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Current Status of Standardization of Inhalant Allergen Extracts in Korea

Kyoung Yong Jeong,[1] Jae-Hyun Lee,[1] Eun-Jin Kim,[2] Joo-Shil Lee,[2] Sang-Heon Cho,[3] Soo-Jong Hong,[4] Jung-Won Park[1]*

[1]*Department of Internal Medicine and Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea*
[2]*Center for Immunology and Pathology, Korea National Institute of Health, Osong, Cheongwon, Korea*
[3]*Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea*
[4]*Department of Pediatrics, Childhood Asthma Atopy Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*

Allergy diagnosis and immunotherapy in Korea rely mostly on imported allergen extracts. However, some allergens that are not important in Western countries are not commercially available, and even the same species of allergen source often displays differences in allergenicity due to amino acid sequence polymorphisms. Therefore, it is essential to prepare allergen extracts that reflect regional characteristics. Allergen standardization has been performed since 2009 with the support of the Korea Center for Disease Control and Prevention. Here, we summarize the current status of allergen standardization, focusing on the house dust mite and cockroach. Pollen allergens that are under investigation are also briefly described.

Key Words: Allergen; allergen extract; standardization

INTRODUCTION

It is essential to standardize allergen extracts for commercial allergy diagnostic and immunotherapeutic reagents.[1] A project for the standardization of allergen extracts has been supported by the Korea Center for Disease Control and Prevention since 2009. Here, we summarize the results from 4 years of this project. A standardization procedure needs to be established in order to ensure the consistency and reproducible preparation of allergen extracts. Allergen standardization can be performed through preparation of standard reference material and determination of the unit that denotes IgE binding potency. The overall potency of the extracts was determined by using an *in vivo* method, measurement of the bioequivalent unit (BAU) by an intradermal skin test. This potency has been used as a reference for the estimation of the potency of extracts from other batches by an *in vitro* method, competitive IgE-binding assay. The concentration of major allergens has also been assessed because it has been known to be proportional to the potency of the extracts.[2] Currently, 7 allergen extracts, including house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), German cockroach (*Blattella germanica*), pollens of *Humulus japonicus*, *Artemisia vulgaris*, *Ambrosia artemisiifolia*, and *Quercus acutissima*, have been prepared or are under investigation. Various recombinant proteins are now being generated and characterized for better allergen standardization.

Standardization of indoor allergens

House dust mite is the most important source of indoor allergens,[3,4] therefore we first focused on the preparation and characterization of house dust mite extract, followed by German cockroach extract. Commercial dust mite extracts are well characterized and are provided at a concentration of 5,000-30,000 allergy units (AU)/mL. The AU indicates that the potency of the extract was measured by *in vitro* methods with the reference standard. To achieve successful immunotherapy for house dust mite allergy, a maintenance dose of 500-2,000 AU/mL is suggested.[5] However, commercial cockroach allergen extracts are not standardized and are provided at a dilution of 1:10 w/v. The suggested dose during the maintenance phase for effective cockroach immunotherapy is the highest tolerated dose.

House dust mite

House dust mite extracts were prepared from Korean isolates.[6] The allergen potency of *D. pteronyssinus* extract was compared to reference material obtained from the Center for Biologics

Correspondence to: Jung-Won Park, MD, PhD, Department of Internal Medicine and Institute of Allergy, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea.
Tel: +82-2-2228-1961; Fax: +82-2-362-8647; E-mail: parkjw@yuhs.ac
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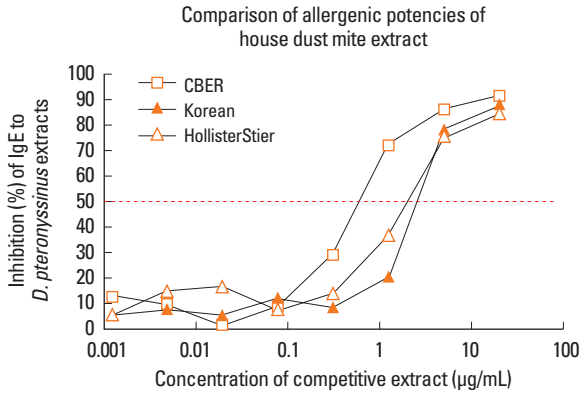

