

# Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp-allergic individuals

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Serum was collected from 77 atopic subjects from Cartagena, Colombia, who had a positive skin test ( $\geq 3$  mm) to either *Dermatophagoides farinae* (Df) (73) or *D. pteronyssinus* (Dp) (70). Twenty-nine healthy volunteers with negative allergy histories and negative skin tests were used as control subjects. RAST and RAST-inhibition assays were used to study the prevalence of specific IgE to *Blomia tropicalis* (Bt), *Lepidoglyphus destructor* (Ld), and Df. RAST results were considered positive when a serum bound  $\geq 1\%$  of the total counts added; 69 atopic subjects (89.6%) were positive to Df, 66 (85.5%) to Bt, and 46 subjects (59.7%) were positive to Ld. RAST results, analyzed by regression analysis, revealed the following correlation coefficients: Df versus Bt,  $r = 0.59$ ; Df versus Ld,  $r = 0.44$ ; and Bt versus Ld,  $r = 0.52$ . RAST-inhibition assays with a serum pool of 11 individuals highly allergic to all three mites (mean specific IgE to all mites,  $\geq 18.2$  percent total counts bound) demonstrated considerable cross-reactivity between Bt and Ld and moderate cross-reactivity between Df and Bt and Ld. Bt, Df, and Ld possess unique, as well as common, allergens. Sensitization to Bt and Ld is very common in mite-allergic individuals in Cartagena, Colombia. (J ALLERGY CLIN IMMUNOL 1991;88:943-50.)

**Key words:** Specific IgE, storage mites, allergic diseases, Cartagena, Colombia

Mites of the family Pyroglyphidae, genus *Dermatophagoides*, especially *Dp* and *Df*, are the main sources of house dust allergens and an important etiologic factor in allergic respiratory diseases.<sup>1-8</sup> Storage mites can also cause allergic respiratory diseases in exposed and sensitized individuals.<sup>9-16</sup> The most common storage mites belong to the families Glycyphagidae (genera, *Blomia*, *Lepidoglyphus*, and *Glycyphagus*), Acaridae (genera, *Tyrophagus*, *Acarus*, *Suidasia*, and *Aleuroglyphus*) and Chortoglyphidae (genus, *Chortoglyphus*).<sup>12, 13, 16-18</sup> There is an increasing interest in the allergenicity of these mites because they

## Abbreviations used

Df: *Dermatophagoides farinae*  
Dp: *Dermatophagoides pteronyssinus*  
Bt: *Blomia tropicalis*  
Ld: *Lepidoglyphus destructor*  
AR: Allergic rhinitis  
PBS: Phosphate-buffered saline  
TCB: Total counts bound

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have been found in house dust samples throughout the world, and there is evidence that sensitization to their allergens is not limited to individuals with occupational exposure.<sup>8, 15, 19-21</sup> Some investigators have found little or no cross-reactivity among *Dermatophagoides* spp and storage mites, suggesting that this lack of cross-reactivity may imply important clinical consequences for individuals sensitized to storage-mite allergens.<sup>9, 22</sup> However, Miyamoto et al.<sup>23</sup> suggested by in vitro neutralization of skin-sensitizing antibodies that house dust and storage mites have common and species-specific antigens. It has been reported that *Dp* and *Df* have two and four common

antigens with *Bt*, respectively.<sup>24</sup> The IgE binding capacity of these antigens has not been established yet. In another study, Griffin et al.<sup>25</sup> concluded that there is limited cross-reactivity among *Acarus siro*, *G. destructor*, and *Dp* and that these mites have common and species-specific allergenic determinants.

