

Investigations of culture medium-free house dust mites

III. Antigens and allergens of body and fecal extract of *Dermatophagoides farinae*

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Crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) were used to characterize antigens (Ags) and allergens derived from *Dermatophagoides farinae* (DF) culture media-free mite body and mite fecal matter extracts. CIE of DF body and DF feces extracts revealed the presence of 35 and 20 Ags, respectively. CRIE experiments demonstrated IgE binding by 14 and seven DF body and DF feces Ags, respectively, when CIE gels were incubated with reference sera from clinically mite-sensitive patients. Binding of specific IgE to the various Ags in the two extracts varied significantly both in frequency and in strength from patient to patient and within the same patient's serum. Sera from some patients demonstrated IgE binding predilection for specific DF body Ags, whereas other sera exhibited greater binding preference for DF feces Ags. Homologous, heterologous, and intermediate gel CIE and CRIE clearly demonstrated that DF bodies and DF feces share some common Ags or epitopes, but the two different extracts also were quantitatively different. Some Ags and allergens originate from mite body material and are not present in mite feces. These results indicate that only extracts containing high concentrations of both body and fecal allergens should be used in clinical testing and therapy. (*J ALLERGY CLIN IMMUNOL* 1987;79:457-66.)

House dust mites, DF and *D. pteronyssinus*, are the sources of multiple important Ags and allergens found in house dust.¹⁻¹³ Binding of specific IgE to the various Ags varies significantly both in frequency and in strength from patient to patient. The mite extract preparations used for research, diagnosis, and immunotherapy vary widely. Often, these extracts are prepared with whole cultures containing mites and CM combined without attempts to separate organic material in CM. Therefore, the concentration or ratio of mite material such as feces, secretions, exoskeletons,

Abbreviations used

CM: Culture medium
CIE: Crossed immunoelectrophoresis
CRIE: Crossed radioimmunoelectrophoresis
DF: *Dermatophagoides farinae*
Ag: Antigen

whole bodies, various life stages, and CM is not determined and could be highly variable. Since some extracts or the cultured mite and culture material from which extracts are produced may contain irrelevant soluble proteins, these may dilute mite Ags or add nonmite Ags. An essential prerequisite of determining source validity, isolation, and characterization of all important mite allergens depends on analysis of well-defined and separated mite material from which extracts are prepared. An important allergen from *D. pteronyssinus*, P₁ (also DP 42 or Dpt 12), or its equivalent from DF, F₁, has been attributed to mite feces.¹⁴⁻¹⁶ A recent study suggests that some *D. pteronyssinus* Ags may originate from only whole mite bodies, and these are Ags not present in pure mite

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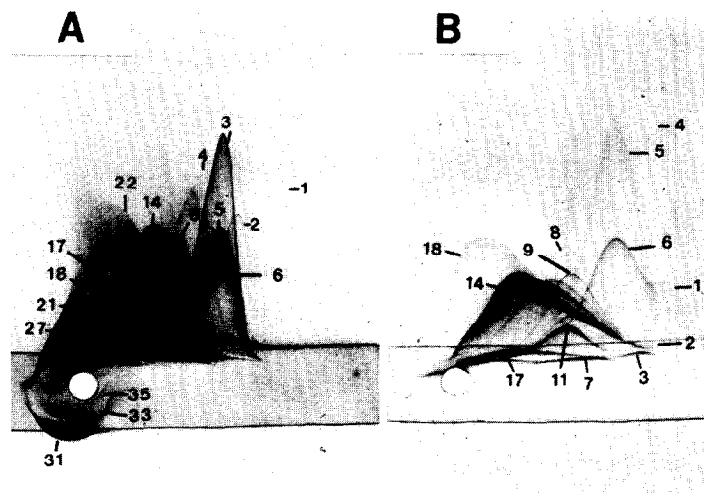


FIG. 1. CIE patterns. **A**, DF body extract reacting with rabbit anti-DF body serum. **B**, DF feces extract reacting with rabbit anti-DF feces serum. Not all numbers for identification of Ags appear on the gels. Each gel contained 9 μ l of extract (1:20 w/v concentrated five times) reacted with 150 μ l/3 ml of agarose (top) and 60 to 75 μ l/ml of agarose (bottom) antiserum that was concentrated 2.5 times.

feces.¹⁷ This possibility had briefly been reported in an earlier comparative study of cross-reactivity between DF and *Tyrophagus putrescentiae*¹; however, it was not fully investigated. There is little direct evidence demonstrating that important allergens originate primarily from DF mite bodies (such as body tissue, hemolymph, and glandular secretions), mite feces (besides F₁), or are common to both. By careful collection methods, it is possible to collect highly concentrated natural mite bodies or mite feces. The purpose of this study was to investigate the antigenic and allergenic relationships between extracts of medium-free mite bodies (DF) and mite fecal matter (DF feces) for the house dust mite, DF.

