cDNA Cloning and Identification of a Major Allergen of *Humulus japonicus* Pollen

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cDNA Cloning and Identification of a Major Allergen of *Humulus japonicus* Pollen

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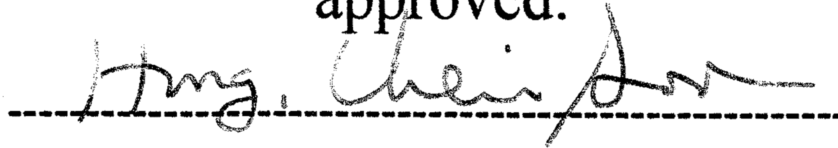
This certifies that the Doctoral Dissertation of Hyun-Sun Jin is approved.



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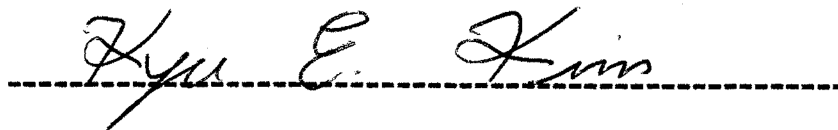

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Abbreviation of the paper

H. japonicus: *Humulus japonicus*

rHum j 1: recombinant *Humulus japonicus* 1

SDS-PAGE: Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis

RT-PCR: Reverse-Transcriptase-Polymerase Chain Reaction

cDNA: complementary DNA

bp: base pair

dT: deoxynucleoside thymidine

kDa: kilo dalton

SPT: skin prick test

E. coli: Escherichia coli

Vol: Volume

AP: Alkaline Phosphatase

PBST: Phosphate Buffered Saline containing Tween-20

TBST: Tri-HCl Buffered Saline containing Teween-20

RACE: Rapid Amplification of cDNA Ends

HPLC: high performance liquid chromatography

IPTG: Isopropyl-β-D-Thiogalactopyranosid

PCR: Polymerase Chain Reaction

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The pollen of *Humulus japonicus* (*H. japonicus)* is one of the common causes of allergic diseases in Korea, Japan and China. However, the molecular structure of *H. japonicus* allergen has not yet been identified. In order to determine the molecular structure of this novel major allergen, an antibody against the major allergen of *H. japonicus* pollen was generated by immunizing a rabbit with a major allergen, eluted protein from SDS-PAGE gel. The rabbit polyclonal antibody showed a specific binding of the major allergen in the *H. japonicus* pollen. The cloning of cDNA for the major allergen of *H. japonicus* pollen was attempted by RT-PCR using degenerate oligonucleotide

primers designed from a partial amino acid sequence reported previously. A cDNA with nucleotide sequence of 468 bp for the major allergen of *H. japonicus* pollen was determined. The recombinant protein for the partial cDNA (320 bp) was produced in *E. coli*, rHum j 1, which had an estimated molecular weight of 19 kDa. rHum j 1 bound to IgE in a positive sera pool of patients allergic to *H. japonicus* pollen, and also bound to IgG in the rabbit antisera against the purified major allergen from *H. japonicus* pollen using the analysis of western blot immunodetection and ELISA assay. Dot-blot analysis was performed with individual sera sensitized to *H. japonicus* pollen and showed that 100% (39/39) of the patients had rHum j 1 specific-IgE binding activity. A maximum of 88% and 74% inhibition of rHum j 1 specific-IgE or native Hum j 1 specific-IgE was obtained by the native Hum j 1 or rHum j 1 of *H. japonicus* pollen in inhibition ELISA. The full nucleotide sequences of the major *H. japonicus* allergen were identified and the immunological properties of rHum j 1 have been characterized.

Key words: *Humulus japonicus*, type I allergy, IgE, allergens

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I. Introduction

Aeroallergens are the most important allergens involved in producing the symptoms of respiratory allergies [1-2]. There have been several reports on the results of skin prick test (SPT) for inhalant allergens in asthma and allergic rhinitis in Korea [3-5]. One study reported that the prevalence of positive SPT for pollen allergens in Korea was caused by sagebrush and Hop Japanese (the English name for *Humulus japonicus*) [6]. *Humulus japonicus* (*H. japonicus*) pollen is a major autumn aeroallergen and

elicits clinical symptoms including rhinitis and conjunctival congestion accompanied by pollen specific-IgE production. *H. japonicus* is widespread in Korea, Japan and China [7-10]. The pollination period of *H. japonicus* pollen lasts about 3 months, starting in August and ending in October, so that *H. japonicus* pollinosis is one of the most important causes of respiratory allergy in autumn in Korea.

Several studies were performed in the 1980s, beginning with two cases of asthmatic patients whose allergy was caused by *H. japonicus* pollen which were reported in 1987 [11], and another study which reported on the results of skin reactivity and the detection of specific IgE to the pollen of *H. japonicus*. In this latter study, 47 (13.8%) patients with respiratory allergy exhibited a positive reaction to the SPT and 37.5% of sensitized patients were serum specific IgE positive out of a total of 340 patients [12]. However, these reports did not receive significant attention due to the patients exhibiting few symptoms.

Recent studies have showed that the number of patients who are SPT positive and specific IgE positive to *H. japonicus* pollen has increased in the Seoul metropolitan area and provided evidence of *H. japonicus* pollinosis in Korea with 6.1% out of 1,287 patients being sensitized by this allergen in 1997 [13]. *H. japonicus* allergen immunotherapy was

carried out by the same group and resulted in an increase in *H. japonicus* allergen specific-IgG4 and IgG1 antibodies and in the reduction of a possible Th 2 lymphocyte marker [14]. Another recent report identified partial amino acid sequences of a major allergen consisting from *H. japonicus* pollen [15]. This research group also purified a protein with a molecular weight of 10 kDa, which is the major allergen of *H. japonicus* pollen, and identified a partial sequence of 20 amino acids contained in this protein. However, the entire sequence of the major allergen of *H. japonicus* have not yet been identified.

The diagnosis and immunotherapy of IgE-mediated allergy requires the production of large amounts of pure, well-defined proteins from pollen allergens. In a recent study, this drawback was overcome by using recombinant DNA technology, which is a convenient tool for accomplishing homogenous protein preparation.

In the present study, the cloning, sequencing and expression of cDNA for the major allergen of *H. japonicus* pollen are reported. In accordance with WHO guidelines on allergen nomenclature [16], the allergen was named Hum j 1. The IgE binding properties of the recombinant protein (rHum j 1) were tested in several in vitro assays and compared with its native homologues.

II. Materials and Methods

1. Characterization of patients' sera

For the western blot immunodetection, the sera pool from 15 patients with a clinical history of allergic reaction to *H. japonicus* pollen were used (positive sera pool). The mean age of the patients was 29.1 years and the male: female ratio was 8: 7. All fifteen subjects had a clinical history of at least one or more symptoms, including rhinitis and asthma. Patients were diagnosed as allergic to *H. japonicus* pollen on the basis of clinical history and the SPT, which was performed with commercial *H. japonicus* extract (Torii Co., Tokyo, Japan). Those patients who had never received any specific immunotherapy were included in this study. The size of the wheal produced by *H. japonicus* extract was at least as large as that of the histamine positive control (1 mg/ml) in all of the patients.

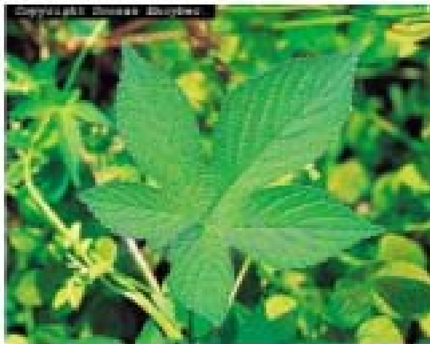
For the dot-blot immunoassay, the sera from 39 patients (including the 15 patients who participated in the western blot immunodetection experiment) of having a respiratory allergy with strong SPT positive reactions were used. A sera pool from 10 patients, who exhibited symptoms of respiratory allergy but gave a negative SPT result, were

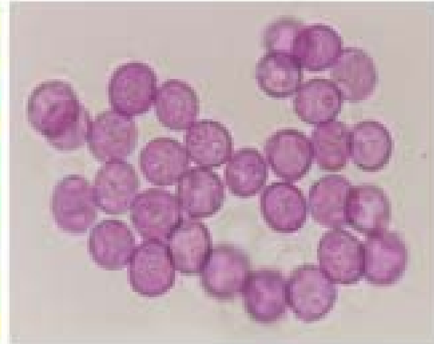
used as the negative control (negative sera pool).

