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Models and approaches to understand the role of airway remodelling in disease

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ABSTRACT

Airway remodelling is a collective term for changes in the amount or organisation of the cellular and molecular constituents of the airway wall. Remodelling occurs in and is associated with the pathophysiology of airways diseases including asthma and chronic obstructive pulmonary disease. The remodelling that occurs in these diseases exhibits both shared and distinct features. Remodelling is generally considered to be deleterious to airway function but recent studies also indicate potential protective effects. However, the true impact of different aspects of the remodelling process on lung function, both negative and positive, is poorly understood. In addition, the genetic susceptibility and processes by which environmental insults drive the cell and molecular events which result in airway remodelling and the potential for therapeutic reversibility are also incompletely understood. The last 10–15 years has seen the development of animal models of airway remodelling which have been refined and modified as new factors such as exacerbations and early life influences have been recognised as being of importance. In addition, invertebrate models have been put forward and complex *in vitro* culture systems and lung slice preparations developed. In parallel, imaging technology has developed to an extent where it is feasible using a combination of techniques to image structural components, cells and proteins in the airway wall as well as to analyse biological processes, cell and receptor activation non-invasively over time. The integration of data from *in vivo* and *in vitro* models together with use of imaging techniques in man and animals should allow validation of models, further our understanding of the pathophysiology of airway remodelling and potentially improve predictive accuracy for the translation of novel therapeutic agents into the clinic.

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1. Introduction

Airway remodelling is the term applied to collective changes in the quantity and/or organisation of the various cell and molecular components of the airway wall. From the luminal side of the airway it includes epithelial damage, goblet cell hyperplasia, enlargement of submucosal mucus glands and mucus hypersecretion. Changes in the subepithelial regions include fibroblast proliferation and activation, altered regulation and deposition of many extracellular matrix (ECM) proteins, angiogenesis and vascular remodelling, airway smooth muscle hyperplasia and hypertrophy. Any or all of these changes may contribute to thickening of the airway wall. Such changes are well recognised as key features of airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) [1–3].

Although airway wall remodelling occurs in both asthma and COPD the nature of the changes is different in the two diseases. In asthma the bronchial epithelium is fragile with variable loss of surface epithelium whereas in COPD squamous metaplasia is often

observed. Thickening of the lamina reticularis occurs in asthma but not in COPD. Whereas, in COPD there is airway wall fibrosis that is not a feature of asthma. There is goblet cell hyperplasia in the large airways, submucosal gland hypertrophy and an increase in bronchial smooth muscle in both asthma and COPD [1,3].

Intuitively, these structural changes would be expected to have a significant pathophysiological impact on airway function and considerable evidence suggests that it does. Many studies, based both on mathematical modelling [4] and experimental data, have linked airway hyperresponsiveness with airway thickening. However there is also evidence to suggest dissociation of these processes with some studies indicating that wall thickening correlates inversely with airway hyperresponsiveness but an increased decline in lung function [2,5]. Airway wall thickening resulting in decreased compliance may therefore also have potentially beneficial effects in limiting hyperresponsiveness or narrowing. Moreover, the genetic and environmental influences that drive the cell and molecular changes leading to remodelling remain poorly understood. This is particularly true of COPD in which research on airway remodelling has lagged somewhat behind that of asthma. In addition, there is conflicting data on the effects that

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current treatments exert on airway remodelling. Experimental models are clearly necessary to develop our understanding of the mechanistic and functional significance of individual remodelling events and their contribution to the overall pathobiology of airway disease. Methods of assessing airway remodelling, or surrogates for airway remodelling, are also required to determine the effects of current and potential novel therapeutics on the components of airway remodelling and their efficacy in limiting or relieving pathological symptoms.

By far, the majority of studies of airway remodelling have been carried out in animal models. Increased understanding of the clinical disease in humans, has lead to the continual development of models to mimic additional factors including, priming and exacerbations, as well as to determine the effects of neonatal and *in utero* exposure to allergens. In addition, technological advances in imaging, development of alternative invertebrate models, as well as new *ex vivo* tissue and cell approaches are likely to provide new insights and higher throughput approaches to airway remodelling. The development of some of these newer methodologies is particularly exciting because they can be applied to the study of humans and animals with the potential to bridge the gap between animal and human investigation, and to validate the animal models upon which such a heavy emphasis has been placed in our understanding of the mechanisms and role of airway remodelling in airway disease pathogenesis. This review aims to provide an overview of the current and developing models (Table 1) and approaches (Table 2) that may be utilized to further understand the mechanisms and role of airway remodelling as well as to develop systems for testing novel agents that will reflect translatability to clinical efficacy in diseases such as asthma and COPD.

