An immunoproteomics approach to identify fungal protein antigens in cystic fibrosis patients with Aspergillus fumigatus colonisation

Juliane Macheleidt, Janis Kruse, Petra Bacher, Claudia Grehn, Alexander Scheffold, Carsten Schwarz, Jan Springer, Jürgen Löffler, Hermann Einsele, Olaf Kniemeyer, Axel A. Brakhage

Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Molecular and Applied Microbiology, Jena; Institute of Immunology, Christian-Albrechts Universität zu Kiel and Universitätshospital Kiel, Kiel; Institute of Clinical Molecular Biology, Christian-Albrechts Universität zu Kiel, Kiel; Department of Pediatrics, Division of Pneumonology and Immunology with intensive Medicine I, Charité, Berlin; Department of Internal Medicine II, University Hospital of Würzburg; Institute for Microbiology, Friedrich Schiller University Jena

Introduction

The opportunistic human pathogenic fungus *Aspergillus fumigatus* is able to cause a variety of diseases ranging from invasive to locally-restricted forms of infection and allergic disorders. In general, diagnosis of *A. fumigatus* related diseases is difficult and therapy options are limited raising the need for new diagnostic and therapeutic approaches. *A. fumigatus* frequently colonizes the airways of patients with cystic fibrosis (CF), which is often accompanied by hypersensitivity responses to this fungus. One of the most sensitive manifestations of fungal allergy in CF patients is allergic bronchopulmonary aspergillosis (ABPA). Its diagnosis in CF patients is often challenging. Elevated IgE and IgG to *A. fumigatus* is one criterion, but the serum IgG/IgE antibody response to crude *A. fumigatus* antigens is in many cases inconsistent. The usage of a defined set of *A. fumigatus* protein antigens may improve the diagnostic accuracy in CF. To gain a deeper insights into the serological response to *A. fumigatus* and to find possible biomarkers, serological proteome analyses (SERPA) for the identification of protein antigens recognized by IgG antibodies from cystic fibrosis or hematological malignancies patients with *A. fumigatus* infections were applied.

**SERPA method**

- A fumigatus secreted proteins
- Immunoblot
- Detection of *A. fumigatus* specific IgG antibodies
- Patient sera
- Image analysis
- 2D gel electrophoresis
- Serological Proteome Analysis
- MS and MS/MS analyses
- Tryptic digest
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**Patient serum samples**

- Invasive Aspergillosis (IA)
  - 8 IA patients
  - 3 time points per patient (before, during, after IA diagnosis)
- Cystic Fibrosis (CF)
  - 16 CF patients with Aspergillus detection
  - 5 CF control patients
  - 4 healthy controls

**The A. fumigatus secretome**

A Secreted proteins of *A. fumigatus* grown on minimal medium with olive oil as additional carbon source were separated on a 2D gel, resulting in 228 individual protein spots (Lightning So3 signal). B Exemplary immunoblots of serum samples from IA and CF patients are shown. Anti-*A. fumigatus* antibodies were detected by an anti-human IgG Alexa 647 conjugate. C Secreted proteins of *A. fumigatus* induce higher frequencies of Th2-responses in CF patients.

**Identified immunogenic proteins**

44 *A. fumigatus* protein antigens were identified by the SERPA approach. The proteins in italics have not been described as antigens, yet.

**Amino acid metabolism**
- Aminotransferase, class V
- 3-Isopropylmalylate dehydrogenase Leu2A

**Cell rescue, defense, and virulence**
- Cu,Zn superoxide dismutase SOD1
- y-Glutamyltransferase

**Central metabolism**
- Mannitol-1-phosphate dehydrogenase
- Oxioreductase, FAD-binding
- Triosephosphate isomerase
- FAD/FMN-containing isocitryl alcohol oxidase MreA
- PDA-like cytochrome P450 monooxygenase
- α-Trehalose glucohydrolase TreA/Ath1
- Transaldolase
- α-L-rhamnosidase C
- Aldose 1-epimerase
- Esterase

**Fungal cell wall carbohydrate metabolism**
- Endo-1,3-β-glucanase EngF1
- Mannosidase MtGid5
- Extracellular cell wall glucanase Cft1 (Asp F9)
- GPI-anchored cell wall β-1,3-endoglucanase EgIC
- Extracellular arabinanase

**Lipid metabolism**
- Extracellular 3-isopropylmalate lipase

**Miscellaneous**
- FG-GAP repeat protein
- Conserved uncharacterised protein
- Cell wall protein
- Cell wall protein PMA
- Conserved uncharacterised protein
- Conserved uncharacterised protein
- Uncharacterised protein
- Cytochrome P450 oxidoreductase PsO2

**Plant cell wall carbohydrate metabolism**
- Pectate lyase A
- Pectate lyase
- Extracellular arabinanase

**Protein biosynthesis**
- Phenylalanine tRNA synthetase α subunit (PodG)
- Histidyl-tRNA synthetase, mitochondrial precursor

**Proteolysis**
- Serine protease
- Aminopeptidase V
- Alkaline serine protease Ap1
- Autophagic serine protease Ap2

**Summary**

- The SERPA method applied to sera from IA and CF patients detected 44 different *A. fumigatus* protein antigens.
- 31 proteins had a N-terminal secretion signal, 22 have not been described as antigens yet.
- Most detected proteins antigens are involved in carbohydrate metabolism, cell wall organisation and biogenesis, cell defense, proteolysis and lipid metabolism.
- The proteins FG-GAP and Ap1 were detected by all patient sera.
- Two so far uncharacterised proteins were recognised by most of the CF patient sera, but by no other tested groups (15/16 and 11/16) and may have diagnostic potential.

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