2. Preparation of crude extract of *H. japonicus* pollen

H. japonicus pollens, as shown in Figure 1, were obtained from the campus of Yonsei University College of Medicine, Seoul, Korea [17]. Five g of *H. japonicus* pollen was defatted with ethylether and extracted in 100 ml of ammonium carbohydrate buffer (0.125 M NH_4HCO_3, 0.015 M NaN_3, pH 7.5, 1: 20 wt/vol) for 24 hr at 4°C with constant stirring. The extract was centrifuged at 5,000 rpm for 1 hr at 4°C and the supernatant was dialysed (the cut off molecular weight was 3.5 kDa; Spectrum, Houston, TX, USA) against distilled water at 4°C for 48 hr. The dialysed supernatant was lyophilized and stored at –20°C until it was used.

A male flower A female flower





A leaf Pollen

Figure 1. *Humulus japonicus* (Hop Japanese)

3. Preparation of purified native allergen of *H. japonicus* pollen

One hundred mg of lyophilized *H. japonicus* extract were resuspended in 20 mM Tris-HCl (pH 8.0) and applied to a diethylaminoethyl (DEAE) cellulose column (10 x 200 mm). The bound proteins were eluted with a buffer containing 0.1, 0.2, 0.3, 0.4 and 0.5 M NaCl from the column and the fractions were pooled, dialysed, and lyophilized. The lyophilized protein was dissolved in a small volume of the buffer (20 mM Tris-HCl pH 8) and fractionated by Sephacryl S-200 HR (Amersham Pharmacia biotech, Uppsala, Sweden) chromatography. The IgE binding activity of the fractions obtained in all of the chromatographic steps was tested by immunoblotting using the positive sera pool. The fractions bound to the positive sera pool were applied onto a Phenyl-sepharose CL-4B column equilibrated in 50 mM Tris-HCl, pH 7.5, containing 0.5 M NaCl. After a thorough washing, a gradient of 0.5-0.0 M NaCl in 50 mM Tris-HCl, pH 7.5, was applied. Finally, an elution step with distilled water was carried out. The eluted proteins were applied to a Nucleosil C-18 column (HPLC) and the elution was performed with an acetonitril gradient (30-60%) in 0.1% trifluoroacetic acid. The protein fractions containing the major allergen of *H. japonicus* were collected and stored at -20°C. The protein contents of each fraction

were determined by Bio-Rad protein assay kit (Hercules, CA, USA).

4. Polyclonal antibody production of the major allergen from *H. japonicus* pollen and immunoblotting

Polyclonal antiserum of the major allergen from *H. japonicus* pollen was raised in a white female rabbit. Firstly, the major allergen from *H. japonicus* pollen was purified from the major protein band that had IgE binding activity, by cutting the band from the SDS-PAGE gel and eluting it from the gel. This procedure was repeated until 1 mg of protein was collected. A primary immunization of 100 μg of purified protein in complete Freund's adjuvant (CFA) was followed by an i.m. booster injection containing 50 μg protein in incomplete Freund's adjuvant (IFA) given on 3 separate occasions at 4 wk intervals. Hyperimmune rabbit serum was harvested at 12 wk.

Total *H. japonicus* pollen extract was prepared as described in the literature. SDS-PAGE of the *H. japonicus* pollen extract was done on 15% gel. The transfer of the pollen proteins onto a nitrocellulose membrane (pore size - 0.45 μm, Amersham, Buckinghamshire, UK) was performed at 350 mA for 1 hr. The membranes were blocked by 3% non-

fat dried milk in TBS-T (50 mM Tris-HCl with 0.1% Tween-20, pH 7.5) and then incubated with polyclonal antibody against *H. japonicus* diluted 1: 500 in TBS-T for 18 hr at 4°C. The membrane was washed 3 times with TBS-T and the goat anti-rabbit IgG conjugated with alkaline phosphatase was diluted 1: 1,000 in TBS-T and incubated for 1 hr at room temperature. After washing with TBS-T, bound goat anti-rabbit IgG antibodies were detected with a BCIP/NBT system (Promega, Madison, WI, USA).

5. RNA extraction and purification

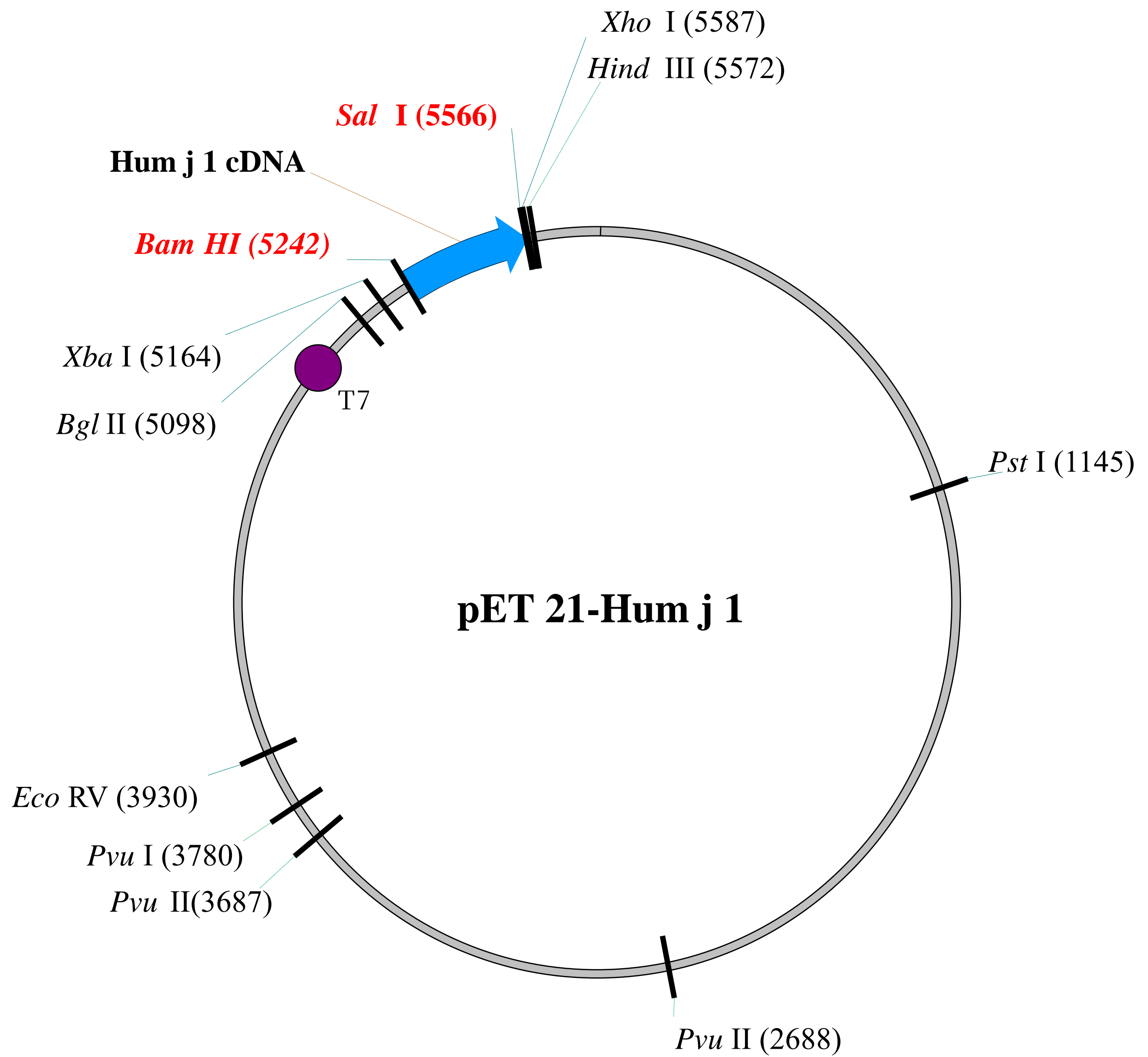
A flower bud of *H. japonicus* was obtained from the campus of Yonsei University College of Medicine, Seoul, Korea at the end of September and frozen immediately in liquid nitrogen, and was subsequently ground in a mortar and pestle under liquid nitrogen. The ground powder was suspended in 6M guanidinium thiocyanate, and total cellular RNA was extracted using the guanidinium thiocyanate-phenol chloroform method developed by Chomoczynski and Sacchi [18] (1987) using Trizol reagent (Life Technologies, Rockville, MD, USA). The quality of the total RNA was evaluated by denaturing formaldehyde agarose gel electrophoresis and the RNA quantity was measured by

spectrophotometer. Poly $(A)^+$ RNA was then purified from the total RNA by oligo dT cellulose affinity chromatography using a poly $(A)^+$ RNA purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

