a diagnostic test. The Pharmacia CAP System (Uppsala, Sweden) is a diagnostic test calibrated against the World Health Organisation standard for IgE, and gives quantitative values for both total and specific IgE [19]. Recombinant allergens from *Aspergillus fumigatus* [20, 21], birch [22, 23], honey bee [24], *Malassezia furfur* [25] and rye grass [26] have been evaluated in the RAST CAP assay with promising results. The two birch pollen allergens, rBet v 1 and rBet v 2, and the timothy grass pollen allergens Phl p 1, Phl p 2, and Phl p 5 are now commercially available in the Pharmacia RAST CAP System.

The aim of this study was to compare the results obtained using the recombinant allergens Lep d 2 (rLep d 2) and Tyr p 2 (rTyr p 2) in the Pharmacia RAST CAP System, with those obtained using commercial whole mite extracts d71 (*L. destructor*) and d72 (*T. putrescentiae*). All analysed sera were from farmers participating in a study of respiratory allergy [27]. Most of these farmers are constantly exposed to mites in their daily work, and about 10% are sensitised to *L. destructor* or *T. putrescentiae*, according to RAST and skin prick tests using whole mite extract. In order to investigate whether additional sensitised subjects could be detected with the recombinant ImmunoCAPs we also analysed sera with negative RAST for d71 and d72. SDS-PAGE of mite extracts followed by immunoblotting was used to compare IgE binding to native (n)Lep d 2 and Tyr p 2 with IgE binding to the recombinant allergens on ImmunoCAPs.

Materials and Methods

Serum Samples

The 461 serum samples selected for this study were obtained from farmers participating in an epidemiological follow-up study of respiratory allergy [27]. All sera had previously been analysed [27] for specific IgE to *L. destructor* (d71) and *T. putrescentiae* (d72) in the Pharmacia RAST® FEIA CAP System (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Forty-five sera were positive for d71 and 43 for d72.

Preparation of rLep d 2 and rTyr p 2

Mite allergens Lep d 2 and Tyr p 2 were both expressed as C-terminal 6×His-tagged recombinant proteins (rLep d 2.01 and rTyr p 2) in *Escherichia coli* as described previously [10, 11]. Recombinant proteins were recovered from inclusion bodies, solubilised and purified by metal affinity chromatography and dialysed against 0.15 M PBS, pH 7.4. Purity was confirmed by SDS-PAGE and amino acid composition analysis. IgE binding properties of the recombinant allergens were evaluated by immunoblotting using sera from mite-allergic farmers. The protein concentration was calculated from the amino acid composition obtained after hydrolysis and amino acid analysis in a Pharmacia-LKB Alpha Plus 4151 instrument. Purified rLep d 2 and rTyr p 2 were kept at –20°C until used.

Extract Preparations

Extracts of *L. destructor* and *T. putrescentiae* were prepared from sieved whole cultures obtained from Allergon (Ängelholm, Sweden). The cultures were extracted in 0.15 M NaCl, 0.02% NaN₃ at 1/10 (w/v) for 16 h at room temperature, centrifuged at 6,600 g, and filtered through a 0.8-µm Millex-HA filter (Millipore, Bedford, Tex., USA). The protein concentration was determined with the Pierce Bicinchoninic Acid Protein Assay Reagent (Pierce Chemical Company, Rockford, Ill., USA). Extracts were kept at –20°C until used.

Recombinant ImmunoCAP Assay

rLep d 2 and rTyr p 2 were immobilised at the same concentration onto cellulose solid phase (ImmunoCAP) by covalent binding. Coupling concentration was adjusted to attain a linear measurement range and a background below 0.35 kilounits of allergen-specific IgE per litre (kU_A/l), the established value for a positive result in the RAST CAP System. The recombinant ImmunoCAPs were used in the Pharmacia automated RAST FEIA CAP System, according to the manufacturer's instructions. Serum samples containing IgE ≥0.35 kU_A/l against the recombinant allergens were analysed twice, as were sera which had a negative RAST to the recombinant allergens but a positive RAST to d71 or d72. The sensitivity of each recombinant assay was defined as a percentage of the number of samples positive to the corresponding commercial assay for whole mite extract.

