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A review of recent immunochemical studies of *Blomia tropicalis* and *Euroglyphus maynei* allergens

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ABSTRACT

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Exposure to mites other than *Dermatophagoides* spp., particularly *Blomia tropicalis* and *Euroglyphus maynei*, has been increasingly recognized as a cause of asthma. Positive skin tests and serum IgE antibodies to *B. tropicalis* have been reported in asthmatic patients from several areas of the world, including São Paulo (Brazil), Hong Kong and Tampa (Florida, USA). Analysis of *B. tropicalis* extracts showed undetectable levels of the major Group I and Group II *Dermatophagoides* spp. allergens. Immunoabsorption experiments showed that most of the IgE antibodies to *B. tropicalis* (~70%) reacted with species-specific allergens. Murine monoclonal antibodies to *B. tropicalis* could present antigens that were recognized by human IgG antibodies. Sensitization to *E. maynei* has been reported in Europe, North and South America and Australia. Analysis of four different *E. maynei* extracts by ELISA and RIA showed that *E. maynei* produces an allergen that is antigenically related to *Dermatophagoides* Group I allergens. The amino acid sequence of this allergen (*Eur m I*) has recently been reported. Further identification and purification of *B. tropicalis* and *E. maynei* allergens is required to develop specific assays for measuring these allergens in dust samples. This will make it possible to investigate the relationship between exposure to *B. tropicalis* or *E. maynei* and the development of sensitization and allergic disease.

INTRODUCTION

In many parts of the world, pyroglyphid mites of the genus *Dermatophagoides* (*D. pteronyssinus*, *D. farinae* and *D. microceras*) account for >90% of the mites found in house dust samples and are the principal cause of mite allergy. However, it is clear that under certain environmental conditions, other

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mite species occur in house dust and can form a significant part of the acarofauna. These include the pyroglyphid mite, *Euroglyphus maynei*, and non-pyroglyphid mites, commonly known as "storage mites", which are primarily encountered in stored food products, grains, barns, hay and straw. Allergic reactions to non-pyroglyphid mites, including *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus* and *Blomia tropicalis*, are usually associated with occupational asthma in grain handlers and farmers (Cuthbert et al., 1979; Ingram et al., 1979; Terho et al., 1985; Van Hage-Hamsten et al., 1985; Blainey et al., 1989). Sensitization to storage mites has been demonstrated by skin tests, RAST and bronchial challenges, and there is increasing interest as to whether household exposure to these mites could play a role in allergic diseases such as asthma and rhinitis. At a recent International Workshop on dust mite allergens, it was proposed that the term "domestic mites" should be used to refer to both pyroglyphid mites and to other mite species found in house dust which induce IgE antibody responses (Platts-Mills et al., 1992).

There have been several problems associated with establishing the clinical and allergenic importance of mites other than *Dermatophagoides* spp. Some mites, e.g. *E. maynei*, grow poorly in culture and it has been difficult to produce good extracts for skin testing, serum antibody assays and allergen analysis. As a result, immunochemical studies of other domestic mites have lagged behind those of *Dermatophagoides*. Perhaps the most difficult problem has been that in cases where patients are exposed to other mites, they are also exposed to large numbers of *Dermatophagoides* spp. Most studies have shown that patients with IgE antibody to *E. maynei*, or to storage mites, also had IgE to *Dermatophagoides* spp., and that the patients were exposed to both storage mite and *Dermatophagoides* allergens in their homes. The key question is whether clinical sensitivity and immune responses to non-pyroglyphid mites occurs as a result of cross-reactivity with *Dermatophagoides* allergens, or whether it reflects concordant sensitization to species-specific allergens. Investigation of these alternatives requires the identification and purification of allergens and immunoabsorption or RAST inhibition experiments using sera from patients with well-defined sensitivities to mite allergens. Previous studies using RAST inhibition suggest that storage mites (e.g. *A. siro*, *T. putrescentiae* and *L. destructor*) produce species-specific allergens (Van Hage-Hamsten et al., 1987; Griffin et al., 1989; Luczynska et al., 1990). In this review, we focus on recent immunochemical studies of *B. tropicalis* and *E. maynei*. Both mites can become the predominant species in house dust samples and cause sensitization in patients with asthma (Hurtado and Parini, 1987; Arlian, 1989; Fernandez-Caldas et al., 1990; Colloff, 1991a,b; Colloff et al., 1991). Extracts of *B. tropicalis* and *E. maynei* have recently been produced by several laboratories and were used in the present studies to assess sensitization and the degree of immunologic cross-reactivity with *Dermato-*

phagoides spp. Establishing the antigenic relationships between the various mite species is important for allergy diagnosis and treatment, and for epidemiologic studies on the role of mite allergens as risk factors for allergic disease.

