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Identification of the Allergen Psi c 2 from the Basidiomycete *Psilocybe cubensis* as a Fungal Cyclophilin

Key Words
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Abstract
Basidiospores are a prevalent and frequent cause of respiratory allergies, yet their allergens remain poorly defined; thus, we have attempted a molecular characterization of representative basidiomycete allergens. A *Psilocybe cubensis* mycelial cDNA library was immunoscreened with patient serum. A clone was isolated that expressed a 23-kD recombinant allergen as a fusion protein and inhibited a 16-kD band (Psi c 2) in immunoprints of *P. cubensis* extract, indicating antigenic identity. Sequence (cDNA) analysis of the clone indicates homology with cyclophilin and the deduced amino acid sequence of Psi c 2 showed 78% identity and 4% similarity with the amino acid sequence of *Schizosaccharomyces pombe* cyclophilin. This recombinant allergen is a useful model for epitope analysis of basidiospore allergens and fungal allergen cross-reactivity, and may provide an improved reagent for basidiospore allergy diagnosis and treatment.

Introduction

The allergenic nature of conidial forms of ascomycetes has long been accepted [1]. Several model species have been studied *(Alternaria alternata, Aspergillus fumigatus)* and their allergens extensively characterized [2, 3]. Basidiomycetes and ascomycetes are both dikaryotic and together comprise the Dikaryomycota, the 'higher' fungi. Basidiomycetes are the large fleshy fungi that are familiar as mushrooms, puffballs, and the tough or leathery conks that decay trees. Although Gregory predicted the allergenic nature of basidiomycetes in 1952 based on their abundance in the air, immunochemical evidence was presented only in the 1980s [4–6]. To further characterize basidiomycete allergens, the mushroom *Psilocybe cubensis* was selected as a model system since the prevalence of skin test reactivity of *P. cubensis* is higher than for other basidiomycetes. The purpose of this study was to clone allergen genes from *P. cubensis* for sequence analysis, and to produce recombinant allergens.

Methods

Since mycelial and spore extracts have similar allergenic activity [7], mRNA was isolated from actively growing (late log phase) *P. cubensis* mycelium and a cDNA library was prepared in the expression vector pcDNAII (Invitrogen). The cDNA inserts (>0.5 kb) were cloned into the *Bst*X/*Not*I restriction site of pcDNAII. The library was immunoscreened with patients' serum for clones expressing IgE-binding proteins. Recombinant allergen expression was induced with isopropyl thiogalactoside, and imprints of colonies were incubated with pooled serum (1:10) overnight, then overlaid with an alkaline-phosphatase-labelled monoclonal antihuman IgE and reacted with NBT/BCIP substrate.

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The sequence of the cDNA insert was obtained using the M13 universal primer, and synthetic primers designed from known internal sequences. Sequence comparisons were conducted between the cDNA sequence and all sequences in the invertebrate, vertebrate and human databases (CDEM33IN, CDEM33VR, CDEM33HU) using the Fast Scan routine of PC Gene (Intelligenetics). The deduced amino acid sequence was compared against the Swiss-Prot database, and aligned with the Clustall routine.

Overnight cell cultures were grown in Luria broth, harvested by centrifugation and lysed by three cycles of freezing in liquid N_2 and thawing at 37°C. Undiluted lysate was resolved on a 10%T/2.5%C SDS-PAGE gel, semidry blots prepared on CNBr-activated nitrocellulose, and reacted with patient serum.

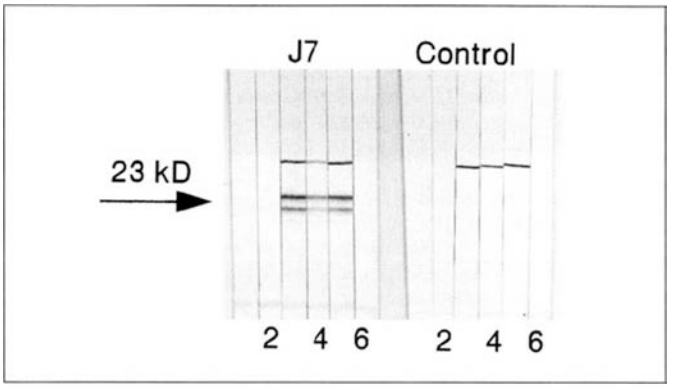

Fig. 1. SDS-PAGE immunoblot analysis of lysate from clone J7, expressing the *P. cubensis* allergen, and from a control clone that was negative by immunoscreening. Lanes 1, 2 and 6 = controls; 3 and 5 = serum pool 1:10; 4 = serum pool 1:20.

