

Localization of a major allergen, Der p 2, in the gut and faecal pellets of *Dermatophagoides pteronyssinus*

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Summary

Background The house dust mite *Dermatophagoides pteronyssinus* is one of the most significant indoor sensitizing agents of allergy. Allergen localization may indicate the importance of secreted materials, faeces, and nonexcreted mite body components as allergen sources.

Objective This study attempted to localize the sites and concentrations of Der p 2 in the cryostat sections of *D. pteronyssinus* using antirecombinant Der p 2 monoclonal antibody.

Methods Male and female mites and mite faeces collected separately from both sexes were used. Live mites were embedded and serial cryostat sections for light microscopy were performed. Anti-recombinant Der p 2 monoclonal antibody previously produced by the authors was used. For immunoprobings, mite cryostat sections were incubated in the following antibody-containing solutions: monoclonal antibody against Der p 2 was initially applied to the sections and fluorescent isothiocyanate conjugated antimouse immunoglobulin G was reacted as the secondary antibody. The faecal pellets were treated the same as described above.

Results Immunofluorescent probing of cryostat sections with the monoclonal antibody showed labelling of the gut lining, gut contents and defecated faecal pellets. No other internal organs were identified as positively labelled.

Conclusion This study suggested that a major allergen, Der p 2, found in the house dust mite *D. pteronyssinus* is derived from the digestive tract and concentrated in the faeces.

Keywords: house dust mite, *Dermatophagoides pteronyssinus*, allergen localization, Der p 2.

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Introduction

Allergy to house dust allergens is very common among patients with bronchial asthma and allergic rhinitis. Two species of the mite genus *Dermatophagoides*, *D. pteronyssinus* and *D. farinae*, are important sources of house dust allergens [1,2]. Recently, several researchers have described the characterization of the allergens from two species of mite, especially group 1 and group 2 allergens. These two allergens are the most significant cause of sensitization, inducing immunoglobulin (Ig) E and IgG antibody responses in 80% to 95% of patients allergic to house dust mites. Each of these

allergens has been cloned and sequenced [3–6]. The 14 kDa group 2 allergen was characterized by immunochemical studies [7–10]. The allergen of Der p 2 was purified as previously reported [9,11]. The cDNA sequences of Der p 2 and Der f 2 showed that each had 129 residues and a calculated MW of 14 kDa [12–15]. Mite faeces have been reported as a major source of house dust allergens [16]. Der p 1 was reported in the epithelium of the posterior end of the mite stomach [17]; in the faecal pellets, mouth and hind-gut tissues [18]; and in the gut lining and gut contents [19]. Anti-group 2 antibodies in *Lepidoglyphus destructor* have been reported to show reactivity associated with digestion and most faecal pellets [20].

In this study, we describe the localization of a house dust mite allergen, Der p 2, in the cryostat sections of *D. pteronyssinus* using antirecombinant Der p 2.

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Methods

Subjects

Stock cultures of *D. pteronyssinus* were maintained at 25 °C and 75% relative humidity in our mite-rearing facilities. The number of mites used in the experiment was 400 males and 400 females. We selected mating mites from the culture, and separate them into females and males. Separated mites were transferred on the slide glass, then set aside for 24 h. The 40 mite faeces were collected with brush, respectively. The live mites were embedded using Tissue Tek OCT compound and stored at -70 °C. Serial sections with 4 mm thickness were performed in a cryostat at -20 °C and the sectioned specimens were moved on glass slides coated with poly L-lysine. They were washed for 7 min in 3 mL of 0.01 M phosphate buffer, pH 7.2, containing 0.15 M saline and then washed for 3 min with distilled water.

Staining and immunoprobings of sections

Anti-recombinant Der p 2 monoclonal antibody, which was made previously [21], was incubated for 1 h at 37 °C. After washing, fluorescent isothiocyanate (FITC) conjugated anti-mouse IgG containing 2.5% bovine serum albumin was reacted for 1 h at 37 °C. The sections were then counter-stained with 0.1% Evans blue solution for 5 min at room temperature for reduction of nonspecific reactions. The slides were observed under a fluorescent microscope (Leitz Dialux 20, Leica Microsystems Wetzlar GmbH, Wetzlar, Germany). The faecal pellets collected from mites were stored at -20 °C until used. The faecal pellets were applied the as same as described above. Twenty nonstained live mites were pressed to confirm the faecal mass inside of the body.