Storage mites are an important source of allergens in the farming environment.<sup>4, 9-13</sup> The glycyphagid mite, *Ld*, is associated with allergic diseases in occupationally exposed individuals.<sup>9-12, 15, 26</sup> In Sweden, van Hage-Hamsten et al.<sup>14</sup> reported a 79.9% prevalence of positive RAST to *Ld* in farmers with a positive RAST to other mite species (*Acarus siro*, *E. maynei*, and *Dp*). Data from Finland,<sup>10</sup> Sweden,<sup>11, 14</sup> and England<sup>12</sup> have demonstrated that *Ld* is the most important sensitizing storage mite in farmers. Positive bronchial challenges have been demonstrated in sensitized farmers in Denmark,<sup>9</sup> Finland,<sup>10</sup> and Sweden.<sup>23</sup> Iversen et al.<sup>15</sup> reported a high prevalence of positive RASTs to storage mites (*Ld*, *T. putrescentiae*, and *Acarus siro*) in 144 Danish farmers. These investigators found a strong association between a positive RAST to house dust mites and a positive RAST to storage mites; 50% of the farmers with a positive RAST to house dust mites also had a positive RAST to storage mites.

Several investigators have studied IgE-mediated sensitization to storage mites in nonfarmers.<sup>19-21, 27-30</sup> Iversen and Dahl<sup>21</sup> reported a 27.5% prevalence of positive RASTs to storage mites in 326 individuals with asthma and with house dust mite allergy. In London, Luczynska et al.<sup>28</sup> studied 196 individuals from an urban environment who were not occupationally exposed to storage mites and found a 24% prevalence of elevated specific IgE to *Dp* and a 14% prevalence of positive RASTs to at least one of the storage mites tested (*Acarus siro*, *Ld*, and *T. longior*). The glycyphagid mite, *Bt*, is very common in some regions of the world and has been detected in 16% to 96% of house dust samples in countries with tropical and subtropical climates.<sup>23, 24, 27, 31-36</sup> *Bt* produced the highest number of positive skin tests among six storage mites in house dust mite-allergic individuals in Hong Kong.<sup>27</sup> Sensitization to *Bt* allergens is very common in Tampa, Fla. *Bt* was identified in 30% of the dust samples analyzed by light microscopy,<sup>32</sup> and specific IgE to *Bt* allergens was detected in 36% of 89 individuals with a positive skin test to *Dp*.<sup>29</sup> In Colombia, *Bt* has been reported as the most common Glycyphagidae species in house dust samples.<sup>36</sup> Two species of the genus *Lepidoglyphus*, *L. michaelli*, and *Ld* have also been identified in house dust samples in different locations in Colombia.<sup>36</sup> Specific IgE to *Bt* and *Ld* has also been detected in mite-allergic subjects with asthma in Bogotá, Colombia (altitude, 2600 m).<sup>37</sup>

Allergic respiratory diseases and house dust-mite sensitivity are common in Cartagena, a tropical city located on the Caribbean on the northwest coast of Colombia. Sensitization to *Df* and *Dp* has been documented in allergic individuals, and the genetic predisposition for developing allergic diseases has been suggested.<sup>35, 38</sup> The role of storage mites in allergic respiratory diseases in Cartagena has not been fully established; therefore, the present investigation studied the prevalence of specific IgE to *Df*, *Bt*, and *Ld* in a population of house dust mite-allergic individuals and the cross-reactivity among these mites.

## MATERIAL AND METHODS

### Patient population

Sera were obtained from 77 individuals (39 male and 38 female subjects; mean age, 29.1 years), 58 of whom had allergic asthma and AR, and 19 had AR alone. All these individuals were evaluated for allergic complaints of the upper and/or lower respiratory tract and were prick skin tested with a battery of allergens (glycerinated extracts of house dust, ragweed mix, cat epithelia, dog epithelia, *Aspergillus* mix, *Penicillium* mix, *Cladosporium* [*Hormodendrum*] *cladosporioides*, *Alternaria tenuis*, and feathers and standardized extracts of *Dp* and *Df* containing 10,000 allergy units [Greer Laboratories, Lenoir, N.C.]). All 77 individuals were skin test positive (wheal  $\geq 3$  mm with erythema 15 minutes after testing) to at least one mite extract; 73 were positive to *Df*, and 70, to *Dp*. Sera from 29 volunteers (20 male and nine female volunteers; mean age, 24.6 years) without a history of AR and/or asthma and negative skin prick test to *Df* and *Dp* were used as negative control subjects. Both patients and control subjects were mulattos, the prevalent ethnic group in Cartagena.

