

Original article

cDNA cloning and immunologic characterization of a novel EF-1 β allergen from *Penicillium citrinum*

Background: We have identified previously that *Penicillium citrinum* is the most prevalent *Penicillium* species in the Taipei area. It is important to delineate the whole spectrum of allergenic components of this prevalent airborne fungus. The purpose of this study was to identify novel *P. citrinum* allergens through molecular cloning of allergen genes using a cDNA library of *P. citrinum* and sera from patients with bronchial asthma.

Methods: A lambda-Uni-ZAP XR-based cDNA library of *P. citrinum* was screened with sera from asthmatic patients. An IgE-binding cDNA clone was isolated and heterologously expressed in *Escherichia coli*. The frequency of IgE-binding to the expressed protein and the IgE reactivity to allergen subunits were analyzed by immunoblotting.

Results: An IgE-reactive cDNA clone (clone B) was isolated by plaque immunoassay. The cDNA insert is 876-bp long and encodes a 228-amino acid polypeptide with a calculated molecular mass of 25 035 Da. Protein database search with the deduced clone B sequence revealed that 121 (53%) and 82 (36%) of the 228 amino acids were identical to those of the elongation factor 1-beta (EF-1 β) proteins from the yeast *Saccharomyces cerevisiae* and the parasite *Echinococcus granulosus*, respectively. His-tagged recombinant clone B proteins were constructed and expressed in *E. coli*. Seven (8%) of the 92 serum samples from patients with bronchial asthma showed IgE-binding to the recombinant clone B protein. Among these seven positive sera, five demonstrated IgE-binding to the C-terminal fragment (aa 119–228) while the other two sera showed IgE reactivity to the N-terminal fragment (aa 1–118) of this newly identified EF-1 β *Penicillium* allergen.

Conclusions: A novel *P. citrinum* allergen (Pen c 24) was identified and characterized in the present study. Results obtained provide more information about allergens of prevalent airborne fungi and a basis to understand more about the IgE responses in human atopic disorders and in parasitic infections.

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Studies of allergens will provide useful information in better diagnosis, treatment and understanding the pathogenic mechanisms of human atopic disorders. Until now, among various environmental allergens, fungal allergens have been least studied.

It was found that among 1000 home samples, the four most frequently isolated fungi were yeasts, *Cladosporium*, *Penicillium* and *Aspergillus* species, which were present in 89.3, 84.5, 72.6, and 50.3% of the homes, respectively (1). We have identified that *P. citrinum* is the most prevalent airborne *Penicillium* species in the Taipei area (2). A survey in Topeka, KS, has found that *P. citrinum*, *P. oxalicum*, *P. chrysogenum*, *P. brevicompactum*, and *P. spinulosum* are the most frequently isolated *Penicillium* species (3). In the analysis of allergens of prevalent *Penicillium* species, our results showed that an alkaline serine protease (4), a

vacuolar serine protease (5), an enolase (6), a peroxisomal membrane protein (7), and a heat shock protein in the hsp70 family (8) are allergens of *P. citrinum* and/or *P. chrysogenum*. However, at least 18 different allergens have already been identified in a taxonomically closely related fungus *Aspergillus fumigatus* (9). It is suggested that more allergens of *Penicillium* species remain to be identified.

In this study, we isolated a novel allergen gene of *P. citrinum* by screening an Uni-Zap XR cDNA library using sera from patients with bronchial asthma. Results from sequence alignment indicated that this newly identified *Penicillium* allergen was an elongation factor 1 β (EF-1 β) protein of *P. citrinum*. Among 92 sera from patients with bronchial asthma, seven (8%) showed IgE-immunoblotting reactivity to the His-tagged

recombinant EF-1 β protein. It was of interest to find that IgE antibodies in patients of *Echinococcus granulosus* infection recognized the EF-1 β protein of the parasite (10). Patients of *E. granulosus* infection recognized predominantly the N-terminal subunit of the EF-1 β protein from the same parasite (10). However, in this study, IgE antibodies of asthma patients reacted mainly with the C-terminal region of the newly identified *Penicillium* allergen (Pen c 24).

