

Cross-allergenicity of the house dust mites *Euroglyphus maynei* and *Blomia tropicalis*

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Background: The domestic mites *Euroglyphus maynei* and *Blomia tropicalis* frequently co-inhabit homes in subtropical/tropical regions around the world. Both species are the source of substances that cause allergic reactions in patients.

Objective: The purpose of this study was to examine the immunologic cross-reactivity between *E. maynei* and *B. tropicalis*.

Methods: Sera of 19 mite-sensitive patients who were skin test positive to *B. tropicalis* and/or RAST positive to *E. maynei* were used to probe immunoblots or crossed radioimmuno-electrophoresis (CRIE) gels.

Results: Western blotting showed that individual sera had IgE that bound to 0 to 17 and 2 to 15 proteins in *E. maynei* and *B. tropicalis* extracts, respectively. Corresponding IgE-binding proteins of 105, 75, 57, 18, and 14 kD were detected in both *E. maynei* and *B. tropicalis* extracts. The majority of IgE-binding proteins did not show corresponding bands in both extracts. Heterologous CRIE showed IgE binding to six of the nine *E. maynei* antigens precipitated by anti-*B. tropicalis* serum with individual sera recognizing 0 to 4 of the six allergens. In the reciprocal reaction, 10 of the 12 proteins of *B. tropicalis* that were precipitated by anti-*E. maynei* serum bound IgE with individual sera binding to 0 to 5 proteins.

Conclusion: This study indicated that *E. maynei* and *B. tropicalis* are the source of both species-specific and cross-reactive allergens, but most allergens in each extract were species-specific.

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INTRODUCTION

The domestic mites *Blomia tropicalis* and *Euroglyphus maynei* co-inhabit homes in the subtropical regions of the United States^{1,2} and in tropical climates around the world.³⁻⁷ Both mite species induce allergic sensitization in patients.⁷⁻¹¹ Significant percentages of mite-sensitive patients give positive skin reactions to both species even when they reside in regions where these mites have not been reported. These mites are the source of multiple allergens and several studies have attempted to characterize the proteins produced by each species. Previous immunoblotting studies reported that *B. tropicalis* is the source of 16 to 25

allergens with four important allergens of 11 to 13, 33, 36, and 64 kD.¹²⁻¹⁴ Another study used crossed radioimmuno-electrophoresis (CRIE) to demonstrate that *B. tropicalis* was the source of 21 allergens of which $\leq$ four were cross-reactive with the other astigmatid mites *Dermatophagoides farinae*, *D. pteronyssinus*, and *Tyrophagus putrescentiae*.¹⁵ Immunoabsorption experiments using *D. pteronyssinus* extracts also showed that approximately 70% of IgE antibody directed at *B. tropicalis* recognized species-specific epitopes.¹⁶ Similar characterization studies have been carried out using *E. maynei* and have determined this mite to be the source of both cross-reactive and species-specific allergens.^{7,17-19} No study, however, has examined the potential cross-reactivity of *B. tropicalis* and *E. maynei*.

The purpose of this study was to directly examine the immunologic

cross-reactivity of *B. tropicalis* and *E. maynei*.

METHODS

Antigen extracts

Extracts were prepared from whole mite bodies collected from pure cultures of *Blomia tropicalis* and *Euroglyphus maynei* as previously described.^{15,17} Briefly, dried mites were ground, defatted, extracted 1:10 (wt/vol) in glass distilled water, centrifuged, and the supernatant (extract) was filter sterilized and stored at 4 °C until used. Protein concentrations were determined by the Bradford method using bovine plasma albumin as the standard.²⁰

Antisera

Antisera to *B. tropicalis* and *E. maynei* were produced previously by immunizing New Zealand White rabbits with extracts prepared from whole mite bodies.^{15,17}

Human Sera

Human sera were obtained from 19 asthmatic individuals (mean age of 30 years) who live in Tampa, FL, an area where both *B. tropicalis* and *E. maynei* are prevalent.^{1,2} Patients were skin test positive to *B. tropicalis* and/or radioallergosorbent test (RAST) positive to *E. maynei*.²¹ Serum was also obtained from two individuals residing in an area where *D. farinae* and *D. pteronyssinus*, but not *B. tropicalis* and *E. maynei* are found. These sera were RAST negative to both *D. farinae* and *D. pteronyssinus* and were used as negative controls.