### Allergen extracts for in vitro studies

Standardized *Dp* and *Df* extracts were obtained from Greer Laboratories, and extracts of *Bt* and *Ld* were prepared in our laboratory. *Bt* mites were cultured for 2 months with autoclaved rodent chow and separated from the food medium with a modified Tullgren apparatus.<sup>39</sup> *Ld* mite bodies were obtained from Biopol Laboratory, Inc., Spokane, Wash.

Whole mite bodies were defatted for 4 hours with anhydrous ether in a Soxhlet extractor and dried at room temperature. Defatted mites were extracted (1:20 wt/vol) in 0.1 mol/L of ammonium bicarbonate for 24 hours at 4° C. The extracts were clarified by centrifugation at 18,000 rpm and dialyzed overnight at 4° C against deionized water with Spectra/Por 3 membranes with a 3500 molecular weight cutoff (Spectrum Medical Industries, Inc., Los Angeles, Calif.). The dialyzed extracts were recentrifuged, filtered with 0.22  $\mu$ m membranes, and stored at -20° C. Protein contents were measured by the bicinchoninic acid method (Pierce Chemical Co., Rockford, Ill.).<sup>40</sup> Protein concentrations in the different mite extracts were as follows: *Df* extract, 3.6 mg/ml; *Bt* extract, 4 mg/ml; and *Ld* extract, 3.2 mg/ml.

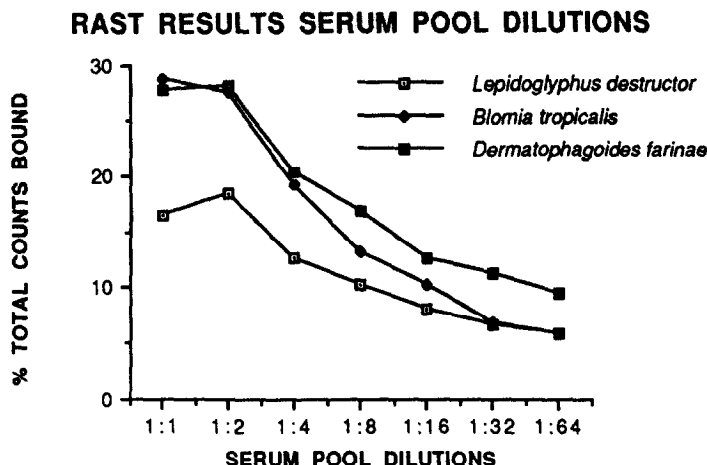


FIG. 1. Serial dilutions of the serum pool and specific IgE binding to *Df*, *Ld*, and *Bt*.

## RAST

Specific IgE to each mite was determined with plastic microtiter plates (Immulon 4, Dynatech Laboratories, Alexandria, Va.). Mite extracts were diluted 1:100 in 0.2 mol/L of sodium carbonate/bicarbonate buffer, pH 9.2, and 100  $\mu$ L was added to each well, incubated overnight at room temperature, and washed three times with RAST wash (potassium phosphate-buffered saline, 1% Tween 20, pH 7.4). One hundred microliters of each serum was then added to each well, incubated overnight in a humid box and washed three times, and 100  $\mu$ L of  $^{125}$ I radiolabeled antihuman IgE (Pharmacia Diagnostics, Piscataway, N.J.) was added. After overnight incubation, the plates were washed, and each well was counted in a gamma counter. The sera were added in duplicate, and the results were expressed as the mean of the percentage of TCB. A RAST was considered positive when a serum bound  $\geq 1\%$  of the total counts added (approximately three times the IgE binding of a negative control).

A serum pool containing aliquots from 11 patients with high specific IgE binding to *Bt*, *Ld*, and *Df* was prepared (Table I). Microtiter wells were coated with allergen, and an optimal serum pool dilution was established by serial dilutions of the serum pool in RAST diluent (potassium phosphate-buffered saline, pH 7.4, 0.1% Tween 20, and 1% bovine serum albumin). A serum dilution of 1:16 (6.25  $\mu$ L of undiluted serum) was used for the RAST-inhibition assays to avoid antibody excess in the fluid phase and enhance the sensitivity of the assay (Fig. 1).

