Sensitization to *Blomia tropicalis* in Patients with Asthma and Identification of Allergen Blo t 5

L. KARLA ARRUDA, LISA D. VAILES, THOMAS A. E. PLATTS-MILLS, ENRIQUE FERNANDEZ-CALDAS, FREDERICO MONTEALEGRE, KEH-LIANG LIN, KAW-YAN CHUA, M. CANDIDA RIZZO, CHARLES K. NASPITZ, and MARTIN D. CHAPMAN

Asthma and Allergic Diseases Center, Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, Virginia; C. B. F. Leti, S. A., Madrid, Spain; Ponce School of Medicine, Ponce, Puerto Rico; Chung Shan Medical and Dental College, Taichung, Taiwan; Graduate Institute of Immunology, College of Medicine, National Taiwan University, Taipei, Taiwan; and Paulista School of Medicine, São Paulo, Brazil

In tropical and subtropical regions of the world, allergens produced by *Blomia tropicalis* are an important cause of IgE-mediated sensitization among patients with asthma. We compared the relative importance of sensitization to the two mite species among asthma patients from Florida, Puerto Rico, and Brazil (n = 83), who were concurrently exposed to *B. tropicalis* and *D. pteronyssinus*, with patients from the United States and from the United Kingdom (n = 56) exposed to *D. pteronyssinus*. In addition, molecular cloning techniques were used to clone and express a major *B. tropicalis* allergen. There were significant differences between IgE antibody responses to *B. tropicalis* and *D. pteronyssinus* that were related to exposure: only 22% of patients exposed to both species had a high ratio (> 10) of IgE *D. pteronyssinus:B. tropicalis*, whereas 68% of patients exposed only to *D. pteronyssinus* had a ratio of > 10 ($p < 0.001$). A major 14-kD allergen (Blo t 5), cloned from a *B. tropicalis* cDNA library, showed 43% sequence homology to *D. pteronyssinus* Der p 5. Recombinant Blo t 5 produced in *E. coli* reacted with 45 to 69% of sera from *B. tropicalis*-allergic asthmatics and induced positive immediate skin tests at 10^{-3} to 1 μg/ml. *In vivo* and *in vitro* comparisons of IgE responses to *B. tropicalis*, *D. pteronyssinus*, rBlo t 5, and rDer p 5, showed that *B. tropicalis* has unique allergens that cause specific IgE responses. The results suggest that *B. tropicalis* is an independent cause of sensitization and that use of recombinant Blo t 5 should lead to a better understanding of the role of *B. tropicalis* in causing asthma in tropical environments. Arruda LK, Vailes LD, Platts-Mills TAE, Fernandez-Caldas E, Montealegre F, Lin K-L, Chua K-Y, Rizzo MC, Naspitz CK, Chapman MD. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5.

AM J RESPIR CRIT CARE MED 1997;155:343–350.

There is increasing evidence that exposure to indoor allergens is a causative factor for the development of asthma among persons who are genetically predisposed to make IgE antibody (ab) responses (1–7). Sensitization to indoor allergens is the strongest independent risk factor associated with asthma (odds ratios > 10). Allergens derived from house dust mites have been recognized as an important cause of IgE ab responses for more than 30 yr, and *Dermatophagoides* species (family PYROGLYPHIDAE) are the predominant fauna in house dust worldwide. At least seven groups of protein allergens have been defined and cloned from *Dermatophagoides* spp, and used in etiologic studies investigating the role of dust mites in asthma (reviewed in References 2 and 8). A recent survey in the United States revealed that, along with *D. pteronyssinus* and *D. farinae*, the most common mite species in the house dust fauna were *Blomia tropicalis* and *Euroglyphus maynei* (9).

Blomia tropicalis (family GLYCYPHAGIDAE) and *D. pteronyssinus* occur with high frequency and at high levels of infestation in houses from tropical and subtropical areas of the world. These regions include the southern states of the United States, Central and South America, Hong Kong, Taiwan, India, and Egypt (9–12). Epidemiologic studies in São Paulo, Brazil, have shown that sensitization to *Blomia* is strongly associated with childhood asthma (13, 14). No significant evidence of sensitization or exposure to cat, cockroach, grass pollens, or *Aspergillus* was found among children in São Paulo, and dust mites appear to be by far the most important allergens associated with asthma in Brazil. A recent multicenter skin testing study in several Latin American countries, including Brazil, Venezuela, and Colombia, confirmed the high prevalence of sensitization to mites, notably, *B. tropicalis*, in patients with asthma (15).

(Received in original form March 11, 1996 and in revised form May 23, 1996)

Supported by Grants AI-34607 and AI-20565 from the National Institutes of Health.

Dr. Arruda is the recipient of an Underrepresented Minority Investigator in Asthma and Allergy Award sponsored by the National Institute of Allergy and Infectious Diseases and by the American Academy of Allergy, Asthma, and Immunology.

Correspondence and requests for reprints should be addressed to L. Karla Arruda, M.D., Asthma and Allergic Diseases Center, Department of Internal Medicine, University of Virginia Health Sciences Center, Box 225, Charlottesville, VA 22908.

Evaluation of the role of *B. tropicalis* allergens in asthma has been complicated by the fact that concurrent sensitization occurs in houses where both *B. tropicalis* and *D. pteronyssinus* are present. In contrast to *D. pteronyssinus*, allergens from *B. tropicalis* are poorly defined, and no major allergen has yet been identified. Previous immunochemical studies showed that *B. tropicalis* does not produce allergens homologous to the Group 1 and Group 2 *Dermatophagoides* spp. allergens. Inhibition studies suggested that *B. tropicalis* allergens were species-specific, and the extent of cross-reactivity with *D. pteronyssinus* was estimated at ~30% (13, 16–19). We have investigated sensitization to *B. tropicalis* and *D. pteronyssinus* among patients with asthma exposed to both mite species or exposed primarily to *D. pteronyssinus*. We have also cloned and expressed an important allergen from *B. tropicalis* (Blo t 5), which shows homology to *D. pteronyssinus* allergen Der p 5. The recombinant Blo t 5 will facilitate clinical and immunologic studies of the role of *B. tropicalis* in asthma, and it offers the prospect of developing new strategies for the diagnosis and treatment of asthma in tropical and subtropical areas of the world.

