

Detection of *Dermatophagoides farinae* in the Dust of Air Conditioning Filters

Zhigang Liu Yu Bai Kunmei Ji Xiaoyu Liu Chengyu Cai Haiqiong Yu
Meng Li Ying Bao Yuyin Lian Bo Gao

College of Life Science, Shenzhen University, Shenzhen, China

Key Words

Dermatophagoides farinae • Allergenic species in air conditioner filters • Der f 1 • Der f 2

Abstract

Background: The allergenic dust mite species *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* generally inhabit warm moist environments. This study tested the hypothesis that these allergenic species may thrive in air conditioner filters. **Methods:** A year-long investigation of the dust mite population densities and species identities living in air conditioner filters in Shenzhen City in Southern China was performed. Additional data describing the levels of major dust mite allergen proteins from samples collected in July and August 2004 were analyzed. Genetic polymorphism analysis of Der f 1 and Der f 2 genes in the collected animals was also conducted. **Results:** Our investigation revealed that larval dust mites started to grow in March, from which time their populations proceeded to steadily increase until reaching their population zenith in July and August. The dust mite populations decreased sharply in October and live dust mites were no longer observed in the winter. Among the mites collected in July and August, 30.1 and 25.8% were of the species *D. farinae*. The concentration of Der f 1 was 3.04 ± 1.75 and 3.21 ± 1.84 $\mu\text{g/g}$ dust in July and August, respectively, and that of Der f 2 was 2.15 ± 0.82 and $2.04 \pm$

1.15 $\mu\text{g/g}$ dust. Four types of Der f 1 and 5 types of Der f 2 cDNA sequences were cloned from collected Der f mites. Their sequences were highly homologous with those previously published in GenBank (No. AB034946.1 and No. AB195580.1). **Conclusions:** This research demonstrated that Der f allergens exist in the dust of air conditioner filters in this area.

Copyright © 2007 S. Karger AG, Basel

Introduction

According to a survey made by the International Study of Asthma and Allergies in Childhood, the asthma rate of teenagers in some Chinese cities has reached 3.3–5.1%, a rate many times greater than the approximately 1% estimated just 1 decade ago [1]. Presently, there are about 10 million children suffering from asthma and more than 50 million allergic rhinitis patients in China [2–3]. It is well known that allergic diseases are increasing in prevalence all over the world and have become a global health problem [4]. Consequently, scientists are investigating why allergic diseases have been rising in recent years and are seeking solutions to this problem.

Various kinds of allergens can cause common allergic diseases like allergic asthma, allergic rhinitis and atopic dermatitis. Dust mites, especially the species *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides*

farinae (Der f), are very important sources of allergens since >50% of allergic diseases observed in clinics have been attributed to them [5].

Previous research has demonstrated that airborne allergenic substances derived from Der f and Der p mites are present in substantial quantities in static indoor dust of beds, sofas, clothing and carpets [5]. The present study examined whether Der f and Der p animals and associated allergens exist in the dust of air conditioner filters in Shenzhen City of Southern China, where air conditioning is widely used in business and home environments from May to October. This research intended to seek a new source of indoor allergens.

Materials and Methods

Dust Sample Collection and Mite Counting

For a period of 1 year from December 2003 to November 2004, a total of 360 dust samples were collected from air conditioner filters of 10 private homes, 10 hotels and 10 offices in the Shenzhen area. A dust sample was collected from each of these 30 sample sites each month. The dust was gently brushed from the air conditioner filter into a salver, collected in a container and weighed. The living dust mites contained in each sample were counted under a light microscope.

Morphologic and Taxonomic Examination of Dust Mites

Water-soluble Berlese medium was used to fix and transparentize the mite bodies in a one-step procedure without any additional dehydration steps [6]. Each mite body was picked up with an anatomic needle, washed in phosphate-buffered saline (PBS, pH 7.4) and placed onto a piece of filter paper. A drop of Berlese medium was placed on a glass microscope slide and the clean mite body was placed into the middle of the drop. The mite's body was adjusted so that it was resting in a flat position with appendages spread out and covered with a glass coverslip. The glass was heated above a flame until the medium began to boil; it was then quickly removed, allowed to cool and kept at 37°C for 24 h. Fixed dust mite bodies were examined under a light microscope and then classified based on their morphology [6].

