

Allergenicity of *Euroglyphus maynei* and its cross-reactivity with *Dermatophagoides* species

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Background: The house dust mite, *Euroglyphus maynei* (EM), is common in homes in England, Europe, the southern United States, and other parts of the world. It is often present in densities greater than 100 mites per gram of dust. EM usually occurs with *Dermatophagoides farinae* (DF) and *D. pteronyssinus* (DP) and it is frequently more abundant than *Dermatophagoides* species. Therefore the allergenicity of EM and the cross-reactivity between EM and *Dermatophagoides* species are important considerations for diagnosis of mite-induced allergy and the use of appropriate immunotherapy.

Methods: Crossed immunoelectrophoresis revealed that EM was the source of at least 33 antigens. Crossed radioimmuno-electrophoresis with 32 different sera from patients with RAST results that were positive to EM and skin test results that were positive to both DF and DP identified 15 allergens. Individual sera recognized two to eight EM antigens as allergens.

Results: Ninety-one percent of the patients showed serum IgE directed at three or more allergens. Eighty-four percent of the patients had moderate or strong levels of IgE directed at antigen no. 33. All 15 allergens showed moderate or strong IgE binding by one or more sera. EM shared four and six cross-reacting allergens with DF and DP, respectively. Therefore 11 and 9 allergens of EM were species-specific and not shared by DF and DP, respectively.

Conclusions: The results of this study clearly indicate that sensitivity to EM should be considered in geographic areas where this mite is abundant in homes. (J ALLERGY CLIN IMMUNOL 1993;91:1051-8.)

Key words: *Euroglyphus maynei*, *Dermatophagoides* species, antigenicity, allergenicity, allergic disease, house dust mites, cross-reactivity, asthma, rhinitis

The dust mite, *Euroglyphus maynei* (EM), is found in homes in some geographic areas in almost all countries where dust mite surveys have been conducted.^{1, 2} In some homes in some locations EM was the dominant species or constituted more than 20% of the total house dust mite population.³⁻¹⁷ For example, in a survey in England, EM was as common as *Dermatophagoides pteronyssinus* (DP) or *D. farinae* (DF) in the homes of individuals with asthma, and it was the predominant species in 48% of beds examined.⁸ A recent study of U.S. homes found that EM oc-

Abbreviations used

EM:	<i>Euroglyphus maynei</i>
DF:	<i>Dermatophagoides farinae</i>
DP:	<i>Dermatophagoides pteronyssinus</i>
CM:	Culture medium
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmuno-electrophoresis
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

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curred in 25% of 90 homes of subjects with asthma that were surveyed in New Orleans, Galveston, Memphis, Delray Beach, and San Diego and was the most abundant species in five homes.¹ In another study, EM was the dominant species in 1 of 20 homes sampled in Atlanta.¹⁷ Generally, worldwide detailed surveys of mite fauna in dust show that most homes with EM are also coinhabited with DF, DP, or both.^{1, 2, 12, 17}

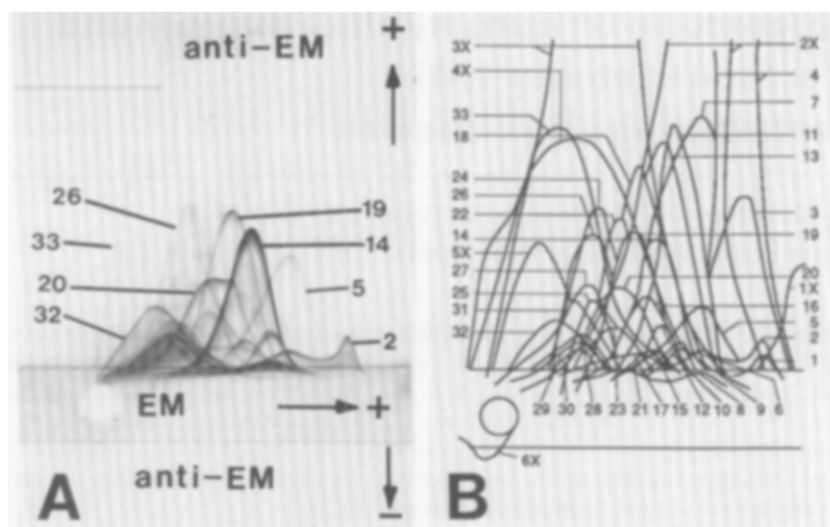


FIG. 1. EM extract reacted with rabbit anti-EM serum. **A**, Homologous CIE antigen pattern (only some antigens are numbered as a reference); **B**, schematic profile with all antigens numbered. Numbers followed by X indicate antigens visualized by CRIE only.

