

Identification of mutations in the genes for the pollen allergens of eastern red cedar (*Juniperus virginiana*)

T. MIDORO-HORIUTI, R. M. GOLDBLUM and E. G. BROOKS

Department of Paediatrics, Child Health Research Center, University of Texas Medical Branch, Galveston, TX, USA

Summary

Background Cedar pollens are important causes of seasonal allergic disease in diverse geographical areas. However, pollens from different families and species vary in their propensity to induce allergic responses.

Objective To compare the structure of potential allergens from eastern red cedar (*Juniperus virginiana*) pollen with those of the highly allergenic cedar (mountain cedar, *J. ashei*) pollens.

Materials and methods The cDNAs for potential pollen allergens, Jun v 1 and Jun v 3, were amplified by reverse transcriptase-polymerase chain reaction, cloned and sequenced. Expression of the native proteins in pollen was characterized by SDS–PAGE and immunoblotting.

Results The cDNA sequence for one potential major allergen, Jun v 1, was highly homologous to those of the other cedar pollens. The second potential allergen, Jun v 3, was also highly homologous to its counterpart in mountain cedar, but a stop codon in the mRNA would result in a protein of only 91 amino acids, which would lack potential N-glycosylation sites and the IgE binding epitopes of the 199 amino acid homologue from mountain cedar pollen, Jun a 3. IgE from the sera of patients with hypersensitivity to cedar pollen bound to eastern red cedar proteins of four different sizes. N-terminal amino acid sequence analysis indicated that two of these proteins (43 and 30 kDa) were either isoforms or processed Jun v 1. No Jun v 3 protein was detected. The N-terminal sequence of an additional 145-kDa allergen, termed Jun v 4, was not homologous to any previously described allergens.

Conclusion These findings suggest that mutations in the genes or post-translational modifications of two potentially allergenic proteins might help to explain why the pollen of eastern red cedar is reported to be less allergenic than those of other members of Cupressaceae and Taxodiaceae families.

Keywords: cedar allergy, eastern red cedar, Jun a 1, Jun a 3, *Juniperus ashei*, Jun v 1, Jun v 3, *Juniperus virginiana*, pollen allergy, pollinosis

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Correspondence: Terumi Midoro-Horiuti MD, PhD, Department of Paediatrics, University of Texas Medical Branch, Route 0366, Galveston, TX 77555-0366, USA.

The protein sequences reported in this study will appear in the Swissprot protein sequence databank under the accession numbers P81825 and P81826. The nucleotide sequences reported in this study have been submitted to the GenBank/EMBL Data Bank, with accession numbers AF151427, AF151428, AF151429 and AF151430.

Introduction

The pollens of conifers in diverse geographical areas are often highly allergenic. We recently reported the cloning of two allergens, Jun a 1 [1] and Jun a 3 [2], from mountain cedar (*Juniperus ashei*, Cupressaceae) pollen. The major protein, Jun a 1, has about 80% homology to Cry j 1 (the major allergen of Japanese cedar, *Cryptomeria japonica*,

Taxodiaceae) and Cha o 1 (the major allergen of Japanese cypress, *Chamaecyparis obtusa*, Cupressaceae).

The second major protein allergen in mountain cedar, Jun a 3, had no homology to Cry j 1, Cry j 2, or any other pollen allergens, but was homologous to the pathogenesis related protein (PR-protein) family, PR-5 group. PR-proteins are made by plants during stress, such as drought, freezing temperature and infection by virus, viroids, fungi, or bacteria [3], and exposure to environmental pollutants, such as ozone [4]. Some members of other PR-protein groups, expressed in fruits, pollen and leaves, have been shown to be allergenic [5,6].

Juniperus virginiana (Cupressaceae), also called eastern red cedar, is the most abundant juniper in the eastern half of the USA. Eastern red cedar pollinates from early February to late March, while mountain cedar pollinates from December to January. Although mountain cedar is well described as a potent allergen, and despite cross-reactivity with eastern red cedar pollen in allergic patients [7–9], eastern red cedar is not reported to produce the severe seasonal allergic symptoms that are so characteristic of mountain cedar pollinosis [9]. We therefore questioned whether differences in the reported allergenicity of these two closely related species were due to differences in the molecular structures of their major pollen allergens. To that end, we cloned the eastern red cedar pollen proteins that were homologous to the mountain cedar allergens, Jun a 1 and Jun a 3. Comparisons between these proteins from the two different species may suggest a role for certain protein structures in the allergenic potency of these pollens.

