EDITORIAL
BASIC SCIENCE

Getting neural about airway gland secretion

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Alterations in host defence functions of the airway epithelium play a central role in chronic airway diseases [1] by contributing to airway mucus obstruction, microbial dysbiosis and chronic airway inflammation. The contribution of altered epithelial functions to the pathogenesis of airway diseases is well illustrated in cystic fibrosis (CF), a monogenetic disease caused by inherited mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [2]. Because of diminished CFTR-dependent chloride and fluid secretion by the airway epithelium, CF patients exhibit airway dehydration, mucus obstruction and decreased mucociliary clearance. In addition, reduced CFTR-mediated bicarbonate transport may decrease the activity of pH-sensitive antimicrobial proteins and peptides (AMPs) due to acidification of the airway surface liquid. Impaired mucus clearance and reduced activity of AMPs may contribute to selective outgrowth of respiratory pathogens in CF airways, which may trigger airway epithelial inflammatory responses. CFTR dysfunction may also promote airway epithelial inflammation, independently of infection [3]. Recent studies have shown that the absent/defective CFTR-dependent epithelial ion transport processes, together with inflammatory responses, contribute to an airway muco-inflammatory milieu in early CF lung disease [4].

In addition to functional alterations of CF superficial airway epithelia, imbalances in host defence properties of submucosal gland cells may contribute to the pathogenesis of CF lung disease. Submucosal glands are mainly located in the upper airways and consist of gland acini, which secrete fluid, mucus and AMPs into the airway surface liquid *via* gland ducts [5]. Besides myoepithelial stem cells and mucous cells, submucosal glands contain serous cells, which are specialised secretory cells. Serous cells are characterised by the expression of CFTR and production and secretion of several AMPs, including lysozyme, lactoferrin and human β-defensin 1. The secretory properties of gland serous cells are controlled by parasympathetic neurons that innervate the airway glands [6]. These neurons release multiples neuropeptides, including vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY). VIP activates CFTR *via* rises in intracellular cyclic adenosine monophosphate (cAMP) levels [7], and alterations of its effects on CFTR-expressing serous cells may have important implications for CF. On the other hand, NPY has been associated with psychological stress-induced Th2 inflammation in patients with asthma [8]. Studies in murine models demonstrated a contribution of NPY to inflammatory responses in

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allergy-induced asthma [9, 10], further suggesting a role for NPY in asthmatic airways. These studies led to the notion that NPY regulation of serous cell function may be a contributing factor in asthma pathogenesis.

In this issue of the *European Respiratory Journal*, McMahon *et al.* [11] conducted an extensive study on secretory properties of serous cells in response to VIP and NPY, which advanced our understanding of how imbalances in neuropeptide activities may contribute to chronic airway diseases, particularly CF. By using freshly excised serous cells and serous cell monolayers cultured under air-liquid interface (ALI) conditions, McMahon *et al.* [11] have shown that VIP induced chloride and bicarbonate transport in serous cells, resulting in increased fluid secretion and regulation of fluid pH. Both responses were absent in serous cells from CF patients or upon pharmacological inhibition of CFTR in non-CF cells, demonstrating the importance of CFTR in serous cell secretory function. In addition, VIP promoted serous cell secretion of AMPs, independently of CFTR activation. Additional studies with NPY demonstrated that it suppressed the upregulation of cAMP levels induced by VIP, resulting in suppression of CFTR activity and reduced secretion of AMPs by serous cells. Because NPY release is not restricted to neurons, and can also be derived from macrophages, the authors performed co-culture studies with ALI-cultured serous cells and macrophages and found that NPY from macrophages could attenuate VIP-induced CFTR activity. Moreover, NPY released from macrophages attenuated the secretion of AMPs and reduced the antimicrobial activity of apical surface washings of serous cell cultures. Further studies focused on the effect of neuropeptides on inflammatory responses by serous cells. NPY amplified microbial- and cytokine-induced expression and release of pro-inflammatory mediators by serous cells. In contrast, VIP displayed anti-inflammatory effects on serous cells by reducing inflammatory responses induced by microbial stimuli or NPY (*e.g.* blunting of NYP-increased release of interleukin (IL)-1β). Pharmacological inhibition of CFTR blunted the anti-inflammatory effects of VIP, supporting the notion that CFTR dysfunction contributes to sterile inflammation [3]. Notably, McMahon *et al.* [11] also reported that CFTR activity inversely correlated with the secretion of IL-1β by serous cells. These findings have implications for the pathophysiology of CF airway disease associated with inflammation and mucus production. For instance, IL-1β has recently been characterised as the predominant promucin secretory cytokine in mucopurulent secretions from CF patients and responsible for induction of robust mucous cell metaplastic responses in superficial airway epithelia [12]. Hence, it can be speculated that CFTR dysfunction in serous cells blunts the anti-inflammatory action of VIP, thereby contributing to mucous cell metaplasia in CF airways *via* increased secretion of IL-1β [4].

The studies by McMahon *et al.* [11] also suggest that imbalances in the functional properties of VIP and NPY on serous cells may be extended to the pathogenesis of other airway diseases. For example, it has been proposed that patients with COPD may suffer from acquired CFTR dysfunction due to the effects of smoking [13]. This view leads to the hypothesis that cigarette smoking may attenuate CFTR function in serous cells, although submucosal glands could be protected from the direct cytotoxic effects of cigarette smoke due to their location underneath the airway surface. Further studies are clearly necessary to examine whether serous cell functions are affected in COPD patients.

