

Immunoblot and radioallergosorbent test inhibition studies of allergenic cross-reactivity of the predatory mite *Amblyseius cucumeris* with the house dust mite *Dermatophagoides pteronyssinus*

Nicolette W. de Jong*; Gis  le C. M. Groenewoud, MD*; Ronald van Ree, PhD†; Astrid van Leeuwen‡; Ab M. Vermeulen‡; Albert W. van Toorenenbergen, PhD‡; Hans de Groot, MD, PhD*; and Roy Gerth van Wijk, MD, PhD*

Background: In 1999, an extensive study among bell pepper growers showed that a predatory mite, *Amblyseius cucumeris*, is a potentially relevant source of occupational allergens because 23% of the population had positive skin prick test reactions.

Objective: To investigate whether cross-reactivity between *A cucumeris* and *Dermatophagoides pteronyssinus* is responsible for the cosensitization to both mite species found in 58.7% of *A cucumeris*-sensitized greenhouse workers.

Methods: Fifteen serum samples from greenhouse workers with work-related inhalant allergy and a positive radioallergosorbent test (RAST) reaction to *A cucumeris* or *D pteronyssinus* were selected for immunoblot analysis using extracts of both mites. A subselection (n = 5) was used for RAST and immunoblot inhibition to investigate potential cross-reactivity.

Results: On immunoblot, 2 distinct patterns were observed: one pattern showed common protein bands in *A cucumeris* and *D pteronyssinus* blots suggestive of cross-reactivity between *A cucumeris* and *D pteronyssinus* and the other pattern showed no shared protein bands. *Dermatophagoides pteronyssinus* RAST inhibition with *A cucumeris* extract was low in 4 serum samples (<25% inhibition) and nearly absent in 1 serum sample; *A cucumeris* RAST inhibition with *D pteronyssinus* extract was high in 1 serum sample (75% inhibition), low in 2 serum samples (35% and <15% inhibition), and absent in 2 serum samples. These results were confirmed by immunoblot inhibition experiments.

Conclusions: *Amblyseius cucumeris*, a new occupational allergen, has species-specific antigens and common antigens that are cross-reactive with the house dust mite *D pteronyssinus*.

Ann Allergy Asthma Immunol. 2004;93:281–287.

INTRODUCTION

Allergens from mites are well-known sensitizers in allergic diseases. One of the most important allergenic mite species is the house dust mite *Dermatophagoides pteronyssinus*. Furthermore, other mite genera, such as spider mites^{1–4} and storage mites,^{5–9} have been shown to be important inducers of allergic reactions as well. In 1999, an extensive study¹⁰ in bell pepper workers showed that predatory mites can also cause allergic disorders. The predatory mite *Amblyseius cucumeris* is used to protect crops against thrips in this branch of horticulture.^{11,12} Thrips are one of the major pests in greenhouses, and they commonly belong to the species *Frankliniella occidentalis* and *Echinothrips americanus*. Numerous studies^{5–9} have shown cross-reactivity between different mites of closely related taxonomy. Although *A cucumeris* is only distantly related to *D pteronyssinus* (Fig 1), there was a high percentage (58.7%) of cosensitization to *D pteronyssi-*

nus in these *A cucumeris*-sensitized greenhouse workers.¹⁰ The aim of this study was to evaluate the allergenic cross-reactivity between *A cucumeris* and *D pteronyssinus*. Cross-reactivity patterns can help elucidate to what extent occupational exposure to *A cucumeris* is an independent risk factor for the development of allergic disorders.

Because greenhouse workers in the Netherlands are mainly exposed to predatory mites as the new biological control agent, we focused primarily on this mite in this study. Future experiments are planned regarding *Tyrophagus putrescentiae* sensitization, which is also found in many greenhouse workers.

MATERIALS AND METHODS

Patients

For this study, serum samples were selected from a cohort of greenhouse workers who had previously participated in a study of bell pepper horticulture.¹⁰ That investigation included 472 employees, of which 109 (23%) were sensitized to *A cucumeris*, as demonstrated by skin prick testing (SPT). Sixty-three of these workers had a positive radioallergosorbent test (RAST) reaction to *A cucumeris* (concordance, 58%). Serum samples from 15 of these *A cucumeris*-sensitized patients (mean age, 38.6 years; range, 25–62 years)

* Department of Allergology, Erasmus MC, Rotterdam, the Netherlands.

† Department of Immunopathology, Sanquin, Amsterdam, the Netherlands.

‡ Department of Clinical Chemistry, Erasmus MC, Rotterdam, the Netherlands.

Received for publication November 28, 2003.

Accepted for publication in revised form April 21, 2004.

