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IgE Binding Patterns to German Cockroach Whole Body Extract in Korean Atopic Asthmatic Children

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It is widely known that the cockroach is an inhalant allergen in atopic asthma and allergic rhinitis. Even though Bla g I and Bla g II are considered as the major allergens, several relatively high-molecular weight (MW) cockroach allergens have also been recently identified by IgE-immunoblot in western countries. However, the environmental control and diagnostic tests mainly focussed on Bla g I and Bla g II. Furthermore there is no data about major IgE-binding cockroach antigens in Korea. We performed this study to identify the major German cockroach allergens in Korean atopic children. By the results of allergy skin tests, 14 children with atopic asthma (9 were cockroach-sensitive and 5 were cockroach-nonsensitive atopics) were enrolled in this study. We conducted IgE immunoblot and autoradiographic analysis using Yonsei-extract of German cockroach antigen produced in our laboratory, individual sera from 9 cockroach-sensitive children, and the pooled sera of 5 house-dust-mites-only-sensitive children. We performed an allergic skin test to cockroach mix, and a radioallergosorbent test (RAST) using German cockroach crude extract on all subjects. German cockroach-specific IgE was detected in 6 out of 9 subjects by RAST. We identified at least 15 IgE-binding protein bands, and among them, the components of MWs of 76, 64, 50, 38, and <14 kilodaltons (kDa) were the major German cockroach allergens in study subjects. Therefore, Bla g I (25~30 kDa) and Bla g II (36 kDa) could not be the absolute indicators of German cockroach sensitization and parameters of environmental control.

Key Words: IgE binding patterns, German cockroach extract, atopic asthmatic children

The cockroach is an insect belonging to the family *Blattaria*, subtype *Blattodea*. There are 4 different types of cockroaches in Korea, the German cockroach, American cockroach, Oriental cockroach and Japanese cockroach. Among them, the German cockroach is the most prevalent throughout the country (Ree *et al.* 1973).

It is widely known that the cockroach is an inhalant allergen in allergic rhinitis and atopic asthma. The allergenicity of cockroach antigens have been proven by skin test or RAST, and asthmatic groups have shown a positivity of 49~61% (Bernton and Brown, 1964; Bernton and Brown, 1970; Mendoza and Snyder, 1970; Bernton *et al.* 1972; Kang and Sult, 1978; Kang *et al.* 1989). By bronchial challenge tests, cockroach antigen has been identified as an important indoor allergen in asthmatic children (Bernton *et al.* 1972; Kang *et al.* 1989). There have been several studies of environ-

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mental exposure to cockroach allergens by monoclonal antibody-based analysis (Pollart *et al.* 1991; Schou *et al.* 1991; Call *et al.* 1992).

A report from Korea by Lee *et al.* showed an 11.4% positivity for cockroach antigen on skin test, placing it among the 5 most common allergens, also including house dust mites, house dust, silk and cat epithelium (Lee *et al.* 1988). In the separate study by Lee *et al.* 41 out of 220 (18.6%) Korean atopic children showed positive skin reaction and/or positive RAST responses (Lee *et al.* 1993a) By the bronchial provocation test with German cockroach extract, Lee *et al.* also confirmed a bronchial constriction in 6 out of 16 (37.5%) asthmatic children tested (Lee *et al.* 1993b). Immunoblot results (Stankus and O'Neil, 1988) identified the presence of significant allergens in German cockroach whole body extract in a MW range of 12.5 kDa to 110 kDa (Stankus and O'Neil, 1988), and it is accepted that the major German cockroach allergens are *Bla g I* (MW range of 25 to 30 kDa), *Bla g II* (MW of 36 kDa) and several high-molecular weight antigens in the USA (Polart *et al.* 1991; Schou *et al.* 1991). But until now, the environmental control and diagnostic tests mainly focussed on *Bla g I* and *Bla g II.* Considering the possibility of differences in major allergenic components among countries and/or individuals, in this study we conducted the IgE immunoblot and autoradiographic analysis using crude German cockroach extract for the purpose of identifying the major German cockroach allergens in Korean ashmatics.

MATERIALS AND METHODS

Subjects

Fourteen children with atopic asthma were involved in this study, 9 showing positive and 5 showing negative skin reactions to commercial cockroach extract. The clinical characteristics of subjects are summarized in Table 1.

Sera were collected from all subjects at the Allergy Clinic, Severance Hospital, Yonsei University College of Medicine. Five sera from cord blood were also obtained and used for negative control sera. All sera were stored frozen at −20°C until use.

