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Immunoblot Analysis and Comparative IgE Responses of Atopic Patients to Extracts of the Domestic Mite *Blomia tropicalis.*

FEDERICO MONTEALEGRE, DVM, Ph D*; KERSTIN GOTH, MT†; BARBARA HART, PhD †

Background. **The domestic mite *Blomia tropicalis* is found in subtropical and tropical environments, and its clinical importance as a sensitizing agent in allergic disease is widely accepted.**

Objective. **To investigate the IgE reactivity to allergens present in extracts of the domestic mite *B. tropicalis,* and compare the IgE responses to these allergens by asthmatics, patients with atopic dermatitis and allergic rhinitis, as well as nonatopic controls.**
Methods. **Extracts from *B. tropicalis* were used for skin tests. The *B. tropicalis* specific IgE in the serum were measured using the FAST Plus Test and immunoblot analysis.**

Results. **A total of 199 volunteers participated in the study. The data show that 18 out of 29 polypeptide bands present in extracts of this mite species were recognized by the allergic and control sera. Of these allergens, four showed a high IgE binding frequency and had relative molecular weights of 104, 80, 68 and 14 kDa. The 14 kDa allergen demonstrated the highest IgE binding frequency.**

Conclusion. **Sera from atopic patients reacted to more allergens than sera from patients controls. Extracts from pure bodies of *B. tropicalis* contain one immunodominant and three important allergens. A common characteristic between all of the sera tested was the high degree of serum IgE reactivity observed to the 14 kDa allergen.**

Key words: Blomia tropicalis, Immunoblot, Major allergens

The domestic mite *Blomia tropicalis* (BT), a member of the family Echymyopodidae, (1) is an important source of aeroallergens in tropical and subtropical parts of the world (2-9). In the United States, BT has been described in household dust in the south coastal regions of the mainland as a codominant species with other mites including *Dermatophaoides pteronyssinus* (DP) (10-11). In a multicenter study conducted in the US mailand, BT was found to be the fourth most common domestic mite in Louisiana, Tennessee, Texas, Florida and California (12).

Evidence supporting the allergenicity and clinical importance of BT in atopic diseases, especially asthma and allergic rhinitis, has been provided by several authors. Arruda *et al,* (13) demonstrated the presence of BT specific IgE antibodies in asthmatic children, and showed that the RAST score in this population was moderate to high,.indicating sensitization to this mite. Similar results have been presented by Fernández-Caldas *et al,* (10) and Gabriel *et al.* (14). In the first attempt to identify BT, Arlian *et al,* (15) used crossed immunoelectrophoresis to identify IgE binding antigens. Of the 27 antigen bands, 21 were allergens and most were species specific. Using immunobloting to study the binding frequency by sera from asthmatic individuals, Caraballo *et al.* (16) demonstrated the presence of one major and three important allergens in extracts from isolated BT bodies.

As the clinical relevance of BT in atopic diseases continues to be documented, consistent and reliable identification of the immunodominant allergens is important in order to select and standardize candidate allergens for immunotherapy, standardize extracts for diagnosis, and provide reagents for environmental monitoring.

The purpose of this study was to investigate, in a population of allergic and control subjects living in Ponce, Puerto Rico, the prevalence of IgE reactivity to allergens present in extracts prepared with food-free bodies of the domestic mite BT, and to compare the IgE responses to these allergens by asthmatics, patients with atopic dermatitis and allergic rhinitis, as well as nonatopic controls.

Methods

Environment. The City of Ponce is located in southern Puerto Rico. The average summer (June) temperature is

*The Ponce School of Medicine, Ponce, Puerto Rico, †The Royal Agricultural College, Cirencester, England

Address correspondence to: Dr. F. Montealegre, DVM, Ph.D, Ponce School of Medicine, P.O. Box 7004, Ponce, Puerto Rico. E-mail:Fmontealegre@psm.edu

31°C (79°F), and the average winter (January) temperature is 20°C (68°F). The year average rainfall is 40 inches, the average year round temperature is 26°C (79°F), and the average humidity is 55% by day and 80% by night.

