

Localization of allergens in the domestic mite *Lepidoglyphus destructor*

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Summary

The non-pyroglyphid domestic mite *Lepidoglyphus destructor* is a major source of allergen causing respiratory symptoms in farming environments. This study is the first to focus on the localization of the allergens in the non-pyroglyphid domestic mite *Lepidoglyphus destructor*. Cryostat-cut sections of *L. destructor* mite bodies and faecal pellets were probed with one of three mouse monoclonal antibodies (MoAbs) raised against *L. destructor* or with patient sera, and stained with immunoperoxidase. Eight sera were RAST-positive to *L. destructor* and *L. destructor* faecal pellets. These eight RAST-positive patient sera labelled the wall of the *L. destructor* gut and two of them also the faecal pellets. The MoAbs additionally labelled parts of the exoskeleton and reacted with a majority of the faecal pellets. Cryostat-cut sections of *Dermatophagoides pteronyssinus* mite bodies and faecal pellets were probed with *L. destructor* MoAbs, which resulted in only slight staining of a few faecal pellets. The results suggest that at least one *L. destructor* allergen is associated with digestion.

Clinical and Experimental Allergy, Vol. 22, pp. 251–256. Submitted 13 June 1991; revised 22 August 1991; accepted 27 August 1991.

Introduction

During the last decades an increasing number of studies have consistently demonstrated the clinical significance of IgE-mediated sensitization to pyroglyphid mites [1,2]. Several investigations of rural and urban populations have likewise shown a high prevalence of allergic symptoms due to glycyphagid and acarid mites [3–8]. In Sweden, the glycyphagid mite *Lepidoglyphus destructor* is the dominating source of allergen, causing respiratory symptoms among farmers [4]. Since mite faeces and fragmented bodies easily become airborne, it is of great interest to know which part of the mite is the major sensitizer. So far localization of allergens in mites has only been studied in the *Dermatophagoides* genus [9,10]. These studies have shown that the allergen(s) are to be found in the gastrointestinal canal. Characterization studies have shown that the allergens found were contained in extracts of mite bodies, culture material and faeces [11]. With respect to non-pyroglyphid mites, Arlian *et al.*, who studied *Tyrophagus putrescentiae*, have found allergens in

mite bodies, faeces and medium with the use of crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE) [12]. Eleven allergen components have been identified by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting in extracts of the non-pyroglyphid domestic mite *L. destructor* [13]. We here report on the localization of allergens in *L. destructor*. Thin sections of *L. destructor* were probed either with sera from mite-allergic farmers or with mouse monoclonal antibodies (MoAbs) raised against *L. destructor*.

Materials and methods

Subjects

Sera from 16 farmers (age 35–71, geometric mean 57 years), who had earlier participated in an epidemiological survey of respiratory diseases among farmers on the Swedish island of Gotland [4], were used in the study. Eight of these sera were radioallergosorbent test (RAST)-positive to *L. destructor* (5.7–93 Phadebas RAST units (PRU)/ml) and eight were non-atopic farmers who lacked IgE antibodies to this mite species (controls) (Table 1).

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Determination of IgE antibodies to *L. destructor* faecal pellets

Specific IgE measurements using RAST, as described by Johansson *et al.* [14], were performed against a sieved culture of *L. destructor* (mesh size $<90\ \mu\text{m}$) which was obtained from Allergon AB (Ängelholm, Sweden). This fraction consists mainly of faecal pellets and is subsequently referred to as faecal pellets. Allergen discs were prepared in the laboratory by coupling $10\ \mu\text{l}$ of a 1/10 w/v extract of *L. destructor* faecal pellets to 40 cyanogen-bromide-activated paper discs as described by Ceska *et al.* [15]. The discs gave no non-specific uptake of labelled anti-IgE when tested against non-atopic serum samples with levels of 20–4000 kU/l. All sera ≥ 0.35 PRU/ml were retested. A positive result was defined as a value ≥ 0.35 PRU/ml.