Materials and methods

Messenger RNA purification, complementary DNA synthesis, and reverse transcriptase-polymerase chain reaction

Eastern red cedar pollen was collected in Texas and Florida in 1999 or purchased (Bayer, IN, USA). Messenger RNA was purified from disrupted pollen with guanidinium thiocyanate, using oligo (dT)-cellulose (Pharmacia Biotech, Uppsala, Sweden) as previously described [1]. The first-strand cDNA was synthesized, using oligo dT primer (GIBCO BRL, Rockville, MD, USA) and reverse transcriptase (GIBCO BRL). Forward (5'-CATATGGATAATCCCA TCGACA GC-3') and reverse (5'-GAATTCTAAGCAAG ATTTAGA GAGAG-3') primers for Jun v 1 and forward (5'-CA TATGGTGAAGTTTGATATAAAG-3') and reverse (5'-G AATTCATAATCCTACGTGGG-3') primers of Jun v 3 were synthesized, based on the Jun a 1 [1] and Jun a 3 [2] nucleotide sequences. Polymerase chain reaction (PCR) amplification was performed for 25 cycles, with each cycle consisting of 45 s at 95°C for denaturation, 30 s at 60°C for Jun v 1 and 55°C for Jun v 3 to anneal primers and 1 min at

72°C for polymerization. The PCR products were analysed by agarose gel and purified by QIAquick gel extraction kit (Qiagen, Hilden, Germany).

CDNA cloning, sequencing, and sequence analysis

The cDNA was ligated into pCR II and pCR2.1 vectors, subcloned and sequenced using a Perkin Elmer/Applied BioSystems model 373A DNA sequencer and primers for T7, SP6 or M13 sites. In order to confirm the fidelity of the nucleotide sequences, they were repeated on three independent RT-PCRs on different preparations of mRNA obtained from pollens from Texas and Florida. To obtain the 5' end of the cDNA sequence, we used primers synthesized based on the leader sequences or 5' untranslated region of Jun a 1 (5'-GAAATAAATTCTATATTCT-GAACCC-3') and Jun a 3 (5'-AATGGCCCCGAGTATC AGAGC-3'). These were paired with forward primers for Jun v 1 and Jun v 3.

Sequences were compared against the GenBank database (GCG) for homology to known sequences (Madison, WI, USA).

Extraction of the eastern red cedar pollen proteins

Crude extract (CE) of eastern red cedar was obtained as described for mountain cedar [2]. Briefly, the pollen grains were extracted in 0.125 M ammonium bicarbonate (pH 8.0, containing 0.02% NaN₃ and 50 µM 4-(2-aminoethyl)-benzenesulphonyl fluoride). A fraction with differential solubility in 40–80% ammonium sulphate was collected.

The molecular weight and isoelectric point determination and Western blotting

The molecular weight and isoelectric point of Jun v 1 and Jun v 4 were determined by SDS-PAGE of reduced samples [10] and non-equilibrium pH gradient electrophoresis in tube gel, with comparison to the coomassie blue and gold (Pierce, Rockford, IL, USA) stained molecular weight and isoelectric focusing standards (BioRad, Richmond, CA, USA).

For Western blot analysis, the proteins were transferred from unstained SDS-PAGE gel to nitrocellulose membranes [11] and incubated with anti-Cry j 1 mouse polyclonal or monoclonal antibodies (Kowa, Tsukuba, Japan [12]) or patients' sera followed by the appropriate biotinylated second antibody and detection with horseradish peroxidase (HRP)-streptavidin (Pierce) and ECL (Pharmacia).

Automated protein/peptide sequence analysis

Primary structure was analysed on protein bands transferred to PVDF sheets, using an Applied Biosystems model 475A protein/peptide microsequencer with an on-line model 120A microbore phenylthiohydantoin-amino acid analyser and a model 900 data processor as previously described [13]. The repetitive yields ranged from 90% to 95% for the peptides.