METHODS

Subjects

A total of 139 patients were enrolled in the present study. Sixty-two were children 3 to 14 yr of age with moderate to severe asthma who attended the Pediatric Allergy Clinic of Paulista School of Medicine, São Paulo, Brazil. Sixteen were asthmatic children from Poole, United Kingdom, who had participated in a previous study (1). Thirty-six were adult patients with asthma: 14 from Ponce, Puerto Rico; seven from Tampa, Florida; 15 from Charlottesville, Virginia. Twenty-five were children or adult patients with atopic dermatitis seen at the University of Virginia, Charlottesville, VA. The patients from Brazil, Puerto Rico, and Florida (n = 83) were selected on the basis of a positive immediate skin test to *D. pteronyssinus* and/or *B. tropicalis*, and a positive radioallergosorbent test (RAST) (> 40 units/ml) to *B. tropicalis* extract. The group of patients with asthma or atopic dermatitis from Charlottesville and Poole (n = 56) were selected on the basis of positive immediate skin tests and RAST (> 40 units/ml) to *D. pteronyssinus*. Serum samples from patients in Brazil, Puerto Rico, and Florida were collected in 1993; the serum samples from Charlottesville and Poole were collected between 1989 and 1994. Serum samples were stored at −20° C. Nine children 7 to 12 yr of age with rhinitis and/or asthma from Brazil, and five nonallergic adult subjects from Manchester, UK (20), underwent skin testing with recombinant allergens.

IgE Antibodies to *D. pteronyssinus* and *B. tropicalis*

Serum IgE antibodies (ab) to *D. pteronyssinus* and *B. tropicalis* were measured by quantitative RAST, as described previously (21). The assay was calibrated using a control curve of *D. farinae* discs and serial 2-fold dilutions of a mite allergic serum pool (UVA 87/01), containing 1,000 units/ml of IgE ab. The UVA serum pool was substandardized against an international reference serum pool (National Institute of Biological Standards and Control: Code No. 82/528), and one RAST unit is equivalent to ~0.1 ng of IgE (21).

Molecular Cloning of *B. tropicalis* Allergens

Total RNA was extracted from 3.8 g *B. tropicalis* whole bodies, using 5 M guanidinium isothiocyanate/0.01 M EDTA, 5% 2-ME/0.05 M TRIS HCl at pH 7.5 (22). Messenger RNA was obtained using a FastTrack kit (Invitrogen, San Diego, CA). A *B. tropicalis* cDNA library was prepared in the UniZAP-XR expression vector (Stratagene, La Jolla, CA), using 12 μg *B. tropicalis* mRNA. The cDNA library (amplified titer, 7×10^9 pfu/ml) was screened with IgE antibodies obtained from eight asthmatic children with a high *B. tropicalis* RAST (600 to 4,700 units/ml, i.e., 60 to 470 ng/ml IgE ab). The *B. tropicalis* library (400,000 pfu) was screened as described previously, and eight positive plaques were identified and cloned (22). Partial nucleotide sequencing revealed that 7/8 cDNA clones were identical. One of the cDNA clones, *bt* 2B (537 bp), was further screened against individual sera from 139 patients by plaque immunoassay. Double stranded sequencing of *bt* 2B DNA was carried out by dideoxynucleotide chain termination using a Sequenase kit (USB Biologicals, Cleveland, OH). The FASTA program was used to search the NBRF, GenBank, and Swiss-Prot databases for sequence homologies (23). The protein encoded by the *bt* 2B cDNA clone was designated Blo t 5, according to the WHO/IUIS allergen nomenclature (24).

Expression of Recombinant Group 5 Mite Allergens

Polymerase chain reaction (PCR) was used to generate a 366 bp Blo t 5 DNA fragment containing BamH I and Xho I restriction enzyme sites, which allowed subcloning into the pGEX-4T1 expression vector (Pharmacia Biotech, Piscataway, NJ). Fifty nanograms of Blo t 5 plasmid DNA were amplified by PCR using *Pfu* DNA polymerase (Stratagene). Reactions were carried out in 50 μl volume, with denaturation at 94° C for 1 min, annealing at 37° C for 1 min, and extension at 72° C for 3 min for 30 cycles. An initial 5-min incubation step at 94° C was performed, and each reaction was terminated for 15 min at 72° C. Primers for PCR were as follows: 5′ CGC GGA TCC CAA GAG CAC AAG CCA AAG 3′ (sense); and 5′ CCG CTC GAG TTA TTG GGT TTG AAT ATC 3′ (antisense). The PCR amplified Blo t 5 DNA did not contain the 5′ noncoding sequence and the predicted signal peptide sequence (nucleotides 1 to 83). Ligation of the 366 bp Blo t 5 DNA into BamHI/Xho I digested pGEX-4T1 and transformation of competent *Escherichia coli* strain TOP10F′ (Invitrogen) were performed as described (25).

Expression of Blo t 5 as a fusion protein with glutathione-S-transferase (GST) was induced with 1 mM IPTG, and purification from bacterial lysates was performed over glutathione sepharose (Pharmacia Biotech) followed by elution with 10 mM reduced glutathione. Digestion with thrombin (10 units/mg protein for 18 h at room temperature) released the recombinant 14 kD Blo t 5 protein (rBlo t 5), which was collected in the flow through after further purification over glutathione sepharose. Recombinant Der p 5 (rDer p 5) was prepared as for Blo t 5. Briefly, *E. coli* strain TG1 transformed with a pGEX-2T Der p 5 construct was grown at 37° C, and expression of rDer p 5 as a fusion protein with GST was induced with 1 mM IPTG. Purification of rDer p 5 from bacterial lysates was carried out over glutathione sepharose, followed by elution with 10 mM reduced glutathione and digestion with thrombin, releasing the 14 kD rDer p 5 protein (26). The purity of rBlo t 5 and rDer p 5 was assessed by silver-stained SDS-PAGE using an 8 to 25% gel on a PhastSystem (Pharmacia), and the N-terminal amino acid sequence was determined by Edman degradation. The yield of recombinant Group 5 allergens was 1.0 to 1.5 mg/L of culture.

