Analysis of cross-reactivity between group 1 allergens from mites

LUZ MARY CRUZ, MS*; FERNANDO LÓPEZ-MALPICA, MD†; ANA MARÍA DÍAZ, DSc*

Mite allergen exposure can lead to sensitization in genetically predisposed individuals, and the development of asthma in previously sensitized individuals. The major allergens of mites belong to *Dermatophagoides spp*. and *Blomia tropicalis* (Bt). Various allergens of Bt have been cloned and sequenced. Some of them show homology sequence with purified allergens from *Dermatophagoides pteronissynus* (Dp). Recently, the allergen group 1 from Bt, Blo t 1, was cloned and sequenced at our laboratory. Recombinant Blo t 1 showed 35 % of identity and 50% of similarity with group 1 allergens as Der p 1 (from Dp), Der f 1 (from *D. farinae*) and Eur m 1 (from *Euroglyphus maynei*) at amino acid level. This would suggest that cross-reactivity between allergens of different mite species could exist. Here, we analyzed the cross-reactivity between group 1 allergens from mites using recombinant proteins and monoclonal antibodies against them. ELISA inhibition assay showed that cross-reactivity between homologous allergens from *Dermatophagoides spp.* is high, but it is low to moderate between mites from different species. IgE-reactivity analysis using serum samples from allergic individuals revealed a strong reactivity of rBlo t 1 for serum samples from subjects with highly positive reaction to Bt extract in skin test, but lack of reactivity of this protein with serum samples from individuals with highly positive reaction to house dust mite extract in the skin test. These results suggest that it is important to include Bt allergens in routine skin test in order to improve the diagnostic accuracy and precision of allergies.

Key words: Dust mites, Allergy, Asthma, Cross-reactivity, Blomia tropicalis, Dermatophagoides spp.

Respiratory diseases of allergic etiology have been dramatically increased in the last years in many regions of the world, including Latin America (1-4). In 2000, according to the Puerto Rico Health Department report, asthma had a prevalence of 15.9% in Puerto Rico and 10.5% in the United States (5). The origin of asthma and allergic rhinitis is not well established; however, there is a tendency for asthma to run in families. Most research supports the idea that allergen exposure is the principal cause of childhood asthma and the most common allergic diseases are asthma and allergic rhinitis (6). Exposure to indoor allergens is one of the main risk factors of allergic sensitization in genetically predisposed patients (7). House dust mites (HDM) are important sources of indoor allergens (8). Preliminary identification of the acarologic fauna in Southern Puerto Rico demonstrated that *Blomia tropicalis* (Bt), *Dermatophagoides pteronissynus* (Dp), *Dermatophagoides farinae* (Df) and *Euroglyphus maynei* (Em) are the dominant domestic mite species found in homes of asthmatic individuals (4). The members of the genus *Dermatophagoides* are predominant in temperate and tropical regions of the world, including North America and Latin America (8-9). The most common species in this genus are Dp and Df both found in temperate as well as in warm and humid climates (10). Bt is one of the most important causes of allergic sensitization in tropical and subtropical areas around the world (9, 11). In a 1997 study in Puerto Rico, Bt was found as the second most common house dust mite (4). Coexistence at high levels between Bt and Dp has been reported (4, 12-13). In recent years, a number of epidemiologic studies have shown that both Dp and Bt sensitize the atopic populations in the world's tropical and sub-tropical regions (14-15).

Investigations about molecular characterization of mite allergens have showed that there is a high percentage of homology sequence between homologous allergens from different mite species, which could be indicative of a possible cross-reaction between these molecules. The structural characteristics of proteins are major determinants of cross-reactivity and the identification and

*Microbiology and Medical Zoology Department and †Internal Medicine Department, University of Puerto Rico, Medical Sciences Campus, School of Medicine.

A Research Grant from MBRS-SCORE (2-S06-GM08224), and the Associate Deanship of Biomedical Sciences, School of Medicine., Medical Sciences Campus, UPR, supported this study.

Address correspondence to: Ana María Díaz, DSc, University of Puerto Rico, Medical Sciences Campus, School of Medicine, Department of Microbiology and Medical Zoology, PO Box 365067, San Juan PR 00936-5067, adiaz@rcm.upr.edu

characterization of allergen IgE epitopes is necessary for the determination of possible cross-reactivity between allergens.

