Mite faeces are a major source of house dust allergens

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The association between house dust allergy and asthma has long been recognized, and it has been demonstrated that a major allergen in house dust is related to the presence of mites of the genus *Dermatophagoides*[1]. Using extracts of mite culture for skin testing, as many as 10% of the population and up to 90% of allergic asthmatics give positive immediate reactions[2]. Although mites may occasionally become airborne during bed-making[3], it has also been demonstrated that they 'secrete or excrete' some allergen[1]. Recently, we have shown that up to three-quarters of the serum IgE antibodies to mites are directed against a major allergen—antigen P_1 (molecular weight 24,000)[4]. Using a radioimmunoassay it is possible to measure the concentration of this glycoprotein in both dust samples and mite cultures. These measurements, which are reported here, show that more than 95% of the allergen accumulating in mite cultures is associated with faecal particles.

When the components of mite culture were separated, whole mites, cuticles and mite faeces contained antigen P_1 but eggs contained very little (Table 1). The number of faecal particles in cultures seemed to be very high from direct microscopy. Using a mean value of 20 faeces per day, we estimated that after 1 month faeces would account for >95% of the antigen P_1 in cultures. These particles are roughly spherical and have a relatively smooth surface (Fig. 1). They are 10–40 μm in diameter and their size is directly proportional to the size of the mites producing them, which vary in length from 170 to 350 μm ($r = 0.91$, $P < 0.001$). The antigen P_1 content of faecal particles, mite culture or house dust elutes very rapidly in saline, whereas the allergen content of live mites is only very slowly eluted (Fig. 2).

Table 1 Quantity of antigen P_1 in mite culture components

Culture components		ng antigen P_1 per 100 components	% Of total antigen P_1
Mites	Female	185	0.6
	Male	105	
	Immature	75	
Cuticles		29	0.4
Eggs		0.4	–
Faeces	Mixed size (22 ± 6 μm)	12	99
	Large (31 ± 8 μm)	17	
	Small (17 ± 4 μm)	3	

Antigen P_1 content of separated components of mite culture. Live mites (Bencard) were grown on Oxoid liver powder for 1 week at 24 °C and 75% relative humidity. Individual components were separated, homogenized, extracted with borate-buffered saline and assayed for P_1 content by double antibody inhibition radioimmunoassay[4]. The contribution of the components to the total P_1 content of culture is based on a mean lifetime for a mite of 3 months involving three changes of cuticle and an estimated rate of defaecation of 20 faeces per day. Two methods were used to measure the rate of defaecation. When the mean time for the passage of coloured food through the mite gut (5 h) was multiplied by the mean number of faeces produced by an isolated fully fed mite (mean 8 faeces, range 6–11), a rate of 40 faeces per mite per day was obtained. Alternatively, counting the number of faeces produced by mites fed on large washed lumps of culture media after 6 days in culture gave a mean rate of defaecation of only 6 faeces per day in these suboptimal conditions. Faecal sizes are means ± s.d.

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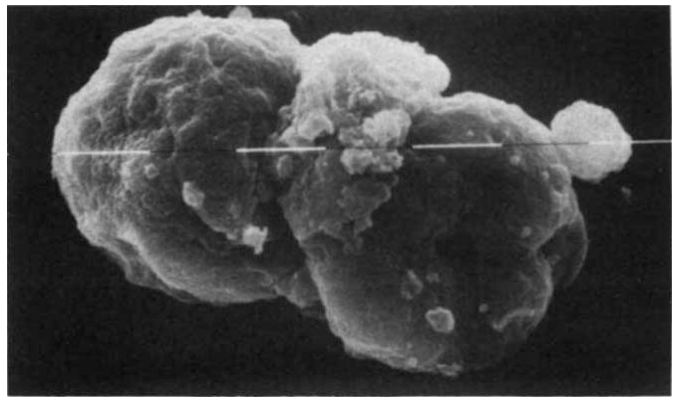

Fig. 1 Electron scanning micrograph of two mite faecal balls. Mite faeces range from 10 to 40 μm in diameter with a mean of 22 ± 6 μm s.d. For a similar species it has been reported[9] that the faeces are produced by compacting three to five foodballs covered in a peritrophic membrane. Scale bar, 10 μm.