MATERIAL AND METHODS

Culture of DF, harvesting of *medium-free* mites (DF) containing all life stages, collection of 96% pure fecal matter (DF feces), and extract preparations were identical to that described in previous articles.^{1, 18}

Mite culture

Pure DF cultures were maintained on a 1:1 ratio of Purina (Ralston Purina Co., St. Louis, Mo.) laboratory chow and Fleischmann's (Standard Brands, Inc., New York, N. Y.) granulated dry yeast at room temperature and 75% humidity. Culture medium-free live mites were aspirated from culture container rims as they migrated from the medium, frozen, desiccated, and stored over Drierite (W. A. Hammond Drierite Co., Xenia, Ohio). The active and pharate life-stage composition of the aspirated mites was 56% adults, 9% tritonymphs, 24% protonymphs, and 11% larvae. Microscopic examination of DF body material revealed no

gross evidence of culture medium. An occasional fecal pellet was visible outside the body, but one to two fecal pellets were visible inside the gut of 48% of the whole bodies. DF feces was collected by sieving mite culture contents of well-established cultures in which most of the food supply had been exhausted. This fecal matter consisted of 96% fecal pellets, 1.6% bodies and exoskeleton fragments, and 2.4% culture medium fragments. The nonfecal particles were no more than two to three times larger than fecal pellets (10 to 30 μ m in diameter).

Mite extract preparation

Dried test material was defatted in anhydrous ethyl ether, placed in a corresponding volume of 0.9% sodium chloride or distilled water to provide a 1:40, 1:20, or 1:10 w/v dilution, ground, cold sonicated, and extracted for 48 hours at 4° C. Extracts were centrifuged (cold), filtered (0.2 μ m Acrodisc, Gelman Sciences, Inc., Ann Arbor, Mich.), and kept at 4° C or -15° C. Protein contents were determined by the standard Bio-Rad (Bio-Rad Laboratories, Rockville Centre, N. Y.) method with bovine gamma globulin as a reference protein. The protein contents were 3.29 ± 0.37 and 1.61 ± 0.33 mg/ml for DF body and DF feces extracts, respectively (1:10 w/v dilution).

Assay of mite extract for CM contamination

Although no CM was visible in DF bodies, and DF feces was 96% pure, CIE and CRIE were performed to demonstrate directly that DF body and DF feces extracts contained no allergens originating from the CM. An extract of pure CM (1:10 w/v) was prepared by methods identical to that for mite extracts. The CM extract was reacted with DF body and DF feces antisera. Likewise, rabbits were immunized with CM extract to demonstrate that CM components would

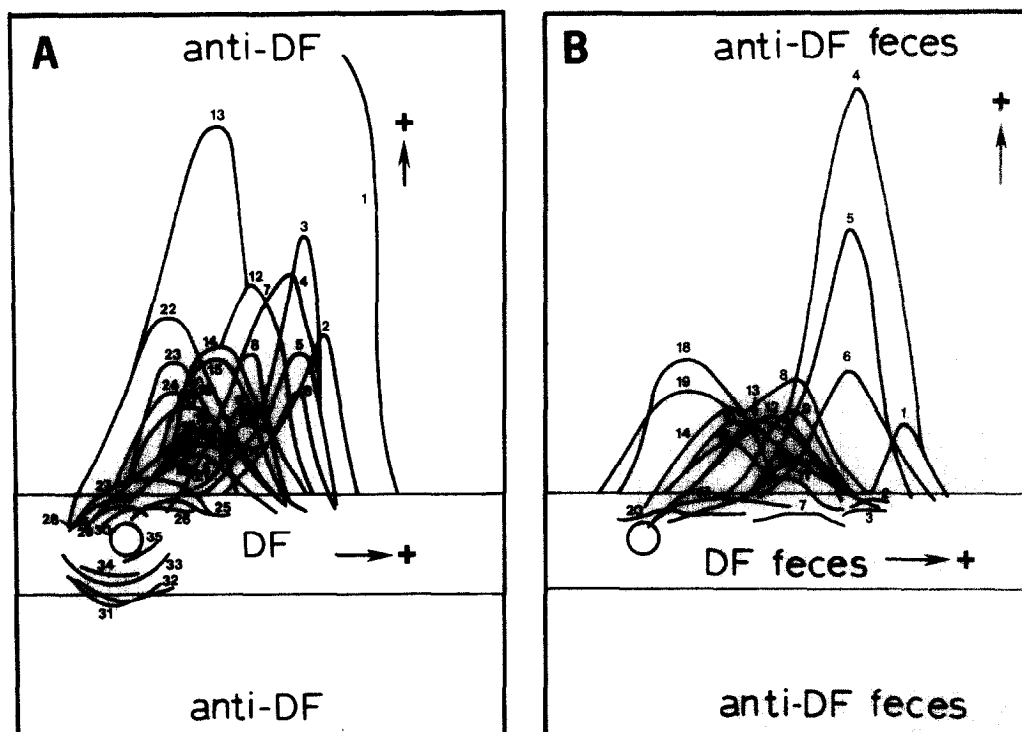


FIG. 2. Reference drawing of CIE precipitin patterns. **A**, DF body extract and rabbit anti-DF body serum. **B**, DF feces extract and rabbit anti-DF feces antiserum.

stimulate antibody production in rabbits had CM been present in the extracts. This CM antiserum was used for additional CRIE reactions with DF body and DF feces extracts.

Rabbit antisera

Antisera to each extract were raised by subcutaneous immunization of three New Zealand white rabbits. All the rabbits were obtained from the same source at the same time, were approximately the same age, and were immunized simultaneously. The immunization procedure was performed essentially as described previously.¹ Individual rabbits were immunized until a high antibody titer was exhibited by at least 30 plus antigen-antibody precipitates by CIE evaluation. The individual antisera for each extract were pooled and stored frozen at -20°C until the sera were required.

