INTRODUCTION

Allergic disorders are one of the most common diseases, affecting approximately 20%-40% of the population in developed countries. Recognized as an important cause of asthma in the last 50 years, cockroach allergy is the second leading allergen in Taiwan, next only to house dust mites. The most common domestic cockroaches associated with allergy are the German cockroach Blattella germanica in the US and Europe, and the American cockroach Periplaneta americana in South America and Asian countries. Since the cloning of Bla g 2, the major allergen of German cockroach, more and more cockroach allergens have been identified. There have been 9 American cockroach allergens, namely Per a 1-7 and Per a 9-10, and 9 German cockroach allergens, namely Bla g 1-8 and Bla g 11, described in current literature. Cockroach allergens are classified into 11 groups in the World Health Organization (WHO) and the International Union of Immunological Societies (IUIS) Allergen Nomenclature database according to their sequence homology and biological functions. Group 1 to 11 cockroach allergens are midgut microvillar proteins, aspartic proteases, arylphorin/hemocyanins, lipocalins, glutathione S-transferases, troponin C, tropomyosins, myosin light chain, arginine kinase, serine protease, and alpha-amylase, respectively. The comparison of allergens from American and German cockroach species has not been studied in detail. However, the po-

Purpose: Cockroaches are the second leading allergen in Taiwan. Sensitization to Per a 2, the major American cockroach allergen, correlates with clinical severity among patients with airway allergy, but there is limited information on IgE epitopes and tissue localization of Per a 2. This study aimed to identify Per a 2 linear IgE-binding epitopes and its distribution in the body of a cockroach. Methods: The cDNA of Per a 2 was used as a template and combined with oligonucleotide primers specific to the target areas with appropriate restriction enzyme sites. Eleven overlapping fragments of Per a 2 covering the whole allergen molecule, except 20 residues of signal peptide, were generated by PCR. Mature Per a 2 and overlapping deletion mutants were affinity-purified and assayed for IgE reactivity by immunoblotting. Three synthetic peptides comprising the B cell epitopes were evaluated by direct binding ELISA. Rabbit anti-Per a 2 antibody was used for immunohistochemistry. Results: Human linear IgE-binding epitopes of Per a 2 were located at the amino acid sequences 57-86, 200-211, and 299-309. There was positive IgE binding to 10 tested Per a 2-allergic sera in 3 synthetic peptides, but none in the controls. Immunostaining revealed that Per a 2 was localized partly in the mouth and midgut of the cockroach, with the most intense staining observed in the hindgut, suggesting that the Per a 2 allergen might be excreted through the feces. Conclusions: Information on the IgE-binding epitope of Per a 2 may be used for designing more specific diagnostic and therapeutic approaches to cockroach allergy.

Key Words: Cockroach allergy; IgE-binding epitope; synthetic peptide; specific immunotherapy; Per a 2
tential for cross-reactivity among homologous allergens from different species is expected.

Recently, we reported the cloning and expression of 8 major American cockroach allergens (Per a 1-7 and Per a 9) in *E. coli* and aimed to identify markers for disease severity among cockroach allergic patients.13 Our results revealed that patients with rhinitis and persistent asthma (AS group) had higher serum levels of the inflammatory cytokines IL-8, MCP-1, CCL-20, and GM-CSF compared to those with allergic rhinitis only (AR group) and non-atopic subjects. Interestingly, we found that the numbers of IgE-binding allergens do not correlate with clinical severity of airway allergy. However, IgE-binding to Per a 2 was more frequent in the AS group, as compared to the AR group (81% vs 45%, *P*<0.05). In contrast, 80% of the AR patients had IgE-binding activity to Per a 9 compared to only 28.5% of the AS patients (*P*<0.01). Our study suggested that IgE-binding allergens are not of equal importance. Sensitization to Per a 2 may be an important indicator for cockroach allergy to develop asthma.

Identification of IgE-binding epitopes of an allergen is helpful in designing sequences for more accurate and safer peptide-based allergen diagnosis and immunotherapeutic agents.14-18 The IgE-binding epitopes of Per a 1 and Per a 3,19,20 as well as Bla g 1, 2, 4, 5, and 6,21-23 have been identified previously. Lee *et al.*,25 reported that the amino acid residues 1-75 and 146-226 to be important for IgE-binding in Bla g 2. However, as there is only 44% of sequence identity between Bla g 2 and Per 2,26-28 studies on Bla g 2 may not be directly applicable to Per a 2.29-30 This study aimed to identify the IgE-binding epitopes and localize the distribution of Per a 2 proteins in the body of American cockroach.

