

Kinetics of Allergen Expression in Cultures of House Dust Mites, *Dermatophagoides pteronyssinus* and *D. farinae* (Acari: Pyroglyphidae)

ELENA ERASO,^{1,2} JORGE A. GUI SANTES,² JORGE MARTÍNEZ,^{1,2} MIRIAM SÁENZ-DE-SANTAMARÍA,² ALBERTO MARTÍNEZ,¹ RICARDO PALACIOS,¹ AND RAMÓN CISTERNA²

J. Med. Entomol. 34(6): 684-689 (1997)

ABSTRACT Laboratory cultures of house dust mites *Dermatophagoides pteronyssinus* (Trouessart, 1897) and *Dermatophagoides farinae* Hughes, 1961 were used to study the population dynamics of the mites and the kinetics of antigen appearance. The analysis of extracts obtained after different incubation periods, carried out by SDS-PAGE, immunoblotting, and enzyme-linked immunosorbent assay, allows for the definition of 3 different growth phases: the latency phase (F1); the exponential growth phase (F2) during which the allergenic proteins, including the Der 1 and Der 2 major allergens, were expressed more intensely and in larger quantities; and a final phase (F3), death, in which the lowest rates of allergenic components with a clearly different pattern were seen. The data obtained from this work demonstrates that mite cultures during the maximum growth phase (F2) contain the largest amount of allergenic components as well as the highest major allergen concentrations.

KEY WORDS *Dermatophagoides* spp., house dust mites, mite allergens, mite growth, Der 1, Der 2

KERN (1921), COOKE (1922), and Storm van Leeuwen (1922) reported the existence of positive cutaneous reactions to dust extracts in allergic patients and of the allergenic components present in house dust. Voorhorst et al. (1964) found that mites of the genus *Dermatophagoides* Bogdanov, 1864, present in house dust, were responsible for house-dust bronchial asthma and Voorhorst et al. (1969) concluded that *Dermatophagoides pteronyssinus* (Trouessart, 1897) was the main source of the allergens responsible for house dust atopy. These findings indicated the importance of the use of entire mite cultures extracts instead of extracts from just the mites themselves and that allergens occur in mite bodies as well as in substances excreted by them. Tovey et al. (1981) demonstrated that mite fecal particles are a major source of house dust allergens.

Another species of the same genus, *Dermatophagoides farinae* Hughes, 1961, was reported as an important source of allergens (Miyamoto et al. 1968).

The importance of *Dermatophagoides* as a main allergenic source in house dust was first verified in Europe by Voorhorst et al. (1964) and later shown by several authors worldwide (Fain et al. 1990, Platts-Mills et al. 1992).

Extracts of mite culture are widely used for the diagnosis as well as for the immunotherapy of house dust allergy. In this regard, there are numerous reports on the allergenic composition of mites of the genus *Dermatophagoides* (Chapman and Platts-Mills 1980, Le Mao et al. 1981, Heymann et al. 1989, Tovey et al. 1989, Lake et al. 1991, Stewart et al. 1992, Ando et al. 1993, Shen et al. 1993, Yasueda et al. 1993). However, despite several existing studies on the population growth and developmental stages of *Dermatophagoides pteronyssinus* (Portus 1975; Andersen 1988, 1991), there is no evidence of the correlation between phases of mite growth in culture and the allergenic expression during this process.

In the current work we investigated and determined the growth pattern of the main allergenic mite species, *D. pteronyssinus* and *D. farinae*, and also evaluated the protein and allergenic expression during the culturing period of both species.

Materials and Methods

Mite Culture. *D. pteronyssinus* and *D. farinae* were reared on a 1:1 (wt:wt) autoclaved mixture of rat and mouse fodder and dried yeast powder. We began with 12 cultures per species, all of them from the same inoculum, incubated at 23–25°C and 75–80% RH, which was maintained by a saturated solution of sodium chloride. Cultures were examined regularly to assess mite growth, and checked to discard any cross-contamination with other mites or

Patients gave their informed consent; our institutional protocol is on file in our laboratory.

