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Research paper

In vitro and *ex vivo* models of human asthma

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ABSTRACT

Asthma is an inflammatory disorder of the conducting airways which undergo distinct structural and functional changes leading to non-specific bronchial hyperresponsiveness (BHR) and airflow obstruction that fluctuate over time. It is a complex disease involving multiple genetic and environmental influences whose multifactorial interactions can result in a range of asthma phenotypes. Since our understanding of these gene–gene and gene–environment interactions is very poor, this poses a major challenge to the logical development of ‘models of asthma’. However, use of cells and tissues from asthmatic donors allows genetic and epigenetic influences to be evaluated and can go some way to reflect the complex interplay between genetic and environmental stimuli that occur *in vivo*.

Current alternative approaches to *in vivo* animal models involve use of a plethora of systems ranging from very simple models using human cells (e.g. bronchial epithelial cells and fibroblasts) in mono- or co-culture, whole tissue explants (biopsies, muscle strips, bronchial rings) through to *in vivo* studies in human volunteers. Asthma research has been greatly facilitated by the introduction of fibreoptic bronchoscopy which is now a commonly used technique in the field of respiratory disease research, allowing collection of biopsy specimens, bronchial brushing samples, and bronchoalveolar lavage fluid enabling use of disease-derived cells and tissues in some of these models. Here, we will consider the merits and limitations of current models and discuss the potential of tissue engineering approaches through which we aim to advance our understanding of asthma and its treatment.

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1. Introduction

Epidemiological studies have shown that the prevalence and severity of asthma has increased remarkably over the last decades both in children and adults. It is estimated that 300 million people suffer from the condition worldwide, with up to 18% of the population being affected in some countries [1]. Furthermore, the asthma related costs, which include direct costs for healthcare as well as indirect costs due to working days lost, have been rising dramatically and, in Europe, are estimated to be around €17.7 billion per annum [2]. A recent assessment of asthma across Europe (Brussels Declaration) has identified a substantial unmet clinical need with those 10% of patients with severest disease accounting for ~50% of the health costs [3]. Asthma prevention has not been achieved; once established there is no cure and there are currently no medications that can alter its natural history. Management is primarily directed towards suppressing inflammation with corticosteroids and relieving bronchoconstriction with bronchodilators.

Asthma is a chronic inflammatory condition defined primarily by clinical characteristics; it is often considered to be a syndrome rather than a disease, as patients exhibit a range of different phenotypes including differences in the type of inflammation or response to therapy [4–6]. Although many studies have been undertaken to investigate the complex mechanisms of asthma, our understanding of the disease is still limited. This makes asthma one of the biggest challenges for researchers and clinicians. The complexity of asthma and the likelihood that the underlying disease mechanisms are multifactorial probably explain why there remains an unmet clinical need for new therapeutic strategies. In order to fully understand the underlying mechanisms of asthma, there is on the one hand a need for detailed characterisation of the subtypes of asthma and on the other hand the need for good experimental models that reflect these different phenotypes. Together, these approaches may result in new therapeutic strategies and more efficient patient-tailored treatments. This review will give an overview of the complexity of asthma and discuss the human tissue/cell based models used to study asthma mechanisms *in vitro* and *ex vivo*.

2. Definition of asthma

The hallmarks of asthma are airway inflammation, airway hyper-responsiveness (AHR), reversible airway obstruction and

