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The Major Allergen of the House Dust Mite, *Dermatophagoides pteronyssinus*, Is Synthesized and Secreted into Its Alimentary Canal

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Abstract. The natural production of *Der p1*, the major faecal allergen from the house dust mite (*Dermatophagoides pteronyssinus*), was investigated. Mite cultures grown on radiolabelled substrate were examined at intervals over a period of 21 days and incorporation of radiolabel into the mite proteins measured over the time course. Mite proteins were extracted, purified by chromatofocussing and characterized by immunodiffusion, SDS-PAGE, electroblotting and autoradiography. The faecal allergen, *Der p1*, did not incorporate a significant level of label until the 14th day ($p < 0.05$). The results suggest that *Der p1* is a protein synthesized and excreted by the house dust mite.

Introduction

Two major allergens and a number of minor allergenic proteins have been identified from house dust mites. Several have been purified and a start made in the process of characterizing their clinical importance. In 1980, Chapman and Platts-Mills purified a clinically relevant allergen now known as *Der p1* from *Dermatophagoides pteronyssinus*, the acarid mite most relevant to atopic disease in England [Warner, 1978]. This allergen is found in high concentration in the mite faeces [Tovey et al., 1981]. *Der p1* is a highly water-soluble protein with a molecular weight of 24,000 daltons. They found that approximately 75% of the serum IgE antibodies to mites in their (adult) mite-sensitive patients were directed against this component [Tovey et al., 1981] and accordingly it can be classified as a major allergen. Lind [1985] has also described the purification of *Der p1* and, in addition, purified another allergen originally named DpX from aqueous extracts of mite bodies, but since renamed *Der p11*. Immunochemical studies have shown this antigen to be chemically different from *Der p1*.

Little is known about the site or mechanism of production of *Der p1* within the mite. It could be present in the faeces either as a by-product of enzymatic digestive processes in the alimentary canal, or as a result of synthesis by the gastrointestinal or excretory system and its subsequent secretion into the alimentary

canal from which the faecal pellets emerge. The following experiments were devised to differentiate between these possibilities.

Materials and Methods

Mite Cultures

Pure cultures of *D. pteronyssinus* are routinely grown in our laboratory on sterile liver powder (Oxoid Ltd.) [Solomon and Cunningham, 1964]. Adult mites were transferred to bovine serum albumin (BSA) as growth media. After several transfers through fresh BSA 98-99% (Sigma Ltd.), the mites were growing well on this food source, and residual liver powder was negligible, being totally replaced by BSA. 15- μ g aliquots of BSA were radiolabelled with 1 mCi of ^{125} I (Amersham Ltd.) by the chloramine T technique [Klinman and Taylor, 1969]. 94% of the activity could be removed by precipitation with trichloroacetic acid (TCA). Small quantities (2 mCi) of radiolabelled BSA were introduced into the cultures. The incorporation of this radioactive marker into mite proteins was examined by purifying the mite proteins from the cultures at 1, 3, 7, 14 and 21 days after the introduction of ^{125} I BSA. The proteins were separated by chromatofocussing and characterized by double immunodiffusion against polyclonal rabbit antisera by the Ouchterlony [1958] method, SDS-PAGE, electroblotting and autoradiography. As a control against nonspecific uptake of radiolabel, ^{125} I BSA was added to a freshly prepared aqueous extract of whole mites. The incorporation of the radioactive marker was also examined in these purified mite proteins over a similar time course.

