CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Dec. 2005, p. 1451–1454 Vol. 12, No. 12
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.12.1451–1454.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Immunoglobulin E Binding Reactivity of a Recombinant Allergen Homologous to α-Tubulin from *Tyrophagus putrescentiae*

Kyoung Yong Jeong,[1] Haeseok Lee,[1] Jae Sik Lee,[1] Jongweon Lee,[1] In-Yong Lee,[1] Han-Il Ree,[1] Chein-Soo Hong,[2] Jung Won Park,[2] and Tai-Soon Yong[1]*

Department of Parasitology and Institute of Tropical Medicine[1] and Department of Internal Medicine and Institute of Allergy,[2] Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea

Received 17 April 2005/Returned for modification 11 July 2005/Accepted 5 August 2005

Storage mites may cause allergic respiratory diseases in urban areas as well as pose an occupational hazard in rural areas. Characterization of storage mite allergens is important for the development of diagnostic and therapeutic agents against mite-associated allergic disorders. Here we report on the cloning and expression of α-tubulin from the storage mite (*Tyrophagus putrescentiae*). The deduced amino acid sequence of the α-tubulin from the storage mite showed as much as 97.3% identity to the α-tubulin sequences from other organisms. The highly conserved amino acid sequences of α-tubulins across different species of mites may indicate that cross-reactivity for this potential allergen exists. The frequency of immunoglobulin E reactivity of this recombinant protein is 29.3% in sera from storage mite-allergic subjects.

It is known that sensitization to allergens derived from storage mites may lead to occupational allergic disorders (2, 3). There has been increasing interest in the allergenicity of storage mites in recent years (13). In epidemiological studies, the predominant species in Korean homes were found to be *Dermatophagoides farinae* (65 to 77%) and *Dermatophagoides pteronyssinus* (8 to 20%) (7, 15). *Tyrophagus putrescentiae* was reported to be the third most common domestic mite, infesting 6.5 to 8.5% of Korean homes (7, 15). In a study with sera from atopic urban inhabitants, a marked inhibition of *T. putrescentiae*-specific immunoglobulin E (IgE) by *D. farinae* and *D. pteronyssinus* extract was demonstrated, while *D. pteronyssinus*- or *D. farinae*-specific IgE was partially inhibited by a *T. putrescentiae* extract (14). Significant cross-reactivity between other arthropods and nematodes was also reported (9). The prevalence of storage mite sensitization was reported in a general adult population in Spain (18), and the allergen-specific IgE (CAP) correlation analysis in Spain also showed allergenic similarity between *Dermatophagoides* spp. and storage mites, such as *T. putrescentiae*, *Glycyphagus domesticus*, and *Acarus siro* (10). Molecular cloning of Tyr p 2 and Tyr p 13 from *T. putrescentiae* has been done (5, 8). In addition, molecular modeling has found many amino acid substitutions at surface residues of Tyr p 2 that still conserve its tertiary structure (17). These substitutions may indicate that there is limited cross-reactivity for group 2 allergens among different mite species. Moreover, group 13 allergens have not been identified from *Dermatophagoides* spp. Identification of more allergens and their characterization in molecular detail will allow us to better understand the cross-reactivity for mite species. In this respect, expressed sequence tag (EST) strategies could be useful for the

