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Original Article

Cytokines in nasal lavages and plasma and their correlation with clinical parameters in cystic fibrosis

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Abstract

Background: Because persistent inflammation plays a dominant role in cystic fibrosis (CF), we assessed systemic and local upper airway responses during and after pulmonary exacerbation.
Methods: We followed a cohort of *Pseudomonas aeruginosa*-infected adult CF patients (n = 16) over time in pulmonary exacerbation and in stable disease. Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17A, IL-22, interferon-γ and TNFα levels were measured in sputum, nasal lavages and plasma.
Results: In CF patients IL-6 and IL-10 levels in nasal lavages were significantly increased in exacerbation compared with stable disease. Systemic IL-6 significantly correlated with CRP levels and FEV1 (%predicted), independently of disease status. Systemic IL-10 also correlated significantly with CRP and FEV1 (%predicted), but only in exacerbation. Other cytokines tested did not discriminate between exacerbation and stable disease.
Conclusions: Determination of IL-6 and IL-10 in nasal lavages may provide a minimally invasive tool in the assessment of an exacerbation in CF.

Keywords: Cystic fibrosis; Cytokines; Inflammation; Nasal lavages; Interleukin

1. Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) defect predisposes CF patients to chronic respiratory infections, resulting in progressive tissue damage due to airway inflammation [1,2]. Airway inflammation in CF, however, begins already in early infancy [3,4] and, although clearly associated with infection, there is still some uncertainty about whether CF lungs are innately primed for a pro-inflammatory response. Recurrent and persistent lung infections with bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are common from infancy and the incidence of infection increases with age [5]. In adulthood, most CF patients are chronically infected with *P. aeruginosa* [6], and this chronic infection results in a neutrophil-dominated lower airway inflammation and progression of obstructive lung disease and bronchiectasis [1,2].

Similar to the lower airways, mucociliary clearance of the upper airways is impaired by the causative CFTR defect [7–9]. Sinonasal involvement in CF has been proposed as a major source for chronic bronchopulmonary infection with opportunistic bacteria [10]. *S. aureus* and *P. aeruginosa* are known to colonise the upper airways in CF patients [11] and therefore this site may function as a gateway and reservoir for subsequent pulmonary infection [12].

The inflammatory activity reported in CF is not restricted to acute pulmonary exacerbations of CF, but has also been demonstrated during phases of clinical stability [13–15]. Inflammatory mediators as markers of the host response to infection may reflect the intensity of the lung injury and may

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relate to changes in clinical status (pulmonary exacerbation or clinical stability). Because airway inflammation plays a crucial role in progressive lung damage in CF, it is important to find reliable markers of its severity. Bronchoalveolar lavage (BAL) probably reflects the airway inflammation most accurately, but bronchoscopy with BAL is invasive and unpleasant for patients. Surrogate markers, collected by minimally or non-invasive procedures, would therefore be of great assistance [16,17]. Nasal lavage is one of these non-invasive methods. However only few studies investigated nasal lavages as a diagnostic method to evaluate airway inflammation [8,18–20], and to our knowledge no study has compared nasal lavage cytokine levels in exacerbation to those in stable disease. Furthermore, since systemic inflammation has also been reported in CF [15,21], collection of peripheral blood may also serve as a minimally invasive tool in the assessment of pulmonary inflammation.

We hypothesized that the inflammatory response in CF is increased in patients in acute exacerbations compared with those in stable disease and that the intensity of this inflammatory response – as reflected by the cytokine production – correlates with disease severity as defined by pulmonary function testing (FEV1 %predicted) and C-reactive protein (CRP) levels. We especially assessed whether local nasal or sputum inflammatory responses are indicative for acute pulmonary exacerbations in CF. To test this hypothesis, we followed a cohort of *P. aeruginosa*-infected adult CF patients over time and measured cytokine levels in stable disease as well as in acute pulmonary exacerbation. Cytokines reflecting innate and adaptive immune activity were determined both locally, in sputum and nasal lavages, and systemically in plasma.