Skin test and detection of serum IgE antibodies

Allergy skin test was performed by a scratch method in all subjects with commercial antigens including *Dermatophagoides farinae (D. farinae), Dermatophagoides pteronyssinus) D. pteronyssinus),* cockroach mix and house dust extracts (Bencard Co., Brentford, UK). The results were read 15~30 minutes after the scratches. The wheal size was

Table 1. Clinical characteristics and results of skin tests of subjects

Subjects[1]	Sex	Age(year)	Diagnosis[2]	HD[3]	DF[3]	DP[3]	CR[3]
A	M	8	ARE	2+	3+	3+	2+
B	F	13	AR	3+	4+	4+	2+
C	M	12	AR	2+	3+	3+	3+
D	F	4	AE	3+	−	−	2+
E	M	4	AE	3+	2+	3+	2+
F	M	14	AR	3+	2+	3+	3+
G	M	10	AR	2+	3+	3+	3+
H	M	4	A	3+	2+	−	2+
I	M	8	A	−	3+	3+	3+
J1	M	4	A	−	3+	3+	−
J2	M	5	A	−	3+	4+	−
J3	F	4	ARE	−	3+	4+	−
J4	M	4	AR	−	3+	4+	−
J5	M	6	AE	−	3+	3+	−

[1]: Cockroach sensitive(A-I) and nonsensitive subjects(J1-J5), [2]: A:asthma, R: rhinitis and E: eczema, [3]: HD: house dust, DF: *Dermatophagoides farinae*, DP: *Dermatophagoides pteronyssinus,* CR: cockroach mix

determined by the maximun diameter and compared with the wheal size from a 0.1% histamine control solution. The rating was 3+ if wheal size was the same as that of the histamine, 2+ if more than half but smaller than the histamine wheal, and 4+ if the wheal was more than twice the size of the hiatamine.

The total serum IgE level was measured by using the paper-radio-immunosorbent test (PRIST, Pharmacia, Upsalla, Sweden), and the result expressed in IU/ml (1 IU=2.42 ng IgE). The detection of German cockroach-specific serum IgE was performed by Phadebas RAST analysis (Pharmacia, Upsalla, Sweden). Individual serum was used without dilution and incubated with an antigen-coated disc for 3 hours. The remaining procedures were done according to routine protocol for RAST analysis.

Antigen for SDS-PAGE and autoradiography

The German cockroaches prevalent in relatively large numbers in Korea, were cultured in a jar and stored at −70°C. The crude extract was obtained by Coca's solution as described by Bernton and Brown (1964). Briefly, the frozen cockroaches were ground and defatted with ether. The extract was then mixed in a waring blender with Coca's solution (NaCl 5 gm, $NaHCO_3$ 2.75 gm, phenol 5 gm/H_2O 1 L) and stored at 4°C for 7 days. This was then centrifuged at 18,000 rpm and the supernatant was filtered through a 0.45 um filter. The supernatant was then dialyzed extensively against distilled water for 3 days. The pore size of dialysis membranes used for all the dialysis steps was 6000~8000 daltons. The extracts were then freeze-dried and the protein concentration of this extract was 450 μg/g of powder by Lowry method. This extract, named Yonsei-extract, was used in SDS-PAGE and autoradiography.

SDS-PAGE and autoradiographic detection of allergens

SDS-PAGE was done using a method modified from Laemmli (1970). A 12% gel was used together with a 4% stacking gel. The antigens were heated at 100°C for 4 minutes in 0.06 M/L of Tris HCl, pH 6.8 containing 2% (wt/vol) SDS, 5% (wt/vol) 2 mercaptoethanol, and 0.02% (wt/vol) bromophenol blue. Electrophoresis was done at 30 mA for 4 hours and the bands were then stained with 0.01% Coomassie Brilliant Blue (Sigma, St Louis, Mo, USA). MWs were calculated in accordance with low-MW protein standards (Bio Rad Lab, Richmond, CA, USA).

After electrophoresis, the protein bands were transferred onto a 0.45 μm nitrocellulose membrane (Millipore, Bedford, M.A.) at 70 V for 2 hours according to the method of Towbin *et al* (1979). The nitrocellulose membranes were then washed in 10% methanol in PBS for 1 hour and soaked in a 10 mM Tris buffer together with 3% bovine serum albumin for 4 hours. This was then washed in 0.9% NaCl and dried at room temperature.