¹ Department of R and D, Industrial Farmaceutica y de Especialidades S.A. Alameda de Urquijo 27, 48008-Bilbao, Spain.

² Department of Immunology, Microbiology and Parasitology, Faculty of Pharmacy, University of Basque Country, P.O. Box 450, 01080-Vitoria, Spain.

molds. The cultures were inoculated and examined in a biological safety cabinet.

At 14-d intervals, a small culture sample (≈ 50 –100 mg) was used to determine the mite concentration in culture (live and dead mites and total mite population) by microscope counts. Cultures were then processed to obtain the extracts.

Extracts Preparation. Extraction from the whole culture was performed under agitation at 4–8°C in phosphate buffer saline (pH 7.2 during 6 h). The extract suspension was clarified by centrifuging at $3,000 \times g$ for 30 min and the supernatant was filtered. Extracts were dialyzed in membranes with a 5,000 Da cut-off at 4°C against distilled water for 24 h, centrifuged at $12,000 \times g$ for 30 min, and then lyophilized.

An extract from the culture medium was also obtained according to the same protocol.

Protein Determination. Protein concentrations were estimated according to the method of Lowry et al. (1951) by using bovine serum albumin as the standard.

Human Sera. Sera from 37 mite-sensitive patients with persistent allergic symptomatic histories and no history of either immunotherapy or other sensitizations were used to perform the immunoblotting. All sera were screened with Phadebas RAST reagents (Kabi, Uppsala, Sweden) and showed high specific IgE levels (RAST class ≥ 3). Control sera were obtained from nonatopic individuals.

Gel Electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was done by using the method of Laemmli (1970), employing 12.5 and 4% polyacrylamide concentrations for separating and stacking gels, respectively. Proteins dissolved in 0.125 M Tris-HCl, pH 6.8 were dissociated with 0.1% SDS and 5% β -mercaptoethanol by treatment at 100°C for 5 min, and 70 mg protein were applied per lane. Electrophoresis was carried out for 1 h at 200 V with an electrode buffer containing 0.025 M Tris, 0.19 M glycine, and 0.1% SDS, pH 8.7. Proteins on the gel were stained with Coomassie Brilliant Blue R-250 (0.1% in methanol, acetic acid, and distilled water 4:1:5), and the molecular weights of the separated proteins were calculated by the low molecular weight electrophoresis calibration kit from Pharmacia Biotech (Uppsala, Sweden). The protein profiles were analyzed by means of the Millipore Bio-Image System (Bedford, MA).

Immunoblotting. Separated protein bands from the polyacrylamide gel were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore), according with Towbin et al. (1979). The blotted gels were stained with Coomassie Brilliant Blue R-250 to evaluate the effectiveness of transfer. Immunochemical staining was performed by first blocking the immobilon membrane with 90% skimmed dry milk in Tris-buffered saline (TBS) 1 h at 40°C. After 4 washes with TBS, blots were incubated with the undiluted human serum pool for 16 h at 4°C. The blots were then washed 4 times with TBS and incubated with 1:25 peroxidase conjugated to

rabbit antihuman IgE immunoglobulins (ϵ -chains) (DAKO, Denmark). Bound peroxidase was detected by the addition of 0.06% wt:vol of 4-chloro-1-naphthol solution and 0.01% H_2O_2 in TBS. After 30 min, blots were rinsed in distilled water and air-dried. Antigen-antibody binding was identified by purple-stain deposition (Shen et al. 1988). Nonspecific binding of the anti-IgE was checked with a negative control serum. The band patterns were analyzed by means of the Millipore Bio-Image System.

Major Allergen Determinations. The measurement of group I and group II allergens was performed by a 2-site monoclonal antibodies (Mab) enzyme-linked immunosorbent assay (ELISA) using Der p 1 ELISA kit (5H8/4C1), Der f 1 ELISA kit (6A8/4C1), and mite group 2 ELISA kit (1D8/7A1) INDOOR Biotechnologies (Chester, U. K.), according to Luczynska et al. (1989).