Human sera. The initial study population was comprised of consecutive 676 volunteers, age range 1-66 years, residing in the City of Ponce, Puerto Rico were grouped according to a physician's diagnosis of either atopic dermatitis (n=173), allergic rhinitis (n=162), asthma (n=208) and nonallergic controls (n=131). Each volunteer was unrelated and represented different households. The diagnosis of each condition was conducted by several specialists which included pulmonologists, dermatologists and an allergist according to established criteria for each condition. After the medical diagnosis was established, the patients were referred from the different outpatient clinics to our laboratory for the study. Each subject was unrelated and represented different households. Volunteers who meet all of the following inclusion criteria were included in the present study:

1) specialist-based clinical diagnosis and active symptoms of either atopic dermatitis, allergic rhinitis, or asthma,
2) a valid skin test (subject responded to the histamine positive control with a wheal greater than 3 mm and without a skin reaction to the negative control;
3) a positive skin test to BT and have donated blood in sufficient amount for completion and optimization of laboratory tests including total IgE, specific IgE to *Blomia tropicalis* and Enzyme Immuno Transfer Blot (EITB or Western Blot),
4) not taking medications, such as antihistamines, that could interfere with the skin prick tests,
5) were skin prick test negative to mite growth media,
6) skin prick test positive to histamine control with 3 mm^2 or more,
7) skin prick test negative to PBS/glycerin and
8) EITB results with clear and defined bands which allowed objective identification of allergens. The control volunteers were individuals without any self reported history of allergy. Prior to testing, all of the participants voluntarily signed the Institutional Review Board approved informed consent forms.

Allergen extracts. Mite extracts for SDS-PAGE and electroblotting were prepared as previously published (17). Briefly, food-free BT bodies were obtained from live cultures by the heat scape technique. The clean mites, which dropped through the mesh, were collected in 50 ml conical centrifuge tubes, immediately placed on a 1" deep x 12" diameter brass sieve pan (Dual Manufacturing Co, Chicago, Ill) and exposed to the 40 watt light for 15 min. The sieve pan was turned face down and gently tapped so that food particles and dead mites were eliminated. The live mites were killed by storing the sieving pan at -70 C, and dried by exposing to a light bulb for 4 hrs as described above. The dead mites were detached from the receiving pan, and sieved using three 8" diameter U.S. Standard sieves (Dual Manufacturing Co, Chicago, Ill) in sequence. The mesh openings used were 425 µm, 300 µm, 250 µm and the dried mites were placed on a sieve shaker (W.S. Tyler Inc, Mentor, OH) and agitated for 4 hrs. The dried mites which stayed between the 300 µm and the 250 µm meshes were considered pure bodies. The purity of the preparation was examined by placing 0.5 mg of dried mites on a 4 x 4 mm plastic dish using a SZ-STBL stereoscope (Olympus, Japan), and was found to be 99.5% pure mite bodies free of food particles, and >95% of the mite bodies showed fecal particles inside their bodies or attached to their legs. Next, fifty grams of food-free BT bodies were placed in 1 liter of 0.2M ammonium bicarbonate and sonicated for 15 minutes, on ice, at full output for 10 cycles, and further incubated overnight under constant agitation at 5EC. The extract was centrifuged at 6500 rpm for 1 hr, and the supernatant extensively dialyzed against water using a membrane with a 3,000 kDa exclusion limit. The extract was concentrated to 50 ml using polyethylene glycol and clarified on a refrigerated centrifuge at 15,000 rpm for 1 hr. The protein concentration of the clear supernatant was determined using an adaptation of the Lowry assay (18). The protein concentration was adjusted to 2 mg/ml, and 1 ml aliquots were stored at -70EC until use. Extracts for skin-prick testing were prepared as described above with the following modifications. A total of 1 gram of BT or *Euroglyphus maynei* (EM) bodies were extracted in 20 ml of 0.2 M ammonium bicarbonate supplemented with 50% glycerol and sterilized using a 0.22 im pore size filter. Mite cultures of EM were kindly provided by Dr. B. Hart.