Material and methods

Serum samples

Serum samples were collected from 92 patients with bronchial asthma and attending the allergy clinics at the Veterans General Hospital-Taipei and the Cheng Hsin Rehabilitation Medical Center, Taipei. After collection, sera were stored in aliquots at -70°C .

Cloning and molecular techniques

Total RNA was extracted from a 2-day-old fungal mat culture of *P. citrinum* strain 52-5 (2). A lambda-Uni-ZAPTM XR-based cDNA library of *P. citrinum* was custom-made by Stratagene (La Jolla, CA, USA). The cDNA clones were screened by plaque immunoassay of the isopropyl- β -D-thiogalactopyranoside (IPTG)-induced library using a serum mixture from asthma patients essentially as described (7, 8). Four serum samples from patients with bronchial asthma with positive IgE reactivity to *P. notatum* (*P. chrysogenum*) (two to three classes) in CAP test (RAST FEIA; Pharmacia AB, Uppsala, Sweden) were selected and used in cDNA cloning. IgE reactivity was detected with alkaline phosphatase-labeled monoclonal anti-human IgE (Pharmingen, San Diego, CA, USA) and developed using the alkaline phosphatase conjugate substrate kit (containing BCIP and NBT solutions; Bio-Rad, Hercules, CA, USA; catalog number 170-6432). The insert of an IgE-binding cDNA clone (clone B) thus obtained was excised from the ZAP vector by super-infecting the *E. coli* SOLRTM strain with helper phage (ExAssistTM helper phage, M13; Stratagene) according to the manufacturer's protocol. The excised phagemid was replicated in *E. coli* and the nucleotide sequence of the cDNA insert was determined with an ABI 377 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Preparation of recombinant proteins

Clone B was expressed as 6x His-tagged protein according to the manufacturer's instructions (Qiagen Inc., Valencia, CA, USA). The cDNA of clone B was amplified through polymerase chain reaction (PCR). The forward primer used in the reaction was clone B-BamHI (Table 1) that contains the BamHI restriction site in addition to the sequence encoding the N-terminal 7 amino acid residues (GFTDFVS) of the protein. The reverse primer clone B-PstI (Table 1) contains a PstI restriction site and the nucleotide sequence encompassing bases 682–701 (including the TAA stop codon) of the cDNA clone. The PCR product was separated by agarose gel electrophoresis and purified with a QIAquick gel extraction kit (Qiagen, Hilden, Germany). It was digested with BamHI and PstI restriction enzymes then ligated into the BamHI and PstI restriction

Table 1. Primers for PCR

Name	Nucleotide sequence (in 5' to 3'-end orientation)
Clone B-BamHI	CGGGATCCGGTTTACCGACTTCGTTTC
Clone B-PstI	AACTGCAGTTACAGCTTTTGCATGGCAG
N-clone B-BamHI	CGGGATCCGGTTTACCGACTTCGTTTC
N-clone B-PstI	AACTGCAGTTAAGCAACGACGGCGGGGTC
C-clone B-BamHI	CGGGATCCGAGCGCAACAAGAACTTGG
C-clone B-PstI	AACTGCAGTTACAGCTTTTGCATGGCAGC

sites of the pQE-30 vector. The resulting vector was transformed into *E. coli* JM109 and M15. The recombinant polypeptide expressed in *E. coli* M15 was affinity-purified, under denaturing condition, from a Ni-NTA resin column (Qiagen). The reactivity of the recombinant protein with IgE antibodies in sera from asthmatic patients was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-immunoblot.

For mapping of IgE-binding regions, His-tagged recombinant proteins of the N- (aa 1–118) and the C- (aa 119–228) terminal subunits of the clone B protein were also prepared essentially as described above. The primers used were N-clone B-BamHI and N-clone B-PstI, as well as C-clone B-BamHI and C-clone B-PstI as described in Table 1.

