Sequence variation and IgE-binding epitope of Bla g 5, the German cockroach major allergen

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Sequence variation and IgE-binding epitope of Bla g 5, the German cockroach major allergen

Directed by Professor Tai-Soon Yong

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This certifies that the Master's Thesis of
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The Graduate School
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대학원에 입학한지가 엊그제 같은데 어느새 몇 달 후의 졸업을 앞두고 있다는 사실이 믿기지 않습니다. 2년이라는 시간 동안 부족한 저를 이끌어주신 용태순 교수님께 진심으로 감사의 마음을 전하고 싶습니다. 그리고 바쁘신 와중에 논문심사를 맡아주시고 조언을 아끼지 않으신 박중원 교수님과 이봉기 교수님께도 감사드립니다.

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Sequence variation and IgE-binding epitope of Bla g 5,
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(Directed by Professor Tai-Soon Yong)

Cockroaches are associated with human habitation and are present in numerous regions throughout the world. Cockroach infestations have been linked to allergic diseases such as asthma in humans. Bla g 5 is the major cockroach allergen that the IgE response value of Bla g 5 is higher than that of any other cockroach allergens. Bla g 5, a 23 kDa glutathione *S*-transferase (GST), is believed to play a role in the detoxification of endogenous and xenobiotic toxic compounds. Although several cockroach allergens have been identified and cloned, information regarding IgE-binding epitopes on the B and T cells of cockroach allergens is limited.

In order to analyze linear IgE binding epitopes of Bla g 5 and full-length and 5 peptide fragments (A, 1-100 amino acid residue; B, 91-200; Ba, 1-125; Bb, 1-150; and Bc, 1-175) were expressed. Twelve (37.5%) of 32 sera from cockroach-sensitized subjects showed positive IgE reactivity to recombinant Bla g 5 (rBla g 5). Six strong positive sera were selected for the epitope study.

Recombinant proteins not containing 176-200 amino acid residues were not react to sera from cockroach sensitive individuals, while rBla g 5 and B fragments were recognized by those sera. These results suggest that the C-terminal region between 176-200 amino acid residues contains most of the IgE-binding epitopes. Despite strong IgE reactivity to rBla g 5, pooled serum from 5 cockroach-sensitized patients did not show IgE reactivity to every synthetic peptide.

These results suggest the possibility that Bla g 5 may have a conformational epitopes in the C-terminal region. GST is the important target for the development of vaccines and diagnostic tools for allergic diseases because of its high cross-reactivity among insect species. This study will help recombinant mutated allergen research on immunotherapy for cockroach allergen and possibly other insect allergens.

Key words: cockroach allergen, epitope, glutathione-*S*-transferase

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I. INTRODUCTION

Cockroaches are associated with human habitation and are present in numerous regions throughout the world. They transport microbes on their bodies, some of which are potentially dangerous to humans[1]. Cockroach infestations have been linked to allergic diseases such as asthma in humans[2]. The most common habitable species of cockroaches are *B. germanica* (German cockroach) and *Periplaneta*. In Seoul, Korea, cockroaches were founded in 62% (107/174) of homes, i.e., 63 with *B. germanica*, 60 with *Periplaneta* spp., which included 16 homes with double infestations[3].

Six allergens of *B. germanica* have been cloned and immunologically characterized: Bla g 1, Bla g 2, Bla g 4, Bla g 5, Bla g 6, and Bla g 7. Bla g 1 has an unusual structure that consists of 7 tandem repeats of 100 amino acid residues and is thought to be related to the digestive organs[4]. It has been reported that Bla g 1 and Per a 1 are cross-reactive and both show 30%

sequence homology[5]. Bla g 2 is an inactive zinc-binding aspartic protease[6, 7]. Bla g 4 is a lipocalin only produced in adult male accessory reproductive glands and transferred to females during copulation[8,9]. Bla g 5 has been identified as a 23 kDa glutathione *S*-transferase (GST) that is believed to play a role in the detoxification of endogenous and xenobiotic toxic compounds[10]. Bla g 6 is a calcium-binding subunit of the troponin complex that regulates skeletal and cardiac muscle contraction[11]. Bla g 7 has a high degree of similarity to tropomyosin, a type of muscle protein[12]. IgE binding frequencies of cockroach allergens have been reported to be 30-50% for Bla g 1, 60% for Bla g 2, 40-60% for Bla g 4, 70% for Bla g 5, 14% for Bla g 6, and 16% for Bla g 7[13].