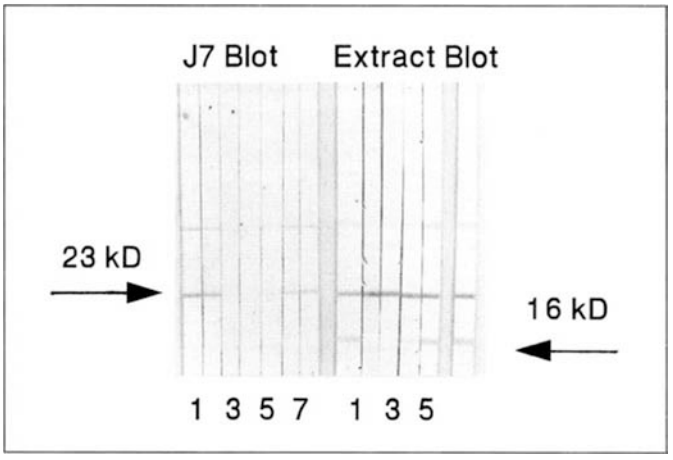

Fig. 2. Reciprocal SDS-PAGE immunoblot inhibitions of lysate from clone J7 and *P. cubensis*. Strips blotted with J7 lysate (left) were inhibited with increasing concentrations of *P. cubensis* extract. Lanes 1 and 2 = controls; 3 = serum pool inhibited with neat extract; 4 = 1:10; 5 = 1:100; 6 = 1:1,000; 7 = 1:10,000. Strips blotted with *P. cubensis* extract (right) were inhibited with increasing concentrations of J7 lysate. Lane 1 = control; 2 = serum pool inhibited with neat lysate; 3 = 1:10; 4 = 1:100; 5 = 1,000; 6 = 1:10,000.

Results

Clone J7 was recovered from the cDNA library and bound IgE from atopic serum. The clone contained an approximately 0.6-kb insert and expressed a 23-kD protein (fig. 1). The 16-kD allergen in *P. cubensis* mycelial extract was inhibited in a dose-dependent fashion by cell lysate from the clone, suggesting that J7 contained cDNA for the 16-kD allergen (fig. 2). The cDNA sequence of J7 contained an open reading frame (ORF) of 88 residues in frame 1, and one of 162 residues (9.72 pI, 17.4 kD) in frame 3, approximately the size of the allergen inhibited in the immunoprint. Sequence analysis of the cDNA insert in J7 indicated a high degree of identity with a number of cyclophilin and cyclophilin-related genes from various species, including tomato, yeast, and human. The deduced amino acid sequence of the ORF in frame 3, the *P. cubensis* allergen Psi c 2, was also very similar to cyclophilin from a variety of species. The best match was 78% identity and 4% similarity (conservative substitutions) between J7 and the amino acid sequence of cyclophilin from the fission yeast *Schizosaccharomyces pombe* (18 kD) [8].

Discussion

Previous reports described the allergens in *P. cubensis*, and identified a 16-kD protein as one of the major allergens in this species, reacting with 82% of sera from skin-test-reactive subjects [7]. Our evidence indicates that the cDNA insert in clone J7 codes for the 16-kD allergen in *P. cubensis* spore and mycelial extracts. On the basis of the cDNA and the deduced amino acid sequences, the cloned allergen is *P. cubensis* cyclophilin.

This is the first report of an allergen DNA sequence and of a recombinant allergen from a basidiomycete fungus. The identification of this allergen as a widely occurring protein (cyclophilin) presents a unique opportunity to study the basis of allergenicity, including B and T cell epitopes, since cyclophilin has been sequenced from a number of species. Although human cyclophilin is not allergenic, cyclophilin from other species, such as *P. cubensis*, is a major allergen. An analysis of the sequence and epitope differences between these homologous proteins should provide clues regarding those sequences or motifs significant for eliciting an IgE or T cell response.

References

1 Salvaggio J, Aukrust L: Mold-induced Asthma. J Allergy Clin Immunol 1981;68:327–346.

2 Vijay HM, Young NM, Curran IHA, Copeland DF, Bernstein IL: A major allergen of *Alternaria alternata* with potential for safe and effective immunotherapy. 1993;91:826–828.

3 Arruda LK, Mann BJ, Chapman MD: Selective expression of a major allergen and cytotoxin, *Asp f* 1, in *Aspergillus fumigatus*. J Immunol 1992;149:3354–3359.

4 Gregory PH, Hirst JM: Possible role of basidiospores as air-borne allergens. Nature 1952; 170:414.

5 Lehrer SB, Lopez M, Butcher BT, Olson J, Reed M, Salvaggio JE: Basidiomycete mycelia and spore-allergen extracts: Skin test reactivity in adults with symptoms of respiratory allergy. J Allergy Clin Immunol 1986;78:478–485.

6 Horner WE, O'Neil CE, Lehrer SB: Basidiomycete Allergens. Clin Rev Allergy 1992;10:191–211.

7 Helbling A, Horner WE, Lehrer SB: Comparison of *Psilocybe cubensis* spore and mycelium allergens. J Allergy Clin Immunol 1993;91: 1059–1066.

8 Harding MW, Handschumacher RE, Speicher DW: Isolation and amino acid sequence of cyclophilin. J Biol Chem 1986;261:8547–8555.