

Sensitization to per a 2 of the American cockroach correlates with more clinical severity among airway allergic patients in Taiwan

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ABSTRACT

Background: In Taiwan, 57.5% of asthmatic patients are allergic to cockroaches, which are a major indoor allergen for immunoglobulin E (IgE)-mediated respiratory diseases.

Objective: To determine whether sensitization to different cockroach allergenic components correlates with different clinical manifestations and severities.

Methods: The complementary DNAs (cDNAs) encoding for Per a 1 through 7 and Per a 9 were generated by reverse transcription polymerase chain reaction and cloned into the *Escherichia coli* expression system. Sixty-four subjects were divided into 3 groups based on the clinical severity of their allergic reaction: those with persistent asthma and rhinitis (AS), those with allergic rhinitis only (AR), and the nonallergic controls (NA). Serum levels of interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), chemokine (C-C motif) ligand 20 (CCL-20), and granulocyte macrophage colony-stimulating factor (GM-CSF) were measured, and the binding frequencies to each recombinant allergen were examined.

Results: Serum levels of IL-8, MCP-1, and CCL-20 were significantly higher in the AS group than in the AR and NA groups. The numbers of IgE-binding allergens did not correlate with the clinical severity of airway allergy to cockroaches. However, 81% in the AS group had IgE-binding activity to Per a 2, which was significantly higher than that of the AR group (45%, $P < .05$). In contrast, 80% of AR patients had IgE-binding activity to Per a 9 compared with only 28.5% of AS patients ($P < .01$).

Conclusions: Allergens from American cockroaches do not have equal importance in terms of pathogenicity. Sensitization to Per a 2 correlates with more severe airway allergy and elevated proinflammatory chemokines. This may help in selecting target allergens for component resolved diagnosis and immunotherapeutic agents.

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Introduction

Cockroach proteins are potent environmental allergens worldwide, especially in America, Europe, Taiwan, Thailand, and South Africa.¹ In Taiwan, 54.9% of patients with airway allergy and 57.5% of the patients with asthma are sensitive to American cockroaches,² and exposure to their allergen in early life is associated not only with asthma development^{3,4} but also with increased morbidity, severity, and hospitalization in children with asthma.^{5–7}

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Cockroach allergy has been recognized as an important immunoglobulin E (IgE)-mediated type I hypersensitivity since 1964.⁸ Except for the traditional IgE-mediated allergic inflammation, increasing evidence shows that cockroaches also induce protease-activated receptors-dependent allergic airway inflammation independent of its IgE binding capacity.^{9–11} However, detecting allergen-specific IgE to cockroach or skin-prick test with crude cockroach extract remains the mainstay of diagnosing cockroach allergy in clinical practice, which leaves room for improving diagnostic sensitivity and specificity. In 2002, Patterson and Slater¹² tested 24 cockroach allergen extracts from 9 manufacturers by competition enzyme-linked immunosorbent assay (ELISA) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).¹² They indicated that commercially available cockroach allergen extracts vary in protein content, electrophoretic banding patterns, and relative potency. Many problems associated with the

Table 1
Sequences of primers used for the PCR-based cloning of *Periplaneta americana* allergens

Allergen/Acc. no.		Nucleotide sequence	Expression sector	Recombinant protein size (kDa)
Per a 1.02/U69957	F	¹ GTGACATGCAGAGATCAGAACAT	pET30b	53.8
	R	² GCGGCCGCTTATGCTCTGGAACCTCCG		
Per a 2/AY792947	F	³ GGATCCGATCCAGTCGTCGTTCTT	pET30a	48.6
	R	⁴ AAGCTTCTACAGTTCTTCTACGGA		
Per a 3.0203/L40821	F	⁵ GAATTCATGAAGACTGCTTTGGTATTC	pET30a	51.7
	R	² GCGGCCGCTTAATGGCTTGGACTCC		
Per a 4/AY792948	F	³ GGATCCGGAGATGATTCA	pET30a	30.6
	R	⁴ AAGCTTTCAAGACTTGTACT		
Per a 5/AY92949	F	³ GGATCCATGACCATCGACTTCTAC	pET30a	28.9
	R	⁴ AAGCTTTCACCTTCTGGCGAGGTT		
Per a 6/AY792950	F	³ GGATCCATGGATGAACTGCCAGAT	pQE30	17.1
	R	⁴ AAGCTTTTATTCTCCGGTCATGAC		
Per a 7/Y14854	F	⁵ GAATTCATGGATGCTATCAAGAAGAAG	pET30a	42.8
	R	² GCGGCCGCTTAGTAACCAAGTTCGGT		
Per a 9/AY563004	F	³ GGATCCATGGTGGACGCCGAGTT	pQE30	41.4
	R	⁴ AAGCTTAGAGCGAGCTCTCCAGCTT		

Restriction sites are in italics, and priming regions are underlined. ¹Sall, ²NotI, ³BamHI, ⁴HindIII, ⁵EcoRI.

use of natural allergenic products can be overcome by using genetically engineered recombinant allergens.