Sensitization to EM has been reported.¹⁸⁻²⁴ Several studies have reported that EM extracts produced positive skin test reactions in a high percentage of patients (51% to 85%) with allergic asthma and/or rhinitis and positive skin reactions to other mites (mainly DF and DP).¹⁸⁻²¹ Similarly, Van Hage-Hamsten and Johansson²² reported that among 64 farmers from the island of Gotland, Sweden, who had RAST results that were positive to one or more of the mites, *Acarus siro*, *Lepidoglyphus destructor*, EM, and DP, 37 had RAST results that were positive to EM. Two studies have characterized the IgE-binding response to protein components of EM extracts after they were fractionated by nonreducing sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).^{23, 24} The two studies identified a high number of allergens but did not agree on the degree of allergenic homology between EM and DP. Hill et al.²³ reported that EM extract had 30 IgE-binding bands that range from 185 kd to less than 14 kd in molecular weight. All but 6 of the 30 IgE-binding bands of EM had comparable IgE-binding bands in DP extracts, which leads to the conclusion that the spectrum of allergens in EM was generally similar to that found in DP. By contrast, Colloff et al.²⁴ identified 25 and 28 IgE-binding components for EM and DP, respectively. However, the homology between IgE-binding components of DP and EM was relatively low. Only 32% of the components were common to both DP and EM, and 30% were unique to EM. Both studies identified EM allergens that were

similar in molecular weight to the group I and II allergens of DP. Platts-Mills et al.,²⁵ using a radioimmunoassay, directly demonstrated that EM was the source of an allergen that cross-reacted with *Der p* I. No studies have examined the relationship between EM and DF.

Despite the abundance of EM in homes throughout the world and preliminary evidence that this species is a source of potent allergens, the clinical significance of EM allergens is still unknown, and EM extracts have not been well characterized. There are inconsistencies in the reports of the amount of cross-allergenicity between EM and DP in studies in which SDS-PAGE and immunoblotting were used. Also, the cross-reactivity between EM and DF has not been investigated. Levels of *Der p* I and *Der f* I in house dust are used to determine the causal relationship between DP and DF allergen exposure and the risk of asthma/rhinitis in mite-sensitive individuals. Such assays may overlook the presence of important species-specific EM allergens. Therefore the purpose of this study was to characterize the allergenicity of EM and determine its immunologic relationship to both DP and DF with the use of crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE).

METHODS

Mite extract

Gram quantities of pure EM, DP, and DF mites were brushed from culture container rims as mites migrated from the culture medium (CM) of thriving cultures.

