

A cDNA Clone Coding for a Novel Allergen, *Cla h* III, of *Cladosporium herbarum* Identified as a Ribosomal P₂ Protein¹

Lei Zhang,* Gauri Muradia,* Ivan H. A. Curran,* Harold Rode,[†] and Hari M. Vijay^{2*}

*Life Sciences Division, Bureau of Drug Research and [†]Bureau of Biologics, Health Canada, Ottawa, Ontario, Canada

Mold allergens represent a major cause of atopic disorders. Progress in the molecular characterization of allergens has been hampered by batch-to-batch variation and poor yields in mold extracts. In the present study, we established a cDNA library in λ ZAP II by using mRNA isolated from a major allergen-producing mold, *Cladosporium herbarum*. From this library, a novel allergen has been cloned and sequenced. The clone encodes a full length protein of 111 amino acids with a molecular mass of 11.1 kDa and pI of 3.94. By using sequence homology analysis, the allergen was found to belong to the ribosomal P₂ protein family. Approximately 60% peptide sequence homology was found between the cloned protein and other known fungal ribosomal P₂ proteins. In addition to conserved C-terminal sequences and serine blocks for phosphorylation in all eukaryotic ribosomal P₂ proteins, this protein also contains a potential N-glycosylation site at position 15–17. Northern blots demonstrated that mRNA molecules of this gene are present even at late stages of culture. The gene was expressed in *Escherichia coli* by using the pMAL-2c system, and murine antisera against the recombinant allergen (rCh2.1) were generated. The antisera revealed that the native allergen corresponding to rCh2.1 was present in extracts prepared by grinding mycelia under liquid nitrogen in the presence of protease inhibitors, but absent in extracts prepared by conventional methods. This result indicates the usefulness of recombinant allergens in characterization and standardization of mold allergenic extracts. *The Journal of Immunology*, 1995, 154: 710–717.

IgE-mediated atopic diseases afflict about 20% of the population. Airborne protein allergens, originating from a variety of sources (1), cause symptoms of rhinitis, conjunctivitis, and asthma in atopic individuals. Certain species of molds constitute a large portion of this inhalant allergen reservoir by airborne spores (2, 3). Although the major species of allergen-producing molds have been well recognized, it has been difficult to characterize their allergenic components because of batch-to-batch variation and because of the presence of minute amounts of proteins in the mold extracts. Carbohydrate molecules account for a major part of the extracts and have been implicated in IgE binding (4). It is well documented that IgE production is under tight control by T cells which recognize only the specific peptide and MHC complex (5–

8). Therefore, the protein moiety of the extracts is the primary factor in the initiation of IgE production.

Molecular characterization of the protein allergens are essential, not only for a better understanding of the pathogenesis of atopic diseases, but also for development of more efficacious and specific diagnostic and therapeutic modalities. A number of mold allergens have been isolated and characterized by physicochemical or reverse immunosorbent techniques (9–12). However, in most cases, further studies of the allergens, such as the analysis of the primary sequences and mapping of the epitopes, are hampered because of the limited amounts of pure allergens obtained. Recently, gene cloning and expression techniques have been employed in allergen characterization. A number of allergens have been cloned, sequenced, and synthesized from a variety of sources (13–18), which in turn facilitated determination of their biologic activities, demarcation of the T and B cell epitopes, and an understanding of the molecular mechanisms by which specific IgE Abs are induced.

Cladosporium herbarum (*C. herbarum*) is abundant in both outdoor and indoor air and is a major source of fungal inhalant allergens (19–21). Two allergens, named *Cla h* I

Received for publication April 26, 1994. Accepted for publication October 4, 1994.

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¹ This study was funded by the Canadian National Biotechnology Strategy.

