

Localization of major allergens in the dust mite *Lepidoglyphus destructor* with confocal laser scanning microscopy

M. VAN HAGE-HAMSTEN, S. OLSSON, A. EMILSON, B. HÄRFAST, A. SVENSSON and A. SCHEYNIUS

Department of Laboratory Medicine, Division of Clinical Immunology, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden

Summary

The dust mite *Lepidoglyphus destructor* is the dominating source of allergens giving rise to asthma and rhinitis among farmers. In a previous study of the localization of allergens in *L. destructor* we demonstrated that the 39 kDa allergen is associated with digestion. Here we describe the localization of the principal 15 kDa allergen and the high molecular weight allergen complex (79 and 93 kDa) in *L. destructor* with confocal laser scanning microscopy (CLSM). Cryostat-cut sections of mite bodies and faecal pellets were probed with mouse monoclonal antibodies (MoAbs) raised against the allergens. The 15 kDa allergen disclosed labelling of the mite body and most of the faecal pellets but left the exoskeleton unlabelled. The binding was widespread, and most intense in the mouth region. However, some staining was also observed around the gastrointestinal tract. In contrast, the 79 and 93 kDa allergen complex stained the exoskeleton and the front part of the mite. Interestingly, we detected no labelling of the faecal pellets with the MoAb against the 79/93 kDa allergen. The study indicates that the 15 kDa allergen is associated with the digestive tract whereas the function of the 79 and 93 kDa allergen complex remains to be elucidated.

Clinical and Experimental Allergy, Vol. 25, pp. 536–542. Submitted 6 July 1994; revised 6 September 1994; accepted 18 October 1994.

Introduction

Exposure to storage (non-pyroglyphid) mites has been increasingly recognized as a cause of asthma or rhinitis [1–5]. Storage mites are distributed worldwide. These mite species are a risk factor for sensitization in occupations such as farming and grainstore work [1–8]. Mites also exist in dust in damp urban dwellings, since humid conditions favour their growth [9]. In Europe *Lepidoglyphus destructor* is the predominant non-pyroglyphid mite species [2,5] whereas sensitization to *Blomia* dominates in subtropical and tropical areas [10–12]. However, in Columbia atopic subjects may have IgE-antibodies to *L. destructor* as [12] well as to the mites *Aleuroglyphus ovatus* and *Chortoglyphus arcuatus* [13].

Allergenic characterization of *L. destructor* has so far revealed 21 IgE-binding components, the principal allergen being localized at 14–15 kDa [14,15]. To further study the allergen composition of *L. destructor* we have raised several monoclonal antibodies (MoAbs), one of which (designated 42B6) is directed against the 39 kDa allergen [16]. This allergen seems to be of importance to rural as well as urban subjects. Nearly half of these individuals were scored positive in a radioimmunoassay (RIA) where MoAb 42B6 was used as a capture antibody [17]. Another MoAb (117F9) identifies two high molecular weight components of 79 and 93 kDa, of which at least one causes IgE-mediated sensitization [18]. As high a proportion as 60% of sera from farmers with specific IgE antibodies against *L. destructor* recognize this allergen complex [18].

In a previous study of the localization of allergens in *L. destructor* we demonstrated that the 39 kDa allergen is

Correspondence: Dr M. van Hage-Hamsten, Department of Clinical Immunology, Karolinska Hospital, S-171 76 Stockholm, Sweden.



Fig. 1. *L. destructor* mite in lactic acid. Light microscope at $\times 150$ magnification.

associated with digestion [19]. To gain more information about the 15 kDa allergen of *L. destructor* and about the high molecular weight allergen complex we have now investigated their labelling pattern and compared it with that of the 39 kDa allergen. In addition to standard light microscopy we used confocal laser scanning microscopy (CLSM) to localize the allergens. The latter technique has several advantages over light microscopy [20], such as significant reduction of stray light — thus improving contrast, and increased resolution in both lateral and vertical dimensions. Furthermore, a specimen can be scanned sequentially at different depths, both horizontally and vertically. The images obtained can be stored on a computer for further processing, thus enabling 3-dimensional (3-D) reconstructions of the object.