2. Methods

2.1. Study design

A prospective study was performed in CF patients with chronic *P. aeruginosa* infection between January and December 2010. Patients admitted for intravenous antibiotic therapy during acute respiratory exacerbation to the Erasmus MC, a CF centre with a total adult population of 120 patients, were enrolled in the study. The medical ethics committee of the Erasmus MC approved of the study. Written informed consent was obtained from each patient.

An airway exacerbation was defined as prescribed previously by Fuchs et al. [22], and was said to have occurred when a patient was treated with parenteral antibiotics for any 4 of the following 12 signs or symptoms: change in sputum; new or increased hemoptysis; increased cough; increased dyspnea; malaise, fatigue, or lethargy; temperature above 38 °C; anorexia or weight loss; sinus pain or tenderness; change in sinus discharge; change in physical examination of the chest; decrease in pulmonary function by 10% or more from a previously recorded value; or radiographic changes indicative of pulmonary infection. Exclusion criteria were known immunodeficiency or autoimmune disease, the use of systemic corticosteroids, and the presence of allergic bronchopulmonary aspergillosis (ABPA) or allergic asthma. In all patients intravenous antibiotics were administered for 21 days. Selection of antibiotics was based on the sensitivity of the cultured bacteria and consisted in all patients of two antibiotics with different mechanisms of action.

Upon admission, we collected venous blood and sputum samples and performed nasal lavages in all patients. Additionally, spirometry and routine biochemical analysis (including CRP) were done. Measurements were repeated approximately 3 months after hospital admission when patients were clinically stable, which was defined as no need for intravenous antibiotics for at least 6 weeks prior to measurements. Chronic *P. aeruginosa* infection was diagnosed if the organism was isolated in at least 3 consecutive sputum samples within a 6-month period.

2.2. Collection of sputa, nasal lavages and plasma

Sputum samples were collected by spontaneous coughing, and were stored at 4 °C for a maximum of 2 h until processing. Samples were processed using Sputolysin (Calbiochem). Briefly, 1 ml of 10% Sputolysin was added per 1 mg of sputum, and incubated for 15 min at 37 °C while vigorously shaking. Subsequently, samples were centrifuged at 600 ×*g* for 10 min at 4 °C, and supernatants were aliquoted.

Nasal lavage was performed essentially as previously described [12]. However, instead of using 10 ml of sterile isotonic saline, we inserted 5 ml into each nostril with a 10 ml syringe with a slightly reclined position of the head during occlusion of the soft palate. Specimens were stored at 4 °C for a maximum of 2 h until processed. Nasal lavages were centrifuged at 1200 ×*g* for 10 min at 4 °C and supernatants were aliquoted. To obtain plasma, venous blood was collected into EDTA containing vials and centrifuged at 1200 ×*g* for 10 min at 4 °C. All samples were stored at −80 °C until cytokine analysis.

2.3. Cytokine level measurements

Levels of IL-6, IL-10, IL-8, IL-1β, TNFα, IL-17A, IL-22, IFNγ, IL-4, IL-5 and IL-2 in sputum samples and nasal lavages were determined using Multiplex assays (FlowCytomix, eBioscience) according to manufacturer's instructions. Levels of IL-6, IL-10, IL-8, IL-1β, TNFα, IL-17A, IL-22, and IL-4 in plasma were assessed by enzyme-linked immunosorbent assay (ELISA) using commercially available assays (IL-8 OptEIA Set, BD Biosciences; all other cytokines Ready-Set-Go kits, eBiosciences). Specific sensitivity levels can be found in the manufacturers' manuals.

2.4. Statistical analysis

Data are shown as mean values (±SD) in cases of normally distributed data or median values with interquartile ranges (IQRs) when not normally distributed. Cytokine levels were not normally distributed and therefore nonparametric tests were used to make comparisons between groups (Kruskal–Wallis test for across group comparison of three or more groups, Mann–Whitney *U*-test for pair-wise analyses). Paired data were tested using the paired Wilcoxon rank test. Correlations were calculated by using Spearman's Rank correlation coefficient.