Identification of the German Cockroach Allergens in Korean Atopy Using SDS-PAGE and Western Blot Analysis

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Background **: Cockroaches are important components of house dust allergens. In spite of significant subject reactivity to cockroach extracts, the specific source of the allergen remains unclear.**

Objective **: This study was performed to identify the important allergens in German cockroach whole body (GWBE), egg (GEE) and fecal (GFE) extracts in Korean atopy, and to compare the reactivity of GWBE and GEE by Western blot inhibition.**

Methods **: Sera from 11 subjects with Korean atopy were used for sodium dodecil sulfate polyacrylamide gel elctrophoresis (SDS-PAGE) and Western blot analysis.**

Results **: Allergenic fractions in German cockroach extracts were numerous and distributed throughout the wide range of molecular weights. The important allergens of GWBE, GFE and GEE were similar to each other by using SDS-PAGE and Western blot analysis. The allergen bands at 55 kd showed the most significant reactivity; in GWBE, GFE and GEE 73%, 82%, 55%, respectively. Other bands exhibiting significant activity were the 67 kd band with 37%, 19% and 19%, the 64 kd band with 64%, 37% and 9% respectively. Furthermore, Western blot inhibition investigations revealed that either GWBE or GEE could almost completely inhibit the reactivity of the other extract.**

Conclusion **: This study confirms that the 55 kd allergen can be considered as the major allergen in Korean atopy and demonstrated that the GWBE and GEE antigens have identical IgE-binding sites. (Ann Dermatol 12(4) 247~251, 2000).**

Key Words : German cockroach allergens, Korean atopy

There are 4 different types of house-hold cockroaches in Korea such as German cockroaches *(Blattella germanica)*, American cockroaches *(Periplaneta americana)*, smoky brown cockroaches *(Periplaneta fuliginosa)* and Japanese cockroaches *(Periplaneta japonica)*. Among them, the German cockroach is the most popular species[1]. It is the smallest species in Korea, adults 12 to 16mm in length, pale yellowish brown with two dark brown longitudinal stripes on the pronotum[1]. German cockroach allergens have been found in whole body, cast skins, egg shells and feces[2]. Considering the possibility of differences in major allergenic components among countries, we conducted the IgE immunoblot using crude German cockroach extract for the purpose of identifying the major

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German cockroach allergens in Korean atopy. Additionally, the reactivity of egg and whole body allergens was compared through Western blot inhibition studies.

MATERIALS AND METHODS

Sera

Sera from 11 patients with atopy (1 with atopic dermatitis, 7 with allergic rhinitis, 3 with asthma) were used for study. Sera from five atopic subjects without skin test reaction to cockroach were served as controls.

Cockroach extracts and Protein Content

Cockroaches were killed by freezing at -70℃. Cockroach whole bodies, fecal pellets and egg cases were identified macroscopically and removed separately with forceps. Cockroaches were then ground in a mortar and pestle or in a food processor to a fine powder. Powdered cockroaches were then extracted with petroleum ether in a Soxhlet extractor for 4 hours in a well ventilated chemical hood. Heating mantle was set at 37℃ for ether vaporization; cold water to condense ether; reflux should take place about every 10-15 minutes; ether should have pale yellow color when peanuts are defatted. Ether extracted cockroachs were allowed to dry until no residual ether odor is noticeable; 1:20 w/v extracts were then prepared in 1 M NaCl to 20 mM sodium phosphate (pH 7.0) for overnight at 4℃. The extract was isolated by centrifugation at 20,000g for 60 minutes at 4℃. The BCA technique (Pierce Chemical Co, Rockford,¤B., USA) was used for protein determinations following the manufacturer's instructions with BSA as a standard.

SDS-PAGE and Western blot

SDS-PAGE was carried out with a 4-20% polyacrylamide separating gel and a stacking gel of 4%. Twenty microliters of a 1mg/ml solution of each fraction was applied to each well. Electrophoresis was performed for 90 minutes at 0.030 A per gel (Novex, San Diego CA) for the 8 cm gels. To ensure proper protein separation and visualization, Colloidal blue stain (Novex, Sandiego, CA) was done on gels. Proteins were transferred from the separating gel to a nitrocellulose membrane in a transfer buffer (tris-glycine) with 0.05% SDS and 40% methanol. The procedure was done in a transblot apparatus (Novex) for 2 hours (0.250A). After removal from the transblot apparatus, the nitrocellulose was placed in TBST with 1% BSA as blocking solution for 1 hour. The nitrocellulose blot was incubated with the pooled cockroach-sensitive IgE serum (1:20 dilution) for 2 hours at room temperature with rocking. After another wash step they were incubated 2 hours with horse anti-human IgE labeled with iodine 125 (Santofi, MN). Autoradiographic detection of IgE binding was done by exposing X-OMAT AR x-ray film (Eastman Kodak, Rochester, N.Y) to the membrane at -70℃. Exposure times were usually overnight. The number and molecular weight of IgE-binding bands were determined by visual inspection of two independent observers.