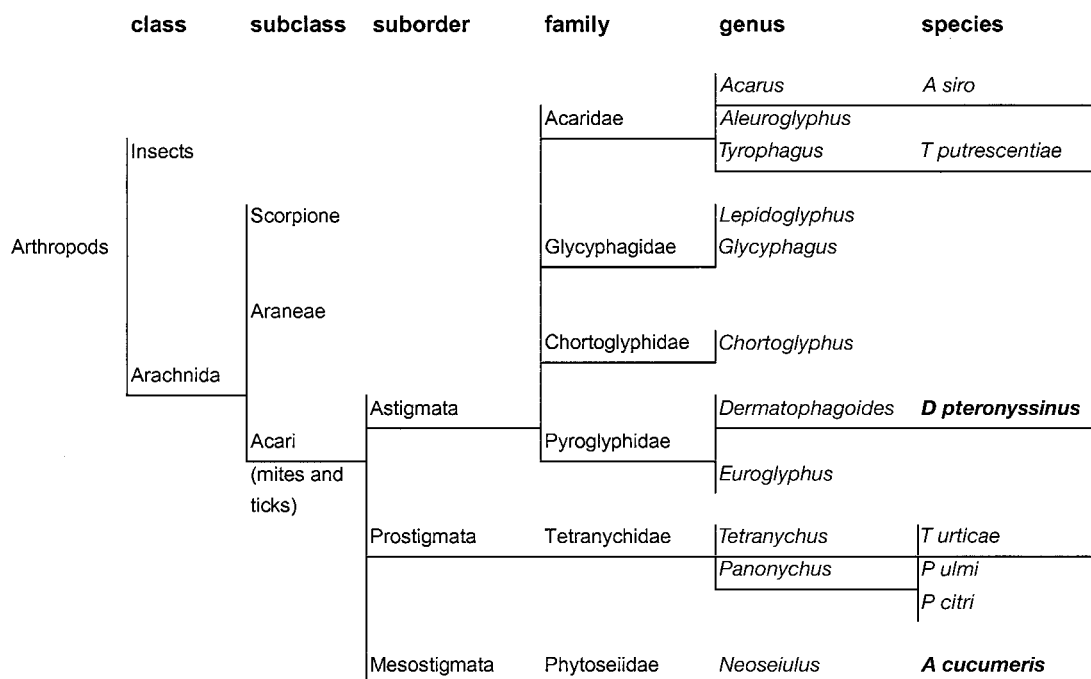


Figure 1. Taxonomic position of the mites.

were selected on the basis of a positive SPT reaction (>3 mm in diameter), a positive RAST reaction (class 1 or better), a case history positive for *A cucumeris*, and enough material for further immunoblot and IgE inhibition studies (Table 1).

Skin Prick Tests

Skin prick tests were performed in employees with work-related symptoms. The symptoms were considered to be work

related when the employees noticed a substantial improvement or complete regression of their complaints on weekends and holidays. SPT was performed by applying 1 drop of allergenic extract to the skin of the volar side of the forearm. Subsequently, the dermis was punctured with a standardized prick test lancet (ALK Abelló, Horsholm, Denmark), and results were read after 20 minutes. Reactions are expressed in

Table 1. Characteristics of the Patients, Results of Skin Prick Testing with Inhalant Allergens, and Serum IgE Values to *Amblyseius cucumeris* and *Dermatophagoides pteronyssinus*

Serum sample No.	Age, y	Sex, M/F	UAS	LAS	Mites					Pollen SPT, mm			Animal SPT, mm	
					Ac SPT, mm	RAST, kU/L	Dp SPT, mm	RAST, kU/L	Tp SPT, mm	Bell pepper	Tree	Grass	Cat	Dog
1	62	M	+	+	10	1.6	12	13.8	0	0	0	0	0	4
2	44	M	+	—	11	4.71	4	<0.35	5	11	0	0	0	0
3	31	M	+	+	6	4.82	4	2.45	5	6	4	4	0	4
4	47	M	+	—	10	3.89	8	29.5	5	9	0	0	4	6
5	25	M	+	—	18	14	7	63.6	4	7	6	10	10	8
6	28	F	+	+	5	10.5	0	<0.35	0	14	5	0	0	0
7	37	M	+	+	6	10.9	8	79.9	0	11	5	10	7	4
8	41	M	+	—	8	46.2	6	18	0	8	5	7	0	0
9	26	M	+	—	9	8.04	9	7.12	0	5	8	10	0	4
10	60	M	+	—	8	2.25	0	2.42	0	6	5	4	6	0
11	34	M	+	—	10	28.3	0	<0.35	4	0	0	0	0	0
12	28	M	+	+	11	10.2	5	2.26	3	0	0	0	0	0
13	29	F	+	—	7	4.79	0	0.51	0	0	0	0	0	0
14	47	F	+	+	4	22.9	4	>100	0	8	0	8	0	4
15	40	M	+	+	8	30.7	4	93	5	3	0	0	9	0

Abbreviations: Ac, *Amblyseius cucumeris*; Dp, *Dermatophagoides pteronyssinus*; LAS, work-related lower airway symptoms; RAST, radioallergen sorbent test; SPT, skin prick test; Tp, *Tyrophagus putrescentiae*; UAS, work-related upper airway symptoms.

millimeters of mean wheal diameter (adding the longest diameter to the orthogonal diameter measured at half of the longest diameter and dividing by 2). A diameter of 3 mm or more was considered positive.⁵ Dilution buffer was used as a negative control, and histamine chloride (10 mg/mL) was used as a positive control. The same investigator performed all of the SPTs.