² Address correspondence and reprint requests to Dr. Hari M. Vijay, Life Sciences Division, Health Canada, Sir F. G. Banting Research Centre, Tunney's Pasture, Ottawa, ON, Canada K1A 0L2.

and *Cla* h II, were partially purified by gel filtration (22, 23). The former is a group of allergens with molecular mass of approximately 13 kDa and pI values ranging from 3.7 to 4.4 (22). The latter is a 20-kDa glycoprotein composed of approximately 80% carbohydrate and 20% protein (23). Our previous studies revealed that the mold produces at least 17 different allergens (24). In the present study, the construction of a cDNA library and gene cloning of a novel allergen of this organism are reported. The cDNA clone, Ch2.1, which codes for the allergen, has been sequenced. Analysis of its deduced amino acid sequence revealed that the allergen belongs to the family of ribosomal P₂ proteins. The allergen is designated as *Cla* h III according to the International Union of Immunological Societies recommendation (25).

Materials and Methods

Establishment of a unidirectional cDNA library

An isolate, IMI 96220, of *C. herbarum* was cultured on liquid synthetic revised tobacco medium at 22°C (26). Mycelia were harvested on days 7, 15, and 23. After three washes with sterile ice-cold PBS, the mycelia were dried and stored at -70°C. The mycelial mass was ground in a mortar and pestle under liquid nitrogen. The powder was suspended in 4.7 mol/l guanidine isothiocyanate containing 1% β -mercaptoethanol and homogenized with a Polytron homogenizer, followed by addition of *N*-lauroylsarcosine to a final concentration of 0.1%. The debris was removed by centrifugation at 10,000 rpm for 10 min. The supernatant was layered on top of 5.7 mol/l cesium chloride and subjected to ultracentrifugation at 25,000 rpm with a SW28 rotor for 24 h. The RNA pellets were extracted twice with phenol/chloroform and precipitated with sodium acetate and ethanol. After determination of the RNA yield, equal amounts of the three different time cultures were pooled.

A cDNA expression library was constructed in λ ZAP II vector (Stratagene, La Jolla, CA) with poly(A) RNA isolated from the total RNA pool by oligo dT cellulose chromatography (27). The first strand DNA molecules were synthesized by using primer *Xho*I(dT)₁₅ and reverse transcriptase, followed by DNA polymerase and RNase H to synthesize the second strand DNA. Potential *Eco*RI sites within the cDNA molecules were methylated before ligation with *Eco*RI adapters. Both the cDNA molecules and the vector were digested with *Eco*RI and *Xho*I. The cDNA was ligated into λ ZAP II vector. The *Escherichia coli* strain XL1-blue was used as host for amplification and screening of the library.

Screening of the cDNA library with human IgE Abs

The cDNA library was screened by using a human serum pool from six *C. herbarum* atopic individuals, as determined by skin test and direct RAST (24, 26). Sera from six non-mold allergic individuals were also pooled and used as a negative control. *E. coli* XL1-blue cells were transfected with the phage particles and plated on Luria-Bertani (LB)³ agar in petri plates. Each plate (90-mm diameter) was plated with approximately 5×10^3 phages and incubated at 42°C for 4 h. Nitrocellulose filters (Schleicher & Schuell, Keene, NH) presoaked with 10 mmol/l isopropyl- β -D-thiogalactopyranoside (Life Technologies, Inc., Burlington, Ontario, Canada) were layered on the top agar and incubated at 37°C for 3.5 h. The filters were lifted and washed three times for 5 min each with Tris-buffered saline containing 0.5% Tween 20. After saturation with 3% blocking reagent (Boehringer Mannheim, Laval, Quebec, Canada) at room temperature for 3 h, the filters were incubated at room temperature overnight with a 1 in 5 dilution of the serum pool preabsorbed with *E. coli* extract. The IgE-bound plaques were detected by ¹²⁵I-labeled anti-human IgE Abs (Kabi Pharmacia Diagnostics, Piscataway, NJ).