Of 4,000 species of cockroaches, the German cockroach (*Blattella germanica*) and American cockroach (*Periplaneta americana*) are the most common household pests and well-documented allergens. Ten allergens have already identified in the American cockroach, namely, Per a 1 to Per a 10, and the sequences of Per a 1 through 7 and Per a 9 have been reported.^{1,13} With improvements in recombinant DNA technology, many studies have shown that recombinant allergens may be used to replace traditional natural allergen extracts for diagnosis and immunotherapy of allergic diseases.^{14,15} The idea of component resolved diagnosis, by testing patient reactivity to individual recombinant allergens, has shown improved diagnostic reliability and differential ability in house dust mite, pollen, carrot, cherry, and some food allergies.^{16–21}

The aim of this study is to determine whether sensitization to different cockroach allergenic components correlates with different clinical manifestations and severities, using 8 cloned American cockroach allergens with known sequence and the *Escherichia coli* expression system to analyze the IgE-binding map and cytokine profiles.

Methods

Subjects and Sera

Subjects were recruited from patients attending the Allergy Clinic of Taichung Veterans General Hospital or those who responded to an advertisement. All subjects were interviewed and examined by an allergist. Patients included as persistent asthma must have symptoms of recurrent episodes of wheezing, shortness of breath, activity-induced cough or wheeze, or nocturnal cough in periods without viral infections more than once per week plus evidences of bronchial hyperresponsiveness by pulmonary function test.²² As for the inclusion criteria of allergic rhinitis, patients must have at least 2 of the typical symptoms of chronic rhinitis, including watery rhinorrhea, itchy nose, stuffy nose, and sneezing

for at least 2 months plus a positive allergen skin prick test or presence of allergen-specific IgE by blood test.²³ Cockroach allergy was confirmed by a positive (>0.35 kU/L) specific IgE to cockroach by ImmunoCAP (Pharmacia, Uppsala, Sweden). The subjects were divided into 3 groups based on their clinical severity of their airway allergy. Group AS was subjects with persistent allergic asthma and rhinitis. Group AR was those with allergic rhinitis only, and group NA was the non-allergic controls.

The hospital's institutional review board approved the study, and all of the subjects were recruited after signing written informed consent.

Serum Chemokine Levels

Quantitative sandwich ELISA kits were used to measure serum levels of interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), chemokine (C-C motif) ligand 20 (CCL-20), and granulocyte macrophage colony-stimulating factor (GM-CSF) in duplicate according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota). The lower limits of detection were 16 pg/mL IL-8; 16 pg/mL MCP-1; 8 pg/mL CCL-20; and 8 pg/mL GM-CSF.

PCR-Based Cloning of *Periplaneta americana* Allergens

The complementary DNA (cDNA) library for *P. americana* was used as a template for polymerase chain reaction (PCR) amplification of the American cockroach allergens.²⁴ For PCR-based cloning, gene-specific primers were designed with restriction sites for cloning into the pET30 (Novagen, Madison, Wisconsin) or pQE30 (Qiagen, Hilden, Germany) expression vectors. Oligonucleotide primers and molecular weight of recombinant allergens are listed in Table 1. After PCR and cloning, DNA sequencing was performed using the ABI PRISM Dye Terminator Kit and ABI 377 sequencer. *E. coli* expression was done as described previously.²⁵

Purification of Recombinant Proteins

Plasmid pET30 and pQE30, both containing the His-tag sequence, expressed a stretch of 6 histidine residues at the N-termi-

Table 2
Subject demographics

Group	Subject number	Age (years) Median (range)	Sex F/M	Symptoms	Specific IgE to Cockroach Median (range) kU/L
AS	21	31 (8–62)	8/13	Persisted asthma and rhinitis	2.37 (0.38–15.9)
AR	20	28 (11–86)	9/11	Allergic rhinitis only	1.80 (0.38–5.9)
NA	23	37 (21–75)	18/5	No allergy	<0.35 kU/L

Abbreviations: AS, asthma with allergic rhinitis; AR, allergic rhinitis only; NA, nonallergic control.

