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IgE-Binding Epitope Mapping and Tissue Localization of the Major American Cockroach Allergen Per a 2

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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

INTRODUCTION

Allergic disorders are one of the most common diseases, affecting approximately 20%-40% of the population in developed countries.[1] 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.[2-7]

The most common domestic cockroaches associated with allergy are the German cockroach *Blattella geranica* in the US and Europe, and the American cockroach *Periplaneta americana* in South America and Asian countries.[8,9] Since the cloning of Bla g 2,[10] 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.[11,12] 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-

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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, [21-25] 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] studies on Bla g 2 may not be directly applicable to Per a 2.[27-30]

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 Anti-sense	GGATCC^{61*} GATCCAGTCGTCGTCGTTCCT^{78*} AAGCTT^{279*} CACGTTATTTGATACAGGCT^{260*}	14.9
N-2 (57-162)	Sense Anti-sense	GGATCC^{169*} GATACGTCATCATACACAACG^{189*} AAGCTT^{486*} CATAACAGTCGGCAGATTAAA^{466*}	17.7
N-3 (87-162)	Sense	GGATCC^{262*} CCTGTATCAAATAACGTGGC^{281*}	14.6
N-4 (87-186)	Anti-sense	AAGCTT^{558*} CTGTCCGTCGGGATATCTTC^{539*}	17.4
N-5 (87-198)	Anti-sense	AAGCTT^{594*} AGGTATTGTGCCTCCGAG^{577*}	18.4
N-6 (87-211)	Anti-sense	AAGCTT^{633*} TACGAGAGGTACGTAAGTGA^{614*}	20.1
C-1 (200-351)	Sense Anti-sense	GGATCC^{601*} TATAGGGGCGACTTCACTTA^{620*} AAGCTT$^{10.56*}$ CTACAGTTCTTCTACGGA^{1039*}	22.3
C-2 (213-283)	Sense Anti-sense	GGATCC^{640*}GATACCTGGAACTTCAAGGT^{659*} AAGCTT^{849*} TGGCACATTGTCGAGTTTA^{831*}	14.5
C-3 (213-298)	Anti-sense	AAGCTT^{894*} TGAGCTGATGTTAAAGGCAA^{875*}	15.9
C-4 (213-309)	Anti-sense	AAGCTT^{927*} GCACAAATCTCCGTTTTGCT^{908*}	17.2
C-5 (281-351)	Sense	GGATCC^{844*} GTGCCATCAGTCACATT^{860*}	13.3

The position of the amino acid sequences of cDNA of Per a 2 (NCBI accession numer GU188391) were placed in parenthesis. Restrictiction sites for BamHI and HindIII were underlined.

*Represents nucleotides 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 *Bam*HI and *Hin*dIII, 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 semidry (Bio-Rad) for 30 minutes at 0.8 mA/cm^2 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 (Applied Biosystem, Bedford, MA, San Antonio, TX, USA). The signals were recorded by exposure to ECL Hyperfilm (Amersham Biosciences, Buckinghamshire, UK).

Peptide synthesis

Three peptides comprising the IgE epitopes of Per a 2 were synthesized on the Applied Biosystems peptide synthesizer model 433A (Genemed Synthesis, San Antonio, TX, USA). The amino acid sequences were ^{61}YTTVIPSASCVSGGCNCANV^{80}, ^{200}YYRGDFTYVPLV^{211}, and ^{299}TYHIQQNGDLC^{309}. The HPLC-purified peptides were coupled to keyhole limpet hemocyanin (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, Maanedorf, 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 dialyzed 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.[31]

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% H_2O_2 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). The plates were incubated for 2 hours at 37°C.

On the other hand, 96-well assay plates were prepared by coating with 0.1 μg/well of rPer a 2 in a carbonate buffer for 2 hours at 37°C. Then, the non-reacted sites were blocked for 1 hour with 3% non-fat milk/PBS. After 2-hour incubation of the inhibition plates to allow antibody reaction with the tested extracts, the preabsorbed Per a 2 antibodies were transferred to 96-well assay plates and incubated for 2 hours at room temperature. After washing, a 1:5,000 dilution of peroxidase-labeled goat anti-rabbit IgG was added and incubated for 1 hour at room temperature, and a colored reaction was developed by the addition of ABTS (55 μg/mL in 0.1 M sodium citrate buffer, pH 4.2, containing 0.03% H_2O_2). The results were read at 415 nm on a Sunrise Reader (TECAN). The concentration of Per a 2 in the tested extracts were determined by comparing the reference standards and expressed in mg/g of extractable protein.