DNA sequencing of the cDNA insert

The positive clones were isolated, replated, and rescreened until pure clones were obtained. An overnight culture of *E. coli* XL1-blue cells was co-infected with the cloned λ phage and helper phage (Stratagene) to convert the phage into the plasmid. Double stranded plasmid DNA was used as a template for DNA sequencing. The plasmid was isolated from an overnight culture of the XL1-blue cells by using the Magic Miniprep Kit (Promega, Madison, WI). Asymmetric PCR was performed to produce different lengths of ssDNA by using 5' fluorescein-labeled M13 reverse and -40 primers, Tth DNA polymerase, and dNTP and ddNTP (28) provided in the Pharmacia Auto-Cycle Kit (Pharmacia, Baie d'Urfe, Quebec, Canada). The PCR cycle employed was 95°C for 36 s, 50°C for 36 s, and 72°C for 84 s, with a total of 25 cycles. A strategy of primer walks was employed for continuation of sequencing. The primers were fluorescein-AGAAGCTTGCTCCGTTCCC and fluorescein-AGGAG-GAGTCCGACG, which were designed according to the obtained cDNA sequence and synthesized by using a Cyclone Plus DNA Synthesizer (Millipore, Bedford, MA). To prevent ambiguity caused by GC-rich regions, a higher annealing temperature was used in the PCR cycle. All sequences were read and recorded by using an automatic ALF DNA sequencer (Pharmacia). The cDNA and deduced amino acid sequences were analyzed by using DNAsis and Prosis programs (Hitachi, San Bruno, CA), respectively.

Northern blots

Total RNA (20 μ g) from *C. herbarum* was subjected to electrophoresis in the presence of formamide in 1.2% agarose/formaldehyde gel. The separated RNA molecules were electrotransferred onto Hybond-N membrane (Amersham, Oakville, Ontario, Canada) by using 1X Tris-acetate-EDTA buffer, pH 7.5, followed by UV cross-linking with the use of a UV Stratalinker (Stratagene). The membrane was blocked in hybridization solution: 5X SSC, 1% blocking reagent (Boehringer Mannheim), 0.1% *N*-lauroylsarcosine, and 0.02% SDS at 65°C for 2 h. The cDNA insert was released from pBluescript SK-vector by using *Eco*RI and another restriction enzyme capable of cleaving a site flanking the 3' end of the insert. ³²P-dATP was incorporated in the insert DNA by using a Random Primer Labeling Kit (Promega) (29), and the probe was incubated with the membrane at 65°C overnight. The membrane was washed twice for 5 min each at room temperature with 2X SSC, 0.1% SDS, followed by two washes for 15 min each at 65°C with 0.1X SSC and 0.1% SDS. The membrane was dried at room temperature and then autoradiographed.

Expression of the cloned allergen

Full length Ch2.1 cDNA clone was amplified by PCR with the use of P1 (GAATTCTC-CGGCAAAAACACCGTCAAGATG) and P2 (CTG CAG-AATACGACTCACTATAGGGCG) primers. P1 and P2 were designed according to the cDNA 5' sequence and pBluescript SK-vector sequence flanking the 3' end of the cDNA. The amplified fragments were subcloned into pCR II vector (Invitrogen, San Diego, CA). The isolated plasmid was digested by using *Eco*RI and *Pst*I to release the insert, followed by ligation into expression vector pMAL-c2 (New England Biolabs, Beverly, MA), which was also prepared by *Eco*RI and *Pst*I digestion. The induction and analysis of the fusion protein was performed according to the manufacturer's instructions. Briefly, TB1 cells were transformed by the recombinant plasmid vectors and plated on ampicillin-containing LB agar. Single colonies were picked to inoculate LB-ampicillin media and grown overnight at 37°C. LB media containing 0.2% glucose and ampicillin was inoculated with the overnight growth culture at a ratio of 1:100 (v/v) and incubated at 37°C. Isopropyl- β -D-thiogalactopyranoside was added to induce synthesis of the fusion protein when the cell density reached an A₆₀₀ of 0.5. The fusion protein, viz recombinant Ch2.1 (rCh2.1) allergen, was isolated from the cells by freeze-thawing and ultrasonication in 50 mM Tris buffer. The recombinant allergen was detected by SDS-PAGE and IgE immunoblot (30). Separation and purification of the rCh2.1 allergen was achieved by SDS-PAGE (31).

³ Abbreviations used in this paper: LB, Luria-Bertani; ORF, open reading frame; BMB, bacterial maltose binding.