* Corresponding author. Mailing address: Department of Parasitology and Institute of Tropical Medicine, Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea. Phone: 82-2-228-1840. Fax: 82-2-363-8676. E-mail: tsyong212@yumc.yonsei.ac.kr.

```
- 114        GCTC GATTGGAAAG GTTTTCTTTG CTGACCTAAC AATTCTCGCC ACTTTGCGAT
- 60   CTTTTCGCCT CACCCTCTTC GACTTCTCAA ACCCACCCGT TTTAACAACC CAACGTCAAC
1      ATGCGCGAAT GTATCTCCGT TCACGTCGGC CAGGCTGGTG TCCAGATCGG CAACGCCTGC
        M  R  E   C  I  S  V   H  V  G   Q  A  G   V  Q  I  G   N  A  C
61     TGGGAGCTGT ACTGCCTCGA GCACGGAATT CAGCCTGATG GACAGATGCC CTCTGACAAG
        W  E  L   Y  C  L  E   H  G  I   Q  P  D   G  Q  M  P   S  D  K
121    ACCATCGGCA CTGGTGACGA CTCCTTTAAC ACCTTCTTCA GCGAGACTGG CTCTGGCAAG
        T  I  G   T  G  D  D   S  F  N   T  F  F   S  E  T  G   S  G  K
181    CACGTTCCCC GTGCTGTGTA CGTCGACTTG GAGCCGACCG TGGTCGACGA GGTGCGCACT
        H  V  P   R  A  V  Y   V  D  L   E  P  T   V  V  D  E   V  R  T
241    GGAACCTACC GACAGCTGTT CCACCCCGAG CAGCTGATCA CCGGCAAGGA GGATGCCGCC
        G  T  Y   R  Q  L  F   H  P  E   Q  L  I   T  G  K  E   D  A  A
301    AACAACTACG CTCGTGGCCA CTACACCATT GGCAAGGAGA TTGTCGACGT TGTTCTCGAT
        N  N  Y   A  R  G  H   Y  T  I   G  K  E   I  V  D  V   V  L  D
361    CGCATTCGCA AGCTGAGCGA CCAGTGCACT GGCCTGCAGG GCTTCCTGAT CTTCCACTCT
        R  I  R   K  L  S  D   Q  C  T   G  L  Q   G  F  L  I   F  H  S
421    TTCGGCGGCG GCACGGGCAG TGGCTTCACC AGCCTCCTGA TGGAGCGTCT CTCTGTGGAC
        F  G  G   G  T  G  S   G  F  T   S  L  L   M  E  R  L   S  V  D
481    TACGGCAAGA AGAGCAAGCT GGAGTTTGCC GTTTACCCTG CCCCGCAGGT CTCCACCGCC
        Y  G  K   K  S  K  L   E  F  A   V  Y  P   A  P  Q  V   S  T  A
541    GTCGTTGAGC CGTACAACAG TATTCTGACC ACCCACACCA CTCTGGAGCA CTCTGACTGC
        V  V  E   P  Y  N  S   I  L  T   T  H  T   T  L  E  H   S  D  C
601    GCCTTCATGG TCGACAATGA GGCCATCTAC GACATCTGCC GTCGCAACCT GGACATTGAG
        A  F  M   V  D  N  E   A  I  Y   D  I  C   R  R  N  L   D  I  E
661    CGCCCCACAT ACACCAACTT GAACCGTCTG ATTGGCCAGA TTGTCTCCTC GATCACCGCC
        R  P  T   Y  T  N  L   N  R  L   I  G  Q   I  V  S  S   I  T  A
721    TCTCTCCGCT TCGATGGCGC CCTCAACGTG GAACTGACTG AGTTCCAGAC CAATCTGGTC
        S  L  R   F  D  G  A   L  N  V   E  L  T   E  F  Q  T   N  L  V
781    CCATATCCTC GCATTCACTT TCCTCTGGTT ACCTACTCGC CAGTCATCTC GGCTGAGAAG
        P  Y  P   R  I  H  F   P  L  V   T  Y  S   P  V  I  S   A  E  K
841    GCCTACCACG AGCAGCTGAC CGTGGCTGAG ATTACCAACA CCTGCTTCGA GCCCCAGAAC
        A  Y  H   E  Q  L  T   V  A  E   I  T  N   T  C  F  E   P  Q  N
901    CAGATGGTCA AGTGCGACCC GCGTCATGGA AAATACATGG CCTGCTGTCT CCTCTACCGA
        Q  M  V   K  C  D  P   R  H  G   K  Y  M   A  C  C  L   L  Y  R
961    GGTGACGTGG TTCCCAAGGA CGTCAACGCC GCCATCGCTG GCATCAAGAC CAAACGTTCG
        G  D  V   V  P  K  D   V  N  A   A  I  A   G  I  K  T   K  R  S
1021   ATTCAGTTTG TGGACTGGTG CCCAACTGGC TTCAAGGTCG GTATCAACTA CCAGCCACCG
        I  Q  F   V  D  W  C   P  T  G   F  K  V   G  I  N  Y   Q  P  P
1081   ACGGTGGTTC CTGGTGGAGA CCTAGCCAAG GTGCAGCGTG CCGTGTGCAT GCTGAGCAAC
        T  V  V   P  G  G  D   L  A  K   V  Q  R   A  V  C  M   L  S  N
1141   ACAACTGCTA TCGCTGAGGC TTGGGCCCGT CTGGACCACA AATTCGACCT GATGTACGCC
        T  T  A   I  A  E  A   W  A  R   L  D  H   K  F  D  L   M  Y  A
1201   AAGCGCGCCT TCGTGCACTG GTACGTCGGC GAGGGCATGG AGGAGGGCGA GTTCAGCGAG
        K  R  A   F  V  H  W   Y  V  G   E  G  M   E  E  G  E   F  S  E
1261   GCTCGCGAGG ATCTCGCTGC CCTGGAGAAG GACTATGAGG AGGTGGGCCT GGACTCCACT
        A  R  E   D  L  A  A   L  E  K   D  Y  E   E  V  G  L   D  S  T
1321   GAAGCCGAAG GAGGCGATGG AGAGGAATTC TAAGTGGTGT GGCTCTTCCA CTACCTACTT
        E  A  E   G  G  D  G   E  E  F
1381   AATTGTGACT AATCTCTACC TGAGAAATGC ACAAGAATTG AATTAAAAAA CTGATTAATT
1441   TCAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
```