PAGE/Immunoblotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (PAGE) and immunoblotting were performed to determine the molecular weights of proteins and IgE-binding allergens contained in

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both mite extracts. Electrophoresis was carried out on 12% acrylamide gels under non-reducing (no reducing agent added to sample buffer) conditions using the method of Laemmli.²² Each lane was loaded with 15- μ g of protein and Kaleidoscope prestained standards (Bio-Rad, Hercules, CA) were used to provide molecular weight references. Proteins on parts of each gel were visualized by silver staining.^{23,24} PAGE-resolved proteins were electrophoretically transferred to Pro-Blott PVDF (polyvinylidene difluoride) membrane (Applied Biosystems, Foster City, CA) in 10 mM CAPS (3-[cyclohexylamino]-1-propanesulfonic acid), 10% methanol, pH 11 according to the manufacturer's recommendations. Blots were cut into pieces containing lanes with standard *B. tropicalis* and *E. maynei*. These strips were blocked overnight with a solution composed of 8 M urea, 0.1 M 2-mercaptoethanol and 5 mg/mL bovine serum albumin (BSA).^{23,24} Each strip was incubated overnight at room temperature in 1 mL serum diluted in 25 mL of 0.1 M phosphate buffer, pH 7.5 with 2 mg/mL of BSA and 1% Tween 20 (RAST A buffer). IgE-binding allergens were visualized as previously described^{23,24} by incubating strips with 1 million cpm ¹²⁵I-labeled anti-human-IgE (KallestadTM AllercoatTM RAST isotope reagent, Sanofi Diagnostics Pasteur, Chaska, MN) diluted in 20 mL buffer. Blots were washed, dried and placed in cassettes with tungsten-enhancement screens and Kodak X-OMAT LS film at -80 °C. Film was exposed for one, three, or ten days. To standardize and to make comparisons, all blots were processed identically in one batch with one lot of reagents.

Following autoradiography, IgG antibody binding to the strips was detected using peroxidase-labeled goat anti-human-IgG (Fisher Scientific, Pittsburgh, PA) at 1:2,000 in RAST buffer. All strips were developed for five minutes using the CND (4-chloronaphthol/3,3'-diaminobenzidine/H₂O₂) chromogenic substrate of Young.²⁵

Several strips were also probed with the mouse monoclonal antibodies 1D8, 7A1 and α DpX (obtained from Dr. Martin D. Chapman, University of Virginia, Charlottesville) to identify group 2 allergens.²⁶ Murine antibody was diluted 1:400 in RAST A buffer and applied to strips for three hours at room temperature. Antibody binding was detected using alkaline phosphatase-labeled goat anti-mouse-IgG (Fisher Scientific) at 1:2,000 followed by development for 30 min with Biomedica BCIP/NBT (5-bromo, 4-chloro,3-indolylphosphate/nitroblue tetrazolium) chromogen (Fisher Scientific). This yielded a purple precipitate that was clearly distinguishable from the red/brown bands produced in the human IgG development reaction.

CIE AND CRIE

Crossed immunoelectrophoresis (CIE) was performed to visualize individual antigens contained in the two mite extracts as previously described.^{15,17} Fifteen microliters of mite extract (53 μ g of *B. tropicalis* and 45 μ g of *E. maynei* protein) was electrophoresed in the first dimension 1% agarose gel for 35 minutes at 200 V. Antigen-containing lanes were excised and placed transversely on a sheet of GelBond. An upper gel (anodic) containing 300 μ L of rabbit antiserum in 3 mL of 1% agarose and a lower gel (cathodic) with 50 μ L of serum in 1 mL of agarose were poured and electrophoresed overnight at 50 V. Both homologous (*B. tropicalis* extract reacted with *B. tropicalis* antiserum and *E. maynei* extract reacted with *E. maynei* antiserum) and heterologous (*B. tropicalis* extract reacted with *E. maynei* antiserum and *E. maynei* extract reacted with *B. tropicalis* antiserum) reactions were performed. Gel plates were washed, pressed, dried and stained with Coomassie Brilliant Blue R-250.

IgE-binding allergens were visualized by crossed radioimmunoelectrophoresis (CRIE) using unstained gels as previously described.^{15,17} Briefly, CIE gels were incubated overnight in 0.5 mL human serum diluted with 20 mL RAST A buffer. Gels were

washed, incubated with ¹²⁵I-labeled anti-human-IgE, washed, dried, placed in cassettes and subjected to autoradiography as described above for immunoblots.