Allergens for SPT

For SPT, living predatory mites (*A cucumeris*) were kindly supplied by Koppert Biological Systems (Berkel en Rodenrijs, the Netherlands). A 10% (wt/vol) extract was prepared in phosphate-buffered saline (PBS) (pH 7.4) containing 0.03% human serum albumin and 0.5% phenol at 4° C.

Pollen from the flowers of the bell pepper plants was collected in a greenhouse. The flowers were in full bloom. A 25% (wt/vol) extract was prepared in PBS-AT (0.82% sodium chloride, 10 mmol/L of phosphate, 0.1% sodiumazide, 0.2% Tween, 0.3% bovine serum albumin, and pH 7.4). The extracts were centrifuged for 10 minutes at 2,000g, and supernatants were passed through a 0.22- μ m Millex GS filter (Millipore, Amsterdam, the Netherlands). All extracts were stored in appropriate aliquots at -20° C until use in skin tests. Before use, extracts were defrosted for 1 hour and then mixed. In addition, SPTs were performed with *T putrescentiae* (SQ 505) and 5 common inhalant allergens from ALK Abelló: *D pteronyssinus* (SQ 503), tree mix (SQ 108), grass mix (SQ 293), dog dander (SQ 553), and cat dander (SQ 555).

Allergens for RAST, RAST Inhibition, and Immunoblotting

The Research Station for Floriculture and Glasshouse Vegetables (Naaldwijk, the Netherlands) kindly supplied the *A cucumeris* mites. A cultivating process to obtain a minimum of contamination with other mites was applied for this study. Usually the mites are cultivated on other mites. For this project, the *A cucumeris* mites were cultivated on a selection of flower pollen. All *D pteronyssinus* mite bodies were obtained from Commonwealth Serum Laboratories (Melbourne, Australia).

The protein concentration was analyzed using the benzenethonium chloride method¹³ on the Hitachi 911 routine clinical chemistry analyzer, according to the manufacturer instructions (Boehringer/Roche, Almere, the Netherlands), in the *D pteronyssinus* and *A cucumeris* supernatant. After extraction of 20 mg/mL in PBS-T (0.82% sodium chloride, 10 mmol/L of phosphate, 0.1% NaN₃, 0.2% Tween, and pH 7.4) for 1 hour at room temperature the protein concentration was 1.34 and 0.250 mg/mL, respectively.

IgE Antibody Measurements

IgE antibodies to *A cucumeris* and *D pteronyssinus* were measured using RAST, as described by Adkinson et al.¹⁴ Briefly, 10 mg of *A cucumeris* and *D pteronyssinus* bodies was extracted with 2 mL of coupling buffer (0.1 mol/L of sodium hydrogencarbonate and 0.5 mol/L of sodium chloride; pH 8.5) for 1 hour at 4° C. After centrifugation for 10

minutes at 1,400g, protein in the supernatant was covalently coupled to 100 mg of CNBr-activated Sepharose 4B (Sigma-Aldrich Corp, St. Louis, MO), according to the manufacturer instructions. Sepharose (4 mg per test)-coupled *A cucumeris* and *D pteronyssinus* was incubated overnight with 50 μ L of serum in a final volume of 550 μ L of PBS-AT. After 4 washes with PBS-T, radioiodinated rabbit anti-human IgE antibodies (Pharmacia, Uppsala, Sweden) were added. After overnight incubation and 4 washes, the percentage of bound radioactivity was measured and was expressed in kilounits per liter when read from the Pharmacia CAP standard curve obtained simultaneously using the same batch of radiolabeled anti-IgE antibodies.

IgE Inhibition Experiments

For IgE inhibition experiments, 10 mg of *A cucumeris* and *D pteronyssinus* was extracted in 1 mL of PBS-AT under rotation for 1 hour at room temperature. After centrifugation, the supernatant was vortexed, and 50 μ L of supernatant was incubated overnight at 4° C with 50 μ L of diluted serum. Dilutions of the serum samples in PBS-AT were made to obtain a concentration of specific IgE of approximately 5 to 10 kU/L. For *D pteronyssinus* inhibition, the serum samples were diluted as follows: serum sample 4, four times; serum sample 7, twenty times; serum sample 8, two times; and serum sample 15, ten times. For *A cucumeris* inhibition, serum samples 4, 7, and 9 were incubated undiluted; serum sample 8 was diluted 7 times; and serum sample 15 was diluted 4 times. All experiments were performed with the same freshly made extract. Some (50 μ L) of the mixture was incubated with Sepharose-coupled *A cucumeris* and *D pteronyssinus*. Serum samples were also incubated with PBS-AT and grass pollen extract (*Dactylus glomerata*, ARTU Biologicals BV, Lelystad, the Netherlands) (10 mg/mL of RAST-AT buffer) as control extract.