IgE Antibodies to Recombinant Group 5 Allergens

IgE ab to rBlo t 5 and rDer p 5 were measured in sera from 138 or 136 mite-allergic patients, respectively, using an antigen-binding RIA (27). Two serum samples were not available for the Der p 5 assay. Briefly, 20 μg rBlo t 5 and rDer p 5 were radiolabeled with 0.5 mCi ^{125}I, using the chloramine-T technique (specific activity, 21.5 μCi/μg and 16 μCi/μg, respectively). Sera diluted 1/2 and 1/10 were incubated with 2 ng ^{125}I-rBlo t 5 or ^{125}I-rDer p 5 (~100,000 cpm added) for 4 h at room temperature. IgE myeloma serum (P.S.) diluted 1:200 was used as a carrier. Immune complexes were precipitated overnight at 4° C with 50 μl sheep antihuman IgE (The Binding Site, San Diego, CA), washed, and counted in a γ-counter. The assays were quantitated using control curves constructed with serial 2-fold dilutions of patients' sera No. 37 (Brazil) or V.P. (Charlottesville), for the rBlo t 5 and rDer p 5 assays, respectively. Each serum was assigned 1,000 units/ml of IgE antibody.

Immediate Skin Testing

Skin prick tests were performed using commercial *D. pteronyssinus* (10,000 AU/ml; Bayer Corp., Spokane, WA). *B. tropicalis* extract was prepared from mites cultured on a medium of autoclaved nude mice food and Brine shrimp (1:1), and separated using a Tullgren apparatus. In this system, the photophobic live mites are induced to pass through six layers of cheese cloth to escape the light and heat produced by a 60-W light bulb, allowing collection of > 95% pure mite bodies. Whole mite bodies were defatted and extracted in 100 mM ammonium bicarbonate. After centrifugation, the supernatant was dialyzed against distilled water and filtered (12). The protein concentration of the *B. tropicalis* extract was 1,900 μg/ml. Purified rBlo t 5 and rDer p 5 were used for prick tests at 5 μg/ml. A wheal of ≥ 4 × 4 mm diameter 15 to 20 min after

TABLE 1

COMPARISON OF THE RATIO OF IgE ANTIBODIES TO DIFFERENT MITE SPECIES IN SERA FROM THE AMERICAS AND EUROPE*

Sera from Patients in:	n	Dpt:Bt RAST Ratio > 10
São Paulo, Brazil	62	17 (27%)
Puerto Rico	14	0 (0%)
Florida	7	1 (14%)
All	83	18 (22%)†
Charlottesville, VA, and Poole, UK	56	38 (68%)†

* IgE antibodies to *D. pteronyssinus* and *B. tropicalis* were measured by quantitative RAST, and the ratio IgE ab to *D. pteronyssinus*:*B. tropicalis* was calculated. The number of sera from different patient groups with a ratio > 10 are shown, with percentage values shown in parentheses.
† $p < 0.001$ (chi-square test).

injection was regarded as a positive prick test. Quantitative intradermal skin tests were carried out sequentially with 0.03 ml of 10-fold dilutions of rBlo t 5 or rDer p 5, from 10^{-5} to 10^{0} µg/ml, as previously described, and a ≥ 6 × 6 mm diameter wheal was considered positive (28). Skin tests using mite extracts and recombinant allergens were approved by the Human Investigation Committees of the University of Virginia and the Paulista School of Medicine.

RESULTS

Analysis of IgE ab to *B. tropicalis* and *D. pteronyssinus*

Sera for these studies were obtained from patients with asthma and/or atopic dermatitis living in selected geographic areas where exposure to *B. tropicalis* and *D. pteronyssinus* are known to differ. Patients living in Brazil, Puerto Rico, and Florida were exposed to both *B. tropicalis* and *D. pteronyssinus* (11). In contrast, patients from Charlottesville and Poole were exposed to *D. pteronyssinus* but not to *B. tropicalis*. Extensive mite counts carried out in the 1980s (in more than 200 homes) failed to detect *B. tropicalis* in Charlottesville or Poole (T. A. E. Platts-Mills and S. Wilkins, unpublished data); indeed, *B. tropicalis* has never been reported in the UK. The 139 sera were analyzed for IgE ab to *B. tropicalis* and *D. pteronyssinus* by RAST to assess the degree of cross-reactivity between both species in exposed and unexposed populations.

Among patients exposed to both *B. tropicalis* and *D. pteronyssinus* who had detectable IgE ab to *B. tropicalis* (RAST > 40 units/ml), 84% also had IgE ab to *D. pteronyssinus*. Conversely, among patients exposed to *D. pteronyssinus* but not to *B. tropicalis*, 50% had detectable IgE ab to *B. tropicalis*. Differences between *B. tropicalis* exposed and nonexposed patients were further assessed by comparing the ratio of IgE ab to *D. pteronyssinus* and *B. tropicalis* (Figure 1). Only 22% of the patients exposed to *B. tropicalis* had a ratio > 10, whereas in those not exposed to *B. tropicalis*, the ratio was > 10 in 68% of patients ($p < 0.001$) (Table 1 and Figure 1). These results showed that although as much as 50% of patients from Charlottesville and Poole had detectable IgE ab to *B. tropicalis*, the levels of IgE ab to *D. pteronyssinus* were 10 to 100-fold higher than to *B. tropicalis*. The Charlottesville/Poole patients included 25 with atopic dermatitis who had remarkably high levels of IgE ab to *D. pteronyssinus* (RAST, 240 to 34,770 units/ml), and 18 of 25 of these patients (72%) had IgE ab to *B. tropicalis*. In contrast, only 32% of mite-allergic asthmatic patients (*D. pteronyssinus RAST*, 78 to 10,000 units/ml) had IgE ab to *B. tropicalis* ($p < 0.05$, chi-square test).

Identification of *Blomia tropicalis* Allergen Blo t 5 by cDNA Cloning

To further examine the antigenic relationship between *B. tropicalis* and *D. pteronyssinus*, molecular cloning was used to identify

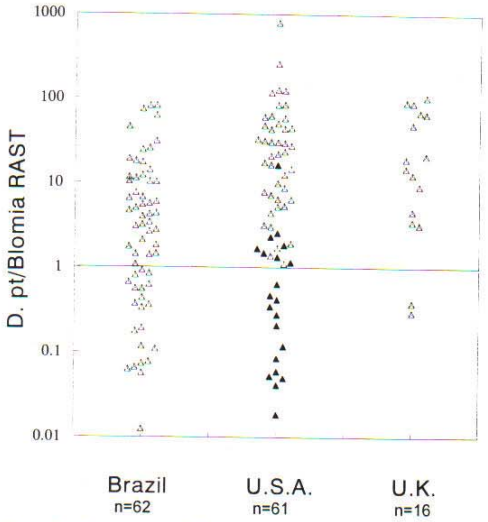

Figure 1. IgE antibodies to *D. pteronyssinus* and *B. tropicalis* (D.pt:Blomia RAST) in sera from patients living in São Paulo, Brazil; Charlottesville, VA, Florida, and Puerto Rico, USA; and Poole, UK. *Closed triangles* represent patients from Florida and Puerto Rico included in the USA group (n = 21).