Western blot inhibition

An IgE-positive undiluted serum sample was mixed individually with GWBE and GEE (20 ug protein of each extract) using end-over-end rotation at 4℃ for 1 hour. The remaining steps were same as in Western blot analysis.

RESULTS

Protein concentrations

Protein determinations for each of the extracts were 13.7, 11.6, 8.5 mg/ml in GWBE, GFE and GEE, respectively.

SDS-PAGE

Allergenic fractions in German cockroach extracts were numerous and distributed throughout a wide range of MWs.

Figure 1. shows an SDS-PAGE gel of the extract proteins. In summary,

1. GWBE revealed a lot of allergenic bands with a MW 5 to 240 kd. The pronounced major bands were stained between 100-65, 47-57, 37, 32, 17-27, 10 and 5kd with many minor staining protein..
2. GFE revealed a lot of allergenic bands with a MW 4 to 110 kd. The pronounced proteins were stained at 57, and between 39-52, 29-36, 4-16 kd.
3. GEE revealed a lot of allergic bands with a MW 5 to 240 kd. The pronounced major bands were stained between 100-67, 57, 50,

36, 27-17, 10 and 5 with many minor staining proteins.

Western blotting

The important allergens of GWBE, GFE and GEE were to similar each other. As schematical illustration in Table 1, two common GWBE allergens were found: one was 55 kd in eight sera, and the other was GWBE allergen in 64 kd in seven of the 11 sera tested. Most common GFE allergen was 55 kd which was detected in nine of the 11 sera tested followed by 64, 45 and 30 kd in 4 of the 11 sera tested. Most common GEE allergen was 55 kd in six of the 11 sera tested. However, no other IgE-binding proteins were detected in control sera.(data not shown)

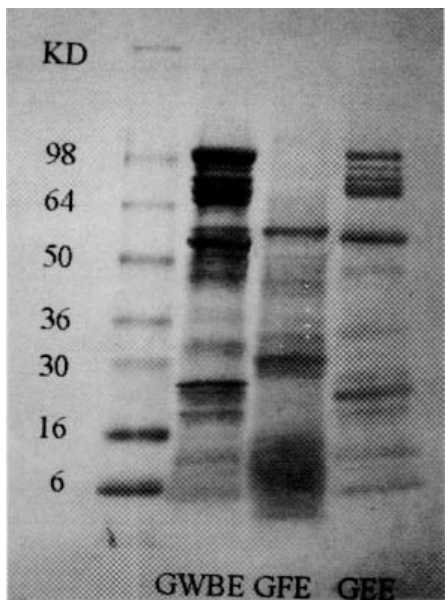

Fig. 1. SDS-PAGE analysis of German cockroach extracts. Allergenic fractions in German cockroach extracts were numerous and distributed throughout a wide range of molecular weights.

Western blot inhibition

The GWBE immunoprint was inhibited by both of homologous (GWBE) and heterologous (GEE) inhibitors. The GEE immunoprint inhibition demonstrated similar results, showing almost equal inhibition by the homologous (GEE) and the heterologous (GWBE) inhibitors. These studies indicated that the allergens in GWBE and GEE contain shared IgE-binding sites (Fig. 2).

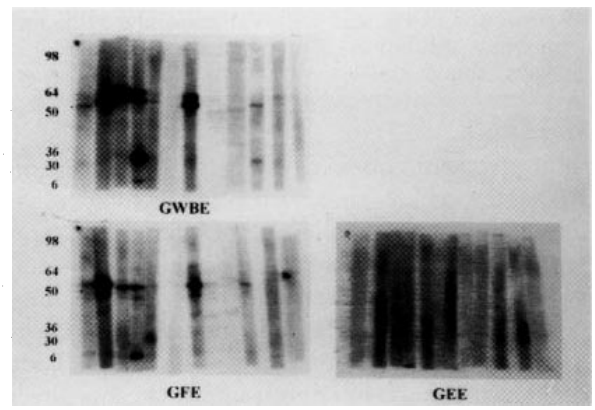

Fig. 2. Western blot of German cockroach extracts. The allergen of 55 kd was detected in most GWBE, GFE and GEE allergens.

