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Major linear IgE epitopes of mountain cedar pollen allergen Jun a 1 map to the pectate lyase catalytic site

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Abstract

Resolution of the 3D structures and IgE epitopes of allergens may identify common or conserved features of allergens. Jun a 1, the predominant allergen in mountain cedar pollen, was chosen as a model for identifying common structural and functional features among a group of plant allergens. In this study, synthetic, overlapping peptides of Jun a 1 and sera from patients allergic to mountain cedar pollen were used to identify linear epitopes. A 3D model of Jun a 1 was produced using the *Bacillus subtilis* pectate lyase (PL) as a template and validated with biophysical measurements. This allowed mappings of four IgE binding sites on Jun a 1. Two of the epitopes mapped to turns or loops on the surface of the model structure. The other two epitopes mapped to the β -sheet region, homologous to the catalytic site of PL. This region of Jun a 1 is highly conserved in the group 1 allergens from other cedar trees as well as microbial PLs. The finding that two out of three major IgE epitopes map to highly conserved catalytic regions of group 1 cedar allergens may help to explain the high degree of cross-reactivity between cedar pollen allergens and might represent a pattern of reactivity common to other allergens with catalytic activity. © 2003 Elsevier Ltd. All rights reserved.

Keywords: Allergen structure; Cedar pollen hypersensitivity; Cry j 1; IgE epitope; Jun a 1; *Juniperus ashei*; Mountain cedar

1. Introduction

Many steps of the cellular and molecular pathways involved in allergic reactions have been clearly elucidated. However, the apparent diversity in allergenic proteins has limited our understanding of the structural requirements for triggering these pathways. For instance, the 3D structure of less than 25 allergens have been resolved and the IgE epitopes of only a few of these have been published. Defining common or conserved features of allergenic proteins could help elucidate structural, and perhaps functional patterns

within groups of allergens. We have chosen Jun a 1, the predominant allergen in mountain cedar pollen as a model for defining structural features that may be common to other plant allergens.

Mountain cedar (*Juniperus ashei*) pollen is a major cause of seasonal hypersensitivity in the central US and pollens of other cedar and cypress trees cause similar illnesses in various regions of the Northern Hemisphere. Although mountain cedar has a limited geographic distribution, the structural similarity between group 1 allergens of all the cedars and the high potency of mountain cedar pollen allergen have made it standard for use in immunodiagnosis and therapy for the cedar/cypress allergic patients (Andre et al., 2000). However, the molecular and structural bases for the high rate of sensitization (Sato et al., 1997) and cross-reactivity between members of the cedar family (Midoro-Horiuti et al., 1992; Schwietz et al., 2000) are poorly understood. We have previously shown that Jun a 1 belongs to a family of plant and microbial pectate lyase (PL) enzymes (Midoro-Horiuti et al.,

Abbreviations: Cry j 1, the major allergen in mountain cedar (*Juniperus ashei*) pollen; FT-IR, Fourier transform-infrared spectroscopy; Jun a 1, the major allergen in Japanese cedar (*Cryptomeria japonica*) pollen; MW, molecular weight; PDB, Protein Data Bank; PL, pectate lyase

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1999b). A high degree of sequence identity among the group 1 allergens of cedar and cypress trees is particularly apparent within the PL active site. Conservation in this region may result from a requirement for PL activity in the pollen to degrade the cell wall of the pistil of the female strobola during fertilization (Schwietz et al., 2000); (Midoro-Horiuti et al., 1999a); (Taniguchi et al., 1995).

The question we ask in the current study is whether allergens have common or conserved features that are commonly recognized by the human immune system. We chose Jun a 1 as a model system for this study. A combination of experimental and computational approaches was used to produce and validate a 3D model of Jun a 1 and to map linear IgE epitopes to its surface. The results suggest that two of the three dominant linear epitopes map near the highly conserved PL active site, and are likely to be shared by the group 1 allergens of other cedars and cypresses, and perhaps PLs from other plants and even microorganisms.