As most particles deposited on the nasal mucosa are swallowed within 10 min, rapid elution of proteins is probably essential for allergenic activity[5]. It is not uncommon for house dust samples to contain over 1,000 mites and 30 μg of antigen P_1 per gramme. Based on the values in Table 1, such samples probably contain over 250,000 faeces per gramme of dust. Our further studies have shown that antigen P_1 is only present in the air of houses during domestic activity and this allergen is associated with particles >10 μm in diameter[6]. In general, particles of this size would be deposited on the mucosa of the nose and oropharynx and only occasional particles would be expected to enter the lungs[7]. In keeping with this, most dust-allergic asthmatic patients are not aware of a direct relationship between dust exposure and the onset of bronchospasm. The concentration of allergen in mite faeces is very high, approximately 10 mg antigen P_1 per ml, assuming 0.1 ng of allergen in a sphere of 20 μm diameter. These particles, whose physical properties are similar to those of pollen grains, would seem to be a very effective way of carrying proteins to the nasal mucosa. In addition, the few particles entering the lungs would be expected to cause localized inflammatory responses because of the high concentrations of allergen[8]. Our results suggest that the relationship between dust mite allergy and bronchial asthma is best

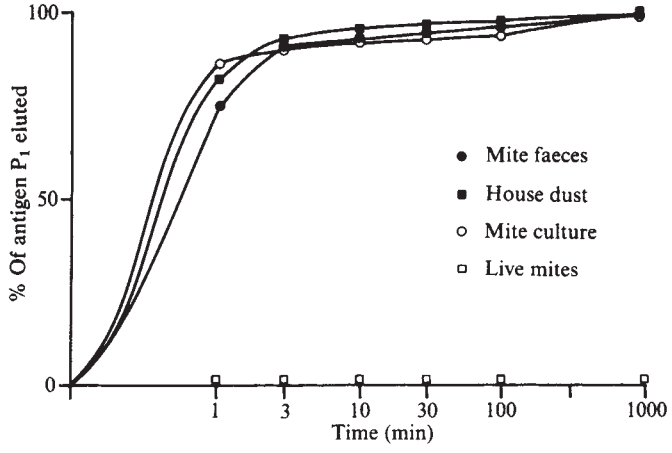

Fig. 2 The elution of antigen P_1 from faeces, live mites, house dust and culture material. Mite faeces or live mites mounted on Cellotape (1 × 5 mm) were contained in a section of capillary tubing and eluted with saline using a peristaltic pump (25 μl min^{-1}). At the end of the elution period 11 of the 60 mites were still mobile and none was observed to defaecate during the elution. Samples of house dust and culture material were repeatedly eluted in filter funnels under vacuum (30 ml, Scinterglass 4, 5 μm pore size). With house dust, culture material and faeces, the quantities of P_1 are expressed as a % of the total eluting over 16 h. With live mites the quantity of P_1 is expressed as a percentage of the quantity obtained by homogenizing the mites.

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explained if inflammatory responses caused by faecal particles have a cumulative effect on the bronchi.

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Active antitoxic immunization by a diphtheria toxin synthetic oligopeptide

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Diphtheria toxin (DT) is a single polypeptide chain of molecular weight 62,000 with two disulphide bridges[1]. Immunization against diphtheria rests on the stimulation of antibodies against detoxified toxin which also combine with the native toxin. Because the antibodies differ functionally from each other, however, only some of them are able to neutralize toxicity[2–4]. We have therefore set out to synthesize part of the amino acid sequence of the toxin whose function as a stimulator of antibodies might be less ambiguous, and have chosen the loop of 14 amino acids subtended by the disulphide bridge nearer the NH_2 terminus of the molecule (Fig. 1). There is reason to think that this loop may be involved in the toxicity and immunological specificity of the molecule[1,5]. We report here our finding that the tetradecapeptide (residues 188–201), when linked covalently with two different carriers, will elicit in guinea pigs antibodies which not only bind specifically with the toxin but neutralize its dermonecrotic and lethal effects. To our knowledge these results constitute the first example of successful active immunization against a lethal bacterial toxin using a synthetic antigen.

The sequences 194–201, 192–201 (synthetic decapeptide SDP), 190–201, 188–201 (synthetic tetradecapeptide STDP) and 186–201 were prepared by solid-phase methodology using *m*-nitrobenzhydrylamine resin and benzhydrylamine resin. The first polymer retains the peptide after hydrogen fluoride (HF) treatment to remove protecting groups of the side chains of the amino acids[6]. Despite certain gaps, such as those caused by destruction of cysteine, the elongation of the sequences can be followed by amino acid analysis performed on the peptidyl resin[7]. With the second polymer, HF treatment cleaves peptides in their carboxylamide terminal form[8]. Peptides were purified by gel filtration and proved homogeneous by TLC. Their amino acid composition agreed with that obtained theoretically. Details of the different syntheses will be published elsewhere.