GSTs are a family of enzymes that carry out various roles, such as the metabolism of toxic substances, protect cells from oxidative stress, and transport several hydrophobic compounds[14]. GST allergen production could be upregulated by the abuse of insecticides. Cytosolic GSTs are classified by classes, as follows: alpha, beta, delta, kappa, mu, omega, pi, sigma, theta, and zeta[15]. The interclass sequence identity is generally lower than 30%[16]. Insect GSTs are classified as 3 distinct classes: delta, sigma, and epsilon[17]. Insect sigma-class GSTs have been discovered in *Drosophila melanogaster*[18], *Bemisia tabaci*[19], *Solenopsis invicta*[20], *Bombyx mori*[21], and *Hyphantria cunea*[22]. Insect delta-class GSTs have been characterized in *Anopheles gambiae*[23], and *Anopheles dirus*[24].

Bla g 5 is such a potent IgE reactive allergen that as little as 3 pg of the recombinant protein is sufficient to cause positive immediate skin test results and IgE responses in 70% of cockroach-allergic patients[10]. The IgE response value of Bla g 5 is higher than that of any other cockroach allergens. The IgE response of Bla g 5 (sigma class) was determined to be higher than delta class GST[25]. Bla g 5 and delta class GST have low amino acid sequence identity (14%) and low cross-reactivity[26]. Bla g 5 was showed to have an amino acid sequence homology of 42-45% to other insect GSTs, and 28% homology to house dust mite GST, Der p 8, *Dermatophagoides pteronyssinus*[10]. The IgE cross-reactivity of insect GSTs had been suggested but this has never been studied.

The identifying IgE binding sites of cockroach allergens is essential in order to understand the antigenic structures and development of novel immunotherapies. Although several cockroach allergens have been identified and cloned, information regarding IgE-binding epitopes on the B and T cells of cockroach allergens is limited. In the present study, we expressed recombinant Bla g 5 (rBla g 5) and produced 5 Bla g 5 peptide fragments, on which IgE reactivity was compared.

II. MATERIALS AND METHODS

1. Cockroach

German cockroaches, *B. germanica*, have been were in the Korea National Arthropods of Medical Importance Resource Bank, Institute of Tropical Medicine, Yonsei University College of Medicine, Seoul, Korea since 1999. Mouse food was used as the diet.

2. Subjects and serum samples

Sera were obtained from 32 patients (21 males, 11 females; mean age, 36.8; range, 7 to 58 years) being treated at the Allergy Clinic of Severance Hospital (Yonsei University College of Medicine, Seoul, Korea). The allergy diagnosis was based on case history and skin prick test results. Sera from patients were tested for the presence of IgE antibodies for *B. germanica* by using the Uni-CAP system (Phadia, Uppsala, Sweden). Sera with CAP results higher than 0.7 kU/l were used for the current study. Fifteen healthy subjects were used as a control.

3. Cloning and sequencing of Bla g 5 cDNA

Total RNA from adult cockroaches was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized and reverse transcribed from 11.5 μl of

total RNA. The primers were designed according to published Bla g 5 cDNA (U92412) as 5'-GGATCCGTATAAACTGACATAC-3' (forward) and 5'-CTCGAGCAGATCTGTAGGAGGA-3' (reverse) which contained restriction enzyme cleavage sites. Underlines indicate *Bam*H Ⅰ site for the forward primer and *Xho* Ⅰ site for the reverse primer. PCR was performed with the initial denaturation at 95℃ for 5 min, 35 cycles at 94℃ for 30 sec, 53℃ for 30 sec, 72℃ for 1 min, and 72℃ for 9 min. The PCR products were separated by 1% agarose gel electrophoresis and then purified. PCR amplified cDNA was ligated into a pGEM-T Easy Vector (Promega, Madison, WI, USA) and nucleotide sequences were analyzed by Solgent, Inc. Sequencing Services (Daejeon, Korea). Alignment of DNA sequences were performed using the BioEdit program (www.nbio.ncsu.edu/BioEdit). A total of 57 DNA clone sequences were translated into amino acid sequences and compared with previously reported Bla g 5.