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symptoms of cough, wheeze and breathlessness. Asthma is associated with episodic exacerbations which can be triggered by a multitude of factors; the most common are viral infections, allergen exposure, exercise and cold air [7]. Considerable effort has been invested into the development of national and international guidelines for the management of asthma in order to improve diagnosis, patient care and treatment [1]. Diagnosis of asthma is mainly based on medical history of symptoms and measurement of lung function. Commonly used methods to evaluate lung function are by spirometry: the measurement of Forced Expiratory Volume in 1 second (FEV₁), Forced Vital Capacity (FVC) and Peak Expiratory Flow (PEF). While lung function tests can enhance the confidence of a symptom-based diagnosis and provide complementary information about asthma control, they are not strongly correlated to the symptoms. In patients with normal lung function but asthma-like symptoms, a diagnosis can also be based on measurement of airway responsiveness to challenges such as inhaled methacholine, histamine, mannitol or exercise. Asthmatic patients normally show a 20% fall in FEV₁ in response to lower agonist concentrations than non-asthmatic subjects, but despite the high sensitivity, these tests lack specificity [8]. Further methods for the diagnosis of asthma can include the measurement of exhaled NO concentration and the number of granulocytes in induced sputum as a marker of airway inflammation [9,10]. In certain cases, a more invasive fiberoptic bronchoscopy can be performed in specialist centres in order to obtain biopsies for histological analysis and bronchoalveolar lavage (BAL) fluid for differential cell counts and analysis of proinflammatory mediators [11].

3. Phenotypes and treatment of asthma

Phenotypes of asthma are very heterogeneous, ranging from mild to severe or from intermittent to persistent airway obstruction. Strictly speaking, there are currently no validated and specific biomarkers based on underlying disease mechanisms which may allow classification of asthma into subtypes and development of more efficient patient-tailored therapies [12,13]. However, the measurement of exhaled NO and the count of eosinophils in sputum have been shown to be of value for prediction of corticosteroid responsiveness [14], and more recently, serum periostin levels have been used to define 'Th2 high' asthmatic subjects who may show treatment benefit with anti-IL-13 [15]. On the basis of clinical characteristics, cluster analysis of asthma patients in primary and secondary care has revealed five clusters divided by symptoms and eosinophilic inflammation: early onset atopic asthma, benign asthma, obese non-eosinophilic asthma, early symptom predominant and inflammation predominant asthma [4]. Other attempts to classify asthma phenotypes are based on symptoms and the responsiveness to drug treatments. For example, asthma phenotypes can be divided into allergic or intrinsic non-atopic asthma; or according to granulocyte cell counts in induced sputum into eosinophilic, neutrophilic, mixed granulocytic, or paucigranulocytic phenotypes. Responsiveness to drugs suggests a corticosteroid insensitive and an aspirin-intolerant subgroup of asthma. For more details about asthma subgroups, the reader is referred to other recent reviews [5,6].

The Global Initiative for Asthma (GINA) has published recommendations and guidelines for the management of asthma. The report 'Global Strategy for Asthma Management and Prevention' is updated yearly in December. Since 2006, the recommendations for asthma management are based on clinical control rather than asthma severity. Asthma medications can be grouped into asthma 'controllers', which are taken on a regular basis to suppress airway inflammation, and 'relievers', which are bronchodilators and are taken only when needed. To date, the most effective controllers of

persistent asthmatic airway inflammation are inhaled corticosteroids. Reliever therapies are inhaled short-acting β_2 -agonists and are predominately used in acute asthma exacerbations in order to relieve bronchospasm. Leukotriene modifiers, such as cysteinyl-leukotriene 1 (CysLT1) receptor antagonists (montelukast, pranlukast, zafirlukast) and 5-lipoxygenase inhibitor (zileuton) are also controllers, but since their effect is less than low doses of inhaled corticosteroids, they are mostly used in combination with corticosteroids. Another class of controllers used in combination with corticosteroids is the long-acting inhaled β_2 -agonist. Combination therapy has a higher efficacy and allows lower doses of inhaled corticosteroids compared to corticosteroids alone. Theophylline in sustained-release formulation can also be used in combination with inhaled corticosteroids but is less effective than long-acting β_2 -agonists. Due to its bronchodilator effect, short-acting theophylline is also used as a reliever, but there is controversy about the benefit in the treatment of asthma exacerbations [1]. For some patients with allergic asthma that remains uncontrolled despite use of high doses of corticosteroids, anti-IgE therapy (omalizumab) can be an option. However, there is still an unmet clinical need for new treatments, especially for patients with severe, uncontrolled asthma. Therefore, there is a need for detailed characterisation of the underlying disease mechanisms that might help in identifying new therapeutic targets.