B. tropicalis allergens. A *B. tropicalis* cDNA library was screened with IgE ab in pooled sera from mite-allergic children. Eight positive plaques were identified, and partial 5′ end-sequencing revealed that seven of eight clones were identical. One cDNA clone (*bt* 2B) was fully sequenced and further screened with 139 individual sera by IgE plaque immunoassay. Among patients from Brazil, Puerto Rico, and Florida, 57 of 83 (69%) had IgE ab to the protein encoded by clone *bt* 2B. These results suggested that the protein encoded by cDNA clone *bt* 2B was an important allergen. In contrast, only 13 of 56 (23%) of the Charlottesville/Poole patients had IgE ab to this protein ($p < 0.001$). Another cDNA clone (*bt* 8) coded for a protein with sequence homology to troponin-C and reacted with 49% of the patients' sera (sequence data on this allergen will be published elsewhere) (Table 2). Nucleotide-sequencing revealed that *bt* 2B cDNA contained

TABLE 2

PREVALENCE OF IgE TO THE PROTEINS EXPRESSED FROM *B. tropicalis* cDNA CLONES*

		Clone Number	
Sera from Patients in:	n	*Bt* 2B (Blo t 5)	*Bt* 8
São Paulo, Brazil	62	42 (68%)	32 (52%)
Puerto Rico	14	9 (64%)	6 (43%)
Florida	7	6 (86%)	3 (43%)
All	83	57 (69%)†	41 (49%)
Charlottesville, VA, and Poole, UK	56	13 (23%)†	ND

* Determined by plaque immunoassay.
† Significant differences between the prevalence of IgE ab in these groups ($p < 0.001$); ND = not done.

```
  1 AA AAC ACT CAC AAT CCA CAA ACT CAA ACA ACA ATG AAG TTC GCC    44
        N   T   H   N   P   Q   T   Q   T   T   M   K   F   A
 45    ATC GTT CTT ATT GCC TGC TTT GCC GCT TCG GTT TTG GCT CAA    86
        I   V   L   I   A   C   F   A   A   S   V   L   A   Q
 87    GAG CAC AAG CCA AAG AAG GAT GAT TTC CGA AAC GAA TTC GAT   128
        E   H   K   P   K   K   D   D   F   R   N   E   F   D
129    CAC TTG TTG ATC GAA CAG GCA AAC CAT GCT ATC GAA AAG GGA   170
        H   L   L   I   E   Q   A   N   H   A   I   E   K   G
171    GAA CAT CAA TTG CTT TAC TTG CAA CAC CAA CTC GAC GAA TTG   212
        E   H   Q   L   L   Y   L   Q   H   Q   L   D   E   L
                •   •   •
213    AAT GAA AAC AAG AGC AAG GAA TTG CAA GAG AAA ATC ATT CGA   254
        N   E   N   K   S   K   E   L   Q   E   K   I   I   R
255    GAA CTT GAT GTT GTT TGC GCC ATG ATC GAA GGA GCC CAA GGA   296
        E   L   D   V   V   C   A   M   I   E   G   A   Q   G
297    GCT TTG GAA CGT GAA TTG AAG CGA ACT GAT CTT AAC ATT TTG   338
        A   L   E   R   E   L   K   R   T   D   L   N   I   L
339    GAA CGA TTC AAC TAC GAA GAG GCT CAA ACT CTC AGC AAG ATC   380
        E   R   F   N   Y   E   E   A   Q   T   L   S   K   I
381    TTG CTT AAG GAT TTG AAG GAA ACC GAA CAA AAA GTG AAG GAT   422
        L   L   K   D   L   K   E   T   E   Q   K   V   K   D
423    ATT CAA ACC CAA TAA AAA TTT AGA ATT GTA CAA TTT TAC ATT   464
        I   Q   T   Q  ***
465    TTT GAT ATG ATT AAA TGT CAA TAA ATG TTC AAT AAA TAA ATT   506
507    CAA TTT TTA ACT ATA17          537
```

Figure 2. Nucleotide and deduced amino acid sequence of cDNA clone *bt* 2B, encoding *B. tropicalis* allergen Blo t 5. The predicted translation initiation codon ATG is typed in bold. The putative signal peptide cleavage site is located between residues A_{17} and Q_{18} (*in bold*). The 17 N-terminal amino acid residues include 13 hydrophobic residues, forming a classic signal peptide sequence (29). A potential N-linked glycosylation site is indicated (*bold dot*) and the stop codon TAA (*asterisks*) is shown. A polyadenylation site (AATAAA) is underlined. Numbering indicates the nucleotide position. The sequence data presented here have been submitted to GenBank under the accession number U59102.

a 432 open reading frame encoding a 134 amino acid residue protein. The sequence included an ATG start codon at positions 33 to 35, and a 17-residue putative signal peptide (nucleotides 36 to 83) with a predicted cleavage site between $Alanine_{17}$ and $Glutamine_{18}$ residue (Figure 2) (29). The mature protein contains 117 amino acid residues, with a predicted molecular mass of 13,876 D.

Homology to *D. pteronyssinus* Allergen Der p 5

Sequence similarity searches of the NBRF, GenBank, and Swiss-Prot databases revealed that the only protein with significant similarity to the deduced amino acid sequence of cDNA clone *bt* 2B was a 14 kD *D. pteronyssinus* allergen, Der p 5. This allergen was originally identified and cloned by Tovey and colleagues (30) and further sequenced and expressed by Lin and colleagues (26). Comparison of the amino acid sequences showed 43% homology between the protein encoded by cDNA clone *bt* 2B and Der p 5 (Figure 3). The *bt* 2B protein is the first *B. tropicalis* allergen that has been cloned and fully sequenced. In accordance with the WHO/IUIS nomenclature, the protein was designated *Blomia tropicalis* allergen 5 (Blo t 5) based on its sequence homology to Der p 5, rather than on the chronologic order of its discovery (24). This also distinguished Blo t 5 from the Group 1 allergens of *Dermatophagoides* spp., which are structurally unrelated (8).