FIG. 1. Nucleotide sequence and deduced amino acid sequence of *T. putrescentiae* α-tubulin. The estimated molecular weight is 50.04 kDa, and the estimated isoelectric point is 4.834.

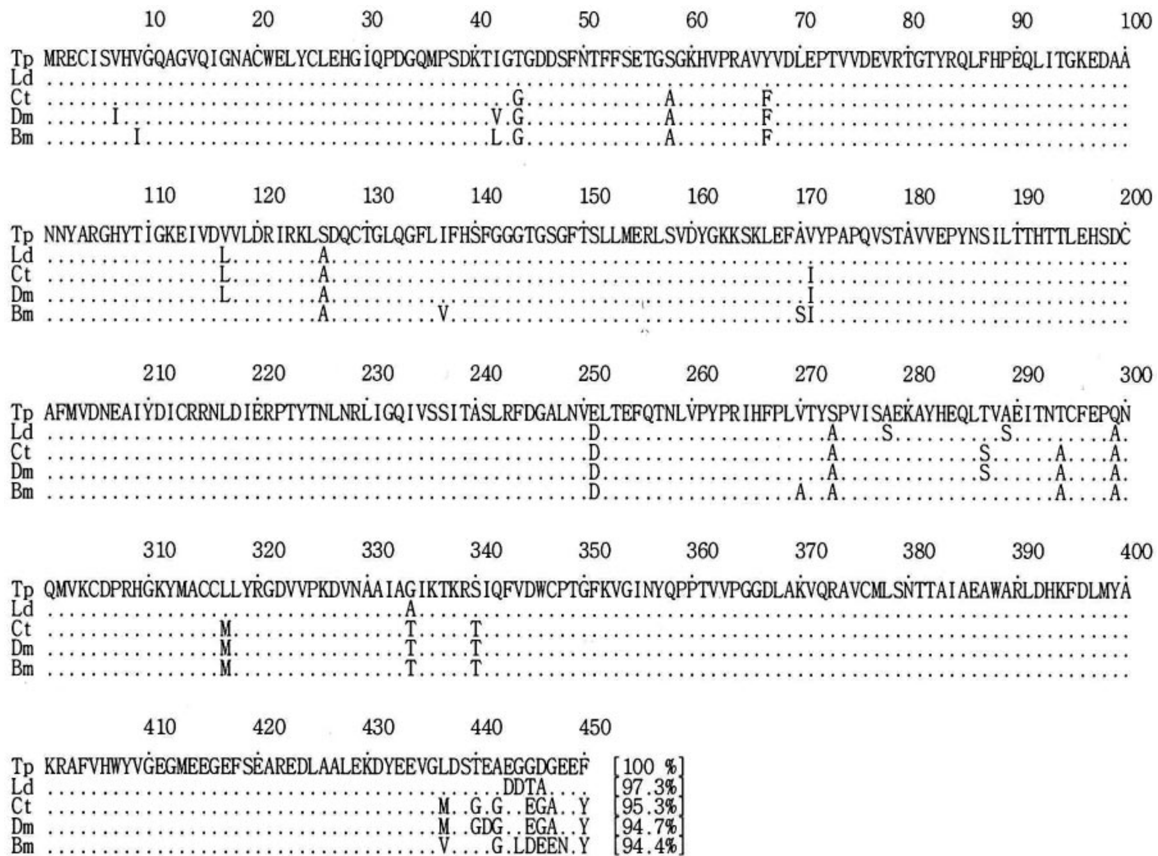

FIG. 2. Amino acid sequence alignments of four arthropod α-tubulins: Ld, *Lepidoglyphus destructor* (GenBank accession number CAD20979); Ct, *Chironomus tentans* (GenBank accession number AAK58683); Dm, *Drosophila melanogaster* (GenBank accession number NP476772); Bm, *Bombyx mori* (GenBank accession number BAB86850). A dot indicates amino acid identity with *T. putrescentiae* (Tp), and the percentage of each sequence identity is given in parentheses.

identification of cross-reactive allergens present in house dust mite and storage mite (1, 8).