4. Pathogenetic mechanisms of asthma

Asthma is characterised by inflammation and structural remodelling of the conducting airways, although with increasing chronicity the small airways can also become affected. The structural changes of the remodelled airways are characterised by goblet cell metaplasia and hyperplasia, subepithelial fibrosis and increased collagen deposition throughout the airway wall, thickening of airway smooth muscle and increased angiogenesis [16]. Asthma is a complex disease that involves both genetic and environmental factors. It was originally thought that asthma is mainly mediated by allergic airway inflammation triggered by exposure to environmental allergens. However, epidemiological studies have shown that up to 40% of the population in industrialised countries are atopic, but only 7% of this group are affected by asthma [17]. These findings imply that there are other underlying mechanisms involved in the development of asthma beyond atopy. New hypotheses are pointing to the importance of the formed elements in the airways, especially the airway epithelium in the development of asthma [18]. This concept is supported by recent studies which have identified a number of asthma susceptibility genes including *PCHD1*, *IL-33* and *ORMDL3* [19] that are expressed in epithelial cells, as well as other genes such as *ADAM33* [20] which are found in mesenchymal cells [21,22].

The airway epithelium forms the first line of defence against inhaled environmental agents and a breakdown in innate defence mechanisms is thought to play a central role in asthma pathogenesis. The barrier properties of the epithelium can be divided into three broad functions: (i) the physical barrier formed by close cell-cell contacts, for example, tight junctions, (ii) the chemical barrier which is formed by the secretions of epithelial cells, for example, mucus, anti-oxidants, etc., and (iii) the immunological barrier which is involved in innate immunity and which interacts with cells of the innate and adaptive immune system through expression of adhesion molecules and release of mediators including cytokines and chemokines [23]. There is evidence that these barrier functions of the epithelium are abnormal in asthma [24], either due to genetic or environmental influences. Although not well explored, environmental factors like pollution or cigarette smoke may cause epigenetic changes which result in altered gene