Allergen Extraction

To determine the concentration of the major allergens Der f 1 and Der f 2 in the dust samples of air conditioner filters, the allergens from the 60 samples collected in July and August were extracted. The dust samples were ground down and sieved through a mesh screen with 300 µm diameter to remove large particles and fibers. In accordance with previously described procedures [7, 8], the allergens in the dust samples were extracted as follows: 20 mg aliquots of dust were dissolved in 0.2 ml PBST (PBS, pH 7.4, with 0.1% Tween-20) and 0.1% bovine serum albumin, shaken overnight at 4°C and then centrifuged at 10,000 g for 10 min at 4°C. The resultant supernatants which contained the water-soluble allergens were collected for assay or stored at -20°C for later use.

Measurement of Der f 1 Concentration by ELISA

The dust supernatants were diluted 20-fold with PBS, and Der f 1 levels in the samples were evaluated with two monoclonal antibody sandwich ELISA kits supplied by Indoor Biotechnologies, Inc. (UK). The ELISA procedure was conducted in accordance with the manufacturer's suggested protocol [8], with the exception that we incubated the antibodies at 37°C instead of at room temperature and used 3,3',5,5'-tetramethylbenzidine (TMB) as substrate instead of 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid). The diluted samples and the serial dilutions of Der f 1 standards were added to ELISA plates coated with anti-Der f 1 monoclonal antibody 6A8 at 200 ng per well and incubated for 1 h at 37°C. A blank well was designated as the negative control. Plates were washed and another biotinylated anti-mite group 1 monoclonal antibody 4C1 was added and incubated for 30 min at 37°C. Then horseradish peroxidase (HRP)-conjugated streptavidin (Kirkegaard and Perry Laboratories, Gaithersburg, Md., USA) was added and incubated for 30 min at 37°C. After the wells were washed, 100 µl of substrate solution, containing 0.11 M sodium acetate (pH 5.5), 0.1 mg/ml TMB (Sigma) and 0.009% hydrogen peroxide (Sigma), was added to each well. After 15 min, the reaction was stopped by adding 50 µl of 3 M sulphuric acid. The absorbance at 450 nm was measured on an ELISA plate reader (Thermo, Sweden). A Der f 1 standard curve was drawn according to the optical density value of the standard Der f 1 serial dilutions. The Der f 1 content in the dust samples was calculated according to this Der f 1 standard curve.

Measurement of Der f 2 Concentration by ELISA

For the measurement of Der f 2 concentration, we prepared recombinant Der f 2 allergen (r Der f 2), immunized rabbits with r Der f 2, collected and purified anti-rabbit polyclonal antibody and then labeled the antibody with HRP [9]. We received Der f 2 monoclonal antibody as a gift from Prof. Tai-Soon Yong at the Yonsei University College of Medicine of Korea [10] and coated this purified monoclonal antibody onto ELISA plates in 50 mM carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C. Plates were washed with PBS and blocked with 2% bovine serum albumin for 2 h at 37°C. The sample and serial dilutions of r Der f 2 standard were held for 1 h at 37°C. A blank well was designated as the negative control. After all the wells were washed, HRP-labeled rabbit anti-Der f 2 polyclonal antibody was added and allowed to incubate for 1 h at 37°C. The plates were washed twice with PBS, and then, TMB enzyme substrate and sulphuric acid reaction termination solutions were added, the plates were read, and Der f 2 protein contents calculated as described for the Der f 1 ELISA above.

Cloning and Genetic Polymorphic Analysis of Der f 1 and Der f 2 cDNAs

A total of 1,000 live Der f mites were collected from 50 of the dust samples (20 mites taken per sample) and grinded together with liquid nitrogen. Total RNA was extracted using a Qiagen animal RNA extraction kit (Qiagen GmbH, Germany). Reverse transcription polymerase chain reaction, cloning and sequence analysis for both Der f 1 and Der f 2 genes were performed according to standard molecular cloning procedures [11]. The sequence data were analyzed with Bioedit software and compared with GenBank data. The primers were designed according to the pub-

lished sequences of Der f 1 (GenBank accession No. AB034946.1) and Der f 2 (GenBank accession No. AB195580.1); primers were synthesized by the Shanghai Biological Engineering Company. The upstream primer of Der f 1 was 5'-atg aaa ttc gtt ttg gcc att gcc tc-3' (44–69 nt), and the downstream primer of Der f 1 was 5'-tca cat gat tac aac ata tgg ata tt-3' (984–1009 nt). The upstream primer of Der f 2 was 5'-atg att tcc aaa atc ttg tgc ctt tca-3' (1–27 nt), and the downstream primer of Der f 2 was 5'-tt aat cac gga tt tac cat ggg-3' (419–441 nt).