Mite allergens are divided by their biochemical composition, sequence homology, and molecular weight (16). Group 1 allergens from mites (i.e. Der p 1 and Der f 1) have a MW of 25KDa and an 80% of homology sequence between them (17). They are cysteine proteases, which can degrade the low affinity IgE-receptor (CD_{23}) on the surface of human B lymphocytes (18). Recently, in our laboratory, Mora and collaborators cloned the group 1 allergen from Bt (rBlo t 1). Recombinant Blo t 1 is homologous to cysteine proteases such as Der p 1, Der f 1 and Eur m 1, showing 53%, 50% and 51% of nucleotide sequence homology with each one, respectively. IgE-reactivity analysis revealed that rBlo t 1 was recognized by 62% of Bt skin test-positive patient's sera, which suggests that this recombinant protein could be categorized as a major allergen from mites (19).

It has been established that group 1 allergens from mites of the same genus, Der p 1 and Der f 1, have a cross–reactivity higher than 80% (10, 20-21). In contrast, several studies demonstrated that there is a moderate degree of cross-reactivity between Bt and Dp (22-26). These results indicate the importance of including Bt allergens in routine diagnostic testing in tropical and sub-tropical situations. Cross-reactivity between group 1 allergens is poorly defined (27). To overcome this limitation, we analyzed the cross-reactivity between Blo t 1 and their counterparts Der p 1, Der f 1 using recombinants proteins, and a panel of monoclonal antibodies against these allergens.

Materials and Methods

Allergens

rBlo t 1: Expression and purification of recombinant Blo t 1 were done as described by Mora et al (19). Briefly, *Escherichia coli* XL1 Blue cells harboring a pDS56 expression vector containing the cDNA for Blo t 1 were cultured overnight in 300 ml of LB medium (10 g Bacto-tryptone, 5 g Bacto-yeast extract, 5 g NaCl/L, pH 7.5) containing ampicillin (50 μg/ml) and tetracycline, (10 μg/mL) at 37 ºC. Two-hundred sixty five mL of the overnight culture were inoculated in 5 L of prewarmed LB medium with the appropriate antibiotics and grown at 37 ºC until the optical density at 600 nm was 0.6. Expression was induced by adding isopropylthiobeta-D-galactoside (IPTG) to a final concentration of 1nM. The culture was grown for an additional 2 hours and centrifuged for cell harvesting (10,000 rpm/10 min). The supernatant was discarded and the cell pellets were re-suspended in 50 ml of lysis buffer B (8M urea, 100mM NaH_2PO_4, 10 mM Tris-HCl, 20 mM imidazole, pH 8.0) and incubated 2 hours at room temperature with mixing. The lysate was centrifuged for 25 min at 4,000 rpm to remove cellular debris and the supernatant transferred to a 25 ml equilibrated Ni-NTA Superflow resin. The mixture was mixed by shaking for 1 hour at room temperature. Then, it was loaded into an empty column (23 cm L x 3 cm W) and the flow-through was collected. After three washes with 80 ml of buffer C (same composition buffer B except for the pH is 6.3), the recombinant protein was eluted 5 times (10 ml each one) with buffer D (same composition as buffer B except for the 20 mM imidazole and the pH is 5.9). The recombinant protein expression and molecular weight was confirmed by SDS-PAGE.

rDer p 1, rDer f 1: These recombinant allergens were commercially purchased from Indoor Biotechnologies (Charlottesville, VA).

Monoclonal antibodies

Anti Blo t 1: A monoclonal antibody which reacts specifically with r Blo t 1 was produced at our laboratory as described by Collazo and Díaz (28).

Anti Der p 1, anti Der f 1: These monoclonal antibodies were commercially purchased from Indoor Biotechnologies (Charlottesville, VA).

Protein concentration determination

Bradford assay was used to determine the protein concentration of recombinant group 1 protein from Bt (rBlo t 1). BSA standard curve was constructed and saved following instructions described by the spectrophotometer manual (SmartSpec 300, Bio Rad, CA). Recombinant protein was serially diluted in PBS 1X. Then, 100 μl of each protein dilution was mixed with 5 ml of Dye Reagent (1:5 dilution) in individuals tubes. The samples in the tubes were incubated for 5 min. at room temperature and the absorbance of the reaction was read at 595 nm.

ELISA

ELISA described by Silva and collaborators (29) was used with some modifications to determine the levels of cross-reactivity between group 1 allergens. Briefly, high-binding microtiter plates were coated with 10 μg/μL of the allergen in PBS 1X and incubated overnight at 4 ºC. Microplates were washed three times with PBS-Tween buffer and blocked with 1% BSA-PBS-Tween for 1 hour at room temperature. Then, 50 μl per well of serial dilutions of monoclonal antibodies was added in duplicate to the wells and incubated for 2 hours at 37 ºC. Plates were washed again five times and incubated with 50 μl per wells of biotinylated goat anti-mouse IgG diluted at 1:1,000 for 1 hour at 37 ºC. Fifty microliters per well of streptavidin-

peroxidase conjugate diluted at 1:1,000 were added and incubated for 30 minutes at room temperature. Plates were washed and 50 μL per well of the enzyme substrate 0.01M 2, 2'-azino-bis-(3-ethyl-benzthiazoline sulfonic acid) (ABTS) (Sigma Aldrich, MO) were added and allowed to react for 10 minutes. Finally, the absorbance was read at 405 nm using an Opsys MR Microplate reader (Thermo Labsystems, MA). For rBlo t 1 allergen, a goat anti-mouse IgG / peroxidase conjugate (Sigma Aldrich, MO) was used as secondary antibody instead of the Biotin-Avidin system.