2. Material and methods

2.1. Allergen preparation

Jun a 1 was purified from mountain cedar pollen (Bayer) using Con-A Sepharose 4B (Pharmacia) chromatography as described previously (Midoro-Horiuti et al., 1999a). The purified Jun a 1 was dialyzed against 0.05 M Tris-HCl buffer, pH 7.8 or 0.5× PBS (0.15 M NaCl and 0.025 M KH_2PO_4 – KHPO_4 at pH 7.1) for analysis. The purity of Jun a 1 was established by SDS-PAGE, reverse-phase HPLC and mass spectrometry.

2.2. Human sera and mouse mAbs

Sera from seven mountain cedar allergic individuals were obtained from patients who were living or had lived in central Texas. The diagnosis of seasonal allergic rhinitis due to cedar sensitivity was established in these subjects by clinical history and CAP-RAST or scratch tests results. Six mAbs against native Cry j 1 (S14, S36, S84, S91, S95 and S131) were prepared as previously described (Sakaguchi et al., 1997).

2.3. Dot blot immunoassay for antibody binding to Jun a 1 and overlapping, synthetic peptides

Individual 13 amino acid peptides, based on the Jun a 1 implied sequence (Midoro-Horiuti et al., 2000), with eight amino acid overlaps were synthesized on a derivatized cellulose membrane using N-(9-fluorenyl) methoxycarbonyl (Fmoc) amino acid active esters by Sigma Genosys (Woodland, TX). The membranes containing synthetic peptides were incubated in blocking buffer with 10% sucrose (Sigma Genosys). After washing, the membranes were incubated

with patient serum, diluted 1:4 in blocking buffer. The sheets were then incubated with a 1:500 dilution of biotinylated goat anti-human IgE (Vector) in blocking buffer. After a further wash, they were incubated with HRP-streptavidin and then ECL (Pharmacia). The intensity of the spots on photographic film, representing serum IgE binding to individual peptides was scored 0–3 visually.

To identify the minimal linear structures required for IgE binding, detailed epitope mapping was performed. Synthetic, overlapping peptides 8 or 13 amino acids in length were designed based on the sequences of the original 13-mer peptides that bound IgE.

2.4. Homology modeling of Jun a 1

A BLAST search (Altschul et al., 1990) of the Protein Data Bank (PDB) (Sussman et al., 1998), using the Jun a 1 sequence as a probe, identified sequence homologies to PLs from bacterial and plant sources. Structure based alignment of three bacterial pectate and one fungal pectin lyase with the amino acid sequence of Jun a 1 identified several areas of conserved sequence and structural similarity (molegos) (indicated by boxes in Fig. 1) (Schein et al., 2002). A region of particularly high sequence identity was found around the putative PL enzymatically active site. The crystal structure (1.80 Å resolution) of the PL from *Bacillus Subtilis* (PDB: 1BN8, EC 4.2.2.2, (Pickersgill et al., 1994)) was chosen as a template for homology modeling, since it had the highest number of identical residues to Jun a 1, in the alignment shown (ca 25%).

The program, EXDIS, [http://www.scsb.utmb.edu/fantom/fm_home.html], developed in our group, was used to extract interatomic distance constraints and dihedral angle constraints from the 3D structure of the template as we described previously (Soman et al., 2000). Structures were generated from these constraints using our self-correcting distance geometry-based program DIAMOD. The program FANTOM was then used with the above constraints to minimize the conformational energy of the protein.