2. Animal models

2.1. Asthma

With the exception of cats and horses, which are not ideal species for experimental research, animals do not spontaneously develop asthma-like disease. Therefore, models that simulate characteristic features of these diseases have been developed.

Animal models that replicate various features of asthma have been extensively used and form the basis for much of our knowledge of mechanisms related to allergic asthma. The archetypal, and most extensively studied, models have tended to involve the use of ovalbumin. Traditionally, acute sensitisation and challenge models have been employed to investigate inflammatory and immune mechanisms. However, the recognition that remodelling of the airways is a key feature of asthma and that it was likely to exert a significant functional impact lead to the development of chronic challenge protocols in order to mimic the features of remodelling in asthmatic airways. These include epithelial cell hypertrophy, goblet cell hyperplasia and sub-epithelial extracellular matrix deposition, providing *in vivo* models for mechanistic and functional investigations.

Considerable progress has been made over the last 10–15 years in the development and refinement of appropriate animal models to investigate and understand the role of remodelling in airway diseases. Models developed by several groups have shown that in adult animals subepithelial fibrosis and airway wall eosinophilia persists for considerable periods following cessation of allergen challenge [6–9] but that goblet cells, luminal inflammation and airways hyperreactivity generally decline more rapidly [7,9]. Such

Table 1
Models for the investigation of airway remodelling.

Model system	Advantages	Disadvantages
<i>In vivo</i>		
Mammalian allergen challenge, e.g. ovalbumin, house dust mite, cockroach, fungus, cigarette smoke.	Development of airway remodelling in whole organism integrating immune, inflammatory, circulatory and neurological systems. Amenable to manipulation, e.g. stage of development or with exacerbating agents.	Time to develop remodelling. Expensive. Analysis often requires retrieval of whole lung.
<i>Drosophila</i>	Simple airway structure amenable to induction of remodelling and infection. Potential for high throughput studies. Relative ease of generating controllable gene mutations.	Lacks an adaptive immune response. Simple airway structure.
<i>In vitro</i>		
Single cell and co-culture systems	Provides information on role and function of specific cells as well as potential interactions between cell types. Use of human cells possible. Amenable to manipulation. Higher throughput.	Does not model complex organ structure and function.
Complex airway culture models	Increased ability to model airway cell, macromolecular and vascular structure. Use of human cells possible. Amenable to manipulation and assessment of effects of airflow and mechanical strain. Long-term culture possible.	Limited modelling of complex organ structure and function.
Lung slice cultures	Ability to analyse airway function. Use of human tissue possible. Amenable to manipulation with bioactive agents.	Limited viability of slices in culture.

Table 2

Analytical approaches to the analysis of airway remodelling.

Analytical approach	Application	Advantages/disadvantages
Microscopic, e.g. histochemical and immunohistochemical staining, in situ hybridisation and morphometric analysis	Visualisation, localisation and quantification/semi quantification of airway structural changes, inflammation, gene and protein expression.	Airway specific. Protein and gene changes only semiquantitative.
Whole lung tissue	Molecular quantification of protein/gene expression, e.g. extracellular matrix proteins, mediators, etc.	Not airway specific. 'Noise' from surrounding lung tissue limits sensitivity of detecting airway specific changes.
Laser capture microdissection	Isolation and molecular analysis of protein and gene expression in enriched populations of airway cells.	More sensitive and specific than analysis of whole lung. Limited by ability to identify and capture specific cell populations. Laborious and time-consuming.
Multiphoton microscopy	<i>Ex vivo</i> imaging of airway elastin and collagen microstructure.	
High resolution computed tomography (HRCT) and micro-CT	<i>In vivo</i> and <i>ex vivo</i> analysis of airway wall thickness in humans and animals.	Repeated measurements <i>in vivo</i> limited by radiation dose and also in animals by mechanical ventilation.
Bronchoscopic and anatomical optical coherence tomography (OCT)	<i>In vivo</i> and <i>ex vivo</i> assessment of airway measurements, response to pharmacological agents and subsurface imaging of airway wall micro-structure.	Higher resolution than HRCT. Does not require radiation. Allows analysis of structure/function relationships in response to stimuli. Invasive bronchoscopy.
Fluorescence and near infrared fluorescence (NIRF) tomography	Use of targeted or activatable fluorescent probes to examine protease activity, cell and receptor activation sequentially <i>in vivo</i> .	Highest resolution imaging modality. Probes not currently available for use in humans.
Bioluminescence imaging	<i>In vivo/ex vivo</i> tracking of cells or gene activation in animals genetically engineered to express luciferase in a cell/tissue specific manner or under the control of a specific gene promoter.	Non-invasive tracking of cell/gene activation. Relatively low resolution.