ELISA inhibition

ELISA inhibition assays were done following the method described by Barletta and collaborators (30) with some modifications. Briefly, antigens were diluted at 5 μg/ml. The working monoclonal antibodies dilution was calculated on the basis of the values producing approximately 80% maximum binding. Prediluted monoclonal antibodies were incubated overnight at 4 ºC with serial dilutions of inhibitors ranging from 0.25 to 20 μg/ml. Then, the ELISA was performed as above.

The percentage of inhibition was calculated according to Kuo et al. (31) as follow:

$$\% \text{ Inhibition} = [1 - (A/B) \times 100]$$

where: A= OD with inhibitor B= OD without inhibitor

Dot blotting

Following the procedure described by Chew and collaborators (32), purified undiluted recombinant proteins (rBlo t 1, rDer p 1 and rDer f 1) were blotted in dots of 2 μl in a nitrocellulose membrane. Nonspecific sites were blocked with 5% nonfat dry milk (blocking buffer). Then, membranes were incubated with the correspondent mAb for 1.5 hr with gentle agitation at room temperature. Following six washes with wash buffer (PBS 1X/ 0.05% Tween-20), membranes were incubated with goat-anti mouse IgG antibody/Peroxidase conjugated (diluted 1/ 100,000 in blocking buffer 1/10). The membranes were stringently washed as before and were subsequently incubated with the Supersignal West Femto Maximum Sensitivity Substrate (PIERCE, IL). Chemiluminescence detection was performed incubating the membranes for 5 min. in Luminol/peroxidase system followed by dry and exposition to the film (X-OMAT, Kodak, NY) at different times (5, 10 sec, 1, 3 min.) Films were developed in developing solution (GBX Developer/Replenisher, Sigma, MO), washed in distilled water, and fixed in fixative solution (GBX Fixer/Replenisher, Sigma, MO).

Serum samples

Sera from non-immunotherapy-treated Puerto Rican patients were used. These patients had been tested positive in previous skin prick test with Bt or HDM extract. Skin test reaction was categorized in a 1-4 scale, where 4 was a highly positive reaction to the extract tested. Sample sera were properly maintained in our laboratory. Sera from non-allergic individuals were used as negative control. These individuals did not have history of atopy and showed a negative skin prick test (0 reaction) to Bt or HDM extracts. The participating subjects have signed informed consent forms.

Dot blotting for serum samples

For these immunoblotting assays, the same protocol as above was followed with some modifications according to the components of the experiment following the procedure described by Fonseca and Díaz (23). After blocking the nonspecific sites with 5% nonfat dry milk (blocking buffer), the blots were incubated overnight with 2 μL of sera (diluted 1/5 in blocking buffer) at 4 ºC and followed by washing with PBS-Tween 20 (0.05%). For IgE detection, the dots were then incubated with horseradish peroxidase anti-human IgE monoclonal antibody (1/200,000 dilution in 10% nonfat dry milk) for 1 hr at room temperature. Detection was performed by the chemiluminescence method as above.

Statistical analysis

For IgE-reactivity, statistical analysis of dot blot and skin test results was done. Following statistical methods, a descriptive matrix form that compared two descriptive variables, sensitivity, specificity, predictive values, and false positive and negatives results of the assays was calculated (33). The following table summarizes the method used to calculate the statistical parameters.

Dot Blot results			
Skin test results	**Positive**	**Negative**	**Total**
Positive	A	B	A + B
Negative	C	D	C + D
Total	A + C	B + D	N

A: Samples with positive results in both test (skin test and dot blot), B: Samples with positive results in skin test, but negative results in dot blot, C: Samples with negative results in skin test, but positive results in dot blot, D: Samples with negative results in both test (skin test and dot blot), N: Number of subjects, Sensitivity = A/(A+B) Specificity = D/(C+D) Positive predictive value = A/(A+C) Negative predictive value = D/ (B+D) False positive = 1-specificity False negative = 1- sensitivity

Results

ELISA

The reactivity of recombinant allergens group 1 allergens from mites (Bt, Dp and Df) with monoclonal antibodies against each one of them were tested using ELISA. Optical density (OD) of the reaction was proportional to reactivity of mAbs with recombinant proteins. It has been established as a positive reaction, OD values 0.2 units greater than OD value for negative control (PBS 1X).