2.5. Fourier transform-infrared (FT-IR) spectroscopy

The secondary structure of Jun a 1 was determined by FT-IR with a Bomen MB-series FT-IR spectrometer (Quebec, Canada) equipped with a dTGS detector and purged constantly with dry air generated by a Balston air dryer (Haverhill, MA). Two microlitres of a 13.1 mg/ml solution of Jun a 1 in 0.05 M Tris-HCl, pH 7.8, or buffer alone were placed in a CaF_2 cell of 6 μm path length and allowed to equilibrate with the temperature in the instrument for 10 min. Two hundred scans of the FT-IR spectrum were collected at room temperature with a resolution of 4 cm^{-1} . The absorption spectrum of the buffer was subtracted from that of the protein sample. The secondary structural content was calculated using the 'Factor Analysis' function in the software PROTA provided by Bomen (Dong and Caughey, 1994).

Jun a 1	DQNRMKLADCAVGFSSSTMGGKGG-----DFYTVTSTDDNPVNPTPGTLRYG-----
1BN8A	ADLGHQTLGSNDGWGAYSTGTTGGSSKASSSNVYTVSMRNQLVSAL----GKET-----
1PCL	-AVETDAAT--TGWATQNGGTTGGAKAA--KAVEVKNISDFKKALNG-----
1QCXA	--AGVVGAA--EGFAH---GVTGGGSA--SPVYPTTIDELVSYL-----
1AIR	-----ATDT--GGYAA---TAGGNVTG-AVSKTATSMODIVNIID-----AARLDANG
Jun a 1	----ATREKALWIIFSQNMNI-----
1BN8A	----NTTPKIIYIKGTIDMNVDDNLKPLGLNDYKDPEYDLDKYLKAYDPSTWGKKEPSGT
1PCL	----T-DSSAKIIKVTGPIDISG--GKAYT-----
1QCXA	----G-DNEPRVIILDQTFDFT---GTEGT-----
1AIR	KKVKG-GAYPLVITYTGNE-----
TARG	-----KLKMPLYVA---
1BN8A	QEEARARS-----QKNQKARVMVDIP---
1PCL	-----S-----FDDQKARSQISIP---
1QCXA	-----ETTTGCAPWGTASQCQVAINLHSCDNYQASAPKVSVDYDKAGILPITVN---
1AIR	-----SLINAAAAANICGQWSKDP--RGVEIKEFT
Jun a 1	GHKITIDGRGADVHLGNGGPCLFMRK--VSHVILHSLHIHCNTSVLGDVLVSESIGVEPV