TAXONOMY AND DISTRIBUTION OF *B. TROPICALIS*

Blomia tropicalis was first described by Van Bronswijk et al. (1973), and was previously regarded as a storage mite. This small (230–465 μm), globular-shaped mite belongs to the *Glycyphagidae* family and is distinguished, at least to the non-acarologist, by the many long setae covering the body (resembling a porcupine!) (Fig. 1). *Blomia tropicalis* occurs in tropical and subtropical regions throughout the world and has been found in dust samples from Hong Kong, Venezuela, Brazil, Japan, Taiwan, Surinam, India, Spain, Egypt and Nigeria (Van Bronswijk et al., 1973; Jorge Neto et al., 1980; Gabriel et al., 1982; Hurtado and Parini, 1987; Fernandez-Caldas et al., 1990). In the

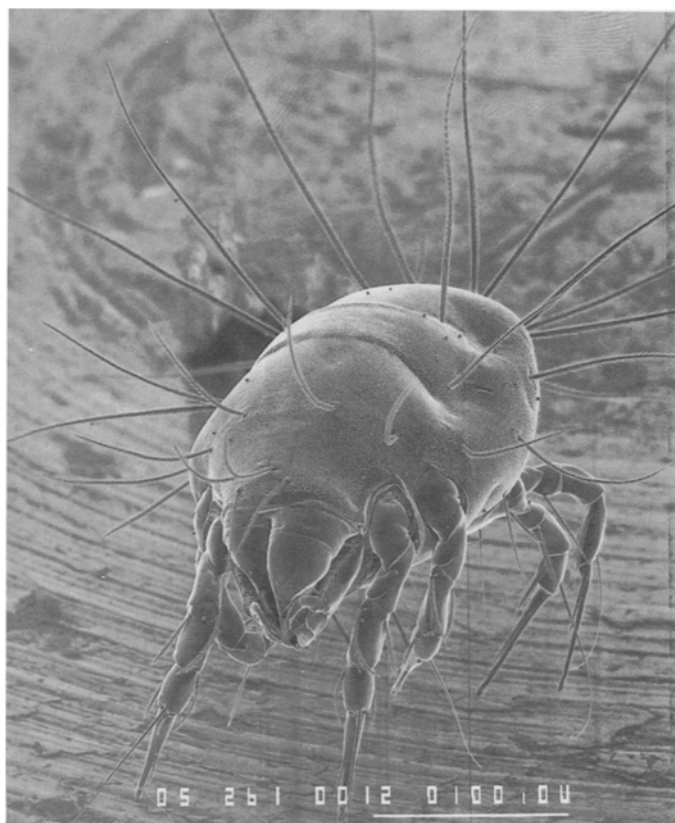


Fig. 1. Scanning electronmicrograph of *Blomia tropicalis*, kindly provided by Dr. Enrique Fernandez-Caldas, University of South Florida, Tampa, FL.

United States, *B. tropicalis* has been found in large numbers (100–3000 mites/g of dust) in mattresses and carpet dust from houses in Tampa, Florida, being the predominant mite species in some samples (Fernandez-Caldas et al., 1988, 1990). *Blomia tropicalis* appears to be an important source of mite allergens in those areas of the world where it is abundant in houses. Exposure to *B. tropicalis* has been assessed by microscopic identification and counting of mites in dust samples.