Identification of structures in sections of mites

The identification of the organs and terminology followed the description of the closely related mite, *D. farinae* [22].

Results

The localization of allergens of *D. pteronyssinus* was demonstrated by cryostat sections of mite bodies (Figs 1a–d) and defecated faecal pellets (Figs 1e,f) in both sexes. They were probed with mouse monoclonal antibody raised against recombinant Der p 2. Sections incubated with normal mouse serum as negative controls demonstrated as weak orange (Fig. 1). The monoclonal antibody bound very strongly to faecal mass and hind-gut lining tissues inside the mite body and defecated faecal pellets

(Figs 1b–d). The mite faecal pellets collected from both sexes separately, showed most intense fluorescence males (Fig. 1e) and females (Fig. 1f) faecal pellets. No other organs, especially male and female reproductive organs, were identified to be reactive to the monoclonal antibody. No difference between male and female mites was noticed.

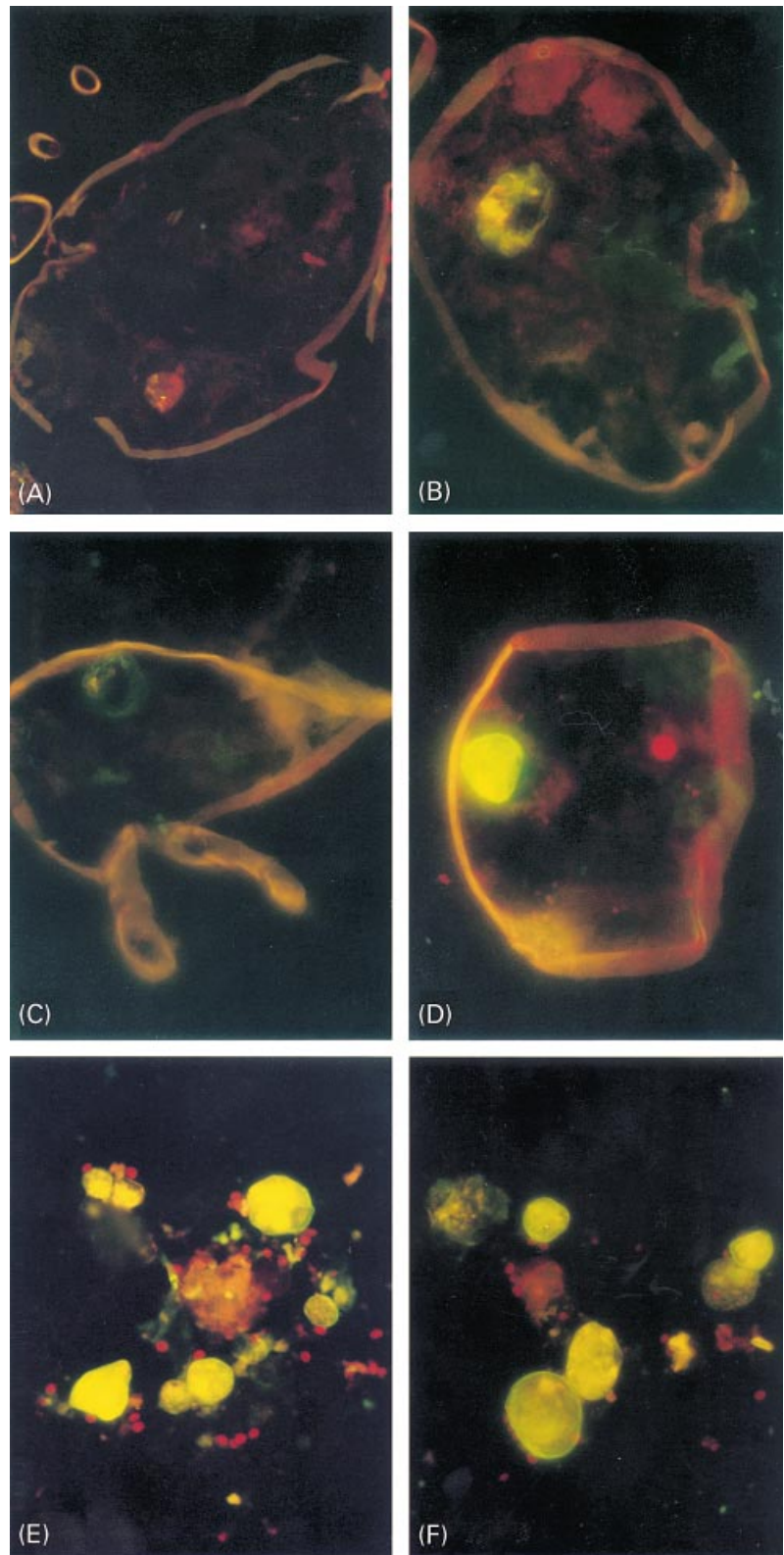
For light microscopic observations, 30 male and 28 female mites were pressed on the slide glass without fixative (Fig. 2). They had faecal mass from one to three within their bodies. Faecal pellets were spherical. They were in the range from 12.69 to 47.94 mm in diameter with a mean of 26.85 ± 5.5 mm in females and from 12.93 to 34.31 mm and 20.89 ± 3.25 mm in males, respectively. Their size was directly proportional to the size of the mite bodies, which varied in length from 165 to 340 mm.