Fig. 1. Inhibition analysis of house dust mite extracts. The specific IgE antibody binding activities of *D. pteronyssinus* extracts were compared by ELISA. □, American reference material from CBER; ▲, Korean reference material; △, HollisterStier extract.

Evaluation and Research (CBER, Food and Drug Administration, USA) (Fig. 1). Interestingly, compared to the CBER reference, the Korean reference material showed only 11.6% allergenic potency in terms of 50% inhibitory concentration, and the commercial extract (HollisterStier, Spokane, WA, USA) showed 14.6% potency. In addition to allergen potency, allergen composition is also important in terms of the consistency of allergen preparations. However, the concentration of major allergens of the house dust mite is known to be variable, even in commercial skin test reagents.[7,8] For example, the ratio of Der p 1 (12-30 μg/mL)/Der p 2 (3-15 μg/mL) ranges from 1.1:1 to 6:1.[7] Mite extracts prepared from raw materials rich in fecal pellets contain higher ratios of mite group 1/2 allergens than those from pure mite bodies.[9] In the extracts from Korean house dust mite isolates, we measured concentrations of 5.0 μg/mg Der f 1, 12.0 μg/mg Der f 2, 11.6 μg/mg Der p 1, and 12.4 μg/mg Der p 2 by 2-site ELISA (Indoor Biotechnologies Inc., Charlottesville, VA, USA).[6]

Quantification of mite allergens, particularly group 2 allergens, is known to be influenced by amino acid sequence polymorphism.[10] Sequence polymorphism analysis of Korean mite isolates revealed the predominant and novel variants of mite group 1 and 2 allergens.[11] Specifically, for group 1, Der f 1.0101 (64.0%) appeared as a predominant variant. Der p 1.0102 (44.0%) was a predominant variant, followed by Der p 1.0105 (14.0%), which contains Ala124 and Gly182. A new variant (GenBank Accession No. JN222803) (8.0%) with Ala124 and Ser182 was also identified. For group 2 allergens, Der f 2.0102 (40.7%) and a new variant (GenBank Accession No. JQ698663) with Gly42 (27.8%) were predominant. The SLD-motif at positions 57-59, along with Ile63 and Phe75, suggest large differences from the European and Thai populations. Two variants of Der p 2—Leu40, Thr49, and Asn114 (26.6%) and Val40, Thr49, and Asn114 (20.0%)—were predominant. Interestingly, all Der p 2 sequences had Thr instead of Lys at position 49. Five new variants of Der p 2 were deposited in GenBank under accession No. JN222805-9. Moreover, most of the variants contained Ser47 which is known to have a higher IgE-binding affinity, and Asn114 which is responsible for strong binding to the monoclonal antibodies 1D8 and 4G7 in the commercial ELISA kit. In a study from Thailand, rabbit polyclonal antibodies were used for the quantification of Der f 1.[12] The problems caused by polymorphisms may be overcome by the production of a regional standard using a mixture of predominant variants and/or development of monoclonal antibodies recognizing non-polymorphic regions. Stability of mite extracts have also been investigated.[13] Storage temperature is the most important factor in the preservation of allergenicity of the extract, and 92.0%-97.0% of IgE reactivity is retained at 1 year regardless of buffer composition when stored at 4°C. The mite extract reconstituted in 50% glycerol retained more than 89.9% IgE reactivity at 1 year even at room temperature.