Skin-prick test (SPT). Skin-prick tests were performed in the volar surface of the right arm by a trained nurse. Volunteers were tested using standardized commercial glycerinated extracts of *D. pteronyssinus* (DP) and *D. farinae* (DF) (Holliester Stier, Spokane, WA). Extracts from BT and EM were prepared in our laboratory as described since commercial extracts are not available for these two species. All of the patients were negative when skin tested with culture medium extract. The skin reaction was recorded at 15 min. The mean of the major diameter and its perpendicular for the wheal were measured and a skin reaction was considered positive was if it had a mean wheal equal or greater than 3 mm5 in the mean diameters.

Positive (histamine 1 mg/ml) and negative (sterile PBS in 50% glycerol) controls, and an extract of the mite culture growth medium were included in the skin testing. For the prick tests, disposable acrylic copolymer needles were used (Morrow Brown, Oakhurst, NJ). All of the participants were tested using the same allergen lot and preparations. Each subjects signed a written informed consent to participate in the study prior to any allergy testing.

Determination of IgE antibodies. Total IgE was determined using the ELISA procedure as recommended by the manufacturer (Endocrine Technologies, CA). Specific IgE measurements were performed using the IgE fluoroallergosorbent test (FAST Plus Test, Bio Whittaker, Walkersville, MD). Briefly, microtiter plates were coated overnight with 100 µl of BT extract at the predetermined protein concentration of 2 mg/ml. After this, the assays were conducted according to the vendor's specifications. A positive FAST result was defined as a value ≥ 0.35 IU/ml (FAST class 0= ≤ 0.20 IU/ml, class 0/1=0.21-0.34 IU/ml, class 1=0.35-0.99 IU/ml, class 2=1.0-2.99 IU/ml, class 3=3.0-6.99 IU/ml, class 4= 7.0-16.99, class 5=17.00-43.99 and class 6=<44.00 IU/ml).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was conducted under non-denaturing conditions as previously described (19). Briefly, a total of 225 µl containing 50 µg/ml of the BT extract were electrophoresed on a new 15% polyacrylamide gel (18 x 8.5 cm) at 60 MA for 45 min. Polypeptide bands were identified in the silver stained gel by densitometric analysis using a Fluor-S-Multimager (Bio-Rad, Richmond, CA).

Electrotransfer and immunoprobing. Polypeptides were electro transferred to PVDF membranes (Immobilon P, Millipore, Bedford, MA) as previously described (20) using a mini-Transblot cells (BioRad, Richmond, CA.) Electrotransfer was carried out for 3 h at 30 V with cooling. The PVDF membranes were cut into 3 mm strips using an Immuno-strip bladeless cutter (Novex, San Diego, CA). Immunoprobing was conducted by incubating the individual strips overnight with 150 il of human serum from allergic subjects and controls. After incubation, the strips were washed twice with PBS/Tween, twice with blocking solution (Boehringer-Mannheim, Indianapolis, IN.), and incubated for 1 hour with rabbit anti-human IgE labeled with peroxidase (Binding Site, San Diego, CA) at a dilution of 1:1000 in blocking solution. After five additional washes, the strips were aligned on a clear plastic sheet protector and impregnated with the Supersignal substrate (Pierce, Rockford, IL), then exposed for 1 and 5 min to Lumi-Film Chemiluminescent Detection Film (Boehringer-Mannheim, Indianapolis, IN).

Data analysis. The relative electrophoretic mobility of each band recognized by individual sera was calculated according to standard methods.[21] Data were analyzed using a commercial statistical software package (Stata, Stata Corporation, College Station,TX). Descriptive analysis including frequencies for all variables were conducted and comparison between mean number of bands recognized by patients with atopic dermatitis, asthma, rhinitis and controls were conducted using one way ANOVA. Reactivity to each individual allergen band by sera from atopic dermatitis, asthma, rhinitis and controls was assessed by the Wilcoxon ranks sum test. The level of significance was established at 0.05%.