Materials and methods

Mites and faecal pellets

Nine batches of live mites and five batches of faecal pellets (mesh size $< 90 \mu\text{m}$) belonging to *L. destructor* (Fig. 1) were obtained from Allergon AB (Ängelholm, Sweden). The mites were embedded in Tissue Tek[®] (Miles Diagnostics Division, Elkhart, IN, USA) and stored at -70°C . Twenty serial $10 \mu\text{m}$ sections per mite

were cut in a cryostat at -18°C and transferred to glass slides coated with chromium gelatine. On each glass slide, between one and four sections of one mite were mounted. Sections of about 60 mites were studied.

Twenty milligram of faecal pellets from *L. destructor* were mixed in 2 ml of 0.05 M phosphate buffer, pH 7.6, containing 0.15 M saline. A total of $200 \mu\text{l}$ of the faecal preparation per glass slide was centrifuged at about 20 g for 5 min (Shandon Cytospin[®] 3, Shandon Scientific Ltd, Cheshire, UK), air-dried and analysed.

Mouse monoclonal antibodies

MoAbs were raised against *L. destructor* as described previously [21]. Hybridoma cells were cultivated in Dulbecco's Modified Eagle's medium supplemented with 5% fetal calf serum and gentamicin $25 \mu\text{g/ml}$. The MoAbs were purified on a Protein-A-Sepharose CL-4B column according to the instructions of the manufacturer (Pharmacia AB, Uppsala, Sweden) and stored at -20°C until used. The two MoAbs used were 1112D8 (IgG1) directed against the 15 kDa component [21] and 117F9 (IgG2a) directed against the 79 and 93 kDa allergen complex [18].

Immunohistochemical staining

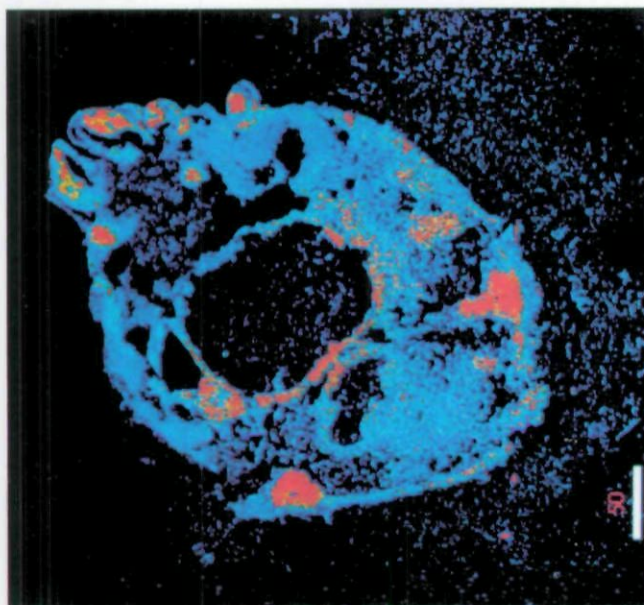
Cryostat-cut non-fixed $10 \mu\text{m}$ sections of the mite bodies were probed with MoAbs against *L. destructor*. The sections were allowed to react with normal rabbit serum (diluted 1/10) for 10 min to reduce non-specific staining. Thereafter, purified MoAbs against *L. destructor* 1112D8 (diluted to $2 \mu\text{g/ml}$) or 117F9 (diluted to $1.7 \mu\text{g/ml}$) were applied for 30 min. Sections were immunostained with the alkaline-anti-alkaline phosphatase (APAAP) complex technique using the chromogenic Fast Red Substrate System (Dakopatts, Copenhagen, Denmark) [22], where rabbit anti-mouse immunoglobulin (diluted 1/40) (Dakopatts) was used as secondary antibody. The intensity of the staining reaction was increased by repeating the incubation steps with the link antibody and APAAP complex. The sections were counterstained with Mayer's haematoxylin. Specificity tests included replacement with subclass-matched irrelevant MoAbs (used in the same concentration as MoAb 1112D8 and 117F9) or with the culture medium for the MoAbs. The cytopsin glasses containing the faecal pellets from *L. destructor* were stained with APAAP as described above.

