

Original article

IgE-mediated sensitization to predatory mites in Swedish greenhouse workers

Background: Predatory mites are used as biological pesticides worldwide for control of spider mites and other pests in greenhouses. The aim of this study was to investigate if predatory and spider mites give rise to sensitization among greenhouse workers who use biological pesticides.

Methods: Blood samples were collected from 31 greenhouse workers from Stockholm area who were using the predatory mites *Phytoseiulus persimilis* and *Hypoaspis miles* for control of pesticides. Immunoglobulin E (IgE) binding to extracts of *P. persimilis* and *H. miles* and of the spider mite *Tetranychus urticae* was analysed with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting.

Results: A total of 16 workers displayed IgE antibodies to one or more of the three mite species, 32% ($n = 10$) to *P. persimilis*, 52% ($n = 16$) to *H. miles* and 26% ($n = 8$) to *T. urticae*. At least 17 IgE binding components in the molecular weight ranging from 28 to > 94 kDa were identified in *P. persimilis*. In *H. miles*, at least 11 components were detected, among them at least one major component at about 70 kDa. Twenty-four IgE binding components were found in *T. urticae*.

Conclusions: In this study we have for the first time shown that the predatory mites *P. persimilis* and *H. miles* can cause IgE-mediated sensitization among greenhouse workers. The clinical relevance of sensitization to predatory mites needs to be investigated in further studies.

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Mites are considered to be a major cause of asthma and other respiratory diseases in many parts of the world (1). The vast majority of studies concerning IgE-mediated reactions to mites have hitherto focused on dust mites and mainly species belonging to the families Pyroglyphidae, Glycyphagidae and Acaridae. Species belonging to these families e.g. *Dermatophagoides* spp., *Lepidoglyphus destructor* and *Acarus siro* respectively, are found in dwellings and/or farming surroundings. However, during the last decade an increasing number of studies have reported occupational allergy to spider mites among greenhouse workers and farmers working in the open-field, and among children living in rural areas (2, 3). Spider mites have been recognized as severe pests on various crops grown in greenhouses and fruit orchards. But they do not merely damage plants: the species *Tetranychus urticae*, *Panonychus ulmi* and *P. citri*, have been shown to elicit IgE sensitization and provoke allergy in humans (3–7).

Another group of mites that humans can be exposed to is predatory mites cultured for commercial use in biological pest control. Out of concern for the environment, mites are now widely used in greenhouses instead of chemicals to protect the crop. Predatory mites are added onto the plants where they actively search for their prey and suck them dry.

Phytoseiulus persimilis, which feeds exclusively on spider mites (*Tetranychus* spp.), and *Hypoaspis miles* which is mainly used for the control of thrips, are two species of predatory mites commonly used in greenhouses. There has so far only been two publications reporting sensitization to cultured predatory mites (8, 9). Groenewoud and colleagues have recently demonstrated that the predatory mite *Amblyseius cucumeris* is an occupational allergen in bell pepper horticulture (8). The authors found that 23% of 472 greenhouse employees using predatory mites had a positive skin prick test to *A. cucumeris*. In another study with focus on the antigenic relationship between the house dust mite *Dermatophagoides farinae* and *P. persimilis*, it was shown that five of 20 *D. farinae* RAST positive sera, were positive to *P. persimilis* in an in-house RAST assay (9). However, the IgE levels to *P. persimilis* were very low and the authors concluded that the results probably reflected cross-reactivity.

The aim of this pilot study was to elucidate whether occupational exposure to the predatory mites *P. persimilis* and *H. miles* causes IgE-mediated sensitization among greenhouse workers and to identify the IgE binding proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting. In addition we investigated the frequency of sensitization to

the spider mite *T. urticae* among Swedish greenhouse workers.

Material and methods

Serum samples

The study comprised sera from 31 greenhouse workers (median age 44 years, range 20–67), employed at nine randomly chosen greenhouses in the Stockholm area, where predatory mites were used to control pests. All but one employee agreed to take part in the study. The sera were collected in November 1999. A total of 15 of 31 subjects had been working > 10 years in greenhouses. The growers were cultivating cucumbers and tomatoes and/or other vegetables, potted plants, herbs and tulips. All growers were exposed to the predatory mites *P. persimilis* and *H. miles*, and spider mites.