Results

Patients. A total of 200 (29.2%) subjects did met all of the inclusion criteria (Table 1) while 479 (70.8%) did not. There were no significant difference between the gender distributions, but the mean age difference between the study subjects and those excluded from the study was 7.08 years (respectively 31.8"15.2 and 38.9"21.6, P<0.0000) was significant.

Table I. Frequency Distribution by Age and Diagnosis of the Study Population

	Diagnosis				
Age group	Atopic dermatitis	Asthma	Allergic Rhinitis	Controls	TOTAL
11-20	22 (36.7)*	15 (25.0)	15 (25.0)	8 (13.3)	60 (30.0)
21-30	11 (20.0)	14 (25.5)	15 (27.3)	15 (27.3)	55 (27.5)
31-40	7 (24.1)	12 (41.4)	2 (6.9)	8 (27.6)	29 (14.5)
41-50	2 (6.7)	9 (30.0)	1 (3.3)	18 (60.0)	30 (15.0)
51-60	1 (8.3)	3(25.5)	4 (33.3)	4 (33.3)	12 (6.0)
>60	-	3 (21.4)	1(7.1)	10 (71.4)	14 (7.0)
TOTALS	43 (21.5)	56 (28.0)	38 (19.0)	63 (31.5)	200 (100)

* Percents in parenthesis

Skin tests. Skin prick testing showed that >98% of the atopic individuals reacted to BT extracts, as compared to 6.35% in the nonatopic control group (Table 2). Most of the atopic patients had positive skin test reactions to the other mite species as well.

Total and specific IgE levels. Of the total of 200 sera analyzed, 28.0% were from asthmatic patients, 21.5% from atopic dermatitis patients, 19.0% from rhinitis patients and 31.5% from healthy controls. Levels of total IgE were significantly higher in the atopic patients when compared to the healthy controls (Figure 1). The mean total IgE levels in patients with atopic dermatitis was 738 ±13 IU/ml (SD), for allergic rhinitis was 854.63±267.44 , for asthma was 708 ±14 IU/ml, and for the controls was 368±41 IU/ml.

Table 2. Positive Skin Reactions to Domestic Mites by Allergic Patients and Controls

Mite	Atopic Species n=43	Asthma Dermatitis n=56	Rhinitis n=38	Control sn=63
Blomia tropicalis	100*	98.2	100	6.35
Dermatophagoides pteronyssinus	90.7	82.14	94.74	11.1
D. farinae	95.35	89.3	97.37	11.1
Euroglyphus maynei	79.07	73.21	84.21	4.76

*Values are given a percentages (%).

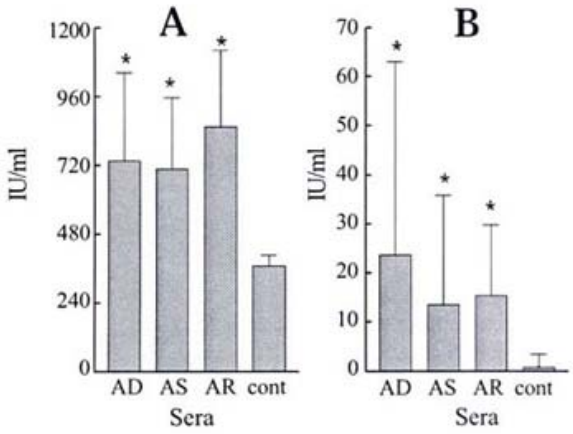

Figure 1. Total and Specific IgE Levels. Insert a Represents the Total IgE Levels, and Insert B Illustrate The Specific IgE Levels. In Both Cases, Allergic Patients had Significantly Higher Levels than the Non-allergic Controls. Asthma (A), Allergic Rhinitis (R), Atopic Dermatitis (A) Patients and Controls (C).

The average of specific levels of IgE for atopic dermatitis was 23.71±39.43 IU/ml, allergic rhinitis 15.47±14.42 IU/ml, asthma 13.63±22.27 IU/ml and controls was 0.88±2.76 IU/ml. The vast majority of the allergic patients had positive specific IgE levels to BT, while 33.5% of the controls. The vast majority of the allergic patients had positive IgE reactions to BT and 33.3% of the controls were FAST Class 1 or greater.