186 190 195 201
-Cys-Ala-Gly-Asn-Arg-Val-Arg-Arg-Ser-Val-Gly-Ser-Ser-Leu-Lys-Cys-

Fig. 1 Sequence of the diphtheria toxin loop within the NH_2-terminal disulphide bridge as inferred from the complete sequence of fragment A (ref. 14) and the data reported for the NH_2-terminal stretch of fragment B (ref. 15).

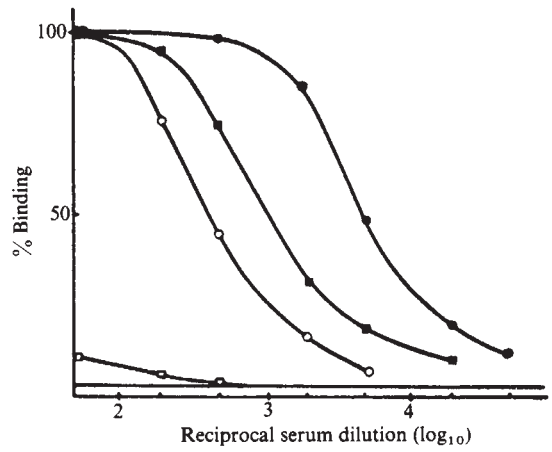

Fig. 2 Binding of radiolabelled diphtheria toxin by anti-SDP and anti-STDP sera. DT was iodinated by the chloramine-T technique. Using appropriate dilutions, 98% of the radioactivity present in the antigenic preparation after labelling was bound by a hyperimmune anti-DT horse serum (gift from M. Mazert). Serial dilutions of anti-toxoid and anti-peptide sera were tested for their capacity to bind ^{125}I-labelled diphtheria toxin. Percentages were obtained in each tube from the formula: c.p.m. found in precipitate ×100/total c.p.m. ●, Anti-toxoid serum corresponding to the pooled sera of group 1 (see Table 1 legend); ■, anti-toxoid serum (pooled sera of group 2); ○, anti-SDP (pooled sera of group 5); □, anti-STDP serum (highest responder of group 6); —, normal rabbit serum.

Octa-, deca- (SDP), dodeca-, tetradeca- (STDP) and hexadecapeptides linked to the *m*-nitrobenzyhydrylamine resin were tested for their ability to combine with anti-DT antibodies. These sera were raised in horses and rabbits, and the immunoglobulins purified and labelled with ^{125}I. Whereas SDP seemed to be the smallest structure capable of binding to antibodies, this property increased strikingly with the hexadecapeptide. Because free SDP and STDP were the only compounds available in sufficient amounts, they were covalently linked to bovine serum albumin (BSA) by their amino groups using glutaraldehyde and administered to guinea pigs in conditions described in Table 1 legend. The resulting sera were tested by passive haemagglutination. Using the SDP–BSA conjugate, only one out of five animals gave detectable response, whereas all but one of the animals challenged with STDP–BSA gave significant

Table 1 Individual response of guinea pigs to toxoid, SDP and STDP

Immunization	Passive haemagglutination titre: Mean	Individual values
Toxoid:		
(1) 3 Lf+FCA	13.8	14 – 14 – 14 – 14 – 13
(2) 3 Lf	11.8	12 – 12 – 12 – 12 – 11
(3) 0.3 Lf	3.8	7 – 4 – 4 – 2 – 2
(4) 0.03 Lf	0.2	1 – 0 – 0 – 0 – 0
(5) SDP–BSA	1.6	8 – 0 – 0 – 0 – 0
(6) STDP–BSA	4.67	10 – 5 – 5 – 4 – 4 – 0

[1] Groups of guinea pigs were injected in each hind footpad with 0.1 ml of the immunizing preparations and 30 days later re-injected in the same conditions. Data represent the log_2 of passive haemagglutination titres of individual sera collected 3 weeks after the second injection, using sheep red blood cells coated with diphtheria toxin by glutaraldehyde in experimental conditions to be described elsewhere. Groups are the following: guinea pigs immunized by diphtheria formalin-toxoid: (1) 3 Lf in FCA; (2) 3 Lf in saline; (3) 0.3 Lf in saline; (4) 0.03 Lf in saline; guinea pigs immunized by peptide conjugates: (5) SDP–BSA 1 mg in FCA; (6) STDP–BSA 1 mg in FCA. 1 Lf = 2.5 μg protein; 1 MLD = 66 ng protein; 1 MRD = 0.1 ng protein.

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