Cockroach

Cockroach is another important source of indoor allergens associated with asthma exacerbation.[14] However, cockroach extract has not been standardized, and manufacturers commonly use the unit of weight (raw material) to volume (extraction buffer) (v/w). Strong protease activity in the cockroach extract makes it difficult to standardize the extract.[15] We prepared an extract of German cockroach and analyzed its allergenic properties.[16] The cockroach population used in this study originated from a single pregnant female captured in Seoul.[17] The cockroach extract showed allergenic potency (94.2%) similar to the commercial extract (Hollister-Stier) in a competitive IgE-binding analysis. However, there was a substantial difference in Bla g 1 and Bla g 2 concentrations between the extracts: the Korean extract contained 404 U/mg Bla g 1 and 273 ng/mg Bla g 2, whereas the Hollister-Stier extract contained 187 U/mg Bla g 1 and 56 ng/mg Bla g 2. A difference in endotoxin concentration between the extracts was also observed (3,440 EU/mL for Korean extract and 6,580 EU/mL for HollisterStier extract); however, endotoxin is not considered essential[18] even though alveolar macrophages play a key role in a cockroach-induced airway inflammation[19] in a mouse model. Interestingly, proteins of approximately 60 and 70 kDa showed strong IgE reactivity in both extracts. Proteomic analysis of the extract showed that 60 and 70 kDa allergens are α-amylase and Bla g 3, respectively (Fig. 2, Table 1). Amylase was also identified as an important allergen in German cockroach fecal extract.[20] An interesting finding of fecal extract analysis is the IgE reactivity of various digestive enzymes, such as α-amylase, trypsin, chymotrypsin, metalloprotease, and midgut carboxypeptidase A. Furthermore, we detected glycinin-like protein, which may have been derived from the cockroach diet and may lead to false diagnosis. Further studies on α-amylase and Bla g 3 allergens are needed because they appear to have various isoforms.

Table 1. Identification of IgE reactive spots from German cockroach extract

Spot	Identification	Species	Molecular weight	Isoelectrical point	Mascot score	Coverage (%)
B1	Bla g 3 isoform	*Blattella germanica*	78687	6.44	193	10
B2	Bla g 3 isoform	*Blattella germanica*	78730	6.35	219	8
B3	α-glucosidase	*Culex quinquefasciatus*	67755	4.90	78	3
B4	α-amylase	*Blattella germanica*	56759	5.74	163	7

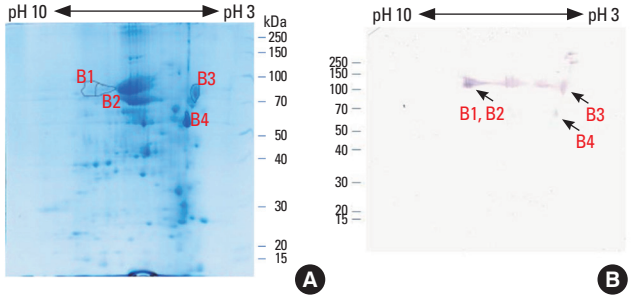

Fig. 2. Proteomic analysis of German cockroach extract. Proteins were separated by 2D-electrophoresis and visualized by Coomassie Blue staining (A), and IgE-reactive components were probed with a pooled serum from cockroach-sensitized subjects (B). Selected proteins were identified by MS/MS analysis (B1-4). The data are summarized in Table.

Standardization of pollen allergens

Pollens from various plants are important causes of seasonal allergic rhinitis. Tree pollens from alder, birch, and oak are known to be important in spring, mainly from March to May, whereas weed pollens from Japanese hop, sagebrush, and ragweed are important in autumn, mainly from August to September.[21] Currently, most of the commercial tree and weed pollen extracts are not standardized and are provided as a protein nitrogen unit (PNU) or weight per volume unit (w/v, 1:10 to 1:40). The recommended maintenance dosage for immunotherapy, the probable effective dose range, was estimated to be 0.5 mL of 1:100 or 1:200 w/v dilution of the non-standardized pollen extract.[5] For short ragweed, a maintenance dose of 6-12 μg Amb a 1 or 1,000-4,000 AU was calculated for effective treatment. Furthermore, most of the pollen extracts are imported from Western countries. Therefore, pollen extracts from trees that are not common or not even found in Korea are currently being used for allergy diagnosis and immunotherapy. A correct diagnosis of pollen allergy is essential because sensitization to pollen allergens may lead to allergic reactions to various fruits.[22]