1BN8A	ANTTIVGSGTNAKVVGGN--FQIK--SDNVIIRNIEFQDAYDYFPQWDP--TDGSSGN
1PCL	SNTTIIGVGSNGKFTNGS--LVIK-GVKNVILRNLYIETPVDVAPHYES--G---DG
1QCXA	SNKSIVGQGTGKGVIKGK--LRVSGAKNVIIQNIATVDINP-----K
1AIR	KGTITIGANG-SSAN-FG--IWIK-KSSDVVVQNMRIGYLPG-----
Jun a 1	HAQDGDAITMRNVTNAWIDHNSLSDCS-----DGLIDVT-LGSTGIT
1BN8A	WNSQYDNITINGGTHIWIDHCTFNDGSRPDSTSPKYYGRKYQ-HHDGQTDA--NGANYIT
1PCL	WNAEWDAVIDNSTNVVDHVTISDGSFTDDKYTTKDGKEYV-QHDGALDIK-KGSDYVT
1QCXA	YVWGGDAITVDDSDLVWIDHVTITARI-----GRQHIVLGTSAADNRVT
1AIR	GAKDGMIRVDDSPNVVDHNELFA--ANHE-CDGT--PDNDTTFESAVIDIK-GASNTVT
Jun a 1	ISNMHFFN-----HHKVMLLGHDDTYD--DDKSMKVTVAFNQFGPNAGQRMPPRA
1BN8A	MSYNYHYD-----HDKSSIFGSSDSKT--SDDGKLKITLHHNRY--KNITVQRAPRV
1PCL	ISYSRFEL-----HDKTILIGHSDSNGSQDSGKLRVTFHNNVF--DRVTERAPRV
1QCXA	ISYSLIDGRSDYSATCNGHHYWGVLN-----GSNDMVTLKGNFY--YNLSGRMPKV
1AIR	VSYNVIHG-----VKKVGLDGSSS--SDTG-RNITYHHNYY--NDVNARLPLQ
Jun a 1	R-YGLVHVANNNDP-----WNIYAIGGSSNPITLSEGSFTAPSE-----SYKKE
1BN8A	R-FGQVHVYNNYEEGSTSSSSYPFSYAWGIGKSSKIYAQNNDIVPGL-----SAAKT
1PCL	R-FGSIHAYNNVYLGDKHSVYPYLYSFLGLTSGSILSESNSFTLSNLKSIDGKNPECSI
1QCXA	QGNTRLHAVNNLFHNF-----DGHAFEIGTGGYVLAEGNVFQD-----VNVV
1AIR	R-GGLVHAYNNLYTN-----ITGSLNVRQNGQALIENNWFEEK-----AINP
Jun a 1	VT-KRIGCESPSAC--ANWWRSTR-----DAFINGAY-FV-----
1BN8A	IS-V-----F--SGGTALYDS-----GTLLNGTQ-IN-----
1PCL	VK-Q-----F--NSKVFSK-----GSLVNGSTTTK-----
1QCXA	VETP-----I--SG-QLFSSPDANTNQQCASFGRSCQLNAFGN--SGS-----
1AIR	VT-S-----RYDGKNFGTWLK-----GNN-----ITKPADFST
Jun a 1	-----SSGKTEETNIY--NSNE--AFKVE--NG-NAAPQLTKNAGV
1BN8A	-----ASAANGLSSSV--GWTP--SLHGSIDASA-NVKSNNVINOAGA
1PCL	-----LD-TCGLTA-Y--KPTL--PYKSAQMTMTSSLATSINNAGY
1QCXA	-----MS--GSD-TSIIISKFAGKTIAAAHPPG-AIAQWTMKNAGO
1AIR	YSITWTADTKPYVNAD-SWTSTG