Sera from two patients with IgE-producing myeloma were used to control for possible unspecific IgE binding to predatory and spider mites in immunoblotting. The sera were diluted to 1000 kU/l with a serum sample lacking IgE antibodies to predatory and spider mites.

IgE antibody measurements

Atopy was defined as a positive Phadiatop (Pharmacia CAP System™ Specific IgE FEIA; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The Phadiatop measures IgE antibodies in serum to the most common aeroallergens in Sweden (birch, timothy, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus* and *Cladosporium herbarum*) and results are expressed as positive or negative.

Total serum IgE was determined using the Pharmacia CAP System™ Total IgE FEIA (Pharmacia & Upjohn). Results are expressed in kU/l and in Sweden the normal range for adults is considered to be 2–122 kU/l.

Mite cultures and extract preparation

All mites were kindly prepared and supplied by Koppert B.V. (Berkel en Rodenrijs, The Netherlands). In order to get pure cultures, the predatory mites *H. miles* and *P. persimilis* were starved for 13 and 24 days, respectively. Dead and living mites were then separated with a Berlese Funnel Technique and the purity of the cultures was checked by microscopy. The spider mite *T. urticae* was isolated from bean leaves. All mite cultures were then kept at –20°C until delivered to our laboratory for extraction.

The mite cultures were extracted at our laboratory with protease inhibitors (Complete™, Mini; Roche Diagnostics GmbH, Mannheim, Germany) at 1/10 (w/v) in 0.15 M NaCl, 0.02% NaN₃, overnight at 4°C. The extracts were then centrifuged and the protein concentration of the supernatant was measured with the Micro BCA Protein Assay Reagent (Pierce Chemical Company, Rockford, IL, USA). The protein concentrations of the extracts were 3.0 mg/ml for *H. miles*, 5.9 mg/ml for *P. persimilis* and 12 mg/ml for *T. urticae*. The extracts were kept at –20°C until tested.

SDS–PAGE and immunoblotting

The SDS–PAGE and electroblotting was performed with the Hoefer® Mighty Small™ Electrophoresis Unit (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the instructions of the manufacturer. Mite extract (10 µg/mm gel) was loaded onto a gradient gel (7.5–20%) and separated under nonreducing condi-

tions. The proteins were then electroblotted to a polyvinylidene difluoride membrane (Immobilon™, Millipore, Bedford, MA), blocked (0.5% Tween-20, 0.2% bovine serum albumin) and cut into strips (2–3 mm), each incubated with a serum sample diluted 1/4. The IgE binding components were visualized as described earlier (10). In brief, the strips were incubated with rabbit anti-human IgE, alkaline phosphatase (ALP)-conjugated goat anti-rabbit immunoglobulin (Dako, Glostrup, Denmark) and finally, substrate for ALP.

Statistical analysis

Serological results are presented as medians with range. The Mann–Whitney *U*-test was used for comparison of total IgE levels between greenhouse workers displaying IgE binding to predatory and/or spider mites, and those lacking IgE binding. Univariate logistic regression analysis was used for calculation of odds ratios. If the 95% confidence interval (CI) did not include 1.0, the odds ratio was statistically significant at $P < 0.05$.

Ethical considerations

The Ethics Committee of the Karolinska Institute, Stockholm, approved the study.

Results

Allergen-specific IgE antibodies and total IgE levels

All 31 greenhouse workers were working inside the greenhouses. A total of 16 workers displayed IgE binding in immunoblotting to at least one of the three mite species *P. persimilis*, *H. miles* and *T. urticae*. The frequency of IgE-mediated sensitization to each of the species was 32% ($n = 10$), 52% ($n = 16$) and 26% ($n = 8$), respectively. Nine of the sensitized individuals had been working > 10 years in greenhouses compared with six among the 15 nonsensitized workers. However, no association was found between long-term exposure (> 10 years) and sensitization to predatory mites (OR = 1.9, 95% CI: 0.46–8.1).

Six greenhouse workers (19%) were atopic as defined by a positive Phadiatop. Five of them were found among the group of 16 greenhouse workers (31%) with IgE to at least one of the three mite species. No association between atopy and sensitization to predatory mites was found (OR = 6.3, 95% CI: 0.65–62.7).