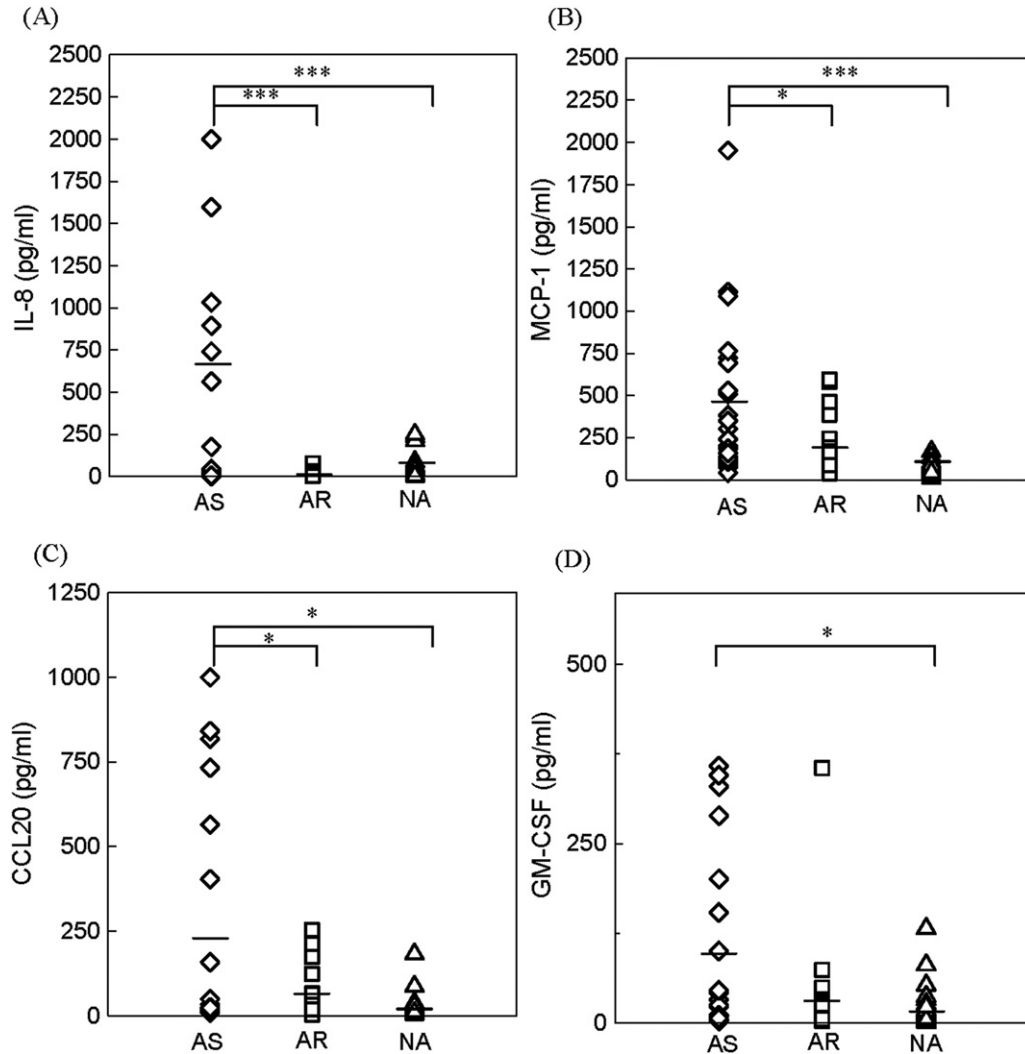


Fig. 1. Serum levels of (A) IL-8; (B) MCP-1; (C) CCL20; and (D) GM-CSF in patients with moderate to severe asthma (AS), allergic rhinitis only (AR), and non-allergic controls (NA) determined by ELISA. * $P < .05$; ** $P < .01$; *** $P < .001$, by one-way analysis of variance with Bonferroni's multiple range test. Lines denote median level of cytokines.

nal ends of the target protein. The sequence of His-tag binding to divalent cations (Ni^{2+}) immobilizing the histidine-binding metal chelation resin (Novagen) and the resulting fusion protein were recovered by elution with 1 M imidazole. Briefly, an overnight culture was diluted 1:100 into 200 mL Luria-Bertani broth containing 25 $\mu\text{g}/\text{mL}$ kanamycin for pET30-BL21 (DE3) or 100 $\mu\text{g}/\text{mL}$ ampicillin for pQE30-M15. Protein expression was induced by isopropyl thio- β -D-galactoside at concentrations of 0.1 to 1 mM. The cells were harvested after 16 h incubation at 37°C. Recombinant proteins were purified using the rapid affinity column chromatography with the pET-His-Tag system as described by the manufacturers (Novagen). Protein concentration was determined using the Bio-Rad Bradford assay (Bio-Rad, Hercules, California). Bovine serum albumin was used as protein standard.

Recombinant Allergen-Based IgE ELISA

Sera from 64 subjects were used to determine specific IgE antibodies to rPer a 1, rPer a 2, rPer a 3, rPer a 4, rPer a 5, rPer a 6, rPer a 7, and rPer a 9. Optimal concentrations of antigen and conjugate were determined by checkerboard titration. Purified recombinant proteins (0.5 $\mu\text{g}/\text{well}$) in coating buffer (0.1 M sodium carbonate, pH 9.6) were used to coat the 96-well plate (Nunc, Denmark) in duplicate and incubated at 37°C for 2 hours. The plate was blocked with 2% bovine serum albumin and incu-

bated overnight at 4°C with a 1:10 dilution of patients' or nonallergic sera.

