

cDNA CLONING AND EXPRESSION OF *Cry j* II, THE SECOND MAJOR ALLERGEN OF JAPANESE CEDAR POLLEN*

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We have isolated and sequenced a cDNA clone coding for *Cry j* II, the second major allergen of Japanese cedar (*Cryptomeria japonica*) pollen. A 1.7-kilobase cDNA clone contained an open reading frame coding for a 514-amino acid protein, including a putative signal peptide of 54 amino acid and three potential glycosylation sites. The deduced amino acid sequence of the *Cry j* II shows significant identities to those of the polygalacturonases associated with fruit-ripening in tomato (40 %) and avocado (43 %) and found in pollen of maize (34 %). *Cry j* II cDNA product expressed in *E. coli* was recognized by human IgE from patients suffering from cedar pollinosis, suggesting that recombinant *Cry j* II might be used as an allergen for the clinical diagnosis of cedar pollinosis. © 1994 Academic Press, Inc.

Pollen from Japanese cedar (*Cryptomeria japonica*) is a potent seasonal aeroallergen in Japan with more than 10 % of the population suffering from cedar pollinosis. Attempts have been made to purify and characterize allergens in the pollen of Japanese cedar. Now, two major allergens have been purified: the first described as Sugi Basic Protein (SBP) by Yasueda *et al.* (1) and designated as *Cry j* I according to WHO allergen nomenclature (2), and the second as *Cry j* II by Sakaguchi *et al.* (3).

Cry j I is a basic glycoprotein with molecular weight of 41 - 45 kDa and pI of 8.9 - 9.2. Taniai *et al.* (4) found a faint band distinct from the *Cry j* I molecule on SDS-PAGE while studying the molecular heterogeneity of *Cry j* I. This protein was isolated as a single band using Mono S column chromatography and designated as *Cry j* II (3) as the basis of its molecular and allergenic differences with *Cry j* I. *Cry j* II was detected on SDS-PAGE as a single band with molecular weight of 37 kDa or 45 kDa under either non-reducing or reducing conditions and showed a pI value of >9.5 on isoelectrofocusing (3).

* The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession number D29772.

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The complete amino acid sequence of *Cry j* I was recently reported by this laboratory (5), whereas only a 10-residue amino acid sequence at the *N*-terminus of *Cry j* II has been reported (3). The small amount of *Cry j* II protein present in the pollen prevents the isolation of the allergen, resulting in few reports concerning the biochemical and immunological properties of *Cry j* II. In the present study, we have isolated a cDNA clone coding for *Cry j* II and determined the complete amino acid sequence of the allergen. Furthermore, we present evidence that human IgE from patients suffering from pollinosis binds to recombinant *Cry j* II (r*Cry j* II) expressed in *E. coli*.

MATERIALS AND METHODS

Plant materials. Pollen of *Cryptomeria japonica* was collected from mature male flowers of Japanese cedar trees and was stored at -70 °C prior to use. Crude extract of the pollen was obtained by dialysis against PBS after extraction of the defatted pollen followed by the precipitation with 80 % saturated ammonium sulfate (1).

Isolation of RNA and construction of cDNA library. Isolation of total RNA from pollen and construction of a lambda-gt10 cDNA library were previously reported (5).

cDNA library screening. The lambda-gt10 cDNA library was screened using a [³²P]-labeled synthetic oligonucleotide probe (5'-GTA-TTT-TTC-(G/C)AC-GTT-GAA-(G/T)AT-GTT-(G/T)AT-(G/T)GC-3'). This probe is complementary to the DNA coding sequences deduced from the *N*-terminal ten amino acid sequence (AINIFNVEKY-) of the native *Cry j* II protein (3). As for the design of the probe, we allowed mismatched T:G at the third letters of the codons, since mismatched T:G base pairs were somewhat more stable than other mismatched base pairs (6). Since there was no A at the third letter of 15 codons for Val in the *Cry j* I cDNA sequence (5), we chose G and C for this position.

The hybridization conditions used were 5 x SSPE, 5 x Denhardt's, 0.1 % SDS, 100 µg/ml denatured salmon sperm DNA, and 1 p mol of [³²P]-labeled oligonucleotide at 48 °C for 14 h. Filters were then washed for 30 min at room temperature, for 5 min at 48 °C and autoradiographed for 3 h. The DNA sequence was determined by fluorescence-based dideoxy sequencing using a 370A DNA sequencer (Applied Biosystems Inc., CA, U.S.A.) (7).