4. Cloning and sequencing of Bla g 5 peptide fragments

In order to analyze linear IgE binding epitopes of Bla g 5, full-length and 5 peptide fragments were amplified with specific primers (A, 1-100 amino acid residue; B, 91-200; Ba, 1-125; Bb, 1-150; and Bc, 1-175) (Fig. 1, Table 1). The PCR was performed with the initial denaturation at 95℃ for 5 min, 35 cycles at 94℃ for 30 sec, 53℃ for 30 sec, 72℃ for 1 min, and 72℃ for 9 min.

Dominant Bla g 5 cDNA was used as a template. The PCR product was separated by 1% agarose gel electrophoresis and then purified. PCR amplified cDNA was ligated into pGEM-T Easy Vector (Promega) and nucleotide sequences were analyzed by Solgent, Inc. Sequencing Services.

5. Expression and purification of rBla g 5 and peptide fragments in *E. coli*

rBla g 5 was ligated into *Bam* HⅠ-*Xho*Ⅰ digested pET-28b(+) vector (Novagen, Madison, WI, USA). Expression of rBla g 5 and the 5 peptide fragments was induced in *E. coli* strain BL21 (DE3) with 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) at 37℃ for 4 hr. The culture was harvested and the cells were resuspended in the binding buffer (6 M urea, 5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9) and lysed using a French press. Recombinant proteins were purified using affinity column chromatography with a pET His-Tag System with Ni-NTA Agarose (Qiagen, Valencia, CA), and protein concentrations were measured with a Bradford assay (Biorad, Hercules, CA, USA) using bovine serum albumin (BSA) as a standard. Protein purity was monitored with 4-20% Tris-Glycine gel electrophoresis (Invitrogen) and stained with Coomassie Blue G-250. Purified recombinant proteins were used for ELISA.

6. IgE-binding reactivity and IgE epitope analysis of rBla g 5 and peptide fragments

The reactivity of serum IgE antibodies to rBla g 5 and the 5 peptide fragments was examined by ELISA in duplicate. The micro titer plate was coated with 10 μg/ml of rBla g 5 and peptide fragments. Wells were washed with washing buffer, phosphate buffer saline (PBS), pH 7.4, which contained 0.05% Tween 20 (PBST). Blocking buffer (PBST containing 3% skim milk) was dispensed to each well and incubated at 37℃ for 1 hr. After washing, 1:4 diluted patient sera were dispensed into the wells, and incubated at 37℃ for 1 hr. Biotinylated goat anti-human IgE (Vector, Burlingame, CA) (1:1000) and streptavidin-peroxidase (Sigma, St Louis, MO, USA) were utilized for detection. The signal was developed by adding 3,3',5,5',-tetramethylbenzidine (TMB) (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) and 0.5 M H_2SO_4 was used as a stop solution. Absorbance was measured at 450 nm with an automatic microplate reader (Tecan, Salzburg, Austria).

7. Overlapping, synthetic peptides and dot blot immunoassay

Fifteen individual amino acid peptides near the C-terminal region of Bla g 5, with 10 amino acid overlaps were designed. The 6 peptides (>80% purity) were synthesized from Peptron (Daejeon, Korea) (Peptide 1, 161-175 amino acid residues; Peptide 2, 166-180; Peptide 3, 171-185; Peptide 4, 176-190; Peptide 5, 181-195; Peptide 6, 186-200). To detect IgE binding epitopes of

Bla g 5, 2 μg of rBla g 5 or 6 synthetic peptides were applied onto nitrocellulose membrane (Novex, San Diego, CA, USA) and left overnight. BSA was used as a control. The dot blots were blocked overnight at 4℃ with 1% BSA in PBST, which were incubated for 1 hr with 1:4 diluted pooled serum of 5 cockroach-sensitized patients. Incubation with secondary antibodies was performed with 1:1000 dilution of biotinylated goat anti-human IgE (Vector) in PBST for 1 hr. The reactions were developed using the nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) system (Promega) as a substrate.

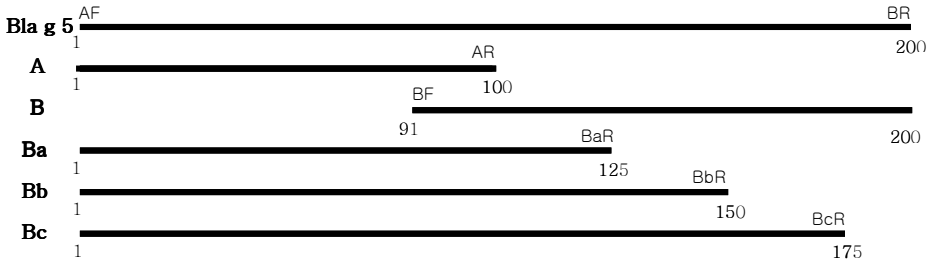

Figure 1. Schematic representation of Bla g 5 fragments for epitope mapping.