### MATERIALS AND METHODS

#### Subjects and sera
A total of 10 cockroach-allergic subjects (P1-P10) and 5 non-allergic controls (N1-N5) were included in this study. Cockroach allergy was confirmed by a positive ImmunoCAP (Phadia, Uppsala, Sweden) result (>0.35 kU/L) and a positive ELISA result to rPer a 2. Serum samples were collected from all subjects after obtaining written informed consent and stored in aliquots at -70°C until use. A serum pool was made by mixing equal aliquots of serum for immunoblotting experiments. The Institutional Review Board of our hospital approved the study protocol (no. C08017).

#### Deletion clones of Per a 2 generated by polymerase chain reaction (PCR) and constructed in an expression vector
The previously cloned cDNA coding for Per a 2.0101 (GeneBank database, Accession number GU188391) was used as a template for PCR amplification of Per a 2 fragments. Gene-specific primers were designed with restriction sites using the software Oligo.exe structure ver. 3.4 program and listed in Table.

The PCR was performed with a hot start at 94°C for 10 minutes using 1×PCR master mix (Hoffman-La Roche, Basel, Switzerland). The subsequent 35 cycles of amplification were under the following conditions: 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes. The PCR products were purified by a

### Table

Primer sequences used for cloning the Per a 2 fragments in pET30

| Overlapping fragment | Nucleotide sequence | MW*(kDa) |
|----------------------|---------------------|----------|
| N-1 (21-93)          | Sense               | GGATCC**+ GATCCAGTCTGTCGTCCCTCT**+  | 14.9   |
|                     | Anti-sense          | AAGCTT**+ CACTTATTTGATCAGCTT**+  |         |
| N-2 (57-162)         | Sense               | GGATCC**+ GATACGTCTCATACACACAG**+  | 17.7   |
|                     | Anti-sense          | AAGCTT**+ CATAACAGTGCCAGATTAAA**+  |         |
| N-3 (87-162)         | Sense               | GGATCC**+ CCTGTATCAAATAACGGTG**+  | 14.6   |
|                     | Anti-sense          | AAGCTT**+ CTGTCGTCCGGGATACCTTC**+  | 17.4   |
| N-4 (87-186)         | Anti-sense          | AAGCTT**+ AGTTATTGTGCTCCGAG**+  | 18.4   |
| N-5 (87-198)         | Anti-sense          | AAGCTT**+ TACGAGAGTAAAGTGAA**+  | 20.1   |
| N-6 (87-211)         | Anti-sense          | AAGCTT**+ TACGAGAGTAAAGTGAA**+  | 22.3   |
| C-1 (200-351)        | Sense               | GGATCC**+ TATAGGGGCGACTTACCTTA**+  | 14.5   |
|                     | Anti-sense          | AAGCTT**+ CTACAGTTTCCTTACCGGA**+  |         |
| C-2 (213-283)        | Sense               | GGATCC**+ TACGACTGGAACTTCAAGGG**+  | 15.9   |
|                     | Anti-sense          | AAGCTT**+ TGGCCACATTGTGAGTTGA**+  |         |
| C-3 (213-298)        | Anti-sense          | GGATCC**+ TGGCCACATTGTGAGTTGA**+  |         |
| C-4 (213-309)        | Anti-sense          | 17.2   |
| C-5 (281-351)        | Sense               | GGATCC**+ TGGCCACATTGTGAGTTGA**+  | 13.3   |

*Represents nucleotide position of the Per a 2; †Represents molecular weight deduced from amino acid sequences of recombinant fragments expressed in BL21 (DE3).
BandPrep kit (Genepure, Taichung, Taiwan) and ligated into pCR2.1 TA vector (Invitrogen, Carlsbad, CA, USA).

Sequences of recombinant cDNA inserts were determined using an automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). After double digestion with BamHI and HindIII, the recombinant fragments were further subcloned into pET30 (Novagen, Darmstadt, Germany) for expression.