Tree pollen

The levels of airborne pollens from pine, oak, and birch were reported to be high in May in Korea.[23] Moreover, the sensitization rates to tree (alder, pine, and oak) and weed pollens (Japanese hop and mugwort) have increased significantly over 10 years (1999 to 2008) in the southern part of Gyeonggi province.[24] In particular, common silver birch (white birch, *Betula verrucosa*) and white oak (*Quercus alba*) are not native to Korea. However, Siberian silver birch (*B. platyphylla* var. *japonica*) is often planted as a landscape tree.[25] In the case of oak species, Mongolian oak (*Q. mongolica*) is known to be a predominant species in Korea,[26] whereas sawtooth oak (*Q. acutissima*) is often found only at human dwellings. Pathogenesis-related 10 proteins (PR-10, Bet v 1 homologues) from the order Fagales are known to be important allergens, but cross-reactivity could be limited.[27] Therefore, there is an urgent need to determine cross-reactivity between pollen extracts from trees native to Korea and the commercially-available extracts from foreign trees. A preliminary study suggested that sawtooth oak is a major cause of oak allergy in Korea. Therefore, we are characterizing the PR-10 protein, a putative Que ac 1, from sawtooth oak and the potency of the sawtooth oak pollen extract is being evaluated by an *in vivo* method.

Weed pollen

Pollens from Japanese hop, sagebrush, and ragweed constitute about 90% of weed pollens in the air in Korea during autumn.[28] In particular, 2 invasive plants, Japanese hop (*Humulus japonicus*) and ragweed (*Ambrosia artemisiifolia* var. *elatior*), have become able to displace native species as a result of global warming.[29] Most importantly, Japanese hop pollen extract is not standardized despite its allergenic importance in East Asia. A study from China reported that profilin (a putative Hum j 2) is a major allergen from the Japanese hop.[30] However, 2 isoforms of recombinant profilin were found to play a minor role in Korea.[31] An allergen with a molecular mass of approximately 11 kDa is believed to be a major allergen.[32,33] We are currently attempting to characterize this allergen of interest. The potency BAU of the allergen extract is also being determined by an intradermal skin test. Detailed information on *in vivo* standardization and characterization of 11 kDa allergen of Japanese hop will be published elsewhere.

At CBER, the potency of the ragweed allergen extract is evaluated through determination of a specific allergen, Amb a 1 by immunodiffusion with sheep polyclonal antibodies. However, a commercial 2-site ELISA kit (Indoor Technologies Inc.) uses monoclonal antibodies for the quantification of Amb a 1. Therefore, it is necessary to compare these 2 methods.

Artemisia vulgaris is commonly used for the diagnosis of mugwort allergy in Korea. However, this species is not found in Korea, and *A. princeps* is reported to be a dominant species.[34] Comparison of 6 different species of *Artemisia* (*A. vulgaris*, *A. sco-*

paria, *A. princeps*, *A. tridentate*, *A. annu*, and *A. campestris*) revealed essentially the same pattern of IgE reactivity both to *A. vulgaris* and *A. princeps*.[35] Therefore, we are conducting *in vivo* standardization of mugwort extract using *A. vulgaris*.