Mites and faecal pellets for immunoperoxidase staining

Live mites and faecal pellets (mesh size $<90\ \mu\text{m}$) belonging to *L. destructor* and *D. pteronyssinus* were obtained from Allergon AB (Ängelholm, Sweden). The mites were embedded in groups of 3–5 mites in Tissue Tek® (Miles Diagnostics Division, Elkhart, Indiana U.S.A.) and stored at -70°C . Sections of mite bodies, $6\ \mu\text{m}$ thick, were cut in a cryostat at -18°C and transferred to glass slides coated with chromium gelatine. On each glass slide, 1–3 sections of 3–5 mites were mounted.

Ten milligrams of faecal pellets from *L. destructor* and *D. pteronyssinus*, respectively, were mixed in 1 ml of 0.05 M phosphate buffer, pH 7.4, containing 0.15 M saline. A total amount of $200\ \mu\text{l}$ of the faecal preparation per glass slide was centrifuged at about 20 g for 5 min (Shandon Elliott Cytospin, Shandon Scientific Co. Ltd., London, U.K.) and air-dried.

Mouse monoclonal antibodies

MoAbs were raised against *L. destructor* as described previously [16]. In the present study, the culture supernatants of three hybridomas were used as sources of MoAbs. To this end, hybridoma cells, $3 \times 10^5/\text{ml}$, were cultivated in Dulbecco's Modified Eagle's medium supplemented with 5% fetal calf serum and gentamicin $25\ \mu\text{g}/\text{ml}$ for 5 days. The cells were subsequently removed by centrifugation and the supernatants were stored at -20°C until used. The three MoAbs used were 42B6 (IgG3), 814C7 (IgM) and 82F3 (IgM).

Immunoperoxidase staining

Cryostat-cut sections of *L. destructor* mite bodies were probed with one of the three *L. destructor* MoAbs or with

patient sera, and stained with the peroxidase-anti-peroxidase method [17]. When pretesting the cryostat-cut sections, we found no need for inhibiting endogenous peroxidase activity, and the best staining result was obtained on non-fixed mite sections. The sections were allowed to react with normal rabbit serum (diluted 1/10) for 10 min to reduce non-specific staining. Thereafter $50\ \mu\text{l}$ of undiluted patient sera or culture supernatant of the MoAbs against *L. destructor* ($5\ \mu\text{g}/\text{ml}$) were applied for 30 min. Human IgE-binding was detected with anti-IgE MoAbs of two different specificities used in combination, anti-D 1 (IgG1) and anti-D 2 (IgG1), diluted 1/80 (kindly provided by Dr C. G. M. Magnusson, Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden). Rabbit anti-mouse immunoglobulin (diluted 1/40) used as a secondary antibody for the *L. destructor* MoAbs (42B6, 814C7, 82F3) and, as a tertiary antibody for the patient sera, preformed complexes of horseradish peroxidase and mouse monoclonal anti-horseradish peroxidase were obtained from Dakopatts, Copenhagen, Denmark. The peroxidase reaction was developed with 3-amino-9-ethylcarbazole [18], and the sections were counterstained with Mayer's haematoxylin. Specificity tests included: (a) omission of the three MoAbs or the patient sera; (b) replacement with normal mouse serum and culture medium for the MoAbs against *L. destructor* or with non-atopic serum samples with IgE levels of 20–4000 kU/ml for the patient sera. Staining was not observed in these tests. Cryostat sections from normal human skin and atopic eczema were included as positive controls for binding of MoAbs (anti-HLA-DR) or detection of IgE, respectively.

The cytospin glasses containing the faecal pellets from *L. destructor* were stained as described above. The cryostat-cut sections and faecal pellets of *D. pteronyssinus* were only probed with the three MoAbs. The examination was performed separately by two investigators (MvHH, AS) under coded conditions.

Statistical analysis

To estimate the strength of the allergenic relationships between positive RAST tests against *L. destructor* and *L. destructor* faecal pellets, Spearman Rank Correlation coefficients were calculated. Estimates of correlation coefficients were tested for a significant departure from zero. For all tests, $P < 0.05$ was considered to be significant.

Results

RAST analysis

The farmers with a positive RAST to *L. destructor* also had specific IgE antibodies to *L. destructor* faecal pellets

Table 1. RAST values and immunoperoxidase labelling results in sections of *L.d.* and *L.d.* faecal pellets of the patient sera.