**Purification of recombinant fragments of Per a 2**

Plasmid pET30 containing insert-transformed *E. coli* BL21 (DE3) was grown at a dilution of 1:100 in 200 mL of Luria-Bertani broth containing 25 μg/mL kanamycin. Target proteins were induced with 0.5 mM isopropylthio-β-D-galactoside and harvested after 16 hours of incubation at 37°C. Total crude proteins were recovered under the denaturing condition from inclusion bodies of the host cells. Recombinant proteins were purified using rapid-affinity column chromatography and eluted with 1 M imidazole according to the manufacturer's recommendation (Novagen). The protein concentration was determined using the Bio-Rad Bradford assay (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as the standard.

**Detection of deletion fragments of Per a 2 by SDS–PAGE and immunoblotting**

Protein samples were loaded on a 4% polyacrylamide stacking gel above a 12% separating gel that was run with discontinuous buffer by Laemmli's method. After electrophoresis, the gels were fixed and stained with 0.2% Coomassie brilliant blue R250. For immunoblotting, the gels were transferred electrophoretically by semidyndry (Bio-Rad) for 30 minutes at 0.8 mA/cm² to nitrocellulose membranes (Millipore Bedford, MA, USA). After the transfer, the membranes were blocked in PBST (10 mM sodium phosphate, pH 7.4; 150 mM NaCl; 0.05% Tween 20) containing 5% skim milk for 2 hours at room temperature. The blots were incubated with a 1:10 dilution of patient serum pool or negative control pool overnight at 4°C, and then washed during three 20-minute periods in PBST and incubated with a 1:2,000 dilution of an anti-human IgE alkaline phosphatase conjugate (PharMingen, San Diego, CA, USA) for 2 hours at room temperature. The reaction was developed using a chemiluminescent substrate solution (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as the standard.

**Peptide synthesis**

Three peptides comprising the IgE epitopes of Per a 2 were synthesized on the Applied Biosystems peptide synthesizer (Genemed Synthesis, San Antonio, TX, USA). The amino acid sequences were YYRGDFTYVPLV, TYHIQQNGDLC, and YTTVIPSASCVSGGCNCANV (KLH) for coating on ELISA plates.

**Immunodetection of synthetic peptides by enzyme-linked immunosorbent assay (ELISA)**

The IgE-binding reactivity of synthetic peptides was evaluated by direct binding ELISA. The optimal concentrations of antigen and conjugate were determined by checkerboard titration. KLH-peptides (1.0 μg/well) in coating buffer (0.1 M sodium carbonate, pH 9.6) were used to coat the micro-titer plate (Maxisorp, Nalge Nunc International, Rochester, NY, USA) in duplicate. The plates were then incubated at 37°C for 2 hours. After incubation, the wells were washed 3 times with PBST, and then filled with 1% BSA and incubated for 2 hours at room temperature. The wells were washed and incubated overnight at 4°C with either a 1:10 dilution of patient or non-allergic sera. After washing, antibody binding was detected using alkaline phosphatase-labeled mouse anti-human IgE monoclonal antibody along with para-nitrophenol phosphate (Sigma Biochemical, St. Louis, MO, USA) as a substrate. Absorbance was determined at 405 nm on a Sunrise Absorbance Reader (TECAN, Manne-dorf, Austria).

**Preparation of rabbit polyclonal antibodies to recombinant Per a 2 (rPer a 2) protein**

The animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Taichung Veterans General Hospital (no. La-101948). Young adult New Zealand white rabbits were injected subcutaneously at 10-20 sites on the dorsum with 150 μL aliquots containing a total of 1.0 mg purified rPer a 2 protein with an equal volume of complete Freund's adjuvant (Sigma Biochemical). Two booster injections were given using 0.5 mg antigen mixed with Freund's incomplete adjuvant (Sigma Biochemical) at 3-week intervals. The rabbits were bled before injection, and antibody reactivity was checked triweekly by indirect ELISA.

Three weeks after the last injection, the rabbits were bled by heart puncture. Anti-sera were precipitated with 18% sodium sulfate, followed by a 14% sodium sulfate precipitation. The partially purified IgG was diazylized against PBS at 4°C and further purified by recombinant Protein A agarose (Invitrogen) affinity chromatography. Rabbit anti-rPer a 2 IgG was applied on immunoblotting of cockroach extract and paraffin-embedded cockroach tissue to determine Per a 2 localization. Anti-Per a 2 antibody was used to quantify Per a 2 in the whole body and fecal extracts by inhibition ELISA using rPer a 2 as the standard, as previously described.