Immunoblotting and Immunoblot Inhibition Experiments

Mite extracts (20 μ g/cm) were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to the method of Laemmli.¹⁵ Gradient gels (10%–20% acrylamide) were prepared using the sodium dodecyl sulfate-polyacrylamide gel system protocol from GIBCO BRL (Research Products Life Technologies Inc, Gaithersburg, MD) and silver stained using the protein silver staining procedure as described by the instruction manual 80-1310-00 (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Western blotting was performed by transferring the proteins after separation (semidry) to nitrocellulose (Protran nitrocellulose transfer membrane [pore size, 0.2 μ m], Schleicher & Schuell Bioscience GmbH, Dassel, Germany) on a Novablot electrophoretic transfer apparatus, according to the protocol of the manufacturer (Invitrogen, Carlsbad, CA). After blocking with PBS-A (10 mmol/L of EDTA and 0.3% bovine serum albumin) for a minimum of 10 minutes, the blots were cut into 3-mm-wide strips. For inhibition, serum samples were allowed a 2-hour preincubation with 150 μ L of

20 mg/mL of mite extracts. Incubation with 150 μ L of PBS-AT served as a control. Immunoprobings of the blot strips took place overnight with 150 μ L of serum (with or without inhibitor) in 3 mL of PBS-AT. After washing away unbound serum with PBS-T, the strips were air-dried. Radio-labeled sheep antibodies against human IgE (Sanquin, Amsterdam, the Netherlands) were used for detection of bound IgE. Blots were exposed to x-ray film (Eastman Kodak Co, Rochester, NY) for 14 days at -70° C, except for the strip incubated with serum sample 15 (1 night) because of the high IgE level.

RESULTS

IgE Antibody Measurements

Of the 15 selected serum samples with IgE antibodies against *A cucumeris*, 12 had IgE antibodies against *D pteronyssinus* (Table 1). Half of these serum samples demonstrated a stronger IgE recognition of *D pteronyssinus* than *A cucumeris* (greater by a factor 2 and up to almost a factor 10). Three serum samples had similar IgE responses to both mites (within a factor 2). For the remaining 6 serum samples (including 3 *D pteronyssinus*-negative), IgE responses to *A cucumeris* were stronger than those to *D pteronyssinus* (greater by a factor 2 and up to at least a factor 25).

Inhibition of IgE Binding in RAST

For RAST inhibition, serum samples were selected from each of the 3 categories: *D pteronyssinus* > *A cucumeris* (serum samples 4, 7, and 15), *A cucumeris* ~ *D pteronyssinus* (serum sample 9), and *A cucumeris* > *D pteronyssinus* (serum sam-

ple 8). *Dermatophagoides pteronyssinus*-RAST inhibition with *A cucumeris* extract (Fig 2) was 25% or less in 4 serum samples (serum samples 7, 8, 9, and 15) and nearly absent in 1 serum sample (serum sample 4). *Amblyseius cucumeris*-RAST inhibition with *D pteronyssinus* extract (Fig 3) was approximately 75% in the case of serum sample 15, 35% for serum sample 7, and less than 15% for serum sample 9. For serum samples 4 and 8, no inhibition of the *A cucumeris* RAST with *D pteronyssinus* extract was observed. The homolog RAST inhibition was effective in all cases (>85%). The results of the RASTs with inhibitor PBS were comparable with the uninhibited RAST results in Table 1. No inhibition of IgE binding to *A cucumeris* and *D pteronyssinus* was seen after preincubation of the diluted serum samples with grass pollen extract (data not shown).

Blotting Experiments

All 15 serum samples were tested on *A cucumeris* and *D pteronyssinus* immunoblots (Figs 4 and 5). No obvious similarities in recognition profiles between *D pteronyssinus* and *A cucumeris* were observed. Serum samples from the *D pteronyssinus* > *A cucumeris* group all showed a similar pattern on *D pteronyssinus* blots, with prominent bands at the major allergens Der p 2/5 (approximately 14 kDa) and Der p 1/7 (approximately 25 kDa).¹⁶ In contrast, on *A cucumeris* blots, most serum samples recognized several high-molecular-weight bands (50–100 kDa). In addition, most of the serum samples also recognized a band of approximately 7 kDa. In the range of 10 to 50 kDa, recognition of *A cucumeris* was highly variable. To identify allergens involved in the

