Airway epithelial cell cultures for studying obstructive lung disease effects of IL-13 and cigarette smoke

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CHAPTER 7

GENERAL DISCUSSION

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are both heterogeneous diseases with various clinical phenotypes and diverse underlying molecular pathways (1, 2). Disease heterogeneity makes treatment of these chronic obstructive diseases challenging, particularly for patients that respond poorly to existing therapies. Consequently, identifying patients using biomarkers indicating towards a specific disease phenotype and subsequent personalized treatment, will become important in improving therapy outcomes. Easy accessible tissues and body fluids allow us to discover novel phenotype specific biomarkers to drive biomarker-guided personalized therapy. Additionally, unravelling the specific molecular pathways underlying the various observed phenotypes will contribute to the discovery of new targets for the development of novel therapeutics. Indeed, the airway epithelium is increasingly in the focus as an important compartment in the development of airway diseases. Cultures of primary cells isolated from human tissue are a particularly relevant tool to study such pathways, because they offer the possibility to closely mimic events occurring in the tissues of patients. Ideally, the cell type investigated is easy accessible, can be isolated from patients and subsequently be cultured *in vitro*, and can be genetically modified to investigate potential new therapeutic targets.

Airway epithelial cells are at the interface of inhaled particles and pathogens, and the underlying tissues. Being at this interface makes airway epithelial cells ideal candidates to report underlying tissue inflammation. Moreover, airway epithelial cells are relatively accessible, can be cultured *in vitro* and can be cryopreserved. Culturing airway epithelial cells *in vitro* to study molecular pathways requires careful consideration of the culture conditions and experimental setup. In **chapter 2** we provide an overview of *in vitro* methods and models available to study asthma and COPD.

AIRWAY EPITHELIAL CELLS AND IN VITRO MODELS: COMPLEXITY, REPRODUCIBILITY OR BOTH?

In vitro models of airway epithelial cells are commonly used to model asthma or COPD. Despite their common use, there is a large discrepancy among the isolation and culture methods described in literature. Both cell lines and primary cells of airway epithelial cells are used, cultured submerged or at air-liquid interface (ALI). Cells are exposed to substances diluted in culture medium or when cells are cultured at ALI, *in vitro* exposures are feasible

that are more representative of exposure of *in vivo* airway epithelium to inhaled substances. In such ALI cultures, cells can be exposed to e.g. aerosolized substances or complex mixtures of gases and particles to mimic the air route of exposure, or substances can be added in culture medium present in the basolateral compartment of the transwell insert. When modeling the *in vivo* epithelium *in vitro*, multiple factors need to be taken into account. *In vivo*, the airway epithelial cells are surrounded by different cell types, both immune cells and structural cells. Additionally, the *in vivo* airway epithelium is not a sterile environment. In contrast, the epithelium is continuously challenged with inhaled microbial species in addition to commensal bacteria, viruses and fungi.

When culturing and/or exposing airway epithelial cells to model asthma or COPD, it is always important to consider the research question asked. Nonetheless, when modeling the *in vivo* epithelium, care should be taken to capture the *in vivo* characteristics as thoroughly as possible to improve the translational potential of the *in vitro* model. Given the complex environment of airway epithelial cells *in vivo*, modeling those characteristics *in vitro* will be very challenging. **Chapter 2** provides a discussion of co-culture models that can be used to mimic interactions of airway epithelial cells and other cell types.

Fully capturing the characteristics of the *in vivo* epithelium is particularly challenging and will add a significant degree of complexity to the *in vitro* model through the various cellular and microbial interactions discussed. Indeed, increasing complexity of the *in vitro* model may result in considerable variation that may introduce differences between experiments. Such reduced reproducibility will result in conflicting data between and also within laboratories. However, oversimplified *in vitro* models may not capture the intricate details of the *in vivo* epithelium, overall resulting in data that cannot be translated to a clinical setting. Finding a balance between complexity and reproducibility should be carefully considered when designing culture models.

