

Short communication

Occurrence of IgE antibodies against the 39-kDa allergen component of the mite *Lepidoglyphus destructor* in urban and rural subjects

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Several species of storage mite have been shown to cause IgE-mediated sensitization. Monoclonal antibodies (mabs) have been raised against the storage mite *Lepidoglyphus destructor*, and one mab (42B6) immunoprecipitated a band of 39 kDa of *L. destructor*. To search for the frequency of IgE antibodies in patient sera against this allergen component, we modified a radioimmunoassay (RIA), 42B6 being used as capturing antibody. Twenty of 43 sera from farmers who were RAST positive to *L. destructor* (46.5%) were scored as positive in this assay. There was a moderate degree of correlation between positive RAST results to *L. destructor* and the 39-kDa allergen. Among 14 sera from Stockholm residents positive in RAST to both *Dermatophagoides pteronyssinus* and *L. destructor*, six detected the 39-kDa allergen component. However, three sera from urban subjects lacking IgE antibodies against both mite species also had IgE antibodies against the 39-kDa allergen. The present study demonstrates that the 39-kDa allergen of *L. destructor* is an important allergen, and that rural as well as urban subjects can become sensitized.

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As *Lepidoglyphus destructor* is the predominant storage mite species both with respect to sensitization and to occurrence in barn dust in Sweden (2, 3), we have concentrated on this mite species. Characterization studies have revealed that the major allergen, detected by SDS–PAGE, is localized at 14–15 kDa (8, 10), and the purification of *Lep d* I (11), as well as the N-terminal amino acid sequence of the major allergen (5), has been reported. However, this is not the only major allergen component, since Johansson et al. have shown that 50% of 20 examined sera detect a band at 53 kDa in *Lepidoglyphus* (8). Using hybridoma technology, we generated monoclonal antibodies (mabs) against *L. destructor*. We found that one mab (42B6) immunoprecipitated a sharp band of 39 kDa of *L. destructor* (1). Further characterization of this mab revealed that it binds to the wall of the *L. destructor* gut, to the exoskeleton, and to most fecal pellets (4). In a modified RAST we

have established that the 39-kDa component is an allergen (1).

To search further for the occurrence of IgE antibodies in patient sera against this allergen component, we modified a radioimmunoassay (RIA) in which an anti-*L. destructor* mab directed against the 39-kDa component was used as capturing antibody. We here report the frequency of IgE antibodies against this component among rural and urban subjects.

Material and methods

Human sera

Seventy-four farmers (55 men and 19 women, aged 16–64 years, mean 35) were chosen for the study. All these farmers had previously participated in an epidemiologic investigation of respiratory disease (2). They were divided into three groups according to

their RAST specificity. The first group comprised 43 atopic persons who were RAST positive to *L. destructor* (0.4–93 kU/l) (Ld + group), and the second, 10 atopic farmers who were positive in RAST only against pollen or animal danders (0.7–2.8 kU/l) (atopic/Ld – group). The control group was composed of 21 nonatopic farmers who lacked IgE antibodies against *L. destructor* (Ld – group).

Forty-two sera which had been sent to our laboratory from the Stockholm city area (urban samples) for RAST against *Dermatophagoides pteronyssinus* were tested for IgE antibodies against the 36-kDa component. Twenty-one of the sera were RAST negative to *D. pteronyssinus* as well as *L. destructor* (Dpt – /Ld –). The other 21 sera were RAST positive to *D. pteronyssinus* (0.4–570 kU/l). Fourteen of these latter sera also had IgE antibodies against *L. destructor* (0.35–7.8 kU/l) (Dpt + /Ld +), and seven were RAST negative to *L. destructor* (Dpt + /Ld –).

Mouse monoclonal antibodies (mabs)

In the present study we used the mouse mab 42B6, which specifically recognizes an allergen component of 39 kDa in *L. destructor* (1).

Mite extracts

L. destructor extract was prepared (8) from whole mite cultures obtained from Allergon, Ängelholm, Sweden. The protein concentration of these extracts was 2 mg/ml.

Determination of IgE antibodies against the 39-kDa *L. destructor* allergen

The RIA was essentially performed as described previously (10). Microtiter plates (Nunc, Roskilde, Denmark) were coated (200 µl/well, 160 µg/ml) with immunosorbent purified goat antimouse immunoglobulin (Dakopatts A/S, Glostrup, Denmark). After washing, the plates were incubated first for 7 h with 100 µl undiluted supernatant of the anti-*L. destructor* mab (42B6) (3 µg/ml), and secondly with 100 µl of *L. destructor* extract (diluted 1/200) overnight. Thereafter, 100 µl of undiluted patient sera and finally 100 µl of ¹²⁵I-labeled rabbit antihuman IgE (diluted 1/2) (Phadebas RAST anti-IgE Tracer, Pharmacia Diagnostics AB, Uppsala, Sweden) were incubated for 24 h, respectively. Bound radiolabeled antibody was eluted with citric buffer, pH 3.0, at 37°C for 30 min, transferred to tubes, and analyzed with a multigamma counter (LKB, Sweden). The assay gave a nonspecific uptake of labeled anti-IgE when tested with a nonatopic serum sample with an IgE level of 4000 kU/l. All sera were tested in triplicate

