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Original Article

Bronchial epithelial cell lines and primary nasal epithelial cells from cystic fibrosis respond differently to cigarette smoke exposure☆

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Abstract

The effects of cigarette smoke extract (CSE) on airway epithelial cells (AECs) from cystic fibrosis (CF) and non-cystic fibrosis (non-CF) individuals are not fully understood. It has been suggested that CSE modulates inflammatory cytokine release from AECs by modulating the epidermal growth factor receptor (EGFR) pathway; these pathways could reveal novel therapeutic targets. We compared the effect of CSE pre-incubation on IL-8 release from CF and non-CF bronchial epithelial cell lines, and separately, with primary nasal epithelial cells (NECs) retrieved from CF and non-CF individuals. We also determined if the EGFR pathway regulates IL-8 release by LPS or cytomix in non-CF and CF AECs at baseline and following CSE exposure.

CF and non-CF cell lines, NECs derived from both CF patients (R117H heterozygous and F508del homozygous), and from healthy subjects, were cultured in the presence or absence of CSE, and subsequently exposed to inflammatory stimuli. In cell lines CSE significantly reduced IL-8 release following inflammatory challenge. Conversely, CSE pre-treatment was pro-inflammatory in primary NECs. In NECs from control subjects, CSE increased cytomix and LPS induced IL-8 release, and for the R117H heterozygous NEC cultures, CSE enhanced basal IL-8 release. Cytomix and LPS induced IL-8 release from F508del homozygous NEC cultures was further heightened following CSE pre-treatment.

EGFR inhibition mitigated IL-8 release from immortalised and primary non-CF and CF AECs, suggesting that constitutive and CSE elicited IL-8 release from AECs is partly regulated via the EGFR pathway.

This study demonstrates the importance of the EGFR cascade in the regulation of constitutive and CSE induced inflammatory mediator release from immortalised and primary AECs. Moreover, it clearly highlights the significance of using primary cells to confirm results obtained from immortalised cell studies, as these model systems may respond very differently to the stimuli under investigation.

Keywords: CSE; Inflammation; Cytokine; Airway; Epithelial; Bronchial; Nasal; Cells; EGFR; IL-8; Release; Immortalised; Primary; Patients; F508del; R117H; Healthy

1. Introduction

Since the identification of the CFTR gene over 25 years ago, significant progress has been made in the understanding and treatment of cystic fibrosis (CF) lung disease. Nevertheless, the mechanisms by which CFTR mutations lead to hyper-inflammation in CF still remain to be elucidated. Various methods to date have been employed to study the effects of reduced/dysfunctional CFTR expression on airway inflammation

Abbreviations: AECs, airway epithelial cells; AEGM, airway epithelial growth media; CSE, cigarette smoke extract; EGFR, epidermal growth factor receptor; NECs, nasal epithelial cells.

☆ Category of study: basic science.

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in CF. These include both the genetic and pharmacological inhibition of CFTR expression and function in non-CF and CF derived AECs via the actions of siRNA [1], and the specific CFTR inhibitor, CFTR(inh)-172, respectively [2]. Findings from these studies suggest that alterations in CFTR expression and function may contribute to the hyper-inflammatory state observed in the CF airway. Nevertheless, due to technical difficulties, such as the silencing of CFTR expression in primary AECs, the majority of research has been conducted using transformed and immortalised AECs which may not be reflective of primary AECs.