SDS-PAGE profile and IgE responses to allergens from the domestic mite B. tropicalis. The Bt extract prepared from food-free mite bodies contained at least 29 polypeptides ranging from 108 to 11 kDa (Fig 2). The polypeptides were electro transferred and probed with individual sera from 137 Bt sensitive atopic patients and

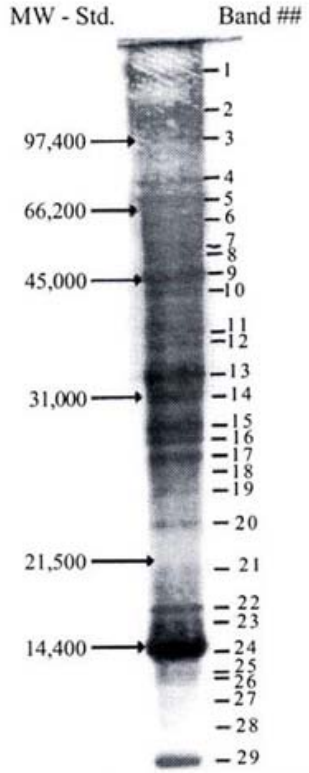

Figure 2. Silver Stained Non-denaturing SDS-PAGE of Food-free *B. Tropicalis* Body Extracts. Arrows Indicate the Relative Mobility of the Molecular Weight Standards, and Band Number Assigned to Each Polypeptide. At Least 29 Polypeptide Bands were Identified.

63 nonatopic controls (Fig.3). Under non-reducing conditions, 18 out of 29 polypeptides reacted with IgE. The allergens most frequently recognized by the atopic sera had relative molecular weights of 104, 80, 68, 64 and

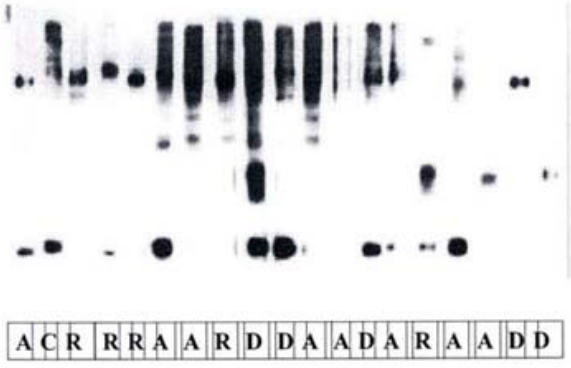

Figure. 3. Profiles of Allergen Recognition by Serum IgE Obtained From Asthma (A), Allergic Rhinitis (R), Atopic Dermatitis (A) Patients and Controls (C).

14 kDa (Figure 4). The 14 kDa allergen had the highest IgE binding frequency followed by the 104 kDa, the 68 kDa, and the 80 kDa allergen. The remaining allergens reacted at lower frequencies ranging from 1.7 to 35.7%. Ninety