A Nunc Maxisorp immunoplate (Nunc, Roskilde, Denmark) was coated with 1 μ g per well of Mab 5H8 (anti-Der p 1, 10 mg/ml), Mab 6A8 (anti-Der f 1, 10 mg/ml), and Mab 1D8 (anti-Der 2, 10 mg/ml) in 50 mM sodium bicarbonate buffer at pH 9.6 overnight at 4°C. The plate was then washed 3 times with phosphate buffer saline containing 0.05% of Tween 20 (pH 7.4) and treated for 1 h at room temperature with 100 μ l per well of the same solution containing 1% of bovine serum albumin (Sigma A-7030, Sigma, St. Louis, MO). Wells were incubated for 1 h at room temperature with 100 μ l of diluted extracts (from 1 to 10^{-5} mg/ml) using double dilutions of a reference antigen (from 250 to 3.9 ng). After washing 5 times, the wells were incubated for 1 h at room temperature with 100 μ l of a 1:1000 dilution of biotinylated Mab 4C1 (anti-Der 1, 10 mg/ml), 1:3000 dilution of biotinylated Mab 7A1 (anti-Der 2, 10 mg/ml) for Der p 2 assay, and 1:1000 for Der f 2 assay. The plate was then washed 5 more times and wells were incubated for 30 min with 1:1000 streptavidin-peroxidase (Sigma, S-5512; 0.25 mg of protein was dissolved in 1 ml of distilled water). Finally, the assays were developed by adding 100 μ l per well of 1 mM ABTS [2,2'-azino-di (3 ethylbenzthiazoline sul-

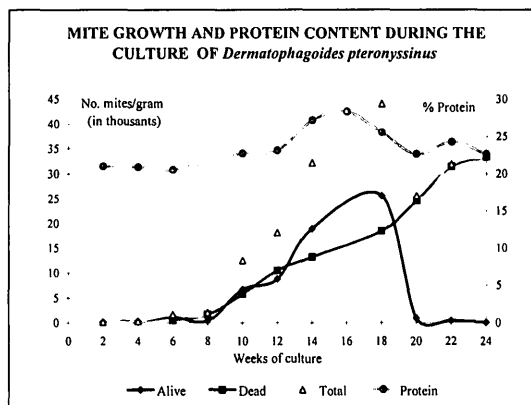


Fig. 1. Mite growth and protein content during *D. pteronyssinus* culturing.

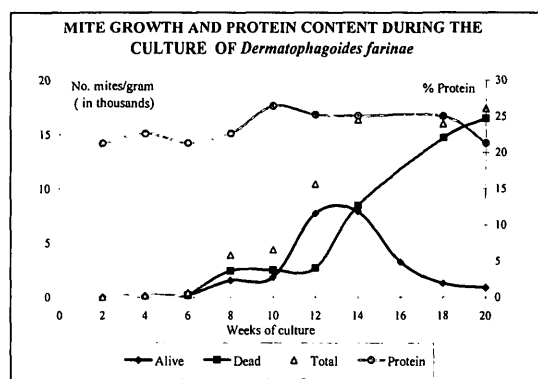


Fig. 2. Mite growth and protein content during *D. farinae* culturing.

fonic acid)] (Sigma, A-1888) and 0.03% hydrogen peroxide in 70 mM citrate-phosphate buffer (pH 4.2). Absorbance was monitored at 414 nm by an ELISA microplate reader.

Results

The growth pattern of *D. pteronyssinus* and *D. farinae* during a 20- to 24-wk period, and its correlation with the protein content of the extracts obtained every 2 wk are shown in Figs. 1 and 2, respectively.

The growth pattern of *D. pteronyssinus* clearly shows 3 phases according to the number of existing live mites: a 1st latency phase (weeks 0–8), a 2nd exponential phase (weeks 10–18), and a 3rd drop stage (weeks 20–24) (Fig. 1). Similarly, the growth pattern of *D. farinae* also exhibits a 1st latency stage, but it differs from the former species because the 2nd phase is shorter and lasts until week 14, and the 3rd phase lasts until week 20 (Fig. 2).