**Localization of Per a 2 by immunohistochemistry**

The cockroaches were fixed in 4% para-formaldehyde for 16 hours at room temperature. After dehydration through graded alcohol, the specimens were embedded in paraffin. Serial sections (5 μm) were cut in the cockroach body. All sections were
collected on glass slides coated with poly-L-lysine. For general morphologic study, sections of the cockroach P. americana were first stained with hematoxylin and eosin (H&E). For immunostaining, the serial sections were de-waxed and immersed in 3% H₂O₂ solution for 5 minutes to block endogenous peroxidase. After blocking unspecific binding with 5% sheep serum for 30 minutes at room temperature, the sections were incubated in 1:2,000 rabbit anti-Per a 2 antibodies, followed by STAT-Q immuno-staining (Innovex Biosciences, Richmond, CA, USA). The section was visualized with 3,3’diaminobenzidine (DAB) for 3 minutes and counterstained with 0.1% Evans blue.

**Preparation of American cockroach whole body and fecal extracts**

American cockroaches were trapped by baited jars with a quarter slice of bread soaked in beer. The feces were collected from the jars. Frozen cockroaches were pulverized in liquid nitrogen and then defatted in precooled ether (1:5, w/v) overnight at 4°C. After vacuum suction, the residual ether was washed away twice with acetone, and the defatted cockroach extract was lyophilized and stored at -70°C. Defatted whole-body powder was extracted overnight at 4°C in phosphate buffered saline (PBS, pH 7.4) (1:20, w/v) containing 10 mM PMSF (Sigma Biochemical). The supernatant was collected by centrifugation at 4°C for 30 minutes at 10,000 g and passed through a 0.45-μm filter (Millipore). A 100 mg sample of feces was mixed with 1 mL of PBS and extracted overnight at 4°C. The preparation was then centrifuged at 10,000 g at 4°C for 30 minutes. The protein contents of the American cockroach whole body and fecal extracts were measured by Bradford assay (Bio-Rad). Finally, the extracts were kept at -70°C until used in an inhibition ELISA for determining the level of Per a 2.

**Inhibition ELISA for quantification of Per a 2**

First, inhibition plates were prepared by blocking with 3% non-fat milk/PBS overnight at room temperature. After washing with PBST, three 2-fold dilutions of whole-body and fecal extracts (100 μL/well) and eight 2-fold dilutions of recombinant Per a 2 proteins were made in duplicate wells. Rabbit anti-rPer a 2 antibodies were added to each sample well (100 μL/well). Mature Per a 2 and overlapping peptide fragments N-1 (Per a 2: 21-93), N-2 (Per a 2: 57-162), N-3 (Per a 2: 87-162), N-4 (Per a 2: 87-186), N-5 (Per a 2: 213-283), C-1 (Per a 2: 200-351), C-2 (Per a 2: 213-298), and C-3 (Per a 2: 281-351)−reacted with IgE antibodies. On the other hand, there was no detectable IgE-binding activity for fragments N-3 (Per a 2: 87-162), N-4 (Per a 2: 87-186), N-5 (Per a 2: 87-198), C-2 (Per a 2: 213-283), or C-3 (Per a 2: 213-298). These results indicated that human linear IgE-binding epitopes of Per a 2 were located at the amino acid sequences 57-86, 200-211, and 299-309. The results of immunoblotting were summarized, and the schematic map of the location of recombinant fragments on the Per a 2 molecule is presented in Fig. 1C.

To show detailed information about IgE-binding epitopes of Per a 2, the 3-dimensional (3D) structure of Per a 2 was generated using the structure of the aspartic protease from Blattella germanica Bla g 2 (Protein Data Bank code 1YG9) as a template. The current model (Fig. 1D) confirmed that the 3 identified IgE-binding epitopes of Per a 2 were located on the protein surface.

**Individual IgE-binding of the synthesized peptides by ELISA**

Three KLH-coupled peptides corresponding to the IgE-binding amino acid sequences of Per a 2 were synthesized. Sera from 10 Per a 2-allergic patients (P1-P10) and 5 non-allergic subjects (N1-N5) were used for analyzing IgE-binding activities to synthetic peptides by ELISA (Fig. 2). An OD level of >0.2 was considered positive. The 3 synthetic peptides showed positive IgE binding to the tested Per a 2-allergic sera, but no antibody
binding was detected in the non-allergic controls.

