Virulence properties of *Exophiala dermatitidis*: cytotoxic activity, interleukin production, biofilm formation and invasion

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*Exophiala dermatitidis* has been associated with cystic fibrosis (CF), but its role on human pathogenicity is still not known. Recently we described the cytotoxic activity of cultured supernatants (CS) of *A. xylosoxidans* to human lung cells (H-460). Among the proteinase studied, lecithinase was the only one detected. Now, the culture supernatants of *A. xylosoxidans* also showed cytotoxic activity on NCI-H292 (human lung mucocoeidemieron carcinoma) and this cytotoxic activity was not affected after heat treatment (100°C for 20 min), showing it to be thermo stable. The protein fractions obtained by ultrafiltration (Amicona), showed biological activity of the extracts with molecular weigh lower than 5kDa. This fraction has also stimulated the IL 8 production by these lung cells of 5 folds more than that detected on the negative controls, determined by ELISA (DuoSet, RD Systems, EUA). These data suggests that the thermo stable cytotoxic activity (cytotoxin), is probably the stimulating agent of the proinflamatory cytokine (IL) that may be related to the pathogenicity for lung cells. *A. xylosoxidans* showed biofilm formation ability on plastic microplates among all the isolates, as verified by flat-bottom microtiter polystyrene plates test. Invasion and intracellular survival in NCI-H292 cells was also observed. The detection of a cytotoxic factor inducing IL 8 production, the bacteria biofilm formation and invasion reinforces the proposal that *A. xylosoxidans* may present a relevant role in the lung parenchyma destruction in cystic fibrosis patients.

**Results:**

- **15** of the **49** sputum samples were PCR positive for *A. fumigatus* species.
- All **PCR** positive samples had a positive specific IgG titre (>40mg/L) but **41%** of PCR negative samples also had a positive IgG titre. **PCR** positive patients had a significantly greater mean specific IgG *Aspergillus* (PCR positive 93mg/L SE 29ºC). The use of a selective medium such as erythritol-chloramphenicol agar (ECA) has been highly recommended for the isolation of *Exophiala dermatitidis*. This study aimed to accurately identify patients with *A. fumigatus* colonisation using Real time PCR and examine the relationship to markers of sensitisation.

**Methods:**

- 49 adult CF patients provided a sputum sample, a blood sample for *Aspergillus* serology and had fungal skin pritck tests. Serological tests included total IgE, specific IgE *A. fumigatus* and specific IgG *A. fumigatus* performed by Phadia ImmunoCAP® assay, and *A. fumigatus* precipitins by counterimmunoelectrophoresis. A commercial Real time PCR kit, MycAssay™ Aspergillus, was used to detect *Aspergillus* in CF sputum samples.

**Results:**

- Over a 2-years period, 9 patients (5.8%) in our CF clinic had at least 1 ED + culture (mean number of cultures/patient/2y: 13, range:10−20; mean % past 12 months with a respiratory culture (criterion 1) [1]; (2) presence of ED in the bronchial tree for at least 6 months, based on at least three positive cultures with at least one month intervals between them (criterion 2), with specific antibody response (criterion 3) [2]. Precipitating antibodies against ED were investigated by agar gel double immunodiffusion. The presence of at least 2 lines of precipitins was considered significant.

**Conclusion:**

Aim: To assess the ability of ED to chronically infect the airways of CF patients.

- By analogy to current definitions of chronic infection by *P. aeruginosa*, chronic infection by ED was defined in 2 ways: (1) ED + culture in ≥50% of the past 12 months with a respiratory culture (criterion 1) [1]; (2) presence of ED in the bronchial tree for at least 6 months, based on at least three positive cultures with at least one month intervals between them (criterion 2), with specific antibody response (criterion 3) [2]. Precipitating antibodies against ED were investigated by agar gel double immunodiffusion. The presence of at least 2 lines of precipitins was considered significant.

- **Results:**

  - Over a 2-years period, 9 patients (5.8%) in our CF clinic had at least 1 ED + culture (mean number of cultures/patient/2y: 13, range:10−20; mean % of ED + cultures: 46%). Criteria 1, 2 and (2+3) were met by 5, 6 and 5 patients respectively. According to criterion 1 or (2+3), 6 patients could be considered as chronically infected by ED. Two lines of precipitins against ED were observed in only 1 out of 9 carefully matched ED− CF patients.

- **Conclusion:** These data suggest that ED can chronically infect the airways of CF patients.

Reference(s)