Human sera

Serum samples were obtained from 13 mountain cedar-allergic patients in Texas. The diagnosis of seasonal allergic rhinitis due to cedar sensitivity was established in these subjects by clinical history and scratch tests results. Serum samples used as negative controls were collected from 12 non-atopic volunteers, who lacked skin test reactivity, by scratch testing with cedar and dust mite extracts (*Dermatophagoides farinae*).

Results

Nucleotide and amino acid sequences of Jun v 1 and Jun v 3

The consensus DNA sequences obtained for Jun v 1 and Jun v 3 from three mRNA preparations are shown in Fig. 1. The complete cDNA coding region of Jun v 1 is shown in Fig. 1a. The sequence of the N-terminal amino acids was identical to that inferred from the nucleotide sequence. Potential N-glycosylation sites were noted at N¹²⁷TS and N¹⁵⁷VT. A TAA stop codon was found at nucleotide position 949, which would result in a mature protein of 346 amino acids.

The cDNA containing the complete coding region of Jun v 3 is shown in Fig. 1b. The 18 nucleotides preceding the ATG start codon are likely to represent a signal peptide or non-coding region, because these amino acids are not found in the purified protein. A TAA stop codon was found at position 274, which would result in a mature protein of only 91 amino acids (Fig. 1b) and an estimated molecular weight (MW) of approximately 10 kDa.

Homology analysis of Jun v 1 and Jun v 3 amino acid sequences

The nucleotide and inferred amino acid sequences of Jun v 1 and Jun v 3 are shown in Fig. 2. The high degree of sequence identity between Jun v 1 and Jun a 1, Cha o 1 and Cry j 1 is shown in Fig. 2a. Potential N-glycosylation sites are indicated by double underlines. The inferred amino acid sequence of mature Jun v 3 protein had only one amino acid difference from Jun a 3. However, a mutation at

nucleotide 272 would lead to a Jun v 3 protein of only 91 amino acids (rather than 199), because of the generation of a stop codon (Fig. 2b).

Molecular weight, antibody reactivity and N-terminal amino acid sequences of eastern red cedar pollen proteins

Figure 3 shows the electrophoresis pattern of a crude extract of eastern red cedar pollen, stained with coomassie blue and colloidal gold. The prominent protein bands had apparent MWs of 145, 43, 30 and 17 kDa. The 43-kDa band stained positively with concanavalin A (Con A) (not shown), anti-Cry j 1 polyclonal antibody, and anti-Cry j 1 monoclonal antibody (not shown), indicating that this band contained a glycoprotein that was immunologically cross-reactive with Cry j 1. No protein of the predicted size of the truncated Jun v 3 was detected on coomassie-stained SDS-PAGE gels or by Western blot analysis with human IgE. Jun v 1 had isoelectric points of 6.2 and > 8.5, while that of Jun v 4 was > 8.5.

The sequences of the first 5 N-terminal amino acids of the 145, 43 and 30-kDa proteins were analysed. The 43-kDa protein (Jun v 1A) was identical to Jun a 1 (DNPID/) and corroborated the nucleotide sequence of Jun v 1. The 30-kDa protein also had an identical N-terminal sequence to Jun a 1 (DNPID/), suggesting that it represented a truncated (or post-translationally modified) form of Jun v 1, which we termed Jun v 1B. The N-terminal amino acid sequence of the 145-kDa protein (ADAKS/) had no significant homology to any known protein, and was thus termed Jun v 4. The 17-kDa band had a N-terminal amino acid sequence of (XEADE/), which also had no significant homology to any known protein.

The IgE from 11 out of 13 (84.6%) of the patients' sera reacted with the 145-kDa protein. Twelve (92.3%) of 13 sera reacted with the 30-kDa, and one (7.7%) was positive to a 17-kDa unidentified protein. Six out of 13 (46.2%) were very weakly positive to the 43-kDa band of Jun v 1 (Fig. 3). Repeated experiments did not improve upon the binding of patient's serum IgE to Jun v 1 in Western blot analysis (data not shown). In fact, Jun a 1 was also not detectable in our IgE Western blot.