6. cDNA cloning of the major allergen of *H. japonicus* pollen and the expression of the recombinant allergen, rHum j 1

RNA was extracted from the flower bud of the *H. japonicus* and cDNA was synthesized by reverse transcription using 400 U Moloney murine leukemia virus reverse transcriptase (GibcoBRL, Life Technologies AG, Basel, Switzerland) and oligo dT primer (5'-TTTTTTTTTTTTTTTTTT-3'). A 20-fold degenerate oligonucleotide primer (5'-TTYGARAAYGGNATGAARGC-3') was deduced from amino acid residues 4-10 of the partial amino acid sequence of the purified *H. japonicus* pollen [15]. First strand cDNA was used as the template and double stranded cDNA was amplified by PCR. One micromolar concentrations of each of the above primers were used in a 50 μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 1.25 U EX *Taq* DNA polymerase (TaKaRa, Japan). A total of 35 cycles were performed by

using a DNA thermal cycler (PerkinElmer Cetus, Norwalk, CT, USA). The cycles were performed, accompanied by annealing at 57°C for 1 min, denaturing at 94°C for 1 min, and extension at 72°C for 1 min. The amplified product was gel-purified using a gel-elution kit (Qiagen, Valencia, CA, USA) and the eluted cDNA was extracted using phenol: chloroform: isoamyl alcohol 25:24:1 (Sigma, St. Louis, MO, USA) and subcloned into the *Bam HI* and *Sal I* sites of a pET 21a vector (Novagen, Madison, WI, USA). The sequence of the cDNA of the major allergen from *H. japonicus* pollen was determined by the cycle sequencing of the plasmid DNA with AmpliTaqDNA polymerase and dye-labeled terminator, using the ABI prism Dye terminator cycle sequencing ready (Applied Biosystems Inc., Doster city, CA, USA) and the T7 sequencing primers. The nucleotide sequence was analyzed with an ABI prism 3100 Genetic Analyzer (Amersham Pharmacia Biotech, Uppsala, Sweden).



This is a schematic representation of Hum j 1 expression vector. Hum j 1 cDNA was inserted into *Bam HI* and *Sal I* sites of pET21 vector.

7. Identification of full nucleotide sequence for the major allergen, Hum j 1, from *H. japonicus* pollen

The SMART^{TM} RACE cDNA amplification kit (BD Biosciences, CA, USA) was used to elucidate the full nucleotide sequence of the major allergen, Hum j 1. A 5'-RACE system was used to identify the upstream region of the message. First-strand cDNA was again produced by reverse transcription (RT) of *H. japonicus* mRNA from a primer designed for the reverse strand, a 5'-RACE CDS primer (5'-$(T)_{25}N_{-1}N$-3') and SMART II A oligo (5'-AAGCAGTGGTATCAACGCAGAGTACGCGGG-3'). PCR was performed with sense (5'-AACTTGTCGACTTGAGAGCCACTTTGAGA-3') and antisense (UPM, Universal Amplification Primer, enclosed in the 5'-RACE system) primers. PCR amplification was performed for 25 cycles with annealing at 68˚C for 10 sec, denaturing at 94˚C for 5 sec, extension at 72˚C for 3 min. For 3'-RACE, first-strand cDNA was produced by reverse transcription of *H. japonicus* mRNA from a 3'-RACE CDS primer A (5'-AAGCAGTGGTATCAACGCAGAGTAC$(T)_{30}N_{-1}N$-3'). PCR was performed with sense primer (5'-AAATGTTGTGGTCAACCTCTTCATTTTGATCG-3') and UPM primer

enclosed in the 3'-RACE system. The PCR amplification condition was the same as that used in the 5'-RACE system. Both amplified fragments were gel-purified, subcloned into pCR2.1-Topo vector and fully sequenced in both directions.

8. Purification of rHum j 1 protein

A 150 mL culture of *E. coli* (BL21 DE3) was transformed with pET 21-Hum j 1 cDNA the rHum j 1 protein was induced with 1 mM isopropyl-β-D-thiogalactopyranosid (IPTG). The cells were harvested by centrifugation at 600 g for 20 min and the harvested cells were suspended in 5 mL of lysis buffer (1% Triton X-100, 1% Tween-20, 0.5 M EDTA, β-mercaptoethanol) and sonicated. The cell lysate was then centrifuged to pellet the debris and the supernatant was added to an Ni-NTA column (Amersham Pharmacia Biotech, Uppsala, Sweden). After washing, the his-tagged protein was eluted according to the manufacturer's instructions.

9. Determination of molecular weight of rHum j 1

rHum j 1 protein was analyzed in 15% SDS-PAGE under reducing

conditions by the method of Laemmli [19]. Proteins were stained with Coomassie blue.

10. IgE reactivity to rHum j 1 by immunoblotting

rHum j 1 protein was analyzed in 15% SDS-PAGE under reducing conditions and electrophoretically transferred onto a nitrocellulose membrane using a current of 350 mA for 1 hr. The membranes were blocked by 3% non-fat dried milk in TBS-T (50 mM Tris-HCl with 0.1% Tween-20, pH 7.5) and then incubated with the positive sera pool (1: 10 diluted in TBS-T, 0.02% NaN_3) or polyclonal antisera (1: 500 diluted in TBS-T, 0.02% NaN_3) at 4°C for 18 hr and washed with TBS-T. Goat anti-human IgE conjugated with alkaline phosphatase (AP) (Sigma, St Louis, MO, USA) or anti-rabbit IgG conjugated with AP (Sigma, St Louis, MO, USA) diluted 1: 2,000 or 1: 1,000 in TBS-T, respectively, were used for incubation with the blots. After washing with TBS-T, the bound antibodies were detected with a BCIP/NBT system (Promega, Madison, WI, USA).

11. Dot-blot immunoassay

Dot-blot immunoassays were prepared by applying 2 μg of the rHum j 1 onto a nitrocellulose membrane. After blocking, the blots were washed with TBST and incubated with 39 individual patients' sera (1: 5 dilution) at 4ºC overnight. Afterwards, these blots were incubated with alkaline phosphatase conjugated anti-human IgE (Sigma, St Louis, MO, USA) for 6 hr at 16ºC. The blots were then washed thoroughly and detected with a BCIP/NBT system (Promega, Madison, WI, USA).

12. IgE reactivity to rHum j 1 by ELISA

Polystyrene micro plates (Costar, Cambridge, MA, USA) were coated with 50 μl of 10 μg/ml rHum j 1 in 0.05 M carbonate buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, 0.2 g NaN_3/L, pH 9.6) incubated overnight at 4°C. Each microplate well was blocked by incubation with 200 μl of 1% bovine serum albumin in PBS-T (137 mM NaCl, 2.68 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, 0.1% Tween-20, pH 7.4) for 1 hr at room temperature. Specific IgE detection was carried out by incubating 50 μl of the positive sera pool for 1 hr at room temperature. After washing three times with PBS-T, 50 μl of 1: 1,000 diluted biotin-labeled goat anti-human IgE (Vector, Burlingame, CA, USA) was added to the each of the

wells and incubated for 1 hr. After washing, the wells were incubated with 1: 1000 v/v streptavidin-peroxidase (Sigma, St Louis, MO, USA) for 30 min, followed by another washing step. The colorimetric reaction was developed with 100 μl of ABTS solution (25 mg of 2,2-azino-bis-3-ethylbenzthiazoline-sulfonic acid in 50 mM citrate phosphate buffer, 50 μl of 30% H_2O_2) for 5 min. The reaction was stopped by the addition of 50 μl of 2 mM NaN_3, and the optical densities were determined in the UV spectrum at 405 nm by an automated spectrophotometer for microplate (Dynatec, Alexandria, CA, USA). All assays were performed in duplicate.

