Early inflammation in the airways of a cystic fibrosis foetus☆

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Abstract

In cystic fibrosis patients, inflammation is often considered to be secondary to chronic infections. In the present study, we show increased levels of pro-inflammatory proteins in the lungs of a cystic fibrosis foetus compared to the lungs of two normal foetuses. Our findings suggest therefore the existence of an early intrinsic pro-inflammatory state in cystic fibrosis airways.

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1. Introduction

Eighty to ninety percents of Cystic Fibrosis (CF) patients succumb to respiratory failure caused by chronic bacterial infection and concomitant airway inflammation [2]. The sequence of events at the onset of pulmonary infection and inflammation is controversial and not fully characterized. At birth, the lungs of CF patients appear normal [3,4]; however, autopsy examination of infants revealed a dilatation of acinar and duct lumens of sub-mucosal glands due to abnormal mucus secretion demonstrating pre-clinical signs in the very first months of life. Several studies also described the presence of neutrophils, elevated levels of elastase and IL-8, in the absence of any pathogen, in broncho-alveolar lavage fluids of CF newborns as compared to healthy individuals, opening the debate on the origin of this inflammation [2,5–7]. Indeed, the abnormal composition of airway secretions and/or their depletion are frequently cited as the host factors that predisposes CF patients to chronic colonization by P. aeruginosa and resultant inflammation. However, a recent study [8] showed that the over-expression of the epithelial Na+ channel ENac in mice bronchiolar epithelium reduced the volume of preciliary liquid leading to neutrophil influx and increased levels of IL-8 in airways. These results indicate that inflammation can arise from disregulated ion transport in airway epithelium in the absence of any infection. Moreover, several reports showed in various CF cell lines [9–12] an increased activation of the transcription factor NF-κB, a central player in inflammation, supporting the idea that CFTR dysfunction can by itself cause the expression of pro-inflammatory mediators [2,5–7,13–15]. Nevertheless, very little evidence for an inflammatory state existing before infection have been brought so far, leaving the debate open.

2. Case report

The examination of a 24 week-old foetus after pregnancy interruption for a hydrocephaly of unknown origin showed pancreatic histology signs evoking CF. There was not any sign of infection in the mother or the foetus. A genetic test highlighted the ΔF508 mutation on both CFTR alleles and
thus confirmed the CF diagnosis. To date, no relation has been established between hydrocephaly and cystic fibrosis.

Two foetuses were obtained after termination of the pregnancies either for a CMV foetopathy (22 weeks of amenorrhoea) or for a Turner syndrome (23 weeks of amenorrhoea) and were used as controls. Lungs were collected after the approval from the Institutional Review Board.

3. Methods

3.1. Immunohistochemistry and immunofluorescence

Studies were performed on paraffin-embedded material. Primary antibodies used were anti-ICAM-1, anti-Gro-β, anti-Gro-γ, anti-p65 (Santa-Cruz), anti-MMP1 (R and D system, France) and anti-COX2 (Cayman, MI).

Immunohistochemistry. A three-step indirect immunoperoxidase technique was used with the LSAB2 kit and DAB + (Dako) as the chromogen after heat-induced antigen retrieval in EDTA buffer (pH 9).

Immunofluorescence. After heat-induced antigen retrieval in sodium citrate buffer (10 mM, pH 6), sections were stained with primary antibody followed by incubation with Alexa-Fluor 488 (Molecular Probes, Leiden, The Netherlands). For nuclear DNA staining, TOTO-3 iodide (Molecular Probes, Leiden, The Netherlands) was added to the secondary antibody solution. Confocal microscopy analyses were performed with a TCS SP confocal microscope (Leica) as described [16].

4. Results and discussion

It is debated whether pulmonary inflammation precedes or follows infection in CF. The lungs of patients with CF are inflamed and infected at a young age, and it is difficult to exclude the presence of all pathogens. Therefore, to investigate a putative early pro-inflammatory state, immunohistological studies were undertaken to compare the expression levels of known pro-inflammatory proteins in the lungs of a 24 week-old CF foetus homozygous for the ΔF508-CFTR mutation and in control lungs from two foetuses at similar development stages. Due to scarcity of such human material, this is the first study of CF foetal lungs.

Since an early pathological hallmark of CF inflammatory response consists of neutrophil influx into the airways, we analysed the expression of the intracellular adhesion molecule-1 (ICAM-1) and the chemokines Gro-β and Gro-γ, three pro-inflammatory molecules known to be involved in neutrophil extravasation and chemotaxis [17,18]. We also studied the expression of the major pro-inflammatory enzyme Cox-2 and of the matrix metalloproteinase 1 (MMP-1) that by degrading extracellular matrix proteins may play a role in airway remodeling.