For mAb anti-Blo t 1 there was a strong reactivity with the homologous system (anti-Blo t 1 / rBlo t 1), but for the heterologous systems (anti- Blo t 1 / rDer p 1 or rDer f 1) the reaction was considered negative. Similar results were obtained for mAb anti-Der p 1 and mAb anti-Der f 1, which obtained a high optical density for homologous systems, but not for rBlo t 1. Recombinant group 1 allergen from Dp and Df reacted strong with anti-Der f 1 and anti-Der p 1, respectively (Table 1).

Table 1. Reactivity of homologous and heterologous systems. Monoclonal antibodies anti-Der p 1 and anti-Der f 1 were diluted 1/1000 in PBS 1X and mAb anti-Blo t 1 was diluted 1/20 in PBS 1X. The antigens in solid phase were diluted at 5μg/ml.

Monoclonal antibody	Antigen in solid phase	Od 405 NM
Anti-Blo t 1	rBlo t 1	0.447 ± 0.1151
	rDer p 1	0.147 ± 0.1045
	rDer f 1	0.056 ± 0.0129
Anti-Der p 1	rDer p 1	0.941 ± 0.1094
	rBlo t 1	0.579 ± 0.0656
	rDer f 1	0.760 ± 0.1424
Anti-Der f 1	rDer f 1	0.837 ± 0.030
	rBlo t 1	0.577 ± 0.160
	rDer p 1	0.909 ± 0.052

Dot blotting assays

Immunoblotting assays reinforced results obtained by ELISA. Figure 1 shows results of dot blot experiments of reactivity of recombinant proteins rBlo t 1, rDer p 1and rDer f 1 with anti-Blo t 1, and anti-Der f 1 mAb.

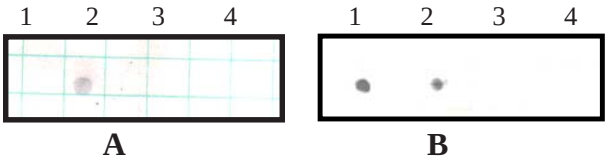

Figure 1. A. Reactivity of anti-Blo t 1 mAb with PBS 1X (dot 1), rBlo t 1 (dot 2), rDer p 1 (dot 3), and rDer f 1 (dot 4). B. Reactivity of anti-Der f 1 mAb with rDer f 1 (dot 1), rDer p 1 (dot 2), rBlo t 1 (dot 3), and PBS 1X (dot 4).

Monoclonal antibodies anti-Der p 1 and anti-Der f 1 reacted strongly with homologous allergens from *Dermatophagoides* spp. (rDer p 1 and rDer f 1), but did not react with rBlo t 1. On the other hand, mAb anti-Blo t 1 failed to react with rDer p 1 and rDer f 1, but reacted with the allergen rBlo t 1.

ELISA inhibition

For rBlo t 1, homologous inhibition (rBlo t 1 as inhibitor with rBlo t 1 in solid phase) achieved a maximum inhibition of 80% at 20 μg/mL, which was statistically significant, and it was considered as a total inhibition. Group 1 allergens from *Dermatophagoides spp*., rDer f 1 and rDer p 1, inhibited, at the highest concentration (20 μg/mL), about 45% and 55%, respectively, when rBlo t 1 was on solid phase; which was considered as a slight to moderate inhibition (Figure 2).

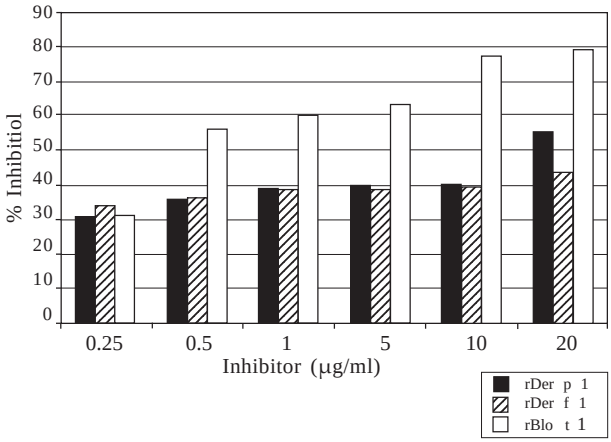

Figure 2. Recombinant protein Blo t 1 was incubated overnight at 5μg/ml in solid phase (high-binding microtiter plates). Six different inhibitor concentrations, ranging from 0.25 to 20 μg/ml, were incubated overnight with anti-Blo t 1 mAb (diluted 1/10000). Then, ELISA was performed as described in Materials and Methods section.