13. Inhibition ELISA assay

Polystyrene micro plates were coated with 50μl of 10 μg/ml of rHum j 1 or native Hum j 1 in 0.05 M carbonate buffer and incubated overnight at 4°C. The diluted positive sera pool (1: 5 dilution) from 15 *H. japonicus* pollen sensitized patients were preincubated with serially diluted native Hum j 1 allergen or rHum j 1 (maximum concentration of 10 μg/ml) and incubated overnight at 4°C. The remainder of the procedure was the same as that used in the case of the ELISA method.

II. Results

1. IgE recognition profile of *H. japonicus* allergen

Serum samples from 15 patients allergic to *H. japonicus* pollen were tested for the prevalence of IgE recognition and the allergenic profiles of *H. japonicus* pollen after SDS-PAGE and electroblotting onto nitrocellulose membrane as shown in Figure 2. Fourteen of 15 patients' sera bound to at least one component of the pollen extract and only one serum did not show any IgE binding to *H. japonicus* pollen. The molecular weight profile of the IgE-binding components varied significantly from one patient's serum to another, and those IgE-binding components with a molecular weight of 10, 13 and 42 kDa were recognized by the IgE antibody. There were weak or strong IgE binding to the low molecular weight components. Sera of 10 patients (66.7%) exhibited IgE binding to the 10 kDa component of the major allergen. Three patients' sera (20%) and 1 patient's serum (6.7%) bound to the 13 kDa and 42 kDa IgE components, respectively.

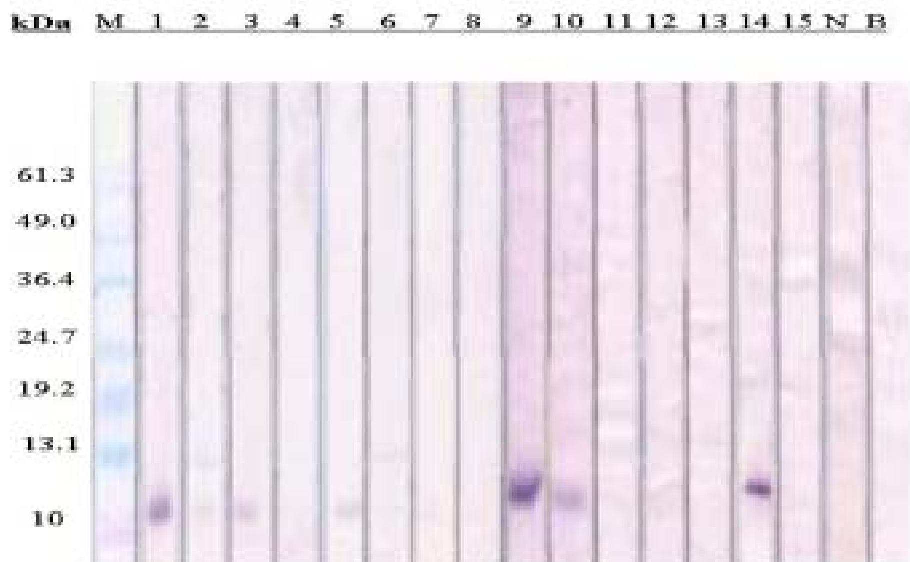


Figure 2. IgE Immunoblotting patterns of *H. japonicus*-reactive patient sera (lanes 1–15), molecular marker (M), negative sera pool, (N), buffer control (B).

2. Characterization of polyclonal antibody

The polyclonal antibody against the major allergen of *H. japonicus* pollen was raised in a rabbit and the immunization of only 50 μg repeated several times was enough to produce the polyclonal antibody. The antibody was tested for specific-Hum j 1 binding activity with *H. japonicus* pollen extract. It showed strong and specific-Hum j 1 binding activity against the extract from *H. japonicus* pollen in the immunoblotting analysis, as shown in Figure 3. These data indicate that the epitope defined by the polyclonal antibody seems to be an important allergenic determinant on the major allergen Hum j 1.

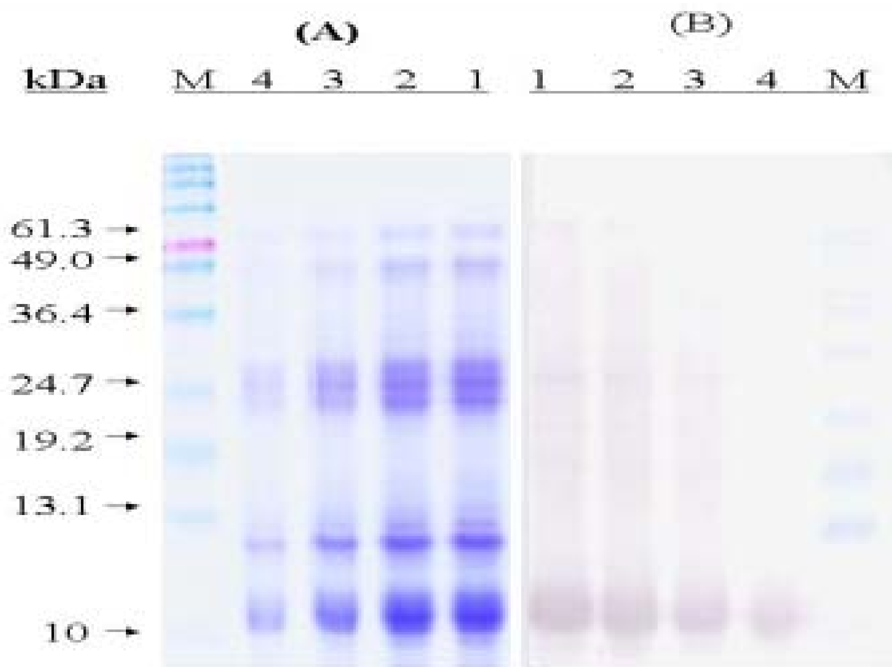


Figure 3. SDS-PAGE (A) and western blot immunostaining (B) of the major allergen of *H. japonicus* pollen. Lane 1: 20 mg/ml crude extract from *H. japonicus* pollen, Lane 2: 15 mg/ml *H. japonicus* crude extract, Lane 3: 10 mg/ml *H. japonicus* crude extract, Lane 4: 5 mg/ml *H. japonicus* crude extract were used.

3. Cloning and sequencing of the cDNA encoding for the major allergen, Hum j 1, from *H. japonicus* pollen

cDNAs encoding a partial sequence of Hum j 1 were obtained from mRNA of *H. japonicus* by specific amplification with the degenerate oligonucleotide primer deduced from the partial amino acid sequences of Hum j 1 reported by Park [15]. The PCR products showed a size of approximately 320 bp (data not shown). The cDNA was subcloned into pET 21a vector for the expression in *E. coli*. The cloned sequence encoded 110 amino acids with a molecular mass of 19 kDa, corresponding to the estimated molecular weight of the recombinant major allergen, is shown in Figure 4. The molecular weight of the expressed protein was determined by SDS-PAGE. The yield of the purified protein was 6.5 mg/L as measured by Bradford assay (Bio-Rad, Hercules, CA, USA).

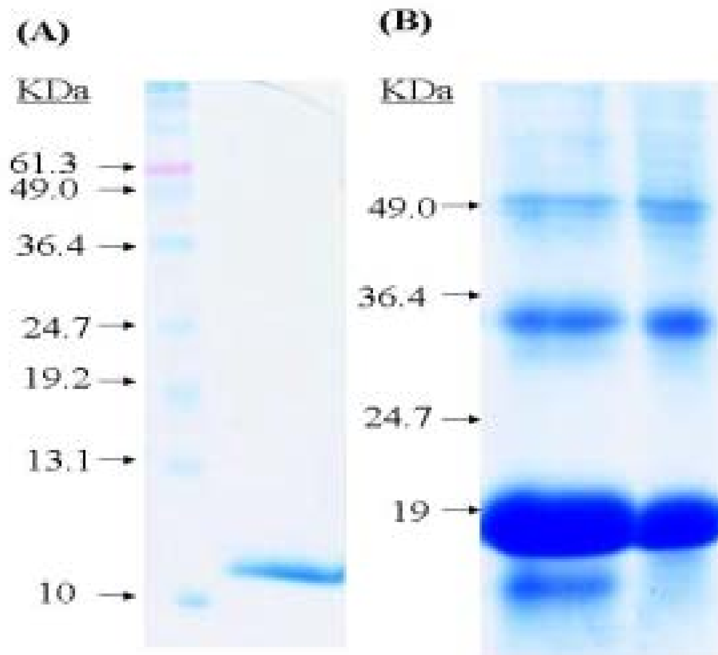


Figure 4. SDS-PAGE of native Hum j 1 (A) and recombinant Hum j 1 (rHum j 1) (B). Native Hum j 1 from *H. japonicus* pollen purified by HPLC and rHum j 1 was expressed in *E. coli.*