Northern blotting. Three microgram of poly (A⁺) RNA from the pollen was electrophoresed through a 1 % formaldehyde-containing gel, blotted onto a Hybond N+ nylon membrane (Amersham, International Ltd., Buckinghamshire, England) and hybridized to [³²P]-labeled cDNA clone according to standard procedures (6).

Expression of *Cry j* II cDNA in *E. coli*. A *Cry j* II cDNA fragment (starting amino acid at position -40) was cloned in-frame into the pGEMEX expression vector (Promega Corp., WI, U.S.A.) to produce a fusion protein with an amino terminal 26 kDa fragment of T7 gene 10 product. The *Cry j* II expression vector was transformed into the BL21(DE3) host bacteria. Expression of the fusion protein was induced when cultures reached OD₆₀₀ 0.7 to 1.0 by 2 mM isopropyl-D-thiogalactopyranoside. After 4 h of further growth, the cells were pelleted, resuspended in 1 mg/ml lysozyme containing buffer (50 mM sodium phosphate pH 8.0, 300 mM NaCl) and incubated for 20 min on ice. The cell suspension was sonicated and the insoluble fusion protein was recovered by low speed centrifugation. The protein was boiled for 7 min in loading buffer and subjected to gel electrophoresis and Western blotting. For IgE binding assay, insoluble materials from the previous step were solubilized in a solution of 0.1 M sodium phosphate and 10 mM Tris-HCl pH 8.0 containing 8 M urea and used as such.

Human sera. Human sera from five patients with allergic rhinitis and from five healthy individuals, were obtained from volunteers working in this institute who approved the use

of their sera for use in this study. All the patients were shown to have specific IgE against *Cryptomeria japonica* pollen by the radioallergosorbent test (RAST; Pharmacia Japan, Tokyo, Japan) with more than 3.5 Pharmacia RAST unit/ml (PRU/ml) (Table 1).

Immunoblot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in 5 - 20 % acrylamide gels. The transfer to PVDF membrane (Nihon Millipore Ltd. Tokyo, Japan) was carried out by the method of Burnette (8). The filters were blocked by incubation in 10 mM Tris-HCl, pH 7.4 and 150 mM NaCl (TBS) containing 0.5 % skim milk and then incubated for 48 h with two fold-diluted human serum with TBS. The blots were washed several times using TBS containing 0.05 % Tween-20, incubated for 16 h with [¹²⁵I]-labeled anti human IgE from sheep (Pharmacia, Uppsala, Sweden) and finally washed once again as before. The blots were then exposed to X-ray film at -80 °C overnight.

Protein concentration determination. Protein concentration was determined using BCA Protein Assay Reagent (Pierce, IL., U.S.A.) according to the protocol included with the kit.

A fluorometric assay for IgE bound to rCry j II. One microgram of the crude extract of pollen or 4 µg of rCry j II were coated on 96-well black plate (Dainippon Pharmaceutical Co., Osaka, Japan) at 4 °C for 16 h and the wells were blocked with Block Ace (Dainippon Pharmaceutical Co., Osaka) at 37 °C for 2 h. Thirty microliter of human serum was diluted to 100 µl with Block Ace, transferred to each well, and incubated at 37 °C for 4 h. The following procedures were performed according to the method of Kato *et al.* (9): the IgE antibody which bound to the solid-phase antigen was detected by β-galactosidase-conjugated anti IgE antibody (Pharmacia, Uppsala, Sweden), 4-methylumbelliferyl-β-D-galactopyranoside was used as the enzyme substrate and the fluorescence arose when the reaction product was detected by Titertek Fluoroskan II (Flow Laboratories Inc., VA., U.S.A.).

