133 Virulence properties of *Achromobacter xylosoxidans*: cytotoxic activity, interleukin production, biofilm formation and invasion

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Achromobacter xylosoxidans 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 mucoepidermoid carcinoma) and this cytotoxic acitivity was not afected after heat treatment (100ºC for 20 min), showing it to be thermo stable. The protein fractions obtained by ultrafiltration (Amiconâ), showed biological activity of the extracts with molecular weigh lower than 5 kDa. 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.

134* Role of real time PCR and specific IgG in identifying patients with *Aspergillus* colonisation

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Purpose: *Aspergillus fumigatus* colonisation is common in cystic fibrosis (CF) but prevalence varies between studies from 12–57%. Colonised patients are not routinely treated with antifungals. 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 prick 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: 15 of the 49 sputum samples were PCR positive for *Aspergillus* species. All PCR positive samples had a positive specific IgG titre (>40 mg/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 93 mg/L SE 6.74 versus PCR negative 47 mg/L SE 4.44 $p \leqslant 0.001$). Using a ROC curve, an IgG level above 67 mg/L gives 85% sensitivity and 90% specificity for positive PCR. No other marker of *A. fumigatus* sensitisation correlated to positive PCR.
Conclusion: Real time PCR can be used to identify patients colonised or infected with *Aspergillus*, including those in whom antifungal therapy is inadequate. A specific IgG titre above 67 mg/L correlates with finding *Aspergillus* in sputum by PCR. Additional data are required to determine which marker(s) is most useful for treatment decision-making.

135 Isolation of *Exophiala dermatitidis* in cystic fibrosis: is ECA the optimal medium?

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Background: The use of a selective medium such as erythritol-chloramphenicol agar (ECA) has been highly recommended for the isolation of *Exophiala dermatitidis* (ED) from respiratory secretions of CF patients. However, using the Sabouraud Gentamicin-Chloramphenicol Agar (SGCA) medium (Becton-Dickinson), we recently observed ED isolation rate in CF patients similar to that mentioned in the mycological literature.
Aim: To compare the ability of ECA and SGCA to detect ED in respiratory cultures from CF patients.
Methods: Over a 8-weeks period, isolation rates of ED from all consecutive sputum samples from non lung-transplanted CF patients in our clinic were assessed using both ECA (incubated 4 weeks at 35ºC) and SGCA medium (incubated 4 weeks at 29ºC).
Results: 235 samples from 132 patients (48% >18 y) were cultured (sputum – when possible – or deep pharyngeal aspirate after a session of physiotherapy). PA and BCC (multivorans or vietnamiensis) were cultured from 22.5% and 2.1% of samples respectively. ED was isolated from the same 11 specimens (4.7%), in 8 patients (6.1%) irrespective of using the ECA or the SGCA medium. With either medium too, ED was detected in all but one case between day 4 and day 8.
Conclusion: In this comparative study, ECA did not yield higher isolation rates of ED than the commercially available and less costly SGCA medium.

136 *Exophiala dermatitidis*: can it chronically colonize and infect airways of patients with CF?

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Background: Exophiala dermatitidis (ED) is considered as an emerging colonizing fungal agent in patients with CF.
Aim: To assess the ability of ED to chronically infect the airways of CF patients.
Methods: By analogy to current definitions of chronic infection by Ps. 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/2 y: 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)

[1] Lee et al. J Cyst Fibros 2003; 2: 23.
[2] Döring et al. Eur Respir J 2000; 16: 749.