CIE and CRIE

CIE and CRIE, as reported by Arlian et al.,^{1,2,18} were used to demonstrate respective extract Ags and allergens. The relative rate of radiostaining of precipitin lines on individual gel plates by use of comparable activity was used as a systematic grading system to estimate the allergenic potency for a particular serum. Specific immunoprecipitates that consistently appeared and elicited clear radiostained images in 3 to 5 days on the x-ray film were classified as moderate to strong allergens. Weak allergens were classified as those that elicited faint radiostaining of an immunoprecipitate in 3 to 5 days relative to the strong images by others

or those that elicited radiostaining only after 5 days of autoradiography on a given gel. Repeated experiments of mite CIE and CRIE systems clearly demonstrated that the results were reproducible. This assessment served to estimate the relative amount of specific IgE bound to various immunoprecipitates.

Patient sera

Sera from 20 DF mite-sensitive untreated patients were selected for study. The patients were selected on the basis of positive prick (≥ 3 mm wheal and flare at 10 mg/ml w/v) or intracutaneous skin tests (3+ or more at a concentration of 1 or 0.1 mg/ml) to DF house dust mite extract and consistent allergic-symptom histories during the heating season. CRIE was performed with individual sera for the 20 patients with DF body extract. Six of the 20 were clearly CRIE positive for DF body allergens. CRIE was also performed with DF feces extract for three of the six sera CRIE positive for DF body extract. CRIE with both DF body and DF feces extracts was also performed with sera pooled from 11 of the 20 patients for which sufficient serum was available. Sera from six normal individuals without allergic history, clinical symptoms, or DF skin test reactivity were used as CRIE controls.

RESULTS

Assay of mite extract for CM contamination

Although the DF body material used to produce the extract contained no visible CM and the DF feces