Homologous inhibition for rDer p 1 at the highest inhibitor concentration (20 μg/ml) was total (approximately 95%). Homologous allergen from Df (rDer f 1) caused approximately 95% inhibition at the highest concentration, when rDer p 1 was on solid phase, which was also considered total. On the other hand, the highest inhibition due to rBlo t 1 at the highest inhibitor concentration was approximately 20% and considered as no inhibition. There was no reduction of reactivity at the first three rBlo t 1 concentrations (0.25 – 1 μg/ml) as inhibitor of the system (data not shown).

When rDer f 1 was on solid phase rDer f 1, as inhibitor, its maximum inhibition was about 98% (total inhibition). rDer p1 as inhibitor showed an approximate 90% inhibition at the highest concentration (20 μg/ml), but rBlo t 1 showed an approximate 20% inhibition in all tested concentrations, which was considered no inhibition. Recombinant Blo t 1 did not inhibit the system at the lowest inhibitor concentration tested (0.25 μg/ml) (data not shown).

Dot blotting for IgE-reactivity

IgE-reactivity of forty seven serum samples from allergic Puerto Rican patients was analyzed by dot blotting assays using recombinant allergens from mites.

Recombinant protein rBlo t 1 reacted with 75% (30/40) of the serum samples from patients showing a positive reaction to Bt extract in skin test, rDer p 1 reacted with 57.5% (23/40) of serum samples from patients with positive reaction to commercial HDM extract in skin test and rDer f 1 reacted with 80% (32/40) of serum samples from patients with positive reaction to commercial extract from HDM.

Recombinant allergen Der p 1 reacted with 26% (6/23) of serum from patients with highly positive reaction to Bt extract on skin test, but less reactive or negative to commercial extract from HDM, and rDer f 1 reacted with 22% (5/23) of serum from patients with highly positive reaction to Bt extract on skin test. On the other hand, rBlo t 1 did not react with any serum from patients with highly positive reaction to commercial HDM extract (Data not shown).

Statistical analysis for IgE reactivity

An analysis of two descriptive variables was performed using a matrix method. Sensitivity, specificity, positive and negative predictive values, and false negative and positive of the tests were calculated in order to analyze and compare the results obtained for both IgE reactivity tests of group 1 allergens from mites. Sensitivity is the probability that a person with a positive skin test is correctly identified by dot blot test, specificity is the probability that a non-allergic patient is correctly identified by dot blot assay, positive and negative predictive values express how often the test is correct or incorrect, respectively, when it is positive or negative, respectively.

Recombinant protein Blo t 1 achieved a high percentage of specificity (100%) and sensitivity (75%). False negative and positive results were considered low, 0% and 25%, respectively. For r Der f 1, similar results were obtained: 80% sensitivity and 85.7% specificity. Positive predictive values were higher than negative predictive values (96.9% vs. 42.8%, respectively), offering low false positive values and increased false negative values, respectively. For r Der p 1, lower sensitivity levels (57.5%) were obtained resulting in higher false negative test results (42.5%). Table 2 shows these statistical results.

Table 2. Statistical analyses

Allergen	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	False Positive	False Negative
rBlot 1	75%	100%	100%	41.17%	0%	25%
rDer p 1	57.5%	85.7%	95.8%	26.1%	14.3%	42.5%
rDer f 1	80%	85.7%	96.9%	42.8%	14.3%	20%

Discussion

It has been established that Bt and *Dermatophagoides* spp. (Dp and Df) are the main mite species in tropical and subtropical areas around the world, and coexistence among them is well documented (12). In addition, they are directly implicated as triggers of allergic responses in sensitized individuals, because these individuals in tropical and subtropical regions of the world are exposed to high levels of Bt, Dp and Df allergens (4).

The major Bt allergens have been identified and have been proven to be clinically important, but cross-reactivity between group 1 allergens is poorly defined (27). To overcome this limitation, cross-reactivity between Blo t 1, Der p 1 and Der f 1 was analyzed, using recombinant allergens and monoclonal antibodies. The results revealed strong reactivity of the positive control for rBlo t 1 indicating that mAb anti-Blo t 1 is specific against rBlo t 1 protein. The mAbs against recombinant allergen from *Dermatophagoides* spp. showed that both reacted relatively strong with both recombinant group 1 allergens from Dp and Df, but no reaction was observed with rBlo t 1 (Table 1). The classification described by Asero and collaborators was used in the analyses of the inhibition assay. These investigators studied cross-reactivity between seasonal airborne allergens, and established that in an inhibition assay, reactivity reductions greater than 75%, 50-75%, 25-50%, and less than 25%, were considered as total, moderate, slight, and no inhibition, respectively (34). Our results showed that there is a high percentage inhibition between recombinant group 1 allergens from *Dermatophagoides* spp. (Der p 1 and Der f 1), but the grade of inhibition of both with group 1 allergen from Bt (Blo t 1) was considered low (Figure 2). rBlo t 1 inhibited rDer p 1 and/or rDer f 1 in solid phase at a low percentage, which was considered as no inhibition.