Immunoadsorption

A pool of mite-allergic serum was prepared using serum from >50 *E. maynei* and/or *B. tropicalis*-allergic subjects (nine subjects from this study). Two 0.5-mL aliquots of this serum were diluted with 20 mL RAST A buffer. One sample was incubated sequentially with 13 *E. maynei* homologous gels for 8 to 24 hours each to

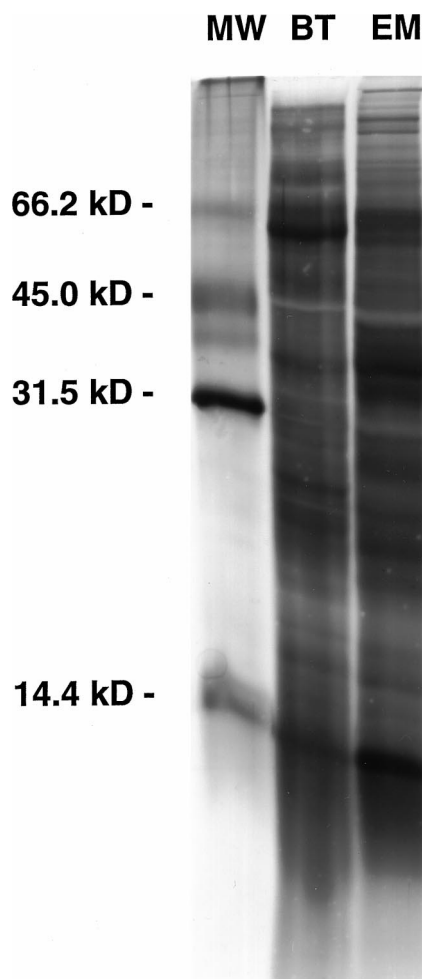


Figure 1. Silver stained, 12% PAGE gel of 15- μ g aliquots of *Blomia tropicalis* (BT) and *Euroglyphus maynei* (EM) extracts run under non-reducing conditions. Locations of molecular weight (MW) markers are shown for reference.

remove *E. maynei*-reactive IgE and IgG while the second sample was depleted of *B. tropicalis*-reactive IgE and IgG by similar incubation with *B. tropicalis* homologous gels. ¹²⁵I-Anti-human IgE binding to the thirteenth gel in each series was negligible. An *E. maynei* homologous gel was then incubated overnight in the *B. tropicalis*-depleted serum while a *B. tropicalis* gel was placed in the *E. maynei*-depleted serum. IgE binding to all gels was visualized as outlined above and all gels were exposed to film at the same time.

RESULTS

Aqueous extracts of both *E. maynei* and *B. tropicalis* showed >50 protein bands when resolved by non-reducing 12% PAGE. Proteins were visible throughout the 5 to 200 kD molecular weight range for each extract (Fig 1). Comparison of the banding profiles of the two mite extracts showed some matched and some unmatched molecular weight proteins. Immunoblots of the PAGE-resolved proteins of both species showed that many of these proteins (allergens) bound IgE present in the serum from the 14 mite-sensitive reference subjects (Figs 2 and 3).

At least 20 proteins of different molecular weights in the *E. maynei* extract bound immunoglobulin. Most bands bound both IgE and IgG however the intensity of binding of these two classes of antibody was generally inversely related. Individual sera contained IgE and IgG that bound to 0 to 17 and 5 to 20 proteins, respectively. Nine of 14 sera had IgE that bound to 13 or more allergens in *E. maynei*. The *E. maynei* allergens appeared relatively evenly distributed from 5 to 200 kD (Figs 2 and 3).