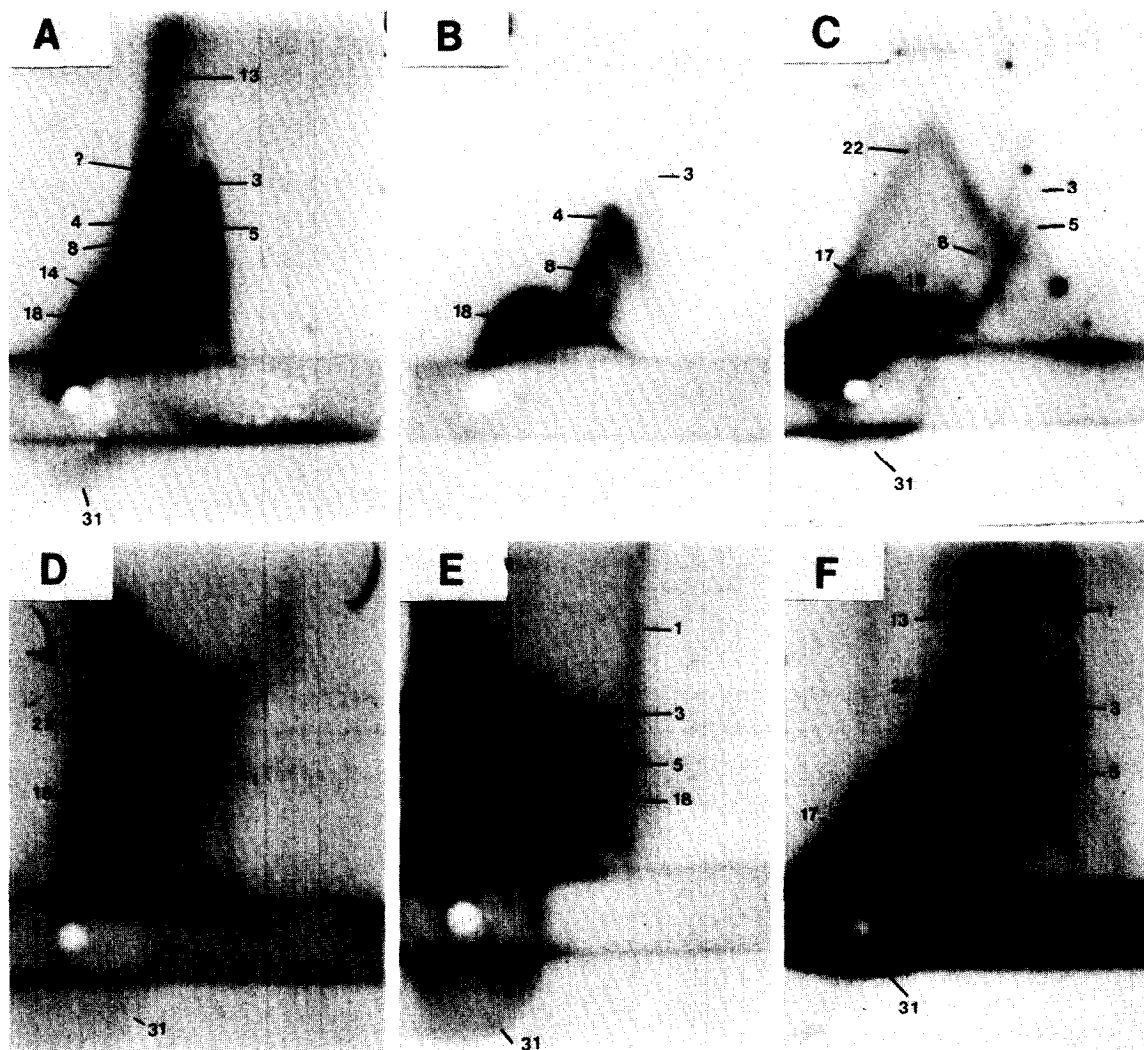


FIG. 3. Autoradiographs (CRIE) of the CIE pattern of DF body extract reacting with rabbit anti-DF body serum. **A-E**, Five individual allergic sera (same as a to e in Table I). **F**, Serum pool of 11 sera from allergic patients. Extract and antiserum quantities are the same as Fig. 1. Autoradiography performed 7, 5, 10, 7, 4, and 7 days for **A** through **F**, respectively.

material contained highly concentrated fecal pellets (96% of particles), it was demonstrated directly by CIE and CRIE that none of the mite and feces Ags and allergens originated from CM contained in the mite gut, remaining in the fecal pellet, or from other invisible sources. CIE and CRIE of CM extract into DF body or DF feces antisera demonstrated no visible Ag-antibody precipitin lines nor radiostaining. CIE of CM extract into CM antiserum demonstrated that rabbits immunized with only CM would produce antibodies to four Ags in the pure CM extract. However, had any of these been present in the extract, none of them were allergens, since radiograms exhibited no radiostaining after incubation of the gels in sera CRIE positive for DF body and DF feces extracts.

Identification of DF body and DF feces Ags reacting with rabbit antisera

Thirty-five Ags were detected after CIE of *medium-free* DF body extract into its corresponding antiserum (Fig. 1, **A**). Anodic and cathodic migration were demonstrated by 30 and five Ags, respectively. Likewise, homologous CIE of DF feces extract and its corresponding antiserum detected 20 anodic Ags (Fig. 1, **B**). A composite reference drawing demonstrating all individual precipitin lines (Ags) obtained after homologous CIE of DF body and DF feces extracts is presented in Fig. 2, **A** and **B**, respectively. Each Ag represented by the precipitin line is numbered consecutively from the fastest to the slowest moving.

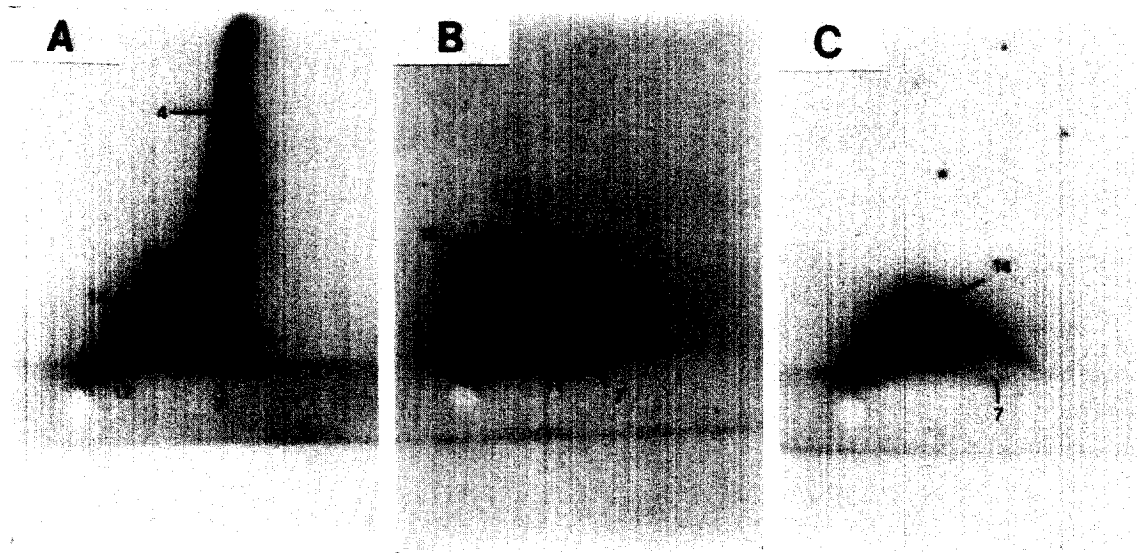


FIG. 4. Autoradiographs (CIE) of the CIE pattern of DF feces extract reacting with rabbit anti-DF feces serum for sera of allergic patients A, B, and C, respectively, of Fig. 3. Extract and antiserum quantities are the same as for Fig. 1. Autoradiographs are 11, 7, and 5 days exposure for A-C, respectively.

TABLE I. Serum IgE binding to individual DF body Ags represented by Ag/antibody precipitates visualized by CIE

Reference sera	Skin test	Antigens visible on CIE/CRIE plates that exhibited IgE binding										IgE binding w/o relation to visible Ag	
a	4+, S	3*	4*	5	8	13	14*	18*		31		1 arc	
b	4+, ID	3	4*		8*			18*					
c	4+, P	3		5	8			17	18*	22	31		
d	4+, P	1	3						18	22	31		
e	4+, P	1	3*		5				18	22*	31*		
f	3+, P		3		5	8	14		18*	21*			
Pooled	†	1	3	4	5	10	13	14*	17*	18*	22	31	

Antigens correspond to numbered Ag/antibody precipitate arcs presented in Fig. 1.