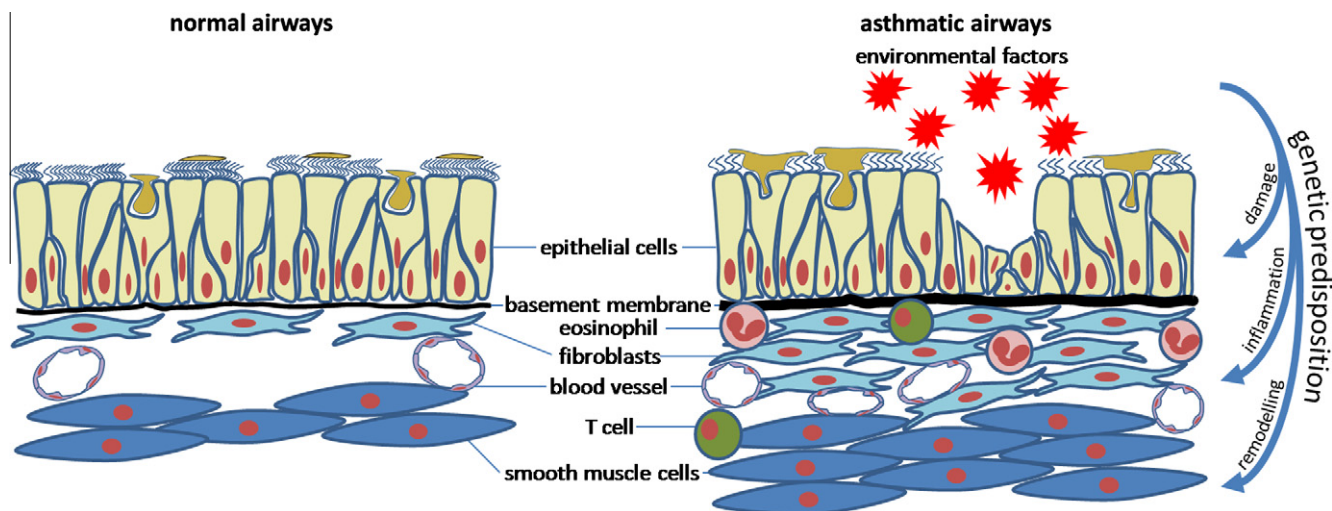


Fig. 1. Environmental factors together with an underlying genetic predisposition trigger processes in the airway mucosa that result in the structural and functional changes observed in asthma. These processes include epithelial damage, airway inflammation and remodelling and result in mucus hyper-secretion, epithelial damage, thickening of the basement membrane, fibroblast and smooth muscle layer, infiltration of eosinophils and T cells and angiogenesis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

expression profiles [25]. A recent paradigm of asthma is based on the hypothesis that breakdown of epithelial barrier functions leads to tissue injury and activation of wound healing processes in the airways [26]. In asthmatic individuals, these repair mechanisms appear to be impaired and cause a chronic wound healing response. This results in activation of the epithelial–mesenchymal trophic unit (EMTU) leading to airway remodelling and maintenance of airway inflammation (Fig. 1).

5. Animal models to analyse asthma mechanisms

For ethical reasons, the majority of *in vivo* experimental studies of asthma have been and will continue to be performed in animals. Such models are also essential for testing the safety and efficacy of new drugs. A variety of species have been used for study of asthma mechanisms, including the fruit fly *Drosophila*, mice, rats, guinea pigs, cats, dogs, pigs, horses and primates [27–31]. Indeed, the knowledge we have today about potential asthma mechanisms, as well as innate and adaptive immune responses, is largely based on observations obtained from experimental animal models. However, it is important to note that most animals used for studying asthma do not spontaneously develop the disease [32]. Asthma-like conditions are only reported in cats [33] with allergic syndrome and heaves in horses [34,35].

Most ‘animal models of asthma’ are models of allergic airway inflammation based on an initial sensitisation phase to an antigen followed by local challenge to induce airways inflammation. In the sensitisation phase, the antigen is usually administered systemically by intraperitoneal injections, mainly in combination with an adjuvant. The use of an adjuvant primes the antigen specific immune response in a certain direction, for example, aluminium hydroxide (alum) promotes a Th2 immune response [36], whereas pertussis toxin favours a Th1 immune response [37]. A variety of antigens have been used, ranging from ovalbumin which is relatively cheap to more disease-relevant allergens such those from house dust mite or fungal spores. However, there is a considerable variability between different models, both in the outcome of cellular immune responses as well as the phenotypes. This may be due to different methods used to initiate a sensitisation, the antigen itself or variation between different species or strains in terms of anatomy, physiology and immunology [38–41]. As a result, direct

comparison of animal studies can be quite difficult and the interpretation of the results into humans must be done with care.

The most commonly used animal to model allergic airway inflammation is the mouse, mainly for practical reasons including relatively low handling costs, short gestation period, litter size and the availability of tools to study them on an immunological and molecular level [42]. Furthermore, it is now straight forward to manipulate mice genetically in order to switch on or off certain genes and thus study specific pathways [43]. Additionally, it is possible to study specific cellular responses by adoptive transfer of cells and tissue from manipulated mice into wild type mice of the same strain or *vice versa* [44].

6. Limitations of animal models and differences to human asthma

Mouse models of allergic airway inflammation have contributed substantially to our understanding of Th1 and Th2 driven immune responses in asthma, and on the basis of these results, new therapeutic strategies targeting Th2 cytokines or pathways have been developed, but these have failed to reach the clinic due to lack of efficacy in the human disease [45]. For example, mouse models of asthma identified that IL-4, IL-5 and IL-13 are key mediators in allergic airway inflammation and highlighted these cytokines and their upstream and downstream pathways as potential therapeutic targets, but disappointingly, drugs interfering with these pathways have failed in clinical trials [46]. However, recent evidence suggests that a subset of ‘Th2’ high asthmatic subjects (defined by elevated serum periostin levels) can be identified and show treatment benefit with anti-IL-13 [15]. While this study highlighted the need for careful patient phenotyping during clinical trials, anti-IL-13 is not useful for corticosteroid refractory disease, where there is a major unmet need. This failure to address therapy resistant disease might be predicted, as the animal models upon which anti-IL-13 was developed are corticosteroid sensitive.

