ELSEVIER

Journal of Cystic Fibrosis 9 (2010) 51 – 58

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Inflammation in *Achromobacter xylosoxidans* infected cystic fibrosis patients☆

C.R. Hansen [a,*], T. Pressler [a], K.G. Nielsen [a], P.Ø. Jensen [b], T. Bjarnsholt [b,c], N. Høiby [b,c]

[a] *Copenhagen CF centre, Pediatric Pulmonary Service, Department of Pediatrics, Rigshospitalet, Denmark*
[b] *Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark*
[c] *Institute of International Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen, Denmark*

Received 27 August 2009; received in revised form 26 October 2009; accepted 27 October 2009
Available online 25 November 2009

Abstract

Background: Achromobacter xylosoxidans infection may cause conspicuous chronic pulmonary inflammation in cystic fibrosis (CF) patients similar to *Pseudomonas aeruginosa* and the *Burkholderia* cepacia complex (*Bcc*). Evolution in lung function was compared in chronically infected patients. Cytokine concentrations in CF patients with and without chronic infection were compared to healthy controls.
Methods: Cytokines in serum and sputum were measured using multiplex bead based immunoassay.
Results: Sixty CF patients, 11 with *A. xylosoxidans*, 11 with *Bcc*, 21 with *P. aeruginosa* and 17 non-infected CF patients were compared to 11 healthy controls. *A. xylosoxidans* patients were younger, but had a FEV_1 decline similar to *P. aeruginosa* patients. *Bcc* patients had the steepest decline in FEV_1.

Serum levels of G-CSF, IL-6 and TNF-α were significantly higher in CF patients compared to healthy controls. Chronically infected CF patients had significantly higher serum levels of IFN-γ and IL-6 compared to non-infected CF patients. *Bcc* patients had significantly lower serum G-CSF and *A. xylosoxidans* patients had significantly higher sputum TNF-α compared to the other groups of chronically infected patients.
Conclusion: A. xylosoxidans can cause a level of inflammation similar to *P. aeruginosa* in chronically infected CF patients. *A. xylosoxidans* is a clinically important pathogen in CF and should be treated accordingly.

Keywords: Cystic fibrosis chronic lung infection; *Achromobacter xylosoxidans*; Inflammatory cytokines; *Pseudomonas aeruginosa*

1. Background

Recurrent and ultimately chronic pulmonary infection is a predominant cause of increased morbidity and mortality in cystic fibrosis (CF) patients [1–3]. Up to 80% of adult CF patients suffer from chronic, pulmonary infection with *Pseudomonas aeruginosa*, but an increasing number of other Gram-negative microorganisms are capable of causing chronic infections in CF patients [4,5].

In CF, the inflammatory response to pulmonary infections is exaggerated [6,7]. Increased levels of inflammatory cytokines have been found in sputum [8], BAL-fluid [9–11] and Exhaled Breath Condensate (EBC) [12] not only in chronically infected patients but even in CF infants without established infections [10,13,14]. Levels of cytokines in BAL-fluid are increased in individuals with airway infection compared to individuals without infection [7,9,15].

Inflammation caused by *P. aeruginosa* has been described through the studies of mouse models of chronic infection; by measurements of cytokines in BAL-fluid, sputum and serum [8,16]; by stimulation of peripheral blood mononuclear cells (PBMC) from patients with chronic *P. aeruginosa* infection [17] and measurements of specific antibody levels [18]. The increased morbidity and mortality in CF patients with this infection is well

☆ Preliminary data have been presented at: the 6th annual Cytokines and Inflammation Congress in Orlando, Florida, January 28–29, 2008; at the 8th International Congress on Pediatric Pulmonology in Nice, France, March 29–31, 2008; at the American Thoracic Society Annual Conference in Toronto, Canada, May 16–21, 2008 and at the 22nd North American CF Conference in Orlando, Florida, October 23–25, 2008.

* Corresponding author. Copenhagen CF centre, 5003, Department of Pediatrics, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. Tel.: +45 3545 4760; fax: +45 3545 6717.
E-mail address: christunte@hotmail.com (C.R. Hansen).

1569-1993/$ - see front matter © 2009 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2009.10.005

known [19]. A rapid increase in specific antibodies is associated to a poor prognosis in these patients [20].

The *Burkholderia cepacia* complex (*Bcc*) affects 3–4% of CF patients [4,21–26]. The prevalence varies greatly in individual centres since some *Bcc* genomovars have shown a high ability to cause cross infections within centres [27–29]. *Bcc* infection is feared due to the ability to cross infect [29,30] and to cause the cepacia syndrome — characterized by a sudden decline in clinical status and a high mortality [26,30–32]. *B. cepacia* lipopolysaccharide (LPS) can strongly stimulate the inflammatory response [33], but a study revealed no difference in levels of inflammatory markers between CF patients with *B. cepacia* and patients with chronic *P. aeruginosa* infection [34].

Another new cause of chronic pulmonary infection is *Achromobacter xylosoxidans* found in around 7% of the CF population [35]. The clinical significance of this infection is still not clearly established. A few studies have shown a negative impact on CF pulmonary disease, especially in patients with a rapid increase in specific antibodies [36], whereas other studies failed to demonstrate any correlation [37–39]. A recent study has shown an overrepresentation of *A. xylosoxidans* infected patients in the group of CF patients in need of lung transplantation compared to prevalence of *A. xylosoxidans* in the general CF population (presented as abstract 359 by Brown et al. at the 22nd NACFC in 2008, Orlando, Florida).

The aim of the present study was to evaluate the inflammatory response to *A. xylosoxidans* by comparing concentrations of inflammatory markers in serum, EBC and sputum from patients chronically infected with *P. aeruginosa*, *Bcc* and *A. xylosoxidans*, respectively, CF patients without chronic, pulmonary infections caused by Gram-negative microorganisms and healthy controls.

2. Methods

2.1. Study design

Cross sectional and retrospective study of patients followed at the Copenhagen CF centre. Patients were invited to participate at outpatient visits and samples for cytokine measurements were collected during a 2 year period.