Discussion

Despite the abundance of the trees, high seasonal concentration of atmospheric pollen and the cross-reactivity with mountain cedar pollen, eastern red cedar is not considered an important aeroallergen [9,14]. In contrast, mountain cedar is a major cause of seasonal pollinosis [15]. In this study, we first examined the pollen from eastern red cedar for mRNAs encoding the two major allergens we cloned from mountain cedar pollen. Using primers designed from the sequence of mountain cedar

(a)				ATG M	-21
GCTTCCCCATGCTTA	ATAGCATTCCTTGTT	TTCCTTTGTGCAATT	GTATCTTGCTGCTCT		
A S P C L	I A F L V	F L C A I	V S C C S		-20
GATAATCCCATAGAC	AGCTGCTGGAGAGGA	GATTTCGAACTGGGGT	CAAAACAGAATGAAG		
<u>D N P I D</u>	S C W R G	D S N W G	Q N R M K		20
CTCGCAGATTGCGCT	GtGGGATTTGGAAGC	TCCACCATGGGAGGC	AAAGGAGGAGATTTT		
L A D C A	V G F G S	S T M G G	K G G D F		40
TACACCGTCACAAGC	GCAGATGATAATCCT	GTGAATCCTACACCA	GGAAC TTTGCGCTAT		
Y T V T S	A D D N P	V N P T P	G T L R Y		60
GGAGCAACAAGAGAA	AAAACACTTTGGATC	ATTTTCTCTCAGAAT	ATGAATATAAAGCTC		
G A T R E	K T L W I	I F S Q N	M N I K M		80
AAGATGCCTTTGTAT	GTTGCTGGACATAAG	ACTATTGACGGCAGG	GGAGCAGATGTTTCAT		
P L Y V A	G H K T I	D G R G A	D V H K L		100
CTTGGCAACGGCGGt	CCCTGTCTGTTTATG	AGGAAAGTGAGCCAT	GTTATTCTCCATGGT		
L G N G G	P C L F M	R K V S H	V I L H G		120
TTGCATATACACGGt	TGTAATACTAGTGTT	TTGGGGGATGTTTTG	GTAAGTGAGTCTATT		
L H I H G	C <u>N T S</u> V	L G D V L	V S E S I		140
GGGGTGGTGCCTGTT	CATGCTCAGGATGGG	GACGCCATTACTATG	CGCAATGTTACAAAT		
G V V P V	H A Q D G	D A I T M	R <u>N V T</u> N		160
GCTTGGATTGATCAT	AATTCTCTCTCCGAT	TGTTCTGATGGTCTT	ATCGATGTTACGCTT		
A W I D H	N S L S D	C S D G L	I D V T L		180
GGTTCCACTGGAATT	ACTATCTCCAACAAT	CACTTCTTCAACCAT	CATAAAGTGATGTTG		
G S T G I	T I ^T F S N N	H F F N H	H K V M L		200
TTAGGACATGATGAT	ACATATGATGATGAC	AAATCTATGAAAGTG	ACAGTGGCGTTCAAT		
L G H D D	T Y D D D	K S M K V	T V A F N		220
CAATTTGGACCTAAT	GCTGGACAACGAATG	CCAAGGGCACGATAT	GGACTTG TACATGTT		
Q F G P N	A G Q R M	P R A R Y	G L V H V		240
GCAACAATAATTAT	GATCCATGGAATATA	TATGCTATTGGTGGG	AGTTCAAATCCAACC		
A N N N Y	D P W N I	Y A I G G	S S N P T		260
ATTCTAAGTGAAGGG	AATAGTTTCACTGCC	CCAATGAGAATTAC	AAGAAGGAAGTAACA		
I L S E G	N S F T A	P N E N Y	K K E K R		280
AAGCGTATAGGGTGT	GAATCAACATCAGCT	TGTGCAAACTGGGTA	TGGAGATCTACACGA		
I G C E S	T S A C A	N W V W R	S T R V T		300
GATGCTTTTTCTAAT	GGAGCTTATTTTGTA	TCCTCGGGGAAAATT	GAAGAGACCAATATA		
D A F S N	G A Y F V	S S G K I	E E T N I		320
TACAATAGTAATGAA	GCTTTCAAAGTTGAG	AATGGGAATGCAGCT	CCTCAATTAACAAAA		
Y N S N E	A F K V E	N G N A A	P Q L T K		340
AATGCTGGAGTTGTA	GCATAA				
N A G V V A *					346

Fig. 1 DNA and amino acid sequences of Jun v 1 (a) and Jun v 3 (b). DNA and amino acid sequences are shown with N-terminal amino acid sequences (underlined). The two potential N-glycosylation sites are shown by double underlining. * indicates a stop codon.