Results

Characterization of Dust Mites Living in Air Conditioner Filters in Shenzhen City

The densities of dust mites found in the air conditioning filter samples collected from December 2003 to November 2004 were determined and are summarized in table 1. The dust mites encountered consisted primarily of the following 7 species: *D. farinae*, *D. pteronyssinus*, *Cheyletus trouessarti*, *Cheyletus malaccensis*, *Spinibdella* sp., *Blomia freemani* and *Lepidoglyphus destructor* [6]. The body morphologies of these species as seen under a light microscope are shown in figure 1.

The larval dust mites in the dust of air conditioner filters began to appear in 10% of 30 air conditioner filters encountered in March 2004. The dust mite number continued to increase in the following months and peaked in July and August, when the proportions of air conditioners with live dust mites to the 30 air conditioners encountered were 63.3 and 56.7%, respectively. The dust mite number and positive rate decreased sharply in October. No live dust mites were found in the dust of air conditioner filters in the winter months (December 2003, January 2004, February 2004 and November 2004).

Der f and Der p were the most widespread species among the variety of dust mites found, and they are associated with potent indoor airborne allergens. Morphologic and taxonomic examination under a light microscope of mites collected during their population peak (July and August) demonstrated that in July, 30.1% of the mites were Der f, 20.2% were Der p, and 49.7% were other species. Similarly, in August, 25.8% were Der f, 18.8% were Der p, and 55.4% were other species.

Contents of Der f 1 and Der f 2 Allergens in the Dust of Air Conditioner Filters in July and August

Because the numbers of dust mites living in the dust of air conditioner filters climaxed in July and August, we determined the levels of the major dust mite major aller-

Table 1. Dust mites in air conditioner filters in the Shenzhen area by month

Month	Sample number	Dust weight, g	Live dust mite positive rate, %	Density mites/g
Dec. 2003	30	0.71 ± 0.31	0	0
Jan. 2004	30	0.63 ± 0.27	0	0
Feb. 2004	30	2.33 ± 0.84	0	0
March 2004	30	1.89 ± 0.72	10	2.12 ± 0.45
April 2004	30	2.80 ± 0.85	26.7	9.23 ± 3.06
May 2004	30	1.83 ± 0.23	30.0	13.89 ± 1.82
June 2004	30	1.87 ± 0.69	40.0	22.39 ± 7.61
July 2004	30	2.45 ± 0.63	63.3	64.92 ± 21.51
Aug. 2004	30	1.57 ± 0.46	56.7	42.75 ± 14.56
Sep. 2004	30	2.14 ± 0.71	33.3	13.69 ± 6.31
Oct. 2004	30	1.07 ± 0.37	6.7	3.10 ± 0.15
Nov. 2004	30	0.78 ± 0.31	0.0	0.00 ± 0.00

Mean values are shown with standard deviations. Sample number = The number of the air conditioners investigated each month; dust weight = the weight of each dust sample collected from one air conditioner; live dust mite positive rate = the proportion of the number of air conditioning with live dust mites to the total 30 air conditioners; density = the density of dust mites in each dust sample collected from one air conditioner.

gens Der f 1 and Der f 2 for these 2 months. The Der f 1 and Der f 2 antigen levels (means ± standard deviations) in July as determined by antibody sandwich ELISA were 3.04 ± 1.75 and 2.15 ± 0.82 µg/g dust, respectively. The Der f 1 and Der f 2 antigen levels in August as determined by antibody sandwich ELISA were 3.21 ± 1.84 and 2.04 ± 1.15 µg/g dust, respectively.

Sequence Analysis of Der f 1 cDNA from Air Conditioner Filter Dust

Der f 1 cDNA clones were sequenced, and analysis of the sequences with BioEdit software enabled us to identify 1 identical variant (100% homology) to the gene described in GenBank (accession No. AB034946.1) and 3 new variants, whose GenBank accession numbers were EF139428, EF139429 and EF139430. The sequence homologies of these gene variants at the nucleotide sequence level were in the range of 99.48–100% relative to the Der f 1 gene sequence published in GenBank (accession No. AB034946.1). Likewise, the deduced amino acid homologies of the 4 Der f 1 gene products were also in the range of 99.48–100% relative to the Der f 1 published in GenBank (accession No. AB034946.1). The new variant sequences had genetic polymorphisms at residues 121, 160 and 259, respectively.