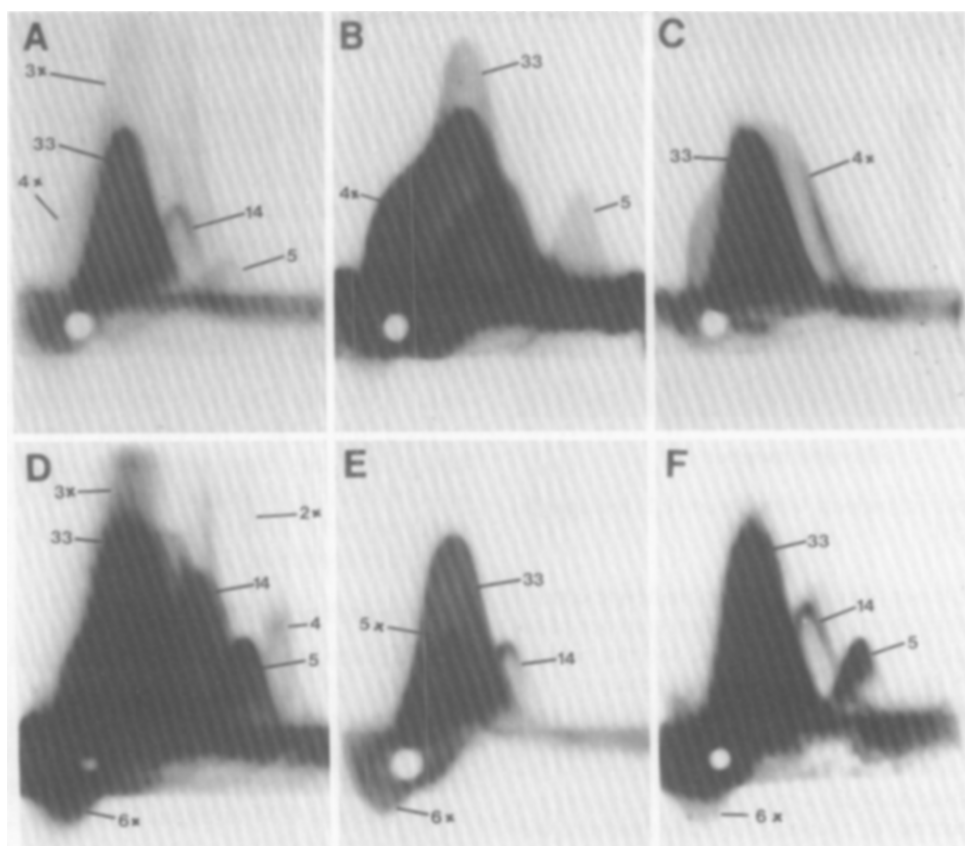


FIG. 2. A to F, Homologous CRIE of EM extract reacted with rabbit anti-EM serum showing IgE binding for six representative sera. Only representative allergens are numbered.

The mites were killed by freezing, desiccated under vacuum at room temperature, and then stored dry at -17°C until extracts were prepared. Aqueous extracts (1:10 wt/vol) of both the CM and dried mites were prepared as previously described.^{26, 27} The protein contents of the extracts made from EM mites and CM were 3.4 and 2.6 mg/ml, respectively. Protein contents of DF and DP were 3.3 and 4.8 mg/ml, respectively. Protein contents were determined by the modified Bradford assay (Bio-Rad Laboratories, Richmond, Calif.) with bovine plasma albumin as the reference protein.

Rabbit antisera

Antisera to EM and CM extracts were produced in New Zealand white rabbits with the same methods as previously reported.^{26, 27} Initial subcutaneous injections contained 2.5 ml of extract (8.5 and 6.5 mg protein for EM and CM, respectively) emulsified in an equal volume of Freund's complete adjuvant. Booster injections of 2.5 ml of extract in Freund's incomplete adjuvant were given in the same manner at approximately 2-week intervals. High-titer antiserum to EM and CM extracts were produced for each rabbit after three boosts. The high-titer antisera for each extract were pooled and frozen. DF and DP antisera were the same as previously reported.^{26, 27}

Human sera

Thirty-two serum samples from patients who had RAST results that were positive (class III or greater) to EM and DP and skin test results that were positive to DP and DF (3+ or 4+ to both) (>15 mm wheal) were used to evaluate the allergen content of the EM extract. All patients had asthma or rhinitis and lived in an area where EM, DP, and DF were prevalent in homes. Eleven individuals who had no history of allergy and no sensitivity to mites (according to RAST or skin test) and who lived in an area where only DF and DP were found served as control subjects.

Immuno-electrophoresis

CIE and CRIE were performed by previously described methods^{26, 27} to visualize respective antigens and allergens in the extracts. For homologous and heterologous CIE, 5–25 μl of extract (1:10 wt/vol) were electrophoresed in the first dimension in a 1% agarose gel with Svendsen's buffer at 200 V for 35 minutes at 13.5°C . Second-dimension anodic and cathodic 1% agarose gels contained 200 to 300 and 60 μl of rabbit antiserum, respectively. Completed gels were stained with Coomassie brilliant blue R-250 to visualize the antigen-antibody precipitates.