## RAST inhibition

Fifty microliters of serial dilutions of the mite extracts (1:8000, 1:6000, 1:4000, 1:2000, 1:1000, 1:500, 1:200, 1:100, and 1:50) was added in duplicate along with 50  $\mu$ L aliquots of diluted pooled serum. The plates were incubated overnight at room temperature and washed, and 100  $\mu$ L of  $^{125}$ I radiolabeled antihuman IgE was added to the wells. After an overnight incubation and wash, individual wells were counted in a gamma counter. The results were analyzed by regression analysis after normalization for background binding.

TABLE I. Specific IgE binding of the 11 sera used in the serum pool, expressed in percent TCB

| Code | Df   | Bt   | Ld   |
|------|------|------|------|
| A6   | 36.5 | 33.6 | 17.1 |
| A17  | 14.2 | 17.6 | 39.8 |
| A21  | 11.9 | 27.3 | 16.4 |
| A32  | 26.3 | 27.2 | 10.3 |
| A40  | 20.8 | 14.5 | 10.5 |
| A41  | 14.3 | 22.0 | 2.5  |
| A42  | 10.5 | 2.5  | 22.5 |
| A50  | 18.2 | 8.8  | 11.5 |
| B12  | 32.4 | 21.3 | 15.2 |
| B17  | 12.6 | 25.2 | 43.0 |
| 85   | 26.9 | 5.8  | 11.4 |

## Total IgE

Total IgE levels in the sera of allergic and nonallergic individuals were measured with the Pharmacia PRIST method.

## Statistical analysis

Graphs and statistical analyses, including regression analysis of RAST and RAST-inhibition results, mean, standard deviations, and Student's *t* test were performed with the StatView 512 software package (BrainPower, Inc., Calabasas, Calif.) and a Macintosh SE 30 computer (Apple Computer, Inc., Cupertino, Calif.).

## RESULTS

A box plot analysis of the positive RAST results to *Df*, *Bt*, and *Ld* is illustrated in Fig. 2. The five horizontal lines on each box plot, where they are visible, illustrate the 10th, 25th, 50th, 75th, and 90th percentiles. The values above and below the 10th and 90th

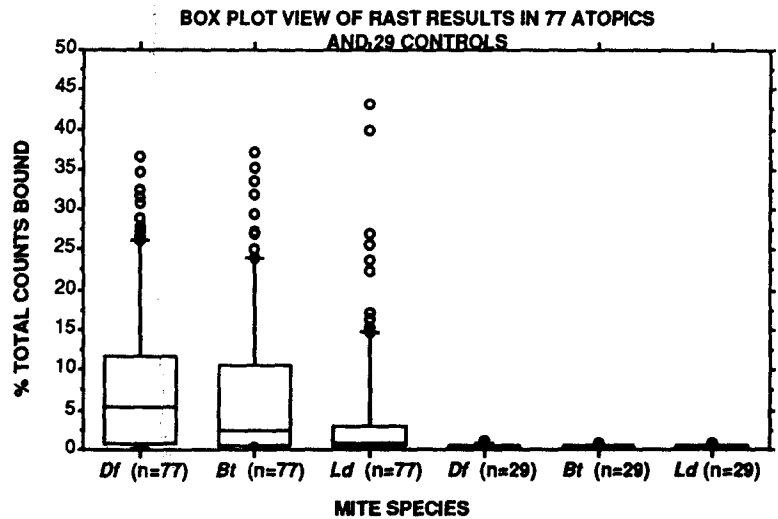


FIG. 2. Box plot view of the RAST results in 77 atopic and 29 control individuals.