After washing, antibody binding was detected using alkaline phosphatase-labeled mouse anti-human IgE monoclonal antibody, with p-nitrophenyl phosphate (Sigma, St. Louis, Minnesota) as a substrate. The optic density (OD) was determined at 405 nm on a Sunrise Absorbance Reader (TECAN, Grödig, Austria). The cutoff value was defined as [mean OD values of 23 nonallergic samples + 2 standard deviations].

Statistical Analysis

Comparison of chemokine levels within groups was performed using one-way analysis of variance. Chi-squared analysis was used for comparison of IgE-binding frequencies of the 8 recombinant allergens between the study groups.

Results

Demographic Data

Table 2 summarizes the demographic data of 64 subjects. The AS group had 21 subjects with moderate to severe asthma who required at least step 3 therapy from the 2006 Global Initiative for Asthma guidelines. The AR group had 20 subjects with allergic

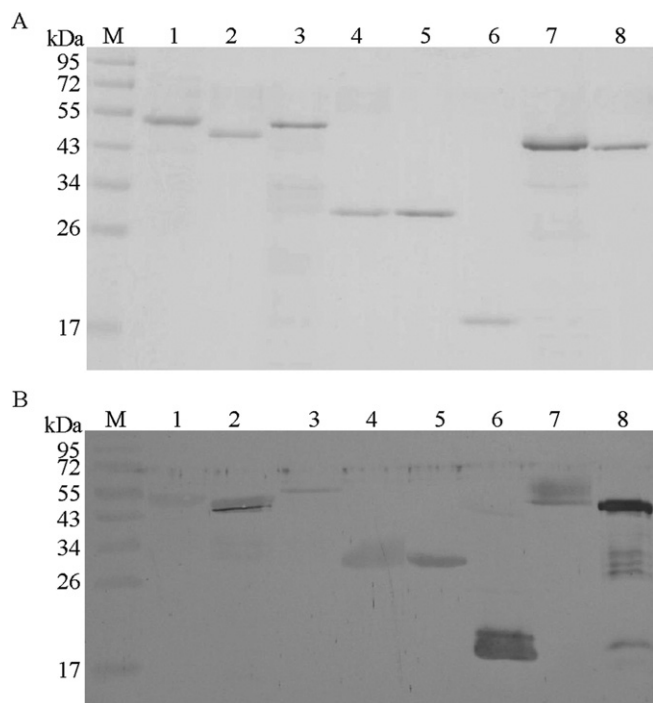


Fig. 2. (A) Coomassie blue-stained SDS-PAGE and (B) immune-blotting of purified recombinant proteins expressed from *E. coli*. Lanes 1–7, Per a 1–7; lane 8, Per a 9; Lane M, protein marker.

rhinitis but without asthma clinically. Twenty-three subjects composed the NA groups.

Serum Levels of IL-8, MCP-1, CCL-20, and GM-CSF

Serum levels of IL-8, MCP-1, CCL-20, and GM-CSF in sera from the 3 groups are shown in Figure 1. Serum levels of IL-8, MCP-1, and CCL-20 in patients of the AS group were significantly higher than those of the AR and NA groups. GM-CSF was significantly elevated in the AS group compared with the NA group. The data suggested that these 3 subject groups not only differed in clinical severity of airway allergy but also in baseline inflammatory chemokines.

SDS-PAGE and Immunoblotting with Pooled Patient Sera

The cDNAs coding for Per a 1, 2, 3, 4, 5, and 7 were inserted into the plasmid pET30 and expressed in *E. coli* BL21(DE3) as recombinant fusion proteins. The cDNAs coding for Per a 6 and 9 were cloned into pQE30 and expressed at high levels in *E. coli* M15 as non-fusion proteins. Both fusion and non-fusion proteins were purified using affinity chromatography, and purified recombinant proteins were subjected to SDS-PAGE and immunoblotted with pooled patient sera to confirm their IgE reactivity. After electrophoresis, all 8 recombinant proteins migrated as a single band and displayed more than 95% purity by Coomassie blue staining (Fig 2A). The molecular weights of recombinant proteins found on SDS-PAGE gels were identical to that calculated from the cDNA. For immunodetection, IgE antibodies from cockroach-allergic patients specifically recognized and bound all 8 recombinant proteins (Fig 2B).

