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Identification and Purification of IgE-reactive Proteins in German Cockroach Extract

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Abstract

Cockroaches have been implicated as a cause of respiratory allergy in urban areas worldwide. IgE-reactive German cockroach proteins were identified with molecular weights (MWs) of 90, 66, 50, 43 and 36 KD by immunoblot analysis in both immune BALB/c mice and sensitized humans. Prominent IgE-reactive proteins were purified using FPLC by ion-exchange chromatography, gel filtration and hydrophobic chromatography. The N-terminal amino acid sequence of a purified protein with a MW of 66 KD on SDS-PAGE was Val-Thr-Leu-Lys-Lys(Val)-Met-Ile-Lys-Thr-Phe-Tyr. No homologous protein was found through a search of GenBank that indicated a novel IgE-reactive protein in German cockroach extract. Another purified protein with a MW of 36 KD reacted strongly with a monoclonal antibody against Bla g 2.

Key Words: German cockroach, IgE, allergens, immunoblot, amino acid sequence

INTRODUCTION

Cockroach-derived proteins in its feces, saliva, eggs and shed cuticles have been implicated as one of the leading causes of asthma in impoverished urban environments throughout the world including Korea, where the German cockroach is the most prevalent species of cockroaches.[1,2]

The allergenicity of cockroach extract has been proven in humans by skin test, bronchial provocation test or RAST.[3-6] Immunoblot analysis studies identified several allergenic components in German cockroaches with molecular weights (MWs) of 12.5 to 110 kDa.[7-9] It is generally accepted, however, that the most focused German cockroach allergens have been Bla g 1 and Bla g 2 in humans.[10,11] However, the allergenicity or IgE-reactivity of German cockroach extract in experimental mice have been poorly studied until now.

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In this study, considering the possible differences in IgE-reactivities between mice and humans, immunoblot analysis was performed to elucidate and compare IgE-binding patterns to German cockroach extracts in immune mice and sensitized human sera. In addition, IgE-reactive German cockroach proteins were purified, and N-terminal amino acid sequencing was performed.

MATERIALS AND METHODS

Cockroach extract

The whole bodies of adult German cockroaches (*Blattella germanica*) were homogenized, defatted with ether, and then extracted in 10 mM phosphate buffered saline (PBS), pH 7.4 by incubation for 10 hours at 4°C. The extract was used for ELISA or immunoblot analysis. On the other hand, lyophilized cockroaches were extracted in the modified Coca solution (0.9% NaCl, 0.25% $NaHCO_3$, 0.4% phenol), and 1 : 40 (w/v) diluted extract was used for the skin test.[1]

Production of antisera in mice

Aluminum hydroxide gel (alum) was prepared as

described.[12] Twenty μg of the cockroach extract in PBS was mixed with 1 ml of alum, and 5 six-week-old female BALB/c mice were immunized intraperitoneally with the cockroach extract-gel (0.1 ml containing 2 μg of the cockroach extract and about 14 mg of alum) at 0, 4 and 8 weeks. At 0, 3, 5, 7 and 9 weeks after the first immunization, the sera were collected.

Enzyme-linked immunosorbent assay (ELISA)

Total serum IgE and German cockroach-specific IgE levels in the immunized mice were measured by ELISA as described previously.[13] To measure the total IgE, the ELISA plates were coated with anti-mouse IgE monoclonal antibody (Pharmingen, San Diego, CA, U.S.A.) at 4°C overnight. The wells were washed, and blocked with 3% bovine serum albumin for 1 hour at 37°C. The immune sera with a 1 : 400 dilution were incubated for 1 hour at 37°C. The purified mouse IgE (Pharmingen) instead of the sample sera was incubated for converting absorbance values to actual concentrations of the total IgE. Wells were washed and incubated with 1 : 2,000 diluted biotinylated anti-mouse IgE (Biodesign, Kennebunk, ME, U.S.A.). After washing, 1 : 8,000 diluted peroxidase conjugated anti-biotin antibody (Vector, Burlingame, CA, U.S.A.) was incubated. Wells were developed with 0.05% orthophenylenediamine and 0.006% hydrogen peroxide in 0.1 M phosphate citrate buffer (pH 5.0). The absorbance was read at 490 nm. To measure the specific IgE, German cockroach extract (5 μg/ml) was coated in the wells and incubated sequentially with immune sera, biotinylated anti-mouse IgE, peroxidase conjugated anti-biotin antibody, and color-developing substrate the same as described above for measuring total IgE.