IMMUNOCHEMICAL STUDIES OF *B. TROPICALIS* ALLERGENS

Blomia tropicalis can be grown on supplemented soybean flour medium or mouse food medium, in cultures maintained at 25°C and 75% RH (Arruda et al., 1991). Extracts of either the isolated mite bodies or the whole culture were used to develop a *B. tropicalis* RAST and have subsequently been used for antigenic comparisons with *Dermatophagoides* spp. allergens. We recently analyzed two *B. tropicalis* extracts, prepared from either mite bodies (Tampa, FL) or whole *B. tropicalis* cultures (São Paulo, Brazil), for their content of Group I and Group II *Dermatophagoides* allergens. Levels of *Der p* I, *Der f* I and Group II, measured by monoclonal-antibody-based immunoassays, were undetectable or < 50 ng/ml in both extracts. The absence of *Dermatophagoides* Group I allergens in the *B. tropicalis* extracts was confirmed using a

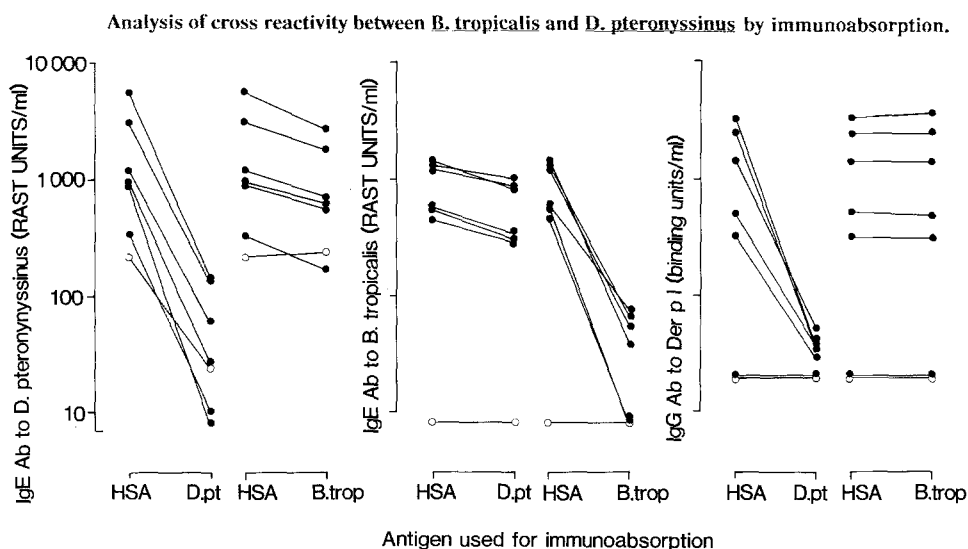


Fig. 2. Evidence from immunoabsorption studies that *B. tropicalis* produces species-specific allergens. Sera were either from mite-allergic patients in Brazil (●) or from a U.S. mite-allergic patient who had never been exposed to *B. tropicalis* (○). [Reprinted from Arruda et al. (1991) with permission of Blackwells Scientific Publishers.]

TABLE 1

Binding of IgE antibodies to antigens presented by monoclonal antibodies to *B. tropicalis*

Patient	<i>B. tropicalis</i> RAST (units/ml)	¹²⁵ I anti IgE bound to antigen presented by anti- <i>B. tropicalis</i> mAb (cpm) ¹			
		1B10	9B12	2A7	5C10
#28	4700	8569	9094	9967	7902
#40	4200	5039	6085	7081	5465
#25	3000	4961	6282	8862	5432
#16	1800	4416	4866	6114	4164
#34	3000	3136	2070	6424	2572
#26	4800	2994	2885	3178	2548
Controls ² (mean, $\pm$ s.d. $n=6$)	< 10	221 $\pm$ 57	232 $\pm$ 62	270 $\pm$ 36	220 $\pm$ 84

¹Cyanogen-bromide-activated cellulose discs were coated overnight with murine monoclonal antibody ascites, diluted 1:100. *Blomia tropicalis* extract 1:50 was added, and the discs were incubated successively with patient's serum and ¹²⁵I anti human IgE ($\sim 110\,000$ cpm/disc). Discs were washed and radioactivity was counted in a gamma counter.

