Credentials
Immuno-Reactive Molecules Identified from the Secreted Proteome of Aspergillus fumigatus

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The secreted proteomes of a three week old culture of an Indian (190/96) and a German (DAYA) Aspergillus fumigatus isolate were investigated for reactivity with IgG and/or IgE antibodies derived from pooled allergic broncho-pulmonary aspergillosis (ABPA) patients’ sera. Two dimensional Western blotting followed by mass spectrometric analysis of the reactive protein spots revealed 35 proteins from the two A. fumigatus strains. There were seven known A. fumigatus allergens among them (Asp f1–4, Asp f9, Asp f10, and Asp f13/15), whereas three proteins displaying significant sequence similarity to known fungal allergens have been assigned as predicted allergens (Dipeptidyl-peptidase-V precursor, Nuclear transport factor 2, and Malate dehydrogenase, NAD-dependent). Eight IgG and IgE reactive proteins were common in both strains; however, 12 proteins specifically reacted in 190/96 and 15 in DAYA. Further testing with sera of 5 individual ABPA patients demonstrated that 12 out of 20 immunoreactive proteins of 190/96 strain of A. fumigatus had consistent reactivity with IgE. Seven of these proteins reacted with IgG also. The 25 of 35 identified proteins are novel with respect to immunoreactivity with ABPA patients’ sera and could form a panel of molecules to improve the currently existing less-sensitive diagnostic methods. Through expressing recombinantly, these proteins may also serve as a tool in desensibilization strategies.

Keywords: Aspergillus fumigatus • proteome • secretory antigen • allergens • diagnostic tool • allergic bronchopulmonary aspergillosis

Introduction

Fungal disorders have emerged as a major threat to public health in the past few decades, and besides Candida, the species of Aspergillus are the most common pathogens involved in such infections.1,2 Aspergillus fumigatus are present worldwide and are known to cause four distinct clinically recognizable forms of hypersensitivity respiratory disorders, that is, allergic bronchopulmonary aspergillosis (ABPA), allergic Aspergillus sinusitis, IgE-mediated asthma, and hypersensitivity pneumonitis.3 ABPA is primarily an immunologically mediated lung disease, which is very frequently associated with asthma. A. fumigatus releases large number of highly antigenic proteins and cell wall polysaccharides during the course of infection.4–6 They include enzymes, toxins, cell wall and growth related molecules, which facilitate A. fumigatus in colonization.7–9 These, often multifunctional molecules may be important for various cellular processes of A. fumigatus and ABPA is caused by an exaggerated hypersensitivity reaction to these antigenic molecules. However, further invasion of A. fumigatus in the deeper body parts leads to more fatal invasive aspergillosis.10

Strategies to detect and diagnose ABPA use recognition of specific immunity toward Aspergillus antigens, especially using crude or partially purified antigens of A. fumigatus. There is a lack of antigenic standardization between laboratories because of the use of local antigen preparations.11 Additionally, these antigen preparations have limited scope due to their low sensitivity and lack of specificity, which make them inept to detect the infection.12 Cross reactivity due to sharing of
Proteomic Characterization of *Aspergillus fumigatus* Treated with an Antifungal Coumarin for Identification of Novel Target Molecules of Key Pathways

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Supporting Information

ABSTRACT: A synthetic coumarin, N,N,N-triethyl-11-(4-methyl-2-oxo-2H-chromen-7-yl)-11-oxo-undecan-1-aminium bromide (SCD-1), having potent activity against pathogenic *Aspergillus* (MIC₉₀ 15.62 μg/mL), was investigated to identify its molecular targets in the pathogen. The proteome of *Aspergillus fumigatus* was developed after treatment with sublethal doses of compound and analyzed. The results demonstrated 143 differentially expressed proteins on treatment with SCD-1. The expression of four proteins, namely cell division control protein, ubiquitin-like activating enzyme, vacuolar ATP synthase catalytic subunit A, and UTP-glucose-1-phosphate uridylyltransferase of *A. fumigatus*, was completely inhibited, whereas there were 13 newly expressed and 96 overexpressed proteins, mainly belonging to stress pathway. The treatment of *A. fumigatus* with SCD-1 also led to attenuation of proteins involved in cell replication and other important biosynthetic processes, including riboflavin biosynthesis, which has been pathogen-specific. In addition to key enzymatic players and antioxidants, nine hypothetical proteins were also identified, seven of which have been novel, being described for the first time. As no cellular functions have yet been described for these hypothetical proteins, their alteration in response to SCD-1 provides significant information about their putative roles in pathogen defense.