SDS-PAGE and immunoblotting

The reactivity of the recombinant proteins and components in crude extracts of *P. citrinum* (6) with IgE antibodies in sera of patients with bronchial asthma was analyzed by SDS-PAGE-immunoblotting essentially as described (4–7). Briefly, the recombinant proteins and the *P. citrinum* components were separated by SDS-PAGE and transferred electrophoretically onto polyvinylidene difluoride (PVDF) membrane (0.45 μm ; Millipore, Bedford, MS, USA). After blocking with 1% skimmed milk, the blots were incubated with diluted human serum samples [1 : 5 dilution in Tris-buffered saline, pH 7.5 containing 0.05% Tween 20 (TTBS) and 1% skim milk] for 16 h at 4°C . After washing, the blots were incubated with alkaline phosphatase-conjugated monoclonal anti-human IgE antibodies (Pharmingen) for 1 h at room temperature. Excessive antibodies were removed by washing, and the blots were developed using an alkaline phosphatase conjugate substrate kit as described above. After washing with water, the deposition of the purple stain that demonstrated antigen-antibody binding was recorded by photography. Sera from healthy laboratory volunteers were used as negative controls.

Peptide synthesis and immunoassay

The most IgE-reactive region of the EGF-1 β / δ protein from the parasite *E. granulosus* was identified to be ¹⁰⁰KGAK-PIRPVVVK¹¹¹ (10). A peptide with an amino acid sequence of the *P. citrinum* EF-1 β (⁸⁴EGSEASANPK⁹³) corresponding to the major IgE epitope of the *E. granulosus* EGF-1 β / δ protein was synthesized commercially (Genesis Biotech Inc., Taipei, Taiwan) on derivatized cellulose sheets. The membrane was blocked and incubated individually with serum samples that showed positive IgE reactivity against the N-terminal subunit protein of the *P. citrinum* EF-1 β . The membrane was washed and incubated with enzyme-labeled anti-human IgE and substrate solution as described above.

Results

cDNA cloning and sequencing

In this study, a lambda-Uni-ZAP XR-derived clone (clone B) was isolated from the *P. citrinum* cDNA library due to its IgE-binding activity by plaque immunoassay. The nucleotide sequence (876 bp, GenBank accession no. AY363911) of the cDNA insert was determined by dideoxy sequencing. The nucleotide sequence of clone B and its deduced amino acid sequence from an open reading frame were shown in Fig. 1. The cDNA insert encodes a 228-amino acid polypeptide (including initiation methionine) with a calculated molecular mass of 25 035 Da. Cysteine residues and putative N-glycosylation sites were not identified on the protein (Fig. 1). A potential polyadenylation signal sequence 'AATATA' was found at 17–22 bases upstream from the polyA tail in clone B. Protein database search with the deduced clone B sequence revealed that 121 (53%) and 82 (36%) of the 228 amino acids were identical to those of the EF-1 β proteins from the yeast *Saccharomyces cerevisiae*

CACCTTCGTTTCATC	14
ATG GGT TTC ACC GAC TTC GTT TCT GAC GCC GGC CTG TCC CTG	56
M G F T D F V S D A G L S L	14
GCC AAC AAC TAC CTG GCT ACC CGC AGC TAC ATC GTT GGC CAC	98
A N N Y L A T R S Y I V G H	28
GCC CCC AGC CAG GCC GAT GTT GTG ACC TAC AAG GCT TTC ACT	140
A P S Q A D V V T Y K A F T	42
GCC TCT CCC GAC GCT GAG AAG TAC CCC CAC GTC GCC CGC TGG	182
A S P D A E K Y P H V A R W	56
TAC AAG CAC ATC GCC TCT TAC GAG AGC GAG TTC CCC ACC CTT	224
Y K H I A S Y E S E F P T L	70
CCT GGT GAC GCC TCC AAG GCT TTC ACT GCC TAC GGC CCC GAG	266
P G D A S K A F T A Y G P E	84
GGC TCT GAG GCC TCT GCC AAC CCC AAG GAC AAG CCC GCC GAG	308
G S E A S A N P K D K P A E	98
GAA GAG GAG GAG GAG GAC CTC TTC GCC TCT GAC TCC GAG GAT	350
E E E E E D L F A S D S E D	112
GAG GAC CCC GCC GTC GTT GCT GAG CGC AAC AAG AAC TTG GAG	392
E D P A V V A E R N K N L E	126
GAG TAC AAG AAG AAG AAG GCC GCT AAG GGA CCC AAG CCC GCC	434
E Y K K K K A A K G P K P A	140
GCC AAG TCC CTG GTC ACC CTG GAA GTG AAG CCC TGG GAT GAC	476
A K S L V T L E V K P W D D	154
GAG ACC AAC CTG GAG GAG CTC GAG GCC AAC GTC CGT GCC ATT	518
E T N L E E L E A N V R A I	168
GAG ATG GAT GGT CTG GTC TGG GGT GCT TCC AAG TTC GTC GCT	560
E M D G L V W G A S K F V A	182
GTT GGC TTC GGT ATC AAG AAG CTC CAG ATC AAC CTG GTC GTC	602
V G F G I K K L Q I N L V V	196
GAG GAC GAG AAG GTC TCC ACC GAC GAG CTC CAG GCT CAG ATC	644
E D E K V S T D E L Q A Q I	210
GAG GAG GAC GAG GAC CAC GTC CAC TCC ACC GAC GTT GCT GCC	686
E E D E D H V Q S T D V A A	224
ATG CAA AAG CTG TAA	701
M Q K L *	228
ATGGGAGTTTGTGCGATAACGTAGAAATGGCGTGATGATGGAATTGATGTTT	876
CCTAGTTACGAACCTAGAACCTAGCCCAAAATTGAGGCTGGTCTGATGAAATATA	
ATATCATGACCCAAAAA	

