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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Surveillance of fungal allergic sensitization using the fluorescent halogen immunoassay

Surveillance de la sensibilisation aux allergènes fongiques par la technique d'immunofluorescence

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Summary
Objective. — Conidia derived from a small number of common fungal genera are widely accepted as the etiological agents responsible for fungal allergic sensitization. The contribution of fungal conidia, spores, airborne hyphae, and subcellular fragments from other uncharacterized fungal genera remains unclear. In this proof-of-concept study, we examined the composition of mycoaerosols that atopic women were exposed and sensitized to in their own indoor environment using the fluorescent halogen immunoassay (fHIA).
Patients and methods. — Mycoaerosols were collected onto mixed cellulose ester protein binding membranes (PBMs) for 30 min with volumetric air sampling pumps. The PBMs were laminated with an adhesive cover slip and indirectly immunostained with individual patient serum IgE using the fHIA. Samples were examined using confocal laser scanning microscopy and immunostained particles were expressed as a percentage of total particles.
Results. — All air samples contained a broad spectrum of fungal spores, conidia, hyphae, and other fungal particulates. Airborne concentrations varied between individual study participant environments. Positively immunostained conidia belonging to moniliaceous amerospores, *Cladosporium*, *Alternaria*, and many unknown species were observed in the majority of air samples. Other fungal genera including *Bipolaris*, *Curvularia*, *Pithomyces*, and *Stachybotrys*, in addition to, ascospore genera and dematiaceous hyphal fragments released detectable allergen. Twelve

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percent of all fHIA haloes quantified in the analysis were directed towards fungal particles. No immunostaining was detected to conidia belonging to *Epicoccum*, *Fusarium*, and *Spegazzinia* species.
Conclusion. — In addition to characterized fungal aeroallergens, we observed a wider composition of fungi that bound human IgE. Field surveillance studies that utilize immunodiagnostic techniques such as the fHIA will provide further insight into the diversity of fungi that function as aeroallergen sources in individual study participant environments.
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MOTS CLÉS
Allergènes ;
Champignons ;
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Cladosporium ;
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Immunoessai

Résumé
Objectif. — Les conidies provenant d'un petit nombre de genres fongiques communs sont généralement connues comme des agents étiologiques responsables de sensibilisation allergique. La contribution de conidies, de spores, d'hyphes aéroportées et de fragments subcellulaires provenant de genres fongiques non identifiés reste obscur. Dans cette étude, nous avons examiné la composition d'aérosols fongiques auxquels des femmes atopiques sont exposées et sensibilisées dans leur propre environnement domestique en utilisant une technique d'immunofluorescence indirecte (fHIA).
Patients et méthodes. — Les aérosols fongiques sont recueillis sur membrane de cellulose (PBMA) pendant 30 minutes avec une pompe volumétrique. Les membranes sont alors recouvertes d'une feuille adhésive et traitées par les IgE sériques de chaque patientes selon la technique fHIA. Les échantillons sont examinés au microscope à balayage laser confocal et les particules immunofluorescentes sont dénombrées en pourcent des particules totales.
Résultats. — Tous les échantillons d'air contiennent un large spectre de spores fongiques, de conidies, d'hyphes et autres particules fongiques. Les concentrations de matériel fongique aéroporté varient selon l'environnement individuel. Des conidies fluorescentes appartenant aux genres *Cladosporium* et *Alternaria* et à de nombreuses espèces inconnues sont observées dans la majorité des échantillons. D'autres genres fongiques comme *Bipolaris*, *Curvularia*, *Pithomyces* et *Stachybotrys*, des champignons ascosporés et des dématiées génèrent des allergènes détectables. Douze pour cent des particules fluorescentes quantifiées dans cette analyse sont d'origine fongique. Aucune conidie appartenant aux genres *Epicoccum*, *Fusarium* et *Spegazzinia* n'a été détectée.
Conclusion. — En plus des allergènes fongiques aéroportés détectés, nous avons observé une large gamme de champignons qui fixent les IgE. Les études de surveillance de terrain qui utilisent les techniques d'immunodiagnostic telles que la fHIA fourniront davantage d'informations sur la diversité des champignons générateurs d'allergènes aéroportés dans l'environnement domestique.
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Introduction

Exposure assessment scientists characterize personal fungal exposure as the inhalation of fungal conidia derived from environmentally abundant and morphologically discernible fungal genera, such as *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*. These fungal genera are the most widely recognized etiological agents associated with fungal allergic sensitization [11], and respiratory morbidity [5,29,49]. However, the contribution of fungal conidia, hyphae, and subcellular fragments from uncharacterized fungal genera remains unclear because of the methodological challenges associated with immunodiagnosis, as well as identifying fungi in environmental samples [19].

Compared to other perennial and seasonal allergens, fungal bioaerosols are viable heterotrophic microorganisms. Some fungal conidia possess unique virulence factors that facilitate colonization by reducing respiratory cilia beat frequency [4,6,10,50]. Depending on the site of deposition, some fungi may also germinate and release a complex assortment of mycotoxins, antigens, and other immunostimulatory macromolecules [15]. Germination is required for fungal colonization (chronic rhinosinusitis and invasive aspergillosis), and may result in further individual exposure to fungal allergens [15,32,48]. Because of these aspects, the characterization of fungal allergens has lagged behind other aeroallergen sources. Complex life cycles, rich biodiversity, and variability of fungal allergens have all hindered understanding fungal allergic sensitization. These confounding factors have also influenced the standardization of commercial extracts available for diagnosis and have limited the availability of diagnostic reagents for many uncharacterized fungi [51].

New developments in immunodiagnostic methodologies, such as the Halogen Immunoassay (HIA), have enhanced the ability to collect, and detect allergic sensitization in patients exposed to mycoaerosols [32,20,21,38,37,52]. The uniqueness of this method is that it provides a method to match the spectrum of an individual's allergic responses with the inherent diversity of bioaerosols collected from the patient's own environment. Compared to other commercially available immunodiagnostic tests, the HIA correlates with Phadia ImmunoCap technologies but less well to skin prick testing [21]. Specifically, eluted surface antigens bind in