4. Full nucleotide sequences of cDNA encoding the major allergen, Hum j 1, from *H. japonicus* pollen

5’-3’ RACE were performed to elucidate the full nucleotide sequence corresponding to the N-terminal and C-terminal end of the major protein and the signal peptide. 5’-RACE with 31 nucleotides length of specific primer was performed and a non-degenerate 32 nucleotides length primer could be designed and used in a subsequent 3’-RACE amplification. The cDNA clone obtained from this 3’-RACE end ranged in size from 350 to 700 bp, because of the distinct length of the poly $(A)^+$ tract and some divergences in the 3’-untranslated region. In Figure 5, the 468 bp corresponding to the complete nucleotide sequences of the cDNA for Hum j 1, including the possible leader and pre-sequences, are given. 320 bp out of the 468 bp nucleotide sequences for the Hum j 1 cDNA seems to be the coding sequences for the mature protein. The sequences of these 468 bp representing the full nucleotide sequence showed encoding 155 amino acid residues. This clone displayed six cysteine residues in the sequence and the molecular weight value estimated for the polypeptide backbone of the Hum j 1 allergen was 6-7 kDa greater than native Hum j 1, possibly due to the inclusion of a signal peptide and a peptide removed from a pre-form of the major allergen. The estimated molecular weight

for recombinant allergen, rHum j 1, was 19 kDa which was also greater than the estimated molecular weight due to a peptide from vector (pET 21) and the 6 x His-tag. The N-terminal of this major allergen is blocked and further study is required to find it. In the proposed translation initiation site, the most critical nucleotide is conserved which is a purine at position –3. The cDNA has a complete 3'-untranslated region that contains the canonical AATAAT polyadenylation signal followed by a poly $(A)^+$ tail. The degenerate oligonucleotide primer sequences are indicated in red, the TATA box is indicated in black, the polyadenylation signal is indicated in blue and the possible translation initiation position is indicated in green, as shown in Figure 5.

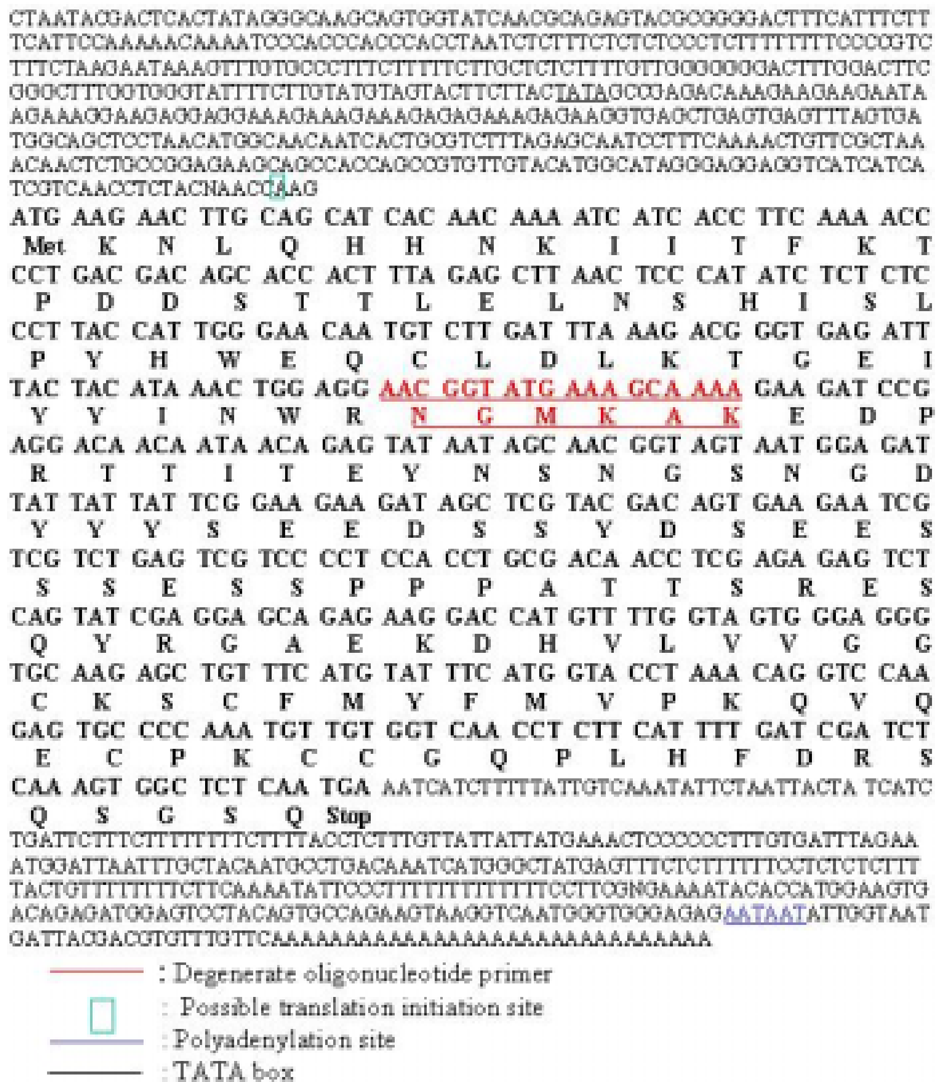


Figure 5. Full nucleotide sequences of cDNA for the major allergen Hum j 1of *H. japonicus* pollen and deduced amino acid sequences.

5. Immunological characterization of rHum j 1

5-1. IgG and IgE reactivity of the rHum j 1 by immunoblot analysis

A western blot employing a rabbit polyclonal antiserum to rHum j 1 is shown in Figure 6. Bacterial extract of pET 21a vector control, IPTG induced pET 21-Hum j 1 and purified rHum j 1 were separated by 15% SDS-PAGE and subsequently electrophoresed, blotted to nitrocellulose membranes and tested with rabbit polyclonal anti-Hum j 1 IgG. Detection was performed with alkaline phosphatase-conjugated mouse anti-rabbit IgG. Total cell lysate of pET 21a vector control (Lane 1) showed no IgG binding activity. Total cell lysate of IPTG induced pET 21-Hum j 1 (Lane 2) and rHum j 1 purified protein (Lane 3) showed strong and specific-Hum j 1 binding activity in the 19 kDa component with rabbit polyclonal antiserum. Lane 4 is used as a negative control.

Another western blot employing the positive sera pool is shown in Figure 7. Bacterial extract of IPTG induced pET 21-Hum j 1 and purified rHum j 1 were separated by 15% SDS-PAGE and electrophoresed, blotted to nitrocellulose membranes and tested with the positive sera pool of patients allergic to *H. japonicus* pollen. Detection was performed with alkaline phosphatase-conjugated goat anti-human IgE. Molecular

standard marker is shown in Lane M, and rHum j 1 purified protein (Lane 1) and total cell lysate of IPTG induced pET-Hum j 1 (Lane 2) showed also strong IgE binding activity in the 19 kDa component with the patients' sera. Controls were performed in parallel with the negative sera pool (Lane 3) and the buffer control (Lane 4).

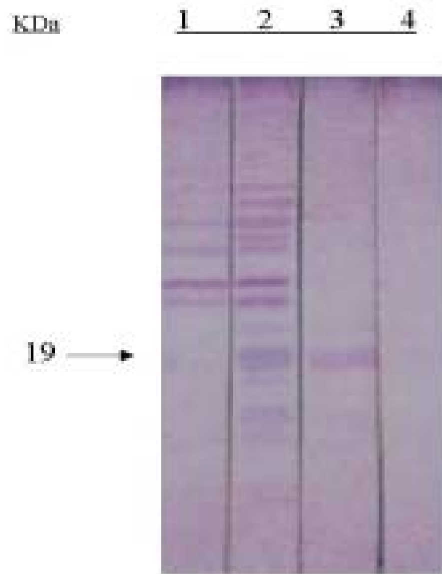


Figure 6. Western blot immunostaining of rHum j 1 using rabbit polyclonal antibody. Bacterial extract of pET 21 vector control and purified rHum j 1 were separated by 15% SDS-PAGE and subsequently electrophoresed, blotted to nitrocellulose membranes and tested with rabbit polyclonal anti-Hum j 1 IgG. Detection was performed with alkaline phosphatase-conjugated mouse anti-rabbit IgG. Lane 1: Total cell lysate of pET 21 vector control, Lane 2: Total cell lysate of IPTG induced pET-Hum j 1, Lane 3: rHum j 1 purified protein, Lane 4: Control.