The median value for the total IgE measurement was higher for the group with IgE to predatory and/or spider mites (92 kU/l; range 8.3–700, $n = 16$) than for the 15 individuals with negative immunoblotting results (15 kU/l; range < 2–170) ($P < 0.01$).

Identification of IgE binding components in extract of *P. persimilis*, *H. miles* and *T. urticae*

The SDS–PAGE and immunoblotting was performed with all 31 serum samples from the greenhouse workers

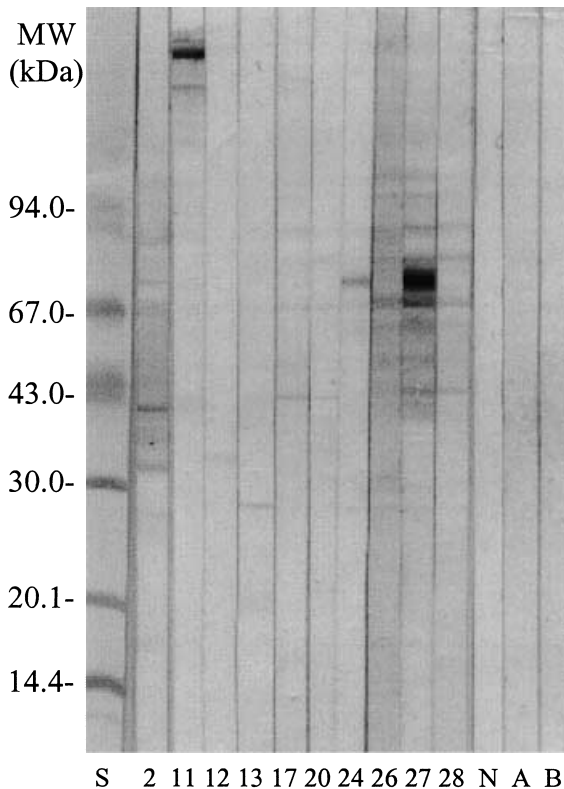


Figure 1. Immunoglobulin E binding components in extract of *P. persimilis* detected on immunoblots by 10 of 31 analysed sera from greenhouse workers 2–28. Lane N represents one of the 21 greenhouse workers with a negative immunoblot. A and B: sera from patients with IgE-producing myeloma, diluted to 1000 kU/l, S: molecular weight standard proteins.

and two serum samples from the patients with IgE-producing myeloma.

A total of at least 17 IgE binding components in the approximate molecular weight ranging from 28 to >94 kDa was detected by 10 serum samples in the extract of *P. persimilis* (Fig. 1). All 10 sera detected more than one component. One serum sample no., 27, showed IgE reactivity to 12 components and strongest to the one at 70 kDa. This component was detected by another two sera, nos 24 and 27. A component at 67 kDa was stained by all the 10 *P. persimilis* positive sera but mostly very weakly. The two myeloma sera, diluted to an IgE concentration higher than any of the other analysed sera (1000 kU/l), were completely negative (A and B in Fig. 1).

Sixteen of the greenhouse workers detected bands in *H. miles* (Fig. 2). Six of the sera displayed IgE binding to a single component and 10 recognized at least two components. The IgE binding was mainly directed to one to three components at about 70 kDa, but at least eight other bands could be seen although they were mostly very weakly stained. Five of them had a molecular weight lower than the 70 kDa component and were detected by sera nos 2, 4, 26 and 27. The other three IgE binding