We previously identified several candidate allergen molecules by analyzing an EST database of *T. putrescentiae* (8). Here we describe the expression of recombinant α-tubulin and its allergenic characterization.

An α-tubulin was first identified as a putative allergen from a phage display *Lepidoglyphus destructor* cDNA library (16). There are multiple α-tubulin genes in most organisms, and they are highly conserved within and among species (4, 11, 12).

A cDNA sequence encoding α-tubulin was obtained by analyzing *T. putrescentiae* EST clones. Both strands of the cDNA were sequenced. The insert cDNA was 1,604 bp in length and contained a single open reading frame of 1,364 bp which started at ATG (nucleotide [nt] 1) and which terminated at stop codon TAA (nt 1351) (Fig. 1). The estimated molecular mass was 50.04 kDa, and the calculated isoelectric point was 4.834. The deduced amino acid sequence of the *T. putrescentiae* α-tubulin showed as much as 97.3% identity to the α-tubulin sequences from other organisms (Fig. 2). A PROSITE search revealed a tubulin consensus sequence, [SAG]-G-G-T-G-[SA]-G (which is known to interact with GTP at positions 142 to 148), and one N-glycosylation site (positions 380 to 382) (6).

A PCR was carried out to introduce a BamHI site upstream of the start codon and a HindIII site just downstream of the stop codon. The following synthetic oligonucleotides were used as primers: GGATCCTATGCGCGAATGTATCTC (forward primer) and AAGCTTCCACTTAGAATTCCTCTC (reverse primer) (underlining indicates restriction sites). The resulting PCR fragment was subcloned into the pGEM-T Easy vector (Promega, Madison, WI) and was then digested with BamHI plus HindIII. The DNA fragment was subsequently ligated into the pET 28b vector. When this construct was used, 34 additional amino acids are added at the N terminus of the α-tubulin. The bacterial transformant was induced to express the recombinant protein by the addition of 1 mM isopropyl-1-thio-β-galactopyranoside after the cells had grown to an A_{600} of 0.6. After sonication, a 56-kDa protein was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the total cell lysate and in the insoluble precipitate. The protein was solubilized in 20 mM Tris, 500 mM NaCl, 5 mM imidazole, and 6 M urea, pH 7.9. It was then purified by using nickel-nitrilotriacetic acid resin (QIAGEN, Valencia, CA) (Fig. 3). The purified protein was desalted and concentrated with a Centriprep filter (YM-10; Millipore, Bedford, MA). The yield of purified recombinant protein was 4.576 mg/liter of bacterial culture.

To test the IgE reactivity of the recombinant protein, an

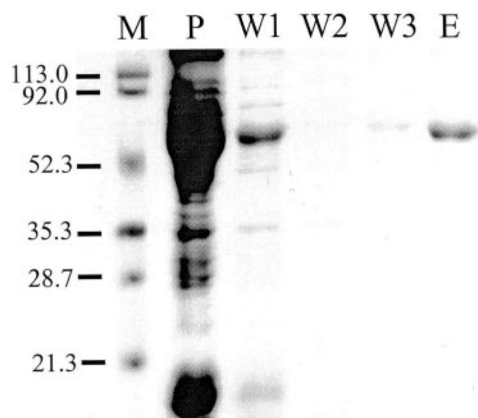

FIG. 3. Purification of recombinant α-tubulin. Proteins were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and stained with Coomassie brilliant blue. Lanes: M, molecular mass marker; P, pass-through; W1, wash with 5 mM imidazole; W2, wash with 20 mM imidazole; W3, wash with 60 mM imidazole; E, eluate in 1 M imidazole. The numbers on the left are in kilodaltons.