Figure 1. Nucleotide and deduced amino acid sequences of the cDNA of clone B encoding the *P. citrinum* elongation factor-1 β allergen (Pen c 24) (GenBank accession no. AY363911). Numbers to the right denote the nucleotide and the deduced amino acid positions. The TAA stop codon is indicated by (*).

	*		**		*	
Pc	MGFTDFVSDA	GLSLANNYLA	TRSYIVGHAP	SQADVVTYKA	FTASPDAEKY	50
Sc	MASTDFSKIE	TLKQLNASLA	DKSYIEGTAV	SQADVTVFKA	FQSA-----Y	
Eg	SAYMRFLSKD	IGKTSATEQK	GDSYLVKEIQ	KVRDNIKSSL	BSNGSWNERM	
		*			*	
Pc	PHVARWYKHI	ASYESEFPTL	PGDASKAFTA	YGPEGSE--A	SANPKDKPAE	98
Sc	PEFFRWFNHI	AFKADEFDSF	PA-----	----ASA--A	AAE-----EE	
Eg	EKLEAIVKEM	SLRVNELEVQ	IQLRGQILPS	ESLKGAKPIR	PVVVKAPPAE	
		***	*	*		
Pc	--EEEEEDLF	ASDSEDEDPA	VVAERNKNLE	EYKKKKAAGK	PKPAAKSLVT	146
Sc	--EDDDVDLF	GSDDEEADAE	AEKWKAEERIA	AYNAKKAAP	AKPAAKSIVT	
Eg	GGDDDDIDLF	GSEDE--GE	ADKCK-QMMA	EQNKAAASKK	EKPVAKSMIV	
		*	*****	*	*	
Pc	LEVVPWDDT	NLEELEANVR	AIEMDGLVWG	ASKFVAVGFG	IKKLQINLVV	196
Sc	LDVKPWDDT	NLEEMVANVK	AIEMEGLTWG	AHQFIPIGFG	IKKLQINCVV	
Eg	LDVKPWDDT	NMAEMEMGVR	AITTDGLLWG	TSKLVLPLVHG	INKLQIACVV	
		***	*	*	*	
Pc	EDEKVSTDEL	QAQIEEDEDH	VQSTDVAAMQ	KL		228
Sc	EDDKVSLDDL	QQSIEEDEDH	VQSTDIAAMQ	KL		
Eg	EDDKVGTDFL	EESIMELEDY	VQSVDVASFN	KL		

Figure 2. Comparison of the deduced amino acid sequences of the elongation factor-1 β proteins from *P. citrinum* (Pc), *S. cerevisiae* (Sc, accession number P32471) and the parasite *Echinococcus granulosus* (Eg, accession no. AAF02297). Consensus amino acid residues are indicated by (*). Dashes (–) denote spaces that have been introduced to optimize the alignment.

(accession no. P32471) (11) and the parasite *Echinococcus granulosus* (accession no. AAF02297) (12), respectively (Fig. 2).