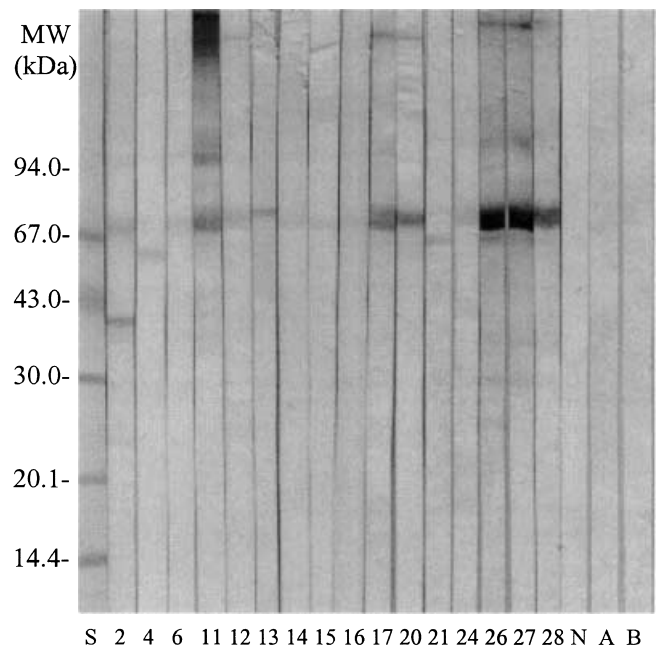


Figure 2. Immunoglobulin E binding components in extract of *H. miles* detected on immunoblots by 16 of 31 analysed sera from greenhouse workers 2–28. Lane N represents one of the 15 greenhouse workers with a negative immunoblot. A and B: sera from patients with IgE-producing myeloma, diluted to 1000 kU/l, S: molecular weight standard proteins.

components were found in the high molecular weight area. No bands were stained by the two myeloma sera (A and B in Fig. 2).

Figure 3 shows the IgE binding components in extract of *T. urticae*. Sera from eight of the 31 greenhouse workers detected a total of at least 24 components and all serum samples recognized multiple bands (at least two). Several bands were strongly stained, for example, at 16 kDa by sera nos 2 and 13, at 30 and 35 kDa by no. 2, at 50 and 51 kDa by no. 28 and at 56 kDa by no. 21. This serum sample also stained a component above 94 kDa strongly. The two myeloma sera (A and B in Fig. 3) were negative.

Discussion

This study describes IgE-mediated sensitization to the predatory mites *P. persimilis* and *H. miles* in a group of greenhouse workers and the first time IgE binding proteins have been identified in these mites. We found that 16 of 31 greenhouse workers displayed IgE antibodies to predatory mites. All 16 reacted with *H. miles*, and 10 of the 16 greenhouse workers reacted also with *P. persimilis*. Although the figure for sensitization to predatory mites was much higher than we expected, we could exclude unspecific IgE binding, as sera from two myeloma patients with high IgE levels were negative in

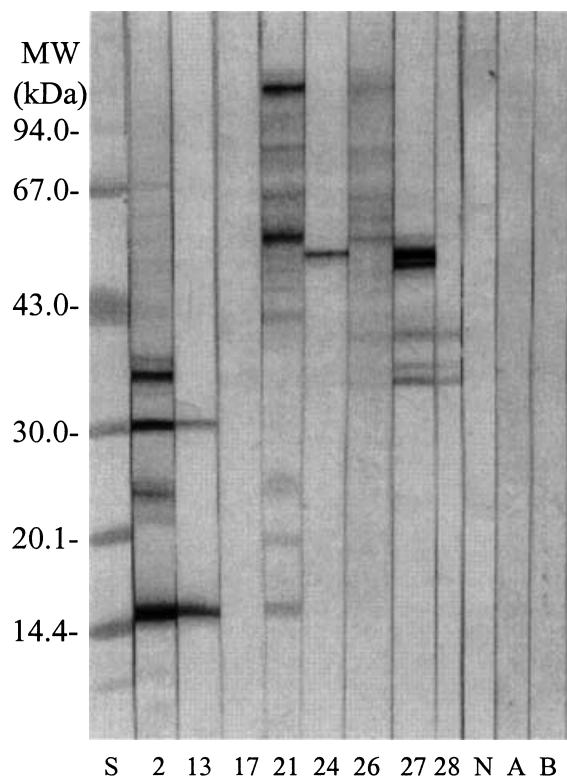


Figure 3. Immunoglobulin E binding components in extract of *T. urticae* detected on immunoblots by eight of 31 analysed sera from greenhouse workers 2–28. Lane N represents one of the 23 greenhouse workers with a negative immunoblot. A and B: sera from patients with IgE-producing myeloma, diluted to 1000 kU/l, S: molecular weight standard proteins.

the blotting experiments. Among the 16 sensitized growers nine had been working > 10 years compared with six among the nonsensitized. Furthermore, five of the six atopic individuals had IgE antibodies to *P. persimilis* and/or *H. miles*. We could not show that long-term exposure or atopy is a risk factor for sensitization to predatory mites when tested in the univariate model. This might be the result of the rather small number of greenhouse workers included in the study. Groenewoud and colleagues, who studied 472 greenhouse employees, could however show that sensitization to the predatory mite *A. cucumeris* was significantly higher in atopic than in nonatopic subjects (8).