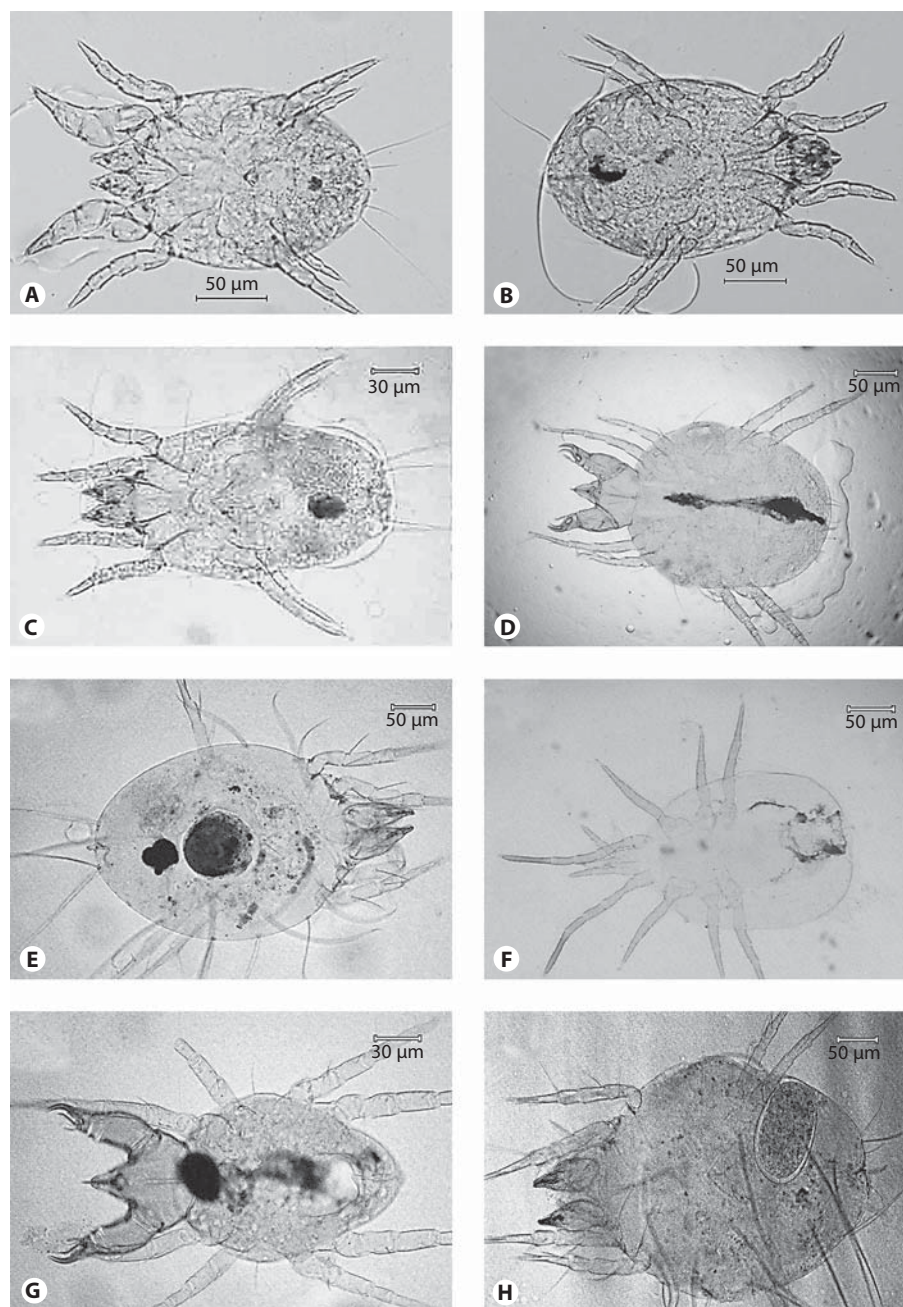


Fig. 1. Photomicrographs illustrating the unique morphology of the 7 species of dust mites encountered in this study. **A** *D. farinae* (male). **B** *D. farinae* (female). **C** *D. pteronyssinus*. **D** *C. trouessarti*. **E** *C. malaccensis*. **F** *Spinibdella* sp. **G** *B. freemani*. **H** *L. destructor*.