Passive cutaneous anaphylaxis

The specific IgE levels in mice were measured *in vivo* by PCA reactions on rat skins.[12] Eight-week-old male Wistar rats were sensitized intradermally with 0.05 ml of a serial dilution of an antiserum. Twenty-four hours after the sensitization, a mixture of 0.5 ml of German cockroach extract (2 mg/ml) and 0.5 ml of 2% Evans blue in PBS was injected intravenously. After 30 min., the rats were sacrificed, the dorsal skin reversed, and the reaction sites were measured.

Human sera

The skin prick test with German cockroach extract was performed for patients who visited the allergy clinic of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. Sera of skin test positives were collected and kept frozen at −20°C. Pooled sera of 10 subjects was used.

IgE-immunoblot analysis

The cockroach extract was electrophoresed on 10% SDS-polyacrylamide gels and electrotransferred to the nitrocellulose (NC) membrane for IgE-immunoblot analysis.[13] Non-specific bindings were blocked with 3% bovine serum albumin and reacted with 1 : 10 dilution of pooled sera of humans or 1 : 20 dilution of pooled sera of immune mice. Washed 3 times, the membranes were incubated with a peroxidase conjugated anti-mouse IgE (Nordic Immunology, Tilburg, Netherlands) or 1 : 500 diluted alkaline phosphatase conjugated anti-human IgE (Sigma, St. Louis, MO, U.S.A.). The color was developed with a substrate for peroxidase, 2 mM diaminobenzidine solution containing 0.003% H_2O_2. For color development of alkaline phosphatase, the substrate consisting of 66 μl of 50 mg/ml of nitrobluetetrazolium and 33 μl of 50 mg/ml of 3-bromo-4-chloro-5-indolyl-phosphate in 10 mM Tris-HCl pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$ was used.

Purification of IgE-reactive proteins from German cockroach extract

Cockroach extract was sequentially fractionated using the FPLC system (Pharmacia, Uppsala, Sweden) by ion exchange chromatography (Resource Q), gel filtration (Superdex 200) and hydrophobic chromatography (Phenyl-Superose). The extract was dialyzed against 25 mM Tris-HCl buffer, pH 8.0, and applied to the Resource Q column. The column was washed and eluted stepwise using a buffer containing 0.1 M, 0.2 M, 0.3 M, 0.5 M and 1.0 M NaCl. Each peak was dialyzed against 25 mM Tris-HCl, pH 7.5 at 4°C using Spectra/por 1 membrane (Baxter, McGaw Park, IL, U.S.A.) with a molecular cut-off of 6−8 KD. IgE-reactivity of the proteins in each peak was assessed by SDS-PAGE and immunoblot analysis. The reactive fractions were applied to a column of

Superdex 200 equilibrated with 25 mM Tris-Cl, pH 7.5. Eluted peaks were concentrated by using Centricon (Amicon, Beverly, MA, USA), and dialyzed against 10 mM potassium phosphate buffer, pH 7.4 containing 0.5 M ammonium sulfate. The IgE-reactive fractions were applied to a Phenyl-Superose column equilibrated with an identical to the previous buffer. The column was eluted with 10 mM potassium phosphate buffer, pH 7.4 containing a decreasing linear gradient concentration of ammonium sulfate from 0.5 M to 0.0 M. Protein fractions and IgE-reactivities were assessed by SDS-PAGE and immunoblot analysis.