SDS-PAGE and Western Blotting

SDS-PAGE and immunoblotting were performed as described earlier [8]. Briefly, crude extracts (1 mg) of *L. destructor* and *T. putrescentiae*, were separated under non-reducing conditions in homogeneous gels (15%) and electroblotted to a polyvinylidene difluoride membrane. After blotting, the membrane was blocked, cut into strips, incubated with serum samples diluted 1:4, and subsequently immunostained to visualise IgE-binding proteins. Sera positive for a 15-kD allergen in *L. destructor* extract and a 16-kD allergen in *T. putrescentiae* extract were scored positive for nLep d 2 and nTyr p 2,

Table 1. Serological results from 58 subjects sensitised to *L. destructor* and/or *T. putrescentiae*

Subject	RAST d 71 ku _A /l	RAST rLep d 2 ku _A /l	Immuno- blot Lep d 2	RAST d72 ku _A /l	RAST rTyr p 2 ku _A /l	Immuno- blot Tyr p 2	Subject	RAST d 71 ku _A /l	RAST rLep d 2 ku _A /l	Immuno- blot Lep d 2	RAST d72 ku _A /l	RAST rTyr p 2 ku _A /l	Immuno- blot Tyr p 2
1	17	0.56	+	5.8	4.3	+	30	0.76	0.94	+	0.35	0.77	+
2	14	29	+	10	19	+	31	0.73	<0.35	–	1.3	<0.35	–
3	12	16	+	5.0	7.3	+	32	0.72	0.84	+	0.43	<0.35	+
4	11	<0.35	–	0.48	<0.35	–	33	0.72	<0.35	–	0.89	<0.35	–
5	11	14	+	6.2	17	+	34	0.69	2.3	+	0.49	0.87	+
6	8.6	17	+	1.5	<0.35	–	35	0.62	1.1	+	0.57	0.81	+
7	8.2	10	+	17	10	+	36	0.62	0.79	+	0.53	0.64	+
8	5.2	5.3	+	4.8	3.9	+	37	0.58	0.90	+	0.62	0.67	+
9	4.9	9.9	+	2.9	6.5	+	38	0.55	0.83	+	<0.35	<0.35	–
10	3.9	6.1	+	<0.35	<0.35	–	39	0.55	<0.35	–	0.66	<0.35	–
11	3.7	4.3	+	2.6	2.7	+	40	0.47	0.39	–	0.97	<0.35	+
12	3.7	4.6	+	2.1	3.1	+	41	0.47	<0.35	–	<0.35	<0.35	–
13	3.2	4.8	+	2.5	3.0	+	42	0.46	<0.35	–	<0.35	<0.35	–
14	3.2	3.7	+	<0.35	<0.35	–	43	0.42	<0.35	–	0.53	<0.35	–
15	2.7	4.7	+	2.3	3.3	+	44	0.39	<0.35	–	0.93	<0.35	–
16	2.7	2.4	+	3.1	3.0	+	45	0.38	<0.35	–	<0.35	<0.35	–
17	2.2	2.5	+	1.8	2.2	+	46	<0.35	0.88	+	1.0	0.77	+
18	2.1	3.4	+	1.7	1.9	+	47	<0.35	0.54	+	<0.35	<0.35	–
19	1.8	13	+	0.95	5.9	+	48	<0.35	<0.35	–	1.2	<0.35	–
20	1.6	2.9	+	0.42	1.9	+	49	<0.35	<0.35	–	0.63	<0.35	–
21	1.4	<0.35	–	1.6	<0.35	–	50	<0.35	<0.35	–	0.44	<0.35	–
22	1.3	6.1	+	3.0	2.4	+	51	<0.35	<0.35	–	0.39	<0.35	–
23	0.98	0.78	+	1.4	0.77	+	52	<0.35	<0.35	–	0.38	<0.35	–
24	0.92	1.3	+	1.1	0.99	+	53	<0.35	<0.35	–	0.35	<0.35	–
25	0.92	<0.35	–	<0.35	<0.35	–	54	<0.35	<0.35	–	<0.35	2.1	–
26	0.87	1.1	+	0.56	0.62	+	55	<0.35	<0.35	–	<0.35	1.1	–
27	0.85	1.1	+	<0.35	<0.35	–	56	<0.35	<0.35	–	<0.35	0.43	–
28	0.84	<0.35	–	0.94	<0.35	–	57	<0.35	<0.35	–	<0.35	0.42	–
29	0.76	0.99	+	<0.35	<0.35	–	58	<0.35	<0.35	–	<0.35	0.41	–