Sequence Analysis of Der f 2 cDNA from Air Conditioner Filter Dust in the Shenzhen Area

Der f 2 cDNA clones were sequenced, and analysis of the sequences with BioEdit software enabled us to identify 5 new variants whose GenBank accession numbers were EF139431, EF139432, EF139433, EF139434 and EF205129. The sequence homologies of these gene variants at the nucleotide sequence level were in the range of 96.8–99.3%

relative to the Der f 2 gene sequence published in GenBank (accession No. AB195580.1). Likewise, the deduced amino acid homologies of the 5 Der f 2 gene products were also in the range of 93.8–98.6% relative to the Der f 2 previously published in GenBank (accession No. AB195580.1). The 5 new Der f 2 variant genes cloned in our experiments had genetic polymorphisms at amino acid residues 71, 74, 75, 76, 80, 92, 93, 105, 110, 128 and 135.

Discussion

Here, we report three sets of experiments conducted on dust samples collected from air conditioning filters in the Shenzhen City area of Southern China, including one that identified dust mite species morphologically, one that assessed the concentrations of the major dust mite allergenic proteins Der f 1 and Der f 2, and one that isolated and compared Der f 1 and Der f 2 cDNA sequences. Together, these experiments provide compelling evidence that the Der f species of dust mites, which produce potent allergens, exist in the dust of air conditioner filters in Shenzhen City. During the year-long investigation, we documented the monthly total numbers of dust mites, their morphologically defined identities and the seasonal changes in their populations. Among the variety of dust mite species observed, the dominant species were Der f and Der p, which are recognized as sources for strong inhaled allergens.

Shenzhen City lies in a subtropical area where it is warm and humid throughout the year (approximately 1,299.4 mm rainfall/year, 77% average relative humidity, 22.5°C average temperature); Shenzhen City's average seasonal temperatures are 16.4°C in January to March, 25°C in April to June, 28°C in July to September and 20.5°C in October to December (www.121.com.cn/index.jsp). This environment is ideal for Der f populations to thrive. Air conditioning is often used from May to October in this area with an operating temperature around 25°C. We found that Der f appeared in the dust of air conditioner filters in the Shenzhen area in March, flourished from June to August, reached their population climax in July, and were no longer present from November through February. This population cycle fits well with what would be expected for the local climate and air conditioner usage.

Among the 30 reported allergens produced by Der f dust mites, Der f 1 and Der f 2 are the major offenders [12]. Der f 1 and Der f 2 are clinically important allergen proteins not only because they are frequently found in high concentrations in house dust, but also because they had >80% immunoglobulin E binding frequency with sera of mite-sensitive patients and accounted for >50% of immunoglobulin E antibodies against total mite extract [12]. Our antibody sandwich ELISA experiments revealed that the average Der f 1 and Der f 2 concentrations in the filter dust in July and August exceeded the risk level (2 µg/g) for inducing allergic sensitization in susceptible persons [13]. While the present findings are consistent with the possibility that dust mites in air conditioner fil-

ters may induce allergic responses when blown into indoor air, further research is needed to determine whether these levels of allergen are sufficient to induce allergic reactions or asthmatic responses.

We provided evidence of genetic diversity of the Der f mites in our sample based on the genetic polymorphisms of the major Der f allergens Der f 1 and Der f 2. We discovered 4 types of Der f 1 cDNA genes and 5 types of Der f 2 cDNA genes. Der f 1 cDNA genetic polymorphisms have previously been reported at amino acid residues 201, 220 and 282 [14]. However, the 4 types of Der f 1 cDNA genes cloned herein revealed new variants with genetic polymorphisms on amino acid residues 121, 160 and 259. Only 1 of the Der f 1 cDNA gene variants we cloned had a nucleotide sequence that matched a sequence previously published in GenBank (accession No. AB034946.1). It has been reported previously that the deduced amino acid sequence of the Der f 2 cDNA gene had genetic polymorphisms on amino acid residues 76, 88, 111 and 125 [15]. However, the 5 types of Der f 2 cDNA gene variants we discovered had genetic polymorphisms on amino acid residues 71, 74, 75, 76, 80, 92, 93, 105, 110, 128 and 135. Interestingly, all 5 of the Der f 2 cDNAs cloned in this study had the same two deduced amino acids, e.g., Asn and Ile, at amino acid residues 110 and 128. Further research may be needed to determine whether this is a regionally distinctive feature of Der f polymorphisms in the Shenzhen area.