RESULTS AND DISCUSSION

N-terminal ten amino acids of the Cry j II allergen has been reported by Sakaguchi *et al.* (3). A synthetic oligonucleotide probe (described under Materials and Methods) was end labeled with [³²P] and used to screen a lambda-gt10 cDNA library of Japanese cedar pollen. Four clones were isolated among 40,000 plaques and they had about 1.7 kilobase cDNA inserts hybridizing to the oligonucleotide probe. The longest cDNA clone (named II-1) was sequenced completely in both strands. Sequence analysis of clone II-1 revealed that the insert is 1733 nucleotides long and has 44 nucleotides preceding the initiator ATG start codon, an open reading frame of 1533 nucleotides coding for a protein of 514 amino acids and a 122-nucleotide untranslated AT-rich region preceding the poly (A) addition site. The position of initiator methionine was confirmed by the presence of an in-frame upstream stop codon (TAG) and by 78 % homology between the plant consensus sequence that encompasses the initiator methionine TAAATGGC (base at positions 41 through 49, Fig. 1) found in Cry j II and the AACAAATGGC consensus sequence for plants (10). The amino acid sequence at positions 55 through 64 from initiator methionine matches the *N*-terminal Cry j II protein sequence previously determined (3), confirming the identity as authentic Cry j II. The *N*-terminal 54 amino acids are probably the signal peptide because the cDNA *N*-terminal sequence is not contained in the mature protein and hydrophobic as has been noted for other signal peptides. The mature protein also contains three potential

AGTTGAGTTCGAGACAAGTATAGAAGAATTTCTTTTATTAAAAATGGCCATGAAA	56
M A M K	-51
TTAATTGCTCCAATGGCCTTTCTGGCCATGCAATTGATTATAATGGCGGCAGCAGAAGATCAATCTGCCCAAATT	131
L I A P M A F L A M Q L I I M A A A E D Q S A Q I	-26
ATGTTGGACAGTGTGTGCAAAATATCTTAGATCGAATCGGAGTTTAAAGAAAAGTTGAGCATCTCGTCATGAT	206
M L D S V V E K Y L R S N R S L R K V E H S R H D	-1
CCTATCAACATCTTCAATGTGGAAAAATATGGCGCAGTAGCGCATGGAAAGCATGATTGCACTGAGGCATTTTCA	281
<u>A I N I F N V E K Y</u> G A V G D G K H D C T E A F S	25
ACAGCATGGCAAGCTCCATGCCAAAAACCCATCAGCAATGTTGCTTGTGCCAGCCAGCAGAAATTTGTTCTAAAC	356
T A W Q A A C K N P S A M L L V P G S K K F V V N	50
AATTTGTTCTTCAATGGGCCATGTCAACCTCACTTTTACTTTTAAAGTAGATGGGATAATAGCTGCGTACCAAAAT	431
N L F F N G P C Q P H F T F K V D G I I A A Y Q N	75
CCAGCGAGCTGAAGAATAATAGAATATGTTGTCAGTTTGTCTAAACTTACAGGTTTACTCTAATGGGTAAAGGT	506
P A S W K N N R I W L Q F A K L T G F T L M G K G	100
GTAATTGATGGCAAGGAAAAATGTTGGGCTGGCCAATGTAAATGGGTCAATGGACGAGAAATTTGCAACGAT	581
V I D G Q G K Q W W A G Q C K W V N G R E I C N D	125
CGTGATAGACCAACAGCCATTAATTCGATTTTCCACGGGTCTGATAATCCAAGGACTGAAACTAATGAACAGT	656
R D R P T A I K F D F S T G L I I Q G L K L M N S	150
CCCGAATTCATTAGTTTGTGGAAATGTGAGGGAGTAAAAATCATCGCCATTAGTATTACGGCACCAGAGAGAC	731
P E F H L V F G N C E G V K I I G I S I T A P R D	175
AGTCCTAACACTGATGGAATTGATATCTTTCATCTAAAAACTTTCACTTACAAAAGAACACCGATAGGAACAGGG	806
S P N T D G I D I F A S K N F H L Q K N T I G T G	200
GATGACTGCGTCTGCTATAGGCACAGGGTCTTCTAATATTGTGATTGAGGATCTGATTGCGGTCCAGGCCATGGA	881
D D C V A I G T G S S N I V I E D L I C G P G H G	225
ATAAGTATAGGAAGTCTTGGGAGGAAAACTCTAGAGCAGAGGTTTCATACCTGCACGTAAATGGGCTAAATTC	956
I S I G S L G R E N S R A E V S Y V H V N G A K F	250
ATAGACACAAAAATGGATTAAGAATCAAAACATGGCAGGGTGGTTACGGCATGGCAAGCCATATAATTTATGAG	1031
I D T Q N G L R I K T W Q G G S G M A S H I I Y E	275
AATGTTGAAATGATAAATTCGGAGAACCCCATATTAATAAATCAATTCTACTGCACTTCAGCTTCTGCTGCCAA	1106
N V E M I N S E N P I L I N Q F Y C T S A S A C Q	300
AACCAGAGGTCTCGGTTCAATCCAAGATGTGACATACAAGAACATACGTGGGACATCAGCAACAGCAGCAGCA	1181
N Q R S A V Q I Q D V T Y K N I R G T S A T A A A	325
ATTCAACTTAAGTGCAGTGACAGTATGCCCTGCAAGATATAAGCTAAGTGATATATCTTTGAAGCTTACCTCA	1256
I Q L K C S D S M P C K D I K L S D I S L K L T S	350
GGGAAAAATTGCTTCCTGCCCTTAATGATAATGCAAAATGGATATTTTCAGTGGACAGTCATCCCTGCATGCAAGAAT	1331
G K I A S C L N D N A N G Y F S G H V I P A C K N	375
TTAAGTCCAAGTGCTAAGCGAAAAGAAATCTAAATCCCATAAACACCCAAAAACTGTAATGGTTGAAAAATATGCGA	1406
L S P S A K R K E S K S H K H P K T V M V E N M R	400
GCATATGACAAGGTAACAGAACACGCATATTTGTTGGGTCGAGGCCCTCCGAATTGTACAAACAAATGTCATGTT	1481
A Y D K G N R T R I L L G S R P P N C T N K C H G	425
TGCAGTCCATGTAAGGCCAAGTTAGTTATTGTTTCATGTTATGATGCCGAGGAGTATTATCCTCAGAGGTGGATA	1556
C S P C K A K L V I V H R I M P Q E Y Y P Q R W I	450
TGCAGCTGTCATGGCAAAATCTACCATCCATAATGAGATACATTGAAACTGTATGTGCTAGTGAATATTCTTGTG	1631
C S C H G K I Y H P *	460
GTACAATATTAGAAGTATATGAAAAATAAATCATCAATGTTTCTAAGGCATTTATAATAGATTATATTAATGGT	1706
TCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1733

