

Antigenic and allergenic properties of the storage mite *Tyrophagus putrescentiae*

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The antigens and allergens in the storage mite Tyrophagus putrescentiae, commonly found in grain and hay and sometimes in house dust, were characterized by crossed immunoelectrophoresis and crossed radioimmunoelectrophoresis. Three mite extracts, T. putrescentiae (TP) mite bodies, feces, and a combination of mites, feces, and culture medium (TP + CM) were studied. TP and TP + CM (primarily feces) extracts exhibited 20 and 18 antigens, respectively. By use of serum from two skin test-positive patients, autoradiograms demonstrated TP contained two allergens and TP + CM contained five allergens, three of which probably originated from feces. CM (whole wheat flour) did not stimulate antibody production in rabbits. TP feces shared 10 antigenic determinants with TP and 14 with TP + CM. Two antigens common to TP feces and TP were also shared allergens. (J ALLERGY CLIN IMMUNOL 74:166-71, 1984.)

Numerous mite species have been reported in the dust from houses. Most of these species with the exception of mites of the genus *Dermatophagoides* are contaminants that occur only sporadically in small numbers. *Dermatophagoides* spp. are established as major sources of allergens in house dust.^{1, 2} Several species of stored-product mites, however, do occur regularly in occupied dwellings, and in specific situations they may occur in significant numbers, particularly when relative humidity is high and cereal and flour products or other food stuffs are available as a source of food.³⁻⁹ The role of these storage mites as allergens is less clear.

Storage mites comprise a wide range of families and species, but those species most often reported are species of *Tyrophagus*, *Glycyphagus*, *Acarus*, *Euroglyphus*, *Lepidoglyphus*, *Chortoglyphus*, and *Go-*

Abbreviations used

CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
TP + CM:	<i>Tyrophagus putrescentiae</i> plus culture medium

hieria.^{4, 9-11} These nonpyroglyphid "storage" mites are also common and numerous in granaries, live-stock-feeding facilities, barns, grain storage/transfer facilities, hay, and straw, and they also contaminate various grain and hay dusts.^{7, 8, 12-19} Significant occupational exposure as well as exposure to dust in homes under certain living conditions can occur. Many reports describe asthma and allergic rhinitis associated with these mites.^{8, 20-24, 25-27} Skin-testing studies demonstrate that many species of storage mites can be important allergens.^{9, 10, 28, 29}

TP is a common storage mite found in homes, grain, hay and straw storage or transfer facilities, and the farming environments. Positive skin test responses to this species have been demonstrated by several studies, and they are reported to have caused asthma in patients in two Ceylon hospitals.^{9, 10, 22, 30} It may, therefore, be an allergenically important mite. The purpose of this preliminary study was to evaluate the antigenic and allergenic properties of this mite by CRIE.

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METHODS

Mite antigens

TP were cultured on whole wheat flour. As cultures became mature and crowded, all stages of mites migrated from the CM to the container rim where live and dead mites were periodically collected by aspiration. Migrating mites that fell out of the culture were collected by brushing the surface area around the culture containers. All collections were immediately frozen or warmed to 50° C for a few hours to kill the mites and were then vacuum dried over Drierite. Dried mite material was used to prepare extracts. Microscopic examination revealed it contained no visible CM and consisted of primarily all life stages and exoskeletons with very little feces. The life-stage composition was 81.3% adults, 16.3% nymphs, and 2.5% larvae. Many of the bodies contained 1 to 3 fecal pellets in the gut, whereas visible fecal matter outside the bodies was rare.

Material that was predominantly mite feces was obtained by sieving (106 micron openings) CM from old thriving cultures in which considerable feces had accumulated. A special antigen mixture was also prepared from material scooped from a thriving TP culture after Berlese-Tullgren extraction.³¹ This antigen, termed TP + CM, contained CM, all life stages of dead mites, eggs, and feces but no live mites because they had crawled out during the Berlese-Tullgren extraction. Collection, concentration, and preparation of mite components were performed by dry techniques. This was considered to be a significant aspect of this research because mites remain dry in the natural environment. Also, collection processes that used solution methods could remove soluble antigens.

Extract preparation

Extracts were prepared according to the method of Arlian et al.³² Briefly, 1 gm of dry mite material was defatted in ether, ground in 20 or 40 ml (1:20 or 1:40 w/v) of 0.9% saline, cold sonicated, extracted at 4° C for at least 48 hr, centrifuged, and filtered (0.45 µm). The protein content of each extract was determined by the Bio-Rad method (Bio-Rad Laboratories, Richmond, Calif.) by use of a bovine gamma globulin standard and was 1421 and 615 µg/ml at 1:40 dilution (w/v) of TP and TP + CM, respectively, and 1130 µg/ml for a 1:20 (w/v) dilution of TP feces.