KEYWORDS: *Aspergillus fumigatus*, therapy, coumarin, antifungal, proteome, molecular targets

INTRODUCTION

*A. fumigatus* has been a highly evolved saprophytic mold bestowed with numerous adaptations which enable it to survive in a multitude of extreme environmental conditions. The morbidity and mortality caused by *A. fumigatus* infection has been significant, as successful management of the disease often becomes complicated as a result of the delay in establishing diagnosis and lack of effective drugs. Current therapeutic options for aspergillosis have been limited to only a few classes of antifungal agents, such as polyenes (amphotericin B and its liposomal formulations), azoles (fluconazole, voriconazole, itraconazole), and echinocandins (caspofungin and anidulafungin). Most of these drugs have been known to target the fungal cell wall and cell membrane. The polyenes bind to the ergosterol and form transmembrane channels leading to efflux of monovalent cations to the exterior, thus disrupting the membrane function. Azoles inhibit the ergosterol biosynthesis by targeting demethylation of lanosterol, leading to accumulation of 14α-methylsterols. This leads to impairment of other enzyme systems, thus inhibiting the cell growth. The echinocandins have been reported to target the fungal cell wall by inhibiting β, 1–3 glucan synthesis and, hence, depleting glucans, which are necessary to maintain its stability. The drugs presently used for treating aspergillosis have been found to be highly toxic and immunosuppressive. The development of resistance in the pathogen against most antifungals has been another major reason for the limited therapeutic success of these drugs.

Owing to the limitations associated with current antifungals, the identification of new molecules to develop improved therapeutic formulations with better efficacy and less toxicity has been emphasized. This can be accomplished by identifying molecules which use pathways different than those used by current drugs to exhibit pathogen-specific activity. Therefore, development of ideal antifungals having novel and specific
Novel Cytoplasmic Allergens of Aspergillus fumigatus Identified from Germinating Conidia

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Aspergillus fumigatus is the common cause of allergic broncho-pulmonary aspergillosis (ABPA) and most of the allergens have been described from its secreted fraction. In the present investigation, germinating conidial cytoplasmic proteins of A. fumigatus were extracted from a 16 h culture. The proteome from this fraction was developed, and immuno-blots were generated using pooled ABPA patients' sera. Well separated Immunoglobulin-E (IgE) and Immunoglobulin-G (IgG) reactive spots were picked from corresponding 2DE gels and subjected to mass spectrometric analysis. As a result, 66 immuno-reactive proteins were identified from two genetically different strains (190/96 and DASYA) of A. fumigatus. Only 3 out of 66 proteins reacted with IgG, and the remaining 63 proteins were found to be IgE reactive. These 63 IgE-reactive cytoplasmic proteins from germinating conidia included 2 already known (Asp f1 and Asp f22) and 4 predicted allergens (Hsp88, Hsp70, malate dehydrogenase, and alcohol dehydrogenase) based on their homology with other known fungal allergens. In view of this, the panel of presently identified IgE-reactive novel proteins holds the potential of providing a basis for the wider diagnostic application in assay for allergic aspergillosis. We could demonstrate that recombinantly expressed proteins from this panel showed consistent reactivity with IgE of individual sera of ABPA patients. The recombinantly expressed proteins may also be useful in desensitization therapy of allergic disorders including ABPA.

Keywords: A. fumigatus • germinating conidia • proteome • allergens • aspergillosis • diagnostics

Introduction

A. fumigatus is an ubiquitous filamentous fungal saprophyte, a primary and opportunistic pathogen, and also a major source of allergens. It is the prototypical airborne pathogen, affecting a wide range of susceptible patient groups.1 Its conidial

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**Credentials**

**Publications**


**Book Chapter**


**Update of International allergen database**

Allergen database allergome (http://www.allergome.org/script/search_step2.php) updated the list of *A. fumigatus* allergens by addition of 20 new immunogenic proteins identified from our study.
**Poster Presentations**


**Bharat Singh, Manish Kumar, Akshat Khanna, V. Yadav, and G. L. Sharma. Identification of a gene encoding for an antifungal protein in from *Escherichia coli*.** 2nd International conference on Trends in Cellular and Molecular Biology held at Jawaharlal Nehru University, Delhi, India from 8th -10th December, 2007.