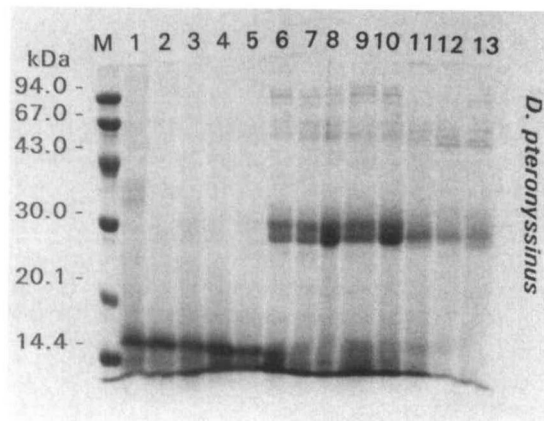


Fig. 3. SDS-PAGE *D. pteronyssinus*. Lane 1, culture medium extract; lane 2, week-2 culture extract; lane 3, week 4; lane 4, week 6; lane 5, week 8; lane 6, week 10; lane 7, week 12; lane 8, week 14; lane 9, week 16; lane 10, week 18; lane 11, week 20; lane 12, week 22; lane 13, week 24. M, molecular weight markers.

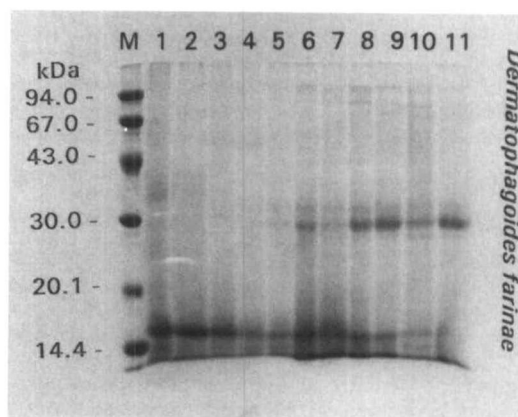


Fig. 4. SDS-PAGE *D. farinae*. Lane 1, culture medium extract; lane 2, week-2 culture extract; lane 3, week 4; lane 4, week 6; lane 5, week 8; lane 6, week 10; lane 7, week 12; lane 8, week 14; lane 9, week 16; lane 10, week 18; lane 11, week 20. M, molecular weight markers.

The protein profiles of the extracts obtained from *D. pteronyssinus* and *D. farinae* at different times during rearing, and achieved by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE), are shown in Figs. 3 and 4, respectively.

These profiles show differences for the extracts obtained at various intervals during mite culturing and growth. In the initial phase, 5–6 bands were identified by SDS-PAGE, 2 of which of 15 and 17 kDa may be related to the culture medium.

From the 8th wk onward, some of these bands decreased in intensity, and new bands of 28–32 kDa and 60–100 kDa began to appear. In *D. pteronyssinus* culture, throughout weeks 10, 12, 14, 16, and 18, bands began to show: 3 bands of high molecular mass, 109, 100, and 90 kDa; 2 bands of 70 and 60 kDa; and prominent bands of 30 kDa, which may be related to the complex Der p 1 (Fig. 3). Two low molecular weight bands of 14 and 15 kDa, respectively, were also observed. Throughout weeks 20, 22, and 24, the high molecular weight components of 90, 100, and 110 kDa disappeared or showed less density, however, a group of 3–4 components of 50–70 kDa appeared. The 28-kDa bands can be seen clearly during this 3rd phase of growth.