An important feature of the *in vivo* epithelium that has received a lot of recent attention and is currently lacking from *in vitro* airway epithelial culture models, is the microbiome. The microbiome comprises of multiple species of micro-organisms of which the composition and total load can vary with health status (3, 4). However, modeling the microbiome *in vitro* will be particular challenging as current sampling methods and microbial culturing methods require optimizing. Also, current *in vitro* culturing methods of airway epithelial cells include the use of antibiotics in the culture medium to prevent infection. *In vivo*, antibiotics are only

encountered during treatment of bacterial infections. In contrast, *in vitro* cultures will always have antibiotics present in the culture medium to prevent infection of susceptible cells. One of the explanations for this increases susceptibility to infection, is that the mucociliary system provided by airway epithelial cells is unable to remove particles in a similar fashion as in the lung, but also the lack of adaptive immunity may help to explain this limitation of the model. It remains however important to consider the effects of antibiotics on *in vitro* cultures of airway epithelial cells and how these may affect experimental readouts. This is illustrated by a recent study showing the impact of antibiotics on growth and differentiation of cultured keratinocytes (5). Using antibiotics in the culture medium will not be feasible in *in vitro* models incorporating the microbiome. Vice versa, controlling outgrowth of particular microbial species under *in vitro* conditions will be particularly challenging. Nonetheless, *in vitro* studies of the gut epithelium combined with complex microbial mixtures have demonstrated the feasibility of such experimental approaches and may provide us with clues to set up an *in vitro* system using airway epithelial cells and the lung microbiome (6, 7).

Another important aspect of the *in vivo* epithelium that has received particular attention to be represented in *in vitro* airway epithelial models is the lung physiology and its associated mechanical forces, including rhythmic breathing patterns, mucociliary clearance and air flow, combined with the 3D environment of the airway epithelial cells containing different cell types such as immune cells and structural cells. Very recently, considerable effort has been made towards including these important features in airway epithelial *in vitro* models (8, 9). Benam and colleagues developed a small airway-on-a-chip model that integrates fully differentiated, pseudostratified small airway epithelial cells, microvascular endothelial cells and immune cells, combined with cyclic mechanical stretching, microfluidics and air flow (9). They used the small airway-on-a-chip to identify COPD-specific biological responses and discovered novel molecular signatures that may serve as potential therapeutic targets or diagnostic biomarkers (8).

In contrast to the aforementioned described complex models, are the oversimplified *in vitro* models of airway epithelial cells that have been widely used, consisting of mostly airway epithelial cell lines that do not develop a fully differentiated, pseudostratified epithelial layer. Whereas transformed or tumor cell lines can easily be grown in large quantities and their use often does not require advanced expertise and expensive culture media, care should be taken in the interpretation of the results since many cellular pathways may be altered in such cell lines. Also, epithelial cell lines are typically cultured submerged, thereby

preventing the archetypal apical to basal polarity of the *in vivo* epithelium. Moreover, the *in vivo* airway epithelium consists of multiple epithelial cell types including basal, secretory and ciliated cells whereas cell lines do not develop these specialized cell types. Ideally, results obtained through cell lines should be validated using air-liquid interface (ALI) cultured primary airway epithelial cells, which have become widely available through commercial sources. Also, culturing methods for ALI cultured primary airway epithelial cells have been thoroughly documented and should not require excessive optimization to culture for *in vitro* validation experiments.