One important feature of human asthma is disease chronicity that appears to be associated with remodelling of the airways, especially in severe asthma. In human asthma, these remodelling processes appear to occur in parallel with allergic Th2 driven immune responses [47] rather than occurring as a consequence of the inflammation, as occurs in the animal models. Crucially, it is

the group of patients with uncontrolled severe asthma and airway remodelling that is in need of new treatments.

It is also important to reflect the anatomical, physiological and immunological differences when translating results from mouse models into the human disease. Due to their small size, it is a technical challenge to determine and characterise the phenotype of asthma in mice by testing lung function, and alternatives such as the enhanced pause (Penh) are often used to estimate airway resistance, but this measure has limitations. Furthermore, all animals used in 'asthma' models are quadrupeds and together with differences in the lobar structure and branching pattern of the bronchi, this might affect ventilation, change airflow distribution and thus aerosol deposition. Additionally, mice lack the ability to cough, one of the hallmark symptoms in human asthma [48]. Another important difference is that in rodents, bronchodilatory nerves are missing [49] and since it is hypothesised that diminished ability to dilate the airway smooth muscles, for example, through deep inspiration, is linked to AHR [50], mouse models of 'asthma' are unable to cover this aspect of the disease. On the immunological level, there are also a few relevant differences between animals and humans to consider. For example, mast cells play an important part in airway inflammation by releasing a variety of mediators after activation, but the pattern of mediators released differs between rodents and humans. Serotonin, released by rodent mast cells, is one of the major mediators of smooth muscle contraction and bronchoconstriction, but human airway mast cells release hardly any serotonin after activation and the human airways are unresponsive to serotonin [32]. In summary, animal models of asthma capture only a part of the many pathways underlying human asthma and the species specific limitations need to be considered carefully when translating results from animal models into human [51].

7. Human tissue-based *ex vivo* and *in vitro* asthma models

While some studies can be undertaken on human volunteers with asthma [52], access to human airway tissue has allowed development of *ex vivo* and *in vitro* models of the human airway that allow mechanistic studies that could not be performed ethically *in vivo* [53]. These studies range in their level of complexity from simple monocultures with little structural organisation to complex 3-D cultures containing many cell types that are organised into a structure that retains (*ex vivo* tissue explants) or mimics (tissue engineered *in vitro* models) some *in vivo* elements (Fig. 2). In centres with appropriate facilities, human airway tissue can be obtained from volunteers at bronchoscopy (small biopsy samples), from surgical lung resections, or post mortem, all being obtained after appropriate ethical approval and informed consent. Commercial companies also supply primary cells grown from lung tissue.

7.1. *Ex vivo* cultures

The simplest human tissue-based models for the study of airways responses are *ex vivo* tissue explants. Tissue from resected lungs can be kept in culture using special media formulations for a limited time period and used for short term experiments. For example, precision cut lung slices have been used to investigate the early phase allergic immune response in the lung [54] and are a useful tool to analyse the effect and metabolism of drugs [55–57]. Biopsy samples obtained from non-asthmatic or asthmatic individuals by bronchoscopy are another source of *in vivo* fully differentiated lung tissue, which can be used *ex vivo* for short term experiments to analyse asthma mechanisms. By taking bronchial biopsies from asthmatic individuals and exposing them to allergen in culture, the involvement of T cells in the allergic inflam-