N-terminal amino acid sequencing

N-terminal amino acid sequencing analysis was performed by Edman degradation method using a solid-phase sequenator from Precise Protein Sequencing System (Applied Biosystems, Foster City, CA, U.S.A.).[14] The phenylthiohydantoin derivatives of amino acids were identified by high performance liquid chromatography. Microsequencing analysis was carried out at the Korea Basic Science Center, Seoul, Korea.

RESULTS

Total IgE and German cockroach-specific IgE levels

Both total and specific IgE levels in sera of immunized mice were gradually increased with the highest levels at 9 weeks after the first immunization measured by ELISA. The total IgE concentration

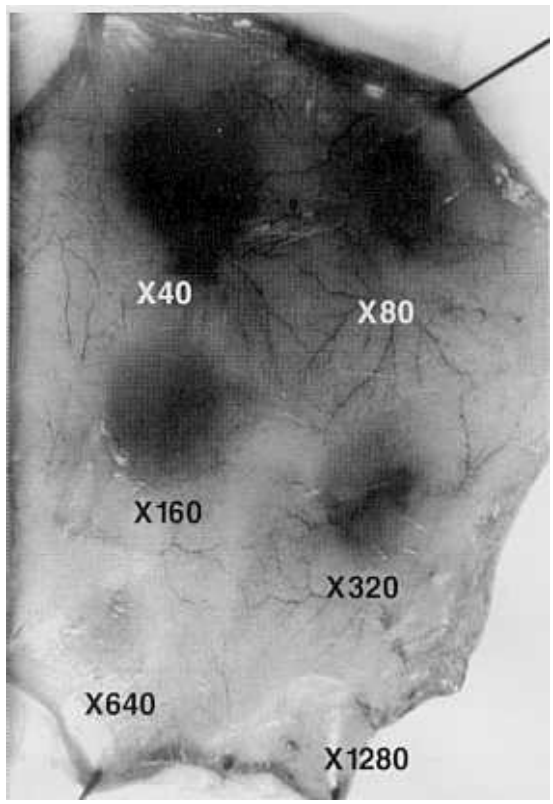

Fig. 2. Passive cutaneous anaphylaxis (PCA) reaction on a rat skin is shown using immune mouse sera and German cockroach whole body extract. The PCA titer was 1 : 640 with sera obtained after 9 weeks of immunization.

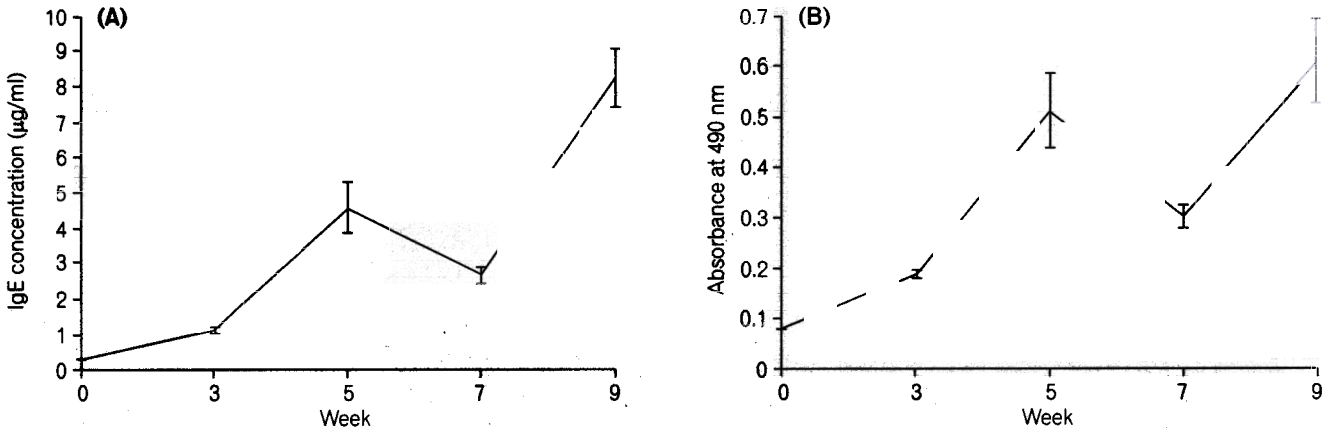

Fig. 1. Increased levels of total IgE and specific IgE in mice immunized with German cockroach whole body extract were noticed by IgE-ELISA. (A) Total IgE was increased from 0.30 μg/ml before immunization to 8.38 μg/ml after 9 weeks of immunization. (B) Mean absorbance of German cockroach-specific IgE increased from 0.08 to 0.60 as a result of immunization.