(b)

TTGCGTTTCTTCTT	GCGGCCACATTGACC	ATCTCTTCACACATG	CAAGAGGCGGGAGCA	-19
A F L L	A A T L T	I S S H M	Q E A G A	
GTGAAGTTTGATATA	AAGAACCAGTGCGGG	TACACAGTCTGGGCA	GCGGGATTGCCCCGA	20
V K F D I	K N Q C G	Y T V W A	A G L P G	
GGAGGGAAGCGGCTT	GACCAGGGGCAGACA	TGGACGGTTAATTTG	GCGGCGGGCAGCG	40
G G K R L	D Q G Q T	W T V N L	A A G T A	
TCGGCAAGGTTCTGG	GGACGAACGGGCTGC	ACTTTCGATGCGAGC	GGGAAAGGAAGCTGC	60
S A R F W	G R T G C	T F D A S	G K G S C	
CAGACCGGTGACTGC	GGCAGGCAACTGAGC	TGCACAGTCTCCGGA	GCAGTTCCCGCGACG	80
Q T G D C	G R Q L S	C T V S G	A V P A T	
CTGGCAGAGTACACG	CAGAGCGACCAGGAC	TACTAAGACGTATCC	CTCGTCGATGGCTTC	91
L A E Y T	Q S D Q D	Y * D V S	L V D G F	
AACATTCCTCTTGCC	ATCAACCCAACGAAT	GCACAGTGCACCGCT	CCTGCCTGCAAGGCT	
	T			
N I P L A	I N P T N	A Q C T A	P A C K A	
	S			
GACATTAATGCAGTG	TGCCCTTCCGAGTTG	AAGGTTGATGGCGGA	TGCAATAGCGCCTGC	
D I N A V	C P S E L	K V D G G	C N S A C	
AATGTCTTCAAACT	GATCAGTATTGCTGC	AGAAATGCGTATGTT	GATAACTGCCCTGCC	
N V F K T	D Q Y C C	R N A Y V	D N C P A	
ACGAATTATTCCAAG	ATATTCAAGAACCAG	TGCCCTCAGGCTTAC	AGCTATGCCAAGGAT	
T N Y S K	I F K N Q	C P Q A Y	S Y A K D	
GACACGGCCACTTTC	GCTTGCGCCTCTGGT	ACCGACTACAGTATT	GTATTCTGCCCTAG	
D T A T F	A C A S G	T D Y S I	V F C P *	
ATATATACCCACGTA	GGATTAGTGAATTC			
I Y T H V	G L V N			

Fig. 1 Continued.

pollen allergens and RT-PCR, both messages, Jun v 1 and Jun v 3, were detected, cloned and sequenced.

The nucleotide sequence of Jun v 3 provided a potential explanation for reduced allergenicity of this potential allergen. Although the deduced amino acid sequence of the mature protein of Jun v 3 would be 99% homologous with the 199 amino acid protein, Jun a 3, a stop codon at nucleotide 274 of Jun v 3 would result in a protein of only 91 amino acids for Jun v 3. Recent structural studies on Jun a 3 suggest that the major IgE epitopes are located within the C-terminal region (amino acid 120–165) of that molecule [16]. Thus, if Jun v 3 were expressed at all, it would lack these important epitopes, which might markedly reduce the allergenicity of eastern red cedar.

The inferred amino acid sequence of Jun v 1 did not

indicate an obvious defect that would reduce the activity of this putative allergen, compared with its highly allergenic homologues from cedar and cypress pollens (Fig. 3). The amino acid sequences of Jun a 1 and Jun v 1 were 95% identical. There were nine non-conservative and two conservative amino acid differences between Jun v 1 and Jun a 1. However, by Western blot analysis, the IgE from a relatively low proportion (46.2%) of the cedar-hypersensitive patients' sera reacted very weakly with the 43-kDa form of Jun v 1A. A much higher proportion, 92.3%, reacted with Jun v 1B (30 kDa). Since the N-terminal sequence of Jun v 1B was found to be identical to Jun v 1A, the shorter length of Jun v 1B suggests that premature termination or post-translational modifications had produced the alternative form of its protein. The fact