factors fit well with the concepts of persistent (subepithelial and subendothelial thickening, extracellular matrix deposition, smooth muscle hypertrophy and vascular remodelling) and variable (allergen, occupational and infection mediated inflammation) airway components which collectively contribute to the regulation of airway hyperresponsiveness and asthma as has been described by Busse [10].

A limitation of many of the models is that they exhibit, in addition to characteristic airway inflammation, extensive parenchymal inflammation that is not a characteristic feature of asthma. In order to overcome this limitation, several groups including our own have developed refined models in which inflammation and remodelling is limited to the conducting airways (e.g. [8,11,12]). Such models are increasingly being used to investigate the role of different cells and mediators in regulating allergen-induced airway remodelling utilizing genetically modified animals, pharmacological agonists and antagonists, and adoptive cell transfer. Recent examples include examination of the role of different TGF- β isoforms, Th17/IL-17 and the M3 cholinergic receptor [13–15].

Recent emphasis on the interactions between the different components of the airway wall in regulating its structure and function as an epithelial-mesenchymal trophic unit [16] has lead to greater interest in the role of the epithelium in asthma (reviewed in [17]). However, the relationship of the epithelium to structural remodelling is only just beginning to be understood [13,14]. Studies by Wang et al. [14] have demonstrated that Th17 cell release of IL-17 in a prolonged ovalbumin challenge model was associated with

increased airway heparin-binding epidermal growth factor (HB-EGF) production, mucus production, airway smooth muscle mass and peribronchial collagen deposition. They further showed that Th17 cells induced epithelial production of HB-EGF and that either EGFR inhibitors or anti-HB-EGF inhibited all the remodelling effects except the peribronchial collagen deposition suggesting that airway epithelial cell production of HB-EGF contributes to allergen-induced mucus production and increased smooth muscle mass. We have presented data suggesting that airway epithelial cell derived TGF- β_1 and TGF- β_2 contribute differentially to airway inflammation and ECM protein deposition in a chronic ovalbumin challenge model [13]. Although not directly linked to asthma, studies by Snyder et al. [18] using naphthalene to selectively injure the airway epithelium have shown that epithelial repair involves dynamic regulation of extracellular matrix metabolism/remodelling. They further showed in transgenic mice that lack the capacity for airway epithelial repair, injury leads to chronic, non-resolving, upregulation of extracellular matrix deposition. Together these data provide strong *in vivo* evidence for epithelial-mesenchymal interaction in maintaining normal airway structure and for dysregulated epithelial repair contributing to fibroproliferation in the airways.

The studies by Snyder et al. examined expression from whole lung homogenates. However, the role of different cells in the regulation of airway remodelling can be investigated by analysis of enriched populations of cells obtained by laser capture microdissection as shown by Kelly et al. [12] who identified the epithelium as a major source of fibroproliferative mediators TGF- β , PAI-1,

CTGF and TIMP-1 in the remodelling airways of ovalbumin sensitised and chronically challenged mice. Additional studies of cationic proteins and their effects on epithelial integrity highlight the critical importance of the tight junctions and epithelial barrier in protection from airway hyperresponsiveness [19,20].

Another issue that has hampered chronic allergen challenge models is the development of tolerance to the allergen [21,22]. Potential explanations for the development of tolerance include deposition of allergen on the fur and ingestion during inhalation challenge protocols. Such problems appear to be overcome by methods which administer the allergen directly to the lung [8,12,13] or by challenging animals with a combination of allergens as described by Goplen et al. who utilized a mixture of dust mite, ragweed and *Aspergillus* [23]. This latter approach may also more closely model exposure in humans where most allergic subjects are known to be sensitised to multiple allergens.

In recent years there has been considerable debate as to the validity of ovalbumin-induced models since it is not a natural inducer of human disease. This has led to the development of more clinically relevant sensitisation and challenge protocols utilizing agents such as house dust mite, ragweed, cockroach and fungal extracts, although not all of these have been investigated in chronic challenge protocols.