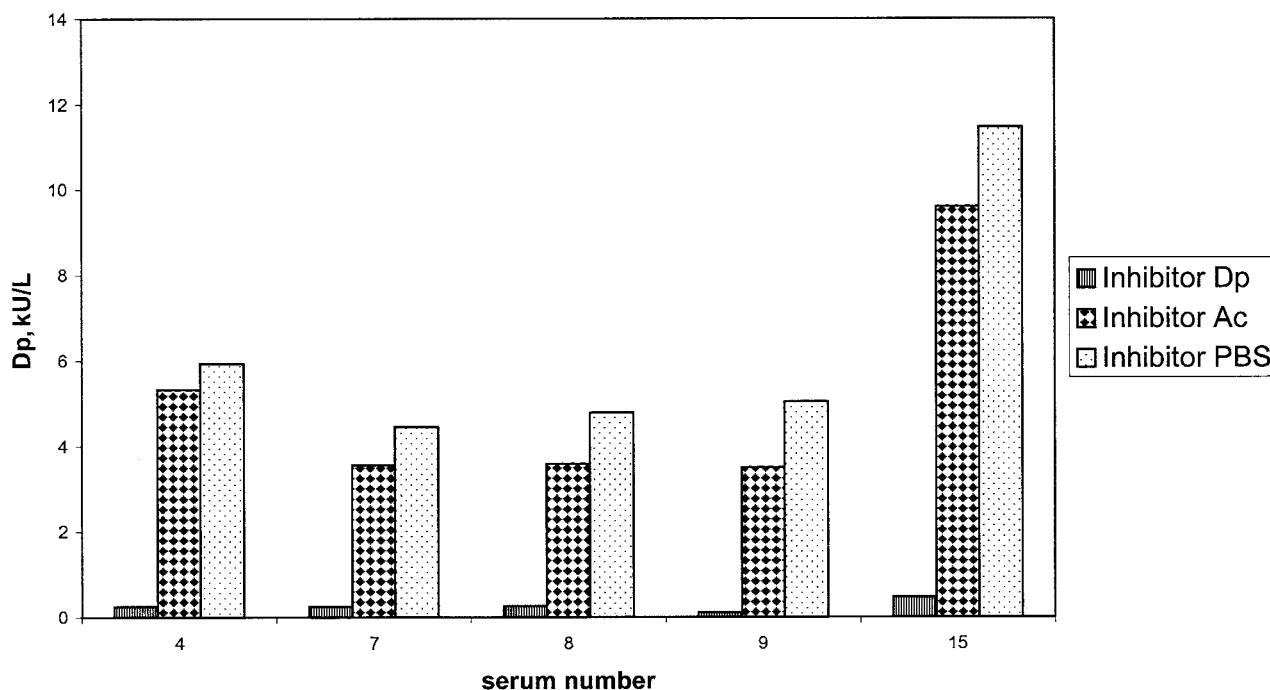


Figure 2. IgE inhibition of *Dermatophagoides pteronyssinus* (Dp) antibodies with Dp, *Amblyseius cucumeris* (Ac), and phosphate-buffered saline (PBS).