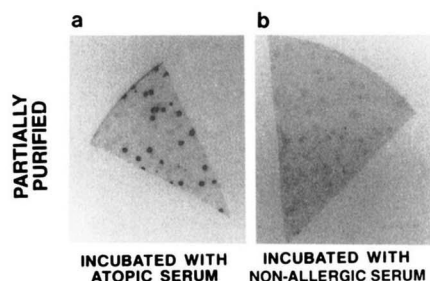


FIGURE 1. Comparison of IgE reactivity between allergen-producing and nonallergen-producing phage plaques. A partially purified Ch2.1 clone was plated and grown on LB agar after transfection. The phage plaques were lifted onto nitrocellulose membrane. Before incubation with ^{125}I -labeled anti-human IgE, the membrane was sliced into small pieces and probed with either the atopic human serum pool (a) or the nonatopic human serum (b).

Immunodetection of the natural allergen corresponding to rCh2.1 allergen in the mold extract

An allergen extract of *C. herbarum* was prepared from mycelia washed with PBS and stored at -70°C . The mycelial mass was ground into fine powder in a mortar and pestle under liquid nitrogen, followed by homogenizer extraction using 50 mM Tris buffer, pH 7.5, containing 0.1% SDS and 100 $\mu\text{g}/\text{ml}$ of PMSF. The debris was removed by centrifugation at 10,000 rpm for 20 min. The supernatant was aliquoted and stored at -20°C . Large polysaccharide components of the extract were precipitated after thawing on ice; these were removed by centrifugation at 13,000 rpm for 10 min. The supernatant was concentrated and dialyzed by using Centricon (Amicon, Beverly, MA) with a 10,000 m.w. cut off. For convenience, this preparation is termed PREP-1. An allergen extract of the mold was also prepared by using a conventional method (PREP-2), i.e., the mycelia were homogenized and extracted in 50 mM Tris buffer overnight (26). Twenty micrograms of protein from each preparation was separated by 12% SDS-PAGE and electroblotted on PVDF membrane. The proteins were probed with either human serum pool or murine sera against rCh2.1 obtained by immunization of BALB/c mice with rCh2.1 allergen extracted as described previously (32). Briefly, 5 μg of rCh2.1 allergen mixed with 2.5 mg dextran sulfate in PBS was given s.c. to each mouse. A similar dose of allergen with 1 mg dextran sulfate was administered 21 days later. The sera were collected 7 days after the second injection.

Results

Identification and isolation of a cDNA clone

The cDNA library was screened with a serum pool from six atopic individuals. One clone, designated Ch2.1, was initially found to react with human IgE Abs, as detected with the use of alkaline phosphatase-tagged goat anti-human IgE conjugate and chromogenic substrate. Its IgE binding activity was confirmed by using ^{125}I -labeled anti-human IgE Abs. The results showed that partially purified plaques containing the recombinant allergen, but not plaques synthesizing other nonallergenic proteins, reacted with the human IgE Abs (Fig. 1a). The normal serum pool showed no binding to any of the plaques (Fig. 1b). The pure clone was obtained by rescreening the allergen-producing phages three times.

Sequence of the cDNA clone and its deduced amino acid sequence

The DNA sequence of cDNA Ch2.1 is shown in Figure 2A. The insert is 630 bp in length and possesses features which indicate that it contains a full length coding sequence. DNA sequence analysis revealed that the cDNA contains several potential start codons, but only those in phase with the open reading frame (ORF) of the β -galactosidase gene of λ ZAP II vector are correct, because these would produce immunoreactive fusion proteins detected by the human IgE Abs. Two such potential start codons are found in this clone, at 20 and 29 bases. The first was considered correct based on the results of the sequence analysis described below. This clone has a complete 3'-untranslated region with a poly(A) tail. The possible polyadenylation signal AATGAA (33) is found 15 bases upstream of the poly(A).

The protein encoded by the gene is 111 amino acid residues in length, and its molecular mass and pI value are calculated to be 11.12 kDa and 3.92, respectively. The amino acid composition of this protein indicates that it is rich in alanine (16.2%), serine (13.5%), glycine (12.6%), glutamic acid (11.7%), and leucine (10.8%). There is a potential N-glycosylation site at residues 15–17 in the hydrophilic region which shows the characteristic Asn-x-Ser/Thr motif. The hydrophobic profile of the cloned allergen was analyzed by using the Kyte and Doolittle method (34) with a window of six amino acids (Fig. 2B). The dominant hydrophilic region of the allergen was demonstrated at its C-terminal sequence. Further analysis with the Tsites program (35) revealed a major amphipathic region at residues 44–63 which may form an α helical structure.