RAST values for d71, rLep d 2, d72 and rTyr p 2 are shown. Immunoblot results are designated + for positive and – for negative detection of nLep d 2 and nTyr p 2.

respectively. Identification of nLep d 2 and nTyr p 2 was based on the results from inhibition of the extracts with corresponding recombinant allergens [manuscript in preparation].

Statistical Analysis

Correlation coefficients were determined with Spearman rank correlation analysis. The median values for IgE are expressed with interquartile ranges.

Results

Evaluation of rLep d 2 in the CAP Assay

A total of 461 serum samples with a known RAST value to *L. destructor* allergens (d71) were analysed for specific IgE to rLep d 2 in the CAP assay. All serological data for subjects with a positive RAST for rLep d 2 (n = 35) and/or d71 (n = 45) are shown in table 1.

The median values for specific IgE to rLep d 2 and d71 for the group with a dual positive RAST were in the same range, 2.9 and 2.1 kU_A/l, respectively (table 2). A significant correlation of IgE values was obtained between the two CAP assays, with an r_s value of 0.70 ($p < 0.001$) (fig. 1). The sensitivity of the rLep d 2 CAP assay was 73.3%, as 33 subjects were positive for rLep d 2 among the 45 with a pos-

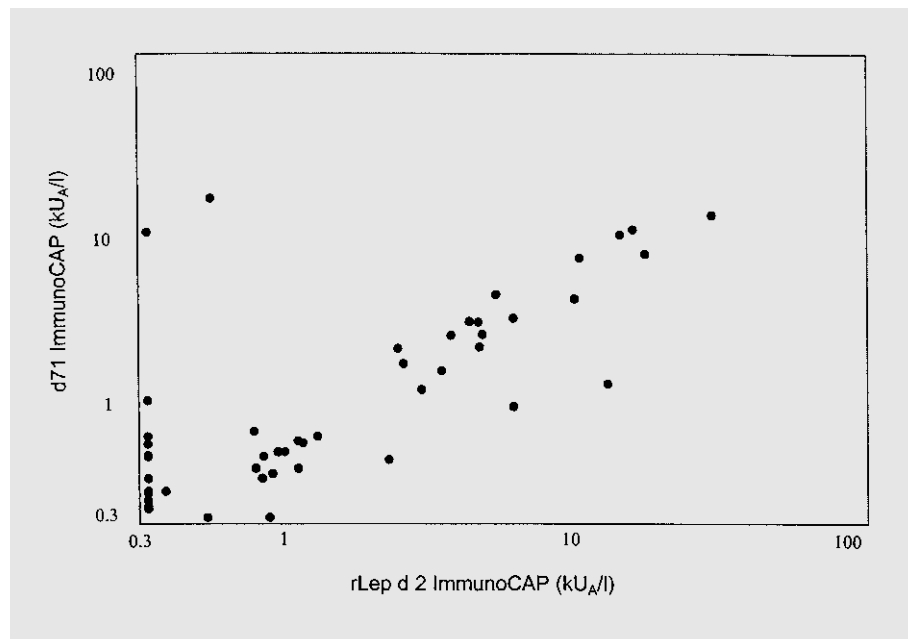


Fig. 1. Correlation of specific IgE values obtained by Pharmacia CAP System. ImmunoCAP d71 is prepared with *L. destructor* extract and ImmunoCAP rLep d 2 with rLep d 2. Plotted values are from 47 subjects positive for at least one of the two ImmunoCAPs. IgE values <0.35 kU_A/l are given as 0.34.