RESULTS

Identification of IgE-binding epitopes of Per a 2 using PCR-derived fragments

First, 3 gene fragments of Per a 2 located at the amino-acid residues 21-93, 87-211, and 200-351 were generated by PCR according to the prediction of domain linkers by amino acid composition (http://armadillo.blueprint.org). The preliminary results showed 3 potential IgE-binding regions within them. Then, we began the process of systematically narrowing down the epitope sites. Finally, 11 overlapping fragments of Per a 2 covering the whole allergen molecule, except 20 residues of signal peptide, were constructed. The oligonucleotide primers and predicted molecular weight of recombinant fragments are listed in Table. Mature Per a 2 and overlapping peptide fragments were expressed as fusion proteins and assayed for IgE reactivity. Affinity-purified recombinant proteins were subjected to SDS-PAGE (Fig. 1A), and the results of immunoblotting using Per a 2-sensitive sera are shown in Fig. 1B. Mature Per a 2 (Per a 2: 21-351), the N-terminus fragments–N-1(Per a 2: 21-93) N-2 (Per a 2: 57-162), and N-6 (Per a 2: 87-211)–and the C-terminus fragments–C-1(Per a 2: 200-351), C-4 (Per a 2: 213-308), and C-5 (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 *Blatella 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

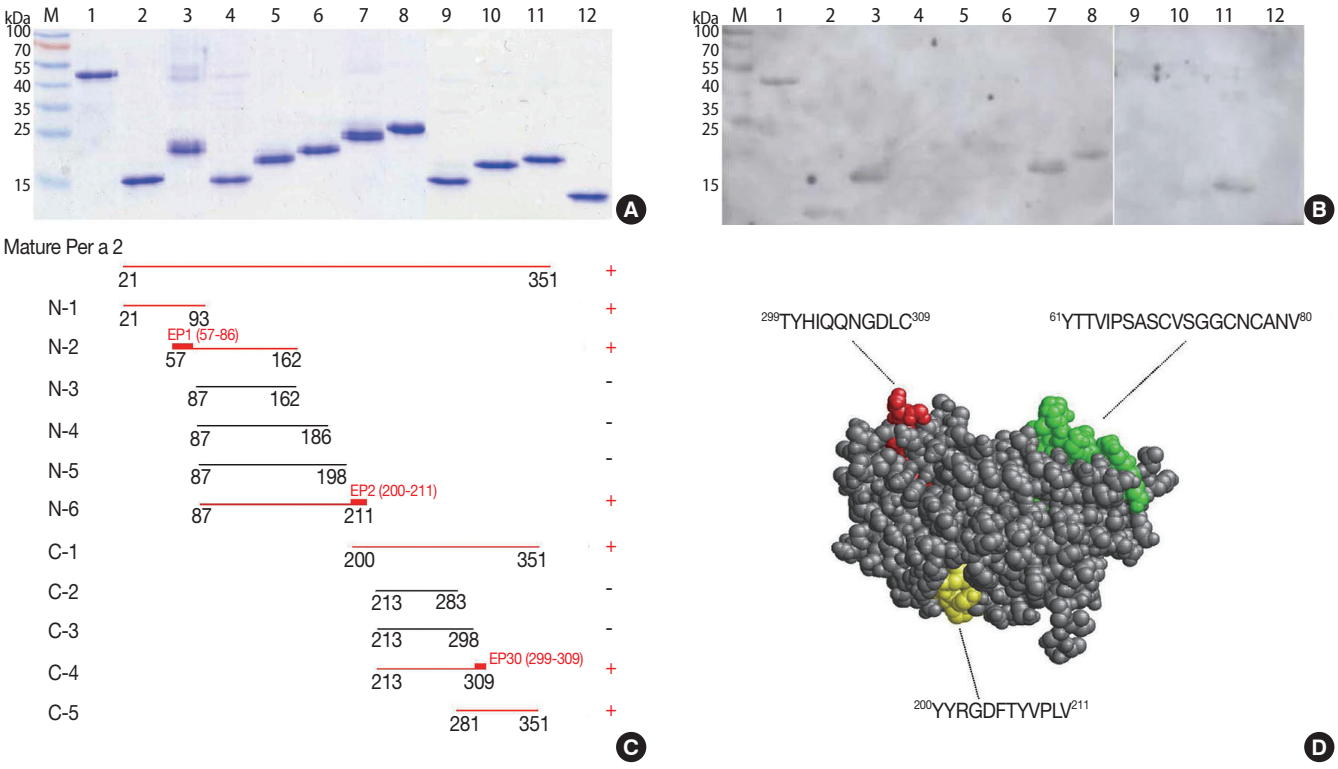

Fig. 1. (A) Coomassie blue-stained SDS-PAGE and (B) immunoblotting of purified proteins expressed from deleted Per a 2 cDNA. Lane 1, Mature Per a 2 (21-351); lane 2, N-1 (21-93); lane 3, N-2 (57-162); lane 4, N-3 (87-162); lane 5, N-4 (87-186); lane 6, N-5 (87-198); lane 7, N-6 (87-211); lane 8, C-1 (200-351); lane 9, C-2 (213-283); lane 10, C-3 (213-298); lane 11, C-4 (213-309); and lane 12, C-5 (281-351). The numbers on the left indicate the sizes of protein markers (kDa, lane M). (C) Schematic map location of deletion mutants on mature Per a 2. The symbol (■) represents the regions recognized by human IgE. (D) Surface diagrams of the Per a 2 molecular model. The IgE-binding epitopes of Per a 2 are colored green (EP1), yellow (EP2), and red (EP3).