²Sera from six healthy non-atopic adult individuals were used as controls.

polyclonal-antibody-based radioimmunoassay (Platts-Mills et al., 1986; Aruda et al., 1991). These results suggested that *B. tropicalis* produced allergens that were antigenically distinct from the major *Dermatophagoides* allergens. This conclusion was also supported by the results of immunoabsorption experiments using sera from Brazilian asthmatic children with high levels of IgE antibody to both *B. tropicalis* and *D. pteronyssinus* (> 200 RAST units/ml). In these experiments, only 36% of the IgE to *B. tropicalis* was removed by absorption with *D. pteronyssinus* extract and similarly, only 32% of the IgE to *D. pteronyssinus* was removed by absorption with *B. tropicalis* extract (Fig. 2). No effect on serum IgG antibodies to *Der p* I was obtained by absorption with *B. tropicalis* extract. These results suggested that most of the IgE antibody to *B. tropicalis* ($\sim 70\%$) was directed against species-specific allergens and that a smaller proportion reacted with antigens common to *B. tropicalis* and *D. pteronyssinus*. The cross-reactivity is associated with allergens other than the Group I or Group II *Dermatophagoides* allergens. In keeping with our results, Van Hage-Hamsten et al. (1990) have recently reported that *D. pteronyssinus* and *B. kulagini*, another *Blomia* species found in Brazil, have predominantly species-specific allergens.

To further identify and define *B. tropicalis* allergens, we began the production of monoclonal antibodies (mAb) to *B. tropicalis* extract. Interestingly, BALB/c mice immunized repeatedly with *B. tropicalis* mite body extract gave predominantly species-specific IgG antibody responses when compared by ELISA (titers of 1/6400–1/25 000 to *B. tropicalis* extract, as compared to $< 1/500$ to *Dermatophagoides* extract). In keeping with this, the majority of hybrids derived from the fusion (71/74, $> 95\%$) produced only IgG antibody-

ies to *B. tropicalis* on screening by ELISA. Six *B. tropicalis*-specific hybrids were cloned and preliminary results using a two-site radioimmunoassay have shown that the mAb can "present" *B. tropicalis* antigens to human IgE antibodies. Representative data showing IgE antibodies in six sera from patients with high levels of IgE anti-*B. tropicalis* binding to antigen presented by four of the mAb are shown in Table 1. The majority of sera from U.S. or British mite-allergic patients, who had not been exposed to *B. tropicalis*, did not show significant binding in this assay. These results suggest that the mAb are directed against *B. tropicalis*-specific allergens and further work is now in progress to determine whether the mAb are directed against the same or different allergens, and to determine the physicochemical and molecular properties of the mAb-defined allergens.

CLINICAL SIGNIFICANCE OF *BLOMIA TROPICALIS*

High levels of IgE antibodies to *B. tropicalis* were found in a group of 20 asthmatic children, living in São Paulo, Brazil (Arruda et al., 1991). These children were selected based on the presence of a positive skin test to *D. pteronyssinus* and/or *D. farinae*, and levels of IgE antibodies to *B. tropicalis* were > 200 RAST units/ml in 11/20 and 13/20 sera, using whole mite culture or mite body *B. tropicalis* extracts, respectively (Fig. 3). Those RAST values have been regarded as moderate to strongly positive, suggesting that *B. tropicalis* is an important cause of sensitization among asthmatic children in Brazil. No significant evidence of exposure or sensitization to cat, cockroach, rye

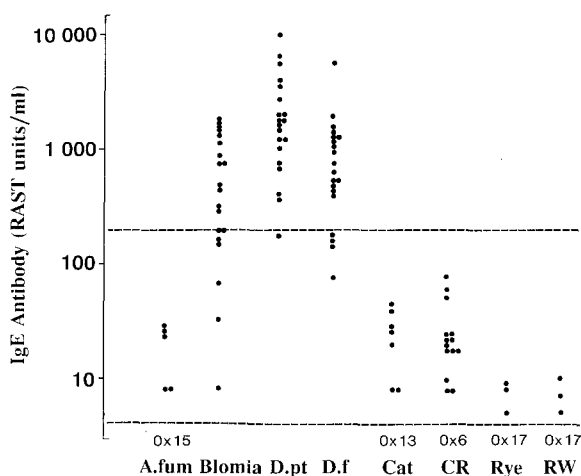


Fig. 3. IgE antibody responses of 20 mite-allergic Brazilian, asthmatic children measured by RAST. Dust mites (*B. tropicalis* and *Dermatophagoides* spp.) were the major cause of sensitization. [Reprinted from Arruda et al. (1991) with permission of Blackwells Scientific Publishers.]