Cut strips of nitrocellulose membranes were incubated with test sera diluted 1 to 3 with 0.1% Tween 20 and 0.1% NaN_3 in PBS for 16 hours at room temperature. Nine strips were incubated with individual sera from 9 cockroach-sensitive subjects, 1 strip was incubated with pooled sera of 5 cockroach-nonsensitive atopic subjects, and 1 strip was incubated with pooled sera of 8 cord blood samples. After washing 3 times with 0.1% Tween 20 in PBS over 3 hours, each strip was then incubated with ^{125}I-labelled anti-human IgE(Pharmacia) for 16 hours at 4°C. After measuring the amount of radioactivity of ^{125}I-labelled anti-human IgE solution, an equivalent of 15,000 cpm per strip was incubated for 16 hours, and washed three times with 0.05% Tween 20 in PBS for 1 hour. These strips were then laid down on X-OMAT x-ray film (Eastman Kodak Co, Rochester, NY, USA) in a light-proof cassette for 7 days at −70°C and the film subsequently developed.

RESULTS

Clinical characteristics of subjects

Nine subjects were cockroach-sensitive children and 5 were cockroach-nonsensitive but house-dust-mite-sensitive atopic controls. Study subjects ranged between 4 and 14 years. All patients suffered from atopic asthma; 7 cases had allergic rhinitis and 5 had eczema. The skin reactivity of cockroach-sensitive

Table 2. Skin reaction to cockroach extract and positivity of German cockroach specific serum IgE by RAST

Subjects[1]	Skin reaction	RAST class	Total IgE (IU/ml)
A	2+	1+	1426
B	2+	1+	3000
C	3+	1+	850
D	2+	4+	20
E	2+	−	788
F	3+	−	840
G	3+	−	866
H	2+	1+	477
I	3+	3+	1708
J1	−	−	221
J2	−	−	2178
J3	−	−	151
J4	−	−	35
J5	−	−	259

[1]: Cockroach sensitive(A-I) and nonsensitive subjects (J1-J5).

subjects was relatively low compared with house-dust-mite-only-sensitive subjects, except in one case (D). Case D was considered a pure cockroach sensitive atopic child.

Specific serum IgE to German cockroach

Using RAST analysis, German cockroach-specific serum IgE was detected in 6 out of 9 cockroach-sensitive subjects, but in none of the house-dust-mite-only-sensitive subjects. But the RAST classes of positive subjects were very low except one case which showed positive skin reactions to house dust and cockroaches but a negative reaction to house dust mites. Furthermore, the degree of skin reaction to cockroaches did not significantly correlate with the RAST class score(Table 2).

SDS-PAGE and autoradiographic analysis

In order to analyze the protein components, the German cockroach crude extract made in our laboratory was separated by 12% SDS-PAGE. As shown in Fig. 1, more than 29 protein bands were observed. Fig. 2 shows the binding of IgE from the individual sera of 9 cockroach-sensitive children, the

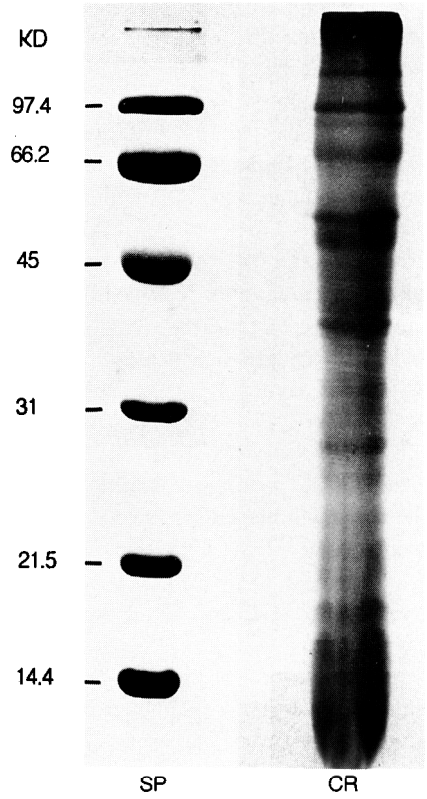

Fig. 1. SDS-PAGE analysis of crude German cockroach (Yonsei-extract). CR: crude cockroach extract. SP: MWs were determined from a standard mixture of known MW, phosphorylase-b(97.4 kDa), bovine serum albumin(66.2 kDa), ovalbumin(45 kDa), carbonic anhydrase(31 kDa), soybean trypsin inhibitor(21.5 kDa) and lysozyme(14.4 kDa).