Table 1. Primers used in PCR and subcloning for expression

Primer	Sequence*
Bg5-AF	5’-GGATCCGTATAAACTGACATAC-3’
Bg5-AR	5’-CTCGAGAGCAATGGCAGCCCTGA-3’
Bg5-BF	5’-GGATCCGACCATCTCTGACTTCAG-3’
Bg5-BR	5’-CTCGAGCAGATCTGTAGGAGGA-3’
Bg5-BaR	5’-CTCGAGGTAAGGAATGGTTTCCTTC-3’
Bg5-BbR	5’-CTCGAGGTCTGCCCATGTCAGCTTT-3’
Bg5-BcR	5’-CTCGAGCAAATTGGGTTGATTGGCC-3’

*Restricted enzyme sites are underlined. *Bam*HⅠ: GGATCC, *Xho*Ⅰ:

CTCGAG

III. RESULTS

1. Amino acid sequence variation of Bla g 5 cDNA

A total of 57 clones of Bla g 5 were obtained by RT-PCR. Amino acid sequence variations of Bla g 5 were compared with previously reported partial sequences of Bla g 5 (accession No. U92412) (Fig. 2). Several amino acid sequence changes were identified at 8 different positions. All 57 clones showed a single amino acid sequence change at position 39, Leu to Lys. The DNA sequence of Bla g 5 cDNA dominant clones (50/57, 87.7%) was deposited in the GenBank database (accession No. EF202178). The dominant form was used for further study.

```
                10        20        30        40        50        60        70        80        90       100
                .         .         .         .         .         .         .         .         .         .
Bla g 5  YKLTYCPVKALGEPIRFLLSYGEKDFEDYRFQEGDWPNLKPSMPFGKTPVLEIDGKQTHQSVAISRYLGKQFGLSGKDDWENLEIDMIVDTISDFRAAIA
5-1      ..........................................K.....................................................
5-5      ..........................................K.....................................R...............
5-12     ..........................................K.....................................R...............
5-24     ..........................................K.....................................................
5-94     ..........................................K.....................................................T.
5-102    ..............................H...........K.....................................................
5-104    ..........................................K.....................................................
5-115    ..........................................K.....................................................

                110       120       130       140       150       160       170       180       190       200
                .         .         .         .         .         .         .         .         .         .
Bla g 5  NYHYDADENSKQKKWDPLKKETIPYYTKKFDEVVKANGGYLAAGKLTWADFYFVAILDYLNHMAKEDLVANQPNLKALREKVLGLPAIKAWVAKRPPTDL
5-1      ..................................................................................... (50/57) [99.5%]
5-5      ..................................................................................... ( 1/57) [99.0%]
5-12     ..................................................................................... ( 1/57) [99.0%]
5-24     ..........................................................................R.......... ( 1/57) [99.0%]
5-94     ..................................................................................... ( 1/57) [99.0%]
5-102    ..................................................................................... ( 1/57) [99.0%]
5-104    ..........................................................S.......................... ( 1/57) [99.0%]
5-115    ..............................G...................................................... ( 1/57) [99.0%]
```

Figure 2. Amino acid sequence variations of Bla g 5. The frequency and sequence identity of each variant are shown in parentheses.

2. SDS-PAGE analysis of rBla g 5 and peptide fragments

rBla g 5 and 5 peptide fragments were subcloned into expression vector pET 28b(+) (Novagen) and were overexpressed in *E. coli* BL21. All recombinant proteins were purified from insoluble fractions. rBla g 5 and peptide fragments in proteins were separated by 4-20% Tri-Glycine gel (Invitrogen) and stained with Coomassie Brilliant Blue (Fig. 3). rBla g 5 has apparently greater molecular mass than the previously reported 23 kDa on SDS-PAGE analysis. Concentrations of proteins were estimated by Bradford assay (Biorad); rBla g 5 34,504 mg/l, A fragment 9,122 mg/l, B fragment 29,524 mg/l, Ba fragment 4,248 mg/l, Bb fragment 1,334 mg/l, and Bc fragment 5,607 mg/l, respectively.