Table 2. Subjects with positive and negative RAST for d71, rLep d 2, d72 and rTyr p 2 (median value, 25th and 75th percentiles)

n	d71	Median kU _A /l	25th–75th percentile kU _A /l	rLep d 2	Median kU _A /l	25th–75th percentile kU _A /l	n	d72	Median kU _A /l	25th–75th percentile kU _A /l	rTyr p 2	Median kU _A /l	25th–75th percentile kU _A /l
33	+	2.1	0.76–3.9	+	2.9	0.99–6.1	26	+	1.9	0.70–3.1	+	2.6	0.83–4.2
12	+	0.64	0.45–0.86	–			17	+	0.66	0.44–0.97	–		
2	–			+		0.63, 0.80	5	–			+	0.43	0.42–1.1
414	–			–			413	–			–		

itive RAST for d71. SDS-PAGE and immunoblotting confirmed this result. Thirty-two of the 33 subjects tested positive for nLep d 2 in extract of *L. destructor* blotted to membrane (table 1). Furthermore, none of the 12 subjects with a positive RAST for d71 who tested negative in the rLep d 2 assay displayed specific IgE to nLep d 2 on immunoblots.

Two subjects among the 416 who had a negative RAST for d71 tested positive in the rLep d 2 CAP assay (No. 46 and 47) (table 1). Both subjects were also positive for nLep d 2 on immunoblots.

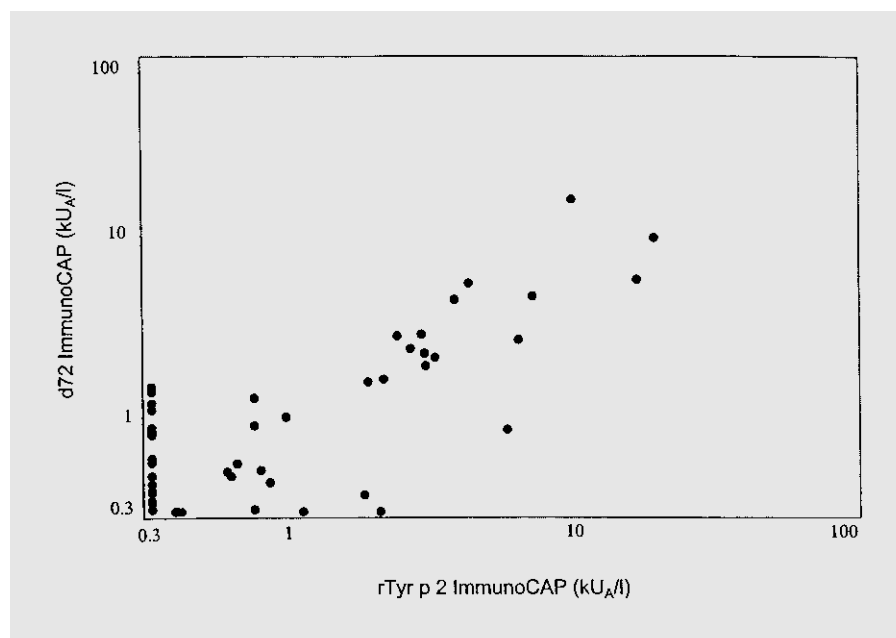
Evaluation of rTyr p 2 in the CAP Assay

All 461 serum samples were analysed for specific IgE to rTyr p 2 in the CAP assay. Serological data for subjects with a positive RAST for rTyr p 2 (n = 31), and/or d72 (n = 43), the commercial *T. putrescentiae* extract in the CAP System, are shown in table 1.

The IgE values obtained with the rTyr p 2 and d72 assay correlated significantly ($r_s = 0.61$, $p < 0.001$) (fig. 2). Among the 43 farmers with a positive RAST for d72, 26 subjects also tested positive for rTyr p 2 (table 2), giving a sensitivity of 60.5% for the rTyr p 2 assay. Seventeen subjects were positive for d72 but negative in the rTyr p 2 assay (table 1). Fifteen of these subjects were also negative for nTyr p 2 in extract of *T. putrescentiae*, as analysed by immunoblotting. This is to be compared with the immunoblotting results of the 26 subjects who were positive in the rTyr p 2 CAP assay. All of them were also positive for nTyr p 2 in extract of *T. putrescentiae* (table 1).