Characterization of recombinant clone B

The complementary DNA fragment of clone B produced by PCR was cloned into the expression vector pQE30. The 6x His-tagged recombinant clone B (EF-1 β) has an apparent molecular weight (MW) of about 33.5 kDa (data not shown). Sera from 92 patients with bronchial asthma were used in the analysis of IgE reactivity to the recombinant protein. Seven (8%) of these 92 sera showed IgE-binding to the recombinant clone B protein (Fig. 3, part a, strips no. 1–7 of panel B). The Allergen Nomenclature Sub-Committee has designated this IgE-reacting clone B protein of *P. citrinum* as the Pen c 24 allergen.

For mapping of IgE-binding regions, our results showed that five of the seven IgE-positive sera reacted with the C-terminal subunit (aa 119–228) of EF-1 β from *P. citrinum* (Fig. 3, part c, strips no. 1–5 of panel B). IgE antibodies in the other two serum samples reacted with the N-terminal subunit (aa 1–118) of Pen c 24 (Fig. 3, part b, strips no. 6 and 7 of panel B). The IgE reactivity to the recombinant Pen c 24 and the N- and C-subunit proteins was not detectable when serum sample from a healthy laboratory volunteer was used (Fig. 3, strips no. 8 of panel B).

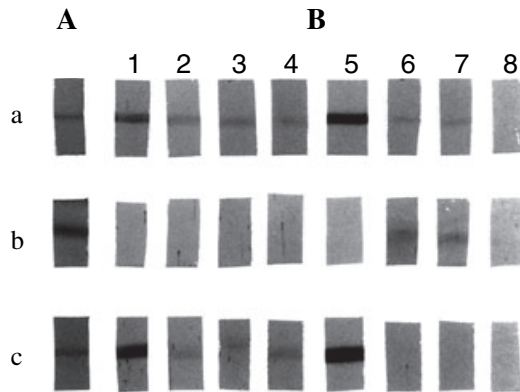


Figure 3. IgE immunoblot reactivity to the recombinant Pen c 24 protein (part a), the N-terminal (part b) and the C-terminal (part c) subunits of the Pen c 24 allergen. Strips in panel A show the Coomassie blue stained recombinant proteins. Strips in panel B show the results of IgE-immunoblotting using seven sera from patients with bronchial asthma (strips no. 1–7) and one serum sample from a healthy laboratory volunteer (strips no. 8).

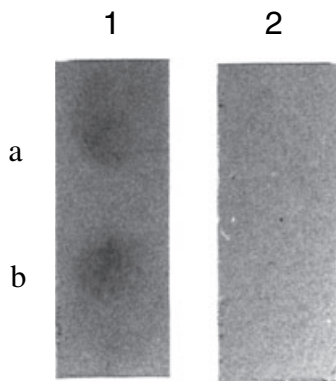


Figure 4. IgE reactivity to the synthetic peptide $^{84}\text{EGSEASANPK}^{93}$ of the Pen c 24 allergen. Dots a and b represent duplicate peptide spots. Strips 1 and 2 were incubated with serum samples no. 7 and 8 as shown in Fig. 3, respectively.

In this study, a peptide with an amino acid sequence of the *P. citrinum* EF-1 β ($^{84}\text{EGSEASANPK}^{93}$) corresponding to the major IgE epitope ($^{100}\text{KGAKPIRPVVVK}^{111}$) of the EF-1 β/δ parasite protein was synthesized on derivatized cellulose sheets. Serum samples no. 6 and 7 in Fig. 3 with IgE-binding to the N-terminal subunit of *P. citrinum* EF-1 β were used in the immunoassay. Our results demonstrate that IgE antibodies in one (serum no. 7) of these two serum samples reacted with the peptide $^{84}\text{EGSEASANPK}^{93}$ (Fig. 4, strip no. 1). For control, IgE reactivity to the peptide was not detectable when serum from the same healthy laboratory volunteer was used (Fig. 4, strip 2).