(a)

Jun v 1	-21 M ASPCLIAFLV FLCAIVSCCS					
Jun a 1	 V S .					
Cha o 1	. . . CT . L . V F .					
Cry j 1	. D V . L . . LSFV . G . . F .					
Jun v 1	DNPIDSCWRG	DSNWGQNRMK	LADCAVGFGS	STMGGKGGDF	YTVTSADDNP	50
Jun a 1		 D			 T	
Cha o 1		. A . . D		. A A .	 S . . D .	
Cry j 1		 A		 L	 NS . . D .	
Jun v 1	<u>VNPT</u> PGTLRY	GATREKTLWI	IFSQNMNIKL	KMPLYVAGHK	TIDGRGADVH	100
Jun a 1		 A				
Cha o 1	. . . A	 RS	. . . K . L	N I . . <u>N . .</u>	 E . .	
Cry j 1	. . . A	 DRP	. . . G	. . . M . I . . Y .	. F Q . Y	
Jun v 1	LGNGGPCLFM	RKVSHVILHG	<u>LHIHGCNTSV</u>	LGDVLVSESI	GVVPVHAQDG	150
Jun a 1		 S			. . E	
Cha o 1		. T	. N	S . N . . I . . AS		
Cry j 1	 V . I	KR . . N . . I . .	. . LY . . S . .	. . N . . IN . . F	. . E . . P . .	
Jun v 1	DAITMR <u>NVTN</u>	AWIDHNSLSD	CSDGLIDVTL	GSTGITISNN	HFFNHHKVML	200
Jun v 1						
Cha o 1	 D	V	S V	A . . V		
Cry j 1	. . L . L . TA . .	I F . N	S V	S . . V	L	
Jun v 1	LGHDDTYDDD	KSMKVTVAFN	QFGPNAGQRM	PRARYGLVHV	ANNYDPWNI	250
Jun a 1			 C			
Cha o 1	. . . S . I . S . .			 I . .	 S .	
Cry j 1	 A . S . .		 C		 T .	
Jun v 1	YAIGGSS <u>NPT</u>	ILSEGENSFTA	PNENYKKEVT	KRIGCESTSA	CANWVWRSTR	300
Jun a 1			. S . S	 P . .		
Cha o 1			. . DSD	R . V P . T	 Q	
Cry j 1			. . S . . Q . .	I KTS . S	. S Q . Q	
Jun v 1	DAFSNGAYFV	SSGKIEETNI	YNSNEAFKVE	NGNAAPQLTK	NAGVVA****	346
Jun a 1	. . . I	 T			 T ****	
Cha o 1	. S . N	. . . N . G	. . N	. . S	. . . LTCILS	
Cry j 1	. V . Y	. . . Y . GG . .	. TKK . . N . .	<u>. . . . T</u>	 LTCSLS	
Jun v 1	****	Identity(%)				
Jun a 1	****	96.5				
Cho a 1	KPCS	85.8				
Cry j 1	KRC*	80.1				

Fig. 2 Homology analysis. Amino acid sequences of Jun v 1 (a) and Jun v 3 (b) are compared with those of Jun a 1, Cha o 1, Cry j 1 and Jun a 3. The only defined IgE epitope of Cry j 1 [17] is underlined. The pectate lyase activity site is dotted underlined. The potential glycosylation sites are double underlined.

(b)

Jun v 3-1				AFLLAATLT	ISSHMQEAGA	-19
Jun v 3-2						
Jun a 3			MARVSE	L.....A	..L.....V	-26
Jun v 3-1	VKFDIKNQCG	YTVWAAGLPG	GGKRLDQGQT	WTVNLAAGTA	SARFWGRTGC	50
Jun v 3-2						
Jun a 3						50
Jun v 3-1	TFDASGKGSC	QTGDCGRQLS	CTVSGAVPAT	LAEYTSQSDQD	Y*DVSLVDGF	91
Jun v 3-2						
Jun a 3		G...			..Y.....	100
Jun v 3-1	NIPLAINPTN	AQCTAPACKA	DINAVCPSEL	KVDGGCNSAC	NVFKTDQYCC	
Jun v 3-2	S.....					
Jun a 3	A.....					150
Jun v 3-1	RNAYVDNCPA	TNYSKIFKNQ	CPQAYSYAKD	DTATFACASG	TDYSIVFCP*	
Jun v 3-2						
Jun a 3						199
Jun v 3-1	Identity (%)					
Jun v 3-2						
Jun a 3	98.9					

Fig. 2 Continued.