Using an appropriate *in vitro* model is important when modeling asthma or COPD *in vitro*. Airway epithelial cells can be isolated from patients or alternatively, airway epithelial cells can be exposed to disease related substances to model disease characteristics *in vitro*. Ideally, patient and healthy control derived airway epithelial cells are used when comparing disease characteristics, but these are difficult to obtain and commercially available patient-derived airway epithelial cells often lack critical clinically information. Therefore, modeling asthma or COPD is typically done with disease related substances. Multiple considerations that need to be taken into account when modeling asthma or COPD *in vitro* have been described in **chapter 2**. In **chapter 3** we used interleukin (IL)-13, a T helper 2 (Th2) cytokine, to model the Th2-high asthma phenotype *in vitro*. Modeling features of asthma *in vitro* is very challenging as the actual cause of asthma is still highly debatable and asthma comprises of multiple phenotypes with different underlying pathways (10). IL-13 has long been recognized to be a pivotal player in asthma pathogenesis, but it has become quite clear that only in a subset of asthma patients IL-13 can be considered as a key player. These patients have been described to have a “Th2-high” phenotype comprising of airway epithelial expression of serpin family B member 2 (SERPINB2), chloride channel accessory 1 (CLCA1) and periostin (POSTN), with POSTN also being detectable in serum (11). Moreover, this epithelial expression pattern can be used to classify asthma patients into Th2-high and Th2-low phenotypes, with Th2-high asthma patients being more susceptible to inhaled corticosteroid treatment (12). The ability to model this gene signature *in vitro* allows us to study factors that could modulate this gene signature.

In **chapter 4** we used ALI cultured primary airway epithelial cells whereby we used disease related substances to induce disease characteristics, representing an *in vitro* model for Th2-mediated inflammation combined with cigarette smoking. Our *in vitro* model showed robust responses that could be reproduced in multiple donors. Of important notice is the

discrepancy between our observed results for mucin 5AC (MUC5AC) and the effects of cigarette smoke exposure. Multiple groups have reported increased expression of MUC5AC by cigarette smoke (13, 14), whereas we did not observe this effect. The aforementioned studies however used cigarette smoke extract, an aqueous solution of cigarette smoke. Cigarette smoke extract does not give a good representation of whole cigarette smoke as discussed in **chapter 2**, but using whole cigarette smoke does come with some challenges for application *in vitro*. We used a custom designed model to expose ALI cultured airway epithelial cells to whole cigarette smoke *in vitro*. Quantifying the actual deposition of whole cigarette smoke on the ALI airway epithelial cells is currently not possible in this model. Despite being the more representable model to expose cells to cigarette smoke *in vitro* compared to aqueous extracts, it remains to be investigated whether our observed effects on MUC5AC represent the *in vivo* situation and that underlying tissue inflammation may indeed be required for the development of mucus hypersecretion in smokers. Alternatively, the exposure setup may require further optimization to recreate the results observed by other researchers using cell lines and cigarette smoke extract.

In **chapter 5** we described an *in vitro* model with viral or bacterial exposure following cigarette smoke exposure. This model could be used as an *in vitro* setup to study viral or bacterial exacerbations in COPD. We used an UV-inactivated lysate of *Haemophilus influenzae* to model bacterial exposure. Using an inactivated bacteria may not fully represent a live infection, but using live bacteria *in vitro* can be particularly challenging and complex as discussed previously for the microbiome. We did however use live human rhinovirus 16 (RV16) infection to model viral exposure as inactivated virus does not induce proper antiviral responses in airway epithelial cells (15-17).

Taken together, *in vitro* models are very heterogeneous and should reflect the *in vivo* epithelium as closely as possible. When designing an *in vitro* model of airway epithelial cells there is a factor of complexity to consider, but it should not go at the cost of reproducibility. There is a vast amount of *in vitro* models and culture methods available for airway epithelial cells ranging from cell lines to primary cells cultured submerged or at ALI. Additionally, modeling asthma or COPD can be achieved in various ways. Given the large variety in culture methods, it should be considered to work towards a more robust standardized culture model for airway epithelial cells. Ideally, the model has to be compatible with the research question asked, has to be representative of the *in vivo* epithelium and should be reproducible.

PREDICTING PATIENT OUTCOMES AND THERAPEUTIC RESPONSES USING AIRWAY EPITHELIAL CELL CULTURES: "SCIENCE"-FICTION?