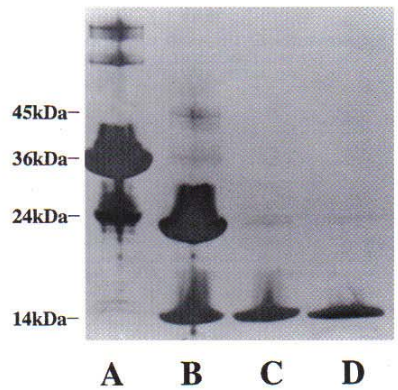

Figure 4. SDS-PAGE analysis of recombinant Blo t 5 expressed in *E. coli* as a fusion protein with glutathione-S-transferase. The fusion protein was purified from *E. coli* lysates by glutathione affinity chromatography (*A*), followed by digestion with thrombin (*B*). Recombinant Blo t 5 was recovered in the flow-through after passage over glutathione sepharose (*C*). Purification of rDer p 5 was carried out under similar conditions (D).

IgE Antibody Responses to Recombinant Group 5 Allergens

Recombinant Blo t 5, i.e., the protein encoded by cDNA clone *bt* 2B, was expressed in *E. coli* and gave a single 14-kD band on SDS-PAGE (Figure 4). In addition, the 8 N-terminal amino acid residues of rBlo t 5 were identical to the predicted sequence from the cDNA. IgE ab to rBlo t 5 was measured in sera from 138 mite-allergic patients by antigen-binding RIA. The prevalence of IgE to rBlo t 5 among patients from Brazil, Puerto Rico, and Florida was 45% (37 of 83), and among patients from Charlottesville and Poole, it was 20% (11 of 55). The levels of IgE ab were also significantly lower in the group from Charlottesville and Poole when compared with the group from Brazil, Puerto Rico, and Florida (GM: 29.2 units/ml and 106 units/ml, respectively; $p < 0.005$) (Figure 5A). In contrast, there was no difference between IgE ab to rDer p 5 in the two groups of patients ($p = 0.38$) (Figure 5B). As much as 41% of radiolabeled rBlo t 5 or rDer

```
                  10         20         30         40         50         60
Blo t 5   MKFAIVLIACFAAS--VLAQEHKPKKDDFRNEFDHLLIEQANHAIEKGEHQLLYLQHQLDELNENKSK
          :::   .:: :.:.  :..    . :: :..:::: ::.:. .. :.:::  :.:::.:.....:. .:
Der p 5   MKF---IIAFFVATLAVMTVSGEDKKHDYQNEFDFLLMERIHEQIKKGELALFYLQEQINHFEEKPTK
                     10         20         30         40         50         60

                70         80         90        100        110        120        130
Blo t 5   ELQEKIIRELDVVCAMIEGAQGALERELKRTDLNILERFNYEEAQTLSKILLKDLKETEQKVKDIQTQ
          :...::. :.:.. :::.:..:.:.: ..: ::.:.:..: : :.   . :: .:::. : .:: :..
Der p 5   EMKDKIVAEMDTIIAMIDGVRGVLDRLMQRKDLDIFEQYNLEMAKKSGDILERDLKKEEARVKKIEV
                70         80         90        100        110        120        130
```

Figure 3. Sequence alignment between *B. tropicalis* Blo t 5 and *D. pteronyssinus* Der p 5 (26, 30). Identical amino acid residues (:) or conserved substitutions (.) are indicated (overall homology, 43%).

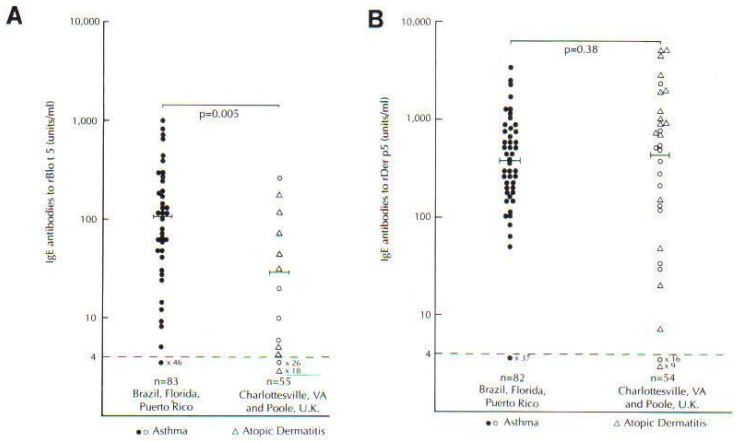

Figure 5. Comparison of IgE antibodies to recombinant Group 5 allergens in sera from patients living in Brazil, Florida, and Puerto Rico exposed to both *D. pteronyssinus* and *B. tropicalis* (*closed symbols*), and patients from Charlottesville, VA, and Poole, UK exposed only to *D. pteronyssinus* (*open symbols*). IgE ab to Blo t 5 (*A*) or Der p 5 (*B*) were measured by antigen binding RIA, and sera from patients with asthma (*circles*) or atopic dermatitis (*triangles*) are indicated. *Horizontal bars* indicate the geometric mean values, and the dashed line is the minimal level of sensitivity of each assay. Student's *t* test was used to analyze differences between patient groups, and the p value is indicated above each figure.

p 5 could be bound by IgE ab from mite-allergic patients (maximum, 46,000 cpm bound), whereas sera from four nonallergic patients consistently gave background binding (< 0.5% radioactivity or 400 cpm bound). The results suggest that IgE ab responses to rBlo t 5 in unexposed patients reflect antigenic cross-reactivity with *D. pteronyssinus* allergen Der p 5.

Linear regression plots were also used to compare IgE ab to the Group 5 allergens in *B. tropicalis*-exposed and -unexposed patients. A high proportion of sera from exposed patients had IgE ab to both rBlo t 5 and rDer p 5 (24 of 58, 41%), and in this group significant numbers of patients had IgE ab specific for either Der p 5 (36%) or Blo t 5 (22%) (Figure 6A). These results suggest that both rBlo t 5 and rDer p 5 are necessary to evaluate IgE ab responses in patients exposed to the two mite species. In contrast, among patients exposed to *D. pteronyssinus* but not to *B. tropicalis*, the majority (66%, 21 of 32) had IgE ab to Der p 5, but not to Blo t5, and only eight sera had IgE ab to both allergens (Figure 6B).