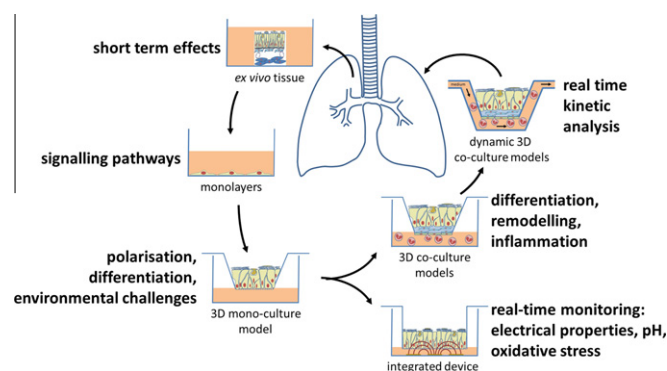


Fig. 2. A variety of experimental models are available to study mechanisms of human asthma. *Ex vivo* tissue is mainly used to analyse short term effects. Isolated primary human lung cells can be used *in vitro* in single cell monolayer cultures for studying signalling pathways. Additionally, the differentiation of primary bronchial epithelial cells can be induced *in vitro* resulting in a 3D model of the epithelial barrier which allows analysing processes involved in polarisation, differentiation and effects of environmental challenges. The development of new devices with integrated functions to measure electrical barrier properties, pH, oxidative stress, etc. will allow real-time monitoring of cellular responses. By combining different cell types together in 3D co-culture models, differentiation, remodelling and inflammation processes of asthma can be analysed in more detail. The integration of a microfluidic flow of medium and immune cells results in a dynamic multicellular 3D co-culture model to mimic more closely the *in vivo* situation. These models are a useful tool to study kinetic processes involved in asthma in real-time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mation and contributing signalling pathways has been analysed [58–61]. While these models benefit from retaining the normal tissue architecture, they are limited by their relatively short term viability and the barrier properties of the epithelial layer are compromised by exposure of the whole excised tissue sample to challenge agents.

7.2. *In vitro* models

The simplest human based models for the study of airways responses are cell lines derived from pulmonary tissue. These can be established from cancers, embryonic lung tissue or by viral transformation of cells from normal lung tissue and include bronchial epithelial cells, smooth muscle cells and fibroblasts. Cell line based models are widely used for analysing cell signalling pathways and basic cellular responses in the airways. For example, the BEAS-2B bronchial epithelial cell line has been used to show that IL-1 β , a cytokine that plays a central role in communication of danger signals from epithelial cells to monocytes, enhances rhinovirus-induced inflammatory responses via MyD88 [62], suggesting that the IL-1 β signalling pathway is a potential therapeutic target. The BEAS-2B model has also been used to show that the release of matrix metalloproteinase-9 (MMP-9), a matrix degrading enzyme, is induced after rhinovirus infection via mitogen-activated protein kinase (MAPK) and Fos-related Ag-1 (Fra-1) signalling pathways and that these pathways are sensitive to β_2 -agonists and corticosteroids. [63]. Furthermore, some airway epithelial cell lines such as the SV-40 transformed cell line 16HBE14o- or the adenocarcinoma cell line CaLu-3 have retained their ability to form an electrically tight barrier by expression of tight junction proteins. These cell lines are widely used for toxicology and drug transport studies, as well as for analysis of pathways involved in barrier function in response to environmental agents including allergens [64,65], particulates [66], cigarette smoke [67,68] or viral infections [69,70]. However, while these cell line based models are readily accessible and easy to manipulate, such immortalised cell lines are not 'normal' and they do not possess the underlying genetic or epigenetic

features that contribute to the disease phenotype(s) in people with asthma.