Materials derived from scurf, cotton fibers, floating dust and smoke accumulate on air conditioner filters with use over time. This provides a hospitable environment for Der f to live. When air conditioning units are operating, the transfer of air through the unit to the indoor environment can bring with it allergenic materials that have accumulated in the filters, including dust mite secretions, excretions and decomposing carcasses. Such tiny airborne allergenic particles are of particular concern as they are obviously more accessible for inhalation relative to static allergens resting in furniture and on household surfaces. It is possible that air conditioning units could serve as a conduit through which live mites could spread indoors.

Although the precise relationship between airborne particles derived from air conditioners and allergic diseases has yet to be defined, our demonstration that robust dust mite allergens do exist in air conditioning units together with the fact that the air-conditioned indoor environment (approximately 25°C with a comfortable level of humidity) is very suitable for dust mites to live and thrive, suggests that the relationship between air conditioning

use and allergenic diseases merits further investigation. Specifically, it should be determined whether the recent popularization of air conditioning is associated with an increasing incidence of allergic diseases, especially those associated with Der f and Der p allergens. If so, the quality of life of individuals susceptible to allergic diseases may be improved by regulated cleaning or replacement of air conditioning filters.

Acknowledgments

This investigation was supported by a series of grants which included the National 863 Program Foundation of China (No. 2002AA214011), the National Natural Science Foundation of China (No. 30271226, No. 30471505) and the Guangdong-Hong Kong Technology Cooperation Funding (No. 20054982207). We thank Dr. Dick Xu and Dr. Ann E. Power for the translation of this paper from Chinese into English. We also thank Prof. Tai-Soon Yong at Yonsei University College of Medicine of Korea for supplying Der f 2 monoclonal antibody.

References

- 1 Zhong N: How to prevent asthma effectively in China. *Chin J Tuberc Respir Dis* 1997;20: 259–260.
- 2 Chen YZ: Prevention and cure for Chinese children suffering asthma. *Chin J Pediatr* 2000;42:81–82.
- 3 Xiao LH, Wang JP, Zheng BZ: Investigation on the epidemiology of juvenile allergic rhinitis and life status of the children. *China J Nurs Sci* 2003;18:415–416.
- 4 Furuta Y, Yoshioka M, Sugiyama T, Miyasaka K: Rapidly increasing allergic diseases. *Hokkaido Igaku Zasshi* 2005;80:3–7.
- 5 Platts-Mills TAE, Chapman MD: Dust mites: immunology, allergic disease and environmental control. *J Allergy Clin Immunol* 1987;80:755–775.
- 6 Hughes AM: *The Mites of Stored Food and Houses* (Chinese Translation). Beijing, China Agriculture Press, 1983.
- 7 Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TA: Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol* 1987;80:184–194.
- 8 Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD: A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p I and Der f I. *J Immunol Methods* 1989;118:227–235.
- 9 Zhu JQ, Liu ZG, Gao B, Ji KM, Xing M: Cloning, expression, purification, and identification of Der f 2 gene and its immunological characteristics. *Chin J Immunol* 2006;22: 213–216.
- 10 Jeong KY, Jin HS, Oh SH, Hong CS, Lee IY, Ree HI, Yong TS: Monoclonal antibodies to recombinant Der f 2 and development of a two-site ELISA sensitive to major Der f 2 isoallergen in Korea. *Allergy* 2002;57:29–34.
- 11 Sambrook J, David Russell: *Molecular Cloning*, ed 3. Cold Spring Harbor, Cold Spring Harbor Press, 2001, vol 1–2.
- 12 Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM: Characterization and immunobiology of house dust mite allergens. *Int Arch Allergy Immunol* 2002;129:1–18.
- 13 Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse RC, Chapman MD: Indoor allergens and asthma: report of the third international workshop. *J Allergy Clin Immunol* 1997;100:1–24.
- 14 Sun JL, Chen J, Zhang HY, Wang HF: Research on molecular biology of dust mite allergen. *Foreign medical sciences (in Chinese)*. *Immunology* 2003;30:196–202.
- 15 Yuuki T, Okumura Y, Ando T, Yamakawa H, Suko M, Haida M, Okudaira H: Cloning and sequencing of cDNAs corresponding to mite major allergen Der f II. *Arerugi* 1990;39:557–561.