grass and ragweed pollens, and *Aspergillus* was found among the Brazilian children, suggesting that mites, including *B. tropicalis*, are the major source of allergen in house dust in this particular environment. Positive skin tests to *B. tropicalis* extracts have been reported among house-dust- and house-dust-mite-allergic asthmatic Chinese subjects living in Hong Kong (Gabriel et al., 1982). Although pyroglyphid mites accounted for 82% of the total mite fauna in the houses of these patients, *B. tropicalis* was the most numerous species among the storage mites. IgE antibody to *B. tropicalis* has also been detected in mite-allergic individuals in Tampa, FL (Fernandez-Caldas et al., 1988, 1990). As part of a continuation of studies in São Paulo, we recently compared exposure of mite-allergic asthmatic children to Group I and Group II *Dermatophagoides* allergens with exposure of a control group of non-atopic, non-asthmatic children, also living in São Paulo. No significant differences in the levels of mite allergens were found among the two groups, both of them being exposed to levels regarded as risk factor for sensitization and development of asthma ($> 2 \mu\text{g/g}$ in $> 90\%$ of cases). However, only the patients with asthma had IgE antibodies to *D. pteronyssinus*, *D. farinae* and *B. tropicalis*. These patients also had serum IgG antibodies to *Der p* I and *Der f* I. In most cases, children in the control group had undetectable levels of both IgE and IgG antibodies. The results of these experiments suggest that *B. tropicalis* should be considered when evaluating immediate hypersensitivity in patients with asthma, living in São Paulo, Brazil, and in other areas of the world where exposure to *B. tropicalis* is likely to occur. Further studies on characterization of *B. tropicalis* allergens and development of immunochemical assays for quantitation of *B. tropicalis* exposure will be useful for evaluating threshold levels for sensitization and for monitoring the efficacy of mite-avoidance measures.

EUROGLYPHUS MAYNEI ALLERGENS

The taxonomy, geographic distribution and clinical significance of *E. maynei* has recently been reviewed in detail (Arlan, 1989; Colloff, 1991a,b). Voorhorst et al. (1969) originally reported immediate skin tests to *E. maynei* extracts and studies carried out largely in the UK, in the 1970s, confirmed their findings and showed that *E. maynei* could occur in large numbers in dust samples and, indeed, could be the predominant species in some cases (Voorhorst et al., 1969; Blythe, 1976; Burr et al., 1980; Walshaw and Evans, 1987). Sensitization and exposure to *E. maynei* has been reported in several parts of the world, including most of Europe, North and South America and Australia, and is usually associated with damp housing (Colloff, 1991a; Colloff et al., 1991). Current evidence suggests that high relative humidity (80%) is an important factor for promoting growth of *E. maynei* over *Dermatopha-*

goides spp., though reproductive, nutritional and other physiological factors are also likely to be involved (Colloff, 1991a,b).

All studies on *E. maynei* have been limited by the fact that this species does not readily adapt to laboratory culture. Until recently, it had not been possible to grow *E. maynei* on a commercial scale and most studies were carried out using mites isolated from house dust samples and maintained with varying degrees of success in short-term cultures. Several acarologists now claim to be able to "grow" *E. maynei* with some consistency and there are more extracts available for analysis. An *E. maynei* RAST has also been marketed in Europe, however, for most of us, growing *E. maynei* remains a mysterious and murky area! Immunochemical studies on *E. maynei* allergens initially involved using extracts prepared from selected house dust samples in which *E. maynei* was the predominant species (> 66% of the total mite population). When the radioimmunoassay for *Dermatophagoides* spp. Group I allergen ("Antigen P₁ equivalent") was developed, it was shown to be inhibited by > 90% by such *E. maynei*-rich dust extracts, thus providing the first evidence for an antigenically related Group I allergen in *E. maynei* (Platts-Mills et al., 1986). Subsequent studies showed that monoclonal-antibody-based immunoassays for *Der p* I or *Der f* I underestimated the Group I allergen content of *E. maynei*-rich dust extracts (Chapman et al., 1987): the explanation being that the mAb recognize species-specific determinants on the *Dermatophagoides* Group I allergens that are not expressed on the *E. maynei* allergen. Recently, those experiments have been repeated using extracts of four *E. maynei* cultures grown in different laboratories. The results of Group I allergen assays are similar to those obtained previously using dust extracts, show-