(mean±standard deviation) was 0.30±0.02 μg/ml before immunization, 1.14±0.08 μg/ml at 3 weeks, 4.57±0.73 μg/ml at 5 weeks, 2.67±0.02 μg/ml at 7 weeks and 8.38±0.82 μg/ml at 9 weeks after the first immunization (Fig. 1A). The mean absorbance± standard deviation at 490 nm of the specific IgE was 0.08±0.02 before immunization, 0.18±0.07 at 3 weeks, 0.51±0.70 at 5 weeks, 0.30±0.21 at 7 weeks and 0.60±0.80 at 9 weeks after the first immunization (Fig. 1B).

Passive cutaneous anaphylaxis

Anti-cockroach IgE-mediated PCA reactions were distinctly visualized with German cockroach extract, indicating allergenicity of the extract in BALB/c mice. Blue spots larger than 5×5 mm on the inner side of rat skin were determined to be positive. The PCA titer was 1 : 640 with sera of 9 weeks after the first immunization, and a negligible blue spot was detected with 1 : 1,280 diluted sera (Fig. 2).

IgE-immunoblot analysis

Using pooled sera of immune BALB/c mice, IgE-reacting protein bands were demonstrated with MWs of >107, 90, 82, 66, 60, 50, 43 and 36 KD on immunoblot analysis (Fig. 3). The reactive bands with MWs of 90, 66, 50, 43 and 36 KD were more prominent than other bands. No IgE-reactive bands were noticed in the control sera. IgE-reactive protein bands were visualized at MWs of >107, 90, 66, 60, 50, 43 and 36 KD on immunoblot analysis using pooled sera of skin test positive humans to German cockroach extract (Fig. 3). The bands with MWs of 90, 66, 50, 43 and 36 KD were recognized prominently in both the immunized BALB/c mice and the skin test positive humans on IgE-immunoblot analysis, which indicated that the same IgE-reactive proteins are recognized by humans and mice sensitized with German cockroaches.

Purified IgE-reactive proteins

Two IgE-reactive proteins with MWs of 36 KD and 66 KD were purified. The protein with a MW of 36 KD was eluted at 0.1 M NaCl from a column of Resource Q. The protein was contained in fraction 3 on a column of Superdex 200, and in the 0.0 M

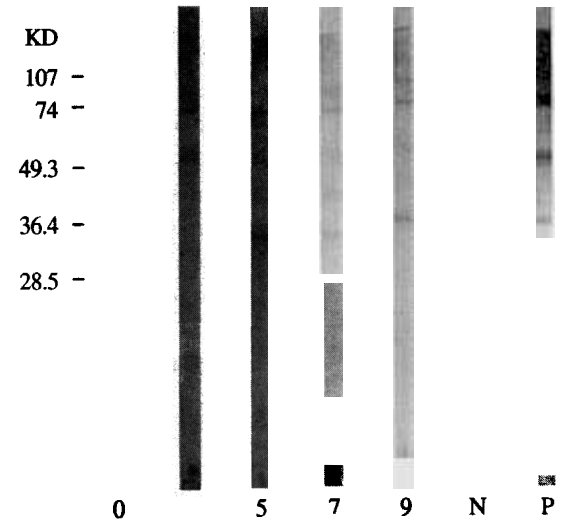

Fig. 3. IgE-reactive protein bands are demonstrated with MWs of >107, 90, 82, 66, 60, 50, 43 and 36 KD using pooled sera obtained from immune BALB/c mice from 3 to 9 weeks of immunization (0, 3, 5, 7, 9) by IgE-immunoblot analysis. No IgE-reactive bands were noticed using the control sera (N). IgE-reactive protein bands were visualized at MWs of >107, 90, 66, 60, 50, 43 and 36 KD on the immunoblot analysis using pooled sera of skin test positive humans to German cockroach extract (P). The bands with MWs of 90, 66, 50, 43 and 36 KD were recognized prominently in both immunized BALB/c mice and skin test positive humans.