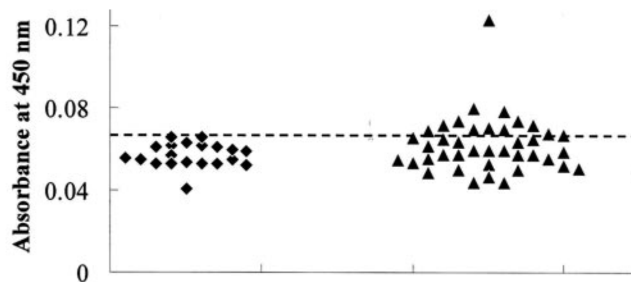

FIG. 4. IgE reactivity of recombinant α-tubulin. The dotted line indicates the cutoff value. ◆, control sera; ▲, *Tyrophagus putrescentiae*-sensitized sera.

enzyme-linked immunosorbent assay (ELISA) was performed with sera from *T. putrescentiae*-sensitized subjects diluted 1:4. Sera were obtained from allergic patients attending the Allergy Clinic of the Severance Hospital, Yonsei University, Seoul, Korea; and the diagnosis was based on the patient's case history and the results of a skin prick test (Allergopharma, Reinbek, Germay). All the subjects were tested for the presence of IgE antibodies against *T. putrescentiae* by using the Uni-CAP system (Pharmacia, Uppsala, Sweden). Those sera with allergen-specific IgE levels (CAP results) between and 17.5 kU/liter were used for the ELISA (n = 41 patients; age range, 7 to 69 years; average age, 32 years). The results of the ELISA with *T. putrescentiae* crude extract showed a good correlation (r^2 = 0.81) with the specific IgE levels investigated by use of the Uni-CAP system. The nonatopic sera from 20 subjects who were not sensitized to the 50 allergens tested were used as negative controls. Informed consent was obtained from the subjects before the experiments with the sera were performed. The optimal condition of the antigen and the antibody concentration were determined by checkerboard titrations. Purified α-tubulin (2.0 μg/ml) in coating buffer (0.1 M sodium carbonate, pH 9.6) was coated onto the microtiter plate (0.1 ml/well), and the plate was incubated overnight at 4°C. The plate was then incubated with human sera for 1 h after it was blocked with 3% skim milk. Antibodies that bound to recombinant α-tubulin were detected by using biotinylated goat anti-human IgE (1:1,000) (Vector, Burlingame, CA) and streptavidin-peroxidase (1:1,000; Sigma, St. Louis, MO). The microtiter plate was washed with phosphate-buffered saline containing 0.05% Tween 20 between each step before color development. The signals were developed by using 3,3′,5,5′-tetramethylbenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD) as the substrate, and the optical density was determined at 450 nm by using an automatic microplate reader (TECAN, Salzburg, Austria). The ELISA showed a frequency of IgE binding to recombinant α-tubulin of 29.3% (12 of 41 samples) when the mean absorbance level for the serum samples from the 20 controls plus 2 standard deviations was used as the cutoff value (P = 0.08) (Fig. 4).

The IgE reactivity may be influenced by a difference between recombinant and native protein folding or the lack of glycosylation, since no posttranslational modifications occur in the *Escherichia coli* expression system. The recombinant α-tubulin derived from *Lepidoglyphus destructor* showed an 11.6% (11 of 95 samples) IgE-binding frequency (16). Inhibition studies for evaluation of the allergenic potency could not be performed due to its low concentration in the whole-body extract.

Here we elucidated the primary structure of α-tubulin, an important potential allergen, from *T. putrescentiae* and expressed the recombinant protein. The clinical relevance of this allergen remains to be evaluated in vivo. The highly conserved amino acid sequences of α-tubulins among different species may indicate that cross-reactivity for this protein exists. To investigate this possible cross-reactivity, molecular cloning of α-tubulins from house dust mites such as *D. farinae* and *D. pteronyssinus*, which are known to be major sources of mite allergens, is needed. The α-tubulin cloned in this study will facilitate examination of the involvement of this class of allergens in the development of mite-associated allergic diseases. It is also hoped that the use of recombinant α-tubulin in combination with other allergens may improve the diagnostic specificity.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been deposited in the GenBank sequence database under accession number AY986760.

This study was supported by the Brain Korea 21 Project for Medical Science, Yonsei University.