The *in vitro* system used can be manipulated in terms of concentrations of inhibitors, monoclonal antibodies and antigen in solid phase, which is not seen in *in vivo* human immune conditions. In addition, temperature can be manipulated, which may alter the maximum inhibition achieved for the protein used as inhibitor. It is important to reinforce that artificial systems were used in this research, which suggest that loss of reactivity could exist. The use of monoclonal antibodies, as an efficient method to analyze cross-reactivity, could result in some disadvantages. These proteins were produced in order to react with high specificity against an antigen, but only immunodominant epitopes are recognized by them. In *in vivo* systems, there are a more complicated and amplified scenarios. Allergens are mainly glycoproteins with globular shape, which show a variety of complex conformations and have many IgE-binding epitopes (multivalent antigen), which increase the probability of recognition between the antigen-antibody system (18). When an individual exposed to an allergen inhales it, that protein enters the body across mucosal membranes as a whole protein stimulating an allergic response. IgE anti-epitopes, present on the surface of mast cells, recognize the inhaled antigen, causing the release of histamine and other inflammatory mediators and the beginning of the manifestation of allergic responses in allergens exposed individuals. Also, these natural allergens bear surface carbohydrates that may be recognized by IgE molecules, but the recombinant allergens may not contain these carbohydrates. This could alter the reactivity of recombinant group 1 allergens from Bt, Dp and Df with monoclonal antibodies. The loss of some of these important molecules by recombinant proteins during the process of expression in a prokaryote system may result in altered patterns of recognition. During the process of recombinant protein production in a prokaryote system, post-transductional modification occurs; carbohydrates and other molecules are lost, which is not the scenario present in an *in vivo* system, where the whole protein enters the body and triggers a hypersensitivity reaction.

The IgE-reactivity of recombinant proteins with serum samples from allergic Puerto Rican patients was analyzed. Skin prick test was previously performed in order to analyze the reactivity of allergic individual with different mite extracts (Bt and HDM extracts). The results obtained showed that recombinant protein Blo t 1 has a strong reactivity (75%) with serum samples from patients with a positive skin test reaction to Bt extracts. Fonseca and Díaz (23) analyzed the IgE reactivity of rBlo t 1 from serum samples, obtaining similar results: 72% IgE response to rBlo t 1 for sera from patients with positive skin test to Bt. This frequency of reactivity was considered statistically significant based on other studies of mite allergens which have shown similar results (22, 31). Recombinant proteins Der p 1 and Der f 1 reacted with 57.5% and 80%, respectively, of the sera of patients positive to HDM extract in skin test. Differences in reactivity between Dp and Df could be due to the use of HDM extract in the skin test that is not unique to a *Dermatophagoides* spp. The HDM extract used in the skin test is composed of an equal mixture of Dp and Df whole body extract that was not defined for any group allergen. The dot blot test used to analyze the IgE reactivity of those serum samples contained recombinant group 1 allergens from *Dermatophagoides* spp., which decrease the probability of reactivity between these recombinant allergens with serum samples. It is possible that the reactivity of Dp and Df could be due to cross-reactive allergens that share common IgE-binding epitopes (35). This can explain the results of the statistical analysis shown in Table 2, mainly for rDer p 1. Moreover, the phenomenon of lymphocyte maturation could influence the discrepancy of the results obtained in both group 1 allergens from *Dermatophagoides spp*. When the immune system interacts with a foreign molecule, B lymphocytes are predominantly involved. The interaction antigen-antibody becomes stronger in such a way that the rate of encounters between them will be more frequent. The skin test, as in an *in vivo* system, indicates that the affinity maturation process of B lymphocytes is at an advanced level, resulting in a rapid recognition of mite allergen epitopes when injected into the skin of allergic patients. Dot blot tests use recombinant allergens that probably are not recognized at the same degree or level by antibodies present in serum samples from sensitized individuals, because *in vitro* systems do not provide all components and opportunities for antigen-antibody encounters and reactions in order to stimulate lymphocyte affinity maturation and an effective and rapid immune response when the body interacts with that antigen again.

Recombinant group 1 proteins from Dp and Df reacted with 26% and 22%, respectively, of the serum from patients with highly positive skin test reactions to Bt extract, but rBlo t 1 failed to react with serum from patients with a highly positive skin test reaction to the HDM extract. This is indicative that cross-reactivity between Bt and Dp or Df is very low.