ammonium sulfate eluted fraction on a column of Phenyl-Superose (Fig. 4A). The other protein with a MW of 66 KD was eluted at 0.3 M NaCl from a column of Resource Q. The protein was contained in fraction 2 on a column of Superdex 200, and was eluted at 0.0 M ammonium sulfate concentration on a column of Phenyl-Superose (Fig. 4B). Purified proteins with MWs of 36 KD and 66 KD were reacted with IgE in both immunized BALB/c mice and skin test positive humans by immunoblot analysis (Fig. 5).

On the other hand, a monoclonal antibody against Bla g 2 provided by Dr. M. D. Chapman reacted strongly with a purified protein with a MW of 36 KD by ELISA.

N-terminal amino acid sequence

N-terminal amino acid sequencing of an IgE-reactive protein with a MW of 36 KD was not possible because the N-terminal amino acid of the protein was blocked. N-terminal amino acid sequence of the protein with a MW of 66 KD was identified

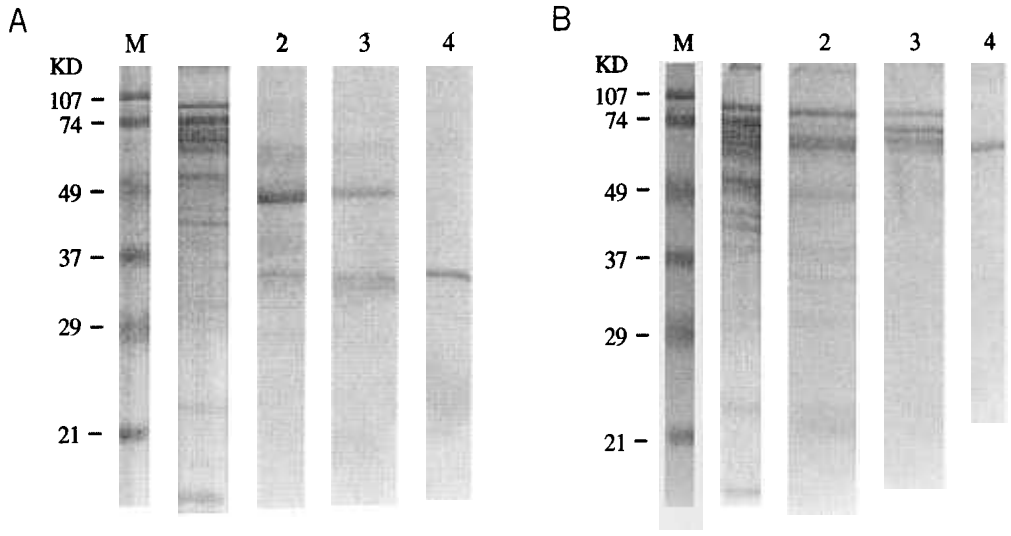

Fig. 4. Purification of IgE-reactive proteins from German cockroach extract: (A) lane 1, crude extract; lane 2, 0.1 M NaCl eluted fraction of crude extract from ion-exchange chromatography on a column of Resource Q (lane 1); lane 3, fraction from the gel filtration of an eluted fraction with 0.1 M NaCl (lane 2) on a column of Superdex 200; lane 4, fraction containing an IgE-reactive protein with a MW of 36 KD eluted with 10 mM potassium phosphate buffer (pH 7.4) containing 0.0 M ammonium sulfate on a column of Phenyl-Superose, (B) lane 1, crude extract; lane 2, 0.3 M NaCl eluted fraction of crude extract from ion-exchange chromatography on a column of Resource Q (lane 1); lane 3, fraction from the gel filtraton of an eluted fraction with 0.3 M NaCl (lane 2) on a column of Superdex 200; lane 4, fraction containing an IgE-reactive protein with a MW of 66 KD eluted with 10 mM potassium phosphate buffer (pH 7.4) containing 0.0 M ammonium sulfate on a column of Phenyl-Superose.