We also analyzed the presence of IgE antibodies against components in a crude extract of *P. citrinum* in the seven serum samples with IgE-binding to the recom-

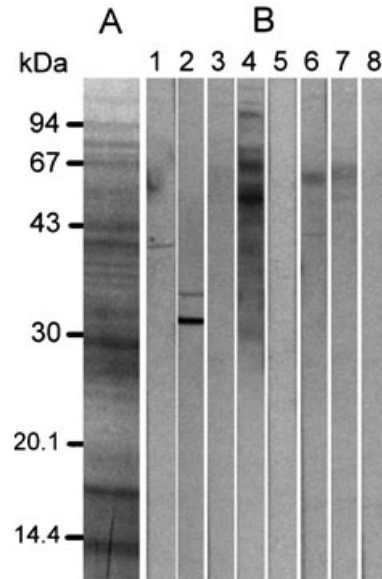


Figure 5. IgE immunoblotting reactivity to components in a crude extract of *P. citrinum*. The strip in panel A shows the Coomassie blue-stained protein profile of the *P. citrinum* extract. Strips no. 1–7 in panel B represent results of immunoblotting by using sera from patients 1–7 in panel B of Fig. 3, respectively. Strip no. 8 represents a control by using serum from a healthy laboratory volunteer.

binant clone B protein. IgE antibodies in five serum samples (sera 1, 2, 4, 6 and 7 in Fig. 3) against *P. citrinum* components which were different in molecular masses were detectable (Fig. 5, strips 1, 2, 4, 6 and 7 of panel B). A very weak IgE-binding to a 28 kDa component was considered for serum 5 (Fig. 5, strip 5 of panel B) that showed a strong IgE-binding to the recombinant clone B protein (Fig. 3, strip 5 of panel B). Results obtained suggest that the Pen c 24 allergen may not be present in the crude *P. citrinum* extract analyzed.

Discussion

In this study, an IgE-binding clone, clone B, was isolated from a *P. citrinum* cDNA library using sera from patients with bronchial asthma. The cDNA of clone B encoded a protein of 228 amino acids which has 53% amino acid sequence identity to that of the EF1- β of *S. cerevisiae*. The result suggests that clone B may encode a *P. citrinum* EF-1 β protein allergen (Pen c 24). The recombinant protein that contains an extra 6x His-tag at the N-terminus of the EF-1 β protein, had an apparent MW of 33.5 kDa on the gel. The molecular mass determined on the gel was significantly higher than the deduced molecular mass of this protein from the ORF (25 035 Da). A discrepancy in the determination of the molecular mass of yeast and *Artemia* EF-1 β , similar to that observed in this study, was also noted (11, 13). The cause for this discrepancy is unknown, but

may reflect some unusual structure features of this protein (11).

The frequency of IgE-binding to recombinant Pen c **24** was about 8% (7/92) when immunoblotting analysis was carried out with sera from 92 asthmatic patients. It indicates that Pen c **24** is a minor allergen of *Penicillium* species. However, strong IgE-binding to recombinant Pen c **24** in two of the asthmatic sera tested suggests that the EF-1 β protein may be an important allergen in these two patients.

The β , δ and γ subunits of EF-1 are conserved, constitutive proteins predominantly located in the endoplasmic reticulum region (14). However, Maneu et al. (15) isolated, from a *Candida albicans* cDNA expression library, a clone encoding the EF-1 β by immunoscreening with a polyclonal antibody against cell wall material. Results from gene disruption experiments suggest that EF-1 β is required for the conversion of EF-1 α -GDP to EF-1 α -GTP and is essential for growth (11). It was also found that in the β and δ subunits of EF-1, the function of GDP-GTP exchange is localized in the C-terminal subunit (12, 16).

In addition to extrinsic allergens, high levels of specific IgE antibodies were also developed in human responses to parasitic antigens of helminthes-infected individuals. In allergic subjects, IgE antibodies are largely responsible for initiating symptoms of immediate hypersensitive reactions. In helminth infections, IgE production may be responsible for the protective immune response to the parasite or immune-mediated pathology or for both events (10, 17–19).