The SDS-PAGE profile of extracts from cultures of *D. farinae* is shown in Fig. 4. The profile corresponding to the 1st stage is similar to the *D. pteronyssinus*. On the 8th wk, several components started to show and these were clearly observed during the 10- to 16-wk period, when SDS-PAGE revealed a group of components of 90–110 kDa, which remained in the culture until week 18. During the growth period, the culture also produced various other components of 40–70 kDa, and 2–3 low molecular weight bands of 14–16 kDa. The most prominent bands were revealed at 27 and 32 kDa and may

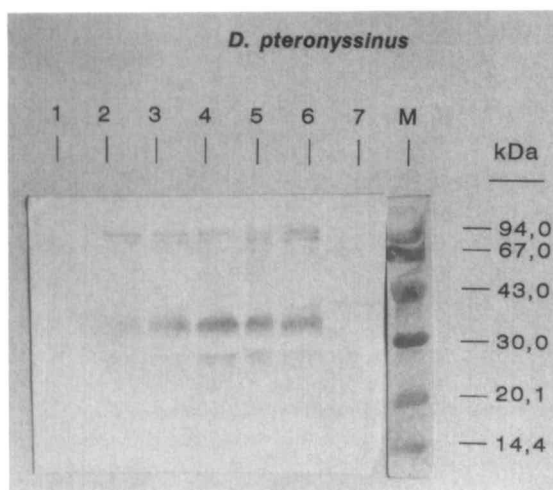


Fig. 5. SDS-PAGE immunoblotting. Lane 1, *D. pteronyssinus* week-8 culture extract; lane 2, week 10; lane 3, week 12; lane 4, week 14; lane 5, week 16; lane 6, week 18; lane 7, week 20. M, molecular weight markers on the immobilon membrane (stained with amido black).

be related to Der f 1. The protein components of 48–104 kDa showed less density during the 20th wk.

SDS-PAGE IgE-immunoblotting of extracts from *D. pteronyssinus* is represented in Fig. 5. This method demonstrated that during the exponential growth stage, the IgE binding proteins present their highest expression, including the components with molecular weight corresponding to the major allergens. The allergens of 28–39 kDa and 95–100 kDa, including the relevant ones, disappeared during the 3rd stage.

SDS-PAGE IgE-immunoblotting of extracts from *D. farinae* is shown in Fig. 6. It can be observed that

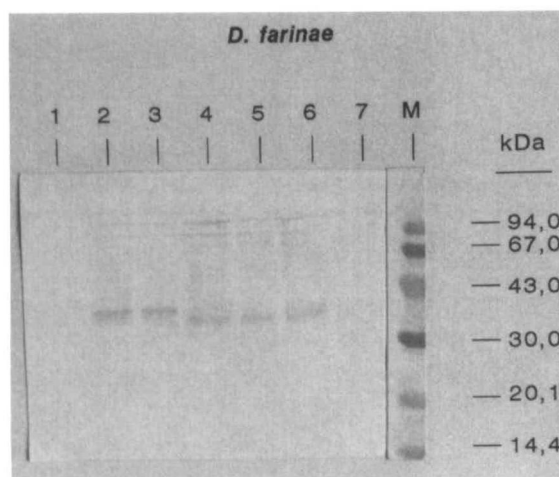


Fig. 6. SDS-PAGE immunoblotting. Lane 1, *D. farinae* week-8 culture extract; lane 2, week 10; lane 3, week 12; lane 4, week 14; lane 5, week 16; lane 6, week 18; lane 7, week 20. M, molecular weight markers on the immobilon membrane (stained with amido black).

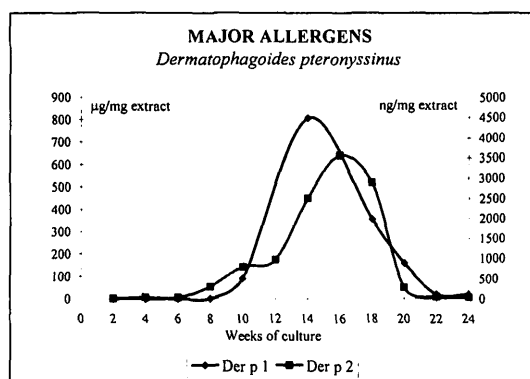


Fig. 7. Der p 1 and Der p 2 levels in *D. pteronyssinus* allergenic extracts.

IgE-binding proteins appeared in the culture on the 10th wk, whereas the allergenic components show their higher density during the 14- to 18-wk period. The allergens of 35 and 60–100 kDa disappeared during week 20.