Microscopic evaluation

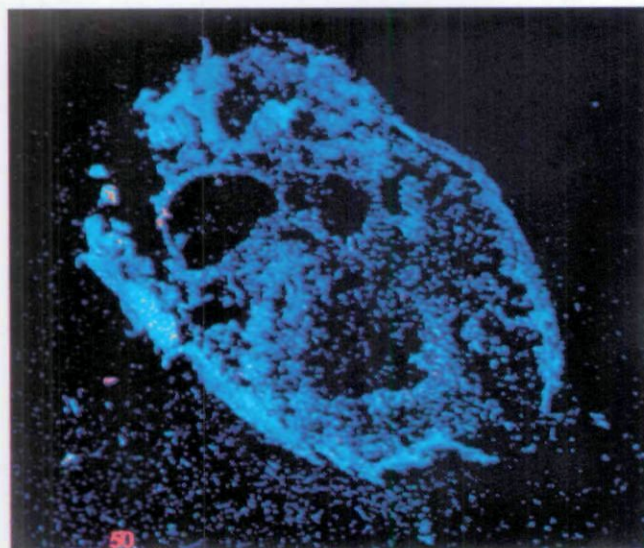
APAAP staining was chosen since it allows assessment



(a)



(b)



(c)

with both ordinary light microscopy and CLSM [22]. After the stained sections of the mite bodies had been evaluated in the light microscope, selected sections were further analysed in a CLSM SARASTRO[®] 2000 system (Molecular Dynamics, Sunnyvale, CA, USA) equipped with an argon ion laser and built around a Nikon Optiphot II microscope. The software for scanning and image processing is implemented on a Silicon Graphics 4D/25 Personal Iris[®] workstation (Silicon Graphics Inc., Mountain View, CA, USA), which runs a UNIX-based operating system. A comprehensive description of CLSM has been published [23]. The 514 nm wavelength was used for excitation of Fast Red [22]. Fast Red was detected with a beam-splitter reflecting at 535 nm, and in front of the detector, a long-pass filter at 535 nm. A confocal aperture of 50 μ m and a $\times 20$ plano-apochromate Nikon objective was used.

Each 10 μ m section was scanned sequentially into nine 'optical sections' separated by a 1.2 μ m, starting at the top. The top was determined from a vertical scan. Extended focus projections were produced by adding all optical sections in the stack with each section in focus. To further visualize the binding pattern of the MoAbs, 'intensity map' projections were obtained where the heights of the peaks were proportional to the pixel values (i.e. registered emitted light).

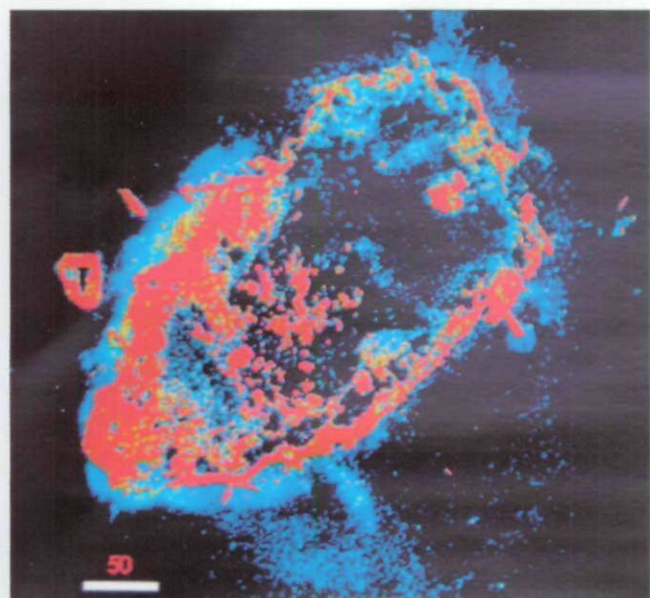
The faecal pellets were evaluated only in the light microscope, due to their autofluorescence at the wavelength (514 nm) used for exciting Fast Red.

