SHORT REPORT

Cystic fibrosis: inflammatory response to infection with *Burkholderia cepacia* and *Pseudomonas aeruginosa*

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Cystic fibrosis: inflammatory response to infection with Burkholderia cepacia and Pseudomonas aeruginosa. J. Hendry, J.S. Elborn, L. Nixon, D.J. Shale, A.K. Webb. ©ERS Journals Ltd 1999.

ABSTRACT: Pulmonary colonization by *Burkholderia cepacia* in cystic fibrosis (CF) may be associated with enhanced deterioration of pulmonary function. This may be due to a more florid host inflammatory response than in colonization by *Pseudomonas aeruginosa*, leading to greater lung injury.

Circulating markers of inflammation were determined during infective exacerbations and periods of clinical stability in an 18 month prospective study in adults with CF colonized by *P. aeruginosa* (n=41). *B. cepacia* (n=13) and in adults who intermittently grew *B. cepacia* (n=6).

There were no differences between the levels of the inflammation markers measured in the three groups (*P. aeruginosa*, *B. cepacia*, *B. cepacia* intermittent) at any of the assessment points. When clinically stable, levels of inflammatory markers in all groups were elevated compared to a matched non-CF population, indicating, continuous inflammation and the potential for lung damage between infective exacerbations.

This study does not support the hypothesis that pulmonary colonization with *Burkholderia cepacia* is associated with a heightened inflammatory response compared with *Pseudomonas aeruginosa* colonization.

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Pulmonary colonization by *Burkholderia cepacia* in cystic fibrosis (CF) may lead to chronic asymptomatic carriage, acceleration of a gradual deterioration in pulmonary function or, a rapid often fatal deterioration with fever, a high peripheral white cell count and progressive consolidation on the chest radiograph [1–3]. The mechanism by which *B. cepacia* causes pulmonary deterioration is unclear. Although virulence factors have been identified, none appears to account for its pathogenicity [4–6]. Furthermore, in animal models, *B. cepacia* is less virulent than *Pseudomonas aeruginosa* and appears to have less potential for tissue invasion [7].

The pathogenicity of *B. cepacia* could be explained by a more vigorous host immune and inflammatory reaction than is provoked by *P. aeruginosa*. This prospective study, compared measurements of inflammatory markers at the onset and end of infective pulmonary exacerbations and during clinically stable periods in CF patients whose lungs were infected with *B. cepacia*, with those of a group of severity matched control subjects whose lungs were infected with *P. aeruginosa*.

Patients and methods

A prospective study was conducted over an 18 month period in the Manchester Adult Cystic Fibrosis Unit. Thirteen patients with CF were chronically infected with *B. cepacia* (growth of the organism in three successive sputum specimens within a 6-month period). All agreed to

participate in the study, forming the *B. cepacia* (BC) group. Patients colonized with *P. aeruginosa* were invited to participate in the study if their forced expiratory volume in one second (FEV₁) % predicted, body mass index and Shwachman score were within the range of the *B. cepacia* group; 34 such patients formed the *P. aeruginosa* colonized (PA) group. Patients who grew *B. cepacia* intermittently, assessed through their sputum, but failed to meet the criteria for chronic infection were also invited to participate in the study, six patients formed the *B. cepacia* intermittent group (BCI).

Study design

The frequency of assessment for each patient was determined by their clinical course. Patients with a pulmonary exacerbation, defined as an increase in pulmonary symptoms (cough, breathlessness, increased daily sputum volume) and decrease in FEV₁ as measured by a spirometer (Vitalograph, Buckingham, UK), were reviewed before commencement of antibiotics and within 48 h of completion of treatment. Intravenous antibiotics were administered for 14 days, which was extended if the patient had a suboptimal response. Patients were reviewed 6 weeks after completion of intravenous antibiotics and thereafter at 6 weekly intervals. Patients who were clinically stable were reviewed at 3 monthly intervals. All patients were asked to attend the clinic promptly for clinical review if they developed features of a pulmonary exacerbation.

Microbiological methods

Samples of fresh sputum were plated onto cystine-lactose-electrolyte-deficient-(CLED) agar (Oxoid, Basingstoke, UK), *Pseudomonas* isolation agar (Difco Labs, Detroit, MI, USA) and *B. cepacia* medium (Mast cepacia agar; Mast diagnostics Ltd, Bootle, UK). Isolates of *P. aeruginosa*, *B. cepacia* and *Staphylococcus aureus* were identified by methods described previously [8]. Routine antibiotic sensitivity testing was carried out using the modified Stokes technique.

Treatment of an infective exacerbation

P. aeruginosa, *B. cepacia* and *Stenotrophomonas maltophilia* were treated with intravenous antibiotics according to the *in vitro* sensitivity of the infecting organism. A multiresistant *B. cepacia* was treated empirically with ceftazidime, meropenem or imipenem with an aminoglycoside based upon previous clinical responses.