TABLE 2

Reactivity of *E. maynei* extracts in immunoassays for *Dermatophagoides* spp. allergens

<i>E. maynei</i>	<i>Der f</i> I ¹ (ng/ml)	<i>Der p</i> I ¹ (ng/ml)	<i>Der p</i> I + <i>Der f</i> I ¹ (ng/ml)	Group I ² (ng/ml)	Group II ³ (ng/ml)
i. (Sweden)	408	< 5	408	9000	< 50
ii. (UK)	2300	1170	3470	3125	< 50
iii. (Italy)	3000	148	3148	13700	< 50
iv. (Italy)	3400	130	3530	17200	< 50

¹Determined by two-site monoclonal immunoassay for either *Der f* I or *Der p* I. Values were calculated using "in house" *D. farinae* (UVA 87/02) and *D. pteronyssinus* (UVA 87/03) reference preparations (Luczynska et al., 1989).

²Inhibition RIA using *E. maynei* extracts to inhibit binding of ¹²⁵I *Der p* I to affinity-purified polyclonal rabbit antibodies to cross-reacting determinants on Group I *Dermatophagoides* spp. allergens (Platts-Mills et al., 1986). Values were calculated using the *D. pteronyssinus* UVA 87/03 reference (2500 ng *Der p* I/ml).

³Determined by two-site monoclonal immunoassay, as described previously (Heymann et al., 1986).

ing in most cases lower reactivity in the mAb-based assays as compared to the inhibition RIA using cross-reacting polyclonal antibodies (Table 2). We have also found that the mAb directed against a cross-reactive epitope on *Der p* I and *Der f* I (clone 4C1) binds to *E. maynei*. The 4C1 mAb can be coated on microtiter wells and used to present the *E. maynei* allergen to affinity-purified, polyclonal rabbit antibodies to *Dermatophagoides* Group I allergen in an ELISA. These experiments confirm that *E. maynei* produces an allergen that is antigenically related to the *Dermatophagoides* Group I allergens and, recently, Hart and colleagues reported the amino acid sequence of the *E. maynei* allergen (*Eur m* I), which they obtained by PCR amplification of *E. maynei* DNA (Hill et al., 1991).

The *E. maynei* extracts showed no detectable binding in immunoassays for *Dermatophagoides* Group II allergens (Table 2). This was surprising because the Group II allergens are more highly conserved, both antigenically and structurally, than Group I. Anti-*Der p* II and anti-*Der f* II mAb bind to common epitopes on these allergens (no species-specific sites have yet been identified) and the amino acid sequences of the *Dermatophagoides* allergens show 88% homology (Heyman et al., 1986; Trudinger et al., 1991). These preliminary results raise the interesting possibilities that either *E. maynei* produces a Group II homologue that is antigenically distinct from *Dermatophagoides* allergens, or that *E. maynei* does not produce Group II allergens. While it would be unusual for proteins as highly conserved as the Group II allergens not to be expressed in these closely related mite species, there is some evidence from RAST inhibition studies that *E. maynei* produces species-specific allergens (Van Hage-Hamsten and Johansson, 1989). The availability of both *E. maynei* extracts and live mites should now enable *E. maynei* allergens to be more thoroughly investigated using immunochemical and molecular techniques.

CONCLUSIONS

There are clearly close parallels between studies on *B. tropicalis* and *E. maynei*: both mites can account for a high percentage of the house dust acarofauna (up to 80%); both cause IgE antibody responses in patients with asthma; and both mites produce some species-specific allergens. *Blomia tropicalis* and *E. maynei* are increasingly recognized as a significant cause of asthma and there has been considerable progress in defining their antigenic cross-reactivity (or lack of) with *Dermatophagoides* spp. These studies have been facilitated by the availability of *B. tropicalis* and *E. maynei* extracts and monoclonal and polyclonal antibodies to the major *Dermatophagoides* allergens. However, further studies are required to identify and purify specific *B. tropicalis* and *E. maynei* allergens and to develop immunochemical assays for measuring these allergens in dust samples. This will make it possible to carry

out epidemiologic studies in different countries (as has been done for *Dermatophagoides* spp.) to establish the relationships between exposure to *B. tropicalis* or *E. maynei* and the development of sensitization and clinical symptoms, and to determine the importance of these species as risk factors for allergic disease.