Results

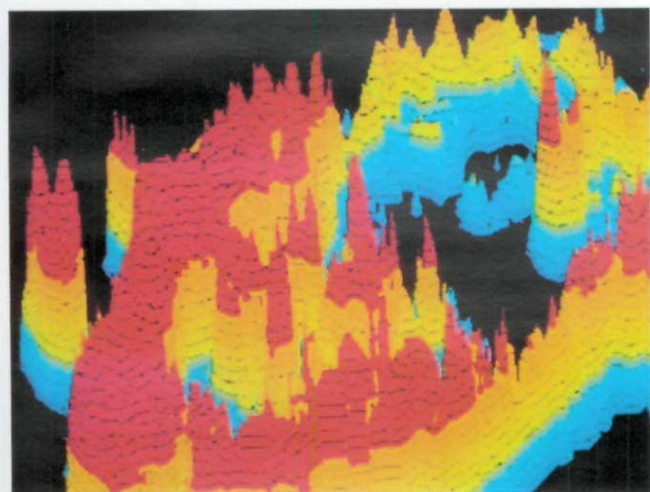
Mite bodies

L. destructor mite bodies express the 15 kDa and the 79/93 kDa allergen components differently. MoAb 1112D8 (anti-15 kDa) disclosed a labelling pattern of the mite body which was widespread, with the most intense area in the region of the mouth (Figs. 2a and b). The

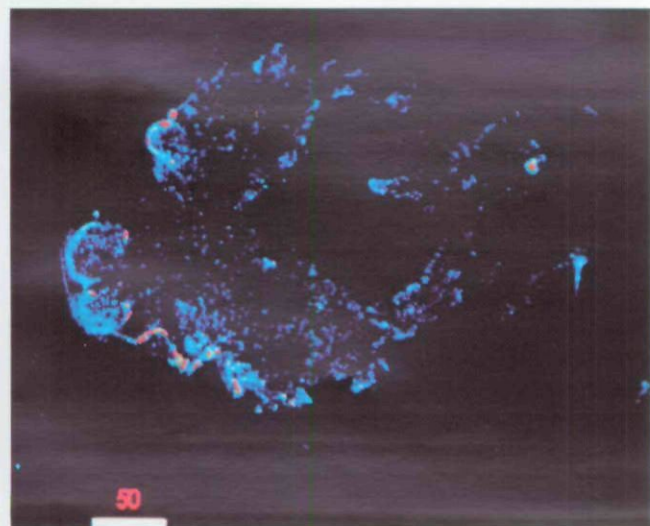
Fig. 2. *L. destructor* mite bodies express a 15 kDa allergen. Cryosections, 10 μ m thick, were probed with (a and b) a MoAb against the 15-kDa allergen of *L. destructor* (1112D8) and stained with the APAAP method using the chromogenic substrate Fast Red. The sections were counterstained with Mayer's haematoxylin. The specificity test comprised replacement with a subclass-matched irrelevant MoAb which showed negative staining (c). In (a) a section is seen in ordinary light microscope ($\times 150$) and in (b) the same section is analysed in CLSM. Extended focus projections are shown in (b and c). The intensity of staining in (b and c) is colour-banded (range red-yellow-green-blue-black, where red is the strongest). The staining is widespread with localized parts in the regions of the mouth and the gut. Bar = 50 μ m.



(a)



(b)



(c)

exoskeleton was left unlabelled. Some staining was also observed around the gastrointestinal tract, but whether its content is labelled cannot be determined since the gut content was generally lost during staining. In contrast, MoAb 117F9 (anti-79/93 kDa) stained the exoskeleton along with the front part of the mite (Fig. 3a). Another way to illustrate the binding of the MoAb (117F9) is shown in Fig. 3b, which is an 'intensity map' projection of Fig. 3a. No labelling was found with the culture medium for *L. destructor* MoAbs or with irrelevant subclass-matched MoAbs (Fig. 2c and 3c). The same staining pattern of MoAbs 1112D8 and 117F9 was obtained in sections from about 30 *L. destructor* mite bodies.

