Comparison of the allergenic potency of spores and mycelium of *Cladosporium*

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ABSTRACT

The allergenic potency of spore and mycelium extracts of *Cladosporium* was estimated by RAST, RAST inhibition and PCA tests. Spores contained a concentration of allergens higher than mycelia. Results of PCA tests suggested that spores contained specific allergens. However, in a comparative study of extracts from different species of *Cladosporium* animal and human models gave different estimates of the allergenic potency of the different species. In spite of these variations it was shown that extracts from spores of *Cladosporium* contained the highest amount of *Cladosporium* allergens.

Key words: Cladosporium. Allergens. Fungal. Spore. Mycelium. Cross-reactions. Passive cutaneous anaphyllaxis test. Radioallergosorbent test.

RESUMEN

El potencial alergénico de los extractos de las esporas y del micelio de *Cladosporium* ha sido valorado por los métodos de RAST, RAST inhibición y PCA. Las esporas contienen una dosis de alérgenos más elevada que el micelio. Los resultados del ensayo PCA sugieren que las esporas contienen alérgenos específicos. Sin embargo, en un estudio comparativo de los extractos procedentes de diferentes especies de *Cladosporium*, el modelo animal y humano han dado diferentes estimaciones del poder alérgenico entre las distintas especies. Aunque haya variaciones, se ha demostrado que los extractos de las esporas de *Cladosporium* contienen una cantidad más elevada de los alérgenos.

Palabras clave: *Cladosporium*. Alérgenos. Espora. Micelio. Reacción cruzada. Prueba cutánea anafiláctica. Prueba de radioalergosorbencia.

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INTRODUCTION

Cladosporium has been known to be one of the most airborne fungi causing respiratory allergic diseases particularly asthma and rhinitis[1]. *Cladosporium cladosporioides* is the most prevalent species[2]. *Cladosporium herbarum* frequently dominates the outdoor mycoflora and it has been extensively studied[3-5]. The direct implication of this fungus in inhalant allergy is now recognized[6-9]. The lack of standardized extracts, the instability and the variability of their antigenes and allergens composition have been the most problem to progress in understanding fungal allergy. Extracts from different strains of *Alternaria alternata*, *Aspergillus fumigatus*, *Candida albicans* and *Epicoccum nigrum* vary greatly in their allergens composition[10-13]. The variability between different batches of *Cladosporium* extracts seems very high[14,6]. This vari-

ability may be due to genetical heterogeneity of fungal isolates, to extraction procedures, to the propagule used for allergen extraction, and to environment conditions (such as, climatic factors, growth media, degree of exposure). Most studies on allergenic moulds are based on culture filtrate extracts obtained from mycelium, rarely on somatic ones[15-17]). Mycelium is the easiest propagule to grow in vitro. However, it is not the fungal propagule inhaled by the patients whereas fungal allergy results from exposure to spores[18]. Airborne fungal spores can penetrate the lower airway and enter in contact with the mucosa. Moreover, previous studies have demonstrated that spores contain high allergenic potency when compared to the mycelium[19,20]. However, a comparison of *Alternaria alternata* spore and mycelium extract found that mycelium extract have greater potency than that of spore extract on the basis of skin test, RAST inhibition and basophile histamine release[21]. It is now acknowledged that the standardized conditions for optimal growth and sporulation and the preparation of metabolic and somatic extracts for both early and late growth phase fungal culture using the best source material containing the most relevant allergens are recommended[9,22].

In order to choose the best source material for the preparation of *Cladosporium* extracts, the allergenic potency of spore and mycelium extracts of different species of *Cladosporium* has been compared using RAST inhibition and PCA tests. RAST inhibition technique has been used in homologous and heterologous inhibitions between fungal extracts of many species of fungi imperfecti and Basidiomycetes[23-25]. In the case of *Cladosporium*, this method has been coupled to PCA tests which have already proven to be very effecient to study the reaginicity of several *Cladosporium* extracts[26,7].

MATERIAL AND METHODS

Preparation of the fungal extracts

The strain LCP 404 of *C. cladosporioides*, LCP 406 of *C. sphaerospermum* (museum national d'histoire naturelle, Paris, France) and IP1679-87 of *C. herbarum* (Unité de Mycologie, Institut Pasteur, Paris, France) were grown on 2 % malt agar medium at room temperature. The spores (> 95 % pure for *C. cladosporioides* and *C. sphaerospermum*, > 60 % for *C.herbarum*) were harvested with a paint brush after 3 to 4 weeks of growth. The mycelium was obtained in a 2 liters Biolafitte fermenter containing 2 % glucose, 1 % peptone (Prolabo) and 0.1 % Rhodorsil 426 R. After 48h of growth at 25 °C, 700 rpm and 0.5 vvm, the mycelium was recovered by filtration, washed with distilled water and stored at –20 °C.

Spores or mycelia were suspended in 10 mM phosphate buffer saline pH 7.2 (PBS) or 50mM Tris pH 9.0 containing 1mM EDTA and 1 % PVP (TEP) or 50 mM NaHCO3 pH 8.0 (Bic) and disrupted in a glass bead (1 mm) MSK Braun cell homogenizer. Bic total fungal extract was stored freeze dried. PBS and TEP extracts were centrifuged (30 min, 15 000 g) and the supernatants were stored at –80 °C. Protein content was measured using the BioRad method.

Sensitization of guinea pigs and mice and PCA test

Guinea pigs were sensitized using Bic total extracts of spore and mycelium of *C. cladosporioides* as previously described[26]. For mice sensitization spores and mycelium extracts from *C. cladosporioides* were used. Six groups of 5 Female Balb/c were sensitized using TEP soluble extracts as previously described[26]. PCA was performed as described by Ogilvie[27] and modified by Ovary[28]. PCA titer was the lowest dilution of serum giving a positive skin reaction.

RAST and RAST inhibition

Extracts of spores of *C. herbarum* and spores and mycelium of *C. cladosporioides* obtained by cell disruption in PBS were used for RAST and RAST inhibition experiments.

RAST

RAST was performed according to Ceska et al[29]. RAST disks were prepared by incubating overnight at 4 °C CNBr activated cellulose disks in 100 µl aliquots containing increasing concentrations of extracts (5 to 500 µg of proteins/ml depending on the extract). After 2 successives incubations (3 and 1 h) of the disks in 0.1 M Tris pH 8.0 and 3 washes in PBS buffer at room temperture, 50 µl of a pool of sera from positive patients were added. Sera were selected on the basis of positive prick tests and RAST to *Cladosporium* commercial extracts. After 3h incubation, washes and successive incubation with 125 I-rabbit anti-IgE were performed according to Phadebas (Pharmacia) technical informations. IgE uptake was expressed as the percentage of total cpm measured with 50 µl of the same 125 I-anti-IgE.