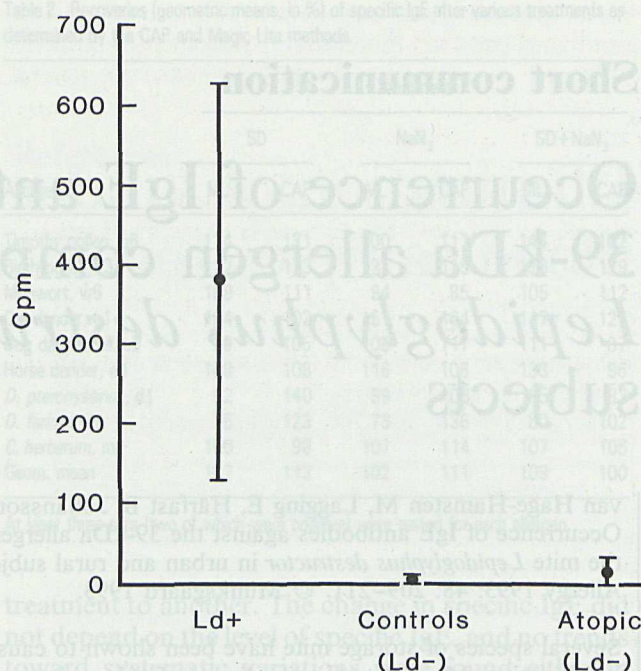


Fig. 1. Distribution of the three groups studied (95% confidence intervals). Ld + = RAST positive to *Lepidoglyphus destructor*; Controls (Ld –) = RAST negative to *L. destructor*; Atopic (Ld –) = RAST positive to pollens or animal danders but RAST negative to *L. destructor*.

and the mean value was calculated. The results were expressed as counts per min (cpm). The cpm values of the samples were corrected by subtracting the cpm obtained in the absence of sera (usually around 50 cpm). A positive result was defined as twice the background value (approximately 100 cpm).

Results

Twenty of the 43 sera belonging to the Ld + group (46.5%) were scored as positive (geometric mean 240, range 103–2437 cpm) against the 39-kDa allergen. The 21 Ld – farmers were all negative in the RIA (geometric mean 7, range 1–59 cpm). The atopic farmers who lacked IgE antibodies against

Table 1. Binding of human IgE in cpm to the 39-kDa allergen component in *L. destructor*

Groups	Proportion of positive patients	Geom. mean (cpm)	Range (cpm)
Ld+	20/43	240	103–2437
Dpt+/Ld+	6/14	1005	250–4100
Dpt–/Ld–	2/21	–	196, 350
Dpt+/Ld–	1/7	–	114
Atopic/Ld–	0/10	6	1–77
Controls	0/21	7	1–59

Atopic=positive in RAST to pollens or animal danders; Ld=*Lepidoglyphus destructor*; Dpt=*Dermatophagoides pteronyssinus*; cpm=counts per min; ≥ 100 cpm=positive result.

L. destructor (atopic/Ld -) were also negative to the 39-kDa allergen component (geometric mean 6, range 1–77 cpm). Fig. 1 shows the distribution of the cpm values of the sera from the three groups (95% confidence intervals).

There was a moderate degree of correlation between the IgE antibody levels against *L. destructor* and against the 39-kDa component ($r=0.50$, $P<0.05$, Spearman rank correlation coefficient). Among the 14 nonrural sera belonging to the Dpt + /Ld + group, six detected the 39-kDa component (geometric mean 1005, range 250–4100 cpm). In addition, 2/21 Dpt - /Ld - and 1/7 Dpt + /Ld - sera were scored as positive (Table 1).

Discussion

The aim of this study was to search for the occurrence of IgE against the 39-kDa allergen in patient sera. We adapted an RIA in which our anti-*L. destructor* mab 42B6 was used as a capture antibody for the allergen. Nearly half of the *L. destructor*-positive farmers' sera detected this allergen component. Interestingly, there was only a moderate degree of correlation between the levels of IgE antibody against *L. destructor*, measured as kU/l by RAST, and the cpm values against the 39-kDa allergen, indicating that sensitization to this allergen component does not depend on the total amount of specific IgE against *L. destructor*. Still, the serum with the highest kU/l level against *L. destructor* gave most cpm.

Our previous finding that patient sera did not detect the 39-kDa band under nonreduced conditions in SDS-PAGE and immunoblotting suggests that the epitopes recognized on this component are susceptible to the treatments involved in SDS-PAGE. In earlier experiments we exposed *L. destructor* extract to various reagents such as protease, trypsin, and periodate, and we concluded that the epitope recognized by mab 42B6 is a very stable structure, of carbohydrate rather than protein nature (7).

We have demonstrated that urban subjects can become sensitized to the 39-kDa component. This is not surprising since storage mites are a part of the house-dust mite fauna (6, 9). Why three sera were positive to the 39-kDa allergen despite being negative in RAST to *Lepidoglyphus* is not altogether clear. It could stem from a low concentration of this component in the RAST assay.

Several interesting issues are raised by the present data. Firstly, the 39-kDa component seems to be an important allergen in *L. destructor*. Secondly, not only farmers but also urban people can become sensitized to this allergen even if they do not have IgE

antibodies against *L. destructor* as measured by RAST based on a "whole extract". Thus, from a clinical point of view, the possibility of sensitization to *L. destructor* among subjects with suspected allergy to house dust should not be overlooked. Finally, a variety of mabs to *L. destructor* will probably detect more allergen components. However, whether these allergens are involved in allergic reactions and whether they will facilitate clinical diagnosis need to be ascertained.

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