Antisera

Two New Zealand white rabbits were immunized to each extract in order to produce anti-TP and anti-TP + CM sera. Each rabbit was initially injected subcutaneously with extract containing 0.5 mg protein emulsified in an equal volume of Freund's complete adjuvant. Second and third injections with one fifth the dose (0.1 mg protein) emulsified in Freund's incomplete adjuvant were made at 2 and 4 wk. Antibody titers were low at this time so that each rabbit was boosted twice at 2-week intervals with 2 mg of (TP or TP + CM) protein emulsified in incomplete adjuvant. At three successive weekly intervals, commencing 2 wk after the last boost, 35 to 40 ml of blood was collected from the

central artery of the ear, and the serum was separated, pooled, and frozen. For all extracts at each immunization, one half the dose was injected postoccipitally; the remainder was equally distributed between the two axillary and inguinal regions. Antiserum titers were determined by the Ouchterlony double-gel diffusion technique. Twofold dilutions of mite extract were diffused against anti-mite sera.

CIE

The number of antigens present in the extracts was examined by CIE. The CIE procedure used was similar to techniques of Anderson and Baer³³ and Weeke.³⁴ Briefly, 8 to 12 µl of extract was electrophoresed with Svendsen's buffer on horizontal glass slides (10 by 10 cm; anode at top) at 200 V for 30 min in 1% agarose gel at 15° C. Antigen containing gel strips (1 by 5 cm) were cut and placed transversely 1.5 cm from the bottom of 5 by 7 cm gel-bond slides. Three milliliters of agarose containing 75 to 200 µl of rabbit anti-serum was poured on the remaining part of the slide above and below the antigen-containing strip. Electrophoresis in the second dimension, perpendicular to the first, was performed with 40 V (anode at the top) at 9° C for 18 to 20 hr. The electrophoresed gels were then pressed, washed, dried, stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories), and destained. If antibody titers were low, the antisera were first concentrated five to 10 times by use of a Minicon concentrator (B125) before combining them with the agarose.

CRIE

Allergen content of extracts was determined by CRIE in a manner similar to Anderson and Baer³³ and Weeke and Lowenstein.³⁵ Briefly, before staining, CIE plates were selected and incubated with gentle shaking for 24 hr in sera from TP, *D. farinae*, and house dust-sensitive patients and RAST A buffer,* and then washed three times for 15 min with RAST B buffer.† Slides were then incubated overnight with ¹²⁵I-radiolabeled anti-human IgE and RAST C buffer,‡ washed as before, pressed, air dried, and placed into x-ray cassettes. The radiolabeled gel slides were placed under two tungsten-enhancement screens. Kodak X-omat (XRP-1) film was located between the two screens. Film was hypersensitized by preexposing it to a single flash of light.³⁶ Film was kept in the x-ray cassettes at -80° C for periods of 4, 7, and 11 days. The gels were then stained with Coomassie brilliant blue R-250 for antigen identification.

CRIE tests for each mite extract were performed at least

*RAST A Buffer: 500 ml 0.1M phosphate buffer (pH 7.5), 1 gm of bovine serum albumin, 5 ml TWEEN 20, 0.5 ml 10% sodium azide (NaN₃).

†RAST B buffer: 1000 ml 0.1M phosphate buffer (pH 7.5), 10 ml TWEEN 20.

‡RAST C buffer: 20 ml calf serum, 5 ml TWEEN 20, 5 ml 10% NaN₃, 470 ml 0.1M phosphate buffer (pH 7.5).

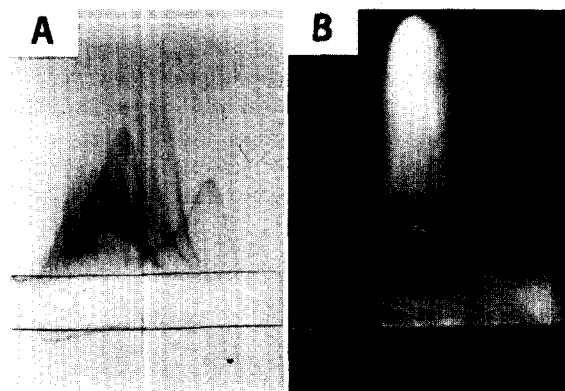


FIG. 1. A, CIE pattern of TP extract reacting with rabbit anti-TP serum. B, CRIE of the CIE pattern with patient 1 serum.

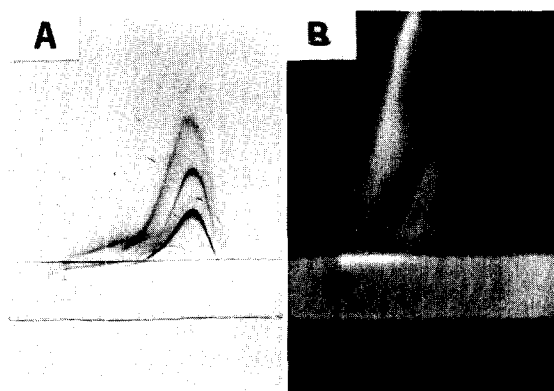


FIG. 2. A, CIE pattern of TP + CM extract reacting with rabbit anti-TP + CM serum. B, CRIE of the CIE pattern with patient 1 serum.