Homology analysis of the protein sequence with all known sequences in the National Biomedical Research Foundation Protein Information Resource data bank revealed that it belongs to the ribosomal P₂ protein family. Sequence homologies to amino acid sequences of other known fungal ribosomal P₂ proteins, *Schizosaccharomyces pombe* (36), *Saccharomyces cerevisiae* (37), and *Dicystostelium discoideum* (38), are 64%, 61%, and 56%, respectively (Fig. 3). These proteins share, in addition to the common features in their amino acid composition, conserved amino- and carboxyl-terminal sequences.

RNA hybridization with Ch2.1 cDNA clone

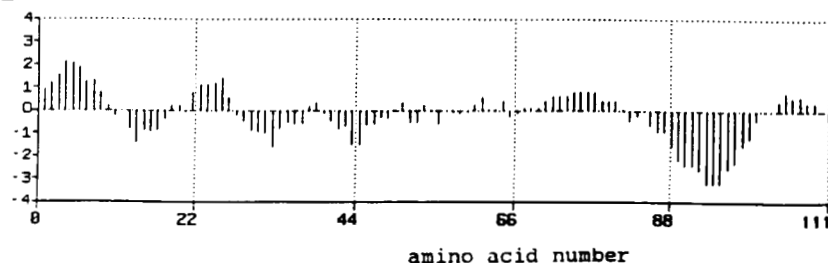
To determine the actual size of the gene transcript, RNA from the organism was hybridized with the ^{32}P -labeled cDNA probe. The results demonstrated that the probe hybridized to a single transcript (Fig. 4). The size of the mRNA coding for the allergen was estimated to be 720 bases. Comparison of RNA isolated from mycelia grown for 7, 15, or 23 days demonstrated that this transcript was present at all stages of the culture. The estimated size of the transcript indicated that about 90 bases were absent in the clone, possibly representing 5'-untranslated regions

A

-19	C	GGC	AAA	AAC	ACC	GTC	AAG	ATG	AAG	TAC	ATG	GCA	GCT
1							M	K	Y	M	A	A	
19	TAC	CTC	CTC	CTC	GGC	TTG	GCC	GGC	AAC	TCC	TCC	CCC	TCT
7	Y	L	L	L	G	L	A	G	N	S	S	P	S
58	GCT	GAG	GAC	ATC	AAG	ACT	GTC	CTC	AGC	TCT	GTT	GGC	ATC
20	A	E	D	I	K	T	V	L	S	S	V	G	I
97	GAC	GCC	GAC	GAG	GAG	CGC	CTC	TCT	TCC	CTC	CTT	AAG	GAG
33	D	A	D	E	E	R	L	S	S	L	L	K	E
136	CTC	GAG	GGC	AAG	GAC	ATC	AAC	GAG	CTG	ATC	TCT	TCC	GGC
46	L	E	G	K	D	I	N	E	L	I	S	S	G
175	TCC	CAG	AAG	CTT	GCC	TCC	GTT	CCC	TCC	GGT	GGC	TCT	GGC
59	S	Q	K	L	A	S	V	P	S	G	G	S	G
214	GCC	GCC	CCC	TCT	GCT	GGT	GGT	GCC	GCT	GCC	GCT	GGT	GGT
72	A	A	P	S	A	G	G	A	A	A	A	G	G
253	GCC	ACC	GAG	GCC	GCC	CCC	GAG	GCC	GCC	AAG	GAG	GAG	GAG
85	A	T	E	A	A	P	E	A	A	K	E	E	E
292	AAG	GAG	GAG	TCC	GAC	GAC	GAC	ATG	GGC	TTC	GGT	CTG	TTC
98	K	E	E	S	D	D	D	M	G	F	G	L	F
331	GAC	TAA	GCG	CCA	ACG	CCA	ACG	ATG	GAT	GCC	TGA	CAT	GAA
111	D	*											
370	AGC	CTG	GCT	TTG	GGA	TGG	ACT	TTG	GTG	GAT	GAA	GGG	CGC
409	AAG	CAA	TTC	TGC	CCC	ACG	GAA	GCC	GGA	GGA	CTA	GAG	GCA
448	TGG	CTA	TGT	CGT	GGA	CTC	ATT	TTT	CGC	AAG	GGT	GGA	AGT
487	CCG	GCG	TCT	GGT	TAT	GCT	CAA	AAT	GAA	CCT	CCT	TCA	TCA
526	GCA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
565	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
604	AAA	AAA	AA										