The use of primary human cells derived from the airways of non-asthmatic and asthmatic individuals offers the potential to compare responses of disease-derived cells and so identify dysregulated pathways that may contribute to disease. These samples can be routinely obtained using fiberoptic bronchoscopy performed on volunteers after ethical approval and informed consent; however, the requirement for bronchoscopy limits availability of this approach to those with access to appropriate clinical facilities. Many protocols have been established for culture of a variety of primary human airway cells *in vitro*, ranging from bronchial epithelial cells grown from bronchial brushings or biopsies [71,72], fibroblasts [73] and smooth muscle cells [74] from bronchial biopsies and immune cells or myofibroblasts from bronchoalveolar lavage [75,76]. Since epithelial cells form the first line of defence against the inhaled environment and many asthma susceptibility genes are expressed in the epithelium [19], there has been a major focus on airway epithelial cells in asthma research. It has been shown that primary bronchial epithelial cells (PBECS) from asthmatic donors retain a distinctive phenotype in culture over several passages [71,72]. This may reflect underlying genetic differences or epigenetic changes that may be a consequence of environmental exposures at critical stages of lung development or early life. Many studies with PBECS from non-asthmatic and asthmatic individuals have highlighted differences in the cellular response after exposure to environmental agents with the potential to cause epithelial damage. For example, it has been shown that monolayer cultures of PBECS derived from asthmatic individuals have a deficient innate immune response to rhinovirus infection by producing less interferon (IFN) β [77] and this is paralleled by higher levels of the Th2 biasing chemokine, thymic stromal lymphopoietin (TSLP) [78]. Based on the observation that exogenous IFN- β restored the anti-viral response and suppressed viral replication [79], clinical trials are currently underway using inhaled IFN- β as a new treatment of virus induced asthma exacerbations.

As an extension of the PBEC monolayer model, the use of *in vitro* differentiated PBECS offers an epithelial model that more closely recapitulates the airways *in vivo*. Placing PBECS at an air–liquid interface (ALI) in the presence of retinoic acid causes the epithelial cells to form a pseudostratified structure with basal cells supporting ciliated cells and mucus-producing goblet cells [80], as occurs *in vivo*. Studies using ALI cultures have provided insights into the effects of the Th2 cytokines, IL-4 and IL-13, on bronchial epithelial cell differentiation, with a marked skewing towards goblet cell hyperplasia, increased mucin gene expression and mucous hypersecretion [81–83]. Furthermore, use of ALI cultures has allowed comparisons of barrier properties of epithelial cells from asthmatic and healthy subjects and the demonstration that tight junctions are reduced in cultures from asthmatic subjects (as observed *in vivo*) with a corresponding increase in ionic and macromolecular permeability [24]. It has also been shown that the permeability of the bronchial epithelial cell layer is only increased in asthmatic subjects after exposure to ozone or nitrogen dioxide [84] and mediator release is altered after exposure of ALI cultures from asthmatic donors to respiratory syncytial virus (RSV) or particulate matter [85]. ALI cultures are also a useful tool to analyse ciliary beat frequency in response to various stimuli [86,87].

While these models contribute significantly to our understanding of asthma mechanisms, they are limited due to the relatively low number of cells that can be collected and grown from each subject and there is the potential for cells to lose their characteristics after long term culture [88]. However, recent advances involving induced expression of human telomerase reverse transcriptase (hTERT) to prevent replicative senescence and expression of the cell cycle protein cdk4 [89] has enabled immortalisation of epithelial

cells in culture without loss of their ability to differentiate when taken to an ALI [90]. Such an approach might be useful to generate cell lines from asthma-derived cells, although any such lines would need careful phenotypic characterisation. As it has been reported that there is an increase in stem cells in the airways in asthma, these cells offer an alternative solution for use in asthma models [91,92]. However, much research is needed to establish optimal conditions for growth of specific cell types and, as with the immortalised cell lines, to characterise their phenotypic properties. In addition to ALI culture models, formation of 3D spheroids can also be achieved using bronchial brushings or primary PBECS. In these cases, the spheroids can be free floating with ciliated cells on the outer surface of the spheroid [93] or embedded in Matrigel to form glandular structures which secrete mucus into the lumen [94]. These models have been used for studying processes involved in morphogenesis and branching [95,96] and may also offer pharmaceutical companies potential for high throughput screening using approaches similar to those used for evaluating anti-cancer drugs [97].

