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Author: Mertens, Tinne  
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CHAPTER 1

GENERAL INTRODUCTION AND
THESIS OUTLINE
INTRODUCTION

Asthma and chronic obstructive pulmonary disorder (COPD) are both chronic lung diseases characterized by chronic inflammation of the respiratory tract and airway obstruction which is often reversible in asthma, but mostly irreversible in COPD (1-3). Allergic asthma has classically been described as a T-helper 2 (Th2)-driven disease with predominantly eosinophilic infiltration, whereas COPD inflammation may involve CD8 cells, B cells, macrophages and neutrophils (3). Traditionally, these two diseases have been considered as two distinct disease entities, however, it has become evident over the last years that both asthma and COPD are very heterogeneous diseases (3-5). Disease heterogeneity in both asthma and COPD is characterized by a wide array of symptoms, variable exacerbation rates, inconsistent clinical findings, pathology abnormalities and diverse patient responses to therapeutics. The large degree of heterogeneity within asthma and COPD suggests that different underlying molecular pathways are contributing to disease pathogenesis. Elucidating these underlying molecular pathways may provide a basis to better delineate various phenotypes within asthma and COPD that respond to specific therapeutics.

Airway epithelial cell dysfunction is an important characteristic of both asthma and COPD, making it a likely candidate for molecular phenotyping to delineate clinical phenotypes (6-8). Because of their localization at the interface between environmental exposures and underlying tissue, airway epithelial cells are ideal candidates as reporters of both environmental exposures as well as underlying tissue pathogenesis. Moreover, airway epithelial cells are reasonably accessible and bronchial brushings can be used to obtain a relatively pure population of epithelial cells. Collection of such bronchial brushings from patients and performing gene expression analysis has proven a successful method to delineate molecular pathways underlying specific disease phenotypes (8, 9). In addition, although considered to be invasive, repeated sampling may allow gene expression analysis at multiple time points during the course of treatment to study intermediate markers of therapeutic efficacy. Airway epithelial cells can be isolated from bronchial brushes or bronchial biopsies collected during bronchoscopy, or from lung tissues resected during surgery. These cells can subsequently be cultured in vitro to study molecular pathways underlying clinical phenotypes, but also to evaluate new potential targets for therapy. Moreover, airway epithelial cells cultured in vitro can be used to evaluate the effect of therapeutics on existing or induced molecular phenotypes representing clinically relevant molecular pathways.
Whereas animal models have contributed significantly to our knowledge of chronic lung diseases, they have been shown to have limited therapeutic predictive value and recreating phenotypes representing clinically encountered phenotypes may not be feasible (10, 11). Therefore, pre-clinical in vitro models using airway epithelial cells representing specific clinical phenotypes are essential to elucidate and unravel molecular pathways. These pre-clinical in vitro models will require extensive optimization and validation, but they could contribute tremendously towards elucidating molecular pathways and discovery of novel therapeutic targets. Also, using in vitro models representing particular features of asthma and COPD, for e.g. exacerbations, can contribute to our knowledge of understating disease progression. An important limitation of airway epithelial cells cultured in vitro is their limited potential for genetic manipulation as these cells are notoriously difficult to transfect. However, using airway epithelial cells isolated from transgenic animals can provide an alternative to human airway epithelial cells, circumventing the need to transfected airway epithelial cells.

**THESIS OUTLINE**

In the studies described in this thesis, in vitro models using airway epithelial cells were used to study molecular pathways involved in asthma and COPD and their disease heterogeneity. Optimized culture methods and a better understanding of these pathways could aid in the development of targeted treatments for these common lung diseases.

In Chapter 2 a review and discussion is provided on epithelial features of asthma and COPD and how these can be modeled in vitro. Additionally, recent developments and the therapeutic potential of these in vitro models are discussed.

In Chapter 3, the effect of the macrolide antibiotic azithromycin on the interleukin (IL)-13-induced gene signature in primary human airway epithelial cells cultured in vitro is evaluated. This IL-13-induced gene signature has previously been described to represent the T helper 2 (Th2) gene signature encountered in a specific subset of asthma patients. An important environmental factor affecting asthma pathogenesis is cigarette smoking. Approximately 20 to 35% of the world population smokes and quite surprisingly, these smoking rates are reported similarly in asthma patients. Because cigarette smoking is an important environmental factor significantly influencing asthma pathogenesis, Chapter 4 is focused on the effects of whole cigarette smoke exposure on the IL-13-induced gene signature in primary human airway epithelial cells cultured in vitro.
An important feature of both asthma and COPD are exacerbations, sudden worsening of disease, often requiring hospitalization. COPD patients have previously been shown to be more susceptible to exacerbations induced by infection. Therefore, in Chapter 5 an *in vitro* model of exacerbations using UV-inactivated *Haemophilus influenzae* exposure or rhinovirus infection in primary airway epithelial cells derived from COPD patients or smoking controls was used to evaluate whether airway epithelial cells from COPD patients respond differently to infection compared to smoking controls.

It is difficult to obtain primary airway epithelial cells, and genetic manipulation of these cells is notoriously challenging. As an alternative, mouse tracheal epithelial cells can be isolated from transgenic animals to circumvent genetic editing of airway epithelial cells. However, these cells are difficult to maintain and expand in culture and numerous animals are needed to set up an *in vitro* experiment. Therefore, in Chapter 6 an alternative and more efficient culture method for mouse tracheal epithelial cells is developed.

Finally, in Chapter 7 the most important findings of the studies described in this thesis are discussed, followed by an outlook on the clinical implications and future perspectives based on the findings described in this thesis.
REFERENCES