Fig. 1. Structure-based alignment of three bacterial pectate and one fungal pectin (1QCX) lyases with Jun a 1. The boxed areas indicate molegos (areas of conserved sequence and structure) in the pectate lyases family, the motifs for which can be seen in the sequences of Jun a 1.

3. Results

3.1. Synthetic overlapping peptides identified four IgE epitopes of Jun a 1

The distribution of the IgE reactivity within the overlapping synthetic peptides suggested that Jun a 1 contains three

or four distinct linear IgE epitopes (or epitopic regions), as shown in Fig. 2A. The IgE in sera from five of the seven mountain cedar hypersensitive patients bound to peptides 15 (Ile⁷¹–Pro⁸³). The same sera reacted to a set of four overlapping peptides 43 (Lys²¹¹–Gly²²³), 44 (Thr²¹⁶–Gln²²⁸), 45 (Gln²²¹–Ala²³³) and 46 (Ala²²⁶–Val²³⁸). Three of these sera also recognized peptide 60 (Trp²⁹⁶–Tyr³⁰⁸).

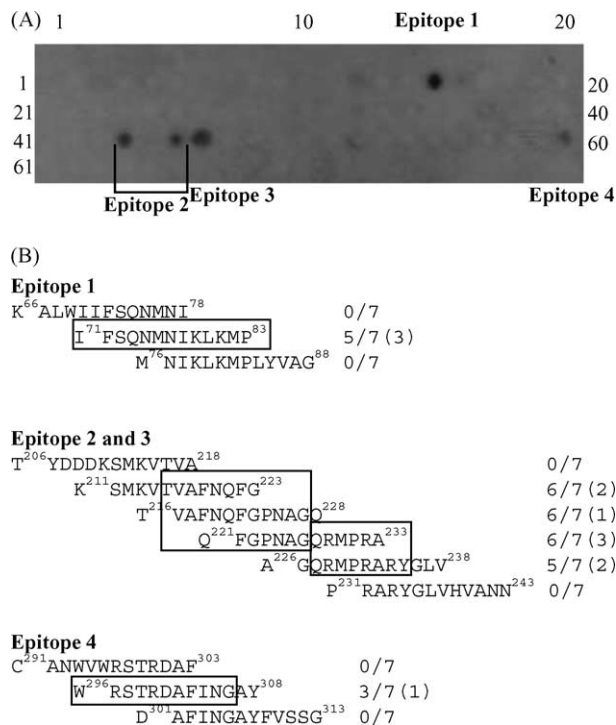


Fig. 2. IgE binding to overlapping peptides. (A) Epitope mapping was performed with the serum IgE from individual mountain cedar allergic patients and overlapping synthetic peptides of Jun a 1 sequence. A representative dot blot is shown. (B) The sequences of the synthetic peptides are shown along with their antibody binding scores (0–3). The proportion of patients that reacted with each epitope are shown as fractions and median intensity in (. The epitope regions are defined with boxes.

3.2. Definition of minimal IgE and mAb epitopes using overlapping peptides

To define the minimum span of amino acids required to form each IgE epitope, we used a pool of sera from patients allergic to mountain cedar to probe a second set of overlapping peptides that represented the sequences around epitope regions 1–4. These peptides differed from each other by only two residues. The results are shown in Fig. 3. The particular pool of sera used reacted less intensely with the original epitope 1 peptide (Ile⁷¹–Pro⁸³), and did not react with the 13-mer peptides differing by two amino acids, which bracketed this peptide. This suggests that the minimal IgE epitope 1 that could be defined by this approach was the 13-mer peptide, Ile⁷¹–Pro⁸³.

Of the ten 8-mers representing the epitope 2–3 region, two peptides, Phe²²²–Arg²²⁹ and Met²³⁰–Leu²³⁷ bound IgE most strongly. There was also weak reactivity with the peptides containing sequences that overlap these two peptides (Fig. 2). Two of the three mouse mAbs to Cry j 1 that reacted with Jun a 1 peptides bound to peptides in this region (results not shown), but they only reacted with peptides within epitope 2, as defined in Fig. 3. This finding suggests that while epitopes 2 and 3 may be contiguous or even overlapping, they are separate epitopes.



Fig. 3. IgE antibody reactivity with overlapping peptides that represent sequences around the epitopes defined in Fig. 2. (A) A membrane with synthetic peptides around the four epitopes and with four peptides designed to mimic the IgE epitopes was probed with sera from Texas patients. (B) Sequences of the peptides to which IgE reacted are shown and the minimal epitopes are indicated by boxes.

IgE from pooled patient sera reacted with a single 13-mer in the epitope 4 region (Fig. 3).

3.3. The model of Jun a 1 structure

The model structure for Jun a 1 has a β -helical core, with several surface loops and helices (Fig. 4). The central β -helix is 40–42 Å wide. At its widest point the molecule is 51 Å. The length is approximately 68 Å. Of the 330 residues of Jun a 1 in the model, ~43% were in a β -conformation in the β -helix, including 42 residues in 18 short strands, 85 residues in β -bends and 16 in β -bridges. Twenty residues (6%) were in two α -helices and three in 3/10 helices. The model was checked in several ways. Procheck (Morris et al., 1992); (Kelley et al., 2000) indicated that the model had good stereochemistry with only six violations (in turns or loop regions) in 330 residues. The final conformational energies, after minimization, were –636 kcal/mol. The Lennard–Jones potential was –1611 kcal/mol. Both of the potential glycosylation sites, Asn¹²⁷ThrSer and Asn¹⁵⁷ValThr, were located on the surface of the model (see Fig. 4).

