Air pollution: Mucus Metaplasia in the Club cells

Contaminación del aire: metaplasia mucoide en las células Club

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Abstract: The Club cells (also called Bronchial Exocrine Cells) are located only in the bronchiolar epithelium, morphologically presenting a dome in the apex, where it stores secretory granules. It produces three Surfactant Proteins and a specific Secretory Protein. This protein is an inducible steroid that maintains the lung's immune response. It has also been utilized as a sensitive biomarker since it can change the permeability of the lung epithelial barrier. Additionally, the Club cell helps metabolize xenobiotics.
On the other hand, these cells can generate different cell types, such as ciliated cells or the Club cells variant positive for mucus. The aim was to evidence the mucus metaplasia from Club cells after inhalation of fine particles (PM2.5 μm). For that, we evaluated in a guinea pig model, the inhalation during 36 h (4 h/9 days) of PM2.5 from Mexico City, which showed the generation of a variant positive of Club Cells for mucus, showing small magenta or pink granules inside it. Exposure to PM2.5 transforms the phenotype from Club cells to mucus-secreting, resulting in mucus metaplasia in the lung. The Club cells are versatile cells that serve as the last defense barrier to metabolize and trap toxic substances, thereby helping to maintain lung health.

Keywords: Bronchioles; Lung; Fine particles; Periodic acid-Schiff; Histopathology.

Resumen: Las células Club (también llamadas Células Bronquiolares Exócrinas) son un tipo celular que se localiza únicamente en el epitelio bronquiolar, morfológicamente presentan una cúpula en su parte apical, en donde almacena gránulos secretores.
Producen tres proteínas tensioactivas y una específica, llamada Proteína Secretora de las células Club. Esta proteína es un esteroide inducible cuya función es regular la respuesta inmune dentro del pulmón. También se ha utilizado como biomarcador de sensibilidad y de daño ya que puede incrementar la permeabilidad de la barrera epitelial pulmonar. Además, las células Club ayudan a metabolizar los xenobióticos. Por otro lado, estas células son capaces de generar diferentes tipos celulares como las células ciliadas, o a sí mismas o la variante de las células Club positivas para moco. El objetivo fue evidenciar la metaplasia mucoide de las células Club después de la inhalación de aeropartículas finas (PM2.5 μm). Para ello, evaluamos en un modelo de cobayo, la inhalación durante 36 h (4 h/9 días) de PM2.5 de la Ciudad de México, la cual mostró la generación de la variante de células Club positivas para moco, evidenciando pequeños gránulos magenta o rosa en su interior. La exposición a antígenos transforma el fenotipo de esta célula en secretora de moco, lo que produce metaplasia mucoide en el pulmón. Las células Club tienen funciones versátiles, pues son la última barrera de defensa ya que metabolizan y atrapan a las sustancias tóxicas, ayudando a mantener la salud pulmonar.

Palabras clave: Bronquiolos; Pulmón; Partículas finas; Ácido peryódico de Schiff; Histopatología.

INTRODUCTION

The Bronchiolar Exocrine Cells (BEC) or Club Cells (CC), previously called Clara Cells (by Max Clara), are nonciliated, nonmucous secretory cells (Harkema *et al.* 2018). CC are located only in the pulmonary bronchioles and show a cubic shape with a basal nucleus (Reynolds and Malkinson 2010; Falcon-Rodriguez 2012). They also feature an apical dome, where they store specific granules surrounded by a membrane, which are released by apocrine secretion (Kuhn *et al.* 1979) to the lumen of the airways (Reynolds and Malkinson 2010; Falcon-Rodriguez 2012).