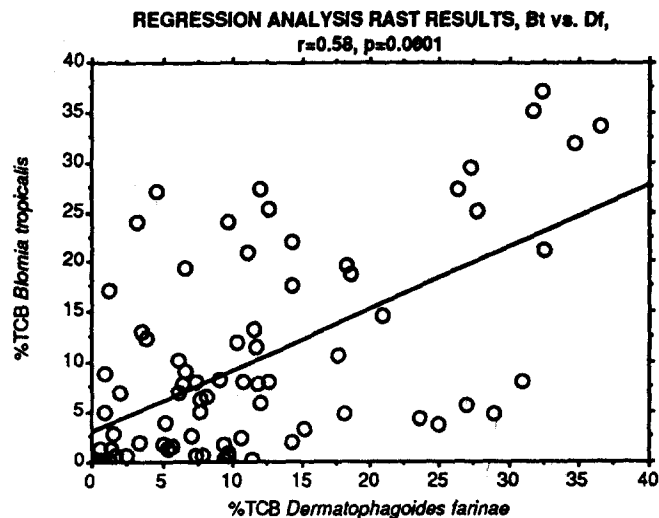


FIG. 3. Regression analysis of the RAST results, *Bt* versus *Df*;  $r = 0.59$ ;  $p = 0.0001$ .

TABLE II. Mean specific IgE binding to the three mite species in the atopic and control individuals

|           | Atopic subjects**† | RAST (+)   | %‡   | Control subjects† | p†     |
|-----------|--------------------|------------|------|-------------------|--------|
| <i>Df</i> | 11.3 ± 9.7         | 12.5 ± 9.5 | 89.6 | 0.4 ± 0.2         | 0.0001 |
| <i>Bt</i> | 10.0 ± 10.1        | 12.4 ± 9.9 | 80.5 | 0.3 ± 0.1         | 0.0001 |
| <i>Ld</i> | 5.5 ± 8.8          | 9.1 ± 10   | 59.7 | 0.3 ± 0.1         | 0.0019 |

\*Includes RAST results of 77 atopic individuals.

†Unpaired two-tail Student's *t* test.

‡Percent individuals with a positive RAST to each mite.

percentile are presented as data points. RAST results to the three mite species in the control and allergic individuals were compared by the Student's *t* test. The results are presented in Table II. In the control

group, mean specific IgE binding to *Df* was 0.4% TCB ± 0.2 (range, 0.22 to 0.99% TCB); to *Bt*, 0.36% TCB ± 0.16 (range, 0.23 to 0.81% TCB); to *Ld*, 0.36% TCB ± 0.12 (range, 0.23 to 0.72% TCB).

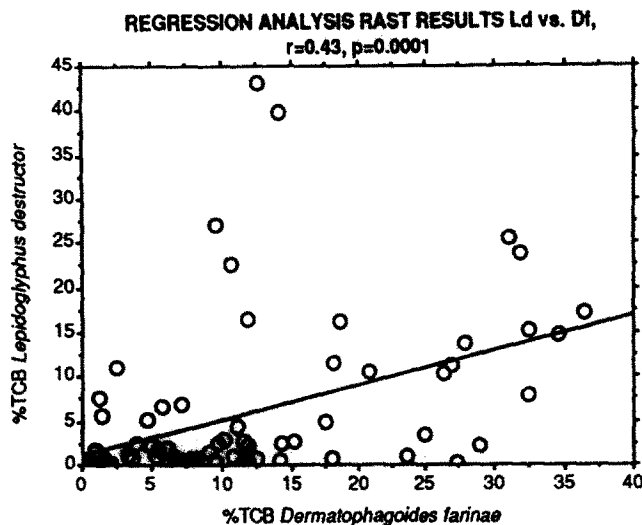


FIG. 4. Regression analysis of the RAST results, *Ld* versus *Df*;  $r = 0.43$ ;  $p = 0.0001$ .