Chronic exposure to house dust mite extract intranasally, without adjuvant, five days a week for up to seven weeks [24] induced chronic, sustained airway eosinophilia together with goblet cell hyperplasia, increased deposition of subepithelial collagen and α -smooth muscle actin positivity, as well as increased methacholine responsiveness. Cessation of allergen challenges lead to resolution of the immunoinflammatory phenotype but sustained structural and functional changes.

Cockroach allergen has also been used as a disease relevant inducer in animals [25]. Mice were sensitised with a single injection of cockroach allergen in incomplete Freund's adjuvant followed 14 days later by four intranasal challenges of allergen each four days apart. This was then followed two days later by two intratracheal challenges of higher doses of allergen two days apart. The remodelling response was characterised by increased airways hyperresponsiveness, peribronchial subepithelial collagen deposition and lung hydroxyproline levels.

Hogaboam et al. [26] developed a model in which mice were sensitised by intraperitoneal and subcutaneous injection of soluble *Aspergillus fumigatus* antigens dissolved in incomplete Freund's adjuvant followed two weeks later by three weekly intranasal challenges with the antigen followed one week later by intratracheal instillation of *A. fumigatus* conidia suspended in 30 μ l of 0.1% Tween-80. Thirty days after challenge with intratracheal conidia they demonstrated increased responsiveness to methacholine was maintained, together with mucus cell metaplasia, subepithelial collagen deposition and lung hydroxyproline levels. The model has recently been further refined through inhalation of the conidia directly from the fungal culture in order to preserve antigenic and hydrophobic characteristics of the spore coat [27]. Whilst subepithelial collagen deposition and also smooth muscle changes were similar, airway hyperresponsiveness and mucus cell changes appeared less robust. However, these features can also be similarly maintained by additional conidia inhalations at 2 week intervals [28].

Thus a range of protocols utilizing clinically relevant allergens now exist for the assessment of airway remodelling. Whilst these natural environmental allergens are capable of inducing airway remodelling there are relatively few studies thus far compared with the more traditional protocols. As such, their advantages in defining the mechanisms involved in airway remodelling and their relationship to human asthma have yet to be established. Indeed, one recent comparator study found no major differences in a range of

remodelling parameters between ovalbumin and house dust mite mediated models [29]. The lack of obvious differences in remodelling between such models does not mean that we should not continue to pursue more naturally disease relevant models but does provide confidence in the wealth of information that has been derived from earlier but now less fashionable models.

2.1.1. Modelling priming and exacerbations

There is considerable evidence that environmental and infectious agents sensitize to or exacerbate allergic responses and asthma. These include viruses, bacteria (e.g. *Mycoplasma pneumoniae* and *Chlamidiphila pneumoniae*), bacterial components, molds and fungal toxins, as well as chemical environmental pollutants such as ultrafine carbon particles and diesel exhaust, tobacco smoke and ozone. Whilst these are known to impact on allergic inflammation (reviewed in [16,30–32]), their effects on allergen-induced airway remodelling have thus far been less extensively studied.

Respiratory syncytial virus (RSV) infection prior, but not subsequent, to ovalbumin challenge in adult mice (6–8 weeks old) not previously sensitised to ovalbumin and which would not normally develop features of airway remodelling, showed evidence of increased lung collagen as well as, peribronchial and perivascular infiltrates and goblet cell hyperplasia, together with increased levels of FGF-2 but not TGF- β 1 [33]. Whereas, RSV did not affect airway remodelling in ovalbumin sensitised and challenged mice whether infected prior to or post-albumin challenge. These studies demonstrate that RSV infection can prime the lungs of mice that have not been systemically sensitised to develop airway remodelling in response to allergen exposure to the airways and that this may involve RSV-induction of FGF-2. In contrast, studies by Becnel et al. [34] using weanling mice (21 days old) indicated that mice infected with RSV followed by exposure to ovalbumin fail to develop airways hyperresponsiveness and remodelling while exposure to ovalbumin followed by RSV infection lead to development of heightened effects compared with ovalbumin sensitisation and challenge alone, including airways hyperresponsiveness, mucus cell hypertrophy and subepithelial fibrosis. Whilst there are differences related to the age of the mice and protocols between these studies, it is clear that RSV infection is capable of priming or exacerbating the response to allergens