Cigarette smoke (CS) exposure has been shown to simulate aspects of CF pathophysiology, such as depletion of the airway surface liquid, leading to the dehydration of the airway epithelium [3]. Additionally, chronic obstructive pulmonary disease (COPD), which is more commonly caused by cigarette smoking in this part of the world, has clinical features similar to that of CF, such as airway bacterial colonization and prolonged and heightened airway inflammation [4,5]. It has been suggested that this CF-like phenotype in COPD can be attributed to CS induced alterations in CFTR expression and function [6,7]. CS is a complex mixture of over 4700 chemicals and oxidants [8–10], many of which can form reactive oxygen species capable of directly modifying CFTR via various chemical reactions, which include oxidation and nitration of CFTR. This leads to a reduction in CFTR expression and function in AECs [11]. Furthermore, CS modulates gene and protein expression of various cytokines, including IL-8 [12]. This ability of CS to regulate cytokine production by the airway epithelium has been attributed to modulation of various signalling cascades, including the epidermal growth factor receptor (EGFR) signalling pathway [13,14]. Interestingly abnormalities in this pathway have also been implicated in CF pathogenesis [15–17], with recent findings suggesting that CFTR inhibition leads to enhanced basal IL-8 secretion, as a consequence of EGFR pathway activation [18]. These findings overall suggest that a reduction in CFTR expression and function, which occurs in CF or via the effects of CS, contributes to heightened airway inflammatory responses (e.g. IL-8 release) through the induction of the EGFR pathway.

In this study we hypothesised that inflammatory responses following cigarette smoke extract (CSE) exposure were similar, both from cultures derived from airway epithelial cell lines, and equivalent cultures from primary nasal epithelial cells. In addition, we aimed to determine if these responses were regulated via the EGFR pathway.

2. Methods

2.1. Cell line culture

Non-CF and CF immortalised bronchial epithelial cell lines; 16HBE14o$^-$ and CFBE41o$^-$ (F508del homozygous) (generous gift from Dr Dieter C. Gruenert, California Pacific Medical Centre Research Institute, San Francisco, California) were grown and maintained in MEM Earl's salt L-Glutamine (200 mM L-Glutamine) medium containing 1% Pencillin/Streptomycin and 10% foetal bovine serum (FBS). For studies 16HBE14o$^-$ and CFBE41o$^-$ cells were plated onto collagen (purecol®) coated 24 well plastic culture plates at 0.8×10^5/ml and 0.6×10^5/ml respectively.

2.2. Subjects

NEC samples were obtained from 20 CF patients; 10 R117H heterozygotes and 10 F508del homozygous individuals, with 10 NEC samples obtained from healthy volunteers. Table 1 provides demographic information on the subjects. Non-CF subjects had no history of persistent respiratory symptoms and were non-smokers. Written consent was obtained prior to sampling with each volunteer receiving a detailed participant information sheet. The study was approved by the Research Ethics Committee of Queen's University Belfast.

2.3. Isolation of primary nasal epithelial cells (NECs)

Nasal brushings were obtained from F508del homozygous, R117H heterozygous and non-CF healthy individuals. NECs were then processed and cultured using methods developed in our laboratory, which resulted in homogenous cultures of basal epithelial cells, as fully described in our previous publication [19].

Viable cells were seeded at a density of 0.8×10^5 onto collagen-coated 24 well plates for the experiments.

2.4. Preparation of cigarette smoke extract (CSE)

CSE was prepared by utilising a protocol described by Comer et al. [20]. For studies with the cell lines only, FBS (at a final concentration of 10%) was added to the 100% CSE suspension. Both cell lines and primary cells were pre-exposed to CSE for 4 h before stimulation.

Table 1
Study participant demographics. Demographics of non-CF and CF participants in the CSE studies. Data represent median (range).

	Group 1: Non-CF, healthy individuals (n = 10)	Group 2: R117H/F508del heterozygous CF patients (n = 10)	Group 3: F5808del homozygous CF patients (n = 10)
% Female	50	40	60
Median age	27 (20, 29.5)	29 (28, 35)	25 (15, 35)
Median BMI	25.3 (22.4, 26.8)	24.5 (21.6, 27.5)	22.3 (20.7, 26.3)
FEV1 (% predicted)	–	82.5 (70, 95.3)	50 (36, 62)
FVC (L)	–	10.02 (8.92, 12.43)	4.03 (2.49, 3.65)
Sputum + ve for *P. aeruginosa*	–	–	10/10

BMI = body mass index.
FEV1 = forced expiratory volume in 1 s.
FVC (L) = forced vital capacity, measured in litres.
– = data not available.
Data shown are median (range).