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Use of airway epithelial cell culture to unravel the pathogenesis and study treatment in obstructive airway diseases

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ABSTRACT

Asthma and chronic obstructive pulmonary disease (COPD) are considered as two distinct obstructive diseases. Both chronic diseases share a component of airway epithelial dysfunction. The airway epithelium is localized to deal with inhaled substances, and functions as a barrier preventing penetration of such substances into the body. In addition, the epithelium is involved in the regulation of both innate and adaptive immune responses following inhalation of particles, allergens and pathogens. Through triggering and inducing immune responses, airway epithelial cells contribute to the pathogenesis of both asthma and COPD. Various *in vitro* research models have been described to study airway epithelial cell dysfunction in asthma and COPD. However, various considerations and cautions have to be taken into account when designing such *in vitro* experiments. Epithelial features of asthma and COPD can be modelled by using a variety of disease-related invoking substances either alone or in combination, and by the use of primary cells isolated from patients. Differentiation is a hallmark of airway epithelial cells, and therefore models should include the ability of cells to differentiate, as can be achieved in air-liquid interface models. More recently developed *in vitro* models, including precision cut lung slices, lung-on-a-chip, organoids and human induced pluripotent stem cells derived cultures, provide novel state-of-the-art alternatives to the conventional *in vitro* models. Furthermore, advanced models in which cells are exposed to respiratory pathogens, aerosolized medications and inhaled toxic substances such as cigarette smoke and air pollution are increasingly used to model e.g. acute exacerbations. These exposure models are relevant to study how epithelial features of asthma and COPD are affected and provide a useful tool to study the effect of drugs used in treatment of asthma and COPD. These new developments are expected to contribute to a better understanding of the complex gene-environment interactions that contribute to development and progression of asthma and COPD.

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1. Introduction

Asthma and chronic obstructive pulmonary disorder (COPD) are common disorders and affect 1 out of 12 people worldwide. Asthma and COPD are chronic inflammatory diseases characterized by airway obstruction which is reversible in asthma and often irreversible in COPD [1]. Another important feature of COPD, and occasionally in severe asthma, is emphysema whereby the alveolar tissue is destroyed, resulting in impaired oxygen exchange [1–3]. Since this review focuses on airway epithelial cells, studies investigating alveolar epithelial cells and their role in the development of emphysema are outside its scope. Inflammation of the airways is present in both asthma and COPD, but in asthma it affects mainly the conducting airways whereas in COPD it affects primarily the small airways, likely reflecting the distribution of inhaled provoking substances, such as allergens in asthma and cigarette smoke in COPD. Despite being different disease entities, both asthma and COPD share an important component of epithelial dysfunction [4,5].

Approximately 20–35% of the world population smokes, with surprisingly similar smoking rates reported in patients with asthma [6–8]. Cigarette smoking has been shown to worsen asthma symptoms, reduce responsiveness to corticosteroid treatment, accelerate lung function decline and increase exacerbation rates [9]. In contrast, various characteristics typically assigned to asthma have also been found in patients with COPD, including reversibility of airway obstruction, atopy and T helper 2 (Th2)- mediated inflammation [1]. Importantly, asthma and COPD share various dysfunctional features of the airway epithelium, in addition to several other disease features [4].

The epithelium of the conducting airways is a pseudostratified epithelial layer that comprises basal, ciliated and secretory cells. The epithelial barrier function in both asthma and COPD has been shown to be decreased, resulting from disrupted intercellular junctional proteins [10,11]. Other shared features of asthma and COPD include goblet cell metaplasia with increased mucus production, altered inflammatory responses, reduced antimicrobial peptide expression and activity, and altered basal function that may lead to defective repair responses following injury [5] [4,10].

Epithelial dysfunction in both asthma and COPD implies an important role for these cells in the development and self-perpetuation of these diseases. Various research models have been applied to investigate the pathogenic mechanisms, diagnostic potential and therapeutic targets of airway epithelial cells in chronic lung diseases. However, very few models have focused on the combined features of both asthma and COPD and how these may interact *in vitro*. In this review, we discuss recent advances and important considerations for *in vitro* models to study airway epithelial cell dysfunction in asthma and COPD.

2. Assessing epithelial function *in vitro*

In contrast to patient studies and *in vivo* models, *in vitro* models allow us to deconstruct multi-layered mechanisms of disease pathogenesis and investigate the contribution of individual cellular components. Epithelial features of asthma and COPD can be investigated *in vitro* using patient derived primary cells, but can also be induced by known invoking substances involved in disease pathogenesis. Such substances can include complex mixtures such as cigarette smoke for COPD or allergen extracts for asthma, but also specific chemicals or proteins known to play a role in specific disease mechanisms can be used. Furthermore, the route of administration of invoking substances can vary. Using the culture media as the vehicle for the compound of interest is the most common approach, but for volatile compounds a more sophisticated technique may be required.

In vitro models can range from simple monolayers of epithelial cells to complex three-dimensional culture models involving multiple cell types. In a pseudostratified epithelium, all epithelial cells are attached to a basement membrane. Therefore, airway epithelial cells can be grown on a variety of different surfaces and careful selection of an appropriate support is warranted. Supports can range from uncoated tissue culture treated plastics to decellularized scaffolds of human tissue. Recent reviews provide an overview of various available supports and scaffolds and will not be revisited here [12–15].

Airway epithelial cells are available as continuous cell lines or as primary cells from various anatomical locations which vary in various characteristics including, but not restricted to apical-to-basal polarization, ciliary development, mucus production or barrier function. Primary epithelial cells can be obtained at a low passage from an increasing number of commercial sources, but can also be isolated from tissue by adequately equipped research laboratories if human samples are available. A major advantage of freshly isolated cells is also that they can be obtained from patients with disease and compared to cells derived from healthy persons. Primary cells can be grown as a submerged monolayer, but also as an air-liquid interface culture with air exposure on the apical side and culture medium on the basolateral side of the membrane. In contrast, most tumour and immortalized cells lines are studied as submerged monoculture, which is partly explained by the fact that they do not differentiate into a pseudostratified epithelial layer at air-liquid interface. Airway epithelial cells can also be grown as organoids, in which cells are grouped and organized in a way similar to the organ they are representing [16,17]. Multiple structural, inflammatory and immune cell types can be included with the airway epithelial cells to create a more complex interacting system involving multiple cell types. Overall, various considerations have to be taken into account when modelling disease features *in vitro*.

2.1. Modelling epithelial changes of asthma and COPD in vitro

Various methods and techniques have been developed to recreate physiological relevant epithelial features of asthma and COPD *in vitro*. Reconstructing these disease features *in vitro* can be done by collecting airway epithelial cells from patients and culturing these cells using different techniques. Interestingly, when primary cells are isolated from asthma or COPD patients, several epithelial features observed *in vivo* are retained *in vitro*, including altered cytokine release, impaired immune responses and increased susceptibility to oxidative stress, suggesting that the epigenetic programming of the airway epithelial cells is retained after isolation [18–22]. Nonetheless, it is important to consider that