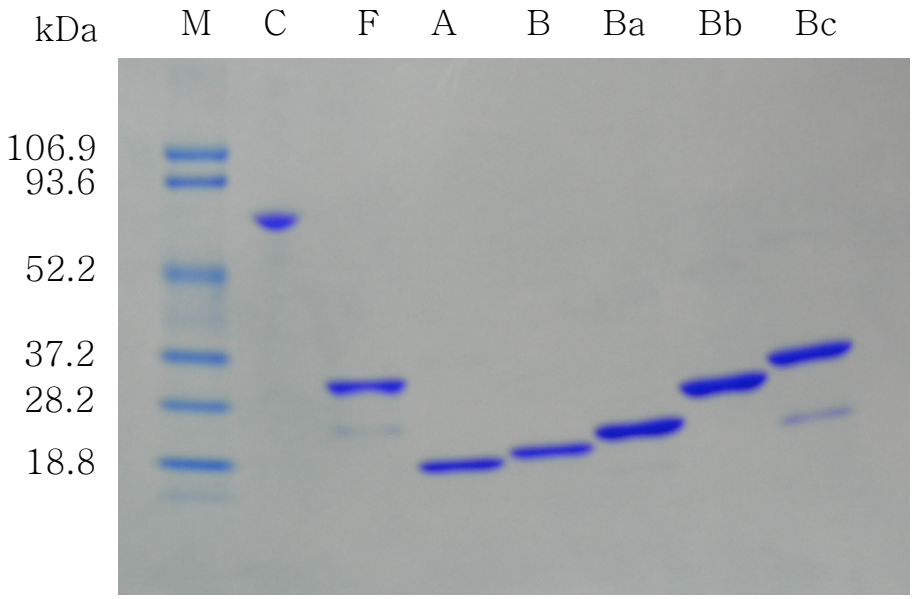

Figure 3. SDS-PAGE of expressed rBla g 5 and 5 peptide fragments. Proteins were separated on 4-20% Tris-Glycine gel and stained with Coomassie brilliant blue. Lanes, M: molecular mass marker, C: BSA, F: rBla g 5, A: rBla g 5 A fragment (1-100), B: rBla g 5 B fragment (91-200), Ba: rBla g 5 Ba fragment (1-125), Bb: rBla g 5 Bb fragment (1-150), Bc: rBla g 5 Bc fragment (1-175).

3. IgE reactivity of rBla g 5

IgE reactivity of rBla g 5 to cockroach-sensitized subjects was determined by ELISA (Fig. 4). Twelve (37.5%) of 32 sera from cockroach-sensitized subjects showed positive IgE reactivity to rBla g 5, but the titer of IgE reactivity was significantly higher in some serum samples. Six strong positive sera were selected for the epitope study. No IgE binding reactivity was observed in 15 sera from normal controls.

4. Identification of IgE-binding epitope of Bla g 5

To study the IgE epitope of Bla g 5, 6 sera that showed strong IgE reactivity to rBla g 5 and 15 healthy pooled control sera were used. Recombinant proteins not containing 176-200 amino acid residues did not react to sera from cockroach sensitive individuals, while rBla g 5 and B fragment were recognized by those sera (Figs. 5, 6). These results suggest that the C-terminal region between 176-200 amino acid residues contains most of the IgE-binding epitopes.