Patient no.	RAST (PRU/ml)		Immunoperoxidase staining	
	(<i>L.d.</i>)	(<i>L.d. f.</i>)	Sections of mite body (<i>L.d.</i>)	Faecal pellets (<i>L.d.</i>)
1	93	22	+++	+++
2	13	2.6	+	—
3	23	2.9	+	+
4	9.1	0.8	+	—
5	5.7	1.2	+	—
6	20	3.4	++	—
7	38	4.8	+	—
8	9.7	2.2	+	—
9–16	<0.35	<0.35	—	—

L.d., *Lepidoglyphus destructor*; *L.d. f.*, *Lepidoglyphus destructor* faecal pellets; —, negative; +, weak; ++, moderate; +++, strong.

(Table 1). The geometric mean of a positive RAST to *L. destructor* was considerably higher than the one to *L. destructor* faecal pellets (geometric mean 17.9, range 5.7–93 PRU/ml vs geometric mean 2.9, 0.8–22 PRU/ml). There was a significant correlation between positive RAST tests against *L. destructor* and against *L. destructor* faecal pellets ($r=0.95$, $P=0.01$).

Immunoperoxidase staining with patient sera

All patient sera containing *L. destructor*-specific IgE antibodies reacted with cryostat-cut sections of *L. destructor*. The serum with the highest PRU level gave the strongest staining (Table 1). The staining was mainly localized around the gut (Fig. 1a). In some sections the content of the gut was also stained. The exoskeleton was not labelled (Fig. 1a).

Only two patient sera (1 and 3) stained the *L. destructor* faecal pellets. Patient serum 1, which displayed the highest PRU value, reacted with most of the pellets, but patient serum 3 with only a few (Table 1). The sera from non-mite allergic farmers gave no staining of the sections (Fig. 1b) or the faecal pellets (Table 1).

Immunoperoxidase staining with mouse monoclonal antibodies against *L. destructor*

A staining pattern similar to that for the IgE specific sera was observed with all three *L. destructor* MoAbs (42B6,

814C7 and 82F3) on sections of *L. destructor*, with additional labelling of parts of exoskeleton (Fig. 1c). However, 42B6 gave a more intense staining of the gastrointestinal region than 814C7 and 82F3 did. The culture medium for the MoAbs gave no staining.

The majority of the faecal pellets from *L. destructor* were labelled by 42B6 (Fig. 1d) and 814C7, in contrast to 82F3 which reacted with few of the *L. destructor* faecal particles. No labelling was obtained with the culture medium for the *L. destructor* MoAbs (Fig. 1e).

Only a few faecal pellets belonging to *D. pteronyssinus* were weakly labelled by 42B6, 814C7, 82F3. On sections of *D. pteronyssinus* there was, however, no clear staining.

Discussion

Domestic mites, which comprise both pyroglyphid and non-pyroglyphid mites, are a widespread cause of inhalant allergy. In 1964 Voorhorst *et al.* found that the *Dermatophagoides* genus was responsible for house dust allergy [19]. In contrast, the non-pyroglyphid mites are more frequently the cause of occupational allergy [3–6], although they may also cause sensitization among urban dwellers [7,8]. We have found that 6.2% of 2578 Swedish farmers had a case history of immediate onset hypersensitivity in barns and a positive RAST (≥ 0.35 PRU/ml) to storage mites [4], in contrast to the rather low figures of allergy to pollens and animal danders [20].

Mite-infested environments contain parts of mite bodies and faecal pellets. These particles are small enough to act as aeroallergens. As regards purification of mite allergens, only the *Dermatophagoides* genus has been studied. The major allergens (Groups I and II) have been isolated and characterized [21,22]. The Group I allergens (24 000 daltons) are associated with faecal allergens [23] in contrast to the Group II allergens (15 000 daltons) which are relatively more concentrated in whole mite bodies [22,24]. The Group I allergen (*Der p* I) occurs in very high concentrations (≈ 0.2 ng per particle) in mite faeces and elutes within 2 min from isolated faecal particles, but very slowly from mite bodies [23]. Recently a third allergen, *Der f* III, with a molecular weight of 29 000 daltons, has been described [22]. Thompson and Carswell have suggested that the major allergen of the house dust mite, *D. pteronyssinus*, is a protein synthesized and exerted into its alimentary canal [25]. In addition, sequence analysis of *Der p* I have shown homology to cysteine proteases and of *Der f* III and *Der p* III to serine proteases [22,27], indicating that they are involved in mite digestion.