A significant limitation of many *in vitro* cell culture models is that they investigate responses only from one isolated cell type, whereas *in vivo*, the interplay between many different cell types is crucial for cellular homeostasis. In order to address these complex interactions in experimental models, considerable effort is currently being invested into the development of co-culture models and 3D tissue constructs integrating cells and extracellular matrix components to more closely mimic the *in vivo* state. A variety of co-culture models have been described using many different combinations of cell types. The simplest co-culture models use differentiated or undifferentiated airway epithelial cells in the apical compartment of transwell support filter units and other cell types in the basal compartment. Adherent cells such as fibroblasts or endothelial cells can also be cultured in close contact with the epithelial cells on the under-surface of the filter insert [98]. These co-culture models represent a valuable tool to analyse epithelial–mesenchymal signalling which is believed to play an important role in airway inflammation and remodelling in asthma. For example, fibroblast activation has been observed in response to epithelial challenges including physical damage [99,100], viral infection [101] or mechanical strain to simulate the effect of bronchoconstriction on the epithelium [102]. Similarly, by co-culturing airway epithelial and endothelial cells, it has been shown that cell–cell communication involving soluble endothelial-derived factor(s) causes a significant reduction in paracellular permeability compared to that seen when epithelial or endothelial monolayers are studied in monoculture [103].

The complexity of these models can be further increased by combining more than two different cell types; however, one critical step in these approaches is optimisation of media compatibility, as different cell types have differing requirements for supplements and co-factors and their behaviour can be markedly modified by changing culture conditions. Using membrane filter supports, a triple cell culture model of the respiratory epithelium has been developed with airway epithelial cells cultured on the upper surface of a transwell until confluent followed by introduction of macrophages onto the apical surface and dendritic cells basolaterally [104,105]. In this model, macrophages and dendritic cells were able to make contact to each other without breaking down the epithelial barrier by expression of tight junction proteins and they were able to pass particulates from the macrophage to the dendritic cell [106]. Another model has been developed using bronchial fibroblasts embedded in extracellular matrix overlaid first with dendritic cells and then with bronchial epithelial cells at the air–liquid interface; this model has been used to show that the structural cells regulate the capacity of dendritic cells to produce the chemokines CCL17, CCL18, and CCL22 [107].

Currently, substantial effort is being invested into the development of new devices and multicellular 3D co-culture models to study airway epithelial barrier functions *in vitro* by using tissue engineering techniques. Since airway epithelial cells are cultured at an air–liquid interface, alternative methods have been developed to monitor barrier functions instead of using chop-stick electrodes that require the cultures to be submerged allowing electrical contact to be made across the epithelial layer. By using electrical impedance, the electrical barrier properties of epithelial cells can be monitored online without submerging the cultures [108]. Another approach to model the pulmonary epithelium is the so called lung-on-a-chip model, a device with alveolar epithelial cells on the apical and endothelial cells on the basolateral side of a porous membrane [109]. In this model, the cells were supplied with nutrition via microfluidic flow of medium which also allowed introduction of immune cells into the model. Additionally, the device was built of flexible materials, which allowed for controlled stretching of the cells on the membrane to simulate the cyclical stretch of the lung tissue during inhalation and exhalation. Other models have used tissue engineered matrices to support airway cells in a 3D tissue equivalent [110]. Using biocompatible hydrogels as an ECM analogue offers the advantage of modulating the ECM stiffness, which is of interest in asthma remodelling processes. For example, it has been shown that increased ECM stiffness induces fibroblast differentiation and results in increased expression of α -smooth muscle actin [111].

8. Conclusion

In vitro cultures of cells from volunteers with asthma offer the potential to model aspects of the disease and to investigate asthma mechanisms in the context of the genetic and epigenetic factors that contribute to the disease. While current models are relatively simple and do not reflect the complexity of lung tissue *in vivo*, rapid advances in tissue engineering approaches will enable development of more involved models that better reflect the *in vivo* situation. The models described here are not limited only to asthma; they can also be used to investigate the pathomechanisms of other airway diseases like chronic obstructive pulmonary disease (COPD) and cystic fibrosis.

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