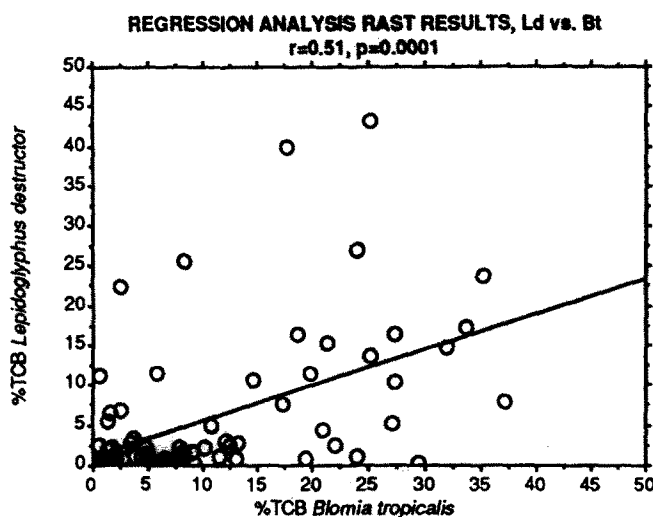


FIG. 5. Regression analysis of the RAST results, *Ld* versus *Bt*;  $r = 0.51$ ;  $p = 0.0001$ .

The geometric mean of the total IgE levels in the allergic individuals was 359.3 kU/L (range, 33.6 to 4600 kU/L), and in the control subjects, 163.63 kU/L (range, 10.5 to 2200 kU/L);  $p < 0.05$ .

There was a high prevalence of specific IgE to the three mites tested in the allergic individuals; 72 individuals (93.5%) were RAST positive to at least one of the three mites tested, and 42 of the 72 individuals (58.3%) were positive to all three mite species. Five individuals were RAST negative to all three mites. Specific IgE to *Df* was detected in 69 individuals (89.6%), to *Bt* in 62 (80.5%), and to *Ld* in 46 individuals (59.7%).

Sixty-four patients (83.1%) had a positive RAST to at least one of the two mites belonging to the Gly-

cyphagidae family. A positive reaction in the same subject to both mites was found in 44 of the 64 cases (68.75%). In contrast, 18 individuals had a positive RAST to *Bt* with negative RAST to *Ld* and two individuals had a positive RAST to *Ld* and a negative RAST to *Bt*. Among patients with a positive RAST to *Df*, 58 (84%) had a positive RAST to *Bt* and 44 (63.7%) to *Ld*. Positive RAST to a single mite species was rare. Seven individuals had a positive RAST exclusively to *Df*, three to *Bt*, and none to *Ld*.

RAST results were compared by regression analysis. Significant correlations were found between the RAST results to *Bt*, *Ld*, and *Df*. In all three cases, a value of  $p = 0.0001$  was obtained (Figs. 3 to 5).