IgE-Binding Profile to Recombinant Allergens by ELISA

Sera from 64 subjects were tested for IgE profiles to 8 purified recombinant cockroach allergens by ELISA. All patients with cockroach allergy reacted to at least 1 recombinant allergen, but no specific patterns were observed between the 2 groups (Table 3). No differences were seen between the AS and AR groups regarding the numbers of IgE-binding allergenic components (mean 4.2 vs 4.3, $P = .979$). A highly variable individual sensitization pattern was

seen among patients. None of the subjects in the NA group reacted to any recombinant allergen tested (data not shown). However, when looking at the IgE-binding frequencies to individual recombinant cockroach allergens, as high as 81% (17 of 21 subjects) of AS patients had IgE-binding activity to Per a 2, which was significantly higher than that in the AR group (45%, $P < .05$). In contrast, 16 of 20 AR patients had IgE-binding activity to Per a 9 (80%) compared with only 6 of 21 AS patients (28.5%, $P < .01$) (Fig 3).

Discussion

Chemokines have been related to the severity of asthmatic inflammation. Interleukin-8 is a potent chemo-attractant for neutrophils, T cells, basophils, and eosinophils and is relevant to the pathogenesis of asthma.¹¹ CCL-20 is the only chemokine known to interact with CC chemokine receptor 6 (CCR6) to form the ligand–receptor pair CCL20–CCR6, which is responsible for the chemo-attraction of immature dendritic cells, effector/memory T-cells, and B-cells, and plays a role in skin and mucosal surfaces under homeostatic and inflammatory conditions.²⁶ GM-CSF is known to stimulate the production of leukocytes and mediate allergic airway inflammation elicited after intranasal exposure of mice to house dust mite.²⁷ Several studies demonstrated that MCP-1 plays a significant role in the pathogenesis of allergic asthma as a potent chemo-attractant for eosinophils, memory T cells, and monocytes.²⁸ Patients of the AS group had both upper and lower airway allergies to cockroaches clinically and had higher serum IL-8, MCP-1, CCL-20, and GM-CSF. This suggests that patients in this group had more severe and systemic inflammatory responses. Patients of the AR group had upper airway allergy only and significantly lower IL-8, MCP-1, and CCL-20 levels.

The current study investigated the IgE-binding activity to 8 American cockroaches from patients with different severities of airway allergy to cockroaches and found that the numbers of IgE-binding allergens do not correlate with clinical severity of airway allergy. However, patients with asthma (AS group) had an 81% prevalence of IgE-binding activity to Per a 2, which was significantly higher than those of patients with allergic rhinitis only (AR group) (45%, $P < .05$). In contrast, 80% of AR patients had IgE-binding activity to Per a 9, compared with only 28.5% of AS patients ($P < .01$). This suggested that IgE-binding allergens are not of equal importance. Sensitization to Per a 2 correlates with more severe airway allergy, and elevated proinflammatory chemokines IL-8, MCP-1, CCL-20, and GM-CSF suggested that sensitization to Per a 2 may be an important indicator for cockroach allergy to develop asthma. However, because patients in our study were those with established diseases, and the in vitro reactivity of the recombinant allergens may not be identical with the corresponding native allergens in vivo, we need more experiments to validate the causal relationship between Per a 2 and allergic asthma.

Little information is available regarding the biological and immunologic characterization of Per a 2. From the literature, Per a 2 is an inactive aspartic protease and, as in the current study (accession no. GU188391), it has only 42 to 44% identity with Bla g 2.^{1,29,30} Studies on Bla g 2 showed that 5 disulfide bridges and a Zn-binding site provide stability to the protein and make this particular allergen able to sensitize patients at lower levels of exposure than other allergens.³⁰ The protease activity in Bla g 2 does not seem to be related to its allergenicity.³¹ Erban et al³² have recently reported that Bla g 2 can exist in cockroach feces for as long as 406 days in an apartment with a room temperature of 25°C and 85% room humidity.³² This warm and humid environment is similar to that in Taiwan, which may encourage the long existence of Per a 2 in the environment. However, no report has addressed the correlation of sensitization to Per a 2 and allergic asthma.

In a recent study, 5 peptide fragments of Bla g 2 were produced and indicated that amino acid residues 1 through 75 and 146

Table 3
Individual IgE-Binding Profiles to Purified Recombinant Allergens by ELISA

