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Monocyte chemoattractant chemokines in cystic fibrosis

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Abstract

Background: Neutrophilic inflammation causes lung damage in cystic fibrosis (CF). Recent data from animal models suggest that the migration of blood monocytes into the airway supports neutrophil-mediated tissue injury. CF may therefore be associated with increased airway levels of chemoattractants for pro-inflammatory monocytes. In this study, we sought to assess the levels of monocyte chemoattractants CCL2 and CX3CL1 in the blood and airways of patients with CF, and expression of their respective receptors CCR2 and CX3CR1 on blood monocytes.
Methods: Blood was obtained from 32 CF patients and 25 healthy controls. Induced sputum was obtained from a further 24 CF patients and 17 healthy controls. Expression of CCR2 and CX3CR1 on CD14++CD16− and CD14+CD16+ blood monocytes was determined by flow cytometry. CCL2 and CX3CL1 levels in blood and induced sputum were determined by ELISA.
Results: Total blood monocyte concentration was not different between CF and controls. CCR2 was absent, and CX3CR1 higher on CD14+CD16+ monocytes from both CF and controls when compared with expression on CD14++CD16− cells. There was no difference in expression of chemokine receptors by either monocyte subpopulation between CF and controls. Blood CCL2, but not CX3CL1, was increased in CF patients ($p = 0.006$). Similarly, CF was associated with increased induced sputum CCL2, but not CX3CL1 (190.6 *vs.* 77.3pg/mL; $p = 0.029$).
Conclusion: CCL2, but not CX3CL1 is increased in the airway and blood of CF patients. Blood monocytes from CF patients are phenotypically competent to respond to CCL2, since they express normal levels of CCR2.

Keywords: CCL2; CX3CL1; Monocytes; Cystic fibrosis; Induced sputum

1. Introduction

Increased numbers of airway neutrophils are a characteristic feature of cystic fibrosis (CF), and contribute significantly to irreversible airway damage and loss of lung function [1,2]. Recent data from a murine model suggest that recruitment of pro-inflammatory blood monocytes into the airway synergistically enhances neutrophil transmigration into the lung [3]. From these data, we hypothesised that monocyte transmigration into the airway is increased in CF [4]. As a first step in testing this hypothesis, we sought evidence for increased levels of monocyte chemoattractants in the blood and airways of patients with CF. The current paradigm for blood monocyte migration into the lung is that CCL2, formally called monocyte chemoattractant protein-1, increases the tissue migration of monocytes with a propensity to initiate and sustain inflammation [5]. CX3CL1, formerly termed fractalkine, in contrast, tends to control the migration of monocytes that will become tissue macrophages and dendritic cells in non-inflamed tissue [5]. CCL2 has been detected in the blood and

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induced sputum from CF patients [6,7]. However, it is unclear whether monocyte chemoattractant chemokines are increased in CF, since no study to date has included normal controls.

Recruitment of blood monocytes into tissue depends on the expression of chemokine receptor on monocyte subsets. In humans, expression of the receptors for monocyte chemoattractant chemokines is not the same for all subpopulations of blood monocytes. Two important populations of blood monocytes are i) the major population of "classical" blood monocytes, which show high levels of cell surface CD14 and no CD16 expression (CD14++CD16− monocytes), and ii) a "minor" population of monocytes, characterised by low level CD14 and co-expression of CD16 (CD14+CD16+ monocytes [8], reviewed in Ziegler-Heitbrock [9]). Studies in the mouse model have shown that the homologues of both subpopulations of monocytes have the capacity to migrate to sites of inflammation [10–13]. Migration studies in man have demonstrated that the CD14+CD16+ subpopulation has a high ability for transendothelial reverse migration [14], and that these cells preferentially respond to CX3CL1 [15]. In contrast, CD14++CD16− monocytes, migrate towards CCL2 [16]. This differential migration behaviour of the two types of monocytes in normal humans corresponds to the differential pattern of chemokine receptors; CD14+CD16+ monocytes lack CCR2 and express high levels of CX3CR1, while the CD14++CD16− monocytes are CCR2 positive and show low levels of CX3CR1 [15,16]. To establish whether blood monocytes are phenotypically competent to respond to CCL2 or CX3CL1 in the CF airway, therefore requires receptor expression on both subpopulations to be assessed.