Chromatofocussing of Mite Culture

Chromatofocussing was performed at room temperature according to the manufacturer's instructions (Pharmacia Ltd.). Briefly, 5 g of whole culture was extracted in 5 ml of 0.025 M imida-

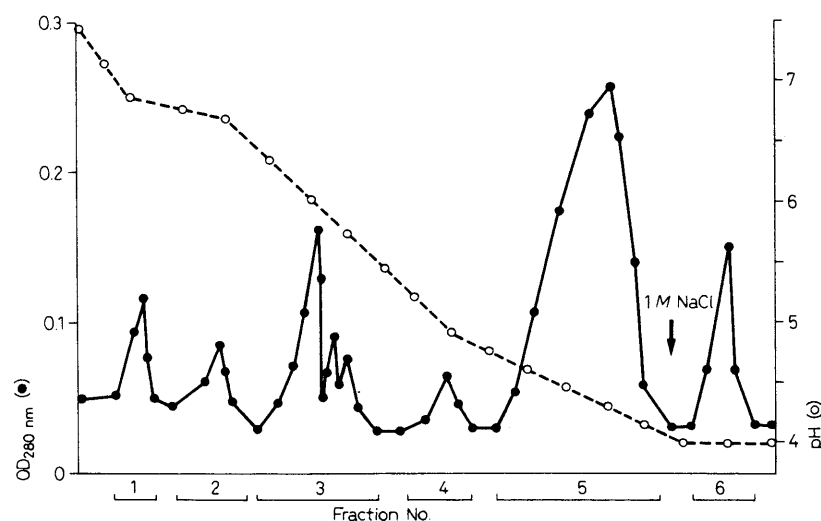


Fig. 1. Chromatofocussed fractions of whole mite culture. The pH and optical density of each sample is shown. Protein peaks were pooled as indicated.

zole buffer pH 7.4; the solution was clarified by centrifugation in a high-speed microcentrifuge for 5 min. 2 ml of the solution was then applied to a 1.0×20 cm column of PBE 94 ion exchange resin equilibrated in the same buffer. Proteins were eluted over the pH range 4–7 using Polybuffer 74 adjusted to pH 4 with HCl. The buffer was pumped at a flow rate of 8 ml/h and 1-ml fractions were collected. Protein elution was monitored by measuring the absorption of the column eluent at 280 nm and the pH profile was constructed by determining the pH of alternate fractions.

Rabbit Antisera

Two NZW rabbits were injected intramuscularly every 2 weeks with 1-mg preparations of *D. pteronyssinus* mite body extract or *Der p1* in 1 ml of PBS pH 7 containing Freund's complete adjuvant. The whole mite body extract had been repeatedly immunoabsorbed to remove *Der p1* cross-reactive proteins as previously described [Carswell and Thompson, 1986]. The IgG fraction was purified by ion exchange chromatography and stored frozen at -20°C until required [Boehringer Mannheim Biochemica, 1985]. The specificities of the antisera raised were clearly distinct from each other when used in inhibition radioimmunoassay studies [Carswell and Thompson, 1987].

Murine Monoclonal Antibodies

Mouse monoclonal antibody to mite allergen *Der p1* was kindly provided by Dr. P. Lind, Copenhagen, Denmark. Anti-DpX monoclonal (*Der pII*) was supplied by Dr. R. Aalberse, Amsterdam, The Netherlands. Both monoclonals were radiolabelled with ^{125}I by the chloramine T technique [Klinman and Taylor, 1969] and used for probing of the nitrocellulose membranes.

Gel Electrophoresis

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis was carried out following the method of Laemmli [1970]. 200 μl aliquots of the aqueous house dust mite extract along with the chromatofocussed fractions, treated with mercaptoethanol, were separated using a 15% running gel and a 5% stacking gel. Electrophoresis was carried out at 330 V for 30 min. Proteins on the gels were stained with Coomassie brilliant blue R-250 and the molecular weight of the separated proteins were calculated using the low molecular weight electrophoresis calibration kit from LKB

Ltd. Duplicate gels were equilibrated for 30 min in Tris/glycine transfer blot buffer to allow protein renaturation and removal of the electrophoretic buffer from the gel prior to Western blotting.