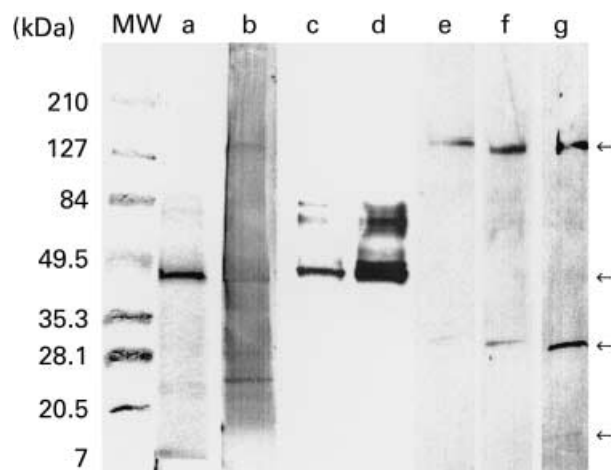


Fig. 3 Electrophoresis pattern and Western blotting of crude extract. The crude extract of the eastern red cedar was analysed by SDS-PAGE under reducing conditions and Western blotting. Lane a, Coomassie blue stain; lane b, colloidal gold stain; lane c, polyclonal anti-Cry j 1 stain, e.g. three typical patterns out of 13 cedar-hypersensitive patients' sera displaying IgE binding; lane d, polyclonal anti-Cry j 1 stain of mountain cedar extract. Western blots of crude extracts of both cedar pollen, developed with monoclonal antibody to Cry j 1, demonstrated the same two (43-kDa and 60-kDa) bands. Arrows indicate the IgE-positive bands.

that some sera contained IgE antibodies that reacted with only the shorter form of Jun v 1 suggests that this allergen may contain one or more cryptic epitopes. In addition, since the IgE binding to the 43-kDa Jun a 1 was readily apparent on dot blots [16], but was difficult to detect in Western blot analysis, the epitopes on the full length Jun a 1 may be conformational and thus disrupted by SDS, reduction of disulphide bands and/or boiling. In the homologous glycoprotein from Japanese cedar, Cry j 1, the C-terminal region contains a major allergic epitope, which is dependent on glycosylation at N³³³ [17]. Jun v 1 is missing this glycosylation site. However, since Jun a 1 and Cha o 1 are also missing this glycosylation site, the loss of this single epitope cannot explain the relatively low allergenicity of Jun v 1.

By Western blot analysis, we found that IgE from cedar-hypersensitive patients reacts with several proteins from eastern red cedar pollen. In fact, the IgE from >90% of the patients' sera reacted to one or more allergens of eastern red cedar pollen. This suggests that the IgE antibodies from the sera of mountain cedar-sensitive patients may react to different epitopes on the homologous allergens of the eastern red cedar and Japanese cedar (Jun v 1 and Cry j 1),

and/or to allergens (e.g. 145 kDa) that are not well expressed in mountain cedar pollen.

Further studies will be necessary to map the IgE epitopes on these related allergens. However, the apparent differences in epitopes between eastern red cedar and its more allergenic relatives might result in differential activation of mast cells and/or basophils and thereby alter the extent of the allergic response. The structure and pathogenic potential of the 145-kDa IgE-binding protein in the pollen of eastern red cedar remain to be investigated.

In conclusion, we have found extensive sequence homology between two proteins from the pollen of eastern red cedar and major allergens of other cedar and cypress pollens. In fact, the IgE from the sera of most mountain cedar-sensitive patients reacted with the eastern red cedar Jun v 1. However, mutations in the genes for Jun v 1 and Jun v 3 from eastern red cedar may disrupt the expression, alter the epitope structure, or otherwise reduce the allergenicity of these proteins, relative to their mountain cedar homologues. These findings may help not only explain the reduced allergenicity reported for the eastern red cedar, but also provide an opportunity to understand some of the structural features of pollen proteins that are critical for allergenicity.

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