The CC is a versatile cell, and studies using an electron microscope have revealed that its large number of mitochondria are close to the apical part. Besides, it has a large amount of smooth and rough endoplasmic reticulum, and the Golgi complex is well developed, located in the apical part of the cell (Rokicki *et al.* 2016). Thanks to the last three cellular organelles, the CC produces Surfactant Protein A, B, and D and other specific proteins called Club Cell Surfactant Protein (CCSP) that contribute to the bronchiole epithelial lining fluid (Harkema *et al.* 2018). The composition of the CCSP is the same as that of uteroglobin produced by the endometrium. In the scientific literature, this protein is known as Club cell protein 16 KDa (CC16), CC10 Club Cell Protein 10 (CC10), Secretoglobin human protein 1 (SCGB), and urine protein 1 (Rokicki *et al.* 2016). CCSP's role is to protect against oxidative stress due to its anti-inflammatory and immunomodulatory properties (Rokicki *et al.* 2016).

The high concentration of CCSP in the lungs has been utilized as a biomarker of lung-blood barrier permeability; CC is essentially in homeostatic function inside the lungs (Reynolds and Malkinson 2010; Falcon-Rodriguez 2012). Besides, CC participates in the biotransformation of many harmful and toxic compounds introduced to the lung by respired air (Rokicki *et al.* 2016) since it contains a detoxification system formed by Cytochrome P-450, monooxygenase, and flavin-containing monooxygenases (Rokicki *et al.* 2016).

Some studies have shown that CCSP is a population biomarker of different types of CC, mainly when the lungs are exposed to environmental pollution. Injury activates quiescent stem cells (vCE) that can self-renew or generate a facultative progenitor of CC (Reynolds and Malkinson 2010; Falcon-Rodriguez 2012). These cells can differentiate into CC of type A, which are mitotically active. Type A cells are divided into two cell types: ciliated cells and maturing Type B cells (Reynolds and Malkinson 2010; Falcon-Rodriguez 2012). Also, we must highlight that some toxic compounds can stimulate mucus secretion by CC and produce a CC-variant positive for mucus (CC-v) (Reynolds and Malkinson 2010; Falcon-Rodriguez 2012). Air pollution, due to its complex composition, can induce mucus metaplasia in bronchiolar structures. To evidence this, we evaluated the mucus metaplasia in Club cells following the inhalation of fine particles (PM2.5 μm).

MATERIALS AND METHODS

The protocol was approved by the Scientific and Bioethics Committees of Instituto Nacional de Enfermedades Respiratorias (INER), approval B17-12. Six animals per group were exposed in real-time to either filtered air or fine particles from Mexico City using a particle concentrator located in the northern zone, for a total of 36 h (4 h/day for 9 days). The lungs were fixed with 3.7% formaldehyde by lung inflation and immersion. Subsequently, the lungs were processed using histological techniques. The histology samples were stained using periodic acid-Schiff (PAS). Schiff reactive dyes polysaccharides, mucinous, and mucopolysaccharides.

RESULTS

The histological study showed the differentiation of CC-variant positive for mucus. We can observe small magenta or pink granules inside this cellular type, corresponding to mucus substance (*Figure 1*).

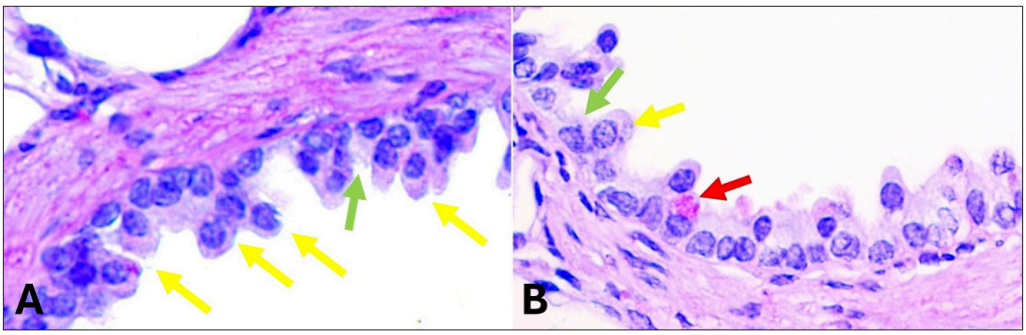

Figure 1. Terminal bronchiolar epithelium of guinea pig. A. Guinea pig exposed to filtered air. B. Guinea pig exposed to fine particles from Mexico City. The green arrow shows the ciliated cell, the yellow arrow points to a CC, and the red arrow points to a CC-variant positive for mucus. Small magenta or pink granules can be observed. PAS, 850X. Original image obtained from our research.