Ortona et al. (10) found that 90% (38/42) of patients with cystic echinococcosis (CE) and with allergic reactions showed IgE-binding reactivity to *Echinococcus granulosus* (Eg) EF-1 β / δ . In addition, Eg EF-1 β / δ could induce cellular reactivity and skewed the Th1/Th2 cytokine balance toward a preferentially Th2 polarization in patients with CE irrespective of the allergic manifestations (10). Their results suggest that Eg EF-1 β / δ is an allergenic molecule and indicate the possible association between allergic disorders related to CE and the presence of IgE specific to a conserved *E. granulosus* protein (EF-1 β / δ). Furthermore, a high percentage (82%) of IgE specific to EF-1 β / δ detected in CE patients who had experienced an anaphylactic shock was also observed. It suggests that EF-1 β / δ , usually hidden within the cyst, could be suddenly released thus participating in the development of anaphylactic shock (10).

Results from Ortona et al. (10) also showed that all 38 patients, whose serum IgE reacted to whole Eg EF-1 β / δ , were IgE-positive in immunoblotting exclusively to the N-terminal subunit (118 aa) of EF-1 β / δ . Epitope mapping identified 100–111 (KGAKPIRPVVVK) as the most IgE-reactive region. It is of interest to find that the 101–109 residues (GAKPIRPVV) of Eg EF-1 β / δ showed in databases 78% identity and 89% similarity with the 5A IgE reactive antigen, residues 50–58, of the parasite *Strongyloides stercoralis* (GPKPIRPVIV) (20). Bearing

similarity to CE, strongyloidiasis infections manifest commonly with cutaneous symptoms and urticaria.

In this study, a sequence identity of 36% was found between the EF-1 β proteins from *P. citrinum* and the parasite *E. granulosus*. It is of interest to analyze the presence of common epitopes of both EF-1 β proteins recognized by IgE antibodies developed in patients of atopic respiratory disorders and of parasitic infections. Our results using seven serum samples showed that IgE epitopes were localized mainly in the conserved C-terminal region of EF-1 β from *P. citrinum*. Only two serum samples showed IgE reactivity to the N-terminal subunit of *P. citrinum* EF-1 β . One of these two serum samples demonstrated IgE-binding against the peptide (⁸⁴EGSEASANPK⁹³) synthesized according to the sequence of *P. citrinum* EF-1 β that corresponds to the major IgE epitope (¹⁰⁰KGAKPIRPVVVK¹¹¹) of the Eg EF-1 β / δ protein as shown in Fig. 2. This result contrasts with data from Ortona et al. (10) that IgE epitopes were localized exclusively in the variable N-terminal region of the *E. granulosus* EF-1 β / δ . Thus, although very similar 3d structures for the highly conserved C-terminal domains (containing C-terminal 90 amino acid residues) of the EF-1 β proteins from *P. citrinum* and *E. granulosus* were found (data not shown), the presence of cross-reactive IgE epitopes between these two EF-1 β proteins might be low. A finding similar to that observed in this study was also found. The cyclophilins are a new family of pan allergens and the mold cyclophilins Mal f 6 and Asp f 11 can be cross-reactive (21). In addition, *E. granulosus* cyclophilin is involved in the allergic symptoms related to CE (22). However, the *E. granulosus* cyclophilin does not cross-react with cyclophilin allergen from other organisms (22).

Results different from those mentioned above were also found. It was revealed that the IgE-binding epitopes of a *Schistosoma japonicum* protein (Sj22.6) exhibit similarities to the IgE-binding epitopes of known allergens (23). Using an overlapping peptide approach, four IgE-binding epitopes were identified in Sj22.6, two of which exhibited similarities to known IgE-binding epitopes from codfish (Gad c 1) and beta-lactoglobulin-related allergens. These findings suggest that allergy and protective immunity to helminth infection may be linked by the structural similarities of epitopes recognized by human IgE antibodies (23). The conserved structure of the IgE-binding peptides might be the common denominator responsible for the dual immune responses against parasites and allergens (22). In the present study, the discrepancies observed in IgE epitopes of the EF-1 β proteins from different sources may be due to differences in the amino acid sequences of the proteins, the routes and the amounts of antigen sensitization of patients with different kinds of diseases.

In conclusion, an obvious hindrance to a reliable diagnosis of atopic allergy is the lack of well-defined allergen preparations. The identification and characterization of novel allergens are of particular importance for organisms whose extracts are difficult to standardize like

fungi. Results obtained from this study provide valuable information and material in the preparation and standardization of diagnostic fungal reagents as well as a basis for studies of immune responses in allergy and human helminth infection.

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