FIGURE 2. DNA and deduced amino acid sequences of cDNA clone Ch2.1.⁴ Nucleotide sequence is the sequence of the *EcoRI* and *XhoI* cDNA insert. A. The deduced amino acid sequence (below the DNA sequence) begins at the first potential in-frame initiation codon and ends with the TAA stop codon denoted by the asterisk. The underlined amino acid sequence is a potential glycosylation site. B. Hydropathic profile. The profile of the deduced amino acid sequence was obtained by using the Kyte and Doolittle method with a window of six amino acids.

B



and/or poly(A), although the cDNA contains the sequence coding for the full length allergen.

Expression of the gene encoding recombinant allergen in E. coli

To further study the allergenicity of the cloned gene product, the recombinant allergen was synthesized as a bacterial maltose binding (BMB)-rCh2.1 fusion protein in *E. coli*, by using the pMAL-c2 gene expression system. Figure 5A shows a Coomassie blue stained gel of the expressed protein, rCh2.1 allergen, which was observed in *E. coli* cells transformed with the expression vector containing the cDNA insert, but not in nontransformed cells nor

in transformants of the nonrecombinant vector. The BMB protein is 42 kDa and the recombinant allergen is 53.6 kDa, as indicated by arrows in Figure 5. Immunoblot analysis demonstrated that the rCh2.1 allergen possesses the ability to bind the IgE Abs, whereas the bacterial proteins and the BMB proteins showed no reactivity to the IgE Abs (Fig. 5B). Of seven sera from *C. herbarum*-allergic individuals, four contained IgE Abs recognizing the allergen (our unpublished observations).

Immunodetection of the ribosomal P₂ protein in the mold extract

The human atopic serum pool (1:5) and a murine serum pool (1:500) of three mice immunized with rCh2.1 allergen were used to detect the ribosomal P₂ protein of the two

⁴ EMBL accession number: X77253.

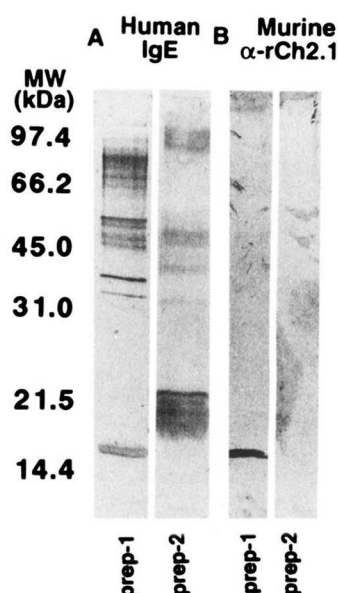


FIGURE 6. Immunodetection of the *Cla h* III allergen from *C. herbarum* extracts by using anti-rCh2.1 Abs. Two preparations of *C. herbarum* allergenic extracts were probed with human IgE (A) and murine anti-rCh2.1 (B) Abs after they were separated by SDS-PAGE and electroblotted onto a PVDF membrane.

3). In addition, matrix searches around ATG codons (39) also support the first ATG as the start codon, because its score is 60 whereas the score of the second ATG at position 29 is only 27. This allergen shares approximately 60% overall homology with other ribosomal P_2 proteins and is rich in alanine and serine residues. Moreover, it shares the most remarkable feature of all ribosomal P_2 proteins of eukaryotic cells (40, 41), i.e. consensual C-terminal sequences. Therefore, we conclude that the allergen belongs to the ribosomal P_2 protein family.