CRIE was performed with 1 ml of human serum

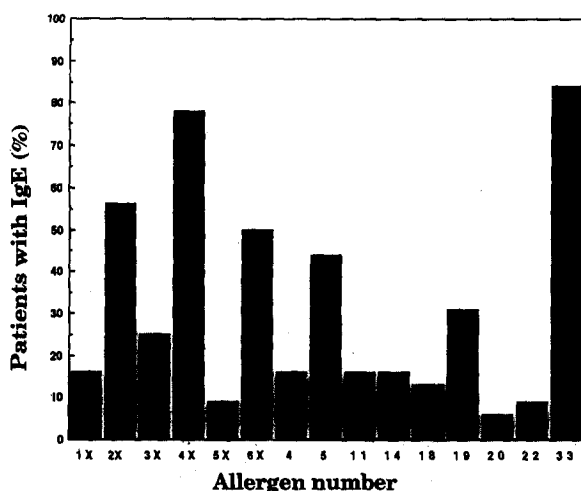


FIG. 3. Percent of patients with serum IgE for specific allergens of EM.

from each mite-sensitive patient or nonallergic control subject. Total radioactivity was 1 million cpm per plate. Autoradiography was performed for specific periods from 1 to 18 days. To standardize and compare IgE binding both within and between sera, all gels were processed identically in one batch with one batch (lot) of reagents and materials (e.g., buffers, iodine-125-labeled anti-human IgE, x-ray film). The system for scoring the potency of allergens for individual sera was similar to that of Lind and Lowenstein.²⁸ An allergen was defined as strong (potent), moderate, or weak if the antigen produced radiostaining on the autoradiographs after 2, 5, or 10 days of exposure, respectively. Allergens that were recognized by $\geq 50\%$ of the reference sera were considered to be major allergens.

RESULTS

Identification of antigens in extracts of EM

The CIE reaction of EM extract with rabbit anti-EM serum resulted in the formation of 33 visible antigen-antibody precipitin complexes that stained with Coomassie brilliant blue (Fig. 1, A). Some precipitin complexes were intensely stained and clearly resolved, whereas others stained only weakly and were only faintly visible. Six additional antigen-antibody precipitin complexes were identified by autoradiography (CRIE). Therefore the total number of antigens in the extract was 39. Fig. 1, B shows a composite reference drawing that demonstrates all individual antigen-antibody precipitin complexes obtained after homologous CIE and CRIE reactions.

It was possible that some of the antigens in the EM extract originated from the mite culture medium that remained in the mite gut or adhered to the mite body. Therefore with the use of CIE, EM

extract was reacted with anti-CM serum to directly determine whether EM extract contained antigens from the CM. This reaction resulted in six visible antigen-antibody precipitin complexes on the CIE gel. The reciprocal reaction produced four antigen-antibody precipitin complexes. These CIE gels were then assayed by CRIE to detect the presence of antigens that did not stain with Coomassie brilliant blue and to determine whether any CM antigens were allergens. Autoradiograms of these heterologous CRIEs exhibited no radiostaining. Therefore all the antigens that originated from the CM were stained with Coomassie brilliant blue. More importantly, none of the visualized antigens from the CM bound IgE in the sera of these EM-sensitive patients. After the six antigens were subtracted for the CM, EM mites were the source of 33 antigens.

Allergens from EM

CRIE was performed with individual sera from the 32 EM-sensitive patients to determine whether serum IgE reacted with antigens of each precipitin complex that resulted from the CIE reaction of EM extract with anti-EM serum. Fifteen different EM antigens bound IgE, and individual sera exhibited varied IgE titers to specific antigens (Figs. 2 and 3). Individual patients had IgE directed at two to eight antigens. Ninety-one percent and 63% of the patients had serum IgE directed at three or more and five or more EM allergens, respectively. Allergen nos. 33, 4x, 2x, and 6x bound IgE in the serum of 84%, 78%, 56%, and 50% of the patients, respectively (Fig. 3). All 15 different allergens showed moderate or strong IgE binding by at least one serum. Eighty-four percent of the patients had moderate or strong levels of IgE directed at antigen no. 33. Forty-seven percent and 34% of the patients had moderate to strong levels of serum IgE directed at allergen nos. 4x and 6x, respectively.