While additional studies are needed to determine whether NPY contributes to CF or COPD airway disease, McMahon *et al.* [11] have shown that NPY enhanced pro-inflammatory responses in serous cells induced by the asthma-relevant Th2 cytokines IL-4 and IL-13. Their findings are in agreement with earlier studies demonstrating similar effects of NPY on inflammatory responses resulting from allergy-induced asthma in mice [9, 10]. The effects of NPY may also have important implications for asthma therapy with β_2 adrenergic agonists, which similar to VIP, act by raising intracellular cAMP. Enhanced NPY levels could suppress the efficacy of β_2 adrenergic agonists, which have been proposed to increase epithelial fluid secretion in a CFTR-dependent manner [14]. However, β_2 adrenergic agonists could also exhibit anti-inflammatory effects on serous cells, suppressing the pro-inflammatory effects of NPY. Further studies are necessary to evaluate whether NPY blunts the efficacy of asthma therapeutics and, thus, may be a therapeutic target for asthma.

Potential therapeutic strategies to recover the function of serous cells have also been addressed by McMahon *et al.* [11], as they demonstrated that activation of the calcium-activated ion channel TMEM16A circumvents impaired VIP-induced secretion due to defective CFTR function. As proof-of-concept, TMEM16A activation restored fluid secretion, airway surface liquid pH regulation and the anti-inflammatory activity of VIP. Therefore, recently generated TMEM16A potentiators [15] may be promising novel therapies to restore impaired serous cell functions in CF.

In summary, the study by McMahon *et al.* [11] furthered the understanding of serous cell functions and how their alterations might contribute to the pathogenesis of airway diseases. A key area of research that remains to be addressed is whether serous cell functions have an impact on the function of other epithelial cells,

including gland mucous cells and the superficial epithelium. The utilisation of complex *in vitro* culture systems will likely enable the assessment of molecular mechanisms that might mediate the crosstalk between serous cells and gland mucous cells or superficial epithelial cells. The recent characterisation of myoepithelial epithelial cells as gland stem cells may enable the development of novel gland models [16, 17]. For instance, gland stem cells can potentially be cultured as three-dimensional organoids, as recently shown with superficial airway epithelial cells [18]. Such a model may not only be used to further the understanding of how imbalanced activities in submucosal glands contribute to chronic airway disease, but may also provide a platform to test therapies, such as TMEM16A potentiators.

Conflict of interest: None declared.

References

1 Hiemstra PS, McCray PB, Bals R. The innate immune function of airway epithelial cells in inflammatory lung disease. *Eur Respir J* 2015; 45: 1150–1162.
2 Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. *N Engl J Med* 2015; 372: 351–362.
3 Montgomery ST, Mall MA, Kicic A, *et al.* Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies. *Eur Respir J* 2017; 49: 1600903.
4 Esther CR Jr, Muhlebach MS, Ehre C, *et al.* Mucus accumulation in the lungs precedes structural changes and infection in children with cystic fibrosis. *Sci Transl Med* 2019; 11: eaav3488.
5 Widdicombe JH, Wine JJ. Airway gland structure and function. *Physiol Rev* 2015; 95: 1241–1319.
6 Wine JJ. Parasympathetic control of airway submucosal glands: central reflexes and the airway intrinsic nervous system. *Auton Neurosci* 2007; 133: 35–54.
7 Lee RJ, Foskett JK. cAMP-activated Ca2+ signaling is required for CFTR-mediated serous cell fluid secretion in porcine and human airways. *J Clin Invest* 2010; 120: 3137–3148.
8 Lu Y, Ho R, Lim TK, *et al.* Neuropeptide Y may mediate psychological stress and enhance TH2 inflammatory response in asthma. *J Allergy Clin Immunol United States* 2015; 135: 1061–1063.
9 Makinde TO, Steininger R, Agrawal DK. NPY and NPY receptors in airway structural and inflammatory cells in allergic asthma. *Exp Mol Pathol* 2013; 94: 45–50.
10 Oda N, Miyahara N, Taniguchi A, *et al.* Requirement for neuropeptide Y in the development of type 2 responses and allergen-induced airway hyperresponsiveness and inflammation. *Am J Physiol Lung Cell Mol Physiol* 2019; 316: L407–L417.
11 McMahon DB, Carey RM, Kohanski MA, *et al.* Neuropeptide regulation of secretion and inflammation in human airway gland serous cells. *Eur Respir J* 2020; 55: 1901386.
12 Chen G, Sun L, Kato T, *et al.* IL-1β dominates the promucin secretory cytokine profile in cystic fibrosis. *J Clin Invest* 2019; 129: 4433–4450.
13 Raju SV, Solomon GM, Dransfield MT, *et al.* Acquired cystic fibrosis transmembrane conductance regulator dysfunction in chronic bronchitis and other diseases of mucus clearance. *Clin Chest Med* 2016; 37: 147–158.
14 Salathe M. Effects of beta-agonists on airway epithelial cells. *J Allergy Clin Immunol* 2002; 110: S275–S281.
15 Danahay HL, Lilley S, Fox R, *et al.* TMEM16A potentiation: a novel therapeutic approach for the treatment of cystic fibrosis. *Am J Respir Crit Care Med* 2020; in press [https://doi.org/10.1164/rccm.201908-1641OC].
16 Tata A, Kobayashi Y, Chow RD, *et al.* Myoepithelial cells of submucosal glands can function as reserve stem cells to regenerate airways after injury. *Cell Stem Cell* 2018; 22: 668–683.
17 Lynch TJ, Anderson PJ, Rotti PG, *et al.* Submucosal gland myoepithelial cells are reserve stem cells that can regenerate mouse tracheal epithelium. *Cell Stem Cell* 2018; 22: 653–667.
18 Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, *et al.* Long-term expanding human airway organoids for disease modeling. *EMBO J* 2019; 38: e100300.