Five subjects with a negative RAST for d72 (n = 418; No 54–58) (table 1), displayed a positive RAST for rTyr p 2. However, they were all negative in immunoblotting for nTyr p 2 in the *Tyrophagus* extract.

Fig. 2. Correlation of specific IgE values obtained by Pharmacia CAP System. ImmunoCAP d72 is prepared with *T. putrescentiae* extract and ImmunoCAP rTyr p 2 with rTyr p 2. Plotted values are from 48 subjects positive for at least one of the two ImmunoCAPs. IgE values <0.35 kU_A/l are given as 0.34.



Discussion

Our goal is to obtain a reliable diagnostic test for the dust mites *L. destructor* and *T. putrescentiae* which uses standardised recombinant allergens with no batch-to-batch variation and has high sensitivity. In this study, we have coupled the recombinant major mite allergens Lep d 2 and Tyr p 2 to ImmunoCAP and used them in the Pharmacia CAP System to screen for specific IgE antibodies among a group of 461 farmers daily exposed to mites. The aim was to evaluate the outcome in comparison to the commercial whole mite extracts d71 (*L. destructor*) and d72 (*T. putrescentiae*).

There was a high correlation between the IgE values to the allergens rLep d 2 and rTyr p 2 and to their corresponding extracts. The correlation coefficients were 0.70 and 0.61, respectively, and this concords with data from other studies involving major allergens [24, 26]. On the other hand, Cramer et al. [20] obtained a correlation coefficient of only 0.31 when comparing IgE values for the commercial *A. fumigatus* extract (m3) and rAsp f 1, one of the major allergens of that fungus. They speculate that rAsp f 1 ImmunoCAP might contain about 200 times more Asp f 1 than m3 ImmunoCAP, since the extract is known to contain low amounts of Asp f 1. This illustrates the difficulty in comparing assays employing recombinant allergens with those that use the commercially available whole extracts. We feel that it is more appropriate to discuss the sensitivity and unspecific binding of an assay to evaluate the possible use in a diagnostic test.

The sensitivity of the rLep d 2 and the rTyr p 2 CAP assays, 73.3 and 60.5%, was calculated with the whole extract as the standard. These results are in good agreement with our earlier results from immunoblotting experiments where we demonstrated that Lep d 2 and Tyr p 2 are indeed the major allergens in extracts of *L. destructor* and *T. putrescentiae* [7, 8]. Furthermore, most subjects were sensitised only to a few allergens other than Lep d 2 and Tyr p 2. Other studies using the CAP assay to compare recombinant major allergens and corresponding whole extracts obtained a sensitivity of the recombinant assays in the same range as we did [20, 24, 26].

To evaluate possible unspecific binding to rLep d 2 and rTyr p 2 in the CAP assay, we used sera from farmers who are frequently exposed to mites who tested negative in the RAST to *L. destructor* and *T. putrescentiae*. Among 416 subjects with negative RAST to *L. destructor* and 418 to *T. putrescentiae*, only 2 were positive for rLep d 2 and 5 to rTyr p 2 (table 2). The 2 subjects who were positive for rLep d 2 were probably sensitised to this allergen, as they also tested positive for Lep d 2 in the *L. destructor* extract with the immunoblotting method. One reason why they were positive in the CAP assay for rLep d 2 but negative for the whole extract could be that the two ImmunoCAPs expose the relevant epitopes to different degrees. The 5 serum samples with a negative RAST for *T. putrescentiae* and a positive RAST for rTyr p 2 were also negative for nTyr p 2 in immunoblotting with extract of *T. putrescentiae*. However, 3 of these sera were very close to the cut-off value of 0.35

kU_A/l. In a previous study [27], these 5 subjects tested negative in the skin prick test using commercial extract of *T. putrescentiae*. A skin prick test with the recombinant allergen might reveal whether they score false-positive in the in vitro test.