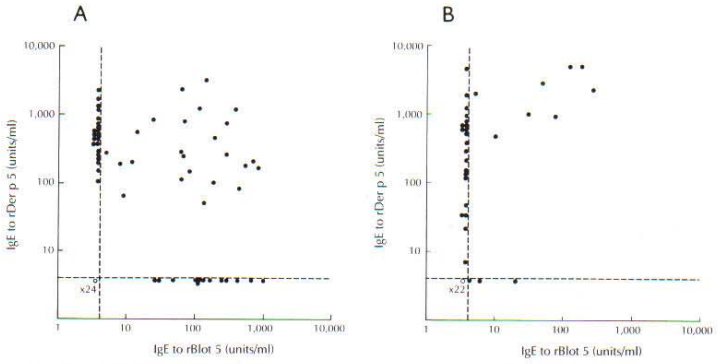

Figure 6. Correlation between IgE antibodies to rBlo t 5 and rDer p 5 in sera from mite-allergic patients. (*A*) Sera from patients in Brazil, Florida, and Puerto Rico with detectable serum IgE ab to Group 5 allergens (n = 58). (*B*) Sera from Charlottesville/Poole patients with IgE ab to Group 5 allergens (n = 32). *Open symbols* indicate sera from mite-allergic patients who had no detectable IgE ab to Group 5. *Dashed lines* indicate the minimal levels of assay sensitivity.

Biologic Activity of Recombinant Group 5 Allergens

To investigate the biologic activity of rBlo t 5, selected mite-allergic patients with asthma and/or rhinitis underwent skin testing with the recombinant allergens. Positive immediate prick tests were obtained using rBlo t 5 at a concentration of 5 μg/ml and intradermal tests at a concentration as low as 10^3 μg/ml, whereas no reaction was observed in nonallergic patients with rhinitis. These results confirmed that the *B. tropicalis* allergen Blo t 5 produced in bacteria retains IgE binding capacity *in vivo*. Similarly, rDer p 5 induced positive skin testing in mite-allergic patients, as previously reported (26) (Table 3).

DISCUSSION

Comparison of IgE ab responses in sera from exposed and unexposed asthma patients, together with the cloning and expression of a major allergen, has made it possible to investigate the molecular basis for sensitization to *B. tropicalis* and *D. pteronyssinus*. Several lines of evidence from the present study suggest that *B. tropicalis* is an independent cause of sensitization in tropical and subtropical countries where patients may be exposed to this species as well as to *D. pteronyssinus*. A significant number of patients living in these areas make higher levels of IgE ab to *B. tropicalis* than to *D. pteronyssinus*; they are more likely to make IgE ab responses to both Group 5 allergens; and ~20% make IgE ab responses to Blo t 5, but no detectable response to Der p 5. *B. tropicalis* appears to be a primary cause of sensitization in Brazil, Florida, and Puerto Rico, and the results would also be expected to apply to other regions where *Blomia* spp constitute a large proportion of the acarofauna in houses. The results show that patients exposed to *D. pteronyssinus* make partially cross-reactive IgE ab responses to *B. tropicalis*. However, the magnitude of the IgE anti-*D. pteronyssinus* response is greater than to *B. tropicalis*, and most of these patients (66%) make IgE ab to Der p 5, but not to Blo t 5. Recent skin test studies on a group of adult patients with asthma in Manchester, UK, have shown positive intradermal skin tests to Der p 5, but not to Blo t 5, in ~50% of *D. pteronyssinus*-sensitive patients (20). Thus, the degree of structural homology between the Group 5 allergens (43%) is not sufficient in most cases to elicit cross-reactive IgE ab responses. The cross-reactivity to *B. tropicalis* observed in *D. pteronyssinus*-sensitized patients usually involved patients with very high IgE ab levels (e.g., from asthma/atopic dermatitis patients), who would be expected to make more polyclonal responses. An important clinical application of our results is that *B. tropicalis* should be included in the panel of allergen extracts used for evaluation and treatment of immediate hypersensitivity in patients with asthma living in areas where *B. tropicalis* is prevalent.

Although several *Dermatophagoides* spp. allergens have been purified, cloned, and sequenced in the past decade, Blo t 5 is the first allergen from *B. tropicalis* to be defined and produced as a recombinant protein. In addition to sequence homology, Blo t 5 and Der p 5 share other structural features. Inspection of two additional Der p 5 cDNA clones revealed that the sequences have 132 amino acid residues, including a 19-residue putative signal peptide sequence and a mature protein containing 113 residues (predicted molecular mass, 13,585 D) (26). Similarly, Blo t 5 is a 134-residue sequence containing a 17-residue putative leader sequence, with a 117-residue mature protein (estimated molecular mass, 13,876 D). Blo t 5 has one potential N-linked glycosylation site and one cysteine residue, neither of which are present in Der p 5. Despite their overall similarities, there are sufficient differences in the sequence and/or potential glycosylation of the two allergens to explain the differences in IgE ab responses. To date, no significant sequence homology between the Group 5 mite allergens and other proteins has been found, and the biologic function of these proteins remains to be determined. Recently, a partial cDNA sequence has been identified that has 78% identity to the C-terminal half of Blo t 5, and it appears to be a truncated form of Blo t 5, or of a Blo t 5 isoform (GenBank accession number, U27702; submitted by L. Caraballo, unpublished).

The results show that the recombinant Group 5 allergens have excellent immunologic activity *in vitro* and *in vivo* and that both allergens can be produced in bacteria with a consistent yield and high degree of purity (> 95%). This raises the possibility that the recombinant allergens could be used for diagnostic and clinical purposes. In keeping with previous studies on Der p 5, the prevalence of IgE reactivity to Group 5 allergens (50 to 70%) was lower than that reported for the Group 1 or Group 2 *Dermatophagoides* spp allergens (~90%) (2). Although the development of a specific test for IgE ab to *B. tropicalis* could not be achieved using rBlo t 5 alone, our results suggest that it would be possible to use rBlo t 5 for diagnostic purposes in combination with another cloned *B. tropicalis* allergen (e.g., the troponin allergen, *bt* 8). The use of recombinant *Blomia* allergens for evaluation of immediate hypersensitivity is currently being investigated as part of a multicenter skin test trial being carried out in the United States, the United Kingdom, Brazil, Argentina, and Singapore.