S = scratch; ID = intradermal; P = prick.

*Indicates moderate to intense radiostaining after 3 to 5 days of autoradiography. Pooled sera includes reference sera a to f plus five other sera selected from skin test positive patients with skin test of 3+ or more with S, ID, or P.

†3+ or more S, ID, or P.

Detection of DF body and DF feces allergens

After incubation of CIE plates with six highly sensitive individual and a pooled sera and ^{125} I-labeled antihuman IgE, autoradiographs revealed that multiple DF body Ags bound mite-specific human IgE (Table I; Fig. 3). By use of CRIE, three sera demonstrating unequivocally positive CRIE results to DF body precipitates were also assayed on CIE gels obtained by electrophoresis of DF feces Ags with rabbit antiserum to DF feces, and they also exhibited clearly positive CRIE results (Fig. 4). A total of 14 and seven precipitin lines formed by homologous CIE

of DF body and DF feces, respectively, bound specific IgE in these patients' sera (Figs. 3 and 4; Tables I and II). The binding properties of mite-specific IgE to the various Ags in both extracts varied significantly from patient to patient (Figs. 3 and 4). Both between and within patients, with comparable conditions, autoradiographic images ranged from intense to faint, indicating that there were interpatient and inpatient variations of sera-specific IgE antibody levels and/or binding affinity to the various Ags of both extracts.

The number of DF body Ags that bound IgE ranged from four to nine among the six strongly reacting

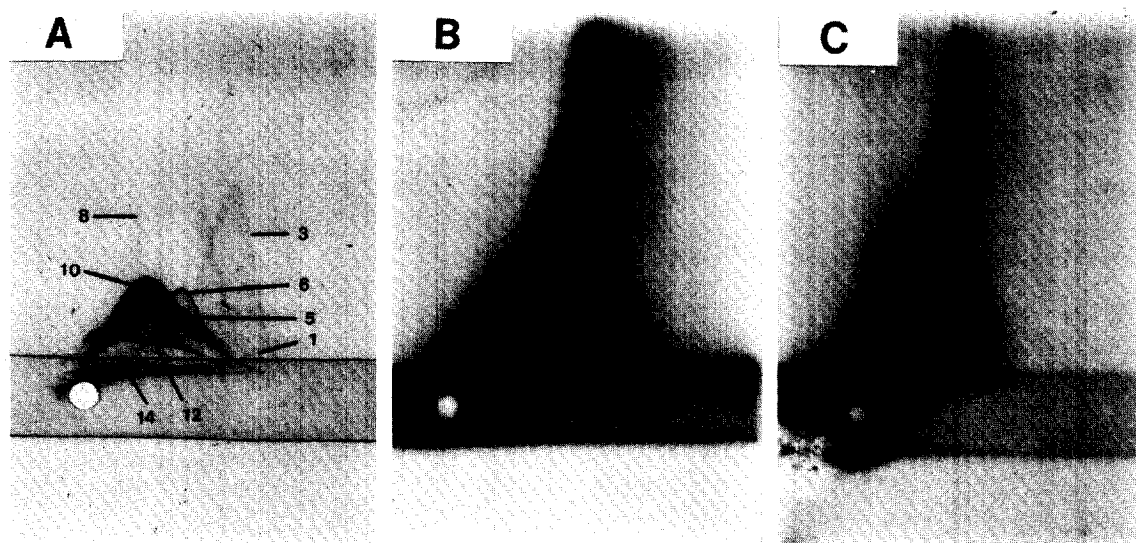


FIG. 5. A, CIE pattern of DF feces extract reacting with rabbit anti-DF body serum. Not all numbers for Ag identification appear on the gels. Extract and antiserum quantities are the same as in Fig. 1. B-C, Autoradiograph (CRIE) of the CIE pattern with sera from allergic patients, sera A and B, respectively, after 5 and 11 days exposure, respectively.

TABLE II. Serum IgE binding to individual DF feces Ags represented by Ag/antibody precipitates visualized by CIE and illustrated in Fig. 2

Reference sera	Antigens visible on CIE/CRIE plate that exhibited IgE binding					
a	4*	7	8*	14*	17	
b		7	8*	11	14*	18*
c		7			14*	
Pooled					14*	

Sera a, b, and c are the same as in Table I; pooled serum is the same as for Table I.

*Indicates moderate to intense radiostaining after 3 to 5 days of autoradiography.

individual sera (Fig. 3; Table I). The Ags that bound IgE in at least one serum were 1, 3, 4, 5, 8, 13, 14, 17, 18, 21, 22, and 31 and one that did not correspond to a precipitin line on the CIE gel. Nine DF Ags, corresponding to Ags 3, 4, 8, 14, 17, 18, 21, 22, and 31 elicited moderately intense to intense radiostaining after 3 to 5 days autoradiography in at least one patient or the serum pool in contrast to the remaining five precipitin lines that elicited only faint images in all test sera after 11 to 20 days of autoradiography. The number of Ags recognized as strong allergens ranged from 0 to 4 in an individual serum with three sera exhibiting strong binding to at least three Ags. All sera exhibited IgE binding to two particular DF Ags (3 and 18), whereas at least 50% of the sera exhibited

IgE binding to 3, 5, 8, 18, 22, and 31. Four of six individual sera exhibited IgE binding to at least six different Ags, and all sera exhibited binding to at least four Ags. One serum exhibited only weak binding to all DF Ags it recognized.