The IgE-immunoblotting of the extract from the culture media revealed no bands.

The kinetics of the concentrations of group I and II allergens of *D. pteronyssinus* and *D. farinae* in culture extracts are represented in Figs. 7 and 8, respectively.

The concentrations of Der 1 and Der 2 are low in the extracts obtained from the initial phase of cultures. After that, the values increase to achieve a maximum during the 14- to 16-wk period and then fall gradually.

Discussion

In our culturing conditions, the mite-growth pattern presented an initial latency period of 6–8 wk, followed by an exponential growth stage that varied depending on the species. Thus, *D. pteronyssinus* reached their highest growth rate in the 18th wk, whereas *D. farinae* did the same in the 14th wk. The

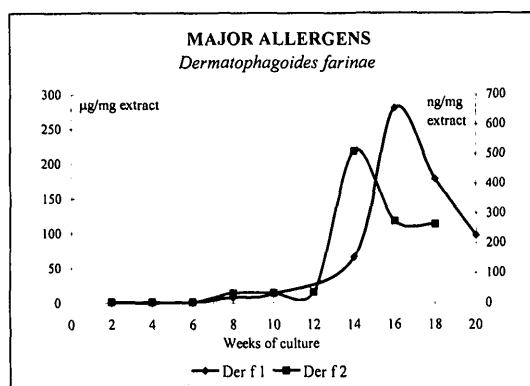


Fig. 8. Der f 1 and Der f 2 levels in *D. farinae* allergenic extracts.

next stage was characterized by a fall in the live mite population of both species, this being near to 0 by the 20th wk, unless culture medium was replaced.

The protein content obtained from various samples increased to a maximum point at 14–18 wk for *D. pteronyssinus* and at 10–14 wk for *D. farinae*. Afterward, it decreased.

SDS-PAGE techniques of the different extracts show 3 stages for both species. During the 1st stage or latency period (0–8 wk), the existing major components are originated by the culture medium. During the 2nd stage, an exponential growth takes place during the 8- to 10-wk period, and the characteristic proteins for each species appear. Major allergens were identified in these proteins: *D. pteronyssinus* showed few bands, mainly around 30 kDa and others of higher molecular weight (60–90 kDa), and *D. farinae* showed a greater number of bands distributed within the whole molecular weight range, but with higher density at 30 kDa. Finally, during the 3rd stage or death period (4 wk), the characteristic components appeared as well but in smaller numbers and with lower density.

In both species, allergens are shown strongly during the maximum growth stage.

In regard to the mite population dynamics, the initial growth is sigmoidal as described for *D. pteronyssinus* by Andersen (1988). Nevertheless, Andersen also described fluctuations in the mite population density during the growth period, which in this case was continuous, because new culture medium was added at all times. In this aspect, our results differ partially from those of Andersen.

There are various studies on the allergenic composition of extracts from *D. pteronyssinus* and *D. farinae* cultures. Thus, by crossed radioimmuno-electrophoresis, >19 allergens were determined (Lind and Löwenstein 1983, Krilis et al. 1984, Stewart et al. 1986), whereas by immunoblotting performed after SDS-PAGE up to 32 components could be described as allergenic (Tovey and Baldo 1987).

The results obtained by the aforementioned technique show that during the 8th wk of growth of *D. pteronyssinus*, the proteinic components were present in minimum quantities showing almost no allergenic activity (Fig. 3). During the 10- to 18th-wk period a constant proteinic pattern was determined by SDS-PAGE, similar to that obtained by IgE-immunoblotting. From week 20 onward, both proteinic and allergenic patterns varied, and although there was a variety of proteinic bands, the number of IgE binding components decreased drastically.

For *D. farinae* extracts, beginning on the 8th wk, the proteinic components start to differentiate clearly and to show their allergenic activities (Fig. 6). During the 10- to 18-wk period a more or less constant pattern for the mite proteinic components appears, which is in accordance with similar patterns of the IgE-binding components, although the latter appear with less intensity, which is probably

the result of their smaller relative concentration (Figs. 2 and 6).