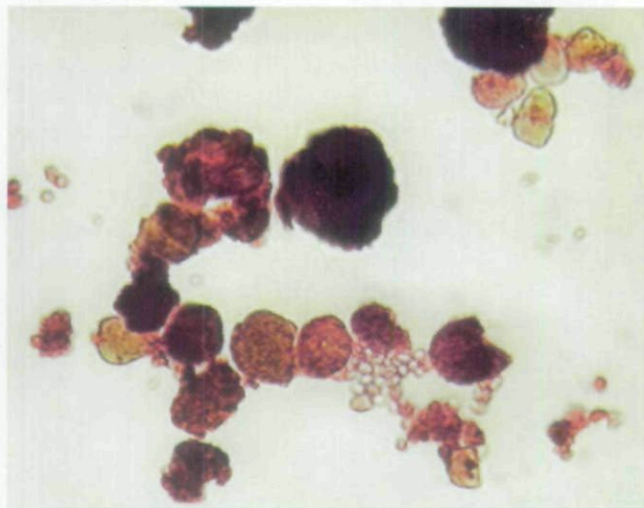
Faecal pellets

The majority of the faecal pellets from *L. destructor* were labelled by MoAb 1112D8 (anti-15 kDa) (Fig. 4a). In contrast, MoAb 117F9 (anti 79/93 kDa) did not react with the faecal pellets (Fig. 4b). No staining was obtained with the irrelevant subclass-matched MoAb (Fig. 4c). The same staining pattern was obtained in five different batches of faecal pellets.

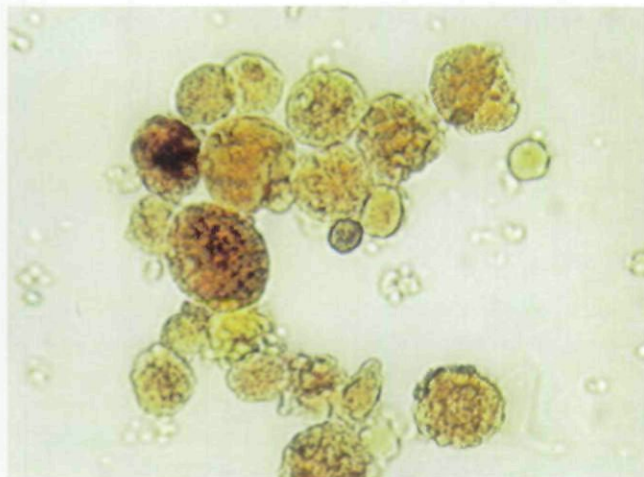
Discussion

L. destructor is the dominating mite species in Northern Europe and the mite species that has so far gained most interest [2,5]. Its principal allergen has a molecular weight of about 15 kDa and is detected by more than 90% of farmers who are RAST positive to this mite species [14]. The N-terminal amino acid sequence of this allergen showed no homology with proteins included in the Swiss data bank [24]. A major allergen of 14 kDa of *Lepidoglyphus* designated *Lep d I* [15], which is probably identical to the 15 kDa allergen, has been purified. We

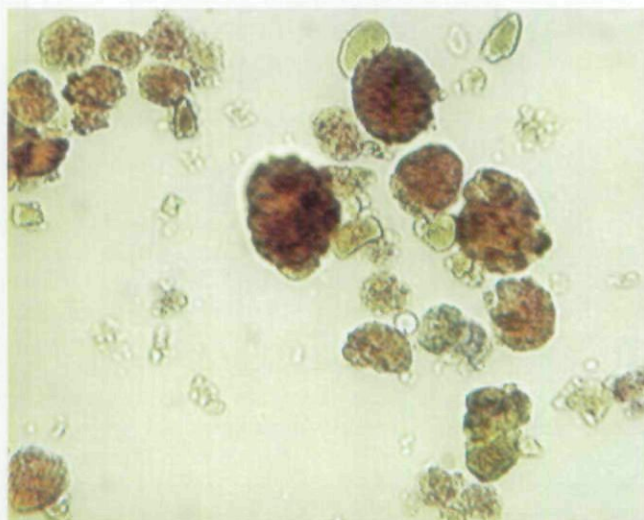
Fig. 3. *L. destructor* mite bodies express a 79/93 kDa allergen. Cryosections, 10 μm thick, were analysed with CLSM after being probed with (a and b) a MoAb against the 79/93 kDa allergen of *L. destructor* (117F9) and stained with the APAAP method using the chromogenic substrate Fast Red. The sections were counterstained with Mayer's haematoxylin. The specificity test comprised replacement with a subclass-matched MoAb which showed negative staining (c). Extended focus projections are shown in (a and c). The intensity of staining is colour-banded (range red-yellow-green-blue-black, where red is the strongest). In (b) the staining is visualized with an 'intensity map' projection of (a). The heights of the peaks are proportional to pixel intensities (i.e. detected emitted light). The staining pattern in the exoskeleton of the mite body is clearly visualized. Bar = 50 μm.