**Tissue location of Per a 2 in sections of the American cockroach nymph**

Polyclonal antibody against rPer a 2 was generated in rabbits, and its specificity for Per a 2 was confirmed by immunoblotting. The SDS-PAGE of cockroach extract and feces showed protein bands ranging from 15 to 100 kDa (Fig. 3A). In immunoblotting analysis, the rabbit anti-Per a 2 polyclonal antibodies bound a specific band, which was similar to purified rPer a 2, in both whole body and fecal extracts (Fig. 3B). The molecular weight of recombinant rPer a 2 is approximately 5 kDa larger than the natural Per a 2 as it is a fusion protein. In the inhibition ELISA experiments, the Per a 2 level was markedly higher in fecal extract than in the whole body extract (12.68 vs 3.24 μg/g of extractable proteins) (Fig. 3C).

Examination of longitudinal sections of an American cockroach nymph using H&E staining revealed general morphologic structures through the body (data not shown). The chitin crust, crop, midgut, and hindgut were clearly demonstrated. The gastrointestinal tract occupied a large part of the abdomen. Under an anatomic microscope, immunoreactive Per a 2 was localized partly in the mouth and midgut. Major staining intensity was observed in the hindgut (Fig. 4). However, there was no Per a 2 immuno-reactivity on the control sections with preimmune rabbit sera.

**DISCUSSION**

In this study, IgE-binding epitope analysis of Per a 2 was performed using overlapping recombinant fragments. The results...
indicated that human IgE-binding epitopes of Per a 2 were located at the amino acid sequences 57-86, 200-211, and 299-309. The findings were further confirmed by IgE-binding profiles using the corresponding synthetic peptides of Per a 2. All the 10 sera from Per a 2-allergic patients were bound to the corresponding synthetic peptides, suggesting that they were linear IgE-binding epitopes of Per a 2. From the results of this study, we found that epitopes 61-80 and 200-211 of Per a 2 seem to correspond to epitopes 1-75 and 146-225 from German cockroach reported by Lee et al.; however, epitope 299-309 seems to be a novel epitope that was not described in Bla g 2.

Our previous study showed that sensitization to Per a 2 correlates with more severe airway disease in patients with allergy to American cockroach. The presence of IgE against Per a 2 may be a clinical indicator for receiving aggressive treatment in the early stage. However, whether the diversity of IgE-binding profiles of Per a 2 has any significance in predicting symptomatic asthma vis-à-vis asymptomatic sensitization like what has been reported in peanut allergy and cow’s milk allergy, requires further studies. The identification of IgE-binding epitopes of Per a 2 in this study opens more knowledge for designing specific and accurate diagnostic methods for American cockroach-induced asthma.

This study also shows that Per a 2 protein is predominantly located in the hindgut of the cockroach body and the level of Per a 2 protein is markedly higher in fecal extract than in the whole body extract (12.68 vs 3.24 μg/g of extractable proteins). This is the first study to investigate the distribution of Per a 2 allergen in the whole body of a cockroach. Per a 2 is predominantly localized in the hindgut. Thus, Per a 2 protein may be excreted into the feces like what has been reported in Per a 1 and Per a 3. Since fecal extract contains almost 4 times the amount of Per a 2 compared to the whole body extract, it is possible that, through fecal passage, the chances of Per a 2 existing in the environment is much higher than those of other allergens that can only exist in body components, as reported in German cockroach. Interestingly, Per a 9, another major American cockroach allergen, which has been correlated with disease exacerbation in Thailand, is not detected in fecal extract but predominantly in the whole body extract (data not shown). Per a 2, by abundantly existing in feces, may be a more important source of allergic sensitization.

It has been reported that Bla g 2, the major Group 2 German cockroach allergen, can exist in an apartment with a half-life of 406 days at proper temperature and humidity. Whether Per a 2 is as robust in the environment as Bla g 2 requires more studies.

Since complete avoidance of cockroach allergen in the environment is extremely difficult, allergen-specific immunotherapy remains the only way to cure cockroach allergy. An asthma murine model for cockroach allergy has been developed in our laboratory, and hopefully we will be able to use Per a 2-derived peptides to treat cockroach allergy using this murine model.