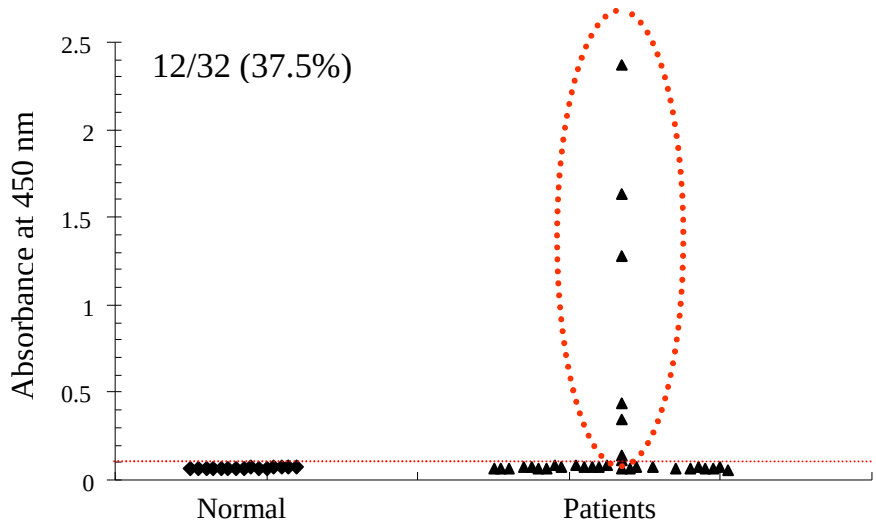

Figure 4. The IgE reactivity of human sera against rBla g 5. Normal: 15 healthy, non-allergic subjects. Patients: cockroach-sensitized 32 patients.

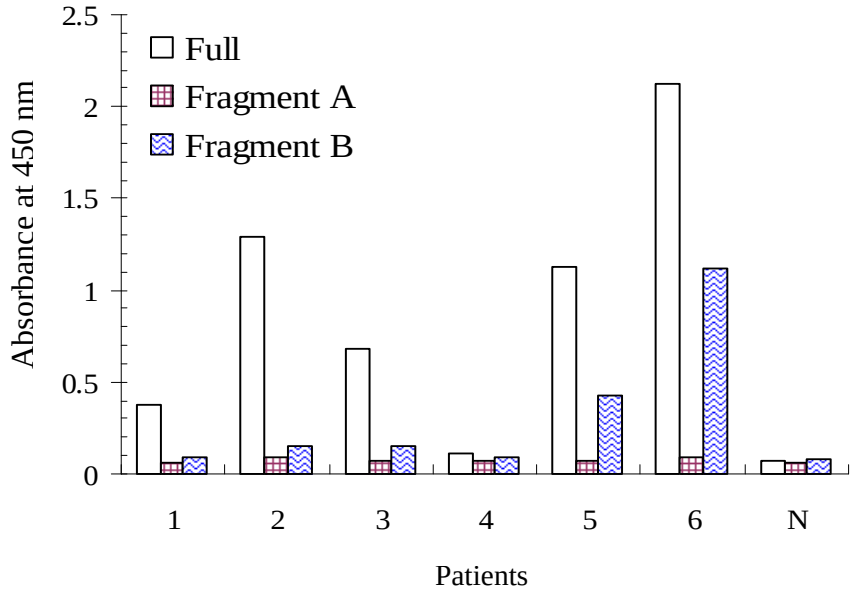

Figure 5. The IgE reactivity of rBla g 5 and peptide fragments; rBla g 5, fragment A, and fragment B. 1-6, serum samples from cockroach-sensitized patients; N, pooled serum from 15 healthy non-allergic individuals was analyzed.

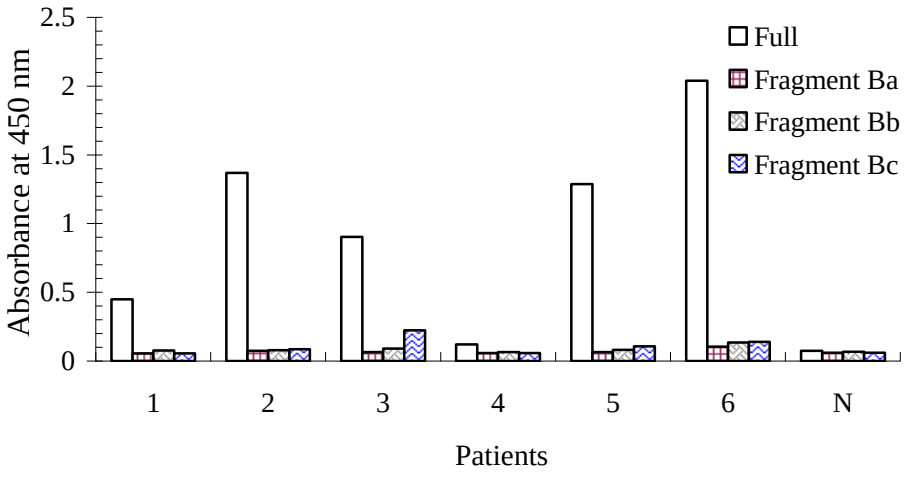

Figure 6. The IgE reactivity of rBla g 5 and peptide fragments; rBla g 5, fragment Ba, fragment Bb, and fragment Bc. 1-6, serum samples from cockroach-sensitized patients; N, pooled serum from 15 healthy non-allergic individuals was analyzed.