A pooled sample of mite-specific IgE sera identified 11 allergens in DF extract (Fig. 3, F; Table I). All but one of these allergens (No. 10) was also detected by performing CRIE with the six individual sera. This additional allergen was probably contributed by sera in the pool that were not assayed in the six individual CRIE tests.

Seven different DF feces Ags, corresponding to precipitin lines 4, 7, 8, 11, 14, 17, and 18, exhibited IgE binding and radiostaining in at least one of three sera from mite-sensitive patients (Nos. a, b, and c, Table II). Two of the three sera had specific IgE that bound to five Ags (Table II). Ags 8 and 14 demonstrated strong IgE binding by at least two of three sera. Of the seven allergens identified in DF feces, four (4, 8, 14, and 18) exhibited significant IgE binding in at least one of the three test sera. All three individual sera and the pooled serum exhibited strong IgE binding to Ag 14.

Although the DF body material used to produce the extract contained no visible CM and the DF feces material contained highly concentrated fecal pellets (96% of particles), it was demonstrated directly by CIE and CRIE that none of the mite and feces Ags and allergens originated from CM contained in the mite gut or remaining in the fecal pellet. CIE and CRIE of CM extract into DF body or DF feces antisera

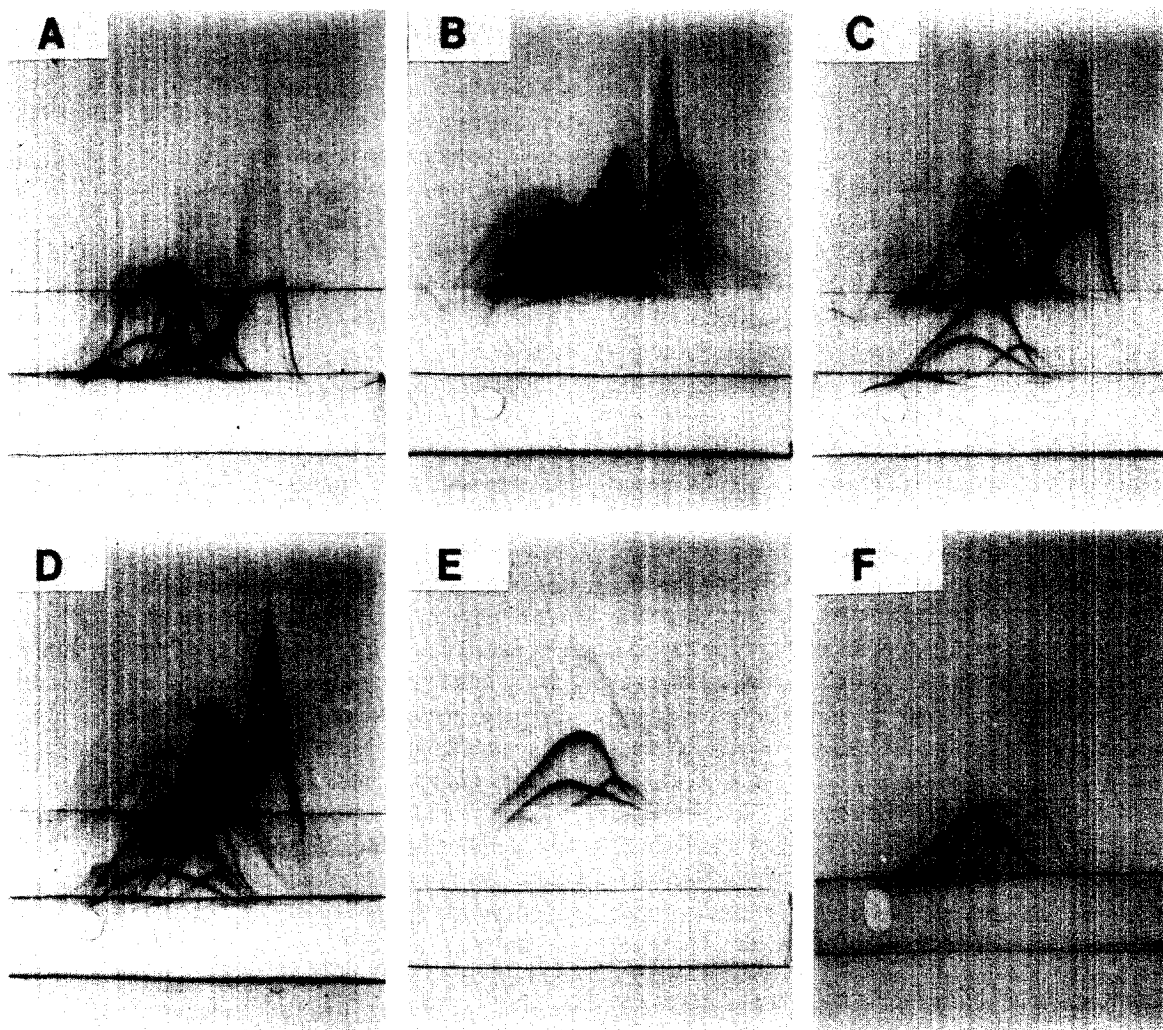


FIG. 6. CIE pattern of DF mite body extract. **A**, Reacting with anti-DF body serum in the intermediate gel and anti-DF feces in the reference (*top*) gel. **B**, Normal serum in the intermediate gel and reference gel containing DF body antiserum. **C**, Anti-DF feces serum in the intermediate gel and reference gel containing DF body antiserum. **D**, DF body antiserum in both the intermediate and reference gels. **E**, Normal serum in the intermediate gel and DF feces antiserum in the reference gel. **F**, CIE pattern of DF feces extract reacting with DF feces in the intermediate gel and DF body antiserum in the reference gel. Extract quantity same as Fig. 1. Reference, intermediate, and bottom gels contained 60, 65, and 60 μ l of antiserum (concentrated 2.5 times) per milliliter of agarose.