In conclusion, information on the IgE-binding epitope of Per a 2 can be used to develop more specific diagnostic and therapeutic approaches to cockroach allergy in the future.
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REFERENCES

1. Holt PG, Thomas WR. Sensitization to airborne environmental allergens: unresolved issues. Nat Immunol 2005;6:957-60.
2. Bernton HS, Brown H. Insect allergy--preliminary studies of the cockroach. J Allergy 1964;35:506-13.
3. Lan JL, Lee DT, Yeh CL. Cockroach hypersensitivity: preliminary study of allergic cockroach asthma in Taiwan. J Allergy Clin Immunol 1988;82:736-40.
4. Kang BC. Cockroach allergy. Clin Rev Allergy 1990;8:87-98.
5. Rosenreith DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gersten P, et al. 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.
6. Dubus JC, Guerra MT, Bodiou AC. Cockroach allergy and asthma. Allergy 2001;56:351-2.
7. Katial RK. Cockroach allergy. Immunol Allergy Clin North Am 2003;23:483-99.
8. Wilson NW, Robinson NP, Hogan MB. Cockroach and other inhalant allergens in infantile asthma. Ann Allergy Asthma Immunol 1999;83:27-30.
9. Sookrung N, Chaicumpa W. A revisit to cockroach allergens. Asian Pac J Allergy Clin Immunol 2010;28:95-106.
10. Arruda LK, Vailes LD, Mann BJ, Shannon J, Fox JW, Vedvick TS, et al. Molecular cloning of a major cockroach (Blattella germanica) allergen, Bla g 2. Sequence homology to the aspartic proteases. J Biol Chem 1995;270:19563-8.
11. Chapman MD, Pomés A. Cockroach allergens, environmental exposure, and asthma. In: Kay AB, Kaplan AP, Bousquet J, Holt PG, editors. Allergy and allergic diseases. 2nd ed. Hoboken (NJ): Wiley-Blackwell; 2008. 1311-45.
12. 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.
13. Lee MF, Song PP, Hwang GY, Lin SJ, Chen YH. Sensitization to Per a 2 of the American cockroach correlates with more clinical severity among airway allergic patients in Taiwan. Ann Allergy Asthma Immunol 2012;108:243-8.
14. Pomés A, Chapman MD. Can knowledge of the molecular structure of allergens improve immunotherapy? Curr Opin Allergy Clin Immunol 2001;1:549-54.
15. Mackenzie KJ, Fitch PM, Leech MD, Ichmann A, Wilson C, McFarlane AJ, et al. Combination peptide immunotherapy based on T-cell epitope mapping reduces allergen-specific IgE and eosinophilia in allergic airway inflammation. Immunology 2013;138:258-68.
16. Worm M, Lee HH, Kleine-Tebbe J, Hafner RP, Laidler P, Healey D, et al. Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy. J Allergy Clin Immunol 2011;127:89-97, 97.e1-14.
17. Mackenzie KJ, Anderton SM, Schwarze I. Peptide immunotherapy for childhood allergy - addressing translational challenges. Clin Transl Allergy 2011;1:13.
18. Matsumoto N, Okochi M, Matsushima M, Kato R, Takase T, Yoshiida Y, et al. Peptide array-based analysis of the specific IgE and IgG4 in cow’s milk allergens and its use in allergy evaluation. Peptides 2009;30:1840-7.
19. Wu CH, Lee MF, Tseng CY. IgE-binding epitopes of the American cockroach Per a 3 allergen. Allergy 2003;58:986-92.
20. Wu CH, Lee MF, Yang JS, Tseng CY. IgE-binding epitopes of the American cockroach Per a 1 allergen. Mol Immunol 2002;39:459-64.
21. Un S, Jeong KY, Yi MH, Kim CR, Yong TS. IgE binding epitopes of Bla g 6 from German cockroach. Protein Pept Lett 2010;17:1170-6.
22. Jeong KY, Joong KY, Kim CR, Yong TS. IgE-binding epitope analysis of Bla g 5, the German cockroach allergen. Protein Pept Lett 2010;17:573-7.