Recombinant allergens

The concentration of major allergens could be affected by environmental stresses. The mite culturing diet[36] and the temperature at which the mites are cultured[37] are known factors that determine allergen concentrations in extracts. The expression of cockroach allergen Bla g 1 is known to be influenced by food intake,[38] whereas excretion of Bla g 2 is increased after exposure to a sublethal dose of boric acid, a common pesticide.[39] Glutathione *S*-transferases, mite group 8 and cockroach group 5 allergens, are thought to be influenced by environmental chemicals because of their detoxification activity.[40,41] A similar situation exists for pollen allergens. Pollen production can be increased by exposure to environmental pollutants, high CO_2 concentrations, and high temperatures.[42] High-quality recombinant allergen should ensure the absence of non-allergenic antigens and adjuvant-like molecules, such as endotoxin, β-glucan, superantigen, and chitin. Even if high-quality allergen extracts are available, production of recombinant allergens is desirable for the quantification of major allergens. Quantification of major allergens is often hampered by polymorphisms. Therefore, it is necessary to identify dominant isoforms and produce regional standard molecules. Currently, various recombinant allergens from house dust mite, cockroach, Asian needle ant, buckwheat, and pollens are being characterized for standardization.

Concluding remarks

Allergen standardization is performed not only by the measurement of IgE-binding potency but also by the quantification of major allergens.[43] However, many of the major allergens from Korean trees and weeds have not been characterized. Furthermore, the clinical value of component-resolved diagnosis has been proven.[44] Continued effort is needed to produce validated recombinant allergens and to develop a quantification system for better standardization.[45] Finally, allergen standardization could facilitate the development of advanced diagnostic and immunotherapeutic reagents.

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REFERENCES

1. Jeong KY, Hong CS, Lee JS, Park JW. Optimization of allergen standardization. Yonsei Med J 2011;52:393-400.
2. van Ree R. Indoor allergens: relevance of major allergen measurements and standardization. J Allergy Clin Immunol 2007;119:270-7.
3. Jeong KY, Hong CS, Yong TS. Domestic arthropods and their allergens. Protein Pept Lett 2007;14:934-42.
4. Jeong KY, Park JW, Hong CS. House dust mite allergy in Korea: the most important inhalant allergen in current and future. Allergy Asthma Immunol Res 2012;4:313-25.
5. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I, Nelson M, Weber R, Bernstein DI, Blessing-Moore J, Khan DA, Lang DM, Nicklas RA, Oppenheimer J, Portnoy JM, Randolph C, Schuller DE, Spector SL, Tilles S, Wallace D. Allergen immunotherapy: a practice parameter third update. J Allergy Clin Immunol 2011;127:S1-55.
6. Jeong KY, Choi SY, Lee JH, Lee IY, Yong TS, Lee JS, Hong CS, Park JW. Standardization of house dust mite extracts in Korea. Allergy Asthma Immunol Res 2012;4:346-50.
7. Meyer CH, Bond JF, Chen MS, Kasaian MT. Comparison of the levels of the major allergens Der p I and Der p II in standardized extracts of the house dust mite, *Dermatophagoides pteronyssinus*. Clin Exp Allergy 1994;24:1041-8.
8. Brunetto B, Tinghino R, Braschi MC, Antonicelli L, Pini C, Iacovacci P. Characterization and comparison of commercially available mite extracts for in vivo diagnosis. Allergy 2010;65:184-90.
9. Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TA. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp). J Allergy Clin Immunol 1989;83:1055-67.
10. Park JW, Kim KS, Jin HS, Kim CW, Kang DB, Choi SY, Yong TS, Oh SH, Hong CS. Der p 2 isoallergens have different allergenicity, and quantification with 2-site ELISA using monoclonal antibodies is influenced by the isoallergens. Clin Exp Allergy 2002;32:1042-7.
11. Jeong KY, Lee IY, Yong TS, Lee JH, Kim EJ, Lee JS, Hong CS, Park JW. Sequence polymorphisms of Der f 1, Der p 1, Der f 2 and Der p 2 from Korean house dust mite isolates. Exp Appl Acarol 2012;58:35-42.
12. Sookrung N, Kamlanghan T, Indrawattana N, Tungtrongchitr A, Tantilipikorn P, Bunnag C, Pattanapanyasat K, Chaicumpa W. Quantification of Der f 1 in houses of patients allergic to house dust mite, *Dermatophagoides farinae*, using a locally produced detection reagents. Asian Pac J Allergy Immunol 2011;29:78-85.
13. Jeong KY, Choi SY, Han IS, Lee JH, Lee JS, Hong CS, Park JW. The effects of storage conditions on the stability of house dust mite extracts. Allergy Asthma Immunol Res 2013;5:397-401.
14. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, Mitchell H, McNiff-Mortimer K, Lynn H, Ownby D, Malveaux F. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997;336:1356-63.
15. Jeong KY, Kim C, Yong TS. Enzymatic activities of allergen extracts from three species of dust mites and cockroaches commonly found in Korean home. Korean J Parasitol 2010;48:151-5.
16. Jeong KY, Choi SY, Lee JH, Lee JS, Yong TS, Hong CS, Park JW. Preparation and characterization of an extract of German cockroach from a Korean source. Allergy Asthma Immunol Res 2013;5:102-5.
17. Jeong KY, Lee H, Shin KH, Yi MH, Jeong KJ, Hong CS, Yong TS. Sequence polymorphisms of major German cockroach allergens Bla g 1, Bla g 2, Bla g 4, and Bla g 5. Int Arch Allergy Immunol 2008;145:1-8.
18. Shin YS, Sohn JH, Kim JY, Lee JH, Cho SH, Hong SJ, Lee JS, Hong