(a)



(b)



(c)

have recently demonstrated that MoAb 117F9 (anti-79/93 kDa) is restricted to *L. destructor* and crossreacts neither with three different storage mites nor with two house dust mite species. Furthermore, the 79/93 kDa allergen complex should not be overlooked as a cause of dust mite allergy, since nearly two-thirds of subjects RAST positive to *L. destructor* detect this complex [18].

In the present study, we detected different locations of the 15 kDa allergen and the high molecular weight allergen complex (79/93 kDa) in *L. destructor* mite bodies and faecal pellets with the use of MoAbs. Our results indicate that the 15 kDa allergen has a widespread distribution on the mite body. Like the binding pattern of one of the major allergens of the dust mite *Dermatophagoides*, *Der p* I [25–27], the major allergen of *Lepidoglyphus* was localized to the head region and to some extent also to the gut lining. The 15 kDa allergen was like *Der p* I [25,27], excreted in the faecal pellets. Since the gastrointestinal tract seemed to be empty in the body sections, it cannot be ruled out that MoAb 1112D8 stained the content of the gut as well. In contrast to the 15-kDa allergen, the high molecular weight allergen components, 79/93 kDa, seem to be located largely in the exoskeleton. The 'intensity map' obtained with CLSM disclosed that the 79/93 kDa allergen complex gave an intense emission from the exoskeleton and the front part of the body. Interestingly, the complex showed negative staining of the faecal pellets.

We earlier reported the localization of the 39 kDa allergen of *L. destructor* by probing mite sections with either MoAbs directed against 39 kDa (42B6) or with patient sera radioallergosorbent test (RAST) positive to *L. destructor* [19]. The sera generally labelled the wall of the *L. destructor* gut and to some extent also the faecal pellets. The MoAb, on the other hand, additionally stained parts of the exoskeleton and reacted with the major part of the faecal pellets. From experiments where we treated *L. destructor* extract with various agents we concluded that the anti-39 kDa MoAb recognizes the carbohydrate part of a glycoprotein [21].

Much interest has been focused on *Dermatophagoides* regarding the function of mite allergens. The *D. ptero-*

Fig. 4. Faecal pellets belonging to *L. destructor* probed with (a) a MoAb against the 15-kDa allergen of *L. destructor* (1112D8) or with (b) a MoAb against the 79/93 kDa allergen (117F9), (c) shows replacement with a subclass-matched irrelevant MoAb 1112D8. The pellets were stained with the APAAP method using the chromogenic substrate Fast Red. Note the red positive staining in (a), and that unstained faecal pellets (b and c) have brownish colour. Magnification $\times 370$ in ordinary light microscope.

nyssinus allergens consist of a catalogue of enzymes (protease, lysozyme, trypsin, amylase and chymotrypsin) [28]. The group I and group II allergens are recognized by nearly all mite-sensitized patients [29]. *Der p* I, which is a cysteine protease [30], can be present in faecal pellets at the very high concentration of 10 mg/ml [31]. The group II allergens probably correspond to lysozyme [32], whereas *Der p* IV corresponds to amylase [33]. Cloning and sequencing of the *D. pteronyssinus* group III allergen has recently confirmed that they are trypsin-like proteins [34]. *Lepidoglyphus* contains carbohydrases, proteinases, phosphatases as well as esterases [35]. The function of the 15 kDa, 39 kDa and 79/93 kDa allergen complex is unclear. However, the presence of the 15 and 39 kDa allergens in faeces indicates that they probably are involved in digestion. Future studies need to focus on recombinant DNA technology for characterization of these allergens. In this way, not only can pure allergens be produced more easily, but the respective amino acid sequences of the allergens might be deduced and compared with those of already described proteins with known function.