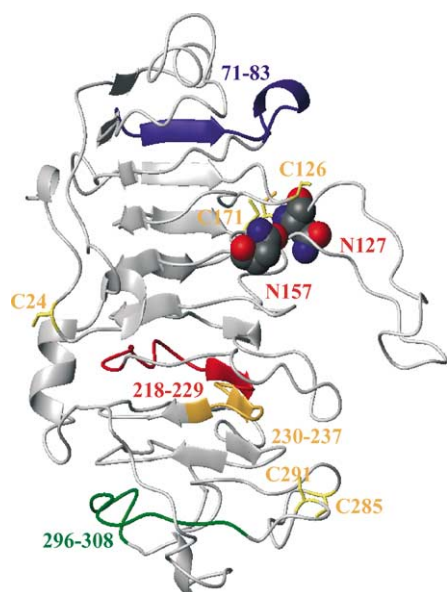


Fig. 4. A ribbon diagram of the Jun a 1 model structure. The glycosylation sites are shown in space filling. Cys are yellow and epitopes are shown as colored regions; epitope 1: IFSQNMNIKMKP (blue); epitope 2: AFNQFGPNAGQR (red); epitope 3: MPRARYGL (yellow) and epitope 4: VWRSTRDAFING (green).

3.4. The FT-IR spectrum of Jun a 1 is consistent with the model structure

The second derivative FT-IR spectrum of Jun a 1 (Fig. 5) indicated that the protein had about 40% β -sheet and about 8% helix content (based on the relative area encompassing the peaks at, 1634 and 1657 cm^{-1} , respectively). The spectrum (Fig. 5) generally agreed with that observed for pectate lyase C (PL-C) and other β -helical proteins (Khurana and Fink, 2000). Thus, the general secondary structural features predicted by modeling are supported by FT-IR analysis. A feature of the Jun a 1 spectrum that is not shared with the bacterial or fungal β -helical proteins is the shoulder at 1646 cm^{-1} , which may indicate a random coil “disor-

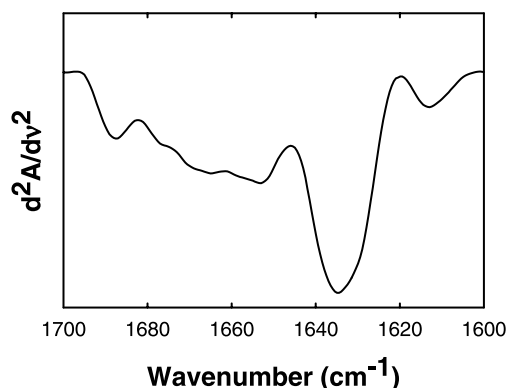


Fig. 5. Secondary structure estimation of Jun a 1. Second derivative FT-IR spectrum (amide I region) for Jun a 1 is shown.

dered structure” (Nevskaya and Chirgadze, 1976) or reflect the N-linked glycans of Jun a 1.

3.5. IgE epitopes map to surface-exposed area on the Jun a 1 model structure

Based on the results of experiments described above, we proposed the minimal numbers of contiguous residues required for each of the four IgE epitopes. We then mapped these epitopes on the model structure (Fig. 4). All four epitopes had areas of surface exposure on the model structure. Epitope 1 was in an area of mixed structure near the N-terminus. Epitopes 2 and 3 were located in a cleft in the β -helix, close to or overlapping with the consensus PL active site. Epitope 4 lies in a β -turn near the C-terminus.

4. Discussion

The limited number of 3D structures available for allergen make functional mapping of the molecules difficult. However Jun a 1, the most potent allergen in mountain cedar pollen (Midoro-Horiuti et al., 1999b), has sequence homology to bacterial PL proteins, for which crystal structures have been determined. All of these PLs have characteristic β -helical core regions, formed by a series of sequence motifs that are conserved throughout this enzyme family. Since Jun a 1 shares many of these motifs, including the characteristic Arg-X-Pro pair known to be involved in catalysis (Herron et al., 2000), we were able to use consensus template matching and homology modeling to produce a validated 3D model for Jun a 1. This model confirmed the resemblance between Jun a 1 and PLs produced by the bacteria (e.g. *Erwinia*) that feed on plants (Herron et al., 2000). The IgE epitopes identified in overlapping, synthetic peptides were mapped to the surface of the model structure.

