Characterization of *Scedosporium boydii* antigens recognized by serum IgGs from cystic fibrosis patients

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INTRODUCTION

Species of the genus *Scedosporium* are emergent pathogens ranking the second, only behind *Aspergillus* spp., among filamentous fungi causing a chronic colonization of the airways of cystic fibrosis (CF) patients. This may lead to chronic inflammation, true respiratory infections like bronchitis, allergic broncho-pulmonary mycoses and fungus ball formations, or even to life-threatening invasive disease following lung transplantation and in cases of severe immunosuppression.

The lack of standardization and the low sensitivity and specificity of the detection methods commonly used result in a delayed diagnosis. Moreover, these poor detection methods also result in an inadequate monitoring and treatment of the patients. In this sense, species identification is essential for the establishment of an appropriate treatment due to species-specific differences in antifungal susceptibility.

OBJECTIVE

To contribute to the finding of new diagnostic targets by identifying the **most immunoreactive antigens** of *Scedosporium boydii* recognized specifically by serum IgGs from **CF patients** colonized by *Scedosporium* spp.

RESULTS

1. Classification of CF patients' sera

2. Serological response against *S. boydii* and *A. fumigatus*

Sera from CF patients colonized by *Scedosporium* spp. showed a greater specific immune response. To compare the serological response against *S. boydii* (A) and *A. fumigatus* (B), an ELISA assay was performed with serial dilutions of the pooled sera against 50 µg/ml of total protein extract of each fungi (growing during 24h in potato dextrose broth at 37°C and disrupted with glass beads in a BeadBeater homogenizer), to calculate the titer of IgG antibodies against both fungal species. Regarding CF patients sera colonized by *Scedosporium*, IgG antibody titer against both fungi was higher (1/2000) than in those with *Aspergillus* (1/500).

3. Characterization of specific *S. boydii* antigens recognized by sera from CF patients with *Scedosporium*

A. Coomassie blue + Schiff staining

B. WB Scedo+

C. Coomassie blue + Schiff staining

D. WB Scedo+

E. WB Scedo+

F. WB Asp+

G. WB Ctrl

SODIUM METAPERIODATE TREATED

IgG serum antibodies of CF patients with *Scedosporium* recognized mainly glycoproteins, between 3-5 pI and 45-250 kDa. *S. boydii* proteome in a 10% acrylamide gel stained with Coomassie blue and Schiff reagent to visualize proteins (blue) and glycoproteins (purple) respectively (A). Western blot of CF patients' sera colonized by *Scedosporium* against *S. boydii* 24 h total protein extract (B). Decreasing acrylamide % to 6 (C, red square) allowed to better visualize high Mr antigens (D). The main reactivity was lost after membrane oxidation with 50 mM sodium metaperiodate. This treatment allowed to visualize strictly protein antigens recognized by the pooled sera from the three groups of CF patients. Sera from CF patients with *Scedosporium* recognized 3 protein antigens with 60-62 kDa and 4.2-4.4 pI (red arrows), which were not detected by sera from the other CF groups (E-G).

CONCLUSION

CF patients colonized by *Scedosporium* recognize specifically **3 antigens** from *S. boydii* with 60-62 kDa and 4.2-4.4 pI, which will be studied as **diagnostic targets** to develop a sensitive and specific serological test.

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