The ribosomal phosphorylated (P) proteins, also called acidic proteins, seem to be involved in the regulation of protein synthesis (42). These proteins have been isolated and characterized from a variety of species (36–38, 40, 41). Sequence analysis defined a potential *N*-glycosylation site in the cloned protein reported here. Whether the protein is actually glycosylated is not clear at present. Because most ribosomal P_2 proteins from eukaryotic cells do not have such a motif, glycosylation of the proteins may not be essential for their common biologic functions. Although ribosomal P_2 proteins from different species share common features, there exists obvious diversity in their amino acid sequences, which may account for differences in their immunogenicity.

To our knowledge, this is the first report that a ribosomal P_2 protein has been identified as an allergen. Protein allergens are a group of Ags which are capable of induction of and binding to IgE Abs under natural conditions. It is still not clear whether any protein allergen contains

unique structures which account for the allergenicity, although recent studies indicate that allergens are different from common Ags in terms of their induction of T cell subsets, cytokines, and Ab isotypes (43–47). Ribosomal P_2 proteins from other sources, including humans, have been reported as Ags capable of inducing IgG Abs (48–50). Differences in the structure of this molecule may contribute to its property of IgE Ab induction. Thus, if there are indeed some structural differences between Ags and allergens, this newly identified allergen will serve as a useful molecular model to facilitate studies toward an understanding of the structure-function relationships of protein allergens.

The Ab-binding sites of the allergen were predicted by its hydropathic profile, according to the deduced amino acid sequence. The carboxyl terminus is highly hydrophilic, indicating the location of a B cell epitope (51). Indeed, previous studies have demonstrated that this consensus sequence is a major determinant recognized by autoantibodies in lupus patients (52). However, immunization of animals with ribosomal P_2 protein from eukaryotic cells of different species may not result in production of Abs against this region (52). Therefore, the proteins were likely to have some other Ab-binding sites besides their C-termini. In this report, three other hydrophilic regions were disclosed within the cloned allergen which may also represent Ab-binding sites. To date, T cell epitopes of the ribosomal proteins have not been defined. From the results of our hydropathic analysis (35), a 20-amino acid region at 44–63 was indicated to be a potential T cell epitope. This region, containing intervals of hydrophobic and hydrophilic amino acids, may form an amphipathic and α -helical structure, which has been described as a sequence feature of T cell epitopes (53).

Availability of the recombinant allergen would facilitate the analysis of its epitopes as well as the antigenicity and allergenicity of the allergen. Because the protein was synthesized in *E. coli* in which no glycosylation occurs, it can be concluded that at least some of the IgE-binding properties of the rCh2.1 allergen are inherent in its amino acid sequence. Moreover, the IgE-binding activities of the allergen were not destroyed when subjected to denaturing conditions, indicating the possibility of employing synthetic peptides to define some of its epitopes.

There is a discrepancy in terms of molecular mass between the cloned protein calculated according to its amino acid composition (11.1 kDa) and the native protein isolated directly from the organism (14.5 kDa). Phosphorylation of the native protein may partially account for such a difference. However, it is unclear whether glycosylation of the protein is also responsible for the discrepancy.

Whether the rCh2.1 represents a major allergen of the organism needs to be further studied. Our previous study demonstrated the diversity of reaction patterns of individual sera to the different allergens in the mold extract (24). Thus, a large number of sera from the mold atopic patients

are required to determine the IgE-reactive frequencies of each individual allergen.

This study revealed that allergen extracts prepared by conventional methods do not contain the ribosomal P₂ protein allergen. The absence of the allergen in such an extract may be a result of two factors. First, we have observed by phase contrast microscopy that the chitinous wall of the mold remained intact, and the cytosolic components remained in the cells after homogenization (our unpublished observations). Hence, it is possible that the ribosomal P₂ protein, which was strongly associated with rRNA in cytosol, was not effectively extracted out. Second, our preliminary study of the natural ribosomal P₂ protein in the extract indicated that it degraded rapidly in the absence of protease inhibitors. Because the mold allergenic extract was well known to possess high levels of protease activity, the protein may be degraded during a relatively long extraction period, such as 24-h extraction and 48-h dialysis. From this viewpoint, recombinant allergens will be essential reagents for the characterization and standardization of mold allergen extracts.

Acknowledgments

We thank Dr. Michael W. De Vouge for reading the manuscript, Dr. Remy Aubin for assistance with the DNA sequence analyses, and Bill Casley for synthesis of the primers.

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