The *L. destructor* allergens assessed in the present study exhibited different labelling patterns. We chose APAAP staining to visualize the 15 and 79/93 kDa allergens since besides being rather permanent it can be evaluated both under the standard light microscope and with CLSM. In a brief preliminary communication we have reported the advantages of CLSM for localizing allergens in mites and yeast cells [23]. The knowledge of the location of allergens clearly adds new information on their biological properties.

Acknowledgments

Financial support for this work was received from the Swedish Work Environment Fund, the Swedish Medical Research Council (grants 16X-105 and 16X-7924), the Swedish Asthma and Allergy Association, the Hesselman Foundation, the Hedlund Foundation, the Konsul Th. C. Bergh Foundation, the King Gustaf V Foundation and the Karolinska Institute.

References

- Cuthbert OD, Brostoff J, Wraith DG, Brighton WD. 'Barn allergy': asthma and rhinitis due to storage mites. *Clin Allergy* 1979; 9:229-36.
- van Hage-Hamsten M, Johansson SGO, Höglund S et al. Storage mite allergy is common in a farming population. *Clin Allergy* 1985; 15:555-64.
- Terho EO, Husman K, Vohlonen I, Rautalahti M, Tukiainen H. Allergy to storage mites or cow dander as a cause of rhinitis among Finnish dairy farmers. *Allergy* 1985; 40:23-6.
- Korsgaard J, Dahl R, Iversen M, Hallas T. Storage mites as a cause of bronchial asthma in Denmark. *Allergol et Immunopathol* 1985; 13:143-9.
- Iversen M, Korsgaard J, Hallas T, Dahl R. Mite allergy and exposure to storage mites and house dust mites in farmers. *Clin Exp Allergy* 1990; 20:211-9.
- Blainey AD, Topping MD, Ollier S, Davies RJ. Respiratory symptoms in arable farmworkers: role of storage mites. *Thorax* 1988; 43:697-702.
- Blainey AD, Topping MD, Ollier S, Davies RJ. Allergic respiratory disease in grain workers: The role of storage mites. *J Allergy Clin Immunol* 1989; 84:296-303.
- Revsbech P, Andersen G. Storage mite allergy among grain elevator workers. *Allergy* 1987; 42:423-9.
- Iversen M, Dahl R. Allergy to storage mites in asthmatic patients and its relation to damp housing conditions. *Allergy* 1990; 45:81-5.
- van Hage-Hamsten M, Machado L, Barros MT, Johansson SGO. Comparison of clinical significance and allergenic cross-reactivity of storage mites *Blomia kulagini* and *Lepidoglyphus destructor* in Sweden and Brazil. *Allergy* 1990; 45:409-17.
- Arruda LK, Rizzo MC, Chapman MD et al. Exposure and sensitization to dust mite allergens among asthmatic children in Sao Paulo, Brazil. *Clin Exp Allergy* 1991; 21:433-9.
- Puerta Llerena L, Fernandez-Caldas E, Caraballo Gracia LR, Lockey RF. Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp.-allergic individuals. *J Allergy Clin Immunol* 1991; 88:943-50.
- Puerta L, Fernandez-Caldas E, Lockey RF, Caraballo LR. Sensitization to *Chortoglyphus arcuatus* and *Aleuroglyphus ovatus* in *Dermatophagoides* spp. allergic individuals. *Clin Exp Allergy* 1993; 23:117-23.
- Johansson E, Borgå Å, Johansson SGO, van Hage-Hamsten M. Immunoblot multi-allergen inhibition studies of allergenic crossreactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1991; 21: 511-8.
- Ventas P, Carreira J, Polo F. Purification and characterization of *Lep d* I, a major allergen from the mite *Lepidoglyphus destructor*. *Clin Exp Allergy* 1992; 22:454-60.
- Ansotegui IJ, Härfast B, Jeddi-Tehrani M et al. Identification of a new major allergen of 39 kilodaltons of the storage mite *Lepidoglyphus destructor*. *Immunol Lett* 1991; 27:127-30.
- van Hage-Hamsten M, Lagging E, Härfast B, Johansson SGO. Occurrence of IgE antibodies against the 39-kDa allergen component of the mite *Lepidoglyphus destructor* in urban and rural subjects. *Allergy* 1993; 48:209-11.
- Olsson S, Härfast B, Johansson SGO, van Hage-Hamsten M. Detection of at least one high-molecular-mass IgE-binding component of the dust mite *Lepidoglyphus destructor*. *Allergy*, 1994; 49:620-5.
- van Hage-Hamsten M, Scheynius A, Härfast B, Wirén A,