Predicting patient outcomes and therapeutic responses in asthma and COPD patients currently largely relies on lung function analysis, clinical information (e.g. exacerbation frequency) and blood analysis (cell differentials and allergy tests), combined with questionnaires. This current gold standard however fails to adequately classify certain patients that may present with a specific phenotype, suggesting the importance of working towards personalized treatment plans for patients. Using patient-specific biomarker profiles, personalized treatment could become the new gold standard, eventually resulting in better patient care and improved cost effectiveness. Airway epithelial cells are the gatekeepers to the lung, making them ideal informers for underlying tissue inflammation. The importance of airway epithelial cells in asthma and COPD pathogenesis has been outlined previously (18, 19). Additionally, the potential of airway epithelial cells as a source for biomarkers to phenotype asthma patients has been shown previously (11, 12). Interestingly, narrowing and disappearance of small conducting airways has been suggested to occur before the onset of emphysema (20, 21). Given the importance of airway epithelial cells in the early pathogenesis of asthma and COPD, these cells are likely candidates for biomarker profiling in patients. Moreover, predicting patient outcomes at an early stage is important to prevent rapid worsening of disease.

Patient phenotyping using airway epithelial cells has become popular over the recent years with the development of more accurate techniques to evaluate genetic profiles. However, predicting patient disease outcomes using airway epithelial cells has not received a lot of attention until quite recently. A recent study identified genes related to emphysema expressed in airway epithelial cells and their expression was associated with lung function (22). Steiling and colleagues identified COPD-related airway epithelial expression pattern changes associated with COPD and continuous COPD-related measures of lung function (23). Several studies have focused on lung function decline using other measures to investigate airway epithelial disease. Bhatt and colleagues used computed tomography (CT) to evaluate small airway disease. They reported that CT-assessed small airway dysfunction was associated with lung function decline (24). Another group used the impulse oscillometry system to assess small airway disease and suggested that small airway disease progressively increased with both old and new GOLD classifications (25). Together these data suggest that airway epithelial cells may have the potential to predict patient outcomes. Current data

is mainly limited to lung function decline, but it may become important to investigate the potential of airway epithelial cells to predict e.g. exacerbation risks in patients.

Airway epithelial cells can be obtained using e.g. bronchoscopy, and therefore patient-specific evaluation using an appropriate airway epithelial *in vitro* model is feasible. Furthermore, repeated sampling during treatment allows for evaluation of patient responses and therapeutic efficacy. The Groningen and Leiden Universities study of Corticosteroids in Obstructive Lung Disease (GLUCOLD) study showed that gene expression profiles of bronchial biopsies taken from COPD patients before and after corticosteroid treatment reflected therapeutic responses in these patients (26). In another study, airway epithelial signatures of COPD reflected lung tissue expression changes and these signatures were similarly affected following inhaled fluticasone treatment (23). Woodruff and colleagues reported a biomarker of corticosteroid responsiveness using *in vitro* airway epithelial cultures. They also showed that corticosteroid treatment could alter the IL-13-induced Th2-high gene signature *in vitro* which reflected *in vivo* patient responses to inhaled corticosteroids (11, 12). Furthermore, because high serum levels of periostin have been shown to be indicative of positive anti-IL-13 treatment responses clinically (27, 28), it is important to evaluate whether factors influencing periostin expression would alter these treatment responses.

In **chapter 3** we evaluated the effect of the macrolide antibiotic azithromycin on the IL-13-induced Th2-high gene signature *in vitro*. Specifically we showed that azithromycin differentially modulates this gene signature in IL-13 treated cultures, as shown by inhibition of SERPINB2 and CLCA1 expression in the absence of inhibition of POSTN. These findings may have implications for the use of the gene product of the POSTN gene, periostin, as a biomarkers in patients with asthma. However, how this effect is translated clinically remains to be investigated.