Cross-reactivity between EM and DP

Heterologous CIE was performed to evaluate the potential cross-reactivity between EM and DP or DP. CIE of EM extract electrophoresed into DP antiserum resulted in at least 15 antigen-antibody precipitin lines (Fig. 4, A). CRIE was then performed with the sera from the 32 EM-sensitive patients and 11 control subjects (Fig. 4, B). Thirty-one of the sera from the 32 EM-sensitive patients contained IgE antibodies that bound to one to six cross-reacting antigens. A

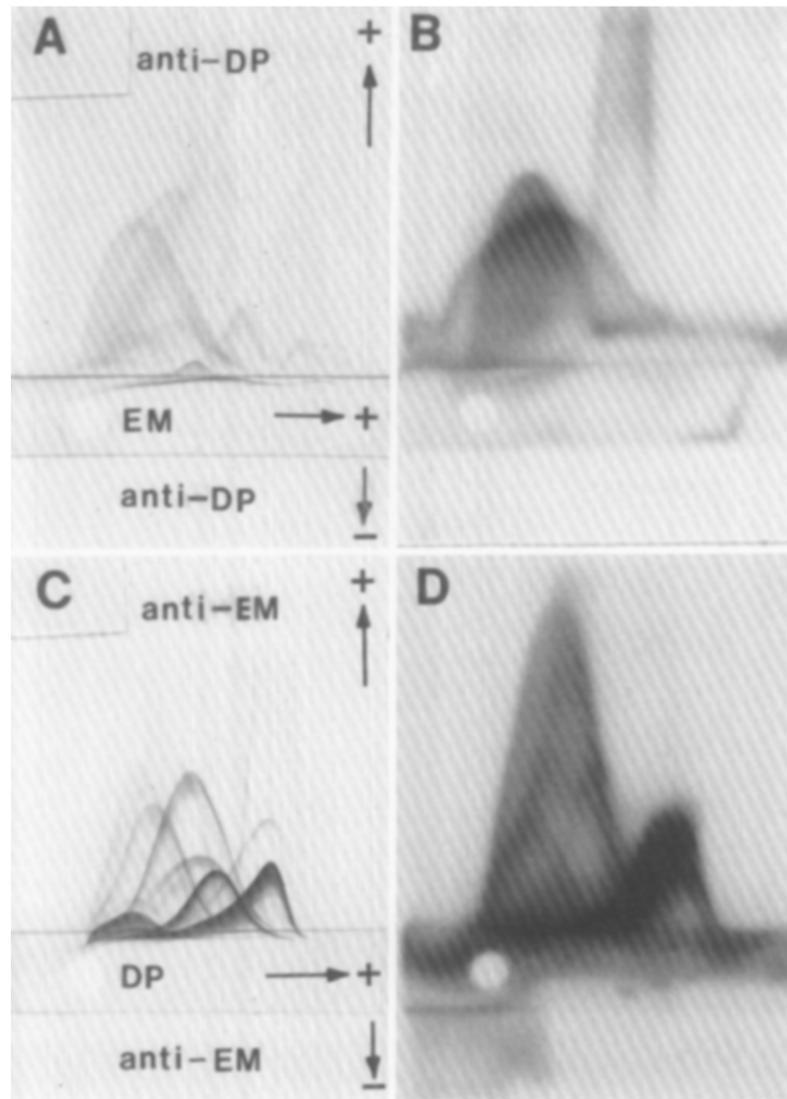


FIG. 4. A, CIE pattern of EM extract reacted with rabbit anti-DP serum; B, autoradiogram of A with a representative serum; C, CIE pattern of DP extract reacted with rabbit anti-EM serum; D, autoradiogram of C with representative serum.

total of six cross-reacting allergens were identified. It should be noted that in this reaction the IgE bound to EM antigens that were precipitated by antibodies raised to DP. Seventy-two percent of the sera contained moderate to high levels of IgE directed at at least one cross-reacting allergen. Nine percent of the sera had moderate to high levels of IgE directed at more than two of the six cross-reacting antigens. Considering all levels of IgE binding (weak to strong), 88% (28 of 32) of the sera had IgE directed at the same two cross-reacting allergens.

CIE of DP extract that was reacted with EM

antiserum (reciprocal of above) also resulted in the identification of 15 cross-reacting antigens (Fig. 4, C). In this case, DP antigens were precipitated by antibodies raised to EM antigens. Six of these cross-reacting antigens bound IgE when probed with the sera of the 32 patients (Fig. 4, D), with the number recognized by an individual patient ranging from zero to six. Forty-seven percent of the sera contained moderate to high levels of IgE directed at three or more cross-reacting allergens. Seventy-eight percent (25 of 32) had IgE directed at the same three cross-reacting allergens.