DISCUSSION

Although house dust mite antigens are also the

Table 1. Allergenic component of the extracts of German cockroach bound with patients' IgE by immunoblot

Protein band No.	Molecular weight(KD)	German cockroach extracts(%)		
		Body	Feces	Eggs
1	92	1(9.1)	1(9.1)	0
2	67	4(36.4)	2(18.2)	2(18.2)
3	64	7(63.6)	4(36.4)	1(9.1)
4	55	8(72.7)	9(81.8)	6(54.5)
5	50	3(27.2)	2(18.2)	0
6	45	0	4(36.4)	0
7	36	3(27.2)	2(18.2)	0
8	30	3(27.2)	4(35.4)	1(9.1)
9	25	2(18.2)	0	0

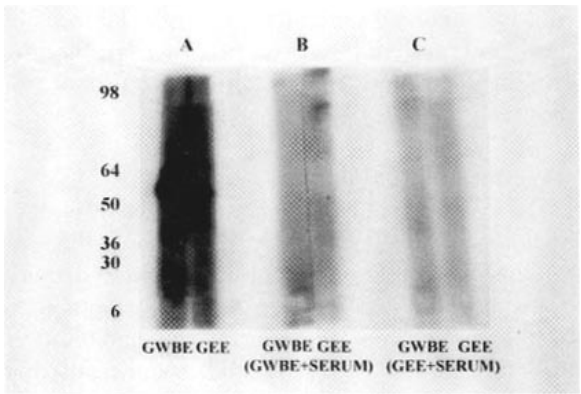

Fig. 3. Western blot inhibition. Lane A represents Western blot. Lane B and C are results after the absorption of GWBE and GEE extracts. The GEE immunoprint inhibition demonstrated similar results, showing almost equal inhibition by the homologous (GEE) and the heterologous (GWBE) inhibitors.

most prevalent components of indoor allergens in Korea, the cockroaches are considered to act as an important common allergen[3]. Cockroaches, present in many households, food establishments and certain occupational settings, represent a serious health problem to susceptible individuals in cockroach-infested environments[4,5]. The whole body cockroach extracts have been shown to cause direct cutaneous sensitivity[6,7] as well as inhalent allergy[8], but potential sources of relevant cockroach allergens in the environment include whole bodies, cast skins, secretions, egg castings, and fecal material. Atopic individuals who live in cockroach-infested housing become sensitized by inhalation to potent cockroach allergens and produce vigorous IgE antibody responses[9]. Several investigators have attempted to identify and isolate major cockroach allergens with different physicochemical and immunochemical techniques[10,11].

In the current study, SDS-PAGE-Western blot analysis demonstrates a similarity in GWBE and GEE IgE-binding analysis. Furthermore, almost complete inhibition of Western blot activity of either GWBE or GEE was obtained by both extracts. Allergenic materials with molecular weights ranging from 6 to 120 kD have been identified from a variety of source materials using serum IgE from cockroach-sensitive individuals by several investigators[12,13]. A group of investigators in New Orleans was able to identify five allergens with MW of 67, 60, 50, 45, and 36 kD that exhibited IgE-binding reactivity in 50 to 80% of 37 subjects' sera tested[13]. Our present study also demonstrates the importance of high-MW German cockroach allergens in Korean atopy. However, there were four allergen bands (MW 67, 64, 55 and 30) that were recognized by pooled sera, regardless of the extract. This allergen can be considered as a similar allergen among the three extracts. Of the 11 sera tested, the protein bands with the highest prevalence of IgE binding were found at 55kd. In Korea, the 55 kd allergen can be considered the major allergen. The band at 55 kd allergen bounded approximately 80% of sera tested in both GWBE and GFE. The disparity in identification of the most clinical relevant allergens in German cockroaches between this study and previous investigations may be due to differences in extract preparation (source material and procedure), separation and isolation techniques, and serum source. Six of 11 sera reacted with GEE, whereas all of 11 sera reacted with GWBE and GFE identified allergens. The disparity in identification of the allergens in German cockroach source may come from a difference in IgE binding.

In conclusion, this study confirmed several common German cockroach allergens, with MWs of 67, 64, 55 and 30 kd in Korean atopy. Especially, 55 kd allergen can be considered to be the major allergen. Additionally, the GWBE and GEE antigens have identical IgE-binding sites.

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