

Isolation of *Basidiomycete* Allergens

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Key Words

Mushroom · *Basidiomycetes* · *Coprinus comatus* ·
Airborne allergens · Cloning · Phage display ·
Recombinant allergens

Background

Basidiomycetes (fleshly mushrooms), the physically largest and most complex fungi are ubiquitous and produce large quantities of tiny spores (3–15 µm) contributing up to 30% of the total spore load [1]. Although fungal spores are generally accepted as causes of respiratory allergies, spores of this fungal class are not yet established as a source of common aeroallergens. The rate of sensitization to three *Basidiomycetes* tested (*Coprinus comatus*, *Boletus edulis*, *Pleurotus pulmonalis*) in our outpatient clinic, in more than 1,200 patients, was 4.0% [2]. This exceeds the sensitization rate for dog, latex and molds. Clinical relevance was demonstrated by nasal challenges [2]. However for an easier and reliable diagnosis of basidiomycete allergy the isolation, characterization and production of the proteoallergenic components is urgently warranted. Here we present the molecular isolation of the first *C. comatus* allergen.

Methods

For the molecular isolation of *C. comatus* allergens a pJuFo library was synthesized according to Cramer et al. [3]. Subsequent screening was performed with a pool of four sera of SPT-positive patients for *Coprinus*. Positive inserts

were recloned into pQE-30 in order to produce recombinant, His-tagged protein in *Escherichia coli* which then was purified over a Ni²⁺-NTA column and refolded by stepwise dialysis against PBS. Purity and molecular weight were assessed by SDS-PAGE. Specific binding of serum IgE from individuals sensitized to *C. comatus* to the recombinant protein was analyzed by immunoblotting [2], allergen-specific ELISA [4] and skin prick tests [2].

Results

The initial complexity of the *C. comatus* pJuFo library was 7.5×10^7 CFU and after five rounds of panning inserts of 0.5, 0.6, 0.9, 1.0, 1.1 and 1.6 kb had amplified. Sequencing of the 0.5-kb insert which did amplify most frequently revealed no significant homology to any deposited DNA sequence. From a 1-liter IPTG-induced culture 64 mg recombinant protein could be isolated which showed no significant contamination with bacterial proteins. ELISA with 114 sera collected from individuals sensitized to one of the three *Basidiomycetes* revealed that 19% of the sera contain specific IgE. None of the basidiomycetes-negative sera of atopic subjects showed IgE binding to this protein.

Immunoblot analysis with patient sera showed that specific IgE recognizes as little as 10 ng of the 12-kD protein when detected with enhanced chemiluminescence. After ethical approval, skin prick tests with the recombinant protein was performed at concentrations of 1 fmol/µl to 25 pmol/µl. Corresponding to the in vitro data, clear positive results could be observed at a concentration of 100 fmol/µl. With reference to the official allergen nomenclature [5] this first *C. comatus* allergen is denominated Cop c 1.

Conclusion

Basidiospores are a ubiquitous source of aeroallergens with the potential to sensitize subjects and to induce respiratory allergies. By immunoblotting it has been demonstrated recently that basidiospores contain proteins which are recognized by patients' serum IgE [2]. Synthesis and screening of a pJuFo library of *Coprinus* once more demon-

strated the reliability of this system for isolating allergens. The isolation of seven cDNAs encoding putative allergens and the full characterization of a minor recombinant allergen thereof, Cop c 1, are the first step towards the urgently needed characterization of the important allergenic components of *basidiomycetes*. This panel of well-characterized allergens will allow to reliably diagnose *basidiomycete*-induced (respiratory) allergies.

References

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