Figure 1.

Nucleotide and predicted amino acid sequences of a *Cryj* II cDNA clone. An ATG initiation codon and a TAA stop codon present bold types and an asterisk, respectively. The putative signal peptide is indicated by negative numbers. Underlined amino acids were identified by amino acid sequencing of *Cryj* II (3). Potential glycosylation sites are boxed. Dotted underlines mean putative polyadenylation signals.

N-linked glycosylation sites corresponding to the consensus sequence N-X-S/T.

The nucleotide sequence and the deduced amino acid sequence of the *Cry j* II were compared to the sequences in the data bases of EMBL (R35.0, June, 1993), GenBank (R77.0, June 1993) and PIR (R37.0, June 1993). The amino acid sequence of *Cry j* II showed 43 %, 40 %, and 34 % identities with those of polygalacturonase associated with fruit-ripening in avocado (11) and tomato (12), and expressed in pollen of maize (13), respectively (Fig. 2). The comparison among the four amino acid sequences reveals the presence of highly conserved domains. A His-residue at position 278 may be a part of the active site of polygalacturonase (14). These results suggest that *Cry j* II protein may belong to the family of polygalacturonase involved in the cell wall degradation associated with pollen tube penetration and expansion into pistils.

As shown in Fig. 2, C-terminal sequence of the *Cry j* II is somewhat longer than those of polygalacturonases from other plants. *Cry j* II polypeptide consisted of 460 amino acids and its molecular weight was calculated to be 50,444 Da, whereas, native *Cry j* II prepared from pollen showed a band at the 45 kDa position under reducing conditions on SDS-PAGE (3). These observations suggest that C-terminal processing of *Cry j* II may occur.

In order to characterize the mRNA for the *Cry j* II allergen, a Northern blot analysis was performed on poly (A⁺) RNA. A single 1.8-kilobase RNA species was detected using the [³²P]-labeled *Cry j* II cDNA probe (data not shown).

In order to define the *Cry j* II protein as an allergen, *Cry j* II cDNA (start Gln at position -40) was cloned into the expression vector pGEMEX, and expressed as a fusion protein with a T7 gene 10 product in *E. coli*. Figure 3A shows SDS-PAGE profiles of the r*Cry j* II expressed in *E. coli*. A 80 to 85 kDa polypeptide was observed (lane 3) and the molecular weight estimated was similar to that expected from the cDNA insert (55 kDa) and the T7 gene 10 (26 kDa). Since the lysate of *E. coli* cells transfected with intact vector did not show any polypeptide bands at this position, the band was concluded to be a fusion protein. Next, we examined whether IgE from a patient with cedar pollinosis bound to the r*Cry j* II molecule by Western blot analysis. IgE from a serum (patient #2) showed the IgE binding to r*Cry j* II but did not bind to control parental bacterial lysate (Fig. 3B, lanes 1 and 3) and normal human sera did not bind to the protein (data not shown). Therefore, r*Cry j* II is capable of binding IgE from a patient with cedar pollinosis.