ELISA inhibition assays showed that there is a high level of cross-reactivity between homologous allergens from *Dermatophagoides spp.*, but the cross-reactivity with Bt is low to moderate. These results probably occur because allergens from Dp and Df have more affinity for a common epitope than rBlo t 1. This fact is indicative that future characterization of IgE-binding epitopes from group 1 allergens from Dp, Df and Bt is necessary for a

complete understanding of immune responses against mite allergens in an *in vitro* system.

The overall results indicate the importance to include Bt allergen in the routine skin test for a more accurate diagnosis and treatment of allergic diseases in tropical and subtropical areas around the world.

Resumen

Los ácaros de polvo doméstico constituyen una importante fuente de alergenos pudiendo sensibilizar a individuos genéticamente predispuestos, y ser un factor desencadenante de asma. Los principales ácaros pertenecen a la especie *Dermatophagoides* y *Blomia tropicalis* (Bt). Varios alergenos de Bt han sido clonados y secuenciados, algunos de los cuales presentan secuencias homólogas con alergenos purificados de *Dermatophagoides pteronissynus* (Dp). En nuestro laboratorio hemos clonado y secuenciado el alergeno del grupo 1 de Bt denominado Blo t 1. La proteína recombinante rBlo t 1 tiene un 35% de identidad y un 50% de similitud con otros alergenos de grupo 1 como Der p 1 (de Dp), Der f 1 (de *D. farinae*) y Eur m 1 (de *Euroglyphus maynei*), esto podría sugerir que existe reactividad cruzada entre estas especies. En este trabajo analizamos la reactividad cruzada entre alergenos del grupo 1 de ácaros utilizando proteínas recombinantes y anticuerpos monoclonales contra ellas. La inhibición del ensayo de ELISA demostró que hay un alto grado de reactividad cruzada entre alergenos homólogos de especies de *Dermatophagoides,* pero es baja o moderada entre ácaros de diferentes especies. El análisis de la reactividad de IgE sérica de individuos alérgicos demostró una fuerte reacción con rBlo t1 en muestras séricas de pacientes con reacción positiva al extracto de Bt en pruebas de piel, pero una falta de reacción sérica en individuos con una reacción de piel positiva al extracto de ácaros de polvo doméstico. Estos resultados sugieren la importancia de incluir alergenos de Bt en las pruebas rutinarias de piel para mejorar la precisión y la certeza del diagnóstico de alergias.

References

1. Strass MD, Ardusso LRF, Crisci CD. Prevalencia de sensibilidad a aeroalergenos en pacientes con rinitis y/o asma en el sur de Misiones y noreste de Corrientes, Argentina. Arch Alergia Immunol Clin 2002;33:47-52.
2. Soares FA, Segundo GR, Alves R, Ynoue LH, Resende RO, Sopelete MC, Silva DA, Sung SS, Taketomi EA Indoor allergen sensitization profile in allergic patients of the allergy clinic in the University Hospital in Uberlândia, Brazil. Rev Assoc Med Bras 2007;53:25-28.
3. Barraza-Villareal A, Sanín-Aguirre LH, Téllez-Rojo MM, Lacasaña-Navarro M, Romieu I. Prevalencia de asma y otras enfermedades alérgicas en niños escolares de Ciudad Juárez, Chihuahua. Salud Publ Mex 2001;43:433-443.
4. Montealegre F, Sepúlveda A, Bayona M, Quiñones C, Fernández-Caldas E. Identification of the domestic mite fauna of Puerto Rico. PR Health Sci J 1997;16:109-116.
5. Ramos P, Rullán J. Guía para el manejo y control del asma en adultos. Estado Libre Asociado de PR. Departamento de Salud. Secretaria Auxiliar para la prevención y control de enfermedades. División de prevención y control de enfermedades crónicas. Rev. 2004
6. Sporik R, Chapman MD, Platts-Mills TA. House dust mite exposure as a cause of asthma. Clin Exp Allergy 1992;22: 897-906.
7. Ardusso LRF. Importancia de Blomia tropicalis como aeroalergeno. Arch. Alergia Immunol Clin 2002;33:37-38.
8. Milián E, Díaz AM. Allergy to house dust mite and asthma. PR Health Sci J 2004;23:47-57.
9. Platts-Mills TAE, Thomas WR, Aalberse RC, Vervloet D, Chapman MD. Dust mite allergens and asthma: report of a second international workshop. J Allergy Clin Immunol 1992;89:1046-1060.
10. Pérez M, García A, Sabina A, Vega M, Macías V. Sensibilización a diferentes tipos de ácaros en pacientes adultos. Rev Cubana Med Trop 2002;41:75-78.
11. De Oliveira AJ, Daemon E. Qualitative and quantitative assessment of mites (Acari) in domiciliary dust in rural dwellings in the "Zona da Mata" region, Minas Gerais, Brazil. Rev Brasilera de Zoología 2003;20:675-679.
12. Arlian LG, Morgan MS, Neal JS. Dust mite allergens: ecology and distribution. Curr Allergy Asthma Rep 2002;2:401-411.
13. Simpson A, Green R, Custovic A, Woodcock A, Arruda LK, Chapman MD. Skin test reactivity to natural and recombinant Blomia and Dermatophagoides spp. allergens among mite allergic patients in the UK. Allergy 2003;58:53-56.
14. Fernández-Caldas E. Allergenicity of Blomia tropicalis. J Investig Allergol Clin Immunol 1997;7:402-404.
15. Yeoh SM, Kuo IC, Wang DY et al. Sensitization profiles of Malaysian and Singaporean subjects to allergens from Dermatophagoides pteronyssinus and Blomia tropicalis. Int Arch Allergy Immunol 2003;132:215-220.
16. Arlian LG, Platts-Mills TAE. The biology of dust mites and the remediation of mite allergens in allergic disease. J Allergy Clin Immunol 2001;107:406-413.
17. Dilworth RJ, Chua KJ, Thomas WR. Sequence analysis of cDNA coding for a major house dust mite allergen, Der f 1. Clin Exp Allergy 1991;21:25-32.
18. Yi FC, Shek LP, Cheong N, Chua KY, Lee BW. Molecular cloning of Blomia tropicalis allergens—a major source of dust mite allergens in the tropics and subtropics. Inflamm Allergy Drug Targets 2006;5:261-266.
19. Mora C, Flores I, Montealegre F, Diaz A. Cloning and expression of Blo t 1, a novel allergen from the dust mite Blomia tropicalis, homologous to cysteine proteases. Clin Exp Allergy 2003;33: 28-34.
20. Pauli G, Bessot JC. The fight against mites. Allergy Today 1998;2:7-9.
21. Sidenius KE, Hallas TE, Poulsen LK, Mosbech H. Allergen cross-reactivity between house-dust mites and other invertebrates. Allergy 2001;56:723-733.
22. Cheong N, Soon SC, Ramos JDA et al. Lack of human IgE cross-reactivity between mite allergens Blo t 1 and Der p 1. Allergy 2003;58:912-920.
23. Fonseca L, Díaz AM. IgE reactivity from serum of Blomia tropicalis allergic patients to the recombinant protein Blo t 1.