In both species studied, allergens were present with greater intensity during the maximum growth stage. This fact coincides with the results obtained by Andersen (1991), who found that the allergenic activity of the extracts obtained from mite cultures at different growth stages correlated positively with the duration of the growth period.

In the current study, the presence and relative concentration of the major allergens of *D. pteronyssinus* and *D. farinae* during the culturing period was determined by enzyme immunoassay with 2 monoclonal antibodies (Figs. 7 and 8). It was observed that for *D. pteronyssinus* the presence and concentration of these allergens were in accordance with the mite population growth pattern, thus presenting a maximum relative concentration of Der p 1 and Der p 2 at the exponential growth stage (weeks 14 and 16). The decrease in the relative concentration of the major allergens coincided with the period where mortality is high (Fig. 7). For *D. farinae* the maximum relative concentration of Der f 1 and Der f 2 coincided with the maximum growth of the culture and the beginning of the decrease of the mite population, although the above-mentioned allergens started to appear around the 8th wk, at the beginning of the exponential growth stage (Fig. 8).

No studies have been published on the evolution of the composition of the major allergens in relation to mite culture growth. In addition, the relationship between the clinical effectiveness, either diagnostic or therapeutical, and the proportion of group I and II allergens has not yet been determined.

The extracts of *Dermatophagoides* obtained during phase II of growth seem to present the greater allergenic and antigenic diversity, as well as the highest concentration of major allergens. Therefore, they can be considered adequate for the diagnostic and therapeutical techniques that exist today.

Acknowledgments

This research was supported by a fellowship from the Spanish Ministry of Education and Science and a grant from the Department of Education, Universities and Research of the Basque Government, Spain (project PI9305).

References Cited

- Andersen, A. 1988. Population growth and development stages of the house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J. Med. Entomol. 25: 370–373.
- 1991. Nutritional value of yeast for *Dermatophagoides pteronyssinus* (Acari: Epidermoptidae) and the antigenic and allergenic composition of extracts during extended culturing. J. Med. Entomol. 28: 487–491.
- Ando, T., R. Homma, Y. Ino, G. Ito, A. Miyahara, T. Yanagihara, H. Kimura, S. Ikeda, H. Yamakawa, M. Iwaki, Y. Okumura, M. Suko, M. Haida, and H. Okudaira. 1993. Trypsin-like protease of mites: purification and char-