Three of the IgE-binding regions (epitopes 1–3) represented major epitopes (recognized by 5–6 of 7 allergic sera) while epitope 4 was a minor epitope (three of seven sera). Two of the three major epitopes of Jun a 1 (2 and 3) were located near or within the PL putative catalytic site. Epitope 3 contains two essential residues for PL catalysis, the equivalents of Arg²¹⁸ and Pro²²⁰ in PL-C from *Erwinia* (Herron et al., 2000). Since the sequence in the putative catalytic region of Jun a 1 (R²¹⁸MPRARYGLVHVANN²⁴⁴) is conserved in Cry j 1 from Japanese cedar, as well as other group 1 cedar allergens (underline in Fig. 6), IgE antibodies directed against this region may be responsible for cross-reactivity among cedar pollens. In fact, two mouse IgG mAbs to Cry j 1 bound to Jun a 1 IgE epitope 2. One of these mAbs (KW-S95) inhibited the binding of IgE from 10 of 21 Japanese cedar-sensitive patients from binding to Cry j 1 (Sakaguchi et al., 1997). These findings suggest that epitope 2 might be a prominent feature of the humoral response to group 1 allergens from different geographic areas and in genetically distinct populations.

Jun a 1:	AF106663			-21 M	ASPCLIAVLV	FLCAIVSCYS	
Jun v 1:	AF151429			.	F..	C.	
Cup s 1:	AF257495			.	D.....		
Cup a 1:	AJ278498			.	V..		
Cha o 1:	D45404			.	..CT.L...	F.	
Cry j 1:	D26544			.	D....V.L..	LSFV.G..F.	
Jun a 1	DNPIDSCWRG	DSNWDQNRMK	LADCAVGFSG	STMGGKGGDF	YTVTSTDDNP	50	
Jun v 1		G.....			A....		
Cup s 1					I....	AE...	
Cup a 1			V.....	L.....	EI...	S....	
Cha o 1		A.....		A.....	A....	S..D.	
Cry j 1		A.....			L....	NS..D.	
Jun a 1	VNPTPGTLRY	GATREKALWI	IFSQNMNIKL	KMPL	LYVAGHK	TIDGRGADVH	100
Jun v 1		T...					
Cup s 1							
Cup a 1		RS...			Y....		
Cha o 1		RS...	K.L.....	N.....	I..N..	E..	
Cry j 1		DRP...	G.....	M.I..Y..	F....Q.Y		
Jun a 1	LGNGGPCLFM	RKVSHVILHS	LHIHG <u>CNTS</u> V	LGDLVLSSEI	GVEPVHAQDG	150	
Jun v 1		G.....			V.....		
Cup s 1		G.....			N.....		
Cup a 1		TA.....					
Cha o 1		T.....			S.N..I..AS	..V.....	
Cry j 1		V.I	KR..N..I.G	LY..S...	N..IN..F	P...	
Jun a 1	DAITMR <u>NVTN</u>	AWIDHNSLSD	CSDGLIDVTL	GSTGITISNN	HFFNHHKVML	200	
Jun v 1							
Cup s 1					S.....		
Cup a 1					C.....		
Cha o 1		D V.....	S...V....	A...V....			
Cry j 1		L.L.TA..	I.....F.N S...	S...V....	S...V....	L.....	
Jun a 1	LGHDDTYDDD	KSMKVTVA	AFN	QFGPNAGQRM	PRARYGL	VHV ANNNYDPWNI	250
Jun v 1							
Cup s 1						Q...	
Cup a 1						Q...	
Cha o 1						S...	
Cry j 1						T...	
Jun a 1	YAIGGSSNPT	ILSEGSNFTA	PSESYKKEVT	KRIGCESPSA	CANWV	WRSTR	300
Jun v 1			N.N.....	T....			
Cup s 1		A...	N.N.....	V.T....			
Cup a 1				T....			
Cha o 1			ND.D...	R.V....T		Q...	
Cry j 1			N...Q...	I...KTS.S	S...	Q..Q	
Jun a 1	DAFING AYFV	SSGKTEETNI	YNSNEAFKVE	NGNAAPQLTK	NAGVVT****	346	
Jun v 1	S.....	I.....			A****		
Cup s 1	S.....		T.....	L.....	A****		
Cup a 1	T.....	A.....		Q.....	****		
Cha o 1	S.N.....	N.G.....	N.....	S.....	L.CILS		
Cry j 1	V.Y.....	Y.GG...	TKK...N..	T.....	L.CSL		
Jun a 1	****	Identity(%)					
Jun v 1	****	96.8					
Cup s 1	****	95.4					
Cup a 1	****	94.8					
Cha a 1	KPCS	85.8					
Cry j 1	KRC*	80.1					