5. Immunodetection of Bla g 5 epitopes

The 6 synthetic peptides were synthesized for detailed IgE epitope analysis. Despite strong IgE reactivity to rBla g 5, pooled serum from 5 cockroach-sensitized patients did not show IgE reactivity to every synthetic peptide (Fig. 7).

(A)

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Bg5E161-175 (Peptide 1) : NHMAKEDLVANQPNL
Bg5E166-180 (Peptide 2) :          EDLVANQPNLKALRE
Bg5E171-185 (Peptide 3) :                   NQPNLKALREKVLGL
Bg5E176-190 (Peptide 4) :                            KALREKVLGLPAIKA
Bg5E181-195 (Peptide 5) :                                     KVLGLPAIKAWVAKR
Bg5E186-200 (Peptide 6) :                                              PAIKAWVAKRPPTDL
```

(B)

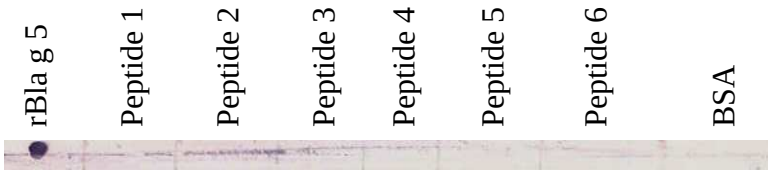

Figure 7. IgE binding to overlapping peptides. (A) Amino acids of 6 synthetic peptides containing overlapping sites that anticipated IgE binding epitopes of Bla g 5. (B) Specific IgE reactivity of dot-blotted 2 μg rBla g 5 and 2 μg synthetic peptides determined with pooled serum from 5 cockroach-sensitized patients.

IV. DISCUSSION

The presence of cockroach allergen is an important risk factor for allergic patients to visit emergency room and hospital admissions[27]. Allergens from different cockroach species have differing characteristics and some allergens are cross-reactive with house dust mite allergens. In this study, I cloned and expressed rBla g 5 from German cockroach to identify IgE binding epitope regions.

All 57 cDNA clones of Bla g 5 were 600 bp and encoded a polypeptide of 200 amino acids with a predicted molecular mass of about 23 kDa. High identity (>99%) was found between the Bla g 5 cDNA sequences (Fig. 2). The sequence polymorphisms in Bla g 5 revealed an amino acid change at position 39 from Leu to Lys compared with the previously described Bla g 5 (U92412) amino acid. These variations may contribute to regional differences and effects on the sensitization and diagnosis of allergic subjects.

I have produced rBla g 5 and it showed 37.5% IgE reactivity among sera from cockroach-sensitized patients (Fig. 4). I also designed and expressed 5 peptide fragments. ELISA results showed that the epitope of Bla g 5 was located in the C-terminal region between 176 and 200 (Figs. 5, 6). However, overlapping peptides did not show IgE binding reactivity (Fig. 7). These results suggest the possibility that Bla g 5 may have a conformational epitope in the C-terminal region. *Drosophila melanogaster* GST, which has a high

sequence homology (46.8%) with Bla g 5[10], was reported to have a large helical C-terminal domain with a wide inter-domain cleft and contained the catalytic site resides[28]. Similarly, Bla g 5 possibly has an inter/inner domain cleft structure in the C-terminal region, which could explain why the synthetic peptides did not detect IgE antibodies.

At present, experimental 3D structures are available in the Protein Data Bank for only 5% (45/829) of all allergens catalogued in the Structural Database of Allergenic Proteins (SDAP, http://fermi.utmb.edu/SDAP)[29]. A better understanding of allergen structures will help to produce successful immunotherapies.