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REFERENCES

- Arlan, L.G., 1989. Biology and ecology of house dust mites, *Dermatophagoides* spp. and *Euroglyphus* spp. Immunol. Allergy Clin. N. Am., 9(2): 339-356.
- Arruda, L.K., Rizzo, M.C., Chapman, M.D., Fernandez-Caldas, E., Baggio, D., Platts-Mills, T.A.E. and Naspitz, C.K., 1991. Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. Clin. Exp. Allergy, 21: 433-439.
- Blainey, A.D., Topping, M.D., Ollier, S. and Davies, R.J., 1989. Allergic respiratory disease in grain workers: the role of storage mites. J. Allergy Clin. Immunol., 84: 296-303.
- Blythe, M.E., 1976. Some aspects of the ecological study of house dust mites. Br. J. Dis. Chest, 70: 3-31.
- Burr, M.L., Dean, B.V., Merrett, T.G., Neale, E., St. Leger, A.S. and Verrier-Jones, E.R., 1980. Effects of anti-mite measures on children with mite sensitive asthma: a controlled trial. Thorax, 35: 506-512.
- Chapman, M.D., Heyman, P.W., Wilkins, S.R., Brown, M.J. and Platts-Mills, T.A.E., 1987. Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, *Der p* I and *Der f* I, and quantitative analysis of the allergen content of mite and house dust extracts. J. Allergy Clin. Immunol., 80: 184-194.
- Colloff, M.J., 1991a. A review of biology and allergenicity of the house-dust mite *Euroglyphus maynei* (Acari: Pyroglyphidae). Exp. Appl. Acarol., 11: 177-198.
- Colloff, M.J., 1991b. Population studies on the house dust mite, *Euroglyphus maynei*, (Cooreman 1950) (Pyroglyphidae). In: R.H. Schuster and P.W. Murphy (Editors), The Acari: Reproduction, Development and Life History Strategies. Chapman and Hall, London, pp. 497-506.
- Colloff, M.J., Stewart, G.A. and Thompson, P.J., 1991. House dust acarofauna and *Der p* I equivalent in Australia: the relative importance of *Dermatophagoides pteronyssinus* and *Euroglyphus maynei*. Clin. Exp. Allergy, 21: 225-230.
- Cuthbert, O.D., Brostoff, J., Wraith, D.G. and Brighton, W.D., 1979. "Barn allergy": asthma and rhinitis due to storage mites. Clin. Allergy, 9: 229-236.
- Fernandez-Caldas, E., Fox, R.W., Trudeau, W. and Lockey, R.F., 1988. Allergenicity of the mite, *Blomia tropicalis*. J. Allergy Clin. Immunol., 88: 270 (Abstract).
- Fernandez-Caldas, E., Fox, R.W., Bucholtz, G.A., Trudeau, W.L., Ledford, D.K. and Lockey,