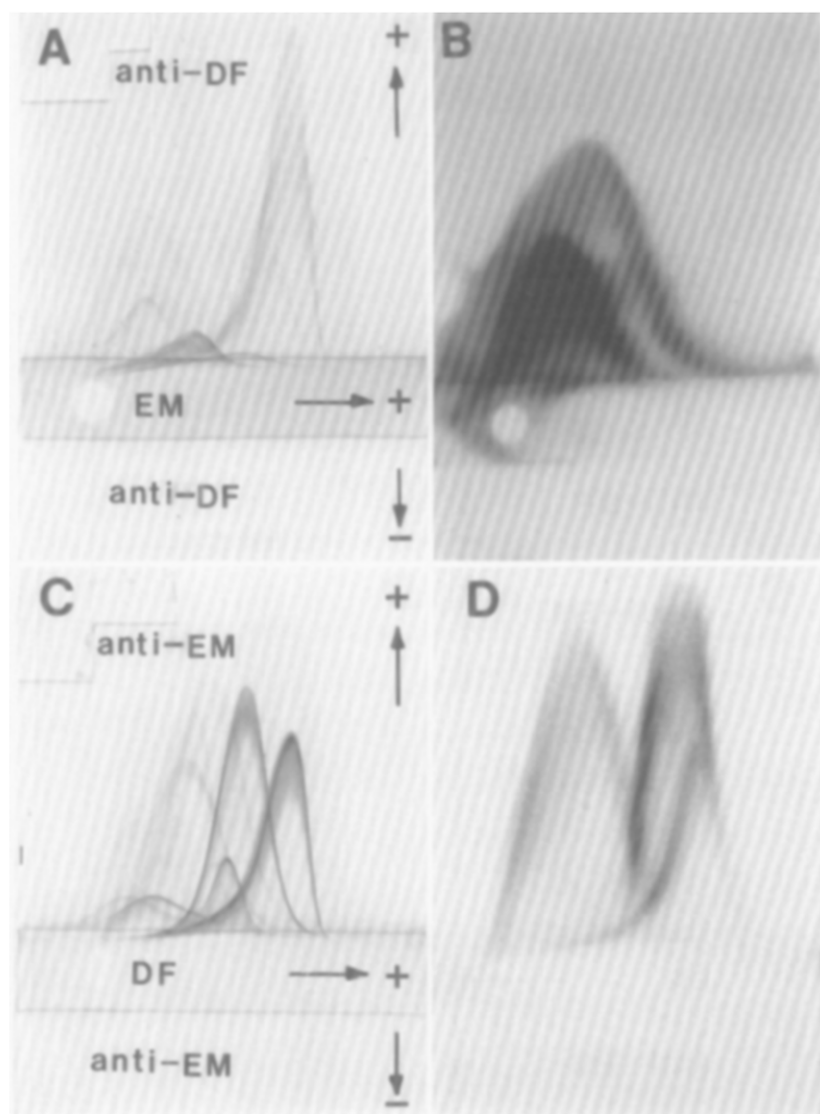


FIG. 5. A, CIE pattern of EM extract reacted with rabbit anti-DF serum; B, autoradiogram of A with a representative serum; C, CIE pattern of DF extract reacted with rabbit anti-EM serum; D, autoradiogram of C with a representative serum.

Cross-reactivity between EM and DF

Heterologous CIE reactions of EM extract with anti-DF serum or the reciprocal reaction (DF extract into anti-EM serum) resulted in the identification of 15 cross-reacting antigens for the two species (Fig. 5, A and C). Autoradiographic analysis with the 32 individual sera demonstrated that all sera had IgE directed at EM antigens that were precipitated by anti-DF rabbit serum (Fig. 5, B). A total of four cross-reacting allergens bound IgE, but the number recognized by individual sera ranged from one to four. Similarly, autoradiographic analysis of DF antigens precipitated by

anti-EM serum also identified four cross-reacting allergens (Fig. 5, D).