	No.	ImmunoCAP-i6 ^a (KU/L)	Per a 1	Per a 2	Per a 3	Per a 4	Per a 5	Per a 6	Per a 7	Per a 9
AS	A-1	0.44	0.327	0.276	0.532	0.207	0.343	0.121	0.168	0.147
	A-2	0.49	0.55	0.288	0.292	0.377	0.476	0.385	0.263	0.182
	A-3	1.07	0.204	0.252	0.481	0.191	0.302	0.123	0.27	0.153
	A-4	1.4	0.143	0.202	0.196	0.218	0.138	0.414	0.137	0.121
	A-5	15.9	0.553	0.273	0.169	0.165	0.439	3.887	0.37	3.803
	A-6	0.62	0.235	0.453	0.308	0.267	0.305	0.081	0.299	0.169
	A-7	2.1	0.496	0.269	0.226	0.476	0.295	0.157	0.253	0.187
	A-8	2.07	0.078	0.162	0.131	0.105	0.407	0.054	0.065	0.062
	A-9	7.11	0.098	0.291	0.137	0.18	0.061	0.056	0.055	0.056
	A-10	2.56	0.231	0.283	0.221	0.568	0.132	0.112	0.108	0.083
	A-11	3.8	0.324	0.299	0.245	0.248	0.23	0.123	0.125	0.147
	A-12	2.87	0.577	0.32	0.326	0.472	0.396	0.292	0.272	0.825
	A-13	0.78	0.389	0.298	0.331	0.37	0.288	0.127	0.174	0.152
	A-14	1.34	0.233	0.9	0.985	0.482	0.768	1.223	0.637	0.8
	A-15	0.38	0.083	0.076	0.12	0.063	0.097	0.629	0.057	0.059
	A-16	0.83	0.148	0.487	0.28	0.165	0.476	0.093	0.26	0.773
	A-17	2.4	0.231	0.168	0.226	0.264	0.809	0.741	1.914	1.08
	A-18	1.41	0.167	0.279	0.379	0.464	0.348	0.262	0.292	0.153
	A-19	0.54	0.545	0.308	0.177	0.231	0.196	0.109	0.139	0.094
	A-20	0.74	0.173	0.296	0.283	0.085	0.228	1.101	0.088	0.093
	A-21	0.85	0.751	0.27	0.253	0.766	0.412	0.274	0.331	0.289
AR	B-1	0.53	0.233	0.295	0.25	0.236	0.225	0.264	0.185	0.121
	B-2	4.27	0.662	0.274	0.25	0.22	0.515	0.301	0.25	0.398
	B-3	2.83	0.427	0.282	0.253	0.373	0.206	0.11	0.135	0.131
	B-4	1.59	0.391	0.186	0.28	0.486	0.301	0.124	0.201	0.261
	B-5	0.53	0.117	0.26	0.251	0.156	0.321	0.322	0.198	0.268
	B-6	1.22	0.099	0.149	0.145	0.165	0.138	1.635	0.123	0.09
	B-7	0.66	0.218	0.114	0.103	0.227	0.159	0.073	0.29	0.089
	B-8	0.86	0.211	0.12	0.132	0.191	0.216	0.084	0.165	0.267
	B-9	0.91	0.512	0.356	0.365	1.473	0.47	0.288	0.479	0.373
	B-10	0.464	0.196	0.155	0.151	0.143	0.203	0.094	0.154	0.313
	B-11	5.99	0.243	0.196	0.253	0.199	0.3	0.144	0.271	0.273
	B-12	0.53	0.267	0.169	0.129	0.346	0.216	0.113	0.131	0.263
	B-13	0.49	0.526	0.354	0.263	0.225	0.499	0.293	0.263	0.561
	B-14	0.54	0.421	0.271	0.361	0.571	0.766	1.625	0.397	0.279
	B-15	0.6	0.14	0.148	0.133	0.12	0.314	0.088	0.108	2.826
	B-16	5.79	0.227	0.118	0.128	0.234	0.237	3.84	3.575	4.61
	B-17	3.09	0.519	0.447	0.277	0.919	0.415	0.289	0.352	0.264
	B-18	3.68	0.337	0.157	0.143	0.245	0.43	0.286	0.252	2.043
	B-19	0.38	0.113	1.688	1.632	1.076	1.685	0.89	0.942	1.382
	B-20	1.07	0.095	0.174	0.169	0.155	0.22	0.164	0.107	0.265

Abbreviations: AS, persisted asthma with allergic rhinitis; AR, allergic rhinitis only.

Data were mean OD values.

Bold data were positive results above the cutoff value (0.25).

^aBy ImmunoCAP.