2.2. Treatment strategy at Copenhagen CF centre

Patients are seen on a regular, monthly basis. At all visits, the clinical status of the patients is assessed by weight, height and lung function parameters, forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) (Masterscreen Pneumo, Jaeger) are obtained according to the ATS guidelines [40], using reference values from Polgar [41]. Lower respiratory tract secretions for microbiological investigation are obtained by coughing or by endo-laryngeal suctioning. Specific, precipitating antibodies are obtained at least once every year, and chronic infection is defined as 6 consecutive positive sputum samples and/or elevated levels of specific, precipitating antibodies [20]. The specificity of the antibody response is determined by absorption experiments [20]. CF patients with chronic, Gram-negative, pulmonary infections are treated with 3-monthly elective courses of 2 weeks of i.v. antibiotics and with continuous inhaled antibiotics.

2.3. Patients

All patients treated at CF centre Copenhagen were eligible for the study. Patients were characterized as non-infected or chronically infected with *A. xylosoxidans*, *Bcc* or *P. aeruginosa*. Preferably, subjects without chronic infections were chosen among patients ≥ 15 years securing their own agreement to give informed consent and better age-match to chronically infected patients. Patients chronically infected with more than one Gram-negative microorganism were not included in analyses to avoid contributing inflammatory stimulation from more than one infectious agent. All patients had a confirmed diagnosis of CF based on abnormal sweat electrolytes, characteristic clinical features and genotype.

Healthy controls were recruited from the staff at the centre.

2.4. Lung function parameters and specific antibodies

Highest value of FEV_1 and highest level of specific antibodies were collected retrospectively for each year of observation, starting the year prior to development of chronic infection, up to 12 years after development of chronic infection. Slope of decline of FEV_1 was calculated as change in % of predicted/year, including all years of chronic infection.

Highest value of FEV_1 in the year prior to development of chronic infection in the *A. xylosoxidans* group was compared to a group of controls without chronic, pulmonary infections. Two CF controls, matched on age (±1 year) and gender, were found for each *A. xylosoxidans* infected patient.

2.5. Biofilms

Gram-stained smears of sputum were searched for biofilms of *Bcc* and *A. xylosoxidans* by microscopy as previously reported in relation to chronic infection with mucoid *P. aeruginosa* [42].

2.6. Samples for cytokine measurements

Samples were taken at least 2 months after a course of i.v. antibiotics to avoid any antibiotic suppression of inflammation [43–45]. The following samples were collected from each participant: serum sample, EBC and, if possible, sputum sample. Age, duration of chronic infection, FEV_1 and level of specific antibodies, were noted at the time of the sample.

Serum: blood samples were allowed to clot. Serum was collected after centrifugation for 10 min at 3000 rpm.

Exhaled Breath Condensate: samples were collected using Ecoscreen® (Jäeger, Hoechberg, Germany). Patients were allowed to breathe normally for 15 min, wearing a nose-clip. After collection, EBC was frozen immediately.

Sputum: Fresh, spontaneously expectorated sputum samples were frozen and stored at −80 °C until preparation. Samples were ultra-centrifuged for 4 h at 38,000 rpm. Non-infected CF

patients were not able to produce sputum, why only sputum samples from the chronically infected groups were analyzed.

All samples were stored at −80 °C prior to analysis.

2.7. Cytokines

Cytokines in serum, EBC and sputum were measured by a multiplexed flow cytometric assay using a human cytokine LINCOplex kit (MPXHCYTO-60 K, Linco Research, St. Charles, MO, USA) and a human high sensitivity LINCOplex kit (HSCYTO-60SK, Linco Research, St. Charles, MO, USA) on a Luminex® 100™ system (Ramcon, Birkerød, Denmark). In serum and sputum, measurements of G-CSF, IFN-γ, TNF-α, IL-6, IL-8 and IL-10 were done. In EBC measurements of IFN-γ, TNF-α, IL-1β, IL-6 and IL-10 were done. Analysis was done according to the instructions of the manufacturer. Samples were measured in duplicates. The range of the standard curves for all cytokines measured was 3.2–10.000 pg/ml. To minimize variation, the same person ran each entire set of samples on the same day. Based on the standard curves, the coefficient of variation (CV) was calculated and did not exceed 20%.

2.8. Microbiology

Bacterial resistance against commonly used antibiotics was tested in isolates from included subjects. A strain was defined as multiresistant if simultaneous resistances to at least 3 different groups of antibiotics were found (aminoglycosides, 3rd generation cephalosporins, colistin, aztreonam, meropenem, ciprofloxacin, tetracycline, chlorampenicol, sulfamethoxazole with trimethoprim and penicillin with β-lactamase inhibitor).

2.9. Statistics

Data and measurements with a normal distribution were compared using one-way ANOVA. More than two groups of data that were not normally distributed were compared using Kruskal–Wallis test. Mann–Whitney Rank Sum test was used for comparison of two groups of data without normal distribution. Correlation of duration of chronic infection to level of FEV_1 and specific antibodies, respectively, was done in the individual groups of chronically infected patients using Spearman's correlation. Level of significance was defined as 0.05 (two-tailed).

3. Results

Sixty-seven CF patients and 11 healthy controls were included. Fifty CF patients had chronic pulmonary infections: 14 patients with *A. xylosoxidans*, 21 with *P. aeruginosa*, 13 with *B. multivorans* and 2 with *B. cepacia*. The last 15 patients were grouped together as the *Bcc* group. Of the chronically infected CF patients, 3 patients in the *A. xylosoxidans* group and 4 patients in the *Bcc* group were co-infected with other Gram-negative microorganisms. Six patients were co-infected with *P. aeruginosa* (2 in the *A. xylosoxidans* group, 4 in the *Bcc* group) and one patient initially having chronic *A. xylosoxidans* infection developed chronic *B. multivorans* infection and continued to have sputum cultures positive for both microorganisms. All 7 patients with co-infections were excluded from further analyses, leaving 11 patients in the *A. xylosoxidans* group and 11 patients in the *Bcc* group. Seventeen CF patients did not have chronic, Gram-negative, pulmonary infection. Demographic data are shown in Table 1. CF patients without chronic infections were younger compared to the *P. aeruginosa* group, the *Bcc* group and the healthy controls, but not when compared to the *A. xylosoxidans* group. Non-infected CF patients had the highest FEV_1 when comparing all groups of CF patients (Table 1). Within the group of chronically infected patients, the *A. xylosoxidans* group was younger ($p<0.003$). The *P. aeruginosa* group had the longest duration of chronic infection ($p<0.002$) (Table 1).

