Cystic fibrosis (CF) is a common lethal inherited disease of Caucasians, with a carrier frequency of 1 in 25 in the U.K. It is characterised by viscous mucus secretions in the pancreatic ducts and lungs, leading to pancreatic malfunction and chronic lung disease. The CF gene protein, CFTR, is a transmembrane conductance regulator (CFTR) that is a membrane protein which acts as a cyclic AMP dependent Cl channel [1]. Our studies [2-4], which have been confirmed by other investigators [5-7] have shown that CFTR also regulates other ion channels, including Na and other Cl channels [8]. At least 500 mutations have been identified in the CF gene of which the most common, which is present on approx. 70% of CF chromosomes in the U.K., results in loss of a phenylalanine residue at position 508 in the protein (ΔF508-CFTR). In cells transfected with wild type or ΔF508-CFTR, it has been shown that ΔF508-CFTR is not fully glycosylated and is incorrectly processed [9]. However, mutant protein reaches the apical surface (AF508-CFTR). In cells transfected with wild type or AF508-CFTR, this has been shown that AF508-CFTR is not fully glycosylated and is incorrectly processed [9]. However, transfected cells overexpress the protein compared to native epithelial cells where studies are somewhat conflicting as to the degree of mislocalisation of ΔF508CFTR and whether any of the mutant protein reaches the apical surface [10-12]. The aim of the present study was to directly immunolocalise AF508-CFTR in native airway epithelial cells from CF patients homozygous for the ΔF508 mutation, using confocal microscopy.

The CFTR antibody used for immunolocalisation of CFTR was raised against a 22 amino acid C-terminal peptide [4,13]. It has been well characterised, showing cross reaction in C127 cells transfected with CFTR but not in mock transfected cells. Surface nasal epithelial cells were digested from nasal polyp tissues with protease XIV for 1-2h at 37°C and cultured for 6-8 days on 0.45μm Falcon cell culture inserts. Cells were fixed with 4% paraformaldehyde, permeabilised with 0.2% Triton X-100 and incubated with first antibody against CFTR (rabbit polyclonal) or the known apical membrane protein CD59 (mouse monoclonal) for 18h at 4°C, followed by labelled second antibodies (FITC-anti-rabbit for CFTR; cy3-anti-mouse for CD59) for 1h at room temperature. Filters were mounted face-up on glass slides, sealed under coverslips and viewed with the confocal microscope.

Fig. 1 shows localisation of ΔF508-CFTR (a) and CD59 (b) in an apical section, 1μm from the cell surface. ΔF508-CFTR is also clearly present on or very close to the apical surface where the apical membrane protein CD59 is located. This data is in agreement with others showing that AF508-CFTR is present at the apical surface of airways cells [11,14,15], although it was not detected at the apical surface of CF sweat duct cells [10]. In a section through the interior of the cell, 8μm from the apical section, a large amount of AF508-CFTR was present whereas CD59 was absent (data not shown).

The first direct demonstration that some ΔF508-CFTR is colocalised with a known apical membrane protein in native airways cells, provides the opportunity to use this as a model for pharmacological manipulation by direct activation of AF508-CFTR and/or by increasing ΔF508-CFTR trafficking.

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