The use of pure recombinant allergens in in vitro diagnosis has been evaluated in several studies, and the general conclusion is that assays using only one single recombinant allergen are more specific, but less sensitive [20, 21, 24]. The decrease in sensitivity is of course usually the result of relying on only one allergen, but it might also be a consequence of the coupling procedure [24]. Therefore, each recombinant allergen to be applied in a diagnostic test has to be individually evaluated.

The number of recombinant allergens needed to achieve optimal sensitivity for a diagnostic assay will probably vary to a great extent. For diagnosis of allergy e.g. to the fungus *A. fumigatus* it will be necessary to use several recombinant allergens, as *A. fumigatus* can produce more than 40 allergens [20, 28]. In contrast, the use of just a few recombinant allergens from tree [29] and grass pollen [30] seems to be

sufficient to diagnose pollen allergy. We have earlier, identified at least 21 allergen components in *L. destructor* with immunoblotting [7]. Several of these are of minor importance and will probably not be necessary in a diagnostic test. Nevertheless, it is obvious that allergens other than rLep d 2 and rTyr p 2 must be added in order to detect as many positive subjects as when using the whole extracts. It also remains to be investigated whether a battery of individual recombinant allergens or a 'cocktail' should be used for optimal results.

Acknowledgments

We thank Maria Lundberg at MIAB, Uppsala, for skillful production of recombinant ImmunoCAPs. Financial support for this study came from the Swedish Council for Work Life Research, the Swedish Foundation for Health Care, Sciences and Allergy Research, the Swedish Asthma and Allergy Association, the Swedish Society of Medicine, the Trygg-Hansa Foundation Fund, the Magn. Bergvall Foundation, the Hesselman Foundation, the Konsul Th.C. Bergh Foundation and the Karolinska Institutet.

References

- Cuthbert OD, Brostoff J, Wraith DG, Brighton WD: 'Barn allergy': asthma and rhinitis due to storage mites. *Clin Allergy* 1979;9:229–236.
- van Hage-Hamsten M, Johansson SGO, Höglund S, Tüll P, Wirén A, Zetterström O: Storage mite allergy is common in a farming population. *Clin Allergy* 1985;15:555–564.
- Terho EO, Husman K, Vohlonen I, Rautalahti M, Tukiainen H: Allergy to storage mites or cow dander as a cause of rhinitis among Finnish dairy farmers. *Allergy* 1985;40:23–26.
- Iversen M, Pedersen B: The prevalence of allergy in Danish farmers. *Allergy* 1990;45:347–353.
- Ebner C, Feldner H, Ebner H, Kraft D: Sensitization to storage mites in house dust mite (*Dermatophagoides pteronyssinus*) allergic patients. Comparison of a rural and an urban population. *Clin Exp Allergy* 1994;24:347–352.
- Luczynska CM, Griffin P, Davies RJ, Topping MD: Prevalence of specific IgE to storage mites (*A. siro*, *L. destructor* and *T. longior*) in an urban population and cross-reactivity with the house dust mite (*D. pteronyssinus*). *Clin Exp Allergy* 1990;20:403–406.
- Johansson E, Borgå Å, Johansson SGO, van Hage-Hamsten M: Immunoblot multiallergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1991;21:511–518.
- Johansson E, Johansson SGO, van Hage-Hamsten M: Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their cross-reactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1994;24:743–751.
- Schmidt M, Olsson S, van der Ploeg I, van Hage-Hamsten M: cDNA analysis of the mite allergen Lep d 1 identifies two different isoallergens and variants. *FEBS Lett* 1995;370:11–14.
- Olsson S, van Hage-Hamsten M, Whitley P, Johansson E, Hoffman DR, Gafvelin G, Schmidt M: Expression of two isoforms of Lep d 2, the major allergen of *Lepidoglyphus destructor*, in both prokaryotic and eukaryotic systems. *Clin Exp Allergy* 1998;28:984–991.
- Eriksson TLJ, Johansson E, Whitley P, Schmidt M, Elsayed S, van Hage-Hamsten M: Cloning and characterization of a group II allergen from the dust mite *Tyrophagus putrescentiae*. *Eur J Biochem* 1998;251:443–447.
- Thomas WR, Smith W: House-dust-mite allergens. Review. *Allergy* 1998;53:821–832.
- Wide L, Bennich H, Johansson SGO: Diagnosis of allergy by an in-vitro test for allergen antibodies. *Lancet* 1967;2:1105–1107.
- Scheiner O, Kraft D: Basic and practical aspects of recombinant allergens. Review. *Allergy* 1995;50:384–391.
- Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, Turner KJ: Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p I*. *J Exp Med* 1988;167:175–182.
- Morgenstern JP, Griffith IJ, Brauer AW, Rogers BL, Bond JF, Chapman MD, Kuo MC: Amino acid sequence of *Fel d I*, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. *Proc Natl Acad Sci USA* 1991;88:9690–9694.
- Chua KY, Kehal PK, Thomas WR, Vaughan PR, Macreadie IG: High-frequency binding of IgE to the *Der p* allergen expressed in yeast. *J Allergy Clin Immunol* 1992;89:95–102.
- Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, Ebner C, Breitenbach M, Rumpold H, Kraft D, Scheiner O: Four recombinant isoforms of *Cor a I*, the major allergen of hazel pollen, show different IgE binding properties. *Eur J Biochem* 1993;212:355–362.
- Axén R, Drevin H, Kober A, Yman L: A new laboratory diagnostic system applied to allergy testing; in: Johansson SGO (ed): Proceedings of a Clinical Workshop on IgE Antibodies and the Pharmacia CAP System in Allergy Diagnosis. Uppsala, Pharmacia, 1988, pp 3–5.
- Cramer R, Lidholm J, Grönlund H, Stüber D, Blaser K, Menz G: Automated specific IgE assay with recombinant allergens: evaluation of the recombinant *Aspergillus fumigatus* allergen I in the Pharmacia CAP System. *Clin Exp Allergy* 1996;26:1411–1419.