3.1. Biofilms

Seven of eight and 6/6 of the CF patients with chronic *A. xylosoxidans* and *Bcc*, respectively, had detectable biofilm-like clusters of Gram-negative rods surrounded by a matrix in sputum (Fig. 1). The remaining patients in these two groups either harboured other Gram-negative rods or only produced pharyngeal

Table 1
Demographic data expressed as numbers (number of patients in groups, genotype and occurrence of CF related diabetes) or median (range) (age, specific antibodies, FEV_1, chronic infection duration).

	CF patients according to infectious status				Healthy controls	*p*-value
	No chronic infection	*A. xylosoxidans*	*P. aeruginosa*	Bcc		
Number (males)	17 (6)	11 (6)	21 (13)	11 (5)	11 (2)	n.s.
Age (years)	17.8 (8.6–25.7) *	20.4 (16.1–26.9) *	29.5 (13.7–43.4)	28.5 (19.2–41.6)	25.7 (21.1–39.1)	$p<0.001$
Number of specific, precipitating antibodies	–	19 (1–43)	23 (3–48)	15 (5–30)	–	n.s.
FEV_1% predicted	83.2 (51.8–97.4) *	83 (30.9–104.2)	59.5 (26.5–109.6)	66.5 (30.9–99.3)	–	$p<0.02$
Chronic infection duration (years)	–	4.6 (2.8–10.4)	16.9 (0.1–30.7) *	8.7 (2–12.8)	–	$p<0.002$
ΔF508 homozygous	10 (7 F508/other)	7 (4 F508/other)	15 (6 F508/other)	9 (2 F508/other)	–	n.s.
CF related diabetes	4/17	2/11	5/21	2/11	0/11	n.s.

All data were assessed at the time of sample collection. All groups were compared using Kruskal–Wallis test (*p*-values given in table). When Kruskal–Wallis test showed a significant result, the groups were compared to each other, two at a time, using Mann–Whitney test.
* $p<0.01$ using Mann–Whitney test.

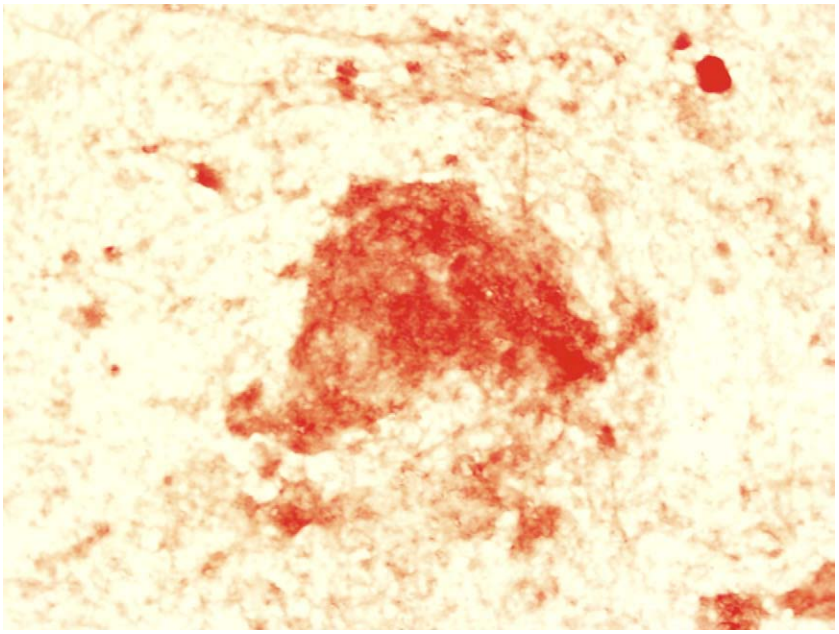

Fig. 1. *A. xylosoxidans* biofilm in CF sputum. Biofilm found in sputum from a CF patient chronically infected with *A. xylosoxidans* for 7.5 years.

secretions dominated by squamous epithelial cells. The CF patients with chronic mucoid *P. aeruginosa* lung infections have previously been shown to have biofilm in sputum [42]. Compared to *P. aeruginosa* biofilms in sputum, the biofilms of *A. xylosoxidans* and *Bcc* looked similar with a dark, condensed appearance where the bacterial cells were located very close to each other in contrast to the biofilms of mucoid *P. aeruginosa* where the abundant light alginate matrix leads to separation of the individual bacterial cells.

3.2. Specific antibodies and change in lung function

In all groups of chronically infected patients, a significant positive correlation between duration of chronic infection and levels of specific antibodies was found (Spearman's rho = 0.587, $p < 0.001$).

When comparing level of FEV_1 in the year prior to development of chronic infection in the 3 groups of chronically infected patients, no difference was found. Patients in the *A. xylosoxidans*, *Bcc* and *P. aeruginosa* groups had a median (range) FEV_1, % of predicted, of 94.7 (55.7 to 118.9), 103.5 (54.9 to 110.8) and 83.5 (51.7 to 116.5), respectively (n.s.). The *A. xylosoxidans* group was compared to a group of controls matched on age and gender. In the year prior to development of chronic infection in the *A. xylosoxidans* group, the CF controls without chronic infections had a median (range) FEV_1, % of predicted, of 94.4 (74.2–126.4) (n.s.).

In all groups of chronically infected patients, a small, but significant negative correlation between duration of chronic infection and levels of FEV_1 decline, % of predicted/year was found (Spearman's rho = −0.287, $p < 0.001$). The *A. xylosoxidans*, *Bcc* and *P. aeruginosa* group had a median (range) change in FEV_1 of −1.7 (−7.5 to +2.3), −3.7 (−7.6 to −0.2) and −0.9 (−9.1 to +2.1), respectively. The *Bcc* group had a greater lung function decline compared to the other groups ($p < 0.005$) (Fig. 2).