In **chapter 4** we extended our knowledge of the Th2-high *in vitro* model and combined it with cigarette smoke, the main contributing environmental exposure to the development of COPD. 20 to 35% of the world population smokes, with surprisingly similar smoking rates reported in asthmatic patients (29-31). Cigarette smoking has been shown to affect asthma patient responsiveness to inhaled corticosteroids (32). We have shown that cigarette smoke differentially affected the IL-13-induced gene signature *in vitro,* whereby POSTN expression was drastically lowered by cigarette smoke exposure and was not recovered upon cigarette smoke cessation. As the presence of the Th2-high gene expression profile has been linked

to sensitivity to inhaled corticosteroid treatment (12), it will be interesting to see how this *in vitro* gene signature may predict *in vivo* patient outcomes. This however remains to be studied and was outside the possibilities of this thesis. Nonetheless, using this Th2 gene signature *in vitro* may allow us to perform preclinical studies to predict *in vivo* effectiveness of existing and new therapeutics.

Current *in vitro* models that accurately predict therapeutic outcomes in patients are lacking, and such models would require extensive validation which thus far has not occurred. In addition, repeated isolation of airway epithelial cells from patients can be challenging and likely more accessible biomarkers are warranted. Blood biomarkers are ideal as they are easily accessible. However, local responses in the lung may not be captured by the blood. Nonetheless, using blood as a window to the lung should be evaluated alongside epithelial signatures to work towards more convenient patient sample retrieval. Alternatively, other sources for biomarkers can be used such as exhaled nitric oxide levels (Fe_{NO}), sputum or even urine. Possibly these aforementioned sources of biomarkers, combined with biomarker profiles of airway epithelial cells, could result in strong, phenotype-specific profiles leading to personalized treatment. Overall, airway epithelial cells cultured *in vitro* have the potential to predict patient outcomes and treatment responses, but they will require extensive validation prior to be used as approved preclinical models.

MOUSE MODELS FOR CHRONIC LUNG DISEASE: REPURPOSING EXISTING MODELS TO IMPROVE PREDICTIVE VALUE?

In vitro cultures are limited in their ability to recapitulate complex interactions between various cell types and tissues observed *in vivo*. To overcome this problem, multiple animal models were developed that recreate certain disease features of chronic lung diseases, including asthma and COPD. *In vivo* mouse models have contributed substantially to our knowledge of underlying disease mechanisms in asthma and COPD, and are essential in the drug discovery process. Despite their widespread use in both academic and industrial research, however, very few compounds that show potential in mouse models of disease have been shown to be successful in clinical studies. The problem is not just the mouse models and understanding how to interpret the findings in these models. Most importantly, poor understanding of the underlying disease mechanisms in human disease hampers proper disease modeling in mice. Also, there is a current lack in funding towards respiratory research, limiting the capacity of research and drug development. Nonetheless, important

differences exist between human and mouse lungs that could attribute to the poor predictive value of current existing mouse models for asthma and COPD.

The basic design of the lung is very similar between mice and human. However, important differences exist in part due to the large differences in body size. The length of the bronchial tree and the total surface area vary greatly between mice and humans. Also, mice are quadrupeds which results in altered physical forces affecting the mouse lung compared to the human lung (e.g. emphysema is generally worse in the lower lobes of COPD patients). Submucosal glands are present in the human airways containing cartilage which reach from the trachea and the intra-lobar airways down to the small bronchioles, whereas in mice the submucosal glands are restricted to only the trachea (33). This particular difference may suggest why mice with the cystic fibrosis-related mutation do not develop the fibrotic lesions in the lung as observed in human cystic fibrosis (34). Additionally, the conducting airways in humans contain basal cells whereas this is not the case in mice, where basal cells are only found in the trachea and main-stem bronchi (33, 35). Instead, mouse airways are lined with a simple epithelium with ciliated and club cells. In these airways, the club cells are mainly responsible for epithelial turnover and repair following injury (36).

Despite some differences between human and mouse lungs, using mice to study asthma and COPD has multiple advantages. Mice are relatively small and inexpensive compared to other available mammalian species to study chronic lung diseases. They are also easy to breed with a short gestational period. There are numerous inbred strains available to work with and importantly, genetic modification and consequent transgenic strains are widespread available. Moreover, the technology to develop transgenic mice is constantly evolving, resulting in more efficient genetic manipulations. Additionally, a large variety of immunological reagents are available, aiding significantly in the analysis of results. Taken together, these combined advantages make mouse models very attractive to discover novel disease mechanisms and to find new therapeutic targets in asthma and COPD.