PR Health Sci J 2003;22:353-357.
24. Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. Allergy 2004;59: 243-267.
25. Huertas AJ, Lavín JR, García-Cervantes AM, Negro JM. Sensibilización a ácaros del género Blomia en el área urbana de Cartagena de Levante. Alergol Inmunol Clin 2004;19: 231-236.
26. Puccio FA, Lynch NR, Noga O et al. Importance of including Blomia tropicalis in the routine diagnosis of Venezuelan patients with persistent allergic symptoms. Allergy 2004;59:753-757.
27. Chua KY, Cheong N, Kuo IC, Lee BW, Yi FC, Huang CH, Liew LN. The Blomia tropicalis allergens. Protein Pept Lett 2007;14:325-333.
28. Collazo M, Díaz AM. Monoclonal antibodies against whole body extract of the dust mite Blomia tropicalis. PR Health Sci J 2003;22:345-351.
29. Silva DA, Gervasio AM, Sopelete MC, et al. A sensitive reverse ELISA for the measurement of specific IgE to Der p 2, a major Dermatophagoides pteronissynus allergen. Ann Allergy 2001; 86:545-550.
30. Barletta B, Afferni C, Thinghino R, Mari A, Di Felice G, Pini C. Cross-reactivity between Cupressus arizonica and Cupressus sempervirens pollen extracts. J Allergy Clin Immunol 1996; 98:797-804.
31. Kuo IC, Cheong N, Trakultivakom M, Lee BW, Chua KY. An extensive study of human IgE cross-reactivity of Blo t 5 and Der p 5. J Allergy Clin Immunol 2003;111:603-609.
32. Chew FT, YI FC, Chua KY, et al. Allergenic differences between the domestic mites Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy 1999;29:982-988.
33. Wayne WD. Biostatistics: a foundation for analysis in the health sciences. 7th Ed. New York: John Wiley & Sons, Inc. 1999; 71-74.
34. Asero R, Mistrello G, Roncarolo D, Amato S. Parietaria profilin shows only limited cross-reactivity with birch and grass profilins. Int Arch Allergy Immunol 2003;133:121-124.
35. Caraballo L, Mercado D, Jiménez S, Moreno L, Puerta L, Chua KY. Analysis of the cross-reactivity between BtM and Der p 5, two group 5 recombinant allergens from Blomia tropicalis and Dermatophagoides pteronyssinus. Int Arch Allergy Immunol 1998;117:38-45.