pooled sera of 5 house-dust-mite-only-sensitive atopic controls, and the pooled sera of 5 cord blood samples to electrophoretically-separated German cockroach components. As schematically illustrated in Fig. 3, of the more than 29 bands transferred from SDS-PAGE gels, 15 bands were bound IgE antibodies in the sera. The common IgE binding bands were components with MWs of 76, 64, 50, 38 and <14.5 kDa. Each of these components bound specific IgE of the sera of more than half the cockroach-sensitive subjects (lanes A to I). Among these 15 IgE-binding bands, the most common component was a molecule of MW 64 kDa. Among the 9 cockroach-sensitive children, subject 'D' was considered the most strongly sensitized patient

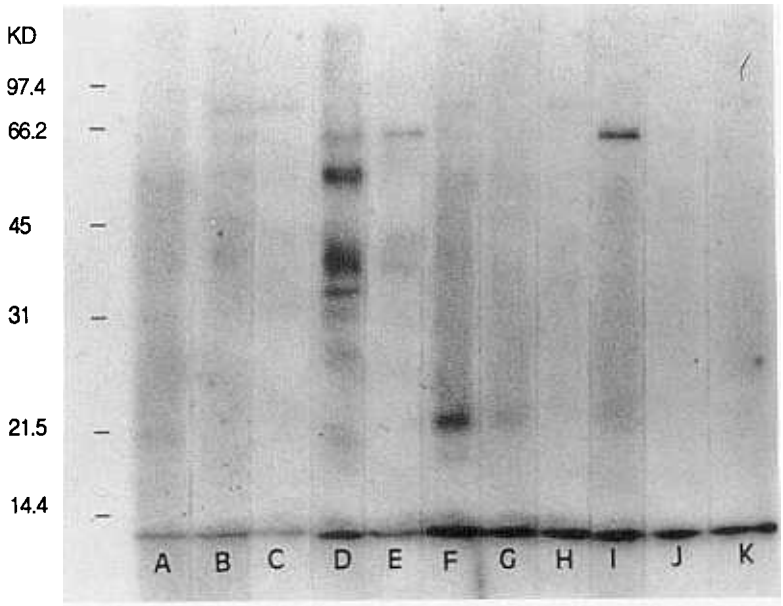

Fig. 2. Autoradiograph of SDS-PAGE-separated components of crude German cockroach (Yonsei extract). Lanes A to I, individual sera from cockroach-sensitive subjects, lane J, pooled sera of 5 house-dust-mites only-sensitive subjects and lane K, pooled sera of 8 cord bloods.

Ige binding component(KD)	Cockroach senstitive subjects A	B	C	D	E	F	G	H	I	Control* J&K	Positive rate(%)
>97.4				●							11.1
76		●	●	●		●		●			55.6
64		●		●	●	●	●		●		66.7
53.5		●		●							22.2
50	●					●	●	●	●		55.6
40				●	●						22.2
38	●			●	●	●			●		55.6
35				●					●		22.2
31				●	●	●					33.3
27.5	●			●							22.2
25		●			●						22.2
23						●	●	●	●		44.4
21.5	●			●							22.2
19											11.1
<14.5		●		●							55.6

Fig. 3. IgE binding components of German cockroach extracts in cockroach-sensitive subjects and controls. (*): lane J, pooled sera from 5 atopic controls and lane K, pooled sera of 5 cord bloods.

according to autoradiographic analysis, and she did not react to house dust mites by allergy skin test. The sera of 3 subjects (E, F, G) showed negative results by RAST, while more than 4 IgE-binding components were detected by autoradiography. No IgE-binding components were visible in the sera of controls.

DISCUSSION

This study was undertaken to identify important German cockroach allergens in Korean atopic children, using the methods of IgE-immunoblot and autoradiographic analysis. Although house dust mite antigens are also the most prevalent components of indoor allergens in Korea, the cockroach is considered an important common allergen(Lee and Kim 1988; Yoon *et al.* 1991; Lee *et al.* 1993a; Kim *et al.* 1994).

In our previous study (Lee *et al.* 1991), we identified up to 13 IgE-binding proteins (MW range of 22 kDa to 110 kDa) by IgE immunoblot with pooled sera from 5 cockroach-sensitive subjects. Then, in this study, we performed IgE-immunoblot and autoradiographic analysis with individual sera from cockroach-sensitive subjects in order to identify the major allergenic components.