DISCUSSION

Many studies have shown that EM is a common mite in homes throughout world and may be the dominant species in some homes.¹⁻¹⁷ Thus individuals may be exposed to large quantities of allergens from these mites. This study clearly demonstrated that EM is the source of at least 33 antigens; at least 15 of these antigens bound circulating IgE in the sera of the EM-sensitive reference patients. Every allergen (15 total) was

recognized as a potent allergen by at least one serum. However, the IgE titer to specific allergens and the number of allergens recognized varied both within and between sera. Most sera (84%) consistently recognized one of the antigens as a potent allergen. In addition, about one half and one third of the patients' sera recognized two other antigens (same ones) as potent allergens. Four antigens were recognized as allergens by 50% or more of the reference sera. Therefore on the basis of the frequency that an allergen was recognized, EM was determined to be the source of at least four major and 11 minor allergens. These results confirm that sensitization to EM mites should be considered in individuals with asthma and rhinitis who live in geographic areas where EM is prevalent.

Hill et al.²³ and Colloff et al.²⁴ found that the majority of patients tested had circulating IgE directed at the same two to three SDS-PAGE-resolved protein components. Our results are consistent with those of Hill et al.²³ and Colloff et al.²⁴ in that the majority of our patients also had high titers of IgE directed at the same three to four CIE-resolved allergens. However, we found only 15 antigens that bound IgE, whereas their studies reported 25 to 30 IgE binding components. There are several possible explanations for this discrepancy. It is possible that the sera of their patients came from individuals with a greater exposure and thus sensitization and specificity to more individual allergens.^{28, 29} EM is the dominant mite species in many homes in England.^{2, 4} The sera for our study came from patients in the Tampa Bay area where EM is present in some homes, but in general it is not the dominant mite species.³⁰ Less likely but possible is that some of the IgE binding epitopes on the antigens in the antigen-antibody complexes on CIE were blocked by the precipitating rabbit antibody, so they did not bind IgE and could not be identified by CRIE as suggested previously for DF and DP.^{28, 29} These same antigens bind IgE when resolved by SDS-PAGE. Likewise, CRIE would not detect allergens to which there was absence of an antibody response in rabbits. Finally, Hill et al.²³ and Colloff et al.²⁴ used nonreducing SDS-PAGE; however, because of enzymatic hydrolysis, some proteins in the extracts may have dissociated into constituent polypeptide chains or subunits, and thus the large number of IgE components they observed were allergenic peptides and not intact allergen molecules.

Our study indicates that 15 EM antigens cross-

reacted with antigens of both DF and DP. All but one of the 32 reference sera that had IgE directed at EM antigens also had IgE binding to one to six cross-reacting antigens. A total of four and six different EM antigens were allergens that cross-reacted with DF and DP, respectively; however, most sera consistently recognized the same two to three cross-reacting allergens. This suggests that there are two to three major cross-reacting allergens and that the majority of EM allergens are species-specific; EM produces 9 and 11 species-specific allergens when compared with DP and DF, respectively. The fact that individual sera showed IgE binding to more EM allergens than cross-reacting allergens also confirms that some EM allergens are species-specific. Our results confirm those of a previous study that reported that some farmers who had RAST results that were positive to EM did not have results that were positive to DP, which suggested that EM was the source of species-specific allergens.²² In the same study²² RAST inhibition experiments showed reciprocal inhibition, which also confirmed that EM and DP possess cross-reactive allergens.

Considerable research is still required in the identification, purification, characterization, and standardization of EM allergens. These are prerequisites for the development of specific allergenic assays (monoclonal antibodies or other) that are needed for clinical evaluations and to quantitate allergenic activity in dust. Whether antigen P 1 equivalent (P 1 eq) is an adequate relative measure of common epitopes on EM allergens and group I allergens of DF and DP must be determined. Preliminary studies indicate that P 1 eq detects an allergen produced by EM.²⁵ The relative importance of the three most frequently recognized potent allergens and the remaining 12 antigens of EM that are strong allergens for some individuals must be determined so that an appropriate extract can be produced. Likewise, the relationship between the two to three major cross-reacting EM allergens and group I, II, and III allergens of DF and DP must be determined. Studies are now needed to determine the prevalence of sensitization to EM and the clinical importance of EM allergens in populations in areas where EM mites are prevalent in house dust.

In conclusion, this study reports that EM is the source of potent species-specific allergens and of allergens that are cross-reactive with DF and DP. Therefore the allergenicity of EM and the cross-reactivity between EM and *Dermatophagoides* species are important considerations for diagnosis of

mite-induced allergy and the use of immunotherapy in areas where EM, DF, and DP are prevalent in homes. EM mites should be considered as a potential cause of asthma and rhinitis in geographic areas where these mites are found.

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