Discussion

Allergens derived from the house dust mite, particularly those of the genus *Dermatophagoides*, are common triggers of allergic disease [23,24] and several allergens from both *D. pteronyssinus* and *D. farinae* have been characterized [25,26]. In Korea, 23 species of house dust mites have been recorded in the literature, of which three species are regarded as being predominant, including *D. farinae* (65.3%), *D. pteronyssinus* (20.6%) and *Tyrophagus putrescentiae* (6.5%) [27].

In the past, there had been a presumption that group 2 allergens are lysozymes, but it is now clear that they are not [28,29]. The localization of major allergens in the dust mite *Lepidoglyphus destructor* has been reported [20], and it showed that the 15 kDa allergen, probably the group 2 allergen in *L. destructor*, was localized in the faecal pellets and gut lining. In this study, we studied the localization of a major house dust mite allergen, Der p 2, in the body of *D. pteronyssinus* by cyrocutting and immunostaining. The immunoreactive components to the anti-Der p 2 monoclonal antibody were located in the faecal pellets and hind-gut lining of the mite. The frozen faecal pellets from both sexes showed strong specific staining. Although Der p 2 has been reported to possibly be an epididymal secretion from the male reproductive tract because of its high sequence homology with a family of epididymal protein [30], we did not observe a specific binding to any other organ of the mite body apart from the digestive tract and faecal pellets. There was no difference in the binding of the monoclonal antibody to Der p 2 between males and females.

In conclusion, we demonstrated that a major allergen, Der p 2, is derived from the digestive tract and is concentrated in faecal pellets of *D. pteronyssinus*.



Figures 1. Cryostat sections and faecal pellets of the house dust mite, *Dermatophagoides pteronyssinus*. The sections were probed with a monoclonal antibody against recombinant Der p 2. (a) The mite body shows a negative controls with weak orange stained. (b) The mite body with logitudinal sectioning shows intense labelling of a mite faecal particle and weak labelling of intestinal tract. (c) The mite body shows labelling at epidermal cells in the gut. (d) The mite body with cross sectioning shows its strongest labelling in a mite faecal mass. The mite faecal pellet from males (e) and females (f) shows intense labelling with a monoclonal antibody against recombinant Der p 2.



Fig. 2. A live house dust mite, *D. pteronyssinus*, by pressing. Arrow indicates faecal pellets. Scale bar 20 μ m.

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