3.3. Serum levels of cytokines

CF patients had higher concentrations of TNFα in serum compared to healthy controls (Fig. 3) ($p < 0.003$). No difference was found when comparing CF patients with and without chronic, Gram-negative infections.

Concentrations of G-CSF were highest in the *A. xylosoxidans* and the *P. aeruginosa* groups compared to all other groups (Fig. 4) ($p < 0.001$). Within the groups of chronically infected patients, the *Bcc* group had lower levels of G-CSF compared to the *A. xylosoxidans* group ($p < 0.02$) and to the *P. aeruginosa* group ($p < 0.01$).

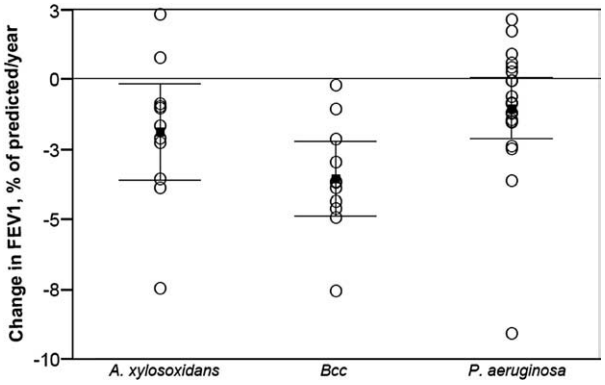

Fig. 2. Change in FEV_1, % predicted/year, up to 12 years of chronic infection. Figure shows individual values, mean and 95% confidence interval: *A. xylosoxidans*: −1.9 (−0.2 to −3.6), *Bcc*: −3.6 (−4.9 to −2.2) and *P. aeruginosa*: −1.1 (−2.2 to +0.05) ($p < 0.005$).

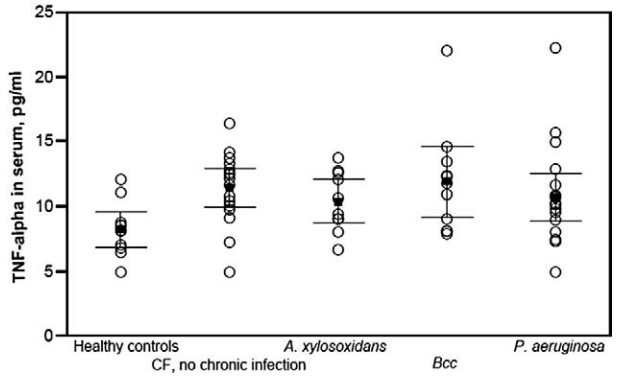

Fig. 3. Level of TNF-α (pg/ml) in serum. Figure shows individual values, mean and 95% confidence interval: Healthy controls: 8.2 (6.9–9.9), CF without chronic infection: 11.4 (9.9–12.8), *A. xylosoxidans*: 10.4 (8.7–12), *Bcc*: 12.7 (9.6–15.8), *P. aeruginosa*: 10.8 (8.7–12.9) ($p<0.003$).

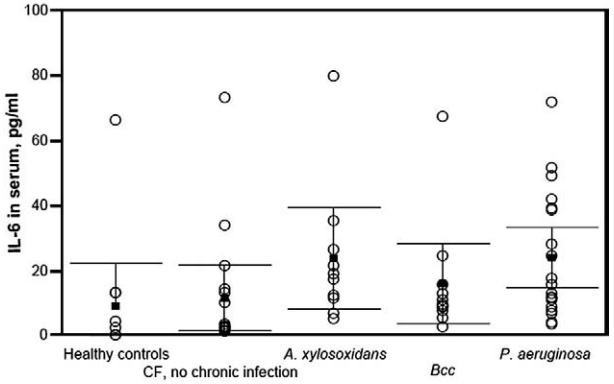

Fig. 5. Level of IL-6 (pg/ml) in serum. Figure shows individual values, mean and 95% confidence interval: Healthy controls: 9.2 (−4–22.4), CF without chronic infection: 11.7 (1.6–21.8), *A. xylosoxidans*: 23.7 (8.1–39.2), *Bcc*: 17.3 (2.1–32.6), *P. aeruginosa*: 25.6 (14.1–37.1) ($p<0.003$).

Concentrations of IL-6 were higher in chronically infected patients when compared to non-infected patients and healthy controls ($p<0.003$) (Fig. 5). We found no difference in IL-6 when comparing non-infected CF patients to healthy controls.

Concentrations of IFN-γ were higher in chronically infected patients compared to CF patients without chronic infections ($p<0.04$) (Fig. 6). Chronically infected CF patients had concentrations similar to healthy controls.

3.4. Exhaled Breath Condensate

No difference in pH between the groups was found (data not shown). Levels of cytokines in the EBC were very low. All samples had TNF-α, IL-1β, IL-6 and IL-10 below detection limit and 14 of 33 samples had IFN-γ below detection limit.

3.5. Sputum levels of cytokines

The *A. xylosoxidans* group had higher concentrations of TNF-α compared to the other chronically infected groups ($p<0.03$) (Fig. 7). G-CSF, IFN-γ, IL-6, IL-8 and IL-10 were measurable in sputum, but no difference in concentrations was found when comparing the 3 groups.

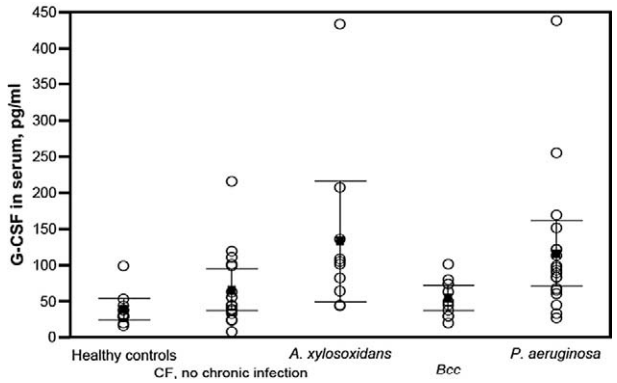

Fig. 4. Level of G-CSF (pg/ml) in serum. Figure shows individual values, mean and 95% confidence interval: Healthy controls: 39 (24–54.1), CF without chronic infection: 66.5 (38.6–94.3), *A. xylosoxidans*: 133 (49.8–216.2), *Bcc*: 60.4 (41.4–79.4), *P. aeruginosa*: 111.1 (57–165.3) ($p<001$).