- 21 Hemmann S, Ismail C, Blaser K, Menz G, Crameri R: Skin-test reactivity and isotype-specific immune responses to recombinant Asp f 3, a major allergen of *Aspergillus fumigatus*. Clin Exp Allergy 1998;28:860–867.
- 22 Zimmermann K, Kober A, Grönlund H, Yman L: IgE antibodies against native birch pollen allergens and recombinant allergens (*Bet v* I and Profilin) measured by Pharmacia CAP system (abstract). ACI News 1994;suppl 2:493.
- 23 Elfman L, Svensson M, Lidholm J, Pauli G, Valenta R: Different profiles in specific IgE to rBet v 1 and rBet v 2 in patients allergic to birch pollen from six countries. Int Arch Allergy Immunol 1997;113:249–251.
- 24 Müller U, Fricker M, Wymann D, Blaser K, Crameri R: Increased specificity of diagnostic tests with recombinant major bee venom allergen phospholipase A2. Clin Exp Allergy 1997;27:915–920.
- 25 Zargari A, Schmidt M, Lundberg M, Scheynius A, Whitley P: Immunological characterization of native and recombinant Mal f 1 yeast allergen. J Allergy Clin Immunol 1999;103:877–884.
- 26 Tamborini E, Faccini S, Lidholm J, Svensson M, Brandazza A, Longhi R, Groenlund H, Sidoli A, Arosio P: Biochemical and immunological characterization of recombinant allergen Lol p 1. Eur J Biochem 1997;249:886–894.
- 27 Kronqvist M, Johansson E, Pershagen G, Johansson SGO, 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.
- 28 Borgå Å: Allergens of *Aspergillus fumigatus*. PhD thesis, Karolinska Institutet, Stockholm, 1990.
- 29 Valenta R, Duchêne M, Vrtala S, Birkner T, Ebner C, Hirschwehr R, Breitenbach M, Rumpold H, Scheiner O, Kraft D: Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. J Allergy Clin Immunol 1991;88:889–894.
- 30 Valenta R, Vrtala S, Ebner C, Kraft D, Scheiner O: Diagnosis of grass pollen allergy with recombinant timothy grass (*Phleum pratense*) pollen allergens. Int Arch Allergy Immunol 1992;97:287–294.

Copyright: S. Karger AG, Basel 1999. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.