- acterization of trypsin-like protease from mite faecal extract *Dermatophagoides farinae*. Relationship between trypsin-like protease and *Der f* III. Clin. Exp. Allergy 23: 777-784.
- Chapman, M. D., and T.A.E. Platts-Mills. 1980. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P₁. J. Immunol. 125: 587-592.
- Cooke, R. A. 1922. Studies on specific hypersensitivity IV. New etiologic factors in bronchial asthma. J. Immunol. 7: 147.
- Fain, A., B. Guérin, and B. J. Hart. 1990. Mites and allergic diseases. Allerbio, Varennes en Argonne, France.
- Heymann, P. W., M. D. Chapman, R. C. Aalberse, J. W. Fox, and T.A.E. Platts-Mills. 1989. Antigenic and structural analysis of group II allergens (*Der f* II and *Der p* II) from house dust mites (*Dermatophagoides* spp.). J. Allergy Clin. Immunol. 83: 1055-1067.
- Kern, A. 1921. Dust sensitization in bronchial asthma. Med. Clin. North Am. 5: 751-758.
- Krillis, S., B. A. Baldo, and A. Basten. 1984. Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. Part II. Identification of the major IgE-binding antigens by crossed radioimmuno-electrophoresis. J. Allergy Clin. Immunol. 74: 142-146.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. Nature (Lond.) 227: 680-685.
- Lake, F. R., L. D. Ward, R. J. Simpson, P. J. Thompson, and G. A. Stewart. 1991. House dust mite-derived amylase: allergenicity and physicochemical characterization. J. Allergy Clin. Immunol. 87: 1035-1042.
- Le Mao, J., J. P. Dandeu, J. Rabillon, M. Lux, and B. David. 1981. Antigens and allergens in *Dermatophagoides farinae* mite. I. Immunochemical and physicochemical study of two allergenic fractions from a partially-purified *Dermatophagoides farinae* mite extract. Immunology 44: 239-247.
- Lind, P., and H. Lowenstein. 1983. Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmuno-electrophoresis with two different rabbit antibody pools. Scand. J. Immunol. 17: 263-273.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.
- Luczynska, C. M., L. K. Arruda, T.A.E. Platts-Mills, J. D. Miller, M. López, and M. D. Chapman. 1989. A two-site monoclonal antibody ELISA for the quantitation of the major *Dermatophagoides* spp. allergens, *Der p* I and *Der f* I. J. Immunol. Methods 118: 227-235.
- Miyamoto, T., S. Oshima, T. Ishizaka, and S. Sato. 1968. Allergenic identity between the common floor mite (*Dermatophagoides farinae* Hughes, 1961) and house dust as a causative antigen in bronchial asthma. J. Allergy 42: 14-28.
- Platts-Mills, T.A.E., W. R. Thomas, R. C. Aalberse, D. Vervloet, and M. D. Chapman. 1992. Dust mite allergens and asthma: report of a second international workshop. J. Allergy Clin. Immunol. 89: 1046-1060.
- Portus, M. 1975. Estudio de la acarofauna del polvo doméstico y su relación con las atopias humanas. Ph.D. dissertation, University of Barcelona.
- Shen, H. D., S. R. Wang, R. B. Tang, F.Z.N. Chang, S. N. Su, and S. N. Han. 1988. Identification of allergens and antigens of Bermuda-grass pollen by immunoblot analysis. Clin. Allergy 18: 401-410.
- Shen, H. D., K. Y. Chua, K. L. Lin, K. H. Hsieh, and W. R. Thomas. 1993. Molecular cloning of a house dust mite allergen with common antibody binding specificities with a multiple components in mite extracts. Clin. Exp. Allergy 23: 934-940.
- Stewart, G. A., A. Butcher, K. Lees, and J. Ackland. 1986. Immunochemical and enzymatic analyses of the house dust mite *Dermatophagoides pteronyssinus*. J. Allergy Clin. Immunol. 77: 14-24.
- Stewart, G. A., L. D. Ward, R. J. Simpson, and P. J. Thompson. 1992. The group III allergen from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. Immunology 75: 29-35.
- Storm van Leeuwen, W. 1922. Diagnosis and treatment of allergic diseases. Neurotherapie 6:1.
- Tovey, E. R., and B. A. Baldo. 1987. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. J. Allergy Clin. Immunol. 79: 93-102.
- Tovey, E. R., M. D. Chapman, and T.A.E. Platts-Mills. 1981. Mite faeces are a major source of house dust allergens. Nature (Lond.) 289: 592-593.
- Tovey, E. R., M. C. Johnson, A. L. Roche, G. S. Cobon, and B. A. Baldo. 1989. Cloning and sequencing of a cDNA expressing a recombinant house dust mite protein that binds human IgE and corresponds to an important low molecular weight allergen. J. Exp. Med. 170: 1457-1462.
- Towbin, H., I. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrilamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 76: 4350-4354.
- Voorhorst, R., M.I.A. Spiekma-Boezeman, and F. Th. M. Spiekma. 1964. Is a mite (*Dermatophagoides* sp.) the producer of the house dust allergen? Allerg. Asthma 10: 329-334.
- Voorhorst, R., F. Th. M. Spiekma, and H. Varekamp. 1969. House dust atopy and the house dust mite *Dermatophagoides pteronyssinus* (Trouessart, 1897). Stafleu, Leiden, The Netherlands.
- Yasueda, H., H. Mita, K. Akiyama, T. Shida, T. Ando, S. Sugiyama, and H. Yamakawa. 1993. Allergens from *Dermatophagoides* mites with chymotryptic activity. Clin. Exp. Allergy 23: 384-390.

Received 30 December 1996; accepted 3 June 1997.