demonstrated no visible Ag-antibody precipitin lines nor radiostaining.

Cross-reactivity of DF body and DF feces Ags and allergens

Heterologous CIE gels of DF feces extract and DF body rabbit antiserum exhibited 15 antigen-antibody precipitin lines that indicated DF body antiserum contained antibodies against DF feces Ags or epitopes (Fig. 5, A). The positions, sizes, and shapes of some of the precipitin peaks of DF feces extract/DF body antiserum system were identical to those of the DF feces/DF feces system (Figs. 1, B, and 5, A). The

reciprocal CIE cross elicited similar results. Five of the 15 cross-reacting or common Ags bound IgE in individual sera from two patients highly sensitive to both DF body and DF feces extracts (Fig. 5, B and C). Autoradiograms of the two sera exhibited nearly identical radiostaining profiles. Radiostaining was intense for two precipitin lines, moderately intense for two others, and faint for one. One allergen migrated toward the cathode.

To determine further the presence of specific and cross-reactive antigenic determinants or common Ags contained in DF body and DF feces extracts, intermediate gel CIE was performed. Several arrangements

of respective Ags and antisera used in these experiments are illustrated in Fig. 6. Interposing an intermediate gel containing DF body antiserum between the first dimensional gel containing DF body antigenic extract and the second dimensional reference gel containing DF feces antiserum resulted in the formation of several precipitin peaks without heads (tops) in the intermediate gel containing the DF body antibodies (Fig. 6, A). In a similar test of DF body extract with a control intermediate gel containing normal rabbit serum (without mite antibodies) and the reference gel containing DF body antiserum, all precipitin peaks formed in the reference gel (above the negative intermediate gel) (Fig. 6, B). Reverse CIE tests with DF body extract in the first dimensional gel, DF feces antiserum in the intermediate gel, and DF body antiserum in the second dimensional reference gel, demonstrated that some precipitates formed only in the reference gel (with no legs extending through intermediate gel); whereas a few formed only in the intermediate gel (Fig. 6, C). A CIE system containing DF body antiserum in both the intermediate and reference gels revealed that precipitin lines that formed in the reference gel on the previous CIE system extended through both intermediate and reference gels (Fig. 6, D). Apart from slight cathodal migration of antibodies, a CIE test with DF body Ags in the first dimensional gel, normal (control) serum in the intermediate gel, and DF feces antiserum in the reference gel demonstrated that all precipitin lines were formed in the reference gel (Fig. 6, E) with the profile and number of precipitins similar to those visualized in the intermediate gel of Fig. 6, C, and the second dimensional gel of Fig. 5, A. This series of tests demonstrated not only that DF bodies and DF feces contained common Ags but also that DF feces lacked or contained only insignificant quantities of some Ags present in DF body extracts.

A similar set of prospective intermediate CIE experiments with DF feces extract in the first dimensional gel suggested that some Ags common to both DF body and DF feces extracts were more concentrated in the DF feces extract. Thus, a CIE test with DF feces extract in the first dimensional gel, DF feces antiserum in the intermediate gel, and DF body antiserum in the reference gel demonstrated that some strong precipitin lines in the intermediate gel lacked strong tops in the reference gel (Fig. 6, F).

DISCUSSION

The results of this study demonstrated that extracts produced from both highly concentrated DF bodies or DF feces contain multiple Ags and allergens. Allergic epitopes were associated with 14 DF body and

seven DF feces antigenic loci, respectively. These results are consistent with other studies that DF is the source of multiple allergens.^{1, 4, 7, 9, 10, 13} However, most previous studies have used extracts produced from whole mite cultures, and thus the relative importance of various mite components could not be assessed.^{4, 9, 10, 13} Results of this study clearly demonstrate that the composition of mite source material used for extract preparation is important and may account for allergenic differences reported by several laboratories.

This study demonstrated that binding of specific IgE to the various Ags in DF body and DF feces extracts varied significantly both in frequency and in strength between and within patients as judged by the varied radiostaining. The varied strength in radiostaining by different precipitin arcs after incubation of a specific gel in an individual serum could reflect either the availability of IgE relevant epitopes on the different precipitin arcs and/or the quantity of specific IgE antibody in individual serum for specific epitopes on the different precipitin arcs. The wide range of radiostaining strengths by the same precipitin arc between patients indirectly indicated a high availability of IgE specific epitopes for most fecal and body Ags in the precipitate complex. Therefore, the variation in radiostaining strength within an individual serum indicated varied levels of specific IgE for most of the specific Ags. In cases in which the different individual sera were reacted with the same Ag and antiserum system, the observed variability in both radiostaining frequency and strength directly indicated varied IgE levels in the sera.