In this study, we sought evidence for increased monocyte chemoattractant activity in the CF airway. We first aimed to determine the levels of blood CCL2 and CX3CL1, and the pattern of receptor expression for these chemokines on blood monocytes. Second, we aimed to confirm findings using induced sputum samples. To assess the relevance of changes in CX3CL1 and CCL2, we also measured chemokine receptor expression on CD14+CD16+ and CD14++CD16− blood monocytes.

2. Methods

2.1. Subjects

CF patients were recruited in tertiary care. Diagnosis of CF was by an elevated sweat chloride level, and all patients had confirmatory testing for CF gene mutations. CF patients were recruited either during a routine outpatient visits, or during a hospital admission for intravenous antibiotic therapy. Clinical details were obtained from a review of the clinical notes. Controls were recruited from healthy volunteers. The study was approved by the local ethics committee and required written consent for adults, and assent and written parental consent for children. We initially compared the absolute counts of different monocyte subpopulations, serum CCL2 and CX3CL1, chemokine receptor expression for CCL2 and CX3CL1 on monocytes subpopulations between CF and healthy control groups. To confirm that changes in chemokines in the blood reflected changes in the airway, we then measured the concentrations of CCL2 and CX3CL1 in induced sputum samples.

2.2. Blood monocyte count

100μL of EDTA whole blood was stained with fluorescent-labeled antibodies for CD14, CD16 and HLADR using CD16 PE (BD Biosciences, Oxford), MY4-FITC (Beckman Coulter, High Wycombe), and HLADR-PC5 (Beckman Coulter) and incubated for 20min at 4°C. Red cells were lysed by 3mL of ammonium chloride based erythrocyte lysis buffer incubated at room temperature for 20min. 100μL of flow-count beads (Beckman Coulter, PN 7507992-E) was added to determine absolute counts. Monocytes were characterised as previously reported [8,9], using flow cytometry (FACSCalibur BD) and the gating strategy outlined in Fig. 1.

2.3. CCR2 expression on blood monocytes

100μL of EDTA whole blood was washed three times with 1mL of phosphate buffer solution (PBS) with 2% fetal calf serum (FCS) at 400g for 5min. Cells were incubated at 4°C for 20min with CD16 FITC (Dakocytomation, Ely), CD14 APC (Beckman Coulter), HLADR PerCP (BD Biosciences), to define CD14++CD16− and CD14+CD16+ subsets. CCR2 expression on the two monocyte subsets was determined using CCR2 PE (R&D Systems), and non specific fluorescence was determined using the isotype control for CCR2 PE; IgG2b PE (R&D systems). Red cells were lysed by adding 3mL of ammonium chloride based erythrocyte lysis buffer incubated at room temperature for 20min. Monocyte subsets were identified as described in Fig. 1. The median fluorescence intensity for CCR2 expression on CD14++CD16− and CD14+CD16+ monocytes was determined in CF and controls by subtracting the fluorescence obtained with the respective isotype controls (Fig. 2 A,B).

2.4. CX3CR1 expression on blood monocytes

100μL of whole EDTA blood was incubated at 4°C for 20min with CD16 PE (BD Biosciences), CD14 APC (Beckman Coulter), HLADR PerCP (BD Biosciences) and with either CX3CR1 FITC (Medical and Biological laboratories Co. LTD), or 20μL rat IgG2b FITC (isotype control for CX3CR1) (Medical and Biological Laboratories Co. LTD). Cells were washed with 1mL of PBS with 2% FCS at 400g for 5min. Red cells were lysed by adding 3mL of ammonium chloride based erythrocyte lysis buffer incubated at room temperature for 20min. Monocyte subsets were identified as described above, apart from the use of a different CD16 monoclonal antibody. The median fluorescence intensity for the CX3CR1 for CD14++CD16− and CD14+CD16+ subsets in CF and controls was determined by subtracting the fluorescence obtained with the respective isotype control (Fig. 2 A,B).

2.5. Blood CCL2 and CX3CL1

Blood CCL2 was determined using the human CCL2 ELISA kit (BD Biosciences, Oxford) according to the manufacturer's instructions. Samples were initially diluted 1:10 for analysis. Repeat analysis was carried out on undiluted serum samples if