CS, Park JW. Endotoxin is not essential for the development of cockroach induced allergic airway inflammation. Yonsei Med J 2012;53:593-602.
19. Kim JY, Sohn JH, Choi JM, Lee JH, Hong CS, Lee JS, Park JW. Alveolar macrophages play a key role in cockroach-induced allergic inflammation via TNF-α pathway. PLoS One 2012;7:e47971.
20. Jeong KY, Kim CR, Park J, Han IS, Park JW, Yong TS. Identification of novel allergenic components from German cockroach fecal extract by a proteomic approach. Int Arch Allergy Immunol 2013;161:315-24.
21. Hong CS, Hwang Y, Oh SH, Kim HJ, Huh KB, Lee SY. Survey of the airborne pollens in Seoul, Korea. Yonsei Med J 1986;27:114-20.
22. Webber CM, England RW. Oral allergy syndrome: a clinical, diagnostic, and therapeutic challenge. Ann Allergy Asthma Immunol 2010;104:101-8.
23. Oh JW, Lee HB, Kang IJ, Kim SW, Park KS, Kook MH, Kim BS, Baek HS, Kim JH, Kim JK, Lee DJ, Kim KR, Choi YJ. The revised edition of Korean calendar for allergenic pollens. Allergy Asthma Immunol Res 2012;4:5-11.
24. Lee JW, Choi GS, Kim JE, Jin HJ, Kim JH, Ye YM, Nahm DH, Park HS. Changes in sensitization rates to pollen allergens in allergic patients in the southern part of Gyeonggi province over the last 10 years. Korean J Asthma Allergy Clin Immunol 2011;31:33-40.
25. Lee JY, Park JS, Kim HR, Kim DY, Noh HS, Lee KE. Analysis of landscape planting in Gangwon-do. J Korean Inst landsc Archit 2011;39:113-26.
26. Kim GS, Song HK, Lee CH, Cho HJ, Lee CS. Ecological comparison of Mongolian oak (*Quercus mongolica* Fisch. ex Ledeb.) community between Mt. Nam and Mt. Jeombong as a Long Term Ecological Research (LTER) site. J Ecol Field Biol 2011;34:75-85.
27. Hauser M, Asam C, Himly M, Palazzo P, Voltolini S, Montanari C, Briza P, Bernardi ML, Mari A, Ferreira F, Wallner M. Bet v 1-like pollen allergens of multiple Fagales species can sensitize atopic individuals. Clin Exp Allergy 2011;41:1804-14.
28. Oh JW. Development of pollen concentration prediction models. J Korean Med Assoc 2009;52:579-91.
29. Song U, Mun S, Ho CH, Lee EJ. Responses of two invasive plants under various microclimate conditions in the Seoul metropolitan region. Environ Manage 2012;49:1238-46.
30. Tao AL, He SH. Cloning, expression, and characterization of pollen allergens from *Humulus scandens* (Lour) Merr and *Ambrosia artemisiifolia* L. Acta Pharmacol Sin 2005;26:1225-32.
31. Jeong KY, Han IS, Choi SY, Lee JH, Lee JS, Hong CS, Park JW. Allergenicity of recombinant profilins from Japanese hop, *Humulus japonicus*. J Investig Allergol Clin Immunol 2013;23:345-50.