3.6. Antimicrobial susceptibility

In the *A. xylosoxidans* group, 8 of 11 strains were resistant to 3 or more groups of antibiotics. In the *Bcc* group, 7 of 11 patients harboured multiresistant strains. In the *P. aeruginosa* group the number was 11 of 21 patients (n.s.).

4. Discussion

Our results show significantly enhanced inflammatory parameters in combination with progressed lung disease in CF patients with chronic *A. xylosoxidans* lung infection. The ability of this microorganism to cause chronic infection or chronic colonization is known, but it has been difficult to establish the clinical significance of this infection. Recent studies have indicated that patients chronically infected with *A. xylosoxidans* may have a more rapid decrease in lung function compared to CF patients without this infection [36] and a higher percentage of *A. xylosoxidans* infected CF patients has been observed among CF patients awaiting lung transplantation compared to the general CF community (Brown et al., abstract 359, NACFC 2008). Furthermore, patients with a rapid increase in specific,

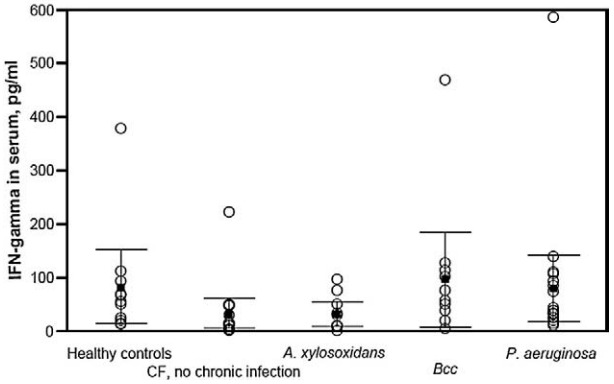

Fig. 6. IFN-γ (pg/ml) in serum. Figure shows individual values, mean and 95% confidence interval: Healthy controls: 83.6 (14.5–152.8), CF without chronic infections: 34.7 (6–63.4), *A. xylosoxidans*: 34.4 (11.1–57.7), *Bcc*: 102.3 (−9.3–213.9), *P. aeruginosa*: 86.8 (7.2–166.4) ($p<0.04$).

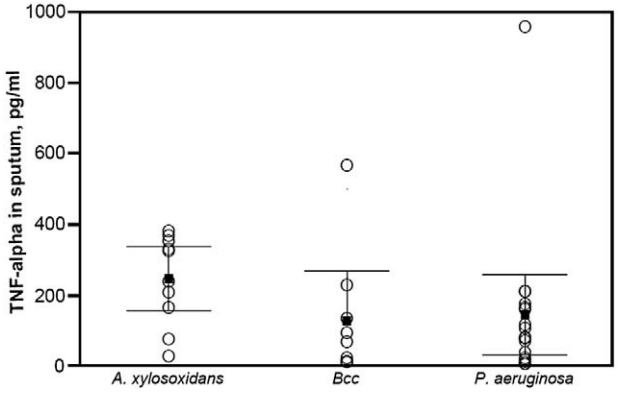

Fig. 7. Level of TNF-α (pg/ml) in sputum. Figure shows individual values, mean and 95% confidence interval: *A. xylosoxidans*: 248.3 (158.1–338.5), *Bcc*: 129.4 (−8.2–267), *P. aeruginosa*: 145.8 (14.4–277.2) ($p<0.03$).

precipitating antibodies to *A. xylosoxidans* had a more rapid decline in lung function [36].

It has been suggested that acquisition of chronic *A. xylosoxidans* infection is associated to a poor pulmonary status measured by level of FEV_1, % of predicted, at time of establishment of chronic infection [37]. In the present study, patients with chronic *A. xylosoxidans* had levels of FEV_1 similar to controls without chronic infection at time of establishment of chronic infection. We found that although patients chronically infected with *A. xylosoxidans* were younger compared to the other groups of chronically infected patients and with duration of chronic infection shorter than the *P. aeruginosa* group, the decline in FEV_1 was as high as in the *P. aeruginosa* group, although lower than in the *Bcc* group.

Biofilms are consortia of bacteria grown in clusters surrounded by a self produced polymer matrix (polysaccharides, proteins, DNA). Both *A. xylosoxidans* and *Bcc* had detectable biofilm-like structures in sputum and this was also the case with mucoid *P. aeruginosa* as reported previously [42]. The biofilms of *A. xylosoxidans* and *Bcc* appeared more dark and condensed, maybe due to the presence of extracellular DNA in the biofilm matrix, and this can maybe explain the reduction of the incidence of such bacteria in sputum of CF patients treated with nebulized DNase [46]. The presence of biofilms in sputum from CF patients infected with *A. xylosoxidans* and *Bcc* could explain the unsuccessful eradication of these chronic infections by antibiotic therapy. Furthermore, similar to chronic *P. aeruginosa* infection, the presence of biofilms can worsen the chronic, probably immune complex-mediated, inflammation and tissue damage in these patients [42], since the production of antibodies against biofilm components such as alginate, LPS, elastase and alkaline protease can lead to formation of immune complexes [47,48].

Increased inflammation in CF patients is well known and high levels of cytokines in BAL-fluid have been shown even in CF infants [7,9,10,14]. Not only in BAL-fluid, but also in sputum, very high levels of IL-1β, IL-6, IL-8 and TNF-α can be found in CF patients [7,8,15,43–45,49–53]. These cytokines increase neutrophilic inflammation in CF airways. Lower than normal levels of IL-10 have been found in CF epithelial cells and airway secretions [49,54], leading to inhibited down-regulation of neutrophilic inflammation. Concentrations of cytokines in serum are low and have been difficult to measure [8,43,53], but some correlation to clinical condition has been found in earlier studies [52,55–57].