We further analyzed the binding activity of IgE to the r*Cry j* II using the fluorometric enzyme-linked immunosorbent assay (Table 1). Five sera from patients bound to crude extract of cedar pollen and three of them bound to r*Cry j* II allergen. The same experiments were done using five sera from healthy individuals but they did not bind to both allergens. Sakaguchi *et al.* (3) previously reported that 10 of 14 sera (71 %) from patient with pollinosis bound to native *Cry j* II allergen. Binding activity of the IgE to r*Cry j* II (3/5, 60 %) in our observation was in good agreement with their results. Therefore it may be possible to use r*Cry j* II as an allergen in

Avocado		MAL	TRLLLPISIL	13
Tomato			M	1
<i>Cry j II</i>		MAMK	LIAPMAFLAM QLIIMAAED QSAQIMLDSV	34
Avocado	WFCFYSSHTI	LQKDPLLICV	NGD-GFDQRA YPTYFGPIL- *EFS*SIMGF	61
Tomato	VIQRNSILL	IIIFASSIST	CRSNVIDDNL FKQVYDNILE -EFAHDFQAY	51
Maize			MACTNN AMRALFLVL	16
<i>Cry j II</i>	VEKYLRSNRS	SRKVEHSRHD	AINIFNVEKY GAVGDGKHDC TEAFSTAWQA	84
Avocado	EPSI*L-LER	FNPVGGPETS	PDTDIS-DDF --R---TD-* -K--EK--KD	109
Tomato	LSYLSKNIE-	NNNIDKVDKN	G-KVI--LSF --K---TYD NI--EQ--NE	101
Maize	FCIVHGEKEE	SKGIDAKASG	PGGS-DIT-L --S-N--T-S -K-VQE--AS	66
<i>Cry j II</i>	ACKNPS*AML	LVPGSKKFVV	NNLFFNGPCQ PHFTFKVDGI IAAYQNPASW	133
Avocado	--SSG-*V*-	I--EN-NYLL	KQIT-S---K SCLR-V-IR-T -E-SSDQSD-	157
Tomato	--SSRTPVQF	V--KN-NYLL	KQIT-S---R SSISV-IF-S LE-SSKISDY	151
Maize	--GGTGKQTI	-I-K*GD-L-	GQ-N-T---K GDV-IQ---N LL-TTDLSEQY	115
<i>Cry j II</i>	*KNNRI*WLQ	FAKLTGFTLM	GKGVIDGQKQ QWWA*GQCKW VNGREICNDR	180
Avocado	VGH--KR-IE	-EDISNL--E	-G-T-N-N-E T--D*SS--* RKKSLP-***K	203
Tomato	*-D*-RL-IA	-DSVQNLVVG	-G-T-N-N-Q V--P*SS--* I-KSLP-***-	195
Maize	*-DH*GN-IE	ILRVDNLVIT	---NL----P AV-SKNS-T* **K*YD-***K	158
<i>Cry j II</i>	DRPTAIKPDF	STGLIIQGLK	LMNSPEFHLV FGNCGEVKII GISITAPRDS	230
Avocado	SA---LT-RS	CKN--VSD-S	IKD-QKM--S -DK-QD-IAS NLMV---EH-	253
Tomato	-A---LT-WN	CKN-KVNN--	SK-AQQI-IK -ES-TN-VAS NLM-N-SAK-	245
Maize	IL-NSLVM--	VNNGEVS-VT	-L--KF--MN MYR-KDMLIK DVTV---G--	208
<i>Cry j II</i>	PNTDGIDIFA	SKNFHLQKNT	IGTGDDCVAI GTGSSNIVIE DLICGPGHGI	280
Avocado	-----H-TG	TQRI-VMNSV	-----IS- ES--KMVIAT NIT-----	303
Tomato	-----VHVS	N TQYIQISDTI	-----IS- VS--Q-VQAT NIT-----	295
Maize	-----BMGD	-SGITITNTV	--V----IS- -P-T-KVN-T GVT-----	258
<i>Cry j II</i>	SIGSLGRENS	RAEVSIVHVN	GAKFIDTQNG LRIK*TWQGG S*GMASHIY	328
Avocado	-----DR--	E-H--G-L-D	-GNLF--T-- ----*----- -*-S-KN-KF	351
Tomato	-----SG--	E-Y--N-T--	E--I-GAE-- V---*----- -*-Q--N-KF	343
Maize	-----YKD	EKD-TDIN-K	DCTLKK-MF- V---AYEDAA -VLTV-K-H-	308
<i>Cry j II</i>	ENVEMINSEN	PILINQFYCT	SASACQNQRS AVQIQDVITYK NIRGTSATAA	378
Avocado	Q-IV-H-VT-	--I-D-Y--D	-KDP-PE-E- --KVS-N-A-M -----SEV	401
Tomato	L----QDVKY	--I-D-N--D	RVEP-IQ-F- ---VKN-V-E --K-----KV	393
Maize	--IK-ED-A-	--F-DMK--P	NKLCTA-GA- K-TVK---F- --T---S-PE	358
<i>Cry j II</i>	AIQLKCSDSM	PCKDIKLSDI	SLKLTSGK** *I*ASCLNDN ANGYFSGHVI	424
Avocado	-VKFD--K-S	--QGYIVGN-	N-VGNG--ET TM**--S-IV QGLLRE-LST	449
Tomato	--KFD--TNF	--EG-IMEN-	N-VGE---PS EA**T-K-VH F-NA**EH-T	439
Maize	-VS-L-TAKV	--TGVTHD-V	NV*EY--TNN KTM-I-T-AK GSTKGC*LKE	406
<i>Cry j II</i>	PACKNLSPSA	KRKESKSHKH	PKTVMVENMR AYDKGNRTRI LLGSRPPNCT	474
Avocado	FLFMKRRVHE	CSY		462
Tomato	-H-TSLEISE	DEALLYNY		457
Maize	L--F			410
<i>Cry j II</i>	NKCHGCSPCK	AKLVIVHRIM	PQEYYPQRWI CSCHGKIYHP	514