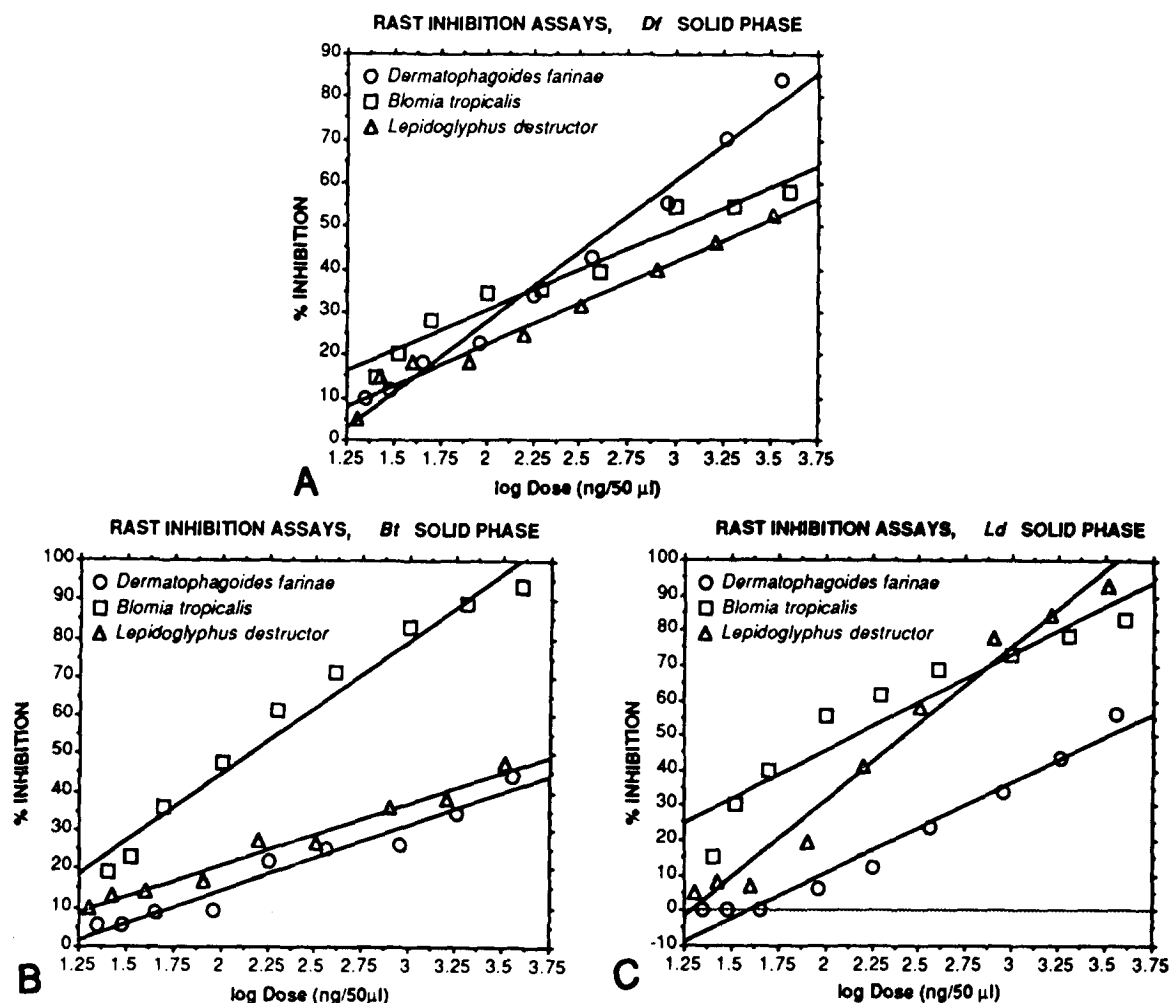


FIG. 6. A, RAST-inhibition assay, *Df* solid phase. B, RAST-inhibition assay, *Bt* solid phase. C, RAST-inhibition assay, *Ld* solid phase.

### Cross-reactivity studies

The RAST results of the individual sera used in the serum pool are presented in Table I. The serum pool contained a high concentration of specific IgE to the three mite species; a similar IgE binding activity was detected when 100 µl or 50 µl was used (Fig. 1). The inhibitory capacities of different concentrations of *Df*, *Bt*, and *Ld* extracts on the serum pool are illustrated in Fig. 6, A, B, and C. When *Bt* was used in the solid phase, *Bt* reached 93.2% inhibition at the highest concentration used (1:50 dilution). At the same dilution, *Df* produced 44.3% inhibition and *Ld*, 47.5% inhibition. When *Df* was used in the solid phase, *Df* produced 84.3% inhibition at the highest concentration used (1:50 dilution). The same dilution of *Bt* inhibited 58%, and of *Ld*, 52.6%. When *Ld* was used in the solid phase, *Ld* inhibited 97.7% at the highest concentrations used (1:50 dilution); at the

same dilution, *Bt* inhibited 83% and *Df*, 56.4%, *Bt* extract revealed a greater inhibitory capacity than *Df* on *Ld* solid phase (Fig. 6, C), and more than *Ld* on *Df* solid phase (Fig. 6, A). *Df* and *Ld* extracts revealed moderate cross-reactivity with *Bt* when *Bt* solid phase was used (Fig. 6, B).