Fig. 6. Comparison of the sequences of the IgE epitopes of Jun a 1 and other group 1 allergens. Four IgE epitopes are shown with boxes. Proven N-glycosylation sites are shown with double underlines and potential N-glycosylation sites are shown with single underlines. The PL active site is underlined. Jun v 1 is from eastern red cedar (*Juniperus virginiana*, Cupressaceae), Cup a 1 from Arizona cypress (*Cupressus arizonica*, Cupressaceae), Cup s 1 from Italian cypress (*Cupressus sempervirens*, Cupressaceae), Cha o 1 from Japanese cypress (*Chamaecyparis obtusa*, Cupressaceae) and Cry j 1 from Japanese cedar (*Cryptomeria japonica*, Taxodiaceae).

Only one IgE epitope was previously identified on the group 1 allergen Cry j 1. This epitope mapped to a glycopeptide from near the C-terminus (Asn³³¹–Leu³³⁸), and the N-linked glycan has required for IgE binding to this peptide (Taniai et al., 1993). However, none of the other five group 1 allergens that have been sequenced share this potential

glycosylation site (Midoro-Horiuti et al., 2001; Aceituno et al., 2000; Sone et al., 1994). Thus, it is unlikely that this epitope is shared by the other cedar species (Aceituno et al., 2000; Midoro-Horiuti et al., 1992; Schwietz et al., 2000).

The finding of major allergenic epitopes in a region of Jun a 1 that is highly conserved in the group 1 allergens of cedars

and PLs may have important implications. These epitopes might contribute to the extensive cross-reactivity between the cedars, previously described with crude pollen extracts in skin testing (Schwietz et al., 2000) and for purified Jun a 1 in ELISA (Soman et al., 2000). In addition, repeated exposures to an extensive array of plant and bacterial PLs might help to explain the high frequency of sensitization to the group 1 allergens of cedar pollens. A more detailed immunological analysis would be required to substantiate this suggestion. However, a similar role was recently suggested for peptide homologues of a T-cell epitopes of the group 1 allergen Cha o 1 (Nakamura et al., 2001).

Antibody responses to the catalytic regions of proteins are well described. In fact, the production of IgG and IgA antibodies after immunization with Streptococcal glucosyl-transferase may prevent dental caries (Taubman et al., 1995). It is possible that catalytic regions are favored as epitopes since the structural requirement to interact reversibly with substrates and antibodies are similar.

In summary, we describe here the results of a combined computational and experimental investigation that allowed us to develop a 3D model structure for the major allergen from mountain cedar pollen and to locate four IgE epitopes on this structure. The location of epitopes within the highly conserved PL catalytic site of Jun a 1 may be a common feature of the IgE response to the group 1 cedar allergens, and may be important for the process of sensitization and/or allergic reactions to this group of potent seasonal allergens.

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