The clinical course of the CF lung disease probably depends on the nature and degree of the inflammatory response. Chronic *P. aeruginosa* infection in CF patients is characterized by a Th2 response with higher serum levels of G-CSF and IL-4 [58], and *P. aeruginosa* infected CF patients have a higher proportion of Th2 lymphocytes in BAL-fluid [59]. Th1 response, characterized by higher serum levels of IFN-γ and GM-CSF, is dominant in CF patients with chronic *P. aeruginosa* infection and milder disease compared to CF patients with chronic *P. aeruginosa* and more severe disease [60,61]. Finally, increasing serum levels of G-CSF are correlated to decreasing levels of FEV_1 in CF patients with chronic *P. aeruginosa* infection [57].

In the present study we have shown that patients chronically infected with *A. xylosoxidans* had levels of G-CSF in serum (Fig. 4) comparable to patients with *P. aeruginosa* and a similar rate of decline of lung function after development of chronic infection (Fig. 2). Patients in the *Bcc* group had lower levels of G-CSF in serum, yet this group had the fastest decline in lung function after development of chronic infection. A possible explanation could be a different inflammatory response in the *Bcc* group.

Furthermore we found higher levels of TNF-α in serum from all CF patients compared to healthy controls. This might be a result of other, intermittent, infections, such as *Staphylococcus aureus* or other microorganisms that all CF patients suffer from and confirms a report of signs of "background" CF inflammation found even in newly diagnosed CF infants or maybe shows the presence of another focus outside the lung, e.g. the paranasal sinuses [62,63].

IL-6 levels were significantly higher in chronically infected CF patients when compared to CF patients without chronic, Gram-negative infections. IL-6 is a pro-inflammatory cytokine that has not been very extensively studied in CF patients. IL-6 gene expression can be induced by *P. aeruginosa* infection in a CF bronchial epithelial cell line [64], and IL-6 levels in BAL-fluid were increased in CF children with increasing number of CF pathogens cultured in BAL-fluid [15]. Finally, IL-6 has been measured in serum in CF patients chronically infected with *P. aeruginosa*. These patients had higher levels of IL-6 compared to healthy controls [8], and even slightly higher levels than the levels found in the present study. Our study confirms that increased concentrations of IL-6 seem to be related to the occurrence of infections.

High cytokine concentrations were found in sputum samples in all chronically infected patients. Concentrations of TNF-α in sputum were higher in the *A. xylosoxidans* group when compared to other infected groups (Fig. 7). The clinical relevance of the augmented TNF-α in sputum of the *A. xylosoxidans* group is uncertain, but may be particularly important at the site of the chronic pulmonary infection.

Intrinsic resistance to many antibiotics characterize the *A. xylosoxidans* and *Bcc* strains. We found similar incidences of

multiresistant strains in all 3 groups of chronically infected patients but different clinical courses in the groups. A direct connection between occurrence of multiresistance and a worse clinical course could not be found in the patients included for this study.

The inflammatory response in *Bcc* infected patients appears to differ from the response found in CF patients chronically infected with *P. aeruginosa*. This might partly explain the different and more aggressive nature of disease related to *Bcc* infection and should be further investigated.

5. Conclusion

A. xylosoxidans is a clinically important pathogen in CF patients causing inflammation and clinical deterioration similar to the changes found in *P. aeruginosa* infected CF patients.

References

[1] Courtney JM, Bradley J, McCaughan J, O´connor TM, Shortt C, Bredin CP, et al. Predictors of mortality in adults with cystic fibrosis. Pediatr Pulmonol Jun 2007;42(6):525–32.

[2] Frangolias DD, Mahenthiralingam E, Rae S, Raboud JM, Davidson AG, Wittmann R, et al. Burkholderia cepacia in cystic fibrosis. Variable disease course. Am J Respir Crit Care Med Nov 1999;160(5 Pt 1):1572–7.

[3] Jones AM, Dodd ME, Govan JR, et al. *Burkholderia cenocepacia* and *Burkholderia multivorans*: influence on survival in cystic fibrosis. Thorax Nov 2004;59(11):948–51.

[4] Spicuzza L, Sciuto C, Vitaliti G, Di Dio G, Leonardi S, La Rosa M. Emerging pathogens in cystic fibrosis: ten years of follow-up in a cohort of patients. Eur J Clin Microbiol Infect Dis Feb 2009;28(2):191–5.

[5] Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am J Respir Crit Care Med Oct 15 2003;168(8):918–51.

[6] Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. Chest Feb 2008;133(2):489–95.

[7] Muhlebach MS, Stewart PW, Leigh MW, Noah TL. Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. Am J Respir Crit Care Med Jul 1999;160(1):186–91.

[8] Kronborg G, Hansen MB, Svenson M, Fomsgaard A, Høiby N, Bendtzen K. Cytokines in sputum and serum from patients with cystic fibrosis and chronic *Pseudomonas aeruginosa* infection as markers of destructive inflammation in the lungs. Pediatr Pulmonol May 1993;15(5):292–7.

[9] Armstrong DS, Grimwood K, Carzino R, Carlin JB, Olinsky A, Phelan PD. Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. BMJ Jun 17 1995;310(6994):1571–2.

[10] Armstrong DS, Grimwood K, Carlin JB, Carzino R, Gutierrez JP, Hull J, et al. Lower airway inflammation in infants and young children with cystic fibrosis. Am J Respir Crit Care Med Oct 1997;156(4 Pt 1):1197–204.

[11] Balough K, McCubbin M, Weinberger M, Smits W, Ahrens R, Fick R. The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis. Pediatr Pulmonol Aug 1995;20(2):63–70.

[12] Bodini A, D'Orazio C, Peroni D, Corradi M, Folesani G, Baraldi E, et al. Biomarkers of neutrophilic inflammation in exhaled air of cystic fibrosis children with bacterial airway infections. Pediatr Pulmonol Dec 2005;40(6): 494–9.