Concerning the non-pyroglyphid mites, the allergens in *T. putrescentiae* and *L. destructor* have been identified and characterized with the use of either CIE, CRIE or SDS-PAGE [12,13]. Arlian *et al.* have shown that two

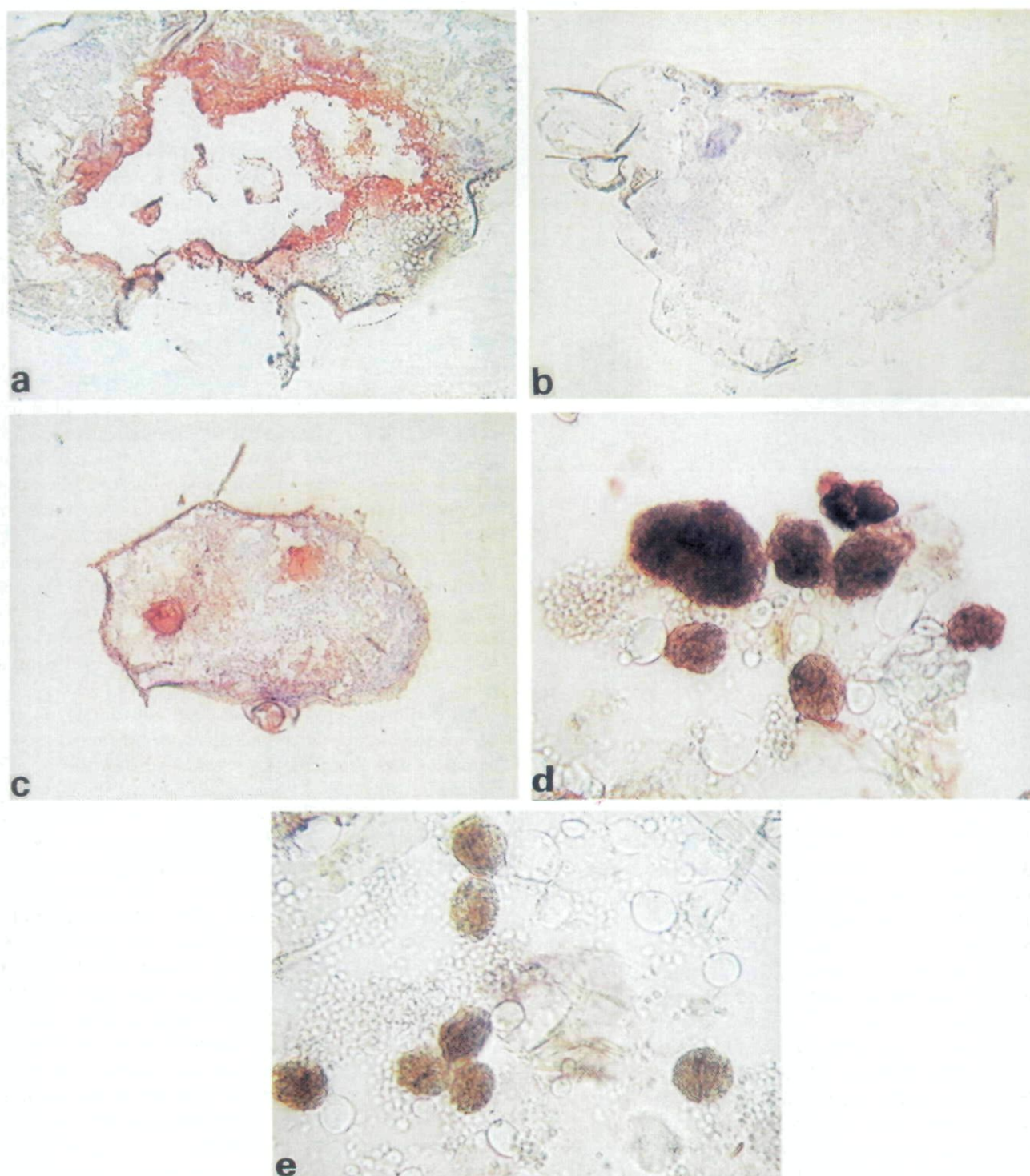


Fig. 1. Cryostat-cut sections of *L. destructor* probed with a serum which is either (a) RAST positive (patient 3) or (b) RAST negative to *L. destructor*, or with (c) a mouse MoAb against *L. destructor* (42B6). (d) shows faecal pellets belonging to *L. destructor* probed with a MoAb against *L. destructor* (42B6) and (e) shows replacement with the culture medium used for the MoAb. The sections and the faecal pellets were stained with immunoperoxidase and counterstained with Mayer's haematoxylin. Red-brown colour is positive staining. Note that unstained faecal pellets in (e) have a light brownish colour. $\times 370$.

antigenic and allergenic determinants are shared by *D. farinae* and *T. putrescentiae* and that two determinants are also shared by the faeces of two species [28]. The results of cross-reactivity studies depend on the quality of the mite extracts and on the populations studied [29]. Although there may be as many as 20 common domestic mite species, it is essential to establish which one is the main provoker of IgE-mediated sensitization in each region. For definition and detailed mapping of allergenic epitopes, the use of MoAbs is probably a useful approach.

The aim of the present study was to localize the allergens in the non-pyroglyphid domestic mite *L. destructor* with the use of mite allergic sera and three MoAbs raised against *L. destructor*. Our results suggest that at least one *L. destructor* allergen is associated with digestion, and this is in accordance with the studies of sections of *D. pteronyssinus* [9,10]. The main labelling occurred around the wall of the gut and on the faecal pellets in *L. destructor*. The *L. destructor* MoAbs stained not only the same parts of the mite body as did patient sera, but the exoskeleton as well. The intensity of the staining obtained with patient sera did not correlate in all cases with the amount of specific IgE antibodies to *L. destructor*. However, the serum with the highest PRU value for both mite body and faecal pellets gave the most intense labelling of both structures.