In SDS-PAGE and immunoblotting we found that the greenhouse workers identified at least 17 IgE binding components in the molecular weight ranging from 28 to > 94 kDa in extract of *P. persimilis*. The most striking components were found at approximately 67 and 70 kDa. The 67 kDa component was detected by all 10 positive sera, but, as the component was only weakly stained, its clinical relevance can be questioned. The component at 70 kDa was strongly stained by one of three of the positive serum samples. As regards *H. miles*, the most prominent bands were also displayed in the molecular

weight area of about 70 kDa. Three different bands were found in this area and at least eight of 16 positive sera stained one or two of them.

It can be discussed to what degree the obtained IgE binding is caused by sensitization to predatory mites or is a consequence of cross-reactivity, as several of the IgE binding components were rather weakly stained. Allergic cross-reactivity between different mite species belonging to the suborder Astigmata has been reported in a number of studies (10–13). Antigenic cross-reactivity between *D. farinae* and *P. persimilis*, belonging to the different suborders Astigmata and Mesostigmata, respectively, has been demonstrated in SDS-PAGE and immunoblotting by using a rabbit antiserum to *D. farinae*. The *D. farinae* antisera reacted with four antigens in extract of *P. persimilis* with molecular weights between 70 and 110 kDa (9). As the authors found that five of 20 *D. farinae* RAST positive sera also displayed IgE binding to *P. persimilis*, they speculated in that allergenic cross-reactivity between the two mite species might also exist. We found that five of the 10 greenhouse workers with IgE antibodies to *P. persimilis* were atopic based on a positive Phadiatop. However, as Phadiatop contains allergens from the mite species *D. pteronyssinus* only, we cannot confirm cross-reactivity between *P. persimilis* and *D. farinae*. Inhibition experiments need to be performed in order to explore whether allergens in *P. persimilis* have cross-reacting allergens with *D. farinae* or other mite species belonging to the suborder Astigmata.

The present study is also the first to investigate the frequency of sensitization to the spider mite *T. urticae* among a group of Swedish greenhouse workers. Among the 31 workers, eight (26%) were scored positive in immunoblotting, a figure comparable with the one Navarro and colleagues demonstrated (14). They investigated 246 greenhouse workers in Spain, recruited directly from the field, of whom 25% were positive in skin prick test. Furthermore, in a Korean survey among 465 apple cultivating farmers, the prevalence of positive skin prick test to *T. urticae* was found to be 16.6% (15). On the other hand, Astarita and colleagues (16) reported a prevalence of positive skin prick test to *T. urticae* of 6% among 960 Italian greenhouse and open-field farmers. The authors claimed that if the data were analysed according to the specific workplace, the prevalence figure was similar to that reported by Navarro et al. (14).

In the immunoblot analysis of *T. urticae*, we found that the greenhouse workers detected 24 IgE binding components, which were in about the same molecular weight range as those reported earlier (17) with the exception that our subjects also displayed a component above 94 kDa. As regards cross-reactivity with other mite species, *T. urticae*, has been demonstrated to have allergens in common with the dust mites *D. pteronyssinus* and *Tyrophagus putrescentiae* (17) although *T. urticae* belongs to the suborder Prostigmata and the dust mites belong to the Astigmata suborder.

In conclusion, this is the first study reporting IgE-mediated sensitization to the predatory mites *P. persimilis* and *H. miles* among greenhouse workers. Several IgE-binding components were found in the two predatory mite species. However, the clinical relevance of sensitization to predatory mites needs to be investigated and their allergenic relationship with other dust mite species explored. Finally, more extensive studies among greenhouse workers are required to elucidate the impact of predatory mites on IgE-mediated allergy.

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