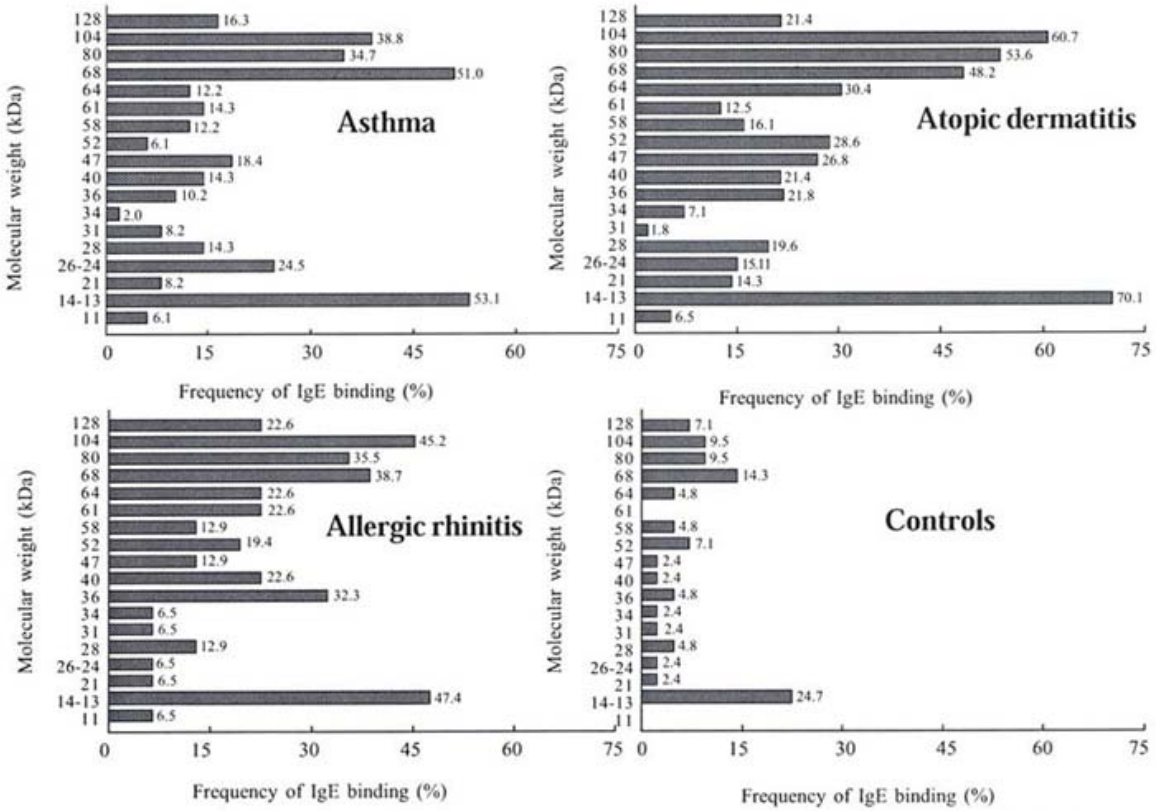

Figure 4. Histograms Demonstrating the Banding Frequency of the Ige Obtained from Atopic Patients and Controls to Individual *B. Tropicalis* Allergens Present in Extracts From Food-free Mite Bodies.

Table 3. Comparison of the Number of Allergens in Mite Extracts Recognized by Serum Ige Obtained from Atopic Patients and Controls

Comparison	N	Mean	SD	Mean difference	p>
Atopic dermatitis vs	43	4.11	3.05	0.65	0.26
Asthma	56	3.46	2.73		
Atopic dermatitis vs	43	4.11	3.05	0.36	0.58
Rhinitis	38	4.47	2.88		
Atopic dermatitis vs	43	4.11	3.05	3.86	0.000*
Controls	63	0.25	1.06		
Rhinitis vs	38	4.47	2.88	0.91	0.12
Asthma	56	3.46	2.73		
Rhinitis vs	38	4.47	2.88	4.22	0.000*
Controls	63	0.25	1.06		
Asthma vs	56	3.46	2.73	3.21	0.000*
Controls	63	0.25	1.06		

two percent of the control sera did no react to any of the polypeptide bands from BT. In contrast, 98.5% of the allergic sera recognized 1 or more allergens. There was a tendency of the sera to recognize polypeptides of medium to high molecular weights, and a lower band of 14 kDa complex. Sixty percent of the sera from patients with atopic dermatitis recognized the 14 kDA allergen followed by 104, 68 and 80 kDa polypeptides. Similar banding patterns were observed in sera from allergic rhinitis and asthma patients. In contrast, sera obtained from the healthy controls reacted to several allergens but -at a-frequencies of 1.6 to 6.3%. Statistically significant differences were observed when the mean number of allergens recognized by each atopic condition were compared to the healthy controls. Patients with AD, AR and AS recognized significantly more bands than the controls, however, no significant differences were observed between AD, AR or AS (data not shown).