Inflammatory markers

At each assessment blood was drawn into disodium ethylenediamine tetraacetic acid (EDTA) for total white blood cell and differential cell counts and separation of plasma to assay neutrophil elastase α -1 antiproteinase complex (NE-APC), the latter samples were kept on melting ice to prevent neutrophil degranulation. Clotted blood was used to separate serum for assay of C-reactive protein (CRP). NE-APC and serum CRP were determined by antigen capture enzyme-linked immunosorbent assays (ELISA) [9]. Tumour necrosis factor (TNF)- α was determined using an immunoenzymic assay kit (TNF- α -EASIA; Medgenix Diagnostics, Fleurus, Belgium). Blood samples were obtained from healthy age matched volunteers, processed and analysed as above to provide "normal ranges" for the inflammatory markers.

Statistical methods

Skewed data are presented as medians and ranges. Normal and approximately normal data are presented as mean $\pm$ SD. Analysis of variance (ANOVA) was used to test differences in clinical parameters between the three groups. The Kruskal-Wallis and Mann-Whitney U-tests were used to compare unpaired non-normally distributed data. Generalized linear interactive modelling was used to examine trends over the study period in the measured clinical parameters for the whole study population and each individual subgroup (BC, PA, and BCI) after log₁₀ transformation. Twenty-nine PA, nine BC, and three BCI patients were included in this analysis. Interpretation of the analysis was restricted to exacerbations 1–4. Statistical significance was accepted when $p < 0.05$.

Results

For the BC, PA and BCI groups respectively, there were no significant differences between the median (range) ages

(20 (17–30), 21 (17–46), 26 (18–36), $p > 0.05$), Shwachmann scores (66 (38–74), 66 (50–88), 67 (59–77), $p > 0.05$), mean $\pm$ SD FEV₁% pred (54.4 $\pm$ 22.0, 59.1 $\pm$ 26.8, 54.3 $\pm$ 28.6, $p > 0.05$), or body mass indices (19.6 $\pm$ 2.7, 19.6 $\pm$ 1.8, 20.2 $\pm$ 2.7, $p > 0.05$).

Sputum culture results of the BC, PA and BCI groups over the follow-up period

Of the 13 BC patients, three grew *B. cepacia* alone, one *B. cepacia* and *S. aureus*, six *B. cepacia* and *P. aeruginosa*, and three *B. cepacia*, *P. aeruginosa* and *S. aureus*. Of the 41 PA patients, 20 grew *P. aeruginosa*, alone, 17 *P. aeruginosa* and *S. aureus*, two *P. aeruginosa* and *S. aureus* and *S. maltophilia*, and one *P. aeruginosa* and *S. maltophilia*. One patient grew no bacterial pathogens throughout the study duration, although they had previously been colonized by *P. aeruginosa*. Of the six BCI patients, three grew *P. aeruginosa*, one *P. aeruginosa* and *S. aureus*, one *P. aeruginosa* and *S. maltophilia*, and one *S. aureus* alone.

Prospective analysis of inflammatory markers in patients who remained stable

Using linear modelling there was no upward or downward trend in the log₁₀ transformed NE-APC, CRP or white cell count (WCC) over the follow-up period for these 10 patients ($p > 0.05$).

Inflammatory markers in patients who experienced pulmonary exacerbations

White cell count. For the whole study population, the geometric mean (95% confidence interval (CI)) WCC count ($\times 10^9$ cells·L⁻¹) at the beginning of the first exacerbation (10.8 (9.5–12.1)) was greater than at the end of the exacerbation (7.9 (7.1–8.9)) or the next visit during clinical stability (9.1 (8.0–10.2)), $p < 0.05$. The geometric mean WCC in stable state was also greater than at the end of the exacerbation, $p < 0.001$. This pattern of variation was repeated in successive exacerbations. Within the BC, PA and BCI groups, similar patterns of variation in the WCC were observed, and at no assessment point were there differences between the three groups, $p > 0.05$.

Neutrophil elastase α -1 antitrypsin complex. For the whole study population the geometric mean (CI) NE-APC (ng·mL⁻¹) value was greater at the beginning of the first exacerbation (79.7 (70.2–90.5)) than at the end of the exacerbation (54.3 (47.8–61.7)) or during a stable clinical state (65.0 (57.2–73.9)), $p < 0.001$ for both. This pattern was repeated in successive exacerbations. Within the BC, PA and BCI groups, the same pattern of variation in plasma NE-APC was demonstrated within and between exacerbations with no differences between the three groups at any assessment point.