Determination of the precise localization of the allergens was restricted by the quality of the cryostat-cut sections. However, cryostat sections of unfixed frozen mite have proved to be the best way to preserve mite allergens [10]. The mites were embedded in groups in order to obtain a sample which included different stages and different parts of the mites. We chose immunoperoxidase staining of the sections instead of immunofluorescence in order to avoid autofluorescence and secondly to obtain permanent stainings.

It is not surprising that all three *L. destructor* MoAbs (42B6, 814C7, 82F3) gave slight staining of the faecal pellets belonging to *D. pteronyssinus*. The two species share at least one allergenic determinant, which was shown in a recent immunoblot multiallergen inhibition study between *L. destructor* and *D. pteronyssinus* [30]. Secondly, in ELISA inhibition assays the *D. pteronyssinus* extract achieved some inhibition of the three MoAbs but over a thousand times less than in comparison with the *L. destructor* extract [B. Härfast *et al.*, submitted]. However, clinically, these mite species do not cross-react since farmers with specific IgE antibodies to *L. destructor* develop <20% fall in forced expiratory volume in 1 s (FEV₁) when challenged with *D. pteronyssinus* [31]. Likewise, patients with asthma caused by *D. pteronyssinus* do not respond to bronchial challenges with *L. destructor* [31].

Since mite faeces and fragmented bodies easily become airborne, it is of great interest to know which part of the mite is the main sensitizer. With thin sections, different structures of the mite body can be studied and various domestic mite species can also be compared regarding localization of allergens. As regards *L. destructor*, our study indicates that the majority of the allergens are involved in digestion. Why the exoskeleton is stained by the *L. destructor* MoAbs but not by the RAST positive sera remains to be settled. Further studies should deal with the biochemical characterization of the allergens in *L. destructor*.

Acknowledgments

The excellent technical assistance of Catharina Johansson is acknowledged. Financial support for this work was received from the Swedish Work Environment Fund (projects 83-1182 and 82-0881), the Swedish Medical Research Council (grants 16X-105 and 16X-7924), the Tore Nilsson Foundation, the Swedish Society of Medicine and the Karolinska Institute.

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