

# Novel insights into the effects of cigarette smoke on the airway epithelial surface – lessons learned at the European Respiratory Society International Congress 2018 in Paris

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The airway epithelium displays a multitude of host defense mechanisms against micro-organisms including mucociliary clearance, antibacterial activity of secreted proteins, and maintenance of the epithelial barrier integrity. Cigarette smoke (CS) exposure of airway epithelial cells is proposed to impair these host defense properties, thereby increasing the susceptibility towards microbial infections, and furthermore initiating epithelial remodeling and airway inflammation. During the 2018 ERS Congress novel data were presented, that corroborate the detrimental outcomes of exposure to CS or the presence of chronic obstructive pulmonary disease (COPD) on the diverse host defense functions of the airway epithelium.

## Novel mechanisms underlying impaired mucociliary clearance

Smoking contributes to the development of chronic bronchitis in COPD patients by impairing mucociliary clearance by airway epithelial cells, leading to mucus accumulation in the lung. This is in part caused by impaired differentiation or function of ciliated cells, which clear mucus via ciliary beating (1). At the ERS Congress, novel evidence was presented for a role of the hedgehog (HH)

signaling pathway in reduced ciliated cell differentiation in COPD (2). Lower expression of HH signaling components was detected in lung tissues from COPD patients, when compared to control subjects. Furthermore, inhibition of HH signaling in air-liquid interface cultured airway epithelial cells attenuated differentiation of ciliated cells, while enhancing secretory cell numbers. These data suggest a novel role of HH signaling in airway epithelial plasticity, regulating luminal cell differentiation. A previous study demonstrated restricted expression of HH signaling components in ciliated cells and a role for non-canonical HH signaling in motile cilia, reducing airway epithelial defense properties (3). The loss of ciliated cells upon HH inhibition provides an additional level of complexity, suggesting a delicate role of HH signaling in controlling airway epithelial differentiation and host defense. Further research is necessary to elucidate the effects of CS and COPD disease status on these distinct regulatory properties of HH signaling in the airway epithelium.

Another presented abstract demonstrated a novel role for adenine nucleotide translocase (ANT) in regulating mucociliary clearance (4). ANT was detected in lung tissues, and in addition to mitochondria, it was located in the cilia of airway epithelial cells. In cultured airway

epithelial cells, overexpression of ANT2 increased the height of the airway surface liquid (ASL), which suggests increased mucus hydration and improvement of mucociliary clearance. Reduced cilia beating in cultured airway epithelial cells exposed to CS was prevented upon ANT2 overexpression. Based on the function of ANT as an ADP/ATP translocator in mitochondria, it can be speculated that this protein regulates ASL volume by promoting ATP transport in motile cilia. Previous studies have shown that extracellular ATP may induce airway epithelial fluid secretion by calcium activated chloride channels. Moreover, conversion of ATP into adenosine, raises cAMP levels by activating the adenosine A2B receptor, which enhances cilia beating and increases the ASL volume [reviewed in (5)]. The precise role of ANT in the regulation of extracellular ATP remains, however, to be elucidated. Moreover, further studies may provide insight whether CS attenuates ANT function, which can be then restored by overexpression of the protein.

### Attenuated host defense properties by airway epithelial cells due to epithelial remodeling

The association between airway epithelial remodeling and impaired mucociliary clearance in COPD has been well established. However, only recently, studies addressed the influence of airway epithelial remodeling on other host defense mechanisms. Studies by Gohy *et al.*, demonstrated impaired expression of the polymeric immunoglobulin receptor (pIgR) by airway epithelial cells of COPD patients (6). This effect was mediated by transforming growth factor (TGF)- $\beta$ 1, a growth factor associated with COPD development (7), which suppressed expression of pIgR in COPD airway epithelial cells by attenuating cell differentiation (8). This mechanism may contribute to microbial colonization and infections in the lungs of COPD patients, as it leads to impaired microbial exclusion by secretory immunoglobulin A (IgA) (9,10). At the Congress (11) it was shown by an abstract that chronic CS exposure of cultured airway epithelial cells impaired expression of pIgR and transcytosis of dimeric IgA, due to aberrant epithelial differentiation. These data were also reported in a recent publication (12). In addition to pIgR, CS suppressed the expression of other antibacterial proteins, i.e., secretory leukocyte peptidase inhibitor (SLPI) and palate, lung and nasal epithelium clone protein (PLUNC) proteins, which are normally expressed in luminal airway epithelial cells. In line with this, CS-exposed airway

epithelial cells displayed reduced antibacterial activity against *Moraxella catarrhalis*. These findings demonstrate impaired production of several host defense proteins, which are restricted to differentiated luminal airway epithelial cells. However, it is still unclear which specific luminal cells are responsible for the constitutive host defense in the lungs. Interestingly, a novel study conducting single cell RNA sequencing of club cells in the small airways (13) demonstrated restricted expression of pIgR, SLPI and LPLUNC1 in this particular luminal cell type. These data suggest a potential novel mechanism underlying the increased susceptibility to microbial infections in COPD, which is caused by a reduced antibacterial defense due to club cell depletion. In line with the expression of pIgR, another abstract in Paris demonstrated that TGF- $\beta$ 1 stimulation of cultured airway epithelial cells could also suppress the expression of other luminal cell-restricted antibacterial proteins (14). Moreover, TGF- $\beta$ 1 also seemed to suppress another antibacterial defense mechanism of airway epithelial cells, mediated by vitamin D. Previous work has shown that active vitamin D could enhance the antibacterial activity of cultured airway epithelial cells, which was likely mediated by increased expression of the cathelicidin-derived antimicrobial peptide LL-37 (15,16). It was furthermore shown that the pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  suppress vitamin D-induced expression of LL-37, due to enhanced expression of CYP24A1 (16). This enzyme inactivates vitamin D, thereby attenuating airway epithelial antibacterial defense. Next to pro-inflammatory cytokines, it was shown at the latest ERS Congress that TGF- $\beta$ 1 could also inhibit vitamin D-induced cathelicidin expression in cultured airway epithelial cells (14). It is likely that this is also mediated by CYP24A1, which was enhanced in cultured airway epithelial cells stimulated with TGF- $\beta$ 1.

### Persisting defects in the airway epithelial barrier in COPD

Initial exposures of epithelial cells to CS may already cause transient alterations in airway epithelial cell differentiation and function (17). These alterations are initially reversible, however CS can induce a permanent reprogramming of epithelial cells in susceptible smokers, leading to COPD development, which is likely due to epigenetic changes (18). The disease phenotype seems to persist in cell culture, as shown by studies demonstrating reduced repair and host defense properties of isolated primary airway epithelial cells (6,19,20). In addition, cultured airway epithelial cells from

COPD patients displayed a reduced barrier integrity and expression of tight junctions, when compared to cultures from control subjects (21). The airway epithelial barrier is a critical host defense property, and a reduced integrity in COPD is postulated to contribute to disease development (22). Another abstract presented at the Congress in Paris 2018 studied how barrier integrity of differentiated airway epithelial cell cultures from COPD patients and control subjects relates to expression of inflammatory mediators and markers of epithelial-to-mesenchymal transition (EMT) (23). In accordance with published data (21), COPD airway epithelial cells displayed a reduced barrier integrity compared to cultures from control subjects. However, no differences were observed in the expression of inflammatory and EMT markers, suggesting that a reduced epithelial barrier might not be directly related to other disease aspects. The decrease of the airway epithelial barrier in COPD can also be related to accelerated aging of the lungs due to smoking (24). In extent of a previous study demonstrating the occurrence of an ageing gene signature in COPD lung tissues (25), it was shown by another ERS highlight abstract that differentiated airway epithelial cells display differences in the epithelial barrier integrity, depending on the age of the donor (26). Furthermore, expression of genes involved in the airway epithelial barrier integrity correlated with donor age as observed in brushed bronchial cells. These findings support the hypothesis of accelerated aging by smoking (24), which may contribute to reduced barrier function in cultured COPD airway epithelial cells.

### Epithelial cell death and inflammation

Airway inflammation is a central hallmark in asthma and COPD. While several cell types from the hematopoietic system contribute to the inflammatory process, lung structural cells, such as airway smooth muscle cells, airway and alveolar epithelial cells as well as endothelial cells also play an important role (27). In this context, airway epithelial cells have been shown to control the release of a plethora of inflammatory mediators through NF- $\kappa$ B activation in the context of asthma (28) and COPD (29). However, the discovery of genes involved in the regulation of NF- $\kappa$ B driven epithelial activation is rapidly increasing, due to the presumably high potential to use the airway epithelium for therapeutic interventions (30). In this context, an abstract presented in the ERS International Congress 2018 has demonstrated that the expression of advanced glycation end-products specific receptor (AGER), the gene encoding

the receptor for advanced glycation-end products (RAGE), was decreased in bronchial biopsies of smokers compared with never smokers. The major isoform of RAGE, (es)RAGE, which is an anti-inflammatory decoy receptor, was however increased (31). These findings highlight that in the early phase of CS exposure, inhibition of the pro-inflammatory gene AGER and increased expression of the anti-inflammatory isoform (es)RAGE contribute to an anti-inflammatory response. Beyond that, another abstract presented during the Congress in Paris 2018 revealed evidence CS exposure-induced damage-associated molecular patterns (DAMPs) are linked to decreased expression of CFLAR, a gene encoding the cell death regulator protein c-FLIP (32). Indeed, the authors showed that CS decreased CFLAR expression, which was followed by apoptosis, necrosis and necroptosis of epithelial cells. This study reinforces the hypothesis that the mechanism underlying CS-induced lung inflammation is not solely driven via the classical simplified induction of pro-inflammatory NF- $\kappa$ B driven genes, but also involves dysregulated cell death pathways (33). In the same way, another abstract presented during the ERS International Congress 2018 showed that S100 protein and adenosine triphosphate (ATP) are also DAMPs capable to induce Mucin5AC (MUC5AC) synthesis and IL-1 $\beta$  release from NCI-H292 airway epithelial cells. These data support the concept that epithelial cells are active players orchestrating the inflammatory response (34).

### Involvement of proteases in emphysema development

Next to chronic airway inflammation, COPD is also characterized by pulmonary emphysema. The latter is defined as a permanent enlargement of airspaces due to a destruction of the alveolar wall, which results in reduced surface for gas exchange, hypoxemia and a significant decrease of lung tissue elastance. In summary, all these factors favor air trapping and thereby account negatively to the prognosis of COPD (35). The complex processes involving emphysema development have partially been attributed to an increased synthesis and release of reactive oxygen species (ROS), and reactive nitrogen species (RNS) and impaired anti-oxidant defense upon CS-exposure (36). In addition, this oxidant/antioxidant imbalance now known as REDOX imbalance controls the activation and deactivation of proteases and anti-proteases, which are molecules centrally involved in the pathophysiology of emphysema (37). Along this line, an abstract presented

during ERS International Conference 2018 showed that a cysteine protease named cathepsin S (CatS), which degrades elastin, was increased in expression and activity in lung tissue of COPD current smokers and in smoker's non-COPD (38). These findings were also confirmed in CS-exposed primary epithelial cell cultures. Taken together, these data reinforce the concept that CS presents a central role in the activation of proteases; which might be involved in the pathophysiology of emphysema. Following the same line, another abstract showed that although CatS activity is stable in COPD patients, it is partially resistant to the effects of the main oxidants derived from cigarette smoke H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide), SIN-1 (peroxynitrite donor), acrolein and formaldehyde (FA); but susceptible to oxidation (39). These interesting observations were attributed to a late formation of sulfinic acid, which supports the rationale that the unexpected stability of CatS sustains its elastinolytic activity.

## Conclusions

In summary, novel concepts on how smoking attenuates the host defense properties of the airway epithelium are emerging. This is supported by *in vitro* studies using cultured airway epithelial cells, exposed to CS or by using cells derived from diseased patients or aging individuals. Alterations in the composition of the airway epithelium due to remodeling such as reduced differentiation and function of ciliated cells may affect mucociliary clearance upon CS exposure. In addition, growth factors such as TGF- $\beta$ 1, may reduce luminal cell-restricted host defense proteins, providing a novel mechanism in which epithelial remodeling promotes colonization and infections of microbes in the lungs of COPD patients. A persistent defect in the airway epithelial barrier integrity in cultured COPD cells and in aging individuals supports the hypothesis that smoking initiates exhaustion of airway epithelial cells related to accelerated lung aging. Furthermore, we have presented links that an aberrant airway epithelial barrier in COPD might contribute to chronic inflammation in asthma and COPD and is also implicated in the protease/anti-protease imbalance in the development of emphysema.

Finally, encouraging novel insights into the complex interaction of CS with airway epithelial cell biology have been presented at the European Respiratory Society International Congress in Paris in 2018, which represent a first step towards the development of novel therapies for chronic lung diseases.

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## References

1. Smaldone GC, Foster WM, O'Riordan TG, et al. Regional impairment of mucociliary clearance in chronic obstructive pulmonary disease. *Chest* 1993;103:1390-6.
2. Belgacemi R, Perotin JM, Coraux C, et al. Involvement of Hedgehog pathway in airway cell differentiation. *Eur Respir J* 2018;52. [Epub ahead of print].
3. Mao S, Shah AS, Moninger TO, et al. Motile cilia of human airway epithelia contain hedgehog signaling components that mediate noncanonical hedgehog signaling. *Proc Natl Acad Sci U S A* 2018;115:1370-5.
4. Nguyen J, Kliment C, Lu Y, et al. From Dictyostelium to human airway epithelium: adenine nucleotide translocase enhances cellular respiration and ciliary function disrupted by cigarette smoke. *Eur Respir J* 2018;52. [Epub ahead of print].
5. Tarran R. Regulation of airway surface liquid volume and mucus transport by active ion transport. *Proc Am Thorac Soc* 2004;1:42-6.
6. Gohy ST, Detry BR, Lecocq M, et al. Polymeric immunoglobulin receptor down-regulation in chronic obstructive pulmonary disease. Persistence in the cultured epithelium and role of transforming growth factor- $\beta$ . *Am J Respir Crit Care Med* 2014;190:509-21.
7. Verhamme FM, Bracke KR, Joos GF, et al. Transforming growth factor- $\beta$  superfamily in obstructive lung diseases. more suspects than TGF- $\beta$  alone. *Am J Respir Cell Mol Biol* 2015;52:653-62.
8. Gohy ST, Hupin C, Fregimilicka C, et al. Imprinting of the COPD airway epithelium for dedifferentiation and mesenchymal transition. *Eur Respir J* 2015;45:1258-72.
9. Polosukhin VV, Richmond BW, Du RH, et al. Secretory IgA Deficiency in Individual Small Airways Is Associated with Persistent Inflammation and Remodeling. *Am J Respir Crit Care Med* 2017;195:1010-21.

10. Polosukhin VV, Cates JM, Lawson WE, et al. Bronchial secretory immunoglobulin a deficiency correlates with airway inflammation and progression of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011;184:317-27.
11. van der Does AM, Amatngalim GD, Schruppf JA, et al. Impairment of respiratory host defence resulting from cigarette smoke-induced changes in airway epithelial cell differentiation. *Eur Respir J* 2018;52. [Epub ahead of print].
12. Amatngalim GD, Schruppf JA, Dishchekian F, et al. Aberrant epithelial differentiation by cigarette smoke dysregulates respiratory host defence. *Eur Respir J* 2018;51. pii: 1701009.
13. Zuo WL, Shenoy SA, Li S, et al. Ontogeny and Biology of Human Small Airway Epithelial Club Cells. *Am J Respir Crit Care Med* 2018. [Epub ahead of print].
14. Schruppf JA, Ninaber DK, Van Der Does AM, et al. TGF- $\beta$ 1 affects epithelial vitamin D metabolism and expression of host defence peptides. *Eur Respir J* 2018;52. [Epub ahead of print].
15. Hansdottir S, Monick MM, Hinde SL, et al. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol* 2008;181:7090-9.
16. Schruppf JA, Amatngalim GD, Veldkamp JB, et al. Proinflammatory Cytokines Impair Vitamin D-Induced Host Defense in Cultured Airway Epithelial Cells. *Am J Respir Cell Mol Biol* 2017;56:749-61.
17. Shaykhiev R, Crystal RG. Early events in the pathogenesis of chronic obstructive pulmonary disease. Smoking-induced reprogramming of airway epithelial basal progenitor cells. *Ann Am Thorac Soc* 2014;11 Suppl 5:S252-8.
18. Shaykhiev R. Airway Epithelial Progenitors and the Natural History of Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2018;197:847-9.
19. Ghosh M, Miller YE, Nakachi I, et al. Exhaustion of Airway Basal Progenitor Cells in Early and Established Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2018;197:885-96.
20. Amatngalim GD, Schruppf JA, Henic A, et al. Antibacterial Defense of Human Airway Epithelial Cells from Chronic Obstructive Pulmonary Disease Patients Induced by Acute Exposure to Nontypeable Haemophilus influenzae: Modulation by Cigarette Smoke. *J Innate Immun* 2017;9:359-74.
21. Heijink IH, Noordhoek JA, Timens W, et al. Abnormalities in airway epithelial junction formation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2014;189:1439-42.
22. Aghapour M, Raee P, Moghaddam SJ, et al. Airway Epithelial Barrier Dysfunction in Chronic Obstructive Pulmonary Disease: Role of Cigarette Smoke Exposure. *Am J Respir Cell Mol Biol* 2018;58:157-69.
23. Carlier F, Detry B, Sibille Y. COPD epithelial phenotype shows partial reversibility in long-term primary epithelial ALI-cultures. *Eur Respir J* 2018;52. [Epub ahead of print].
24. Ito K, Barnes PJ. COPD as a disease of accelerated lung aging. *Chest* 2009;135:173-80.
25. de Vries M, Faiz A, Woldhuis RR, et al. Lung tissue gene-expression signature for the ageing lung in COPD. *Thorax* 2017;73:609-17.
26. Muizer K, De Vries M, Timens W, et al. The effect of age on lung epithelial barrier function. *Eur Respir J* 2018;52. [Epub ahead of print].
27. Barnes PJ. Cellular and molecular mechanisms of asthma and COPD. *Clin Sci* 2017;131:1541-58.
28. Hammad H, Lambrecht BN. Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity* 2015;43:29-40.
29. Wang Y, Liu J, Zhou JS, et al. MTOR Suppresses Cigarette Smoke-Induced Epithelial Cell Death and Airway Inflammation in Chronic Obstructive Pulmonary Disease. *J Immunol* 2018;200:2571-80.
30. Gras D, Chanez P, Vachier I, et al. Bronchial epithelium as a target for innovative treatments in asthma. *Pharmacol Ther* 2013;140:290-305.
31. Pouwels SD, Vermeulen CJ, Guryev V, et al. AGER gene expression and alternative splicing in bronchial biopsies of smokers and non-smokers. *Eur Respir J* 2018;52. [Epub ahead of print].
32. Pouwels SD, Faiz A, Heijink IH, et al. Cigarette smoke exposure decreases CFLAR expression in bronchial epithelium, augmenting susceptibility for cell death and DAMP release. *Eur Respir J* 2018;52. [Epub ahead of print].
33. Lambrecht BN, Hammad H. Death at the airway epithelium in asthma. *Cell Res* 2013;23:588-9.
34. Chung IY, Kim HJ, Kim K, et al. Role of NLRP3 inflammasome and ATP in S100A12-induced MUC5AC production in airway epithelial cells. *Eur Respir J* 2018;52. [Epub ahead of print].
35. Segal LN, Martinez FJ. Chronic obstructive pulmonary disease subpopulations and phenotyping. *J Allergy Clin Immunol* 2018;141:1961-71.
36. Boukhenouna S, Wilson MA, Bahmed K, et al. Reactive

- Oxygen Species in Chronic Obstructive Pulmonary Disease. *Oxid Med Cell Longev* 2018;2018:5730395.
37. Hendrix AY, Kheradmand F. The Role of Matrix Metalloproteinases in Development, Repair, and Destruction of the Lungs. *Prog Mol Biol Transl Sci* 2017;148:1-29.
38. Wartenberg M, Andrault PM, Schamberger AC, et al. Cigarette smoke induces overexpression of cathepsin S in active smokers with and without COPD. *Eur Respir J* 2018;52. [Epub ahead of print].
39. Wartenberg M, Andrault PM, Saidi A, et al. Deciphering molecular mechanisms of cathepsin S resistance to major chemical oxidants of cigarette smoke. *Eur Respir J* 2018;52. [Epub ahead of print].

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