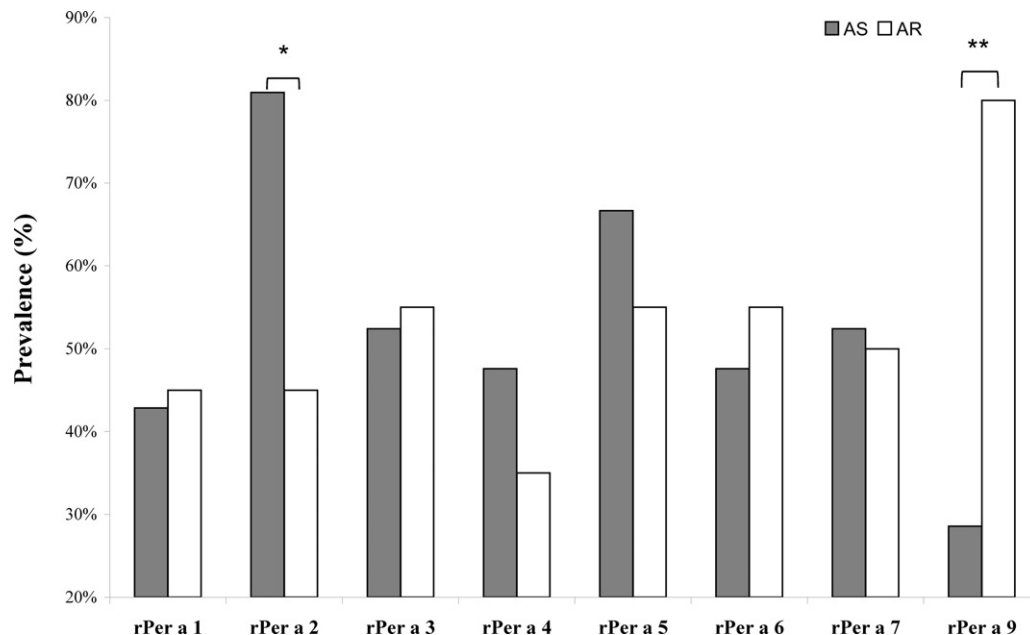


Fig. 3. IgE-binding prevalence to 8 recombinant allergens in sera of patients with moderate to severe asthma (AS, $n = 21$) or with allergic rhinitis only (AR, $n = 20$). * $P < .05$; ** $P < .01$, by χ^2 analysis.

through 226 were important for IgE-binding.³³ However, the structurally diverse feature of group 2 cockroach allergens and their allergenicity warrant further investigation. Except for the potential environmental aspect, the mechanisms by which Per a 2 sensitizes patients also need further investigation.

Per a 9 is an arginine kinase that has been identified as a pan-major allergen of many insect and crustaceans.^{34–39} The seasonal levels of Per a 9 in Bangkok, Thailand, correlate with disease exacerbation.⁴⁰ However, this study does not specify whether the disease exacerbation is of the upper airways only or both the upper and lower airways. In the current study, subjects sensitized to Per a 9 seem to have less lower airway sensitization and lower serum pro-inflammatory chemokines. The role of Per a 9 in airway allergy requires further investigation.

In conclusion, not all allergens from American cockroaches have equal importance as regards pathogenicity. Sensitization to Per a 2 correlates with more severe airway allergy and elevated pro-inflammatory chemokines. This finding may be helpful in selecting a target allergen for component-resolved diagnosis and for immunotherapeutic agents.