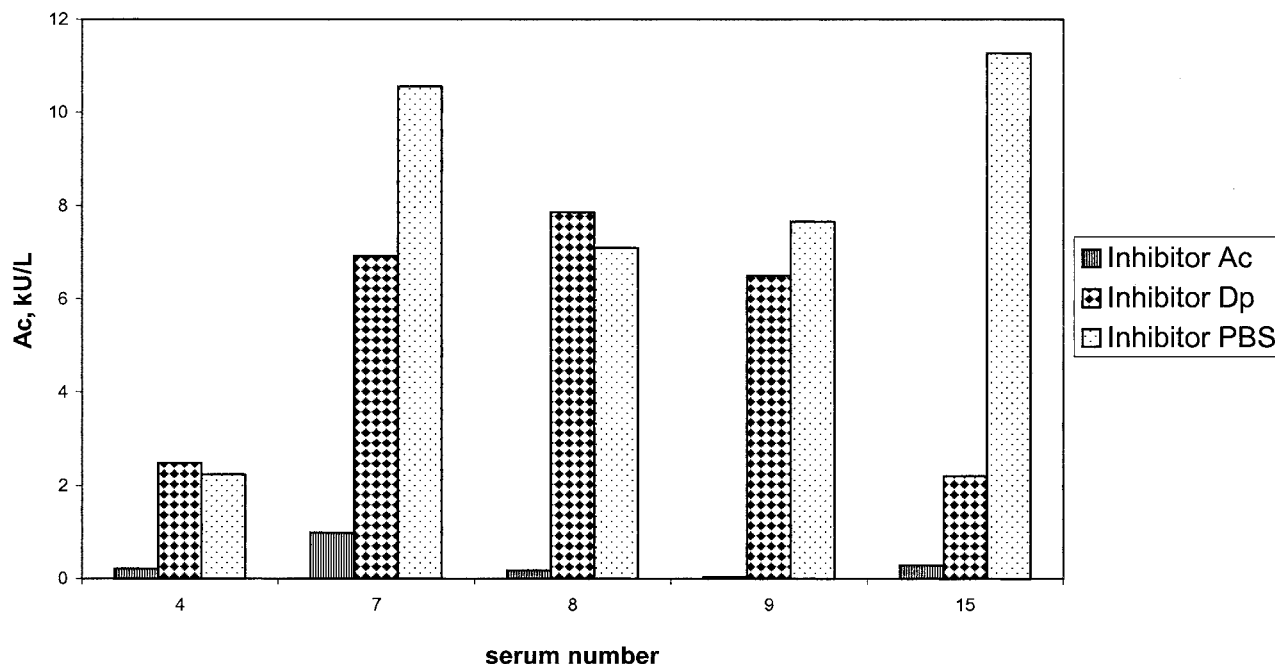


Figure 3. IgE inhibition of *Amblyseius cucumeris* (Ac) antibodies with Ac, *Dermatophagoides pteronyssinus* (Dp), and phosphate-buffered saline (PBS).

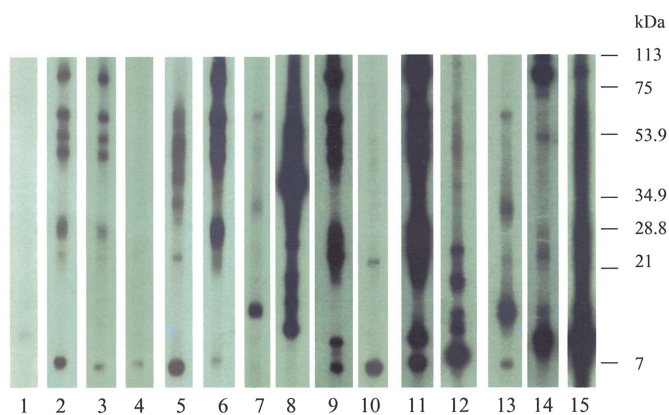


Figure 4. Western immunoblotting using 15 serum samples on *Amblyseius cucumeris* mite extracts. Binding was visualized by using radiolabeled sheep antibodies against human IgE.

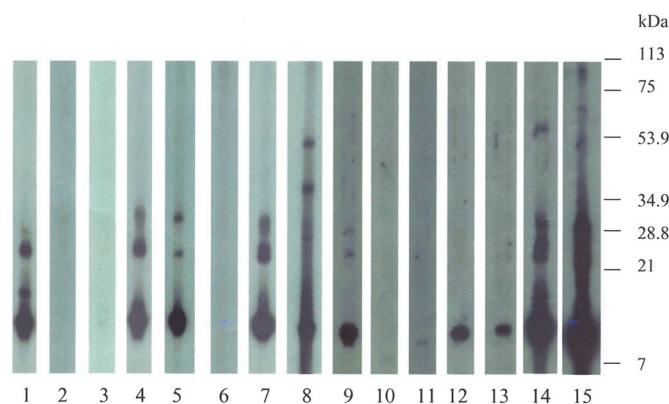


Figure 5. Western immunoblotting using 15 serum samples on *Dermatophagoides pteronyssinus* mite extracts. Binding was visualized by using radiolabeled sheep antibodies against human IgE.

possible cross-reactivity between *A cucumeris* and *D pteronyssinus*, blot inhibition experiments were performed in 4/5 serum samples (samples 7, 8, 9, and 15), which were also used in RAST inhibition experiments (Fig 6).

In accordance with the partial cross-reactivity observed by RAST inhibition, *D pteronyssinus* extract clearly inhibited IgE binding of serum sample 7 (*D pteronyssinus* > *A cucumeris*) to several allergens of *A cucumeris*. *Amblyseius cucumeris* did not inhibit binding of IgE to *D pteronyssinus* blots. Serum sample 8 (*A cucumeris* > *D pteronyssinus*) showed no cross inhibition on immunoblot, as was the case by RAST inhibition. For serum sample 9 (*A cucumeris* ~ *D*

pteronysinus), almost complete inhibition by *D pteronyssinus* of low-molecular-weight bands on *A cucumeris* blots (5–14 kDa) was observed. Prominent IgE binding around 25 kDa and between 50 and 100 kDa was unaffected. This partial inhibition was in line with the results obtained by RAST inhibition (approximately 15%). *Amblyseius cucumeris* did not affect binding to *D pteronyssinus* blots. Finally, serum sample 15 (*D pteronyssinus* > *A cucumeris*) demonstrated complete inhibition by *D pteronyssinus* of binding to *A cucumeris* blots, which was even more efficient than the homologous inhibition. As expected, *A cucumeris* did not affect binding on *D pteronyssinus* blots.