At least 20 PAGE-resolved proteins in *B. tropicalis* extract bound IgE and IgG antibody. Again, most bands bound both antibody types. Individual sera from these subjects showed IgE binding to 2 to 15 *B. tropicalis* allergens and IgG binding to 5 to 20 *B. tropicalis* proteins. Most sera had IgE to 8 or more allergens. The proteins that bound IgE most frequently had

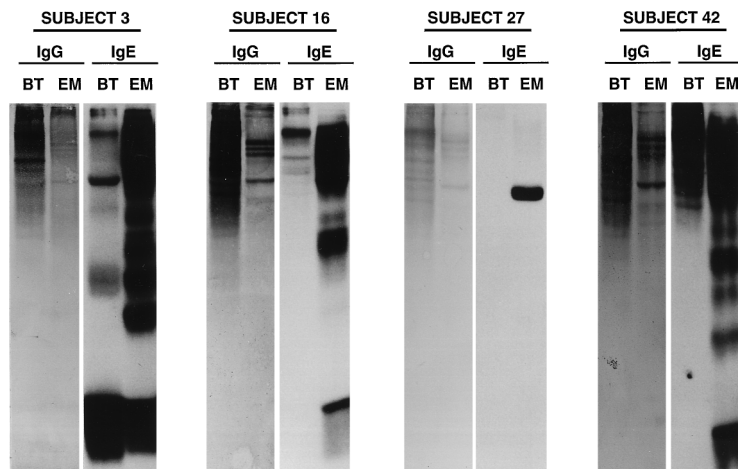


Figure 2. Representative immunoblots of *Blomia tropicalis* (BT) and *Euroglyphus maynei* (EM) extracts resolved on 12% PAGE gels under non-reducing conditions. Blots were incubated in serum from mite-allergic subjects and were probed for IgG and IgE binding as indicated in the "Methods."

molecular weights of 5 to 20 kD and 40 to 150 kD (Figs 2 and 3).

Comparing the two extracts, only five immunoglobulin-binding bands of the same molecular weights were observed in both *E. maynei* and *B. tropicalis* extracts (Fig 3). Proteins of approximately 105, 75, 57, 18, and 14 kD were detected in both *E. maynei* and *B. tropicalis* lanes by 2 (14%), 7 (50%), 5 (36%), 1 (7%), and 7 (50%) of the 14 subjects, respectively. These five pro-

teins with corresponding molecular weights bound both IgE and IgG. Three of the subjects (21%) did not recognize any of the cross-reactive proteins and none of the subjects had immunoglobulin that bound to all five proteins. When blots were subsequently probed with the mouse monoclonal antibody 1D8 (that recognizes Der p 2 but not Der f 2²⁶), this antibody bound to the 14 kD band in *E. maynei* identifying it as the allergen Eur m 2.

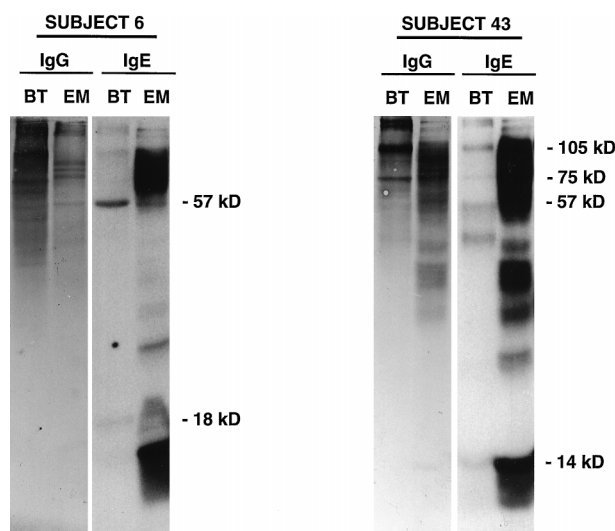


Figure 3. Representative immunoblots of *Blomia tropicalis* (BT) and *Euroglyphus maynei* (EM) extracts resolved on 12% PAGE gels under non-reducing conditions. Blots were incubated in serum from mite-allergic subjects and were probed for IgG and IgE binding as indicated in the "Methods." Molecular weights are indicated for those IgE-binding allergens visualized in both the BT and EM lanes.

No binding was observed in the *B. tropicalis* lane using this or two other anti-group 2 monoclonals (7A1 or α DpX²⁶).

Serum from 17 mite-allergic individuals was then used to probe for *E. maynei* and *B. tropicalis* allergens using homologous and heterologous CRIE gels (Fig 4). Individual subjects had circulating IgE that bound to three to ten of a possible 15 *E. maynei* allergens precipitated in homologous CRIE¹⁷ and to one to eight of a possible 21 *B. tropicalis* allergens precipitated in homologous CRIE.¹⁵

When *E. maynei* extract was reacted with *B. tropicalis* antiserum, nine different cross-reactive antigen/antibody precipitates were detected on the Coomassie blue stained CIE gels (Fig 5). Six of these bound IgE and were detected on autoradiograms (Fig 4). Individual sera showed IgE binding to zero to four of the six cross-reactive allergens. Each cross-reactive allergen was recognized by 5 (29%) to 9 (53%) of the subjects.