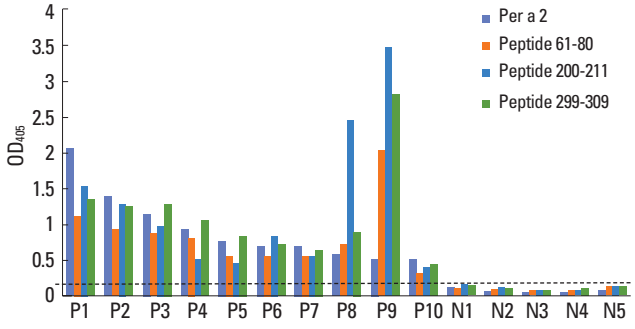

Fig. 2. Binding profiles of IgE antibodies to Per a 2 and synthetic peptides by ELISA. Ten Per a 2-allergic sera (P1-P10) and 5 non-allergic subjects (N1-N5) were analyzed. The cutoff value was 0.2 (dotted line).

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

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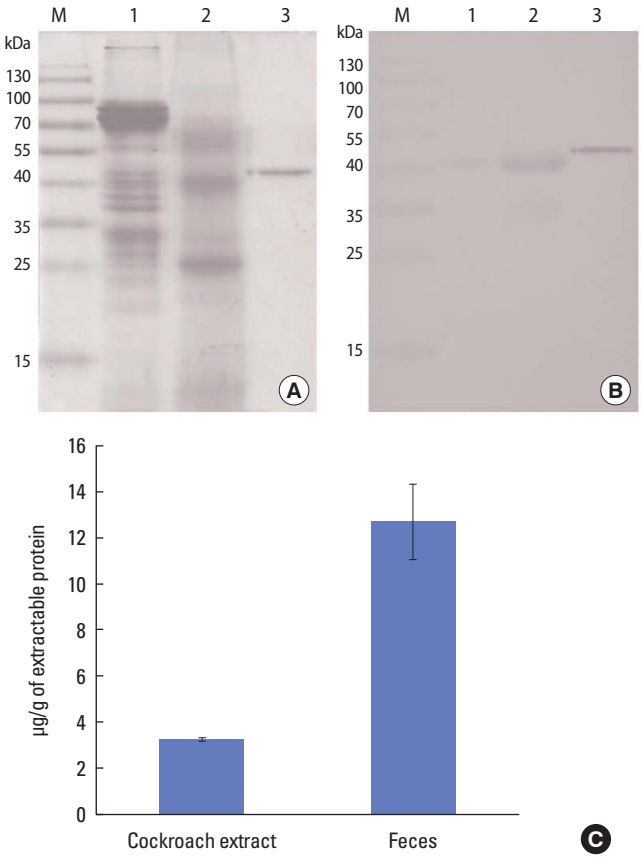

Fig. 3. (A) Coomassie blue-stained SDS-PAGE and (B) immunoblotting with rabbit anti-Per a 2 antibodies. Lane1, cockroach extract; lane 2, fecal extract; lane 3, purified recombinant Per a 2; and lane M, protein marker. The numbers on the left indicate the sizes of protein markers (kDa). (C) Per a 2 levels in cockroach extract and feces by competition ELISA.

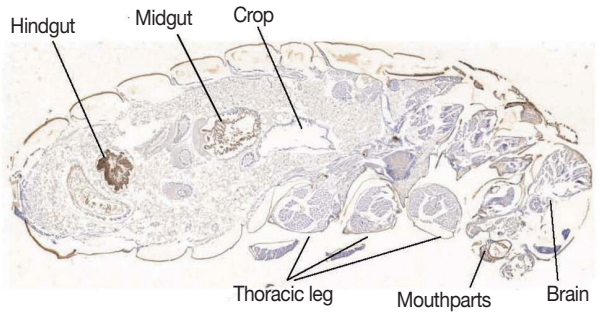

Fig. 4. Immunohistochemical staining of the longitudinal section from the American cockroach nymph with rabbit anti-Per a 2 antibodies.

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 ^{61}YTTVIPSASCVSGGCNCANV^{80}, ^{200}YYRGDFTYVPLV^{211}, and ^{299}TYHIQQNGDLC^{309}, 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.*[25]; 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.[13] 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.[18,32] 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 predominately localized in the hindgut. Thus, Per a 2 protein may be excreted into the feces like what has been reported in Per a 1[33] and Per a 3.[34] 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.[35] Interestingly, Per a 9, another major American cockroach allergen, which has been correlated with disease exacerbation in Thailand,[36] 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,[37] can exist in an apartment with a half-life of 406 days at proper temperature and humidity.[38] 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.[39] 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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