Figure 2.

Sequence comparison among *Cry j II* and polygalacturonases associated with fruit-ripening in tomato and avocado and expressed in pollen of maize. Horizontal bars mean identical amino acids. The asterisk indicates a blank that has been inserted to maximize identity.

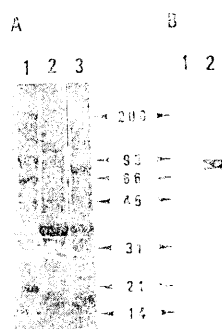


Figure 3.

Detection of a *Cryj* II fusion protein by staining and Western blotting. Cell lysates of *E. coli* with or without a *Cryj* II cDNA expression plasmid were separated by SDS-PAGE (5-20% gel) and the gel was stained by Coomassie blue (A). The proteins in the gel were transferred to a PVDF membrane and analyzed by Western blotting with serum from a patient #2 (B) as described under Materials and Methods. Molecular weight markers (A, lane 1), parental *E. coli* (A, lane 2 and B, lane 1) and *E. coli* harboring a *Cryj* II cDNA expression plasmid (A, lane 3 and B, lane 2).

place of native *Cryj* II prepared from the pollen for clinical diagnosis of cedar pollinosis in the future.

In the present study, we have reported the amino acid sequence of *Cryj* II, the second major allergen of Japanese cedar pollen. Sequence information of *Cryj* II may facilitate study and the determination of T and B cell epitopes of the allergen by use of synthetic overlapping peptides. We also note the possibility that r*Cryj* II expressed in *E. coli* may be used as an allergen in place of native *Cryj* II for the diagnosis of cedar pollinosis and immunotherapy.

Table 1. Binding Activities of IgE to Crude Extract of Cedar Pollen and r*Cryj* II

Sera	RAST (PRU/ml)	Allergens	
		Crude Extract	r <i>Cryj</i> II
Patient#1	> 17.5	509	3,000 ^{a)}
Patient#2	> 17.5	578	709
Patient#3	> 17.5	319	4
Patient#4	3.5 - 17.5	640	4
Patient#5	3.5 - 17.5	100	426
Control#1	< 0.35	5	5
Control#2	< 0.35	4	5
Control#3	< 0.35	6	5
Control#4	< 0.35	4	5
Control#5	< 0.35	5	4

^{a)} values are relative fluorescence units as a patient#1 serum corresponds to 3,000 units.

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