We identified more than 29 protein bands by SDS-PAGE analysis of crude extracts of cockroach. Among them, 15 bands bound IgE antibodies in the subjects' sera, and the common IgE-binding bands were components of 76, 64, 50, 38 and < 14.5 kDa in MW. Each component bound with IgE antibodies in more than half of the individual sera studied, then these were considered the major German cockroach allergens in Korea. Moreover, 67% of tested sera contained IgE specific to the 64 kDa molecule, which could be considered the most common allergen. There was no visible IgE-binding band on the autoradiogrph with pooled sera of 5 house-dust-mite-only-sensitive children and cord blood. Autoradiographic findings for each child demonstrated a different IgE-binding pattern, thus indicating the diverse and complex antibody response to cockroach allergens in each subject. But unfortunately, because we did not perform the monoclonal antibody-based immunoblot study for each allergenic band, we could not confirm the exact bands corresponding to *Bla g I* and *Bla g II*. So we only suspect from this study that the component of 38 kDa was *Bla g II* and that one of the components of 23 kDa, 25 kDa and 27.5 kDa was *Bla g I*. And the component with MW of <14.4 kDa was considered to be broken-down fragments of *Bla g I*.

Regarding the allergenicity of the American cockroach, in an earlier study by Wu and Lan(1988), two components of MW 78 and 72 kDa identified the major allergens that bound 100% of the individual sera of 8 atopic patients tested. In another study by Twarog *et al.* (1977), components of MW 63~65, and 25.5 kDa revealed major American cockroach allergens by 70% skin test positivity, and were thought to be major cockroach allergens. Regarding the German cockroach in the investigation by Musmand *et al.* (1995), the results confirm high-MW allergens and proved strong evidence of the importance of these large allergens relative to previously-characterized *Blatella* allergens. In the largest study for identification of German cockroach allergens (Musmand *et al.* 1995), protein band MWs of 67, 50, 45 and 36 kDa bound more than 50% and the band of 60 KD bound approximately 80% of the 37 tested sera. Our results also demonstrate the importance of high-MW German cockroach allergens in Korean atopic children. Then the previous information about *Bla g I* and *Bla g II*, as the most important major allergenic components of German cockroach, would have to be modified in Korea and some areas of the USA. Furthermore, the diagnostic materials should contain large amount of those major allergens considering the real environment, and these high-MW proteins should be used for environmental control and assays.

Cockroach antigens are considered a major source of allergens contained in house dust, and appear to be a significant cause of asthma, particularly in urban areas of the USA (Bernton and Brown, 1970; Mendoza and Snyder, 1970; Bernton *et al.* 1972; Kang and Sult, 1978; Kang *et al.* 1989). Recently, many authors have studied the characteristics of cockroach allergens and confirmed the significant role of environmental exposure to the cockroach antigen for sensitization (Stankus *et al.* 1990; Lehrer *et al.* 1991; Pollart *et al.* 1991; Schou *et al.* 1991).

But there were variations in the sensitizing components from individual to individual or from country to country. To initiate environmental controls of allergic diseases, the identification of major allergens in a local area is very important.

There are some differences in cockroach allergy between Korea and the USA. First, there is a relatively low level of cockroach infestation in Korea. Second, there are fewer cockroach-sensitive subjects. And finally, there are low correlations between skin reaction and RAST class scores (Lee *et al.* 1993a). The discrepancies between skin tests and RAST, or RAST and immunoblot study, were also seen in this study. We can't explain the cause of this result exactly, but there are several possibilities. While the RAST disc contains only German cockroach extract, the skin-test antigen contains German and American cockroach extracts since we could not obtain the skin-test antigen containing only German cockroach. For that reason, there might be a discrepancy between the skin test and RAST in this study. As for the discrepancy between RAST and imuunoblot study, we can suggest that the RAST disc was lacking several allergenic components which were contained in our own German cockroach extract. Another possibility is that there were several cross-allergenicities between several bands of German and American cockroach (Wu *et al.* 1997), and the positive results of skin tests and immunoblot study back up this suggestion in cases E, F and G. Therefore, the study of cockroach allergy is difficult in Korea, but we suggest that cockroach sensitization might be an important triggering factor in Korean asthmatic children. As we are at an early stage of study on cockroach sensitization, further work is needed to develop a more specific diagnostic method and environmental assays for major cockroach allergens, and control methods.

In summary, we identified several common German cockroach allergens, with MWs of 76, 64, 50, 38, <14.4 kDa in Korean atopic children, and we suggest that these components could be included in the investigation of cockroach allergy in Korea.

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