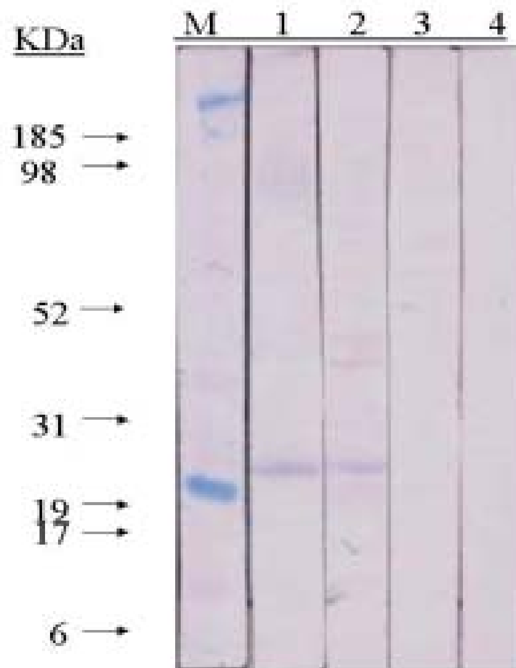


Figure 7. Western blot immunostaining of rHum j 1 using the positive sera pool of patients allergic to *H. japonicus* pollen. Bacterial extract of pET 21 vector control and purified rHum j 1 were separated by 15% SDS-PAGE and subsequently electrophoresed, blotted to nitrocellulose membranes and tested with pooled sera of patients allergic to *H. japonicus* pollen. Detection was performed with alkaline phosphatase-conjugated mouse anti-Human IgE. M : Molecular standard marker, Lane 1: rHum j 1 purified protein, Lane 2: Total cell lysate of IPTG induced pET-Hum j 1 Lane 3: Normal sera, Lane 4: Buffer control.

5-2. Dot-blot immunodetection of IgE specific to rHj 1 for individual patients

rHum j 1 was tested for binding to IgE in sera of 39 individual *H. japonicus* pollen-allergic patients by dot-blot analysis (Figure 8). The buffer control (B) and the negative sera pooled from non-allergic patients (N) are shown and they showed no rHum j 1 specific-IgE binding activity. The result of the positive sera pool from *H. japonicus* pollen sensitized patients is shown in P (P) and showed strong rHum j 1 binding activity. Thirty nine individual patient's serum IgE activity against rHum j 1 is shown from patient number 1 (P1) to patient number 39 (P39). Thirteen sera (33.3%) of the patients showed strong IgE binding activity with rHum j 1, 17 sera (43.5%) of the patients showed mild IgE binding activity against rHum j 1 and 9 sera (23%) showed weak IgE responses with rHum j 1. Therefore, 39 of 39 patients' sera showed rHum j 1 specific-IgE binding activity on dot-blot immunodetection.

B -	P4	P10	P16	P22	P28	P34
N -	P5	P11	P17	P23	P29	P35
P +	P6	P12	P18	P24	P30	P36
P1	P7	P13	P19	P25	P31	P37
P2	P8	P14	P20	P26	P32	P38
P3	P9	P15	P21	P27	P33	P39

0.000	2.555	2.191	2.209	2.539	2.775	1.529
0.012	2.226	2.165	2.376	2.442	1.852	1.301
3.397	1.279	2.283	3.260	3.172	3.414	3.260
2.502	1.960	1.654	2.134	2.785	3.059	2.776
2.230	1.724	1.604	1.851	2.329	2.197	1.924
2.961	2.361	3.059	2.035	1.330	2.522	3.089

Figure 8. Dot-blot immunodetection of IgE specific to rHum j 1 for individual patient. B: Buffer control, N: Negative sera pool from non-allergic patients, P: Positive sera pool from *H. japonicus* pollen sensitized patients and P1 to P39: individual patient's serum from patient number 1 to patient number 39 was used.

5-3. ELISA binding assay of rHum j 1 to IgE in the serially diluted sera of patients allergic to *H. japonicus* pollen

ELISA was performed to test the allergenicity of rHum j 1 with the serially diluted the positive sera pool. In Figure 9, the dose-response curves obtained in the ELISA with the positive sera pool of *H. japonicus* sensitized patients and rHum j 1 was not bound with the negative sera pool used as a control.

5-4. Inhibition ELISA of rHum j 1 and native Hum j 1

In order to elucidate whether rHum j 1 share common IgE-binding epitopes with native Hum j 1, the positive sera pool from *H. japonicus* pollen allergic patients was tested for IgE-binding to rHum j 1 or native Hum j 1 in an inhibition study as shown in Figure 10. A maximum of 88% or 74% blocking of IgE binding to rHum j 1 or native Hum j 1 were observed after preincubation with serially diluted native Hum j 1 or rHum j 1, respectively. The 50% in inhibition concentration of rHum j 1 or native Hum j 1 allergen were 0.23 μg/ml and 0.06 μg/ml, respectively. These data suggested that rHum j 1 shared IgE-binding epitopes with

native Hum j 1.

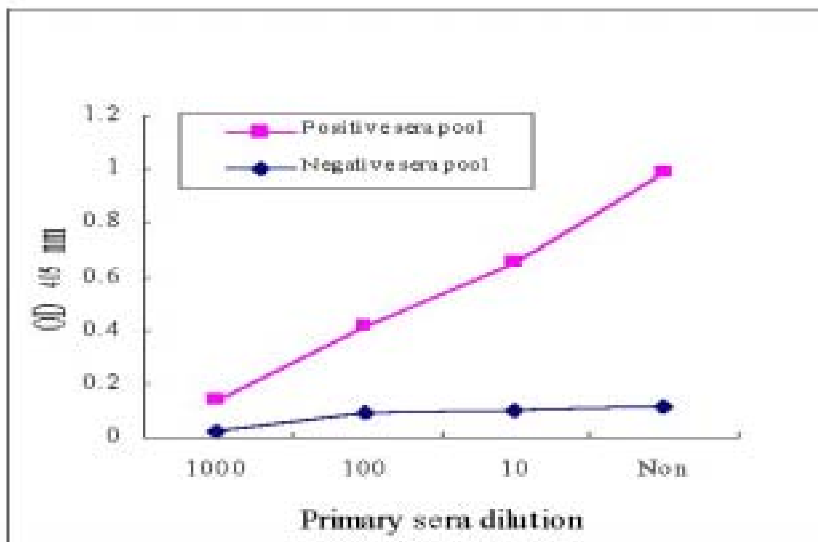


Figure 9. ELISA assay of IgE binding reactivity of the positive sera pool from patients allergic to *H. japonicus* pollen to rHum j 1 and the negative sera pool from non-allergic individuals.

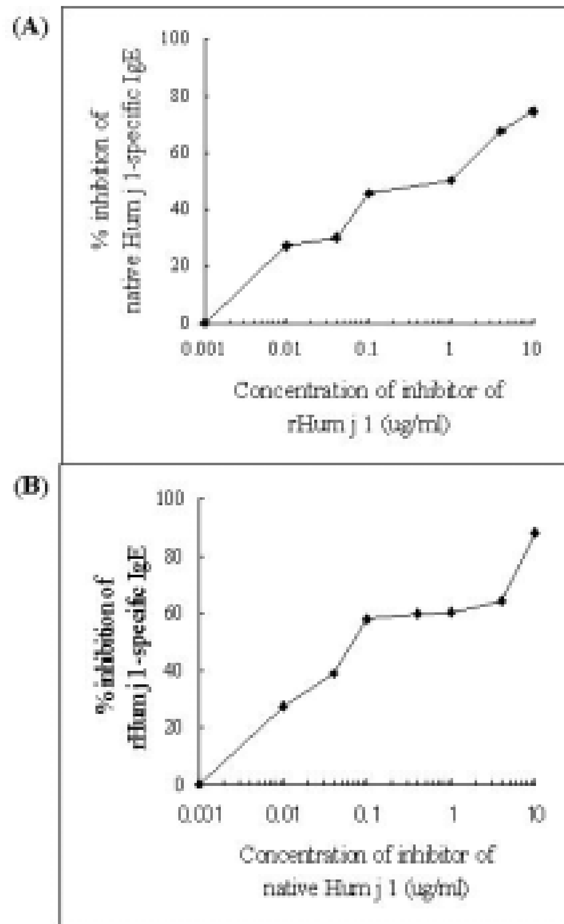


Figure 10. Inhibition ELISA of native Hum j 1 specific-IgE by rHum j 1 (A) and rHum j 1 specific-IgE by native Hum j 1 (B). The positive sera pool from patients allergic to *H. japonicus* pollen was tested for IgE-binding to native Hum j 1 or rHum j 1 after preincubation with the serially diluted rHum j 1 or native Hum j 1, respectively.