- Johansson SGO. Localization of allergens in the domestic mite *Lepidoglyphus destructor*. *Clin Exp Allergy* 1992; 22:251–6.
- 20 Wilson T. ed. *Confocal Microscopy*. London: Academic Press, 1990.
 - 21 Härfast B, van Hage-Hamsten M, Ansotegui IJ et al. Monoclonal antibodies to *Lepidoglyphus destructor*: delineation of crossreactivity between storage mites and house dust mites. *Clin Exp Allergy* 1992; 22:1032–7.
 - 22 Murdoch A, Jenkinson EJ, Johnson GD, Owen JJT. Alkaline phosphatase-Fast Red, a new fluorescent label. Application in double labelling for cell surface antigen and cell cycle analysis. *J Immunol Methods* 1990; 132:45–9.
 - 23 Scheynius A, Emilson A, van Hage-Hamsten M. Confocal laser scanning microscopy in the study of allergens and Langerhans' cells. *Allergy Clin Immunol News* 1993; 5:135–8.
 - 24 van Hage-Hamsten M, Bergman T, Johansson E et al. N-terminal amino acid sequence of principal allergen of storage mite *Lepidoglyphus destructor*. *Lancet* 1992; 340:614.
 - 25 Tovey ER, Baldo BA. Localization of antigens and allergens in thin sections of the house dust mite *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *J Med Entomol* 1990; 27:368–76.
 - 26 Thomas B, Heap P, Carswell F. Ultrastructural localization of the allergen *Der p* I in the gut of the house dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Appl Immunol* 1991; 94:365–7.
 - 27 Rees JA, Carter J, Sibley P, Merrett TG. Localization of the major house dust mite allergen *Der p* I in the body of *Dermatophagoides pteronyssinus* by Immustain®. *Clin Exp Allergy* 1992; 22:640–1.
 - 28 Thomas WR. Mite allergens groups I–VII. A catalogue of enzymes. *Clin Exp Allergy* 1993; 23:350–3.
 - 29 Yaseuda H, Mita H, Akiyama K et al. Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993; 23:384–90.
 - 30 Chua KY, Stewart GA, Thomas WR et al. Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p* I: homology with cysteine proteases. *J Exp Med* 1988; 167:175–82.
 - 31 Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981; 289:592–3.
 - 32 Stewart GA, Bird CH, Thompson PJ. Do the group II dust mite allergens correspond to lysozyme? *J Allergy Clin Immunol* 1992; 90:141–2.
 - 33 Lake FR, Ward LD, Simpson RJ, Thompson PJ, Stewart GA. House dust mite-derived amylase: Allergenicity and physicochemical characterization. *J Allergy Clin Immunol* 1991; 87:1035–42.
 - 34 Smith WA, Chua KY, Kuo MC, Rogers BL, Thomas WR. Cloning and sequencing of the *Dermatophagoides pteronyssinus* group III allergen, *Der p* III. *Clin Exp Allergy* 1994; 24:220–8.
 - 35 Bowman CE. Comparative enzymology of economically important astigmatid mites. In: Griffiths DA, Bowman CE, eds. *Acarology VI*. Chichester: Ellis Horwood 1984; 2:993–1001.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.