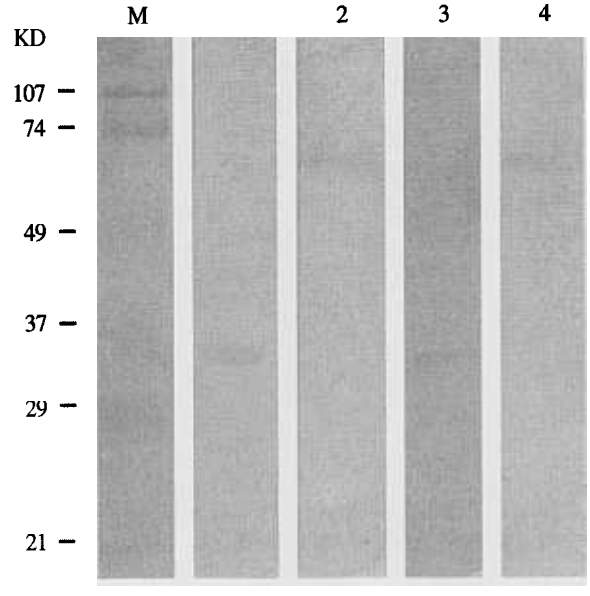

Fig. 5. IgE-immunoblot analysis of purified IgE-reactive proteins with MWs of 36 KD (lane 1, 3) and 66 KD (lane 2, 4) using pooled sera of immunized BALB/c mice (lane 1, 2) and skin test positive humans (lane 3, 4).

to be Val-Thr-Leu-Lys-Lys(Val)-Met-Ile-Lys-Thr-Phe-Tyr. No homologous protein was found through a search of GenBank.

DISCUSSION

Previous studies from various geographic areas have documented that cockroach allergens are present in house dust and that they are significant etiologic factors in allergic asthma, particularly in large urban areas. Cockroach-specific IgE were routinely measured in inner-city Chicago residents with asthma in the U.S.A.[15] Levels of airborne cockroach allergens in low-cost housing in Strasbourg in France was high, so they recommended that patients with asthma living in housing prone to cockroach infestation in urban areas of Europe should be evaluated for sensitization and exposure to cockroach allergens.[16] Cockroach allergen has also been considered as an important allergen in house dust for asthmatic children in Korea.[9]

Several investigations based mostly on immunoblot analysis data have identified allergens or IgE-reactive

components in German cockroach extract as follows. Cockroach-sensitive individuals revealed IgE-binding bands with apparent molecular weights of 36 and 80 KD.[17] Smith et al. revealed 17 IgE-reactive bands with MWs from 14 to 263 KD in 16 serum specimens by immunoblot analysis.[18] Musmand et al. reported that protein bands at 67, 60, 50, 45 and 36 KD bound IgE in more than 50% of the sera tested.[8] Wu et al. reported that the components with apparent MWs of 60, 52, 49, 38 and 12 KD from both American and German cockroaches bound to IgE.[19] German cockroach glutathione S-transferase (GST) purified from whole body extract was reported to show excellent IgE-binding activity, and the immune response to GST plays an important role in allergic diseases.[20] Although Bla g 1 (25−30 KD) and Bla g 2 (36 KD) had been previously documented as major allergens, Lee et al. insisted that those could not be the absolute indicators of German cockroach sensitization and parameters of environmental control because they found many other IgE-reactive bands besides Bla g 1 and Bla g 2 with higher MWs on immunoblot analysis.[9] This study using immune mouse sera showed both the similarities and differences compared with previous results obtained using human sera. The IgE-reactive protein bands at 90, 66, 50, 43 and 36 KD were evident, but others were not. Proteins with relatively high MWs were observed distinctly on immunoblot analysis, implicating genetic differences in immune reactions, especially IgE-reactivities between mice and humans.

