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Original Article

# Progress in understanding mucus abnormalities in cystic fibrosis airways☆

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**Abstract**

Normal airways below the carina maintain an essentially sterile environment via a multi-pronged innate defence system that includes mucus clearance via mucociliary clearance and cough, multiple antimicrobials and cellular components including macrophages and neutrophils. In cystic fibrosis (CF), loss of CFTR function compromises these defences, and with present standard of care virtually all people with CF eventually develop mucus accumulation, plugging and chronic infections.

This symposium focused on how mucus is affected by CFTR loss.


*Keywords:* Cystic fibrosis; Mucus; Mucociliary clearance; $HCO_3^-$; Hypertonic saline; Submucosal glands; Microscopic optical coherence tomography; Ferret; CF piglet; β-ENaC transgenic mouse

## 1. Introduction

Normal airways below the carina maintain an essentially sterile environment via a multi-pronged innate defence system that includes mucus clearance via mucociliary clearance and cough [3], multiple antimicrobials [4] and cellular components including macrophages and neutrophils. In cystic fibrosis (CF), loss of CFTR function compromises these defences, and with present standard of care virtually all people with CF eventually develop chronic airway infections, mucus accumulation and plugging. It is now clear from work on CF animal models that mucus abnormalities precede infection and provide the conditions for it, but once started, infection induces an additional cascade of events that further exacerbates mucus abnormalities leading to increased production of altered mucus that is even more difficult to clear. Thus, high amounts of stagnate mucus accumulate in later stages of cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) in association with goblet cell hyperplasia and metaplasia. Such alterations are induced in part by neutrophil elastase, which is increased in both CF and COPD. Given the 'vicious cycle' of infection-inflammation-mucus stasis-and back to infection, one goal of CF therapy is to mobilize airways mucus [5,6].

Numerous recent articles have examined mucus structure and transport in airways [6–8]. This review is mainly focused on recent studies of how mucus is affected by CFTR loss; for a thorough review of earlier work see ref [9]. Mucus properties are determined by the types of mucins that are assembled from mucin monomers (in airways MUC5B and MUC5AC), by their concentrations, and by other ions and proteins. For defence of the airways from infection, MUC5B is essential [10]. Mucins are exceedingly long molecules that display a vast array

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of polysaccharide side chains. These mucins together with other proteins form mucus that trap bacteria and other particles and, in healthy airways, transport them out of the airways. In airways mucins are produced by surface club cells, goblet cells and mucus cells of submucosal glands, in varying degrees depending upon airway level and species. In CF there is as yet no evidence that mucin polymers are affected, but their unfolding is likely dramatically changed by the loss of anion-mediated fluid secretion [11] as also observed in the small intestine [12], possibly by anion transport across mucin granule membranes [13], and by surface dehydration [14].

## 2. Gland secretion and mucus clearance

In airways, mucociliary clearance is driven by the continuous beating of the cilia that move the airway surface liquid (ASL) cephalically. Because fluid absorption in CF airways continues (or is possibly increased [15]) while fluid secretion is diminished, the net effect is mucus with a higher concentration of solids, increased viscoelasticity, and decreased ability to be cleared [16], all of which is expected to further exacerbate the initially defective mucin unfolding alluded to above. In addition, tethering of mucus to gland openings and other regions of the airway surface further reduce clearance [12,17]. Mucus stasis can also be produced by mutations that increase fluid absorption via increased ENaC activity [18]. Although diminished mucociliary clearance in CF has been studied by many laboratories, there is still no consensus about the relative importance of the multiple processes that follow the loss of CFTR function. The view proposed here is that the major effect of impaired CF airway mucus clearance is to predispose them to chronic airway infections. The dire consequences of chronic CF airway infections are well known, and are the main reason for early death in CF, with milder pathology resulting from sterile inflammation caused by mucus plugging.