Specific immunotherapy is the only effective treatment[30]. The mechanism of immunotherapy expands allergen-specific T_H1 immunity and suppresses T_H2 response. However, a drawback of immunotherapy based on natural extracts is the risk of IgE-mediated anaphylactic side effects. The crude extract is unpredictable because of various factors (e.g., protein degradation, endotoxin, or proteolytic enzymes). Recombinant allergens could overcome the disadvantages of crude extract with purity. Researchers suppose that immunotherapies using specific recombinant allergens or hypoallergens that mutated at IgE binding epitopes of antigens were more safe and effective. However, it may be technically difficult to obtain a recombinant allergen because the protein with correct folding[31] and small protein fragments may not only be hard to express but may also induce low levels of protective IgE/IgG

antibodies[32]. Also, for the generation of epitope-specific vaccines, detailed knowledge of antibody-binding sites (e.g., allergen epitopes) is required. Recently, an interesting study reported that *Alternaria alternate* GST mutated with 2 amino acids that estimated IgE-binding epitope region showed reduction of IgE allergenicity and airway inflammation[33]. Unfortunately, up-to-date, cockroach immunotherapy is not considered to have proven efficacy[34].

I have demonstrated that the epitope of Bla g 5 is the important target for the development of vaccines and diagnostic tools for allergic diseases because of its high cross-reactivity among insect species. This study will help recombinant mutated allergen research on immunotherapy for cockroach allergens and possibly other insect allergens.

V. CONCLUSION

1. Using molecular cloning techniques, I have identified that all 57 clones of Bla g 5 showed amino acid change at position 39 from Leu to Lys (accession No. EF202178).

2. Twelve (37.5%) of 32 allergic sera showed positive IgE reactivity to rBla g 5.

3. The C-terminal region between 176-200 amino acid residues was found to contain conformational IgE-binding epitope site of Bla g 5.

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ABSTRACT (IN KOREAN)

독일바퀴의 주요한 알레르겐인 Bla g 5의 서열 다양성과 IgE 항체 반응성

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정 경 진

바퀴는 세계 많은 지역에서 사람들과 가깝게 함께 주거하며 살아가는 곤충이다. 바퀴는 사람에게 천식과 같은 알레르기 질환을 유발하는 알레르겐을 생산한다. 바퀴 알레르겐들의 B 세포와 T 세포의 IgE-binding epitope에 대한 연구는 진단과 면역치료에 중요하지만 아직까지 연구가 미흡하다.

Bla g 5 (glutathione *S*-transferase)는 여러 다른 바퀴 알레르겐 중에서 가장 강력한 알레르겐으로 알려져 있다. Bla g 5의 IgE-binding epitope를 분석하기 위해 재조합 Bla g 5와 5개의 단편을 제작하여 대장균에서 발현시켰다 (A 단편, 1-100 아미노산; B 단편, 91-200; Ba 단편, 1-125; Bb 단편, 1-150; Bc 단편, 1-175). 바퀴에 감작된 환자들을 대상으로 재조합 Bla g 5에 대한 IgE 반응을 ELISA로 분석하였다. 총 32명의 환자 혈청 중 12명 (37.5%)에서 재조합 Bla g 5에 대하여 양성반응을 보였다. Epitope study를 위하여 12명의 환자혈청 중에서 강한 반응을 보인 6명의 환자혈청을 선택하였다. 재조합 Bla g 5와 B단편에서 6명의 환자 혈청이 IgE반응을 보인 반면에 Ba,

Bb, Bc 단편 모두에서 환자들의 IgE 반응이 나타나지 않았다. 이러한 결과를 보아 1-175 아미노산 잔기를 포함하는 재조합 단백질에서는 IgE반응이 일어나지 않았음을 알 수 있었다. Bla g 5의 176-200 아미노산을 포함하는 C-terminal 부분에 epitope를 포함하고 있을 것이라 예상되어 12개의 아미노산으로 이루어진 펩타이드를 epitope 예상부분에 6개 아미노산이 겹치게 합성하여 dot blot를 실시하였으나 IgE반응이 전혀 나타나지 않았다. 이런 결과를 통하여 Bla g 5의 IgE-binding epitope는 C-terminal 에 가까운 176-200 아미노산 잔기를 포함하는 부분에 conformational epiope로서 존재하고 있다는 것으로 예상된다.

이 연구결과는 바퀴 알레르겐 뿐만 아니라 여러 다른 알레르겐에 대한 특정한 알레르기 백신을 위한 좀더 안정되고 믿을 수 있는 면역치료제 및 진단개발에 이용될 수 있다.

핵심 되는 말: 독일바퀴, 알레르겐, 면역치료제

PUBLICATION LIST

1. Sequence Polymorphism of Major German Cockroach Allergens Bla g 1, Bla g 2, Bla g 4, and Bla g 5. Int Arch Allergy Immunol 2008;145:1-8.

2. Allergenicity of Sigma and Delta Class Glutathione *S*-Transferases from the German Cockroach. Int Arch Allergy Immunol 2009;148:59-64.