C-reactive protein. For the whole study population, the geometric mean (CI) CRP (mg·L⁻¹) value was greater at the beginning of the first infective exacerbation (133

Table 1. – Inflammatory markers within 4 weeks and 6 months of death

Subject No.	Group	NE-APC ng·mL ⁻¹		CRP mg·L ⁻¹		WCC × 10 ⁹ cells·L ⁻¹	
		4 weeks	6 months	4 weeks	6 months	4 weeks	6 months
1	BC	85.4	63.6	32.4	13.4	13.8	8.9
2 [#]	BC	171.4	77.8	138.4	4.78	16.0	8.1
3	BC	63.8	67.3	28.6	31.7	NA	11.4
4	*	109.0	175.5	225.8	21.3	19.9	12.4
5	PA	91.6	72.7	76.6	42.2	14.9	12.4

Data presented as geometric mean. NE-APC: neutrophil elastase α -1 antiproteinase complex; CRP: C-reactive protein; WCC: white cell count; BC: *Burkholderia cepacia*; PA: *Pseudomonas aeruginosa*; NA: not available. [#]: Patient who died from a fulminant pneumonic illness; *: patient initially colonized with *P. aeruginosa* died 10 weeks after becoming infected with *B. cepacia* (excluded from prospective study).

(5.8–304.0)) than at the end of the exacerbation (0.6 (0.3–1.4)) or the next visit during a clinically stable state (3.5 (1.6–8.1)), and the geometric mean CRP was greater during the clinically stable state than at the end of the exacerbation, $p < 0.001$. There was no difference in the pattern of variability between successive exacerbations.

The geometric mean CRP value at the end of the first exacerbation was lower in the PA group compared to the BC group ($p < 0.05$), although this was the only difference between the groups.

Tumour necrosis factor- α . Data on this variable were collected only at the time of the first infective exacerbation after the onset of the study. Complete data were available for eight BC patients, 25 PA patients and five BCI patients. The median (range) TNF- α level in PA, BC and BCI group patients, respectively, were 19.5 (3.3–69.1), 21.3 (12.2–31.8) and 18.7 (5.0–28.3) at the beginning of the exacerbation, 17.8 (3.0–37.7), 19.2 (12.4–40.0), 17.7 (13.1–28.8) at the end of the exacerbation and 6.5 (6.6–34.2), 17.4 (13.2–28.7) and 15.8 (6.1–34.8) during a stable clinical state. There were no differences between the TNF- α levels in the three patient groups at the beginning and end of the exacerbation or when clinically stable ($p > 0.05$).

Inflammatory markers preceding death

In five of the eight patients who died, data on WCC, NE-APC and CRP were available for the month preceding death and for a time of clinical stability within the preceding 6 months. There was no difference between the NE-APC values within 4 weeks of death and 6 months of death, $p > 0.05$ (table 1). Similarly, there were no significant differences for CRP or WCC ($p > 0.05$, for both).

Discussion

The design of this has allowed the observation and comparison of the host inflammatory response during 30 infective exacerbations in *B. cepacia* colonized patients, 81 in *P. aeruginosa* colonized patients, and 10 in patients with intermittently isolated *B. cepacia* from their sputum. No differences were demonstrated for the WCC, NE-APC and CRP between the BC, PA and BCI groups. There was a trend towards greater levels of NE-APC at the beginning of an exacerbation in the BC group. This difference may be

genuine, but relatively small numbers of patients were compared and "signal variability" may be masking that difference. No difference in TNF- α levels in plasma could be demonstrated between the three groups. All of these observations fail to support the hypothesis of greater inflammatory activity in patients colonized with *B. cepacia* compared to those colonized with *P. aeruginosa*, although two important points should temper this conclusion. Firstly, measurement of blood cytokines may not be an accurate representation of cytokine activity within pulmonary tissue, although the circulating and airways changes in CRP, NE-APC, interleukin (IL)-6 and TNF- α have been reported [10]. Secondly, recent work has demonstrated that *in vitro*, lipopolysaccharide (LPS) from both *B. cepacia* and *P. aeruginosa* stimulates the release of TNF- α from monocytes, but LPS from *B. cepacia* is nine times more potent [11]. Using a similar model the release of TNF- α is reduced when a mixture of *B. cepacia* and *P. aeruginosa* are used as the stimulants rather than *B. cepacia* alone [12]. This suggests that *P. aeruginosa* may ameliorate the pro-inflammatory effect of *B. cepacia* and, by extrapolation, may have an impact upon the outcome of *B. cepacia* infection in CF. However, no significant difference was found between the TNF- α levels in the two patients studied who were colonized solely by *B. cepacia* compared to the six patients studied colonized both with *B. cepacia* and *P. aeruginosa*. Had the study group been formed from patients colonized by *B. cepacia* alone, potential interactions between organisms would have been avoided. This was precluded by small numbers of such patients ($n = 3$). Furthermore, such a group would not be representative of the majority of patients in clinical practice who are colonized with both *B. cepacia* and *P. aeruginosa*.

In keeping with a previous study [13], the results demonstrate that prior to death, inflammatory activity does not fall with antibiotic treatment. This may represent a period of intense, refractory inflammation causing damage to pulmonary tissue.

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