The predictive value of drugs currently reaching the market for cardiovascular and HIV/AIDS-related research is 6 to 14% respectively, whereas in respiratory research this is only 3% (37). Whereas the problem cannot be fully attributed to poor predictive value of mouse models, it does remain an important point for improvement. Mouse models of asthma are typically induced by either ovalbumin (OVA) combined with an adjuvant or by house dust mite (HDM) extract. Whereas other mouse models for asthma exist, the OVA and HDM are

the most commonly used, whereby a mouse is initially sensitized followed several weeks later by single or multiple challenges to induce features of allergic airway inflammation. An extensive overview and discussion of mouse models of allergic airway disease is available elsewhere (38-41). Mouse models of COPD are most commonly induced by cigarette smoke exposure over a duration of several months to induce features of COPD. Other mouse models of COPD are discussed elsewhere (42, 43). Despite the common use of these mouse models, even with the use of similar exposure compounds, discrepancy between laboratory outcomes exist. This is likely due to differences in e.g. exposure protocols, inbred strains of mice, delivery systems and intensity in exposure. Moreover, these mouse models typically only recapitulate certain features of disease. In allergic airway disease models, airway remodeling is typically limited to subepithelial fibrosis with limited bronchial smooth muscle hyperplasia or hyper-proliferation (41). In COPD models, even after several months of cigarette smoke exposure, disease features are typically mild, unlike the disease severity observed in COPD patients (following decades of smoking).

A major pitfall is that both asthma and COPD are very complex syndromes comprised of multiple clinical phenotypes with different underlying pathologies which we have just started to unravel. Mouse models can only reflect our current knowledge of human disease and can only model a particular phenotype of the disease. Therefore, it is plausible that we should strive towards understanding human disease better before trying to model it *in vivo*. Stratifying patients into clinical phenotypes has shown to improve therapeutic responses to novel treatments. Anti-IL-5 treatment has been shown to have beneficial effects in a subset of asthmatic patients with high numbers of sputum eosinophils (44, 45). Similarly, a subset of asthmatic patients with high serum levels of periostin, indicating a Th2-high phenotype, were found to be more responsive to anti-IL-13 treatment compared to the overall population of asthmatic patients (27, 28). Overall, stratifying patients into clinical phenotypes may improve predictive values of novel therapeutics. Also, understanding the underlying disease mechanisms and working towards patient specific biomarkers becomes particularly important, especially with an industry working towards personalized medicine.

Improved understanding of the mechanisms underlying asthma and COPD and the clinically observed phenotypes will allow us to better understand the existing mouse models of chronic lung disease. As multiple clinical phenotypes exist for both diseases, it is conceivable that the various mouse models of asthma and COPD could be classified to reflect specific phenotypes, rather than the disease as a whole. It is well accepted that the OVA mouse

model of allergic airway disease is mainly driven by a Th2 phenotype, whereas the HDM mouse model is a more mixed response of Th2 and Th17-mediated disease (46, 47). For mouse models of COPD, the distinction of different phenotypes is not as obvious. However, the commonly used elastase mouse model of COPD is likely more reflective of the genetic form of COPD whereby a mutation in the alpha-1 antitrypsin gene causes unrestricted activity of neutrophil-derived elastase (48). Nonetheless, whereas classifying mouse models into specific phenotypes may improve their predictive value, it should also be noted that the existing models could be improved to reflect human disease more closely.

Both asthma and COPD are complex syndromes influenced by both genetics, lifestyle and environment. Single stimuli to induce features of disease in mouse models likely do not reflect the complex interactions observed in human disease. Therefore, mouse models of asthma and COPD should perhaps also include lifestyle effects on these conditions, such as high fat diet or physical activity. Likewise, environmental factors such as air pollution could also be considered. Exacerbations have also been modeled in mouse models of both asthma and COPD (49-51), although differences in pathogenicity of microbes between mice and humans should be taken into consideration. Additionally, it is important to consider that mice are kept in a clean and controlled environment, which is in stark contrast to human lifestyle. Another point to consider is that typical readout parameters used in mice studies are inflammation and remodeling, whereas in the clinic, changes in lung function are typically used. Various groups have started to include lung function data of mice studies, but it should always be considered that mouse lung physiology does not always reflect human lung physiology.