Epidemiologic studies using monoclonal antibody (mAb)-based assays for measuring *Dermatophagoides* and other allergens in house dust have provided strong evidence that indoor allergen exposure is a major risk factor for asthma (1–3, 6, 13, 14). Assessment of exposure to *Blomia*, on the other hand, has been based on a few published reports of the prevalence of *B. tropicalis* and *B. kulagini* in addition to our own acarologic studies (unpublished). The availability of rBlo t 5 will enable immunoassays to be developed for monitoring allergen exposure, for allergen purification, and for structural and functional studies of the allergen. Several mAb to rBlo t 5 have recently been produced in our laboratory and a mAb-based assay for measuring Der p 5 has also been developed (31). Thus, it will soon be possible to make quantitative comparisons of the levels of exposure to these homologous allergens in exposed and unexposed patients.

The use of recombinant Group 5 allergens also has applications in cellular studies of asthma. These include studies of allergen accumulation in the airways after bronchial challenge, as has recently been demonstrated with Der p 1, and analysis of mediator release and cellular recruitment after allergen challenge (32). The sequence information is an essential prerequisite for studying T-cell proliferation in patients with asthma and for defining the epitopes involved. Increasingly, T-cell-based peptide vaccines are being considered as a new therapeutic approach to allergen immunotherapy, and preliminary trials with T-cell peptides derived from Fel d 1 have shown promising results in asthmatic patients allergic to cats (33). An alternative approach is the development of modified allergens with decreased IgE-binding capacity (also dependent on having expressed, recombinant allergens) that contain a broad repertoire of T-cell epitopes (34). These novel treatment strategies will now become applicable to *Blomia* spp using Blo t 5 and other allergens identified using molecular cloning techniques (35).

In conclusion, the present study provides strong evidence for an important role of *Blomia tropicalis* allergens as an independent cause of sensitization among patients with asthma living in tropical areas of the world. Detailed comparisons of IgE reactivity to *B. tropicalis* and *D. pteronyssinus* supports the view that *B. tropicalis* contains unique allergens, and therefore should be included in the evaluation of immediate hypersensitivity in patients exposed to both mite species. The identification of Blo t 5 as a major *B. tropicalis* allergen, and its production as a recom-

TABLE 3

SKIN TESTS AND SERUM IgE ANTIBODIES TO *B. tropicalis* AND *D. pteronyssinus* ALLERGENS IN CHILDREN FROM BRAZIL

	B. tropicalis		*D. pteronyssinus*		rBlo t 5		rDer p 5	
Patient No.	Skin Test*	RAST (*units/ml*)	Skin Test	RAST (*units/ml*)	Skin Test	IgE† (*units/ml*)	Skin Test	IgE† (*units/ml*)
1	10 × 6	1,460	10 × 8	> 10,000	6 × 4	175	10^{-3}	480
2	6 × 6	1,605	4 × 5	1,500	6 × 5	425	10^{-2}	< 4
3	7 × 8	336	15 × 9	10,000	10^{-3}	< 4	4 × 5	108
4	10 × 5	40	10 × 7	> 10,000	10^{-3}	< 4	5 × 5	154
5	7 × 7	70	10 × 12	1,800	7 × 5	10	7 × 7	120
6	5 × 8	680	15 × 7	> 10,000	10^{-1}	< 4	10^{-3}	< 4
7	6 × 6	30	10 × 11	> 10,000	10^{-4}	< 4	7 × 7	276
Control subjects, n = 7‡	Neg	< 10	Neg	< 10	Neg	< 4	Neg	< 4

* Skin tests are reported as either the wheal diameter (in mm) on prick test or as the concentration of allergen (in μg/ml) that caused a positive reaction (> 6 × 6 mm wheal diameter) 15 min after intradermal injection.

† Measured by antigen-binding RIA.

‡ Controls were two children with rhinitis and negative skin tests to *D. pteronyssinus* and *B. tropicalis* and five nonallergic adult subjects who consented to quantitative intradermal skin tests with rBlo t 5 and rDer p 5 (20). The control children had either negative skin prick tests to Group 5 allergens or gave negative reactions (< 6 × 6 mm wheal diameter) on intradermal skin testing using 1 μg/ml rBlo t 5 or rDer p 5.

binant protein, will make possible investigation of the structure of Group 5 mite allergens, the localization of IgE and T-cell epitopes on these molecules, and further epidemiologic studies of the role of *B. tropicalis* in allergic asthma.

Acknowledgment: The writers are grateful to Dr. Wayne R. Thomas (Western Australian Research Institute for Child Health, Perth, Western Australia) for providing the pGEX Der p 5 construct. They also thank Nancy Malone for assistance with the preparation of the manuscript.