### DISCUSSION

A high prevalence (83.1%) of specific IgE to storage mites in 77 atopic individuals in Cartagena, Colombia, was detected. The highest prevalence of positive RASTs was detected to *Df*. This finding was expected since the patients were selected based on their skin test sensitivity to *Dermatophagoides* spp. In subjects with a positive RAST to *Df*, the prevalence of positive RASTs to *Bt* and *Ld* was 85.5% (59/69) and 63.7% (44/69), respectively. These findings support the idea that sensitization to storage mites is generally asso-

ciated with sensitization to house dust mites.<sup>15, 20, 21</sup> However, the IgE binding patterns to the three mite species varied among the different individuals (Table I). The high prevalence of specific IgE to *Bt* in atopic individuals can be explained by the widespread occurrence of this mite species in the environment. *Bt* was found in 80% of homes of allergic patients in Cartagena and is the most common glycyphagid mite in Colombia.<sup>35, 36</sup> The climate in this tropical region, with a mean annual temperature of 28° C and a mean relative humidity of 82%, offers good conditions for the growth of this mite species.<sup>41</sup> In this study, a higher prevalence of positive RASTs to *Bt* than to *Ld* was found that could reflect the higher prevalence of *Bt* in house dust samples in Cartagena.

A fairly high correlation coefficient between RAST results to *Df* and *Bt* ( $r = 0.59$ ) was detected. This correlation coefficient was similar to that reported by van Hage-Hamsten et al.<sup>42</sup> between *Dp* and *B. kulagini* in Brazil. These findings suggest that in tropical regions the genus *Blomia* may have an allergenic role as important as the *Dermatophagoides* spp and that sensitization to storage mites may be more common than in other geographic areas.

Based on RAST-inhibition assays, the cross-reactivity between *Df* and *Bt* appears to be greater than that between *Df* and *Ld*. Information concerning cross-reactivity between *Df* and *Ld* is very limited. In Denmark, Korsgaard et al.<sup>9</sup> reported a correlation coefficient of  $r = 0.3$  between *Df* and *Ld* by skin testing allergic individuals. More information is available about the cross-reactivity between *Dp* and *Ld*. Studies on the cross-reactivity of *Dp* and *Ld* have produced contradictory results. Although some studies reveal strong association by skin test ( $r = 0.93$ )<sup>12</sup> or by RAST ( $r = 0.66$ ),<sup>13</sup> other studies found no significant correlation in RAST analyses ( $r = 0.18$ ).<sup>20, 23</sup> In this study, regression analysis of RAST results suggested some degree of cross-reactivity between *Df* and *Ld* ( $r = 0.49$ ). This was confirmed by RAST-inhibition studies (Fig. 6, A and C). The findings of a positive RAST to *Ld* and negative to *Df* (two individuals) and a positive RAST to *Df* and negative to *Ld* (24 individuals) support the existence of specific antigens in both mites. Based on these observations, sensitization to *Ld* involves exposure to *Ld* allergens. These findings indicate that there is considerable cross-reactivity between *Bt* and *Ld* and moderate between *Df* and *Bt* and *Df* and *Ld*. It is possible that cross-reactivity among mite species may account for some positive RAST results.

*Bt*, *Df*, and *Ld* are important sensitizers in Cartagena and surrounding areas. The immune responses to *Bt* and, to a lesser extent, to *Ld* were comparable

to responses to *Df*, indicating that *Bt* and *Ld* mites are of clinical importance, *Bt* should be considered as a mite of major clinical importance in Cartagena, along with *Dermatophagoides* spp, because of its common occurrence in house dust and the high prevalence of specific IgE to *Bt* among allergic individuals. The presence of unique allergens in house dust mites and in storage mites is supported by the existence of subjects with positive RAST against either storage mites or *Df*. The high prevalence of positive RASTs to various mites may be the result of the presence of multiple mite species and favorable growing conditions in Cartagena and can be favored by an appropriate genetic background among the population.

This study documents a very high frequency of positive RASTs to storage mites in allergic individuals. Additional characterization studies of different mite species should be conducted to clarify the importance of their allergens. Likewise, genetic studies investigating susceptibility genes controlling the IgE response to mites could be useful in understanding the high prevalence of positive RAST observed in the population studied. Storage mites should be considered for immunotherapy in countries in which they are associated with IgE-mediated sensitivity.

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