In the reciprocal reaction, *B. tropicalis* extract was reacted with antiserum raised against *E. maynei*. Crossed immunoelectrophoresis gels showed eight Coomassie blue staining antigen-antibody precipitates (Fig 5). Using the 17 subjects' sera and autoradiography, ten IgE binding allergens were identified (Fig 4). Six of these allergens were visible on both autoradiograms and Coomassie blue stained CIE gels while four were only visible by autoradiography. Two antigen-antibody precipitates were detected with Coomassie blue staining but did not bind IgE (were not seen on autoradiograms); therefore, at least 12 proteins from a *B. tropicalis* extract could be precipitated by *E. maynei* antiserum. Individual sera showed IgE binding to zero to five of the ten cross-reactive allergens. Each cross-reactive allergen was recognized by one (6%) to seven (41%) of the 17 subjects.

One subject showed antibody binding to three species-specific *E. maynei* and two species-specific *B. tropicalis* antigens but did not have circulating immunoglobulin that recognized any

of the cross-reactive allergens. Two non-mite-allergic subjects were used as negative controls in this study. Neither of these sera showed any IgE binding to any of the homologous or heterologous CRIEs.

Homologous CIE gels were also used to deplete a sample of pooled allergic serum of cross-reactive IgE and IgG. When a *B. tropicalis* homologous CRIE gel was incubated in serum that had been depleted of *E. maynei*-specific IgE and IgG by sequential incubation with 13 homologous *E. maynei* gels, all of the *B. tropicalis* allergens visualized using fresh serum were still visible (Fig 6). The reciprocal experiment, incubation of a homologous *E. maynei* gel in serum depleted of *B. tropicalis*-specific IgE and IgG, yielded even more striking results

since more IgE-binding allergens were visualized on the autoradiogram of this gel than were seen using fresh serum (Fig 6).

DISCUSSION

Using PAGE/immunoblotting we determined that extracts of *Blomia tropicalis* and *Euroglyphus maynei* had five corresponding IgE-binding allergens with molecular weights of 105, 75, 57, 18, and 14 kD in both *B. tropicalis* and *E. maynei* extracts, although each extract appeared to have > 20 immunoreactive (IgE and/or IgG) bands. A monoclonal antibody probe for Der p 2 demonstrated that the 14-kD protein in *E. maynei* had the same antigenic epitope as Der p 2 and was the group 2 allergen Eur m 2. This monoclonal antibody did not react with epitopes on