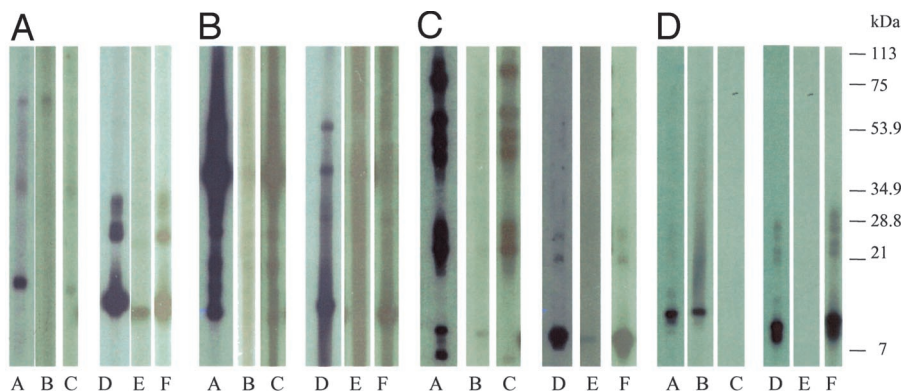


Figure 6. Blot inhibition of IgE binding to *Amblyseius cucumeris* (Ac) and *Dermatophagoides pteronyssinus* (Dp). Serum sample 7 was used in A, serum sample 8 was used in B, serum sample 9 was used in C, and serum sample 15 was used in D. Lane A indicates Ac blot without inhibition; lane B, Ac blot after homologous inhibition; lane C, Ac blot after inhibition with Dp; lane D, Dp blot without inhibition; lane E, Dp blot after homologous inhibition; and lane F, Dp blot after inhibition with Ac.

DISCUSSION

In a recent study performed by our clinic,¹⁰ 109 (23%) of 472 employees in bell pepper greenhouses were shown to have positive SPT reactions to the predatory mite *A cucumeris*. The predatory mite *A cucumeris* is a relatively new allergen in horticulture. Until now, only 1 other study, by Van Hage-Hamsten et al,⁶ described IgE-mediated sensitization to these mites among greenhouse workers. They reported 4 patients with IgE binding to *A cucumeris* extract.

In the bell pepper study,¹⁷ 81 (74.3%) of the 109 employees sensitized to the *A cucumeris* mite were also sensitized to 1 or more common inhalant allergens. The sensitization rate to *A cucumeris* was significantly higher in atopic employees than in nonatopic employees, illustrating a clear association between sensitization to *A cucumeris* and IgE-mediated allergy to inhalant allergens (prevalence rate ratio [PRR], 4.82; 95% confidence interval [CI], 3.27–7.10). Of all common inhalant allergens, sensitization to the house dust mite and grass pollen were most prevalent in our study population and in the subgroup of *A cucumeris*-sensitized employees. The association between sensitization to house dust mite and sensitization to *A cucumeris* (PRR, 4.08; 95% CI, 2.96–5.62) was stronger than the association between grass pollen and *A cucumeris* (PRR, 2.56; 95% CI, 1.87–3.50).

Earlier studies have shown cross-reactivity of *D pteronyssinus* and storage mites such as *T putrescentiae*, *Acarus siro*, and *Lepidoglyphus destructor*.^{5–8} These mite species are, however, near relatives of *D pteronyssinus* compared with the predatory mites in our study (Fig 1). *Dermatophagoides pteronyssinus* belongs to the suborder Astigmata, whereas *A cucumeris* belongs to the suborder Mesostigmata. Comparative studies between mites of different families are rare. Kim et al¹ studied cross-reactivity between spider mites (Prostigmata) and house dust mites (Astigmata). They suggested that common allergens exist in these distantly related mite species. In the present study, we demonstrate that *A cucumeris* sensitization is largely *D pteronyssinus* independent. Furthermore, 3 *A cucumeris*-positive serum samples were negative for *D pteronyssinus*. Cross-reactivity between both mites exists in some cosensitized patients but is of secondary importance. Only for 1 patient, inhibition studies revealed that

most IgE against *A cucumeris* was cross-reactive to *D pteronyssinus* (serum sample 15). The nature of the specific and cross-reactive allergens of *A cucumeris* was not revealed in this study. Certainly, the recognition profile on *A cucumeris* immunoblot was completely different from that observed for *D pteronyssinus*. In general, the recognition of *D pteronyssinus* allergens was normal, ie, recognition of allergens around 14 kDa (Der p 2 and possibly Der p 5) and around 25 kDa (Der p 1, Der p 7, and the serine-proteases Der p 3, 6, and 9). In contrast, several *A cucumeris* allergens seemed to be of higher molecular weight (> 50 kDa), although also allergens between 5 and 50 kDa were recognized. There was 1 patient (8) with strong binding to a 36-kDa allergen, which could point toward tropomyosin. This muscle protein has been considered an important cross-sensitizing invertebrate panallergen.¹⁸ This allergen has been found in several organisms, including the house dust mites *D pteronyssinus* (Der p 10) and *Dermatophagoides farinae* (Der f 10). A RAST for tropomyosin did not confirm the identity of this allergen as tropomyosin (data not shown).