IV. Discussion

H. japonicus is widespread in Asia as well as in some tropical and subtropical zones [12]. It has been considered to be one of the major causative pollens of autumn pollinosis in Korea. The pollen counts of the *H. japonicus* recorded in Seoul are greater than those of mugwort and ragweed [10]. Despite the importance of causative allergen of pollinosis in Korea, no major molecular studies on *H. japonicus* pollen have been done. Several reports showed that the major allergic proteins in *H. japonicus* pollen are the 10, 16, 20, 42 kDa [15] or 13, 74, 88 kDa peptides [13] as identified by the binding of IgE in the sera of patients allergic to *H. japonicus*. A possible explanation for this discrepancy is based on the different geographical areas from which both the sera and pollen were collected. IgE profiles recognized by patients allergic to *H. japonicus* pollen in our data demonstrated that the protein with a molecular weight of 10 kDa showed a strong IgE binding activity in most patients, and that the proteins with a molecular weight of 13 and 42 kDa were also frequently bound to IgE in some patients' sera. Only the low molecular weight IgE reactive proteins of *H. japonicus* pollen was detected. These findings are associated with those of a previous report [15] in that the major allergen in *H. japonicus* pollen is a protein with a molecular weight of 10

kDa and bind strongly with IgE in the patients' sera.

The major allergic component of *H. japonicus* pollen was eluted from the SDS-PAGE gel and the polyclonal antiserum to the major allergen of *H. japonicus* pollen was produced by immunizing a rabbit with the eluted major protein. Western immunodetection confirmed that the anti-serum was specific to the major allergen in *H. japonicus* extract and recombinant protein expressed from cDNA for the major allergen. Therefore, polyclonal antibody could be useful to the identification of the major allergen of *H. japonicus* pollen. The methodology used eluting the major allergen from SDS-PAGE gels was simple with some modifications of conventional method, and the eluted allergen was useful for producing a polyclonal antibody that is specific to the allergen we targeted.

In order to determine the full nucleotide sequences of the cDNA of the major allergen from *H. japonicus* pollen, the construction of the cDNA library was first attempted, however no clones were bound to IgE in the patients sera or polyclonal antibody of the major allergen of *H. japonicus* pollen, indicating that the conventional selection method for finding novel genes might not be applicable due to the importance of the 3-dimentional structure of the *H. japonicus* allergen. Then, a simple and

efficient method has been used to clone the *H. japonicus* major allergen using the partial amino acid sequences [15, 20]. A PCR strategy based on the known sequence, in combination with the 5' – 3' RACE, provided the complete cDNA. Firstly, RT-PCR was performed with a degenerated oligonucleotide primer deduced from the partial amino acid sequences reported previously, and the recombinant protein was produced in *E. coli* from the 320 bp cDNA inserted into the pET 21a expression vector. The recombinant protein had a molecular mass of 19 kDa and this was greater than the estimated protein size, due to the pET 21 vector, several side of restriction enzyme site and 6 x His-tag. This protein was evaluated for its immunological properties by western immunodetection with the patients' sera and the polyclonal antibody. It has been shown that the recombinant protein has allergenicity to the patients' sera allergic to *H. japonicus* pollen. Secondly, 5' – 3' RACE were performed to find the full nucleotide sequence of the major allergen in *H. japonicus* pollen. From a study of 211 eukaryotic messages, a consensus sequence has been identified at the initiation site. This sequence contains a purine (probably an A) at position –3, a G at position +4, and a predominance of C at position –1, -2, -4 and –5. A possible translational initiation site was selected at nucleotide position of –3 (A) relative to the ATG initiation

codons [21] and at position –4 was C. Another study showed the –1 and –3 rule for the prediction of the leader sequence cleavage site, which states that these positions should be small and neutral, hold for Hum j 1 with a –1: C and –3: A [22]. At the 3' end, the untranslated region does not have the AATAAA polyadenylation signal observed in most animal sequences located 9-23 bases upstream from the poly (A) tail. However, a variant sequence of the polyadenylation signal, AATAAT, is located 28 bases from the poly (A) tail in the 3'-untranslated region [22]. The motif ATTTA, which may be associated with mRNA stability, was also found in the 3'-untranslated region. The full nucleotide sequence of the cDNA for the major allergen of *H. japonicus* has a unique nucleotide sequence of 468 bp that include the possible leader and pre-sequences. The estimated molecular weight of the protein might be 6–7 kDa greater than that of the native Hum j 1 protein. This might imply that the N- or C-terminal site might be removed during the processing of the peptide from the immature to the mature state. A comparison of the nucleotide sequence, which was obtained, and the deduced amino acid sequence of Hum j 1 with the EMBL and Swissprot data base showed no significant homology with other sequences. However, a possibility of cross-reactivity with a food allergen has been reported with the results of SPT [24].

In the present study we used different techniques (Dot-blot immunoassay, ELISA, and Inhibition ELISA) to confirm the immunological integrity of purified rHum j 1. Dot-blot immunoassay was performed using the serum of individual patients allergic to *H. japonicus* pollen. 33.3% of the patients showed strong positive reactions with rHum j 1 and another 43.5% or 23% of the patients' sera showed mild or weak rHum j 1 specific-IgE reactivity, respectively. These data indicate that rHum j 1 is the major allergen in *H. japonicus* pollen with 100% of IgE bindings. ELISA assay revealed that recombinant allergen had the IgE binding reactivity with patients' sera and inhibition ELISA results showed that the native Hum j 1 protein was able to inhibit a maximum of 88% and 74% of the IgE binding in solid-phase bound rHum j 1 and native Hum j 1, respectively. Amounts as low as 0.06 μg/ml for the native Hum j 1 resulted in 50% inhibition of IgE binding to rHum j 1, and 0.23 μg/ml for rHum j 1 resulted in 50% inhibition of IgE binding to native Hum j 1. No inhibition of patients' IgE binding to rHum j 1 was observed when the sera were preincubated with BSA (data not shown). These results lead to the conclusion that the native Hum j 1 contains some, but not all, of the epitopes present on the rHum j 1, and that the Hum j 1 epitope is well preserved during the expression of Hum j 1 in *E. coli*.

In conclusion, cDNA for the major allergen from *H. japonicus* pollen was cloned. We report here, the full nucleotide sequence of the cDNA was 468 bp including possible leader and pre-sequences. The recombinant protein of the major allergen from cDNA of *H. japonicus* (rHum j 1) was produced in *E. coli*. The estimated molecular weight of rHum j 1 was 19 kDa and it showed strong Hum j 1 specific-IgE binding activity. The rHum j 1 molecule may contribute to the standardization of the allergens used for diagnosis and immunotherapy. Functional studies of this novel major allergen will be performed in near future.

References

1. Wilson AF, Novey HS, Berke RA, Surprenant EL. Deposition of inhaled pollen extract in human airway. N Eng J Med 1973; 288: 1056-60.

2. Schaeppi G, Taylor PE, Pain MCF, Cameron PA, Dents AW, Staff IA, Suphioglu C. Concentrations of major grass group 5 allergens in pollen grains and atmospheric respirable particles: implications for hayfever and allergic asthma suffers sensitized to grass pollen allergens. Clin Exp Allergy 1999; 29: 633-41.

3. Hong CS, Huh KB, Lee SY. Pollinosis in late summer and early fall. J Korean Soc Allergol 1981; 1-8: 160.

4. Hong CS, Lee JH, Kim KR, Lee HC, Huh KB, Lee SY. A study of intradermal test of inhalant allergens to the patients with asthma and allergic rhinitis. J Korean Soc Allergol 1982; 2: 8-15.