23. Yi MH, Jeong KY, Kim CR, Yong TS. IgE-binding reactivity of peptide fragments of Bla g 1,02, a major German cockroach allergen. Asian Pac J Allergy Immunol 2009;27:121-9.
24. Shin KH, Jeong KY, Hong CS, Yong TS. IgE binding reactivity of peptide fragments of Bla g 4, a major German cockroach allergen. Korean J Parasitol 2009;47:31-6.
25. Lee H, Jeong KY, Shin KY, Yi MH, Cantula G, Hong CS, et al. Reactivity of German cockroach allergen, Bla g 2, peptide fragments to IgE antibodies in patients’ sera. Korean J Parasitol 2008;46:243-6.
26. Pan QR, Wang SM, Shang HS, Chew FT. Identification and characterization of Per a 2, the Bla g 2 allergen homologue from American cockroach (Periplaneta americana). J Allergy Clin Immunol 2006;117:S115.
27. Gustchina A, Li M, Wünschsmann S, Chapman MD, Pomés A, Wlodawer A. Crystal structure of cockroach allergen Bla g 2, an unusual zinc binding aspartic protease with a novel mode of self-inhibition. J Mol Biol 2005;348:433-44.
28. Giesner J, Wünschsmann S, Li M, Gustchina A, Wlodawer A, Himly M, et al. Mechanisms of allergen-antibody interaction of cockroach allergen Bla g 2 with monoclonal antibodies that inhibit IgE antibody binding. PLoS One 2011;6:e22223.
29. Li M, Gustchina A, Giesner J, Wünschsmann S, Vailes LD, Chapman MD, et al. Carbohydrates contribute to the interactions between cockroach allergen Bla g 2 and a monoclonal antibody. J Immunol 2011;186:333-40.
30. Li M, Gustchina A, Alexandratos J, Wlodawer A, Wünschsmann S, Kepley CL, et al. Crystal structure of a dimerized cockroach allergen Bla g 2 complexed with a monoclonal antibody. J Biol Chem 2008;283:22806-14.
31. Lee MF, Wang NM, Han JL, Lin SJ, Tsai JJ, Chen YH. Estimating allergenicity of latex gloves using Hev b 1 and hevamine. J Investig Allergol Clin Immunol 2010;20:499-505.
32. Lin J, Bruni FM, Fu Z, Maloney J, Bardina L, Boner AL, et al. A bioinformatics approach to identify patients with symptomatic peanut allergy using peptide microarray immunoassay. J Allergy Clin Immunol 2012;129:1321-1328.e5.
33. Sookrung N, Khetsuphan T, Chaisri U, Indrawattana N, Remattong O, Chaicumpa W, et al. Specific B-cell epitope of per a 1: a major allergen of American cockroach (Periplaneta americana) and anatomical localization. Allergy Asthma Immunol Res 2014;6:325-32.
34. Wu HQ, Liu ZG, Gao B, Li M, Ran PX, Xing M. Localization of novel IgE-binding epitopes of the American cockroach Per a 1 allergen. Mol Immunol 2002;39:459-64.
35. Yun YY, Ko SH, Park JW, Lee IY, Ree HI, Hong CS. Comparison of...
allergenic components between German cockroach whole body and fecal extracts. Ann Allergy Asthma Immunol 2001;86:551-6.
36. Tungtrongchitr A, Sookrung N, Indrawattana N, Sae-Lim J, Puduang S, Phonrat B, et al. Seasonal levels of the major American cockroach allergen per a 9 (arginine kinase) in Bangkok and their relevance for disease severity. Asian Pac J Allergy Immunol 2009; 27:1-7.
37. Pomés A, Chapman MD, Vailes LD, Blundell TL, Dhanaraj V. Cockroach allergen Bla g 2: structure, function, and implications for allergic sensitization. Am J Respir Crit Care Med 2002;165:391-7.
38. Erban T, Stejskal V, Aulicky R, Krizkova-Kudlikova I, Nesvorna M, Hubert J. The influence of environmental temperature and humidity on temporal decomposition of cockroach allergens Bla g 1 and Bla g 2 in feces. J Med Entomol 2010;47:1062-70.
39. Zhou B, Yuan J, Zhou Y, Yang J, James AW, Nair U, et al. The attenuation of cockroach allergy by DNA vaccine encoding cockroach allergen Bla g 2. Cell Immunol 2012;278:120-4.