Immunoblot inhibition showed that cross-reactive structures are mainly found among the low-molecular-weight allergens (serum samples 7, 9, and 15). The nature of these allergens with molecular weights between 7 and 12 kDa remains to be established.

In summary, *A cucumeris* is a new occupational allergen, responsible for work-related complaints in greenhouse workers. Sensitization to this mite is not simply a cross-reactive phenomenon related to house dust mites. The results of this study may have consequences for the extensive use of biological control in horticulture. With the knowledge that indoor and outdoor mites are frequent causes of allergic diseases, the use of predatory mites as biological control agents should be critically evaluated. Furthermore, the benefits of biological control should be weighed carefully against the increased risk of employees developing an occupational allergy.

ACKNOWLEDGMENTS

We thank Pierre Ramakers, entomologist at the Research Station for Floriculture and Glasshouse Vegetables (Naaldwijk, the Netherlands), for kindly supplying *A cucumeris*.

REFERENCES

- Kim YK, Son JW, Kim HY, et al. Citrus red mite (*Panonychus citri*) is the most common sensitizing allergen of asthma and rhinitis in citrus farmers. *Clin Exp Allergy*. 1999;29:1102–1109.
- Kim YK, Lee MH, Jee YK, et al. Spider mite allergy in apple-cultivating farmers: European red mite (*Panonychus ulmi*) and two-spotted spider mite (*Tetranychus urticae*) may be important allergens in the development of work-related asthma and rhinitis symptoms. *J Allergy Clin Immunol*. 1999;104:1285–1292.
- Kim YK, Cho SH, Min KU, Kim YY. Spider mites: common outdoor allergens among individuals living in rural areas. *Clin Exp Allergy*. 2000;30:1364–1370.
- Kim YK, Oh SY, Jung JW, Min KU, Kim YY, Cho SH. IgE binding components in *Tetranychus urticae* and *Panonychus ulmi*-derived crude extracts and their cross-reactivity with domestic mites. *Clin Exp Allergy*. 2001;31:1457–1463.
- Sidenius KE, Hallas TE, Poulsen LK, Mosbech H. Allergen cross-reactivity between house-dust mites and other invertebrates. *Allergy*. 2001;56:723–733.
- van Hage-Hamsten M, Kolmodin-Hedman B, Johansson E. Predatory mites, *Phytoseilus persimilis* and *Amblyseius cucumeris*, used for biological crop protection, cause sensitisation among greenhouse workers [Abstract]. *Allergy*. 2000;55:30.
- van der Heide S, Niemeijer NR, Hovinga H, de Monchy JG, Dubois AE, Kauffman HF. Prevalence of sensitisation to the storage mites *Acarus siro*, *Tyrophagus putrescentiae* and *Lepidoglyphus destructor* in allergic patients with different degrees of sensitisation to the house-dust mite *Dermatophagoides pteronyssinus*. *Allergy*. 1998;53:426–430.
- Johansson E, Aponno M, Lundberg M, van Hage-Hamsten M. Allergenic cross-reactivity between the nematode *Anisakis simplex* and the dust mites *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Dermatophagoides pteronyssinus*. *Allergy*. 2001;56:660–666.
- Johansson E, Borga A, Johansson SG, van Hage-Hamsten M. Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy*. 1991;21:511–518.
- Groenewoud GC, de Graaf in 't Veld C, van Oorschot-van Nes AJ, et al. Prevalence of sensitization to the predatory mite *Amblyseius cucumeris* as a new occupational allergen in horticulture. *Allergy*. 2002;57:614–619.
- van Schelt J. Biological control of sweet pepper pests in the Netherlands. *IOBC Bull*. 1999;22:217–220.
- van Houten YM, van Rijn PCJ, Tanigoshi LK, van Stratum P, Bruin J. Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. *Entomol Exp Appl*. 1995;74:225–234.
- Luxton RW, Patel P, Keir G, Thompson EJ. A micro-method for measuring total protein in cerebrospinal fluid by using benzenethonium chloride in microtiter plate wells. *Clin Chem*. 1989;35:1731–1734.
- Adkinson NF, Rose NR, Friedman H. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. In: *Manual of Clinical Immunology*. 2nd ed. Washington, DC: American Society for Microbiology; 1980:789–793.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature*. 1970;227:680–685.
- Thomas WR, Smith W. Towards defining the full spectrum of important house dust mite allergens. *Clin Exp Allergy*. 1999;29:1583–1587.
- Groenewoud GC, de Jong NW, van Oorschot-van Nes AJ, et al. Prevalence of occupational allergy to bell pepper pollen in greenhouses in the Netherlands. *Clin Exp Allergy*. 2002;32:434–440.
- Reese G, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate panallergen. *Int Arch Allergy Immunol*. 1999;119:247–258.

Requests for reprints should be addressed to:

Nicolette W. de Jong

Department of Allergology

Erasmus MC

PO Box 2040

3000 CA Rotterdam

The Netherlands

E-mail: n.w.dejong@erasmusmc.nl