Recently, molecular cloning of major German cockroach allergens have been reported. Arruda et al. performed a cloning of Bla g 2 gene, and elucidated that the DNA sequence was homologous to the aspartic protease.[21] They have also cloned Bla g 4 (ligand binding protein or calycin), Bla g 5 (glutathione transferase) and Bla g 6 (troponin). Recombinant Bla g 2, Bla g 4 and Bla g 5 have been over-expressed in bacteria and used for immunologic tests for measuring serum IgE. The results showed that recombinant allergens retain biologic activity, and suggest that cocktails of two to four recombinant allergens could be used for diagnostic or therapeutic purposes.[2] Helm et al. have isolated and characterized a major allergen, that is a MW of 90 KD with multiple IgE binding domains (Bla g Bd90K).[22] A purified protein with a MW of 36 KD, which was identified as Bla g 2, reacted prominently on IgE-immunoblot analysis with immune mouse and human sera in this study. The other purified protein would be a novel German cockroach allergen. Mouse immune sera could be useful to identify or purify allergens with relatively high MWs, and to clone the corresponding allergen genes.

Experimental asthma in a guinea pig model was developed by room-air contamination with cockroach allergen.[23] The development of anaphylactic cockroach sensitivity in guinea pigs was dependent on the levels of cockroach antigen exposure during sensitization, and the cockroach antigen-sensitized animals showed antigen-specific airway inflammation along with airway smooth muscle contractions.[24] Chen et al. immunized a guinea pig and they found leukotrienes appeared to play a significant role in the cockroach antigen-specific airway contractions.[25] Until now, BALB/c mice have not been used in studies on cockroach allergens. As shown in this study, the immunized mice showed antibody responses to major IgE-binding German cockroach proteins similar to sensitized humans, and immune mouse sera were shown useful to identify IgE-reactive allergenic components in German cockroach extract. Experimental mice of a certain strain could be useful as an animal model of allergy, not only for providing antiserum but also for the development of anaphylactic cockroach sensitivity in the future.

In summary, IgE-reactive German cockroach proteins were identified with molecular weights of 90, 66, 50, 43 and 36 KD by immunoblot analysis in both immune BALB/c mice and sensitized humans. The most prominently reactive proteins in mice were purified by FPLC. A purified IgE-reactive protein was identified as Bla g 2, which is a specific German cockroach allergen previously known in humans. The other one was a novel IgE-reactive component in German cockroach extract. Studies are needed to perform cloning of the appropriate allergen gene and to provide further characterization in the near future.

REFERENCES

1. Lee KY, Kim KE, Jeaung BJ, Lee SY, Kim DS. An immunological study on allergic antigenicity of *Blattella germanica* in Korea. J Korean Soc Allergy 1991;11:30-8.
2. Chapman MD, Smith AM, Vailes LD, Arruda LK. Defined epitopes: in vivo and in vitro studies using recombinant allergens. Int Arch Allergy Immunol 1997;113:

102-4.
3. Bernton HS, Brown H. Insect allergy: Preliminary studies of the cockroach. J Allergy Clin Immunol 1964;35:506-13.
4. 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;82:736-40.
5. Lee KY, Kim KE. A study on the method of exclusion of unnecessary allergens from the vaccines for immunotherapy. J Korean Soc Allergy 1988;8:150-64.
6. Kang BC, Chang JL, Johnson J. Characterization and partial purification of the cockroach antigen in relation to house dust and house dust mite (*D.f.*) antigens. Ann Allergy 1989;63:207-12.
7. Stankus RP, O'Neil CE. Antigenic/allergenic characterization of American and German cockroach extracts. J Allergy Clin Immunol 1988;81:563-70.
8. Musmand JJ, Horner WE, Lopez M, Lehrer SB. Identification of important allergens in German cockroach extracts by sodium dodecylsulfate-polyacrylamide gel electrophoresis and western blot analysis. J Allergy Clin Immunol 1995;95:877-85.
9. Lee SY, Kim DS, Kim KE, Jeaung BJ, Lee KY. IgE binding patterns to German cockroach whole body extract in Korean atopic asthmatic children. Yonsei Med J 1998;39:409-16.
10. Pollart SM, Mullins DE, Vailes LD, Hayden ML, Platts-Mills TA. Sutherland WM, et al. Identification, quantitation, and purification of cockroach allergens using monoclonal antibodies. J Allergy Clin Immunol 1991;87:511-21.
11. Arruda LK, Vailes LD, Benjamin DC, Chapman MD. Molecular cloning of German cockroach (*Blattella germanica*) allergens. Int Arch Allergy Immunol 1995;107:295-7.
12. Kawai K, Konishi K. Fundamental studies on chironomid allergy III. Allergen analyses of some adult Japanese chironomid midges (Chironomidae, Diptera). Jpn J Allergol 1988;37:944-51.
13. Ree HI, Lee SH, Kim YK, Jeon S, Chang JK, Kim YS. Identification and characterization of allergens of *Chironomus flaviplumus* adults (Chironomidae, Diptera) in mice. Korean J Parasitol 1996;34:35-47.
14. Celis JE. Cell Biology, A laboratory handbook. 2nd ed. New York: Academic Press; 1998. vol. 4. p.514-5.
15. Kang BC, Johnson J, Veres-Thorner C. Atopic profile of inner-city asthma with a comparative analysis on the cockroach-sensitive and ragweed-sensitive subgroups. J Allergy Clin Immunol 1993;92:802-11.
16. De Blay F, Sanchez J, Hedelin G, Perez-Infante A, Verot A, Chapman M, et al. Dust and airborne exposure to allergens derived from cockroach (*Blattella germanica*) in low-cost public housing in Strasbourg (France). J Allergy Clin Immunol 1997;99:107-12.
17. Helm RM, Burks W, Williams LW, Milne DE, Brenner RJ. Identification of cockroach aeroallergens from living cultures of German or American cockroaches. Int Arch Allergy Immunol 1993;101:359-63.
18. Smith DL, Baldwin LD, Amend MJ, Kordash TR. Biologic potency and immunoblotting studies of extracts of three cockroach species. Ann Allergy Asthma Immunol 1995;75:317-23.
19. Wu CH, Luo SF, Wong DW. Analysis of cross-reactive allergens from American and German cockroaches by human IgE. Allergy 1997;52:411-6.
20. Arruda LK, Vailes LD, Platts-Mills TA, Hayden ML, Chapman MD. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (*Blattella germanica*). J Biol Chem 1997;272:20907-12.
21. Arruda LK, Vailes LD, Hayden ML, Benjamin DC, Chapman MD. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses. J Biol Chem 1995;270:31196-201.
22. Helm R, Cockrell G, Stanley JS, Brenner RJ, Burks W, Bannon GA. Isolation and characterization of a clone encoding a major allergen (*Bla g* Bd90K) involved in IgE-mediated cockroach hypersensitivity. J Allergy Clin Immunol 1996;98:172-80.
23. Kang BC, Zhou K, Lai YL, Hong CB. Experimental asthma developed by room air contamination with cockroach allergen. Int Arch Allergy Immunol 1996;111:299-306.
24. Zhou D, Chen G, Kim JT, Lee LY, Kang BC. A dose-response relationship between exposure to cockroach allergens and induction of sensitization in an experimental asthma in Hartley guinea pigs. J Allergy Clin Immunol 1998;101:653-9.
25. Chen G, Zhou D, Kim JT, Wu Z, Lee LY, Kang BC. Contractile responses to allergen and cholinergic stimulation in bronchus and trachea of cockroach allergen-sensitized guinea pigs. Int Arch Allergy Immunol 1998;116:132-9.