Overall, the results demonstrated that individual patient sera of each allergic patient may have IgE antibodies with predilection toward specific Ags in either extract. This wide variation of specific IgE binding coupled with predilection toward specific Ags by different serum antibodies indicated that specific allergens originating from both mite bodies and mite feces demonstrate clinical specificity in certain individuals. Likewise, specific allergens of one extract (body or fecal) may be more important than those of the other in individual patients. Comparison of specific IgE binding to Ags in both DF body and DF feces extracts with the same test serum clearly demonstrated the differences of relative importance between the allergens from the two different extracts as well as differences in composition of the two extracts. For example, serum a and c demonstrated IgE binding to more body Ags than fecal Ags (Figs. 3, A and C, and 4, A and C). Conversely, serum b demonstrated IgE binding to more fecal Ags than body Ags (Figs. 3, B, and 4, B).

These widely varied responses to both body and fecal Ags are important in considering the composition of extracts used for clinical testing and immunotherapy, particularly if some mite components contain or are the sources of allergens either not present or present in only small quantities in other mite components. Unlike extracts of *D. pteronyssinus* in which P_1 is reportedly more highly concentrated in fecal material, on a weight basis, DF body and fecal extracts contained different allergens that appeared to be equally potent. Determination of the relative importance of each DF extract allergen in this study was limited by the small number of patients tested. To define major or minor allergens more precisely based on the frequency and quantity of IgE binding by CRIE or other methods, it will be necessary to analyze sera from a larger cross section of mite-sensitive patients.

The heterologous CRIE experiments clearly demonstrated both cross antigenicity and allergenicity between the DF body and DF feces extracts and also qualitative differences between the two extracts. This cross-reactivity between the two extracts could be due to common components in both extracts or similar antigenic determinants among different components. Electrophoresis of the same quantity of DF feces extract into both DF feces and DF body antiserum (Figs. 1, B, and 5, A) demonstrated several identical precipitin peaks (position and size) for the two different antisera. The reciprocal system in which the same quantity of DF body extract was electrophoresed into both DF body and DF fecal antisera elicited similar results. These results indicate that both extracts that were prepared on an identical weight/volume basis contain some common Ags and that these were present in similar concentrations. This cross-reactivity between mite body and feces extracts may be derived from the peritrophic membrane that is present in the mite gut and also encapsulates fecal pellets, other fecal components such as bacteria and altered mite food material, or mite secretory products from the supracoxal, lateral opisthosomal, and/or salivary glands that may become dispersed through a mite culture.

It is probable that most of the Ags and allergens originating from fecal material were also present in extracts prepared from medium-free bodies. This was evidenced by the fact that heterologous CIE tests with DF feces extract and DF body antiserum identified 15 precipitin lines, whereas homologous CIE tests with DF feces extract and DF feces antiserum identified only five additional ones. Moreover, the intermediate gel CIE systems demonstrated that none of the DF feces precipitates were specific for DF feces extract alone. The five Ags that did not appear in the heterologous CIE may in fact have been present in the

DF body extract but in such low concentrations that they did not stimulate significant antibody production in the rabbit; thus, they were not visualized by CIE in DF body antiserum. This is likely because a small quantity of fecal material is naturally present in the gut of many of the mites harvested for extract preparation. In this study, for example, 48% of the specimens used to prepare DF body extract contained one to two fecal pellets in the gut. Because of this possibility, some quantitative differences in IgE binding exhibited by patients in this study may also have reflected higher concentrations of fecal allergens in the DF fecal extract than in the DF body extract.

Despite the cross-reactivity of body and fecal extracts, mite body extracts clearly contained specific Ags and allergens that originated from body components and secretions not associated with the gut, feces, or peritrophic membrane. The fact that homologous CIE of DF bodies identified 35 precipitin lines, whereas homologous CIE of DF feces identified only 20 lines, coupled with the fact that sera from mite-sensitive patients recognized up to 14 allergens in body extract but only seven allergens in feces extract, support this conclusion. Direct substantive evidence of this interpretation was provided by intermediate gel CIE experiments. The fact that CIE of DF body and DF feces Ags into various arrangements of DF body and DF feces antisera in the intermediate and reference gels resulted in the formation of precipitin peaks without well-developed tops in the intermediate gels or precipitin peaks only in the reference gels above the intermediate gels clearly confirmed the presence of mite body-specific antigens. Although possible concentration effects of common body Ags could account for different precipitin profiles of DF body and DF feces extracts in the heterologous CIE experiments, the intermediate gel CIE results indicate that body Ags not associated with feces or the peritrophic membrane from the mite midgut may be of primary importance for some patients. Study of sera from a larger population of mite-sensitive patients will be required to determine if any of these DF body-specific Ags are clinically important allergens.

The results of this study pose several considerations that may have future clinical relevance. Because of the quantitative and qualitative variability in IgE binding by the same patient and by different patients, only extracts containing high concentrations of both body and feces allergens should be used for clinical testing and immunotherapy. Currently, only extracts of mite bodies or whole cultures are commercially available. Commercial mite extracts should not contain ill-defined quantities of organic CM because such materials may dilute clinically significant mite allergens, be a

source of irrelevant allergens, or sensitize patients to new allergens.

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