Table 4. Comparison of the Reactivity to *B. Tropicalis* Individual Allergens by Allergic Sera and Controls

Allergens (kDa)	Atopic dermatitis vs asthmatics P value*	Atopic dermatitis vs rhinitis P value	Atopic dermatitis vs controls P value	Rhinitis vs asthmatics P value	Rhinitis vs controls P value	Asthmatics vs controls P value
128	0.37	0.96	0.0004**	0.35	0.0003**	0.0046**
104	0.64	0.29	0.0000**	0.50	0.000**	0.000**
80	0.39	0.67	0.000**	0.71	0.0000**	0.000**
68	0.85	0.93	0.000**	0.8	0.000**	0.000**
64	0.37	0.56	0.001**	0.13	0.000**	0.001**
61	0.88	0.15	0.005**	0.09	0.000**	0.007**
58	0.59	0.69	0.001**	0.92	0.003**	0.004**
52	0.40	0.40	0.002**	0.8	0.001**	0.01**
47	0.39	0.25	0.02**	0.07	0.0009**	0.002**
40	0.39	0.09	0.02**	0.32	0.0001**	0.002**
36	0.22	0.06	0.01**	0.07	0.000**	0.005**
34	0.19	0.57	0.03**	0.06	0.008**	0.28
31	0.85	0.12	0.22	0.06	0.008**	0.28
28	0.17	0.06	0.36	0.54	0.003**	0.01**
26-24	0.88	0.57	0.05**	0.65	0.02**	0.007**
21	0.09	0.06	0.00002**	0.22	0.19	0.01**
14-13	0.12	0.81	0.0000**	0.20	0.000**	0.000**
11	0.73	0.75	0.15	0.98	0.29	0.25

*Wilcoxon ranks sum test, ** Significant values.

Discussion

In this study, we analyzed the IgE reactivity to polypeptides present in food-free body extracts of the domestic mite *Blomia tropicalis* using sera from atopic patients with a confirmed diagnosis of either asthma, atopic dermatitis or allergic rhinitis. Sera from healthy nonatopic controls were also analyzed. The results clearly demonstrate that at least 18 out of 29 polypeptide bands present in extracts from BT were recognized by the allergic sera tested. Since immunodominant allergens must have an IgE binding frequency of at least 50%, two BT allergens qualify in this category: namely the 104 and 14 kDa molecules. Of these, the 14 kDa allergen demonstrated the highest IgE binding frequency in all of the sera tested. Other important allergens include the 80, and the 68 kDa polypeptides, which were recognized by patient=s sera at frequencies of 44.1% and 47.3% respectively.

There are limited number of published data on the identification of BT allergens. Previous observations by Caraballo *et al*, (16) demonstrate that BT whole crude body extracts contained one immunodominant allergen of 11-13 kDa, and three other important allergens with apparent molecular weights of 64 kDa, 36kDa, 33 kDa. Tsai *et al* (22) showed that BT extracts had at least 30 polypeptides, and of these, 14 were allergens. Using sera from 23 allergic patients, the authors also identified three major allergens, 14.3 kDa, 106 kDa and a 94 kDa polypeptides. With the exception of the 14 kDa component, our results differ to those obtained by these researchers. These divergences in the electroblotting analysis of mite allergens have been previously documented. For example, in an analysis of allergens from DP, Tovey reported a total of 20 allergens recognized by human IgE (23) while Baldo and others demonstrated allergenicity in 28 to 37 allergens (24-25). Similar discrepancies exist in studies of IgE binding to allergens of the domestic mite *E. maynei*. Morgan *et al* reported a total of 46 individual allergens in this mite species, (26) while Collof *et al* and Nakinishi report 25 allergens (27-28). The discrepancies between our results and those obtained by Caraballo *et al* (16) and Tsai *et al* (21) may be due to several reasons. First, differences in the mite growth conditions, specially the growth media in which BT was cultured could may induce quantitative and qualitative differences in the expression of allergens. Although there is no published data addressing the influence of substrate on allergen expression, it is possible that dust mites may express neo-allergens resulting in different IgE reactivity patterns. Secondly, the genetic background and gender of the patients in both studies may be different thus producing different reactivity pattern to BT allergens. In a recent study conducted in Barbados, Manoli *et al*., have shown that IgE heritability to react to a major allergen from *B. tropicalis* was consistently greater for male offspring than for female offspring (29). Thirdly, since dust composition may vary from house to house, dust mites will feed on different substrates which in turn will lead to the expression of different allergenic molecules. As a results, each sensitized patient may have different IgE patterns. Fourth, it is possible that methods used to

