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Effect of cystic fibrosis exacerbations on neutrophil function

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Abstract

In cystic fibrosis (CF), inflammation is caused by persistent bacterial infection from *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* in the lung and is characterised by the persistent infiltration of massive numbers of neutrophils which leads to lung injury. The aim of this present study was to investigate the effects of CF exacerbations on the reactivity of peripheral blood neutrophils compared to data from a normal healthy control population. Peripheral blood neutrophils were isolated from control subjects and CF patients before and after an exacerbation of their lung disease. Isolated neutrophils were stimulated with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol 12-myristate 13-acetate (PMA) and the rate of superoxide generation and elastase activity measured and compared with neutrophils from healthy age-matched controls. Neutrophils from CF patients spontaneously generated higher levels of superoxide after resolution of the exacerbation compared to control neutrophils. The stimulated generation of superoxide from control neutrophils was not significantly different from neutrophils isolated from CF patients either before or after resolution of the CF exacerbation. Neutrophils from CF patients spontaneously released more elastase than control neutrophils but released less elastase than control neutrophils in response to fMLP. The stimulated release of elastase from neutrophils was not significantly different before compared to after resolution of the exacerbation. Neutrophils from CF patients displayed a different pattern of response than those from control subjects; however, CF exacerbations did not appear to modulate neutrophil function.

Keywords: Cystic fibrosis; Neutrophil function; Superoxide; Elastase

1. Introduction

Cystic fibrosis (CF) is characterised by chronic progressive bronchiectasis, exocrine pancreatic insufficiency, sterility in males, and abnormally high concentrations of sodium chloride in the sweat. Water reabsorption from the CF airway lumen outweighs limited Cl^- secretion resulting in the airway secretions becoming viscid and clearance of secretions is impaired causing airway obstruction [1]. This is further compounded by colonisation of the airway with organisms such as *Pseudomonas aeruginosa* (*P.*

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aeruginosa) and *Burkholderia cenocepacia* (*B. cenocepacia*) which are associated with a poor prognosis [2,3].

Inflammation caused by infection in the lungs plays a central role in the vicious cycle that leads to lung destruction. The most characteristic feature of inflammation in the CF lung is the persistent infiltration of massive numbers of neutrophils into the airways [4,5]. Neutrophils in the airways undergoing necrosis in situ are a major source of DNA, which makes CF sputum so tenacious [5]. The excessive accumulation of activated neutrophils in the lungs can lead to lung damage [6]. Proinflammatory chemoattractants have been found to be elevated in bronchoalveolar lavage fluid (BAL) from CF, including IL-8, IL-1, IL-6 and tumour necrosis factor alpha (TNFα) [7], leukotriene B_4 (LTB_4), complement factors, such as C5a, and bacterial products also act as potent chemoattractants for neutrophils [2]. Neutrophil elastase (NE) has also been shown to induce gene activation and secretion of IL-8 and digestion of C3b receptors on neutrophils, which limits the phagocytosis of pathogens [8]. In addition, levels of the anti-inflammatory cytokine IL-10 are decreased in BAL from CF patients [9]. These events in combination recruit more neutrophils to the lungs, which in turn recruit even more neutrophils setting up a perpetual inflammatory process, which ultimately causes irreparable lung damage in CF.

Neutrophils isolated from CF patients display a different pattern of response to inflammatory mediators to that observed from normals [10,11]. For example, neutrophils and eosinophils isolated from CF patients have been shown to have an altered arachidonic acid turnover [12], and an increased release of myeloperoxidase (MPO), eosinophil cationic protein (ECP), and eosinophil protein X (EPX) [13]. In addition, isolated peripheral neutrophils from CF patients generated higher levels of elastase when exposed to stimuli such as IL-8 and TNFα compared to neutrophils isolated from controls [14]. The chemotactic responsiveness of neutrophils to certain cytokines is also significantly lower in CF neutrophils compared to normal neutrophils [15–17]. Studies on bacterial products, such as *B. cepacia* lipopolysaccharide (LPS), have shown that the inflammatory nature of the *B. cepacia* infection in CF patients may contribute to increased neutrophil recruitment and priming of the neutrophil respiratory burst [17]. This study uses *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), a compound which mimics bacterial chemotaxins and acts via a cell surface receptor, and phorbol 12-myristate 13-acetate (PMA), an analogue of diacylglycerol which activates protein kinase C directly. These compounds were chosen to investigate the receptor- and nonreceptor-activated stimulation of superoxide generation and elastase release from peripheral blood neutrophils isolated from CF patients before and after an exacerbation compared to neutrophils from normal healthy age and sex matched controls.

The hypothesis for this study was that neutrophil activity, as evidenced by the spontaneous and stimulated release of neutrophil elastase and superoxide production, would be greater in CF patients before resolution of the exacerbation. Therefore, the aim of the present study was to elucidate the effects of cystic fibrosis exacerbations on the reactivity of peripheral blood neutrophils using fMLP and PMA to investigate the rate of stimulated superoxide generation and elastase release compared to neutrophils from a normal healthy control population.

2. Methods

2.1. Subjects

Patients were recruited from those admitted to the adult CF unit of the Belfast City Hospital for a pulmonary exacerbation defined as: increased purulent sputum, decrease in FEV_1 of 10% or greater from previous best, weight loss, and decreased energy. Patients also had a documented sweat test and/or genetic analysis confirming the CF diagnosis. Patients were studied before and after resolution of the CF exacerbation. CF patients were treated with antibiotics for a minimum of 2 weeks until lung function had significantly improved and had returned to a normal level for CF. All young normal volunteers were recruited from healthy students working in the Queen's University of Belfast, who were not taking any medication. Written informed consent was obtained from all participants and the local ethics committee approved the study. As far as we are aware, none of the antibiotics used to treat the CF patients influenced neutrophil function.