References

1. Sporik, R., S. T. Holgate, T. A. E. Platts-Mills, and J. Cogswell. 1990. Exposure to house dust mite allergen (*Der p* 1) and the development of asthma in childhood: a prospective study. *N. Engl. J. Med.* 323: 502–507.
2. Platts-Mills, T. A. E., W. R. Thomas, R. C. Aalberse, D. Vervloet, and M. D. Chapman. 1992. Dust mite allergens and asthma: report of a second international workshop. *J. Allergy Clin. Immunol.* 89: 1046–1060.
3. Peat, J. K., E. Tovey, B. G. Toelle, M. M. Haby, E. J. Gray, A. Mahmic, and A. J. Woolcock. 1996. House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am. J. Respir. Crit. Care Med.* 153:141–146.
4. Burrows, B., F. D. Martinez, M. Halonen, R. A. Barbee, and M. G. Cline. 1989. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N. Engl. J. Med.* 320:271–277.
5. Sears, M. R., B. Burrows, E. M. Flannery, G. P. Herbison, C. J. Hewitt, and M. D. Holdaway. 1991. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N. Engl. J. Med.* 325:1067–1071.
6. Gelber, L. E., L. H. Seltzer, J. K. Bouzokis, S. M. Pollart, M. D. Chapman, and T. A. E. Platts-Mills. 1993. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am. Rev. Respir. Dis.* 147:573–578.
7. Sporik, S., J. M. Ingram, W. Price, J. H. Sussman, R. W. Honsinger, and T. A. E. Platts-Mills. 1995. Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude. Tickling the dragon's breath. *Am. J. Respir. Crit. Care Med.* 151:1388–1392.
8. Thomas, W. R. 1993. Mite allergens groups I-VII. A catalogue of enzymes. *Clin. Exp. Allergy* 23:350–353.
9. Arlian, L. G., D. Bernstein, I. L. Bernstein, S. Friedman, A. Grant, P. Lieberman, M. Lopez, J. Metzger, T. Platts-Mills, M. Schatz, S. Spector, S. Wasserman, and R. S. Zeiger. 1992. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. *J. Allergy Clin. Immunol.* 90:292–300.
10. Fernandez-Caldas, E., R. W. Fox, G. A. Bucholtz, W. L. Trudeau, D. K. Ledford, and R. F. Lockey. 1990. House dust mite allergy in Florida. Mite survey in households of mite-sensitive individuals in Tampa, Florida. *Allergy Proc.* 11:263–267.
11. Arruda, L. K., and M. D. Chapman. 1992. A review of recent immunochemical studies of *Blomia tropicalis* and *Euroglyphus maynei* allergens. *Exp. Appl. Acarol.* 16:129–140.
12. Fernandez-Caldas, E., L. Puerta, D. Mercado, R. F. Lockey, and L. R. Caraballo. 1993. Mite fauna, Der p I, Der fl and *Blomia tropicalis* allergen levels in a tropical environment. *Clin. Exp. Allergy* 23:292–297.
13. Arruda, L. K., M. C. Rizzo, M. D. Chapman, E. Fernandez-Caldas, D. Baggio, T. A. E. Platts-Mills, and C. K. Naspitz. 1991. Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin. Exp. Allergy* 21:433–439.
14. Rizzo, M. C., L. K. Arruda, M. D. Chapman, E. Fernandez-Caldas, D. Baggio, T. A. E. Platts-Mills, and C. K. Naspitz. 1993. IgG and IgE antibody responses to dust mite allergens among children with asthma in Brazil. *Ann. Allergy* 71:152–158.
15. Fernandez-Caldas, E., C. E. Baena-Cagnani, M. Lopez, C. Patino, H. E. Neffen, M. Sanchez-Medina, L. R. Caraballo, J. H. Lopez, S. Malka, C. K. Naspitz, and R. F. Lockey. 1993. Cutaneous sensitivity to six mite species in asthmatic patients from five Latin American countries. *J. Invest. Allergol. Clin. Immunol.* 3:245–249.
16. van Hage-Hamsten, M., L. Machado, M. T. Barros, and S. G. O. Johansson. 1990. Immune response to *Blomia kulagini* and *Dermatophagoides pteronyssinus* in Sweden and Brazil. *Int. Arch. Allergy Appl. Immunol.* 91:186–191.
17. Arlian, L. G., D. L. Vyszenski-Moher, and E. Fernandez-Caldas. 1993. Allergenicity of the mite, *Blomia tropicalis*. *J. Allergy Clin. Immunol.* 91:1042–1050.
18. Stanaland, B. E., E. Fernandez-Caldas, C. M. Jacinto, W. L. Trudeau, and R. F. Lockey. 1994. Sensitization to *Blomia tropicalis*: skin test and cross-reactivity studies. *J. Allergy Clin. Immunol.* 94:452–457.
19. Caraballo, L., L. Puerta, B. Martinez, and L. Moreno. 1994. Identification of allergens from the mite *Blomia tropicalis*. *Clin. Exp. Allergy* 24:1056–1060.
20. Smith, A., R. Green, A. Custovic, A. Woodcock, K. Arruda, and M. Chapman. 1996. *In vivo* cross reactivity between IgE Ab to *Blomia* and *Dermatophagoides* spp and to the recombinant Group 5 allergens among mite allergic patients living in the UK (abstract). *J. Allergy Clin. Immunol.* 97:420.
21. Pollart, S. M., M. D. Chapman, G. P. Fiocco, G. Rose, and T. A. E. Platts-Mills. 1989. Epidemiology of asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J. Allergy Clin. Immunol.* 83:875–882.
22. Arruda, L. K., L. D. Vailes, M. L. Hayden, D. C. Benjamin, and M. D. Chapman. 1995. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses. *J. Biol. Chem.* 270:31196–31201.
23. Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. U.S.A.* 85:2444–2448.

24. King, T. P., D. Hoffman, H. Lowenstein, D. G. Marsh, T. A. E. Platts-Mills, and W. Thomas. 1994. Allergen nomenclature. *Int. Arch. Allergy Immunol.* 105:224–233.
25. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
26. Lin, K.-L., K.-H. Hsieh, W. R. Thomas, B-L. Chiang, and K-Y. Chua. 1995. Characterization of *Der p* V allergen, cDNA analysis, and IgE-mediated reactivity to the recombinant protein. *J. Allergy Clin. Immunol.* 94:989–996.
27. Chapman, M. D., and T. A. E. Platts-Mills. 1980. Purification of the major allergen from *Dermatophagoides pteronyssinus* antigen P_1. *J. Immunol.* 125:587–592.
28. Pollart, S. M., L. D. Vailes, D. E. Mullins, M. L. Hayden, T. A. E. Platts-Mills, W. M. Sutherland, and M. D. Chapman. 1991. Identification, quantitation and purification of cockroach allergens using monoclonal antibodies. *J. Allergy Clin. Immunol.* 87:511–521.
29. von Heijne, G. 1986. A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* 14:4683–4690.
30. Tovey, E. R., M. C. Johnson, A. L. Roche, G. S. Cobon, and B. A. Baldo. 1989. Cloning and sequencing of a cDNA expressing a recombinant house dust mite protein that binds human IgE and corresponds to an important low molecular weight allergen. *J. Exp. Med.* 170: 1457–1462.
31. Li, C.-S., C.-W. Hsu, K.-W. Chua, K.-H. Hsieh, and R.-H. Lin. 1996. Environmental distribution of house dust mite allergen (Der p 5). *J. Allergy Clin. Immunol.* 97:857–859.
32. Ferguson, P., and D. H. Broide. 1995. Environmental and bronchoalveolar lavage *Dermatophagoides pteronyssinus* antigen levels in atopic asthmatics. *Am. J. Respir. Crit. Care Med.* 151:71–74.
33. Wallner, B. P., and M. L. Gefter. 1994. Immunotherapy with T-cell reactive peptides derived from allergens. *Allergy* 49:302–308.
34. Smith, A. M., and M. D. Chapman. 1996. Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen *Der p* 2. *Mol. Immunol.* 33:399–405.
35. Caraballo, L., A. Avjioglu, J. Marrugo, and D. Marsh. 1994. Sequence and immunological studies on a cDNA clone of *Blomia tropicalis* (abstract). *J. Allergy Clin. Immunol.* 93:205.