Electrophoretic Transfer of Proteins to Nitrocellulose

Protein bands were transferred to nitrocellulose (trans-blot 0.45 μm , Biorad Ltd.) using a modification of the method of Towbin et al. [1979] as described by Sutton et al. [1985] using a Biorad trans-blot cell according to the manufacturer's instructions for 3 h at 70 V. During this procedure, the contents of the cell were continuously stirred to assist cooling of the transfer buffer (0.025 M Tris, 0.192 glycine, pH 8.3, 20% v/v methanol). Nitrocellulose membranes were stained with amido black (0.15% in water, methanol, acetic acid, 65:25:10) for 5 min to visualize the transferred proteins. Duplicate blots were washed for 1 h in 10% methanol in phosphate-buffered saline (PBS, pH 7), followed by immersion in 0.1% Tween 20 in PBS at 4°C to block unbound membrane sites [Batteiger et al., 1982]. All nitrocellulose membranes were air dried and stored at 4°C .

Immunological Detection

Immunodiffusion. The technique of Ouchterlony [1958] was followed. The chromatofocussed fractions were tested against the polyclonal rabbit antisera in a Petri dish using a 1.5-mm layer of 1% Agar-Noble (Difco Labs.) in PBS, pH 7.0.

Probing of the Nitrocellulose Membranes with Monoclonal Antibodies. The radiolabelled monoclonal antibodies against *Der p1* and *Der pII* allergens were diluted with PBS to a specific activity of approximately 50,000 cpm/ml. Nitrocellulose membranes were incubated with these solutions for 24 h. This directly detected the protein bands in the separated fractions corresponding to the *Der p1* and *Der pII* allergens. After extensive washing (1 h) with 0.5% Tween 20 PBS, the nitrocellulose membranes were air dried at room temperature and then loaded into a Kenex cassette with high-speed intensifying screens (both from Genetic Research Instrumentation Ltd.) together with photographic film (Fuji RX Safety) for 3–10 days prior to development.

Incorporation of ^{125}I Label. 0.5 ml of the separated and characterized proteins were counted on a LKB rack gamma counter at 1, 3, 7, 14 and 21 days after the introduction ^{125}I BSA. The samples were counted in triplicate and the coefficient of variation of 20 re-

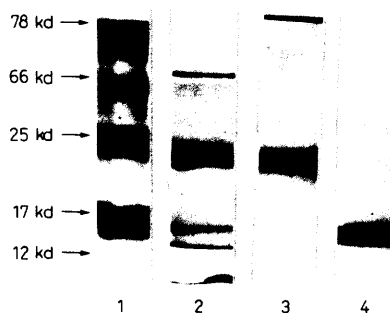


Fig. 2. SDS-PAGE and autoradiographic analysis. Tracks: 1 = MW markers (stained with Coomassie brilliant blue); 2 = whole mite culture (stained with Coomassie brilliant blue); 3 = chromatofocussed fraction 3: nitrocellulose blot probed with monoclonal anti-*Der p1*; 4 = chromatofocussed fraction 4: nitrocellulose blot probed with monoclonal anti-DpX (*Der pII*).

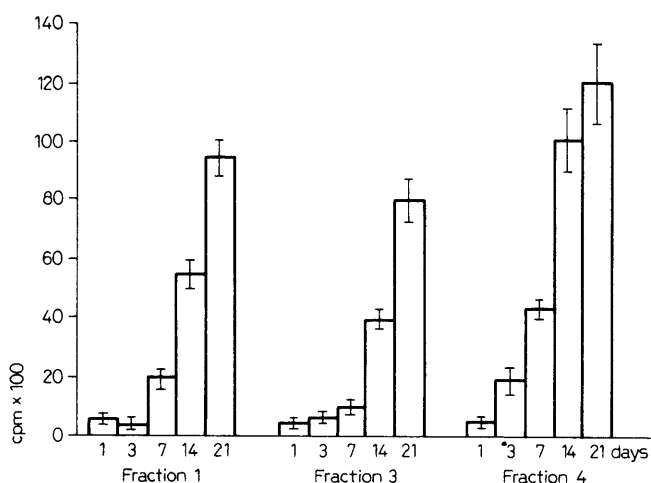


Fig. 3. The incorporation of ^{125}I label into the characterized mite proteins over the stated time course. Mean $\pm$ SE values shown. Fractions 1 and 4 are proteins derived from mite body. Fraction 3 is allergen *Der p1*.

plicate measurements of fraction 3 at 14 days was 2%. There was no significant incorporation of radioactivity, over a similar time course, into the *Der p1* or mite body proteins when radiolabeled BSA was mixed with aqueous mite extracts.