32. Park HS, Nahm DH, Suh CH, Lee SM, Choi SY, Jung KS, Lee SY, Park K. Evidence of Hop Japanese pollinosis in Korea: IgE sensitization and identification of allergenic components. J Allergy Clin Immunol 1997;100:475-9.
33. Park JW, Ko SH, Kim CW, Jeoung BJ, Hong CS. Identification and characterization of the major allergen of the *Humulus japonicus* pollen. Clin Exp Allergy 1999;29:1080-6.
34. Park HS, Hong CS, Choi HJ, Hahm KS. Identification and partial purification of pollen allergens from *Artemisia princeps*. Yonsei Med J 1989;30:346-54.
35. Brandys J, Grimsoen A, Nilsen BM, Paulsen BS, Park HS, Hong CS. Cross-reactivity between pollen extracts from six *Artemisia* species. Planta Med 1993;59:221-8.
36. Avula-Poola S, Morgan MS, Arlian LG. Diet influences growth rates and allergen and endotoxin contents of cultured *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* house dust mites. Int Arch Allergy Immunol 2012;159:226-34.
37. Yella L, Morgan MS, Arlian LG. Population growth and allergen accumulation of *Dermatophagoides farinae* cultured at 20 and 25°C. Exp Appl Acarol 2013;60:117-26.
38. Gore JC, Schal C. Expression, production and excretion of Bla g 1, a major human allergen, in relation to food intake in the German cockroach, *Blattella germanica*. Med Vet Entomol 2005;19:127-34.
39. Zhang YC, Perzanowski MS, Chew GL. Sub-lethal exposure of cockroaches to boric acid pesticide contributes to increased Bla g 2 excretion. Allergy 2005;60:965-8.
40. Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 2001;360:1-16.
41. Enayati AA, Ranson H, Hemingway J. Insect glutathione transferases and insecticide resistance. Insect Mol Biol 2005;14:3-8.
42. Bartra J, Mullol J, del Cuvillo A, Dávila I, Ferrer M, Jáuregui I, Montoro J, Sastre J, Valero A. Air pollution and allergens. J Investig Allergol Clin Immunol 2007;17 Suppl 2:3-8.
43. Chapman MD, Ferreira F, Villalba M, Cromwell O, Bryan D, Becker WM, Fernández-Rivas M, Durham S, Vieths S, van Ree R; CREATE consortium. The European Union CREATE project: a model for international standardization of allergy diagnostics and vaccines. J Allergy Clin Immunol 2008;122:882-9.e2.
44. Treudler R. Update on in vitro allergy diagnostics. J Dtsch Dermatol Ges 2012;10:89-97.
45. Jeong KY, Hong CS, Yong TS. Recombinant allergens for diagnosis and immunotherapy of allergic disorders, with emphasis on cockroach allergy. Curr Protein Pept Sci 2006;7:57-71.