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References

- [1] Wilson NW, Robinson NP, Hogan MB. Cockroach and other inhalant allergies in infantile asthma. *Ann Allergy Asthma Immunol*. 1999;83:27–30.
- [2] Lan JL, Lee DT, Wu CH, Chang CP, Yeh CL. Cockroach hypersensitivity: preliminary study of allergic cockroach asthma in Taiwan. *J Allergy Clin Immunol*. 1988;82:736–740.
- [3] Eggleston PA, Rosenstreich D, Lynn H, et al. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J Allergy Clin Immunol*. 1998;102:563–570.
- [4] Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TA. Risk factors for asthma in inner city children. *J Pediatr*. 1992;121:862–866.
- [5] Rosenstreich DL, Eggleston P, Kattan M, 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;336:1356–1363.
- [6] Gruchalla RS, Pongracic J, Plaut M, et al. Inner City Asthma Study: relationships among sensitivity, allergen exposure, and asthma morbidity. *J Allergy Clin Immunol*. 2005;115:478–485.
- [7] Rabito FA, Carlson J, Holt EW, Iqbal S, James MA. Cockroach exposure independent of sensitization status and association with hospitalizations for asthma in inner-city children. *Ann Allergy Asthma Immunol*. 2011;106:103–109.
- [8] Bernton HS, Brown H. Insect allergy: Preliminary studies of the cockroach. *J Allergy Clin Immunol*. 1964;35:506–513.
- [9] Hong JH, Lee SI, Kim KE, et al. German cockroach extract activates protease-activated receptor 2 in human airway epithelial cells. *J Allergy Clin Immunol*. 2004;113:315–319.
- [10] Arizmendi NG, Abel M, Mihara K, et al. Mucosal allergic sensitization to cockroach allergens is dependent on proteinase activity and proteinase-activated receptor-2 activation. *J Immunol*. 2011;186:3164–3172.
- [11] Lee MF, Wang NM, Liu SW, Lin SJ, Chen YH. Induction of interleukin 8 by American cockroach allergens from human airway epithelial cells via extracellular signal regulatory kinase and jun N-terminal kinase but not p38 mitogen-activated protein kinase. *Ann Allergy Asthma Immunol*. 2010;105:234–240.
- [12] Patterson ML, Slater JE. Characterization and comparison of commercially available German and American cockroach allergen extracts. *Clin Exp Allergy*. 2002;32:721–727.
- [13] International Union of Immunological Societies Allergen Nomenclature Sub-Committee. *Allergen Nomenclature*. 2010. Available at: <http://www.allergen.org>. Accessed on February 7, 2012.
- [14] Valenta R, Linhart B, Swoboda I, Niederberger V. Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens. *Allergy*. 2011;66:775–783.
- [15] Vrtala S. From allergen genes to new forms of allergy diagnosis and treatment. *Allergy*. 2008;63:299–309.
- [16] Valenta R, Twaroch T, Swoboda I. Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. *J Invest Allergol Clin Immunol*. 2007;17:36–40.
- [17] Ballmer-Weber BK, Wangorsch A, Bohle B, et al. Component-resolved in vitro diagnosis in carrot allergy: does the use of recombinant carrot allergens improve the reliability of the diagnostic procedure? *Clin Exp Allergy*. 2005;35:970–978.
- [18] Pittner G, Vrtala S, Thomas WR, et al. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy*. 2004;34:597–603.
- [19] Ballmer-Weber BK, Scheurer S, Fritsche P, et al. Component-resolved diagnosis with recombinant allergens in patients with cherry allergy. *J Allergy Clin Immunol*. 2002;110:167–173.
- [20] Heiss S, Mahler V, Steiner R, et al. Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol*. 1999;113:830–837.
- [21] Kidon MI, Chiang WC, Liew WK, et al. Mite component-specific IgE repertoire and phenotypes of allergic disease in childhood: the tropical perspective. *Pediatr Allergy Immunol*. 2011;22:202–210.
- [22] Bateman ED, Hurd SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J*. 2008;31:143–178.
- [23] Bousquet J, Van CP, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol*. 2001;108:S147–S334.
- [24] Wu CH, Lee MF, Liao SC, Luo SF. Sequencing analysis of cDNA clones encoding the American cockroach Cr-PI allergens: homology with insect hemolymph proteins. *J Biol Chem*. 1996;271:17937–17943.
- [25] Wang NM, Lee MF, Wu CH. Immunologic characterization of a recombinant American cockroach (*Periplaneta americana*) Per a 1 (Cr-PII) allergen. *Allergy*. 1999;54:119–127.
- [26] Schutyser E, Struyf S, Van DJ. The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev*. 2003;14:409–426.
- [27] Cates EC, Fattouh R, Wattie J, et al. Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J Immunol*. 2004;173:6384–6392.
- [28] Chan CK, Kuo ML, Yeh KW, et al. Sequential evaluation of serum monocyte chemotactic protein 1 among asymptomatic state and acute exacerbation and remission of asthma in children. *J Asthma*. 2009;46:225–228.
- [29] Wunnschmann S, Gustchina A, Chapman MD, Pomes A. Cockroach allergen Bla g 2: an unusual aspartic proteinase. *J Allergy Clin Immunol*. 2005;116:140–145.
- [30] Gustchina A, Li M, Wunnschmann S, Chapman MD, Pomes 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;348:433–444.
- [31] Pomes 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;165:391–397.
- [32] 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;47:1062–1070.
- [33] Lee H, Jeong KY, Shin KH, et al. Reactivity of German cockroach allergen, Bla g 2, peptide fragments to IgE antibodies in patients' sera. *Korean J Parasitol*. 2008;46:243–246.
- [34] Shen Y, Cao MJ, Cai QF, et al. Purification, cloning, expression and immunological analysis of *Scylla serrata* arginine kinase, the crab allergen. *J Sci Food Agric*. 2011;91:1326–1335.
- [35] Bobolea I, Barranco P, Pastor-Vargas C, Iraola V, Vivanco F, Quirce S. Arginine kinase from the cellar spider (*Holocnemus pluchei*): a new asthma-causing allergen. *Int Arch Allergy Immunol*. 2010;155:180–186.
- [36] Liu Z, Xia L, Wu Y, Xia Q, Chen J, Roux KH. Identification and characterization of an arginine kinase as a major allergen from silkworm (*Bombyx mori*) larvae. *Int Arch Allergy Immunol*. 2009;150:8–14.
- [37] Garcia-Orozco KD, Aispuro-Hernandez E, Yepiz-Plascencia G, Calderon-de-la-Barca AM, Sotelo-Mundo RR. Molecular characterization of arginine kinase, an allergen from the shrimp *Litopenaeus vannamei*. *Int Arch Allergy Immunol*. 2007;144:23–28.
- [38] Sookrung N, Chaicumpa W, Tungtrongchitr A, et al. *Periplaneta americana* arginine kinase as a major cockroach allergen among Thai patients with major cockroach allergies. *Environ Health Perspect*. 2006;114:875–880.
- [39] Binder M, Mahler V, Hayek B, et al. Molecular and immunological characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate pan-allergen. *J Immunol*. 2001;167:5470–5477.
- [40] Tungtrongchitr A, Sookrung N, Indrawattana N, 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.