determine the relative molecular weights may also have influenced the data. Immunodominant allergens in these and our reports may be different, there was an agreement in the high degree of reactivity to a 14 kDa allergen. However, this should be interpreted with caution, since previous reports support the existence of several polypeptides with similar molecular weights (30-32). Lastly, we used chemiluminescent methods for the detection of IgE which is less sensitive than the isotopic

In our study, we performed the *in vitro* determinations of total and specific IgE. Significant differences were observed between the levels found in atopic and controls. The data showed that the levels of total and specific IgE in both, the allergic and control subjects were elevated. However, these results should be carefully interpreted in particular those related to the control population. It has been demonstrated that there is considerable overlap in total IgE levels between atopic and nonatopic individuals, making the test results difficult to interpret (33).
Grundbacher FJ. Causes of variation in serum IgE levels in normal populations. J Allergy Clin Immunol 1974; 56:104, (34-37). Controls and atopic patients included in this study were also skin positive to other mite species, including DP, DF, and EM. In the atopic population studied, the skin reactivity observed is a reflection of the mite fauna of Puerto Rico which consists of several species including BT, DP, DF, *Dermatophagoides sibonei* and EM (38). It is most likely that the allergic individuals and controls included in this study have been exposed to all of these mite species and their products in the indoor environment. There is also likely to be some degree of crossreactivity between allergens from these mite species. Previous reports have shown that there is significant crossreactivity between *Dermatophagoides* and *Euroglyphus* species (39-40). Others indicate limited-to-moderate crossreactivity between BT, DP *and* DF (41-42). Furthermore, Johansson *et al* has shown the existence of antigenic crossreactivity between *Lepidoglyphus destructor* (LD) and BT (43). The latter may not be important in our study since LD has not been found in Puerto Rico (31). Despite the crossreactivity between mite species, BT expresses sufficient species specific allergens to validate our results (44,45).

In conclusion, we have shown that extracts from BT bodies have up to 18 allergens and of these, two appear to be immunodominant, and two are important. The fact that there are significant discrepancies in the present and other studies, emphasizes the need for standardized culture conditions, growth media and procedures to validate the issue of BT allergenicity. The possibility that neo allergen expression may influence the banding pattern must be addressed.

Resumen

El presente trabajo tuvo como objetivo investigar la reactividad de la IgE humana a alergénos del acaro *Blomia tropicalis*. Además, se comparó la respuesta IgE de pacientes con asthma, dermatitis atópica, rinitis alérgica, y controles no alérgicos. Para esto, la reactividad de la IgE se estudió por medio de la inmuno- electro- transferencia (Western Blot). Un total de 199 voluntarios participaron en el estudio. Los datos nos muestran que 18 de 19 bandas de polipeptídeos presentes en los extractos del ácaro, fueron reconocidas por sueros de los participantes alérgics y controles. De estos alergenos, cuatro de ellos mostraron una alta frecuencia de reactividad con la IgE. El peso molecular de los cuatro alérgenos es de of 104, 80, 68 y 14 kDa. El alergéno de 14 Kda tuvo la frequenciua de reactividad mas alta de todos los alergénos identificados. Podemos concluir que los sueros de pacientes atopicos reaccionaron a mas alergénos que los sueros de controles. Extractos de cuerpos puros de *B. tropicalis* contienen un alergéno inmunodominante, y tres alergénos importantes. La caracteristica en común entre todos los sueros analizados, fue la alta reactividad de la IgE hacia el alergéno de 14 kDa.

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