REFERENCES

1. **Angus, A. C., S. T. Ong, and F. T. Chew.** 2004. Sequence tag catalogs of dust mite-expressed genomes: utility in allergen and acarologic studies. Am. J. Pharmacogenomics **4:**357–369.
2. **Arlian, A. G., D. L. Vyszenski-Moher, S.G. Johansson, and M. van Hage-Hamsten.** 1997. Allergenic characterization of *Tyrophagus putrescentiae* using sera from occupationally exposed farmers. Ann. Allergy Asthma Immunol. **79:**525–529.
3. **Armentia, A., A. Martinez, R. Castrodeza, J. Martinez, A. Jimeno, J. Mendez, and R. Stolle.** 1997. Occupational allergic disease in cereal workers by stored grain pests. J. Asthma **34:**369–378.
4. **Dutcher, S. K.** 2001. The tubulin fraternity: alpha to eta. Curr. Opin. Cell Biol. **13:**49–54.
5. **Eriksson, T. L. J., E. Johansson, P. Whitley, M. Schmidt, S. Elsayed, and M. van Hage-Hamsten.** 1998. Cloning and characterization of a group II allergen from the dust mite *Tyrophagus putrescentiae*. Eur. J. Biochem. **251:**443–447.
6. **Falquet, L., M. Pagni, P. Bucher, N. Hulo, C. J. Sigrist, K. Hofmann, and A.**

Bairoch. 2002. The PROSITE database, its status in 2002. Nucleic Acids Res. **30:**235–238.

7. **Jeong, K. Y., I. Y. Lee, J. Lee, H. I. Ree, C. S. Hong, and T. S. Yong.** Effectiveness of health education for the control of house dust mites and cockroaches in Seoul, Korea. Med. Vet. Entomol., in press.
8. **Jeong, K. Y., W. K. Kim, J. S. Lee, J. Lee, I. Y. Lee, K. E. Kim, J. W. Park, C. S. Hong, H. I. Ree, and T. S. Yong.** 2005. Immunoglobulin E reactivity of recombinant allergen Tyr p 13 from *Tyrophagus putrescentiae* homologous to fatty acid binding protein. Clin. Diagn. Lab. Immunol. **12:**581–585.
9. **Johansson, E., M. Aponno, M. Lundberg, and M. van Hage-Hamsten.** 2001. Allergenic cross-reactivity between the nematode *Anisakis simplex* and the dust mites *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Dermatophagoides pteronyssinus*. Allergy **56:**660–666.
10. **Marcos Bravo, C., I. Luna Ortiz, A. Outon Soto, and R. Gonzalez Vazquez.** 1999. Allergy to storage mites. Allergy **54:**769–770.
11. **McKean, P. G., S. Vaughan, and K. Gull.** 2001. The extended tubulin superfamily. J. Cell Sci. **114:**2723–2733.
12. **Oakley, B. R.** 2000. An abundance of tubulins. Trends Cell Biol. **10:**537–542.
13. **Olsson, S., and M. van Hage-Hamsten.** 2000. Allergens from house dust mite and storage mite: similarities and differences, with emphasis on the storage mite *Lepidoglyphus destructor*. Clin. Exp. Allergy **30:**912–919.
14. **Park, J. W., S. H. Ko, T. S. Yong, H. I. Ree, B. J. Jeoung, and C. S. Hong.** 1999. Cross-reactivity of *Tyrophagus putrescentiae* with *Dermatophgoides farinae* and *Dermatohpagoides pteronyssinus* in urban area. Ann. Allergy Asthma Immunol. **83:**533–539.
15. **Ree, H. I., S. H. Jeon, I. Y. Lee, C. S. Hong, and D. K. Lee.** 1997. Fauna and geographical distribution of house dust mites in Korea. Korean J. Parasitol. **35:**9–17.
16. **Saarne, T., L. Kaiser, O. Rasool, S. Huecas, M. van Hage-Hamsten, and G. Gafvelin.** 2003. Cloning and characterization of two IgE-binding proteins, homologous to tropomyosin and α-tubulin, from the mite *Lepidoglyphus destructor*. Int. Arch. Allergy Immunol. **130:**258–265.
17. **Smith, A. M., D. C. Benjamin, N. Hozic, U. Derewenda, W. A. Smith, W. R. Thomas, G. Gafvelin, M. van Hage-Hamsten, and M. D. Chapman.** 2001. The molecular basis of antigenic cross-reactivity between the group 2 mite allergens. J. Allergy Clin. Immunol. **107:**977–984.
18. **Vidal, C., O. Boquete, F. Gude, J. Rey, L. M. Meijide, M. C. Fernandez-Merino, and A. Gonzalez-Quintela.** 2004. High prevalence of storage mite sensitization in a general adult population. Allergy **59:**401–405.