Results

The experiment was repeated on three separate occasions and the results were not significantly different.

Separation of Mite Proteins

The chromatofocussed elution profile of a whole culture is illustrated in figure 1. Fractions 1, 2, 3, 4

and 6 are house dust mite proteins: fraction 5 is the BSA substrate. On immunodiffusion of chromatofocussed eluates, fraction 3 formed a densely staining precipitate with anti-*Der p1* antisera. This was identical to the precipitin line formed by *Der p1*. A faint precipitin line was also produced by fraction 2 with this antisera. Similarly, fractions 1 and 4 reacted with anti-mite-body antisera.

SDS Gel Electrophoresis and Transfer of Separated Mite Components to Nitrocellulose

The capacity of polyacrylamide gel electrophoresis to separate components of a mite extract is demonstrated in figure 2, track 2, where at least 15 different protein bands could be seen on staining with Coomassie brilliant blue. SDS-PAGE analysis showed fractions 2 and 3 to be electrophoretically similar and contained material with apparent molecular weights of 24,000 daltons. The other chromatofocussed fractions were of different electrophoretic mobility. The mite body protein present in fraction 4 had a molecular weight of 15,000 daltons. After electrophoretic transfer to nitrocellulose, probing with the monoclonals anti-*Der p1* and anti-*Der pII* demonstrated that these allergens were primarily associated with the chromatofocussed fractions 3 and 4 respectively (fig. 2, tracks 3 and 4).

Incorporation of ^{125}I Label

Figure 3 illustrates the incorporation of the radiolabel into the mite proteins present in fractions 1, 3 and 4. The results are the mean of the three separate replicate experiments. The two proteins (1 and 4) associated with the body of the mite acquired significant radioactivity ($p < 0.05$, Fisher's test) from the 7th day, whereas the activity in the *Der p1* protein peak (fraction 3) did not attain significant radioactivity until the 14th day ($p < 0.05$).

Discussion

Iodination of proteins by the chloramine T technique preferentially labels tyrosine molecules. Therefore, the increase in radiolabel was probably due to the incorporation of iodotyrosine residues into the mite proteins. Although this implies that tyrosine residues are present in the allergen *Der p1*, there is no evidence that this amino acid is particularly important for the protein's allergenic nature.

The present study was designed to answer the question: Is *Der p1* a by-product of food digestion or a synthesized secreted protein?

From the first hypothesis that *Der p1* is directly produced by digestion of food, it would be expected to accumulate rapidly in the faecal particles. As the mites' life cycle is approximately 25 days [Voorhorst et al., 1964] it would be predicted that the turnover from substrate to faecal matter would occur rapidly. From the alternative hypothesis that *Der p1* is a mite-synthesized protein which is secreted into the mite's alimentary canal, it would be predicted that digestion, absorption, synthesis and secretion of the allergen into faecal matter would take considerably longer. The fact that homologous mite allergen has been purified by different laboratories, from mites cultured on different substrates including bovine liver powder [Carswell and Thompson, 1986], human dander and yeasts [Spieksma, 1970], supports the second theory. This present study also suggests that *Der p1* is a protein synthesized by the mite.

The site of production of *Der p1* within the mite is unknown although Stewart [1980] was able to demonstrate that fluorescent labelled polyclonal antisera against *Der p1* stained the stomach wall of the mite. The factors controlling its synthesis/output are completely unknown although it would be predicted that as with other secreted products, some variation would occur naturally. Further investigation of the natural process of synthesis/secretion is necessary because of the extensive occurrence of mite allergen in atopic disease [Warner and Price, 1978; Carswell and Thompson, 1987] and the failure to remove the mite by simple cleaning methods [Carswell et al., 1986].

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