TABLE I. CIE and CRIE comparisons of TP extracts and antisera

Extract	Antiserum	Precipitates					
		CIE		CRIE			
		A	B	Patient 1		Patient 2	
				A	B	A	B
TP	TP	18	2	2	0	0	0
TP	TP + CM	5	0				
TP + CM	TP + CM	18	0	5	0	0	0
TP + CM	TP	0	0				
CM	TP + CM	0	0				
TP Feces	TP + CM	13	1	2	0	0	0
TP Feces	TP	10	0	2	0	0	0

A and B = number of precipitin lines in the anodal or cathodal direction, respectively.

twice with two different sera obtained from patients intradermally skin test positive (3+ or higher) to house dust and the storage mite TP extracts (1:100,000). An individual skin test negative (no wheal or erythema) to the two extracts served as a control.

RESULTS

CIE patterns for TP and TP + CM extracts electrophoresed into their corresponding antisera demonstrated both contained 18 anodically migrating antigens (Table I, Figs. 1 and 2). However, the antigen precipitin line profiles for each of these CIE determinations were very different. These profiles indicated that the antigens in the extract containing primarily mite bodies (TP) was different in composition from the extract containing TP + CM (primarily feces) (Figs. 1 and 2). CRIE immunoplates of TP extract and TP antiserum incubated with serum from the skin test-positive patients and ^{125} I-labeled anti-human IgE indicated two of the antigens from TP were allergens for one patient, whereas none of the antigens were

allergens for the second patient (Fig. 1). Similarly, CRIE plates for TP + CM extract and TP + CM antiserum demonstrated that of the 18 visible antigens, five were allergens when serum was used from patient 1 (Fig. 2). Since no antibodies could be produced to fresh unused CM and CIE and CRIE tests of CM extract and TP + CM antiserum revealed no visible precipitin lines or radiostaining, the antigens and allergens visualized in homologous TP + CM immunoplates were derived only from mite components of the TP + CM material. Since only two allergens were visualized with TP extract and five were visualized with TP + CM extract when the serum from the same patient was used, it can be concluded that three of the allergens visualized in the homologous TP + CM immunoplates were derived from mite feces. Furthermore, at least three of the five allergens visualized in TP + CM extract were in a different position on the autoradiograph that indicated these three were not immunochemically similar to the two allergens visualized in homologous CRIE of TP extract. There-

fore, the three allergens were unique and specific to feces.

CIE of TP extract into TP + CM antiserum resulted in five weak precipitin lines that indicated five antigens were common in the two extracts, 13 antigens were not, and TP was a weak antigenic component of TP + CM. The reciprocal CIE experiment by use of TP + CM extract and TP antiserum did not demonstrate visible precipitation lines. This further suggested TP + CM extract contained either weak or dilute TP antigens and confirmed that most of the antigens in TP + CM were originating from mite feces.

TP feces extract electrophoresed into TP or TP + CM antiserum produced 10 and 13 anodic precipitin lines, respectively (Table II, Fig. 3). With the exception of one precipitin line in the TP feces extract and TP + CM antiserum CIE, all were weak and made these gels difficult to interpret. A difference of five precipitin lines between the homologous CIE of TP + CM and the heterologous CIE of TP feces extract and TP + CM antisera was attributed to the mite bodies in TP + CM. Five precipitin lines were visualized when TP was electrophoresed into TP + CM. A larger number of weak precipitin lines in the TP feces extract/TP antisera experiment than for the TP + CM extract/TP antisera experiment was attributed to the fact that the feces were much more concentrated in the TP feces extract. Sufficient TP feces was not obtained to produce antiserum so that a feces extract/feces antiserum CIE could not be performed. CRIE patterns for TP feces extract and TP antiserum demonstrate two of the antigens common to these two extracts were also allergens that actively bound IgE in patient 1 serum (Table II). The CRIE experiment of TP feces extract versus TP + CM rabbit antiserum revealed two allergens common to TP feces and TP + CM when patient 1 serum was used (Table II, Fig. 3).

DISCUSSION

In this preliminary study, we produced precipitating rabbit antibodies directed against various types of TP antigens. Identification of allergens in test extracts was also achieved by the use of TP-specific serum from a mite skin test-positive patient and a radio-labeled anti-human IgE marker. Thus both antigens and allergens clearly originate from the various components of the storage mite TP. This study also demonstrated that skin and CRIE tests may not always correlate. Only one of two TP skin test-positive patients demonstrated evidence of circulating specific IgE by CRIE.