ICAM-1 expression was higher in CF bronchial epithelial cells as compared to control non-CF foetal lung epithelia; ICAM-1 was mainly localized at the apical membrane of these bronchial cells (Fig. 1A and B). High ICAM-1 protein levels were previously observed in adult CF lungs, small intestines and proliferating bile-duets [19–21]. Increased Gro-γ expression was observed in CF foetal lungs, specifically in monocytes and endothelial cells (Fig. 1G and H). In contrast, Gro-β expression was identical in CF and control foetal lungs (Fig. 1E and F). The role of monocytes in CF inflammation has been suggested by Zaman et al. [22] who showed that basal and LPS-induced IL-8 expression by CF monocytes is higher than IL-8 secretion by normal monocytes.

The enzyme Cox-2 plays a central role in inflammation [23–25]. Its over-expression was observed in bronchiolar epithelial cells from CF lungs in comparison with development-matched control lungs (Fig. 1C and D). MMP-1 was found to be expressed in the CF pulmonary cartilage while it was not detected in the cartilage of control foetuses (Fig. 1I and J). Although an over-expression of MMP-1 has never been described in CF patients, increased levels of this matrix metalloproteinase is associated with several human diseases such as cancer, autoimmune disorders, COPD and asthma [26–28]. Moreover, an increased MMP-1 expression has also been shown in cartilage tissue of patients with osteoarthritis [29].

Interestingly, despite of the absence of any histological sign of inflammation, the CF foetal lungs thus over-expressed MMP-1, Groγ, ICAM-1 and Cox-2, four molecules known to be regulated by NF-κB [30–33]. NF-κB is an inducible transcription factor that plays a central role in the regulation of immune and inflammatory responses. While NF-κB is required for cell survival and immunity, prolonged NF-κB activation seems essential for the persistence of chronic inflammatory diseases [34–36]. Constitutive activation of this inflammatory transcription factor has been already associated with CF [10–12]. We therefore determined whether the CF foetal lungs displayed higher NF-κB activation than control lungs. For this purpose, subcellular immunolocalization analyses of p65 were performed (Fig. 2A and B); these analyses revealed a cytoplasmic localization of p65 both in CF (Fig. 2B) and non-CF (Fig. 2A) lung cells. A nuclear staining of p65 was only observed in the CF lung epithelial cells (Fig. 2B). This result indicated that NF-κB is activated in the CF foetal lungs. Our findings therefore suggest that, already in CF foetal lungs, CFTR dysfunction leads to NF-κB activation which enhances the expression of specific pro-inflammatory proteins.

To our knowledge, this is the first report of in vivo evidence for a pro-inflammatory process initiated very early in the pathogenesis of cystic fibrosis before any infection. This intrinsic inflammation is probably mild and likely results from a deregulated NF-κB activation which may be the starting point for subsequent pathological pulmonary dysfunction. The identification of the molecular mechanisms linking mutated CFTR to the NF-κB activation pathway will be crucial to define novel anti-inflammatory strategies for CF patients. Indeed, as inflammation and neutrophil influx are two major pathophysiological features of pulmonary CF disease, any therapy that reduces the production of pro-
inflammatory molecules could, if applied early, prevent the chronic inflammation and the degradation of lung function. Many NF-κB inhibitors are currently being developed and tested in various conditions. Our observations and previous reports provide a rationale for further studies on in vivo NF-κB activity before infection in CF lungs and possibly for clinical trials with specific and tolerable NF-κB inhibitors.

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References


Fig. 1. Over-expression of pro-inflammatory proteins in a CF foetal lung. Control (A, C, E, G, I) and CF (B, D, F, H, J) foetal lungs were stained for ICAM-1 (A, B), COX-2 (C, D), Gro-β (E, F), Gro-γ (G, H) and MMP-1 (I, J) expressions. Arrows indicate over-expressed pro-inflammatory molecules in bronchiolar epithelial cells (A–D), in endothelial cells (G, H) or in cartilage (I, J). Arrowheads indicate Groγ expression in monocytes. Original magnification, ×200 (A–F, I–J), ×400 (G, H).

Fig. 2. Nuclear localization of p65 in the CF foetal lung. Control foetal lungs (A) and ΔF508-CFTR foetal lungs (B) were stained for p65 (A, B) localization. Cell nuclear DNA was stained with TOTO-3 (C–D). Original magnification, ×63.