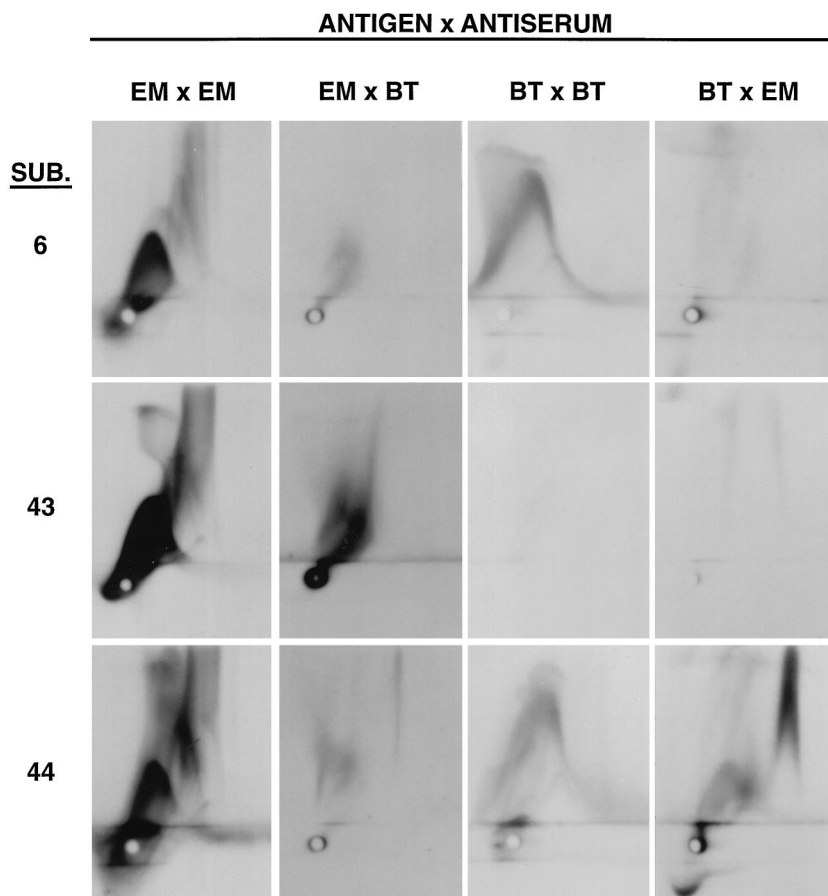


Figure 4. Homologous (EM x EM and BT x BT) and heterologous (EM x BT and BT x EM) CRIE showing IgE binding for three representative sera.

any other protein of *E. maynei*. In contrast, the matching 14-kD *B. tropicalis* allergen did not contain the corresponding Der p 2 antigenic epitope nor did this antibody bind to *B. tropicalis* proteins of other molecular weights.

The heterologous CRIE reactions revealed that *E. maynei* extract contained at least six allergens that were precipitated by anti-*B. tropicalis* serum while *B. tropicalis* extract contained ten allergens which were precipitated by *E. maynei* antiserum. Most of these cross-reactive allergens were recognized by fewer than half of the patients studied. Previous CRIE characterizations of the allergens of these mites demonstrated that *E. maynei* and *B. tropicalis* were the sources of 15 and 21 allergens, respectively^{15,17}; therefore, about half of the allergens of each species had cross-reacting epitopes. Removal of antibody directed at cross-reactive epitopes from serum by immunoadsorption resulted in increased IgE binding to species-specific allergens and IgE binding to a greater number of allergens. This was presumably due to removal of cross-reactive IgG (in addition to IgE) that had otherwise prevented binding of IgE to nearby species-specific epitopes. These heterologous CRIE and immunoadsorption studies clearly indicate that both *E. maynei* and *B. tropicalis* are the source of both species-specific and cross-reactive allergens.

The discrepancy in the numbers of cross-reactive allergens detected by PAGE/blotting (five allergens) versus CIE/CRIE (six to ten allergens) is not surprising. Proteins of different molecular weight can have the same epitopes. Likewise, as we illustrated with the monoclonal antibodies, a protein of the same molecular weight may possess different allergenic epitopes. In addition, proteins may exhibit a large amount of amino acid sequence and epitope homology and yet not run with the same mobility on PAGE due to differences in size, glycosylation, etc. Additionally, multiple proteins of similar size but different pI (isoelectric point) may run with the same Rf (relative electrophoretic mobility). This

could cause several individual allergens to behave as though they were a single protein. Two-dimensional electrophoresis was not performed to evaluate this possibility. Crossed immunoelectrophoresis remains a better technique for the evaluation of antigenic and allergenic cross-reactivity since in it proteins in one sample are precipitated by antibodies raised against a second sample. This means that only those proteins of the first sample possessing epitopes shared by proteins in the second sample are precipitated.

Demonstrating that these two mites each possess both unique and cross-reactive proteins has important implications for the diagnosis and control of mite-induced allergy. The majority of reference sera contained IgE directed at species-specific allergens and not cross-reactive allergens. Also, this study demonstrated that more than half the allergens produced by each mite

are unique while the others have both shared and unique epitopes. Skin test, RAST, ELISA, immunoblot, and CRIE studies had earlier shown that both *B. tropicalis* and *E. maynei* had proteins which cross-react with the Dermatophagoides mites as well as proteins that are unique.^{7,8,10,12,15-18,21,27-32} Taken together, this and previous studies reinforce the assertion that in areas where these mites (*D. farinae*, *D. pteronyssinus*, *E. maynei*, and *B. tropicalis*) are found, successful control of mite allergy will only be achieved when patients are diagnosed and treated with extracts of all mite species found in the local environment.