Reassigning mouse models to better reflect human disease phenotypes could improve their predictive value. Whereas there are genomic differences between mice and humans, the underlying molecular pathways and disease mechanisms are likely to be similar, particularly if specific disease phenotypes are considered. Given the current standing of *in vitro* models, *in vivo* models and patient-based studies, more effort should be put into finding translational links in those disease mechanisms and underlying molecular pathways using e.g. bioinformatics or –omics data. Changes in molecular signatures or pathways associated with functional and physiological data could further enhance the predictive value of mouse models of asthma and COPD. Another important feature of mouse models of chronic lung disease is that mouse airway epithelial cells can be isolated at different time points to study disease progression and perhaps how certain phenotypes develop over time. The

pseudostratified mouse tracheal epithelium contains basal cells, which are lacking in the lower airways of mice. Therefore, mouse tracheal epithelial cells give the best representation of the human pseudostratified airway epithelium. A major setback in isolating these cells however, is that current techniques to isolate mouse tracheal epithelial cells are particularly inefficient.

In **chapter 6** we developed a new culturing technique to expand and differentiate mouse tracheal epithelial cells. Expanding mouse tracheal epithelial cells *in vitro* for subsequent ALI differentiation allows a much more efficient use of animals, which is particularly interesting when mouse tracheal epithelial cells are isolated from difficult-to-breed mouse strains. Also, our culture method can decrease technical and biological variation between experiments and will ultimately lead to a reduction in experimental animal use and related costs. Furthermore, human tissue can be difficult to obtain and genetic modification of primary human bronchial and tracheal epithelial cells has proven to be very difficult (52). Mouse tracheal epithelial cells isolated from disease models or transgenic animals can offer a good alternative for primary human airway epithelial cells. Furthermore, since studies in patients with COPD and asthma have shown that some epithelial features are maintained in culture, mouse tracheal epithelial culture models can help to provide insight into the (epigenetic) mechanisms that may explain the persistence of disease specific features of epithelial cells in culture when cells are no longer present in the tissue of origin.

Overall, repurposing and improving existing mouse models should improve their predictive value. However, more effort should be put into understanding clinical phenotypes underlying human disease and how mouse models may correlate with these phenotypes. Therefore, combining data from *in vitro* models using airway epithelial cells with data from *in vivo* models, focusing on specific phenotypes and translating these results towards the clinic may overall improve respiratory research towards asthma and COPD.

CONCLUSIONS

The studies presented in this thesis were aimed at developing and using *in vitro* models that could benefit research towards understanding asthma and COPD. We used an *in vitro* model representing a Th2-high gene signature and studied how this gene signature may be affected by external factors such as cigarette smoke or drugs. Using these *in vitro* airway epithelial cell models may help to predict clinical outcomes, although they will require

extensive validation. We also investigated the possibility of using primary human airway epithelial cells to model bacterial and viral exacerbations. Whereas this model is currently still under investigation, it could be particularly useful to study possible biomarkers of exacerbations and how these may be affected by external factors. Additionally, we also developed a new method to expand and differentiate mouse tracheal epithelial cells *in vitro*. Whereas these cells do not fully represent human cultures, they have the major advantage that these cells can be isolated during a time course to investigate disease progression. Additionally, transgenic animals can be used to study the influence of particular genes on disease mechanisms. Overall, studying airway epithelial cells may provide important clues for understanding disease pathogenesis, lead to identification of new treatment targets, and may provide important biomarkers. Using airway epithelial cells and their derived biomarkers could significantly improve our understanding in disease phenotypes of asthma and COPD. Additionally, with increasing knowledge of the disease phenotypes, we could better address the unmet need in treatment of asthma and COPD.

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