[13] Armstrong DS, Hook SM, Jamsen KM, Nixon GM, Carzino R, Carlin JB, et al. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. Pediatr Pulmonol Dec 2005;40(6):500–10.

[14] Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med Apr 1995;151(4):1075–82.

[15] Sagel SD, Gibson RL, Emerson J, McNamara S, Burns JL, Wagener JS, et al. Impact of *Pseudomonas* and *Staphylococcus* infection on inflammation and clinical status in young children with cystic fibrosis. J Pediatr Feb 2009;154(2):183–8.

[16] Jones AM, Martin L, Bright-Thomas RJ, Dodd ME, McDowell A, Moffitt KL, et al. Inflammatory markers in cystic fibrosis patients with transmissible *Pseudomonas aeruginosa*. Eur Respir J Sep 2003;22(3):503–6.

[17] Moser C, Kjaergaard S, Pressler T, Kharazmi A, Koch C, Høiby N. The immune response to chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients is predominantly of the Th2 type. APMIS May 2000;108(5):329–35.

[18] Johansen HK, Nørregaard L, Gøtzsche PC, Pressler T, Koch C, Høiby N. Antibody response to *Pseudomonas aeruginosa* in cystic fibrosis patients: a marker of therapeutic success? — A 30-year cohort study of survival in Danish CF patients after onset of chronic *P. aeruginosa* lung infection. Pediatr Pulmonol May 2004;37(5):427–32.

[19] Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol Aug 2002;34(2):91–100.

[20] Høiby N, Flensborg EW, Beck B, Friis B, Jacobsen SV, Jacobsen L. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. Scand J Respir Dis Apr 1977;58(2):65–79.

[21] Cystic Fibrosis Foundation. Patient registry 2006 annual report; 2008.

[22] Courtney JM, Dunbar KE, McDowell A, Moore JE, Warke TJ, Stevenson M, et al. Clinical outcome of *Burkholderia cepacia* complex infection in cystic fibrosis adults. J Cyst Fibros Jun 2004;3(2):93–8.

[23] Ledson MJ, Gallagher MJ, Jackson M, Hart CA, Walshaw MJ. Outcome of *Burkholderia cepacia* colonisation in an adult cystic fibrosis centre. Thorax Feb 2002;57(2):142–5.

[24] Muhdi K, Edenborough FP, Gumery L, O´Hickey S, Smith EG, Smith DL, et al. Outcome for patients colonised with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic and the end of an epidemic. Thorax Apr 1996;51(4):374–7.

[25] Whiteford ML, Wilkinson JD, McColl JH, Conlon FM, Michie JR, Evans TJ, et al. Outcome of *Burkholderia* (*Pseudomonas*) *cepacia* colonisation in children with cystic fibrosis following a hospital outbreak. Thorax Nov 1995;50(11):1194–8.

[26] Isles A, Maclusky I, Corey M, Gold R, Prober C, Fleming P, et al. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. J Pediatr Feb 1984;104(2):206–10.

[27] Cystic Fibrosis Foundation. Patient registry 2007 annual report; 2009.

[28] Govan JR, Brown PH, Maddison J, Doherty CJ, Nelson JW, Dodd M, et al. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. Lancet Jul 3 1993;342(8862):15–9.

[29] LiPuma JJ, Dasen SE, Nielson DW, Stern RC, Stull TL. Person-to-person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. Lancet Nov 3 1990;336(8723):1094–6.

[30] Ledson MJ, Gallagher MJ, Corkill JE, Hart CA, Walshaw MJ. Cross infection between cystic fibrosis patients colonised with *Burkholderia cepacia*. Thorax May 1998;53(5):432–6.

[31] Blackburn L, Brownlee K, Conway S, Denton M. 'Cepacia syndrome' with *Burkholderia multivorans*, 9 years after initial colonization. J Cyst Fibros Jun 2004;3(2):133–4.

[32] Simmonds EJ, Conway SP, Ghoneim AT, Ross H, Littlewood JM. *Pseudomonas cepacia*: a new pathogen in patients with cystic fibrosis referred to a large centre in the United Kingdom. Arch Dis Child Aug 1990;65(8):874–7.

[33] Bamford S, Ryley H, Jackson SK. Highly purified lipopolysaccharides from *Burkholderia cepacia* complex clinical isolates induce inflammatory cytokine responses via TLR4-mediated MAPK signalling pathways and activation of NFkappaB. Cell Microbiol Feb 2007;9(2):532–43.

[34] Hendry J, Elborn JS, Nixon L, Shale DJ, Webb AK. Cystic fibrosis: inflammatory response to infection with *Burkholderia cepacia* and *Pseudomonas aeruginosa*. Eur Respir J Aug 1999;14(2):435–8.

[35] Burns JL, Emerson J, Stapp JR, Yim DL, Krzewinski J, Louden L, et al. Microbiology of sputum from patients at cystic fibrosis centers in the United States. Clin Infect Dis Jul 1998;27(1):158–63.

[36] Hansen CRønne, Pressler T, Høiby N, Gormsen M. Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients; a retrospective case control study. J Cyst Fibros Dec 2006;5(4):245–51.