5. Joo YJ. Studies on pollinosis: Pollen survey in Seoul. Korean J Otolarygol 1965; 8: 11-22.

6. Yoon YW, Lee MK, Park HS, Hong CS. The results of the skin prick test and serum IgE level from allergic patients. J Asthma, Allergy and Clin Immunol 1989; 9: 385-89.

7. Yin J, Ye S, Gu R, et al. A preliminary investigation into the main allergenic pollens during autumnal seasons in Beijing area (in Chinese). Chinese J Microbiol Immunol 1996; 16: 31-35.

8. Hong CS, Hwang Y, Oh SH, Kim HJ, Huh KB, Lee SY. Survey of

the airborne pollens in Seoul, Korea. Yonsei Med J 1986; 27: 114-20.

9. Kim HS, Lee MK, Park HS, Kim HJ, Hong CS. Pollen counts in the air of Seoul during 88 Olympics (in Korean). Korean J Allergol 1989; 9: 564-70.

10. Park HS, Jung DH, Joo Y. Survey of airborne pollinosis in Seoul. J Korean Med Sci 1994; 9: 42-46.

11. Kim SK, Park HS, Oh SH, Hong CS. Cases of bronchial asthma induced by pollen extract of *Humulus japonicus* (in Korean). Korean J Allergol 1987; 7: 224-29.

12. Nahm DH, Park HS, Oh SH, Hong CS. Skin reactivity and the detection of specific IgE to the pollen of *Humulus japonicus*. Korean J Int Med 1988; 35: 213-27.

13. Park HS, Nahm DH, Suh CH, Lee SM, Choi SY, Jung KS, Lee SY, Park K. Evidence of Hop Japanese pollinosis in Korea: IgE-sensitization and identification of allergenic components. J Allergy Clin Immunol 1997; 100: 475-79.

14. Park HS, Nahm DH, Kim HY, Suh YJ, Cho JW, Kim SS, Lee SK, Jung KS. Clinical and immunological changes after allergen immunotherapy with Hop Japanese pollen. Ann Allergy Asthma Immunol 2001; 86: 444-48.

15. Park JW, Ko SH, Kim CW, Jeoung BJ, Hong CS. Identification and characterization of the major allergen of the *Humulus japonicus* pollen. Clin Exp Allergy. 1999; 29: 1080-86.

16. King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA. Allergen nomenclature. WHO/IUIS Allergen Nomenclature Subcommittee. Int Arch Allergy Immunol 1994; 105: 224-33.

17. Ministry of Education Republic of Korea. Illustrated Encyclopedia of fauna and flower of Korea (in Korean), Vol. 5: Tracheophyta. Seoul: Samhwa publishing; 1965. p.402.

18. Chomczynski P and Saccihi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 151-59.

19. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-85.

20. Aceituno E, Del Pozo V, Minguez A, Arrieta I, Cortegano I, Cardaba B, Gallardo S, Rojo M, Palomino P, Lahoz C. Molecular cloning of major allergen from *Cupressus arizonica* pollen: Cup a 1. Clin Exp Allergy 2000; 30: 1750-58.

21. Tuetcke HA, Chow KC, Mickel FS, Moss KA, Kern HF, Scheele GA. Selection of AUG initiation codons differs in plant and animals. The EMBO Journal 1987; 6: 43-48.

22. Birnstiel ML, Busslinger M, Strub K. Transcription termination and 3’ processing: The end is in site! Cell 1985; 41: 349-59.

23. von Heijne G. How signal sequences maintain cleavage specificity. Mol Biol 1984; 173: 243-51.

24. Park HS, Jung KS, Jee SY, Hong SH, Kim HY, Nahm DH. Are there any links between Hop Japanese pollen and other weed pollens or food allergens on skin prick tests? Allergy Asthma Proc 2001; 22: 43-46.

한삼덩굴 주알레르겐의 cDNA 클로닝과 특성 규명

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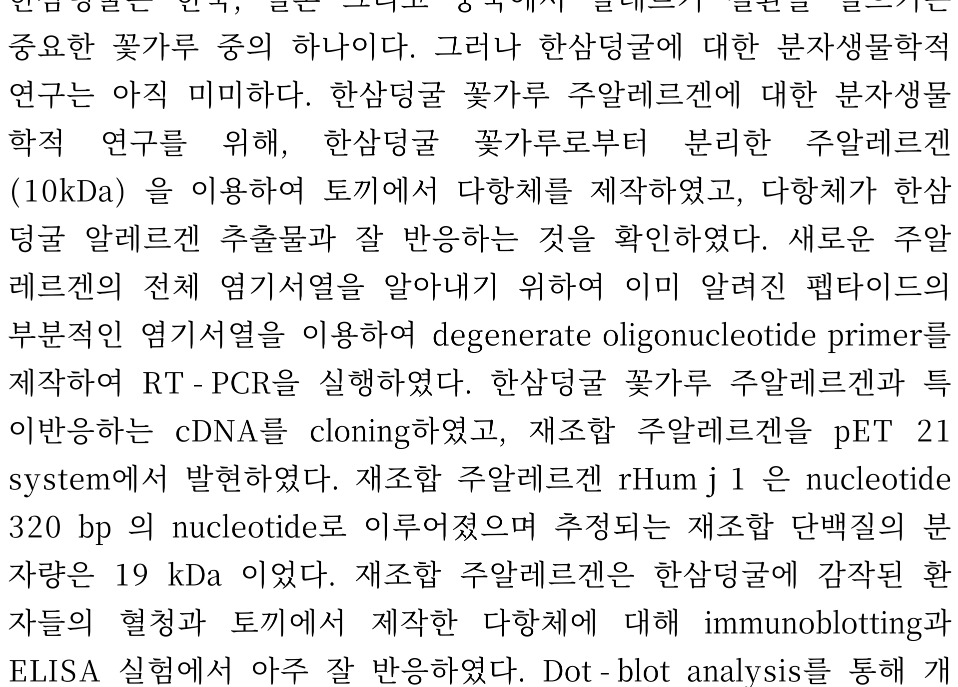
진 현선



한삼덩굴은 한국, 일본 그리고 중국에서 알레르기 질환을 일으키는 중요한 꽃가루 중의 하나이다. 그러나 한삼덩굴에 대한 분자생물학적 연구는 아직 미미하다. 한삼덩굴 꽃가루 주알레르겐에 대한 분자생물학적 연구를 위해, 한삼덩굴 꽃가루로부터 분리한 주알레르겐(10kDa) 을 이용하여 토끼에서 다항체를 제작하였고, 다항체가 한삼덩굴 알레르겐 추출물과 잘 반응하는 것을 확인하였다. 새로운 주알레르겐의 전체 염기서열을 알아내기 위하여 이미 알려진 펩타이드의 부분적인 염기서열을 이용하여 degenerate oligonucleotide primer를 제작하여 RT - PCR을 실행하였다. 한삼덩굴 꽃가루 주알레르겐과 특이반응하는 cDNA를 cloning하였고, 재조합 주알레르겐을 pET 21 system에서 발현하였다. 재조합 주알레르겐 rHum j 1 은 nucleotide 320 bp 의 nucleotide로 이루어졌으며 추정되는 재조합 단백질의 분자량은 19 kDa 이었다. 재조합 주알레르겐은 한삼덩굴에 감작된 환자들의 혈청과 토끼에서 제작한 다항체에 대해 immunoblotting과 ELISA 실험에서 아주 잘 반응하였다. Dot - blot analysis를 통해 개

별 환자의 (39명) 혈청에서 IgE level을 측정하였는데, 환자 혈청의 약 100%가 재조합 주알레르겐과 반응하는 것을 알 수 있었다. Inhibition ELISA를 통하여 재조합 주알레르겐이 순수 분리된 자연 알레르겐을 잘 억제시키는 결과를 얻었고 재조합 주알레르겐이 자연 알레르겐과 같은 알레르겐임을 확인하였다. 본 연구는 한삼덩굴의 주 알레르겐의 cDNA 전체 염기서열을 처음으로 밝혀내었고, 재조합 주 알레르겐의 allergenicity를 규명하였다.

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핵심되는 말: 한삼덩굴, 제1형 과민반응, IgE, 알레르겐