- R.F., 1990. House dust mite allergy in Florida. Mite survey in households of mite-sensitive individuals in Tampa, Florida. *Allergy Proc.*, 11: 263–267.
- Gabriel, M., Cunnington, A.M., Allan, W.G.L., Pickering, C.A.C. and Wraith, D.G., 1982. Mite allergy in Hong Kong. *Clin. Allergy*, 12: 157–171.
- Griffin, P., Ford, A.W., Alterman, L.A., Thompson, J., Parkinson, C., Blainey, A.D., Davies, R.J. and Topping, M.D., 1989. Allergenic and antigenic relationship between three species of storage mite and the house dust mite, *Dermatophagoides pteronyssinus*. *J. Allergy Clin. Immunol.*, 84: 108–117.
- Heymann, P.W., Chapman, M.D. and Platts-Mills, T.A.E., 1986. Antigen *Der fI* from the dust mite *Dermatophagoides farinae*: structural comparison with *Der p I* from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J. Immunol.*, 137: 2841–2847.
- Hill, M.A., Kent, N.A., Holland, P.W.H. and Hart, B.J., 1991. Characterisation of *Euroglyphus maynei* allergens. In: Dust mite allergens and asthma: Report of a second international workshop. U.C.B. Institute of Allergy, Brussels, pp. 65–67.
- Hurtado, I. and Parini, M., 1987. House dust mites in Caracas, Venezuela, *Ann. Allergy*, 59: 128–130.
- Ingram, C.G., Jeffrey, I.G., Symington, I.S. and Cuthbert, O.D., 1979. Bronchial provocation studies in farmers allergic to storage mites. *Lancet*, ii: 1330–1332.
- Jorge Neto, J., Croce, J. and Baggio, D., 1980. Acaros da poeira domiciliar da cidade de São Paulo. Nota previa. *Rev. Bras. Alerg. Immunopatol.*, 2: 140–145.
- Luczynska, C.M., Arruda, L.K., Platts-Mills, T.A.E., Miller, J.D., Lopez, M. and Chapman, M.D., 1989. A two site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, *Der p I* and *Der fI*. *J. Immunol. Methods*, 118: 227–235.
- Luczynska, C.M., Griffin, P., Davies, R.J. and Topping, M.D., 1990. Prevalence of specific IgE to storage mites (*A. siro*, *L. destructor*, and *T. longior*) in an urban population and crossreactivity with the house dust mite (*D. pteronyssinus*). *Clin. Exp. Allergy*, 20: 403–406.
- Platts-Mills, T.A.E., Heymann, P.W., Chapman, M.D., Hayden, M.L. and Wilkins, S.R., 1986. Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for antigen P₁ equivalent in house dust and dust mite extracts. *J. Allergy Clin. Immunol.*, 78: 398–407.
- Platts-Mills, T.A.E., Thomas, W.R., Aalberse, R.C., Vervloet, D. and Chapman, M.D., 1992. Dust mite allergens and asthma: report of a second international workshop. *J. Allergy Clin. Immunol.*, 89: 1046–1060.
- Terho, E.O., Husman, K., Vohlonen, I., Rautalahti, M. and Tukiainen, H., 1985. Allergy to storage mites or cow dander as a cause of rhinitis among Finnish dairy farmers. *Allergy*, 40: 23–26.
- Trudinger, M., Chua, K.Y. and Thomas, W.R., 1991. cDNA encoding the major mite allergen *Der fII*. *Clin. Exp. Allergy*, 21: 33–38.
- Van Bronswijk, J.E.M.H., de Cock, A.W.A.M. and Oshima, S., 1973. The genus *Blomia* Oudemans (Acari: Glycyphagidae). I. Description of *Blomia tropicalis* sp. n. from house dust in tropical and subtropical regions. *Acarologia*, 15: 477–489.
- Van Hage-Hamsten, M. and Johansson, S.G.O., 1989. Clinical significance and allergenic cross-reactivity of *Euroglyphus maynei* and other non-pyroglyphid and pyroglyphid mites. *J. Allergy Clin. Immunol.*, 83: 581–589.
- Van Hage-Hamsten, M., Johansson, S.G.O., Hoglund, S., Tull, P., Wiren, A. and Zetterstrom, O., 1985. Storage mite allergy is common in a farming population. *Clin. Allergy*, 15: 555–564.
- Van Hage-Hamsten, M., Johansson, S.G.O., Johansson, E. and Wiren, A., 1987. Lack of allergenic cross-reactivity between storage mites and *Dermatophagoides pteronyssinus*. *Clin. Allergy*, 17: 23–31.

- Van Hage-Hamsten, M., Machado, L., Barros, M.T. and Johansson, S.G.O., 1990. Immune response to *Blomia kulagini* and *Dermatophagoides pteronyssinus* in Sweden and Brazil. *Int. Arch. Allergy Appl. Immunol.*, 91: 186–191.
- Voorhorst, R., Spieksma, F.Th.M. and Varekamp, H., 1969. House dust atopy and the house dust mite *Dermatophagoides pteronyssinus* (Trouessart 1897). Stafleu's Scientific, Leiden, 159 pp.
- Walshaw, M.J. and Evans, C.C., 1987. The effect of seasonal and domestic factors on the distribution of *Euroglyphus maynei* in the homes of *Dermatophagoides pteronyssinus* allergic patients. *Clin. Allergy*, 17: 7–14.