In healthy airways, mucus secretion from glands and mucus transport rates are stimulated synergistically by combined agonists that increase cytosolic cAMP and $Ca^{2+}$. Mucus from submucosal glands, which is rich in antibacterial compounds is critical for keeping the airways clean [19]. In CF, glands are defective in at least 4 ways: they display decreased anion-mediated fluid into the glands [20], decreased $HCO_3^-$ that leads to lower pH and altered mucus properties [11], decreased antimicrobial effectiveness [21] and decreased mucus clearance—due in part to tethering [17]. Indeed, gland secretion to VIP or other $[cAMP]_i$–elevating agonists is entirely missing in human CF glands [20].

Stimuli that increase gland secretion also stimulate mucociliary clearance velocity (MCCV) ex vivo [22]. Agonists that increase $[Ca^{2+}]_i$ and $[cAMP]_i$ each stimulate gland secretion, and synergistically increase secretion when combined [20]. Synergy has multiple mechanisms [23], and some synergy persists in $CFTR^{-/-}$ pig glands [24]. These results were extended by investigating agonist effects on MCCV of the ferret trachea, looking for the involvement of CFTR and ENaC in MCCV and the possibility that synergy is also seen in MCCV. Ferrets have abundant submucosal glands, large trachea, and $CFTR^{-/-}$ ferrets develop CF-like lung infections [25]. Both forskolin and carbachol stimulated MCCV. $CFTR_{inh}$-172 inhibited forskolin-stimulated but not carbachol-stimulated MCCV, while inhibition of ENaC with benzamil increased MCCV produced by either agonist. Most remarkably, combination of a low dose (0.3 μM) of carbachol with forskolin synergistically increased MCCV to ~35 mm/min, more than two times the additive effects of the two agonists [2]. Of interest, this value is similar to the additive effects of the two agonists given singly in the presence of ENaC inhibition, suggesting that inhibition of ENaC might play a role in synergy. Indeed, carbachol produces a long-lasting inhibition of ENaC-mediated fluid absorption in airway epithelium of several species [1]. Preliminary data (Joo, unpublished) show that the same agonist combination synergistically increased gland mucus secretions and decreased ENaC-mediated $I_{sc}$ (a marker of fluid absorption), but did not induce bronchoconstriction. An overview is shown in Fig. 1.

## 3. Role of mucus bundles

The traditional view of mucus clearance has been that a more or less continuous blanket (or film) of mucus floats at the air/liquid interface and is propelled by cilia. However, recent observations expand this view by showing that mucus bundles, formed in submucosal glands, sweep and clean the normal lung surface [26,27]. How are these strands formed? MUC5B mucin of the tracheobronchial submucosal glands is converted from a densely packed form in the mucin producing cell granules into long, linear polymers by the flow generated from the serous acinar cells in the distal gland. The linear MUC5B polymers bundle into thick bundles with > 1000 polymers when they appear at the submucosal gland openings as visualized with Alcian blue staining [26,28]. In contrast to pig and human that have numerous submucosal glands, mice which lack submucosal glands below the uppermost trachea, instead reveals Alcian blue stained mucus ‘clouds’ (Ermund, unpublished), presumably representing unbundled mucins originating from mouse club cells [29].

In wild type (WT) piglet trachea, the mucus bundles clean the normal lungs and are transported ventrally and cephalically by the cilia [28]. However, these bundles are essentially unmovable in new-born piglets lacking a functional CFTR channel (CF piglets) or in WT tracheas analysed in buffer lacking $HCO_3^-$ [12]. By using antibody and lectin staining, the mucus bundles were shown to have a core made up of the MUC5B mucin. Interestingly, these bundles were coated with MUC5AC mucin from goblet cells found in the last part of the gland ducts and on the tracheal surface. The reason for non-movable mucus bundles in CF pigs is still not fully understood, but it is suggested that the MUC5AC mucin traps the CF mucus strands to surface goblet cells [28].

As mentioned above, elevated neutrophil elastase increases goblet cell production of MUC5AC and contributes to mucus stasis. This phenomenon was studied in a mouse model where inhaled elastase induced goblet cell hyperplasia and mucus-mediated airway obstruction (Fernandez et al., unpublished). Proteomic analysis of the mucus plugs in this model revealed high levels of MUC5B and dramatically increased levels of MUC5AC

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