[37] de Baets F, Schelstraete P, van Daele S, Haerynck F, Vaneechoutte M. *Achromobacter xylosoxidans* in cystic fibrosis: prevalence and clinical relevance. J Cyst Fibros Jan 2007;6(1):75–8.
[38] Raso T, Bianco O, Grosso B, Zucca M, Savoia D. *Achromobacter xylosoxidans* respiratory tract infections in cystic fibrosis patients. APMIS Sep 2008;116(9):837–41.
[39] Tan K, Conway SP, Brownlee KG, Etherington C, Peckham DG. Alcaligenes infection in cystic fibrosis. Pediatr Pulmonol Aug 2002;34(2):101–4.
[40] American Thoracic Society. Standardization of spirometry, 1994 update. Am J Respir Crit Care Med Sep 1995;152(3):1107–36.
[41] Polgar G, Promadhat V. Pulmonary function testing in children: techniques and standards. Philadelphia: W.B.Saunders; 1971.
[42] Bjarnsholt T, Jensen PO, Fiandaca MJ, Pedersen J, Hansen CR, Andersen CB, et al. *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. Pediatr Pulmonol Jun 2009;44(6):547–58.
[43] Colombo C, Costantini D, Rocchi A, Cariani L, Garlaschi ML, Tirelli S, et al. Cytokine levels in sputum of cystic fibrosis patients before and after antibiotic therapy. Pediatr Pulmonol Jul 2005;40(1):15–21.
[44] Karpati F, Hjelte FL, Wretlind B. TNF-alpha and IL-8 in consecutive sputum samples from cystic fibrosis patients during antibiotic treatment. Scand J Infect Dis 2000;32(1):75–9.
[45] Ordonez CL, Henig NR, Mayer-Hamblett N, Accurso FJ, Burns JL, Chmiel JF, et al. Inflammatory and microbiologic markers in induced sputum after intravenous antibiotics in cystic fibrosis. Am J Respir Crit Care Med Dec 15 2003;168(12):1471–5.
[46] Frederiksen B, Pressler T, Hansen A, Koch C, Høiby N. Effect of aerosolized rhDNase (Pulmozyme) on pulmonary colonization in patients with cystic fibrosis. Acta Paediatr Sep 2006;95(9):1070–4.
[47] Pressler T, Pedersen SS, Espersen F, Høiby N, Koch C. IgG subclass antibody responses to alginate from *Pseudomonas aeruginosa* in patients with cystic fibrosis and chronic *P. aeruginosa* infection. Pediatr Pulmonol Sep 1992;14(1):44–51.
[48] Høiby N, Döring G, Schiøtz PO. The role of immune complexes in the pathogenesis of bacterial infections. Annu Rev Microbiol 1986;40:29–53.
[49] Bonfield TL, Panuska JR, Konstan MW, Hilliard KA, Hilliard JB, Ghnaim H, et al. Inflammatory cytokines in cystic fibrosis lungs. Am J Respir Crit Care Med Dec 1995;152(6 Pt 1):2111–8.
[50] Salva PS, Doyle NA, Graham L, Eigen H, Doerschuk CM. TNF-alpha, IL-8, soluble ICAM-1, and neutrophils in sputum of cystic fibrosis patients. Pediatr Pulmonol Jan 1996;21(1):11–9.
[51] Schuster A, Haarmann A, Wahn V. Cytokines in neutrophil-dominated airway inflammation in patients with cystic fibrosis. Eur Arch Otorhinolaryngol 1995;252(Suppl 1):S59–60.
[52] Nixon LS, Yung B, Bell SC, Elborn JS, Shale DJ. Circulating immunoreactive interleukin-6 in cystic fibrosis. Am J Respir Crit Care Med Jun 1998;157(6 Pt 1):1764–9.
[53] Wolter JM, Rodwell RL, Bowler SD, McCormack JG. Cytokines and inflammatory mediators do not indicate acute infection in cystic fibrosis. Clin Diagn Lab Immunol Mar 1999;6(2):260–5.
[54] Becker MN, Sauer MS, Muhlebach MS, Hirsh AJ, Wu Q, Verghese MW, et al. Cytokine secretion by cystic fibrosis airway epithelial cells. Am J Respir Crit Care Med Mar 1 2004;169(5):645–53.
[55] Augarten A, Paret G, Avneri I, Akons H, Aviram M, Bentur L, et al. Systemic inflammatory mediators and cystic fibrosis genotype. Clin Exp Med Oct 2004;4(2):99–102.
[56] Downey DG, Martin SL, Dempster M, Moore JE, Keogan MT, Stacher B, et al. The relationship of clinical and inflammatory markers to outcome in stable patients with cystic fibrosis. Pediatr Pulmonol Mar 2007;42(3): 216–20.
[57] Jensen PØ, Moser C, Kharazmi A, Pressler T, Koch C, Høiby N. Increased serum concentration of G-CSF in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* pneumonia. J Cyst Fibros Aug 2006;5(3):145–51.
[58] Brazova J, Sediva A, Pospisilova D, Vavrova V, Pohunek P, Macek M, Jr., et al. Differential cytokine profile in children with cystic fibrosis. Clin Immunol May 2005;115(2):210–5.
[59] Hartl D, Griese M, Kappler M, Zissel G, Reinhardt D, Rebhan C, et al. Pulmonary T(H)2 response in *Pseudomonas aeruginosa*-infected patients with cystic fibrosis. J Allergy Clin Immunol Jan 2006;117(1):204–11.
[60] Moser C, Jensen PØ, Kobayashi O, Hougen HP, Song Z, Rygaard J, et al. Improved outcome of chronic *Pseudomonas aeruginosa* lung infection is associated with induction of a Th1-dominated cytokine response. Clin Exp Immunol Feb 2002;127(2):206–13.
[61] Moser C, Jensen PØ, Pressler T, Frederiksen B, Lanng S, Kharazmi A, et al. Serum concentrations of GM-CSF and G-CSF correlate with the Th1/Th2 cytokine response in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. APMIS Jun 2005;113(6):400–9.
[62] Muhlebach MS, Miller MB, Moore C, Wedd JP, Drake AF, Leigh MW. Are lower airway or throat cultures predictive of sinus bacteriology in cystic fibrosis? Pediatr Pulmonol May 2006;41(5):445–51.
[63] Roby BB, McNamara J, Finkelstein M, Sidman J. Sinus surgery in cystic fibrosis patients: comparison of sinus and lower airway cultures. Int J Pediatr Otorhinolaryngol Sep 2008;72(9):1365–9.
[64] Borgatti M, Bezzerri V, Mancini I, Nicolis E, Dechecchi MC, Lampronti I, et al. Induction of IL-6 gene expression in a CF bronchial epithelial cell line by *Pseudomonas aeruginosa* is dependent on transcription factors belonging to the Sp1 superfamily. Biochem Biophys Res Commun Jun 15 2007;357(4):977–83.