In antigenicity studies of mites, the questions of extract purity and CM antigenicity are important but

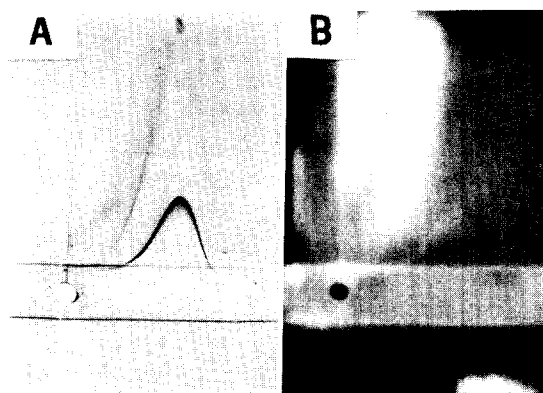


FIG. 3. A, CIE pattern of TP-feces extract reacting with rabbit anti-TP + CM serum. B, CRIE of the CIE pattern with patient 1 serum.

frequently not considered. In this study, mite antigenic extracts used contained only antigens and allergens derived from mites and mite products. The TP extracts were compared to a crude mixed extract containing TP, TP feces, and CM (TP + CM) and an extract of pure CM and the TP + CM extract, which, as primarily feces, was compared to extracts of TP and CM for reference purposes. Such studies revealed that the presence of culture material in these extracts did not contribute significantly to overall antigenicity. CIE and CRIE experiments with and without intermediate gels indicated that CM (whole wheat flour) did not stimulate specific antibody production in rabbits, and CM extract electrophoresed into TP or TP + CM antisera produced no precipitin lines nor radiostaining.

CIE results indicate that TP, TP + CM, and TP feces extracts were complex and contained numerous antigens. Both mite and feces extracts contained heterogeneous mixtures of proteins as indexed by the fact that as many as 20 antigens were directly demonstrated in CIE experiments. Apparently large differences in the quantity and properties of each antigen in the extracts exist since considerable variation in staining intensity and area, shape, and position of precipitin lines on the gels was observed.

Most of the 18 antigens in the TP + CM extract were derived from feces, five were derived from TP material (mite bodies and secretions), and the remaining 13 were from mite feces. CIE of TP feces extract and TP + CM antiserum exhibited 13 anodic precipitin lines and confirmed this conclusion. This was not unexpected since the mite cultures were first subjected to Berlese-Tullgren funnel extraction, which removed most live mites and left primarily feces and CM. Therefore, the TP + CM extract was weak in TP components but strong in feces antigens, and it, along with the less crude feces material, served as a suitable

material to examine the antigenic and allergenic properties of feces.

Despite identification of some antigens specific to either mite feces or mite bodies, all heterologous crosses indicated a close antigenic relationship between feces and whole-body TP extracts. Whether this represents common antigens in the different mite components or a common contaminant was not determined. TP bodies were collected in areas where live mites had crawled from the CM, and it was possible that some defecation did occur in the area where they were aspirated. Possibly common antigens originated from a small amount of feces that was aspirated along with the live mites. Microscopic examination of the material used to produce TP extract demonstrated insignificant feces outside the mite bodies, but a high percentage (73%) of the bodies contained fecal pellets inside the gut. More likely therefore common antigens probably originated from feces remaining in the posterior intestine of the mites when they were collected or possibly from the proteinaceous peritrophic membrane always present in the midgut. This membrane encapsulates the fecal pellets before they are expelled. It is also possible that antigens originate from mite secretory products. This material would become distributed throughout a culture and contaminate all mite components. Separated mite bodies and feces, as was done in this study, would still contain similar secretory products that could be antigenic.

Clearly, however, these mites and components contain important specific allergens. Autoradiograms indicated that TP contained two allergens, and TP + CM (primarily feces) contained five allergens. Of the five allergens in TP + CM extract, two could be identified as originating from TP bodies, and three originated from TP feces in this material. CRIE gels indicated that TP feces and TP extracts also contained common allergens. Similarity in position and shape of radiostaining profiles in the CRIE gels suggest they are immunochemically similar allergens. However, because of the possibility of a contaminating product as discussed above, it is not known if these were molecules common to both mite bodies and feces or simply molecules contaminating both.

Both CIE and CRIE results revealed that there are multiple antigens and allergens in TP and their feces. The large number of specific antibodies produced in rabbits and the identification of five of these as allergens suggest that this mite species provides a large antigenic pool for potential hypersensitization. The mite and its feces each possess specific antigens and allergens, but they also reveal some cross antigenicity and cross allergenicity. Patients with house dust al-

lergy or storage-food mite allergy could produce allergic antibodies against different specific allergens from either mites or their feces or against allergens common to both.

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