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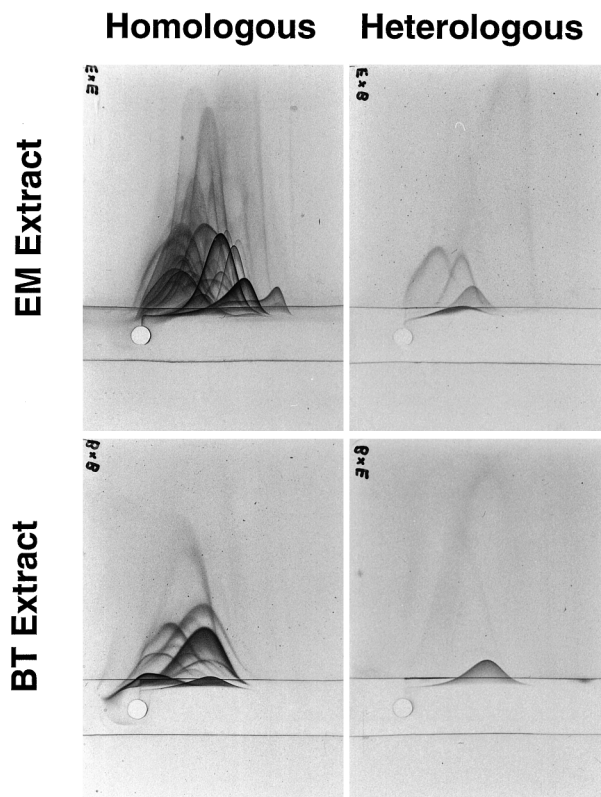


Figure 5. Coomassie blue stained homologous (EM x EM and BT x BT) and heterologous (EM x BT and BT x EM) CIE gels.

ANTIGEN x ANTISERUM

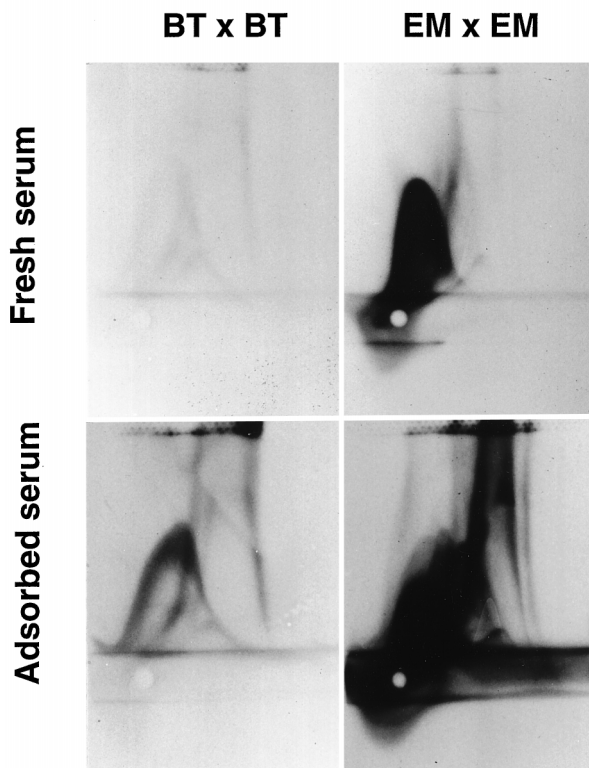


Figure 6. Homologous BT and EM CRIEs incubated in fresh serum (top row) or serum which had been adsorbed to remove cross-reactive IgE and IgG (bottom row).

the monoclonal antibodies used in this study.

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INTERLEUKIN-13 ALLERGIC RHINITIS

One of the functions of interleukin (IL)-13 demonstrated in vitro is the stimulation of IgE and IgG4 synthesis in B cells in the presence of CD40 ligand, similar to IL-4. This study investigated IL-13 gene expression in the nasal mucosa of atopic patients with dust mite-sensitive perennial allergic rhinitis (PAR) compared to subjects with chronic infectious rhinitis and to normals. The cellular localization of IL-13 protein was also determined.

Interleukin-13 gene expression was found in 18 of 19 patients with PAR but was not detectable in patients with infectious rhinitis or in normals. The primary cellular sources were lymphocytes and mast cells. There was a strong correlation between gene expression and total as well as antigen specific IgE. Gene expression was not found in peripheral blood mononuclear cells.

The authors conclude that IL-13 gene expression and its correlation with serum IgE suggest an important role in the pathogenesis of PAR, probably through enhancement of IgE production. The absence of gene expression in the peripheral blood suggests a locally active effect.

—JGE

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