DISCUSSION

The constant exposure to irritants in the airway can transform the phenotype from CC to CC-v mucus-secreting, known as mucus metaplasia. Mice exposed to a mixture of antigens such as lipopolysaccharides or viruses developed mucus metaplasia in these cells, specifically in the proximal and distal bronchioles but not in terminal bronchioles and bronchioalveolar-duct junction, where Club Cell presents both biomarkers for mucus and CCSP+ (Evans *et al.* 2004). In allergic asthma, CC-variant mucus can be found after contact with an allergen (OVA) (Nesterova *et al.* 2019). Additionally, exposure to toxic compounds such as metals (Falcon-Rodriguez 2012), ozone (Kumagai *et al.* 2017), cigarette smoke, and acrolein (Voynow *et al.* 2004) generates mucus metaplasia showing CC both CCSP+ and mucin PAS+ (Periodic acid-Schiff stain). However, the localization of CC-v is different, depending on the animal model and toxic compounds. CC from the proximal bronchioles is transformed to CC-v due to the high amount of neutrophil elastase in the pulmonary alveoli and bronchioles (Voynow *et al.* 2004). In mice exposed to vanadium inhaled for 12 weeks, the proximal and terminal bronchioles and bronchioalveolar duct junction showed two subtypes of CC, a CC population CCSP+ and another CCSP+ and PAS+ (mucin+) (Falcon-Rodriguez 2012).

All environmental contaminants produce oxidative stress and damage in the pulmonary system; therefore, the pulmonary system contains different structures, such as submucosal glands and goblet cells. Both structures help trap particles that can migrate to the alveoli. However, the bronchioles lack submucosal glands and goblet cells; for this reason, the CC could generate a CC-variant mucus, which helps capture all irritant elements that have reached the bronchioles, maintaining a suitable microenvironment in the lung.

Various events in the lung play an essential role in mucus production, either in the submucosal glands (Voynow *et al.* 2004), goblet cells, or the Club cells. A common way is a Th2 response and activation of IL-4 and IL-13, which are synthesized by inflammatory infiltration of eosinophils. Neutrophils also release a lot of elastase protein (Kuperman *et al.* 2005). CC releases IL-13 via STAT-6 and NOTCH2 signaling and can induce CC-v (Nesterova *et al.* 2019). The CC positive for CCSP and Th2 response can upregulate the IL-13, Muc5ac (Evans *et al.* 2004; Kumagai *et al.* 2017), Muc5b, Gob5 (Clca1), and Ym2 (Chi3/l4) (Kumagai *et al.* 2017), which can also be regulated by elastase protein (Evans *et al.* 2004; Kuperman *et al.* 2005). Interestingly, Sox 9 produces mucus metaplasia following the Kinase Insert Domain Receptor (KDR) inhibition in mouse and human Club cells. Also, in asthmatics and cystic fibrosis patients, KDR expression is reduced while SOX9 expression is increased (Jiang *et al.* 2021). The ultrastructural observation from CC-v showed a high amount of rough endoplasmic reticulum and an increased number of secretory vesicles of mucin after the antigen challenge (Evans *et al.* 2004; Falcon-Rodriguez 2012).

CONCLUSION

Air pollution is caused by various chemical compounds that exhibit oxidative effects on the airways, primarily in the bronchioles. To counteract this, Clara Cells undergo mucus metaplasia to prevent pollutants from reaching the deeper regions of the lungs. The Club cells are versatile cells that serve as the last defense barrier to metabolize and trap toxic substances through mucus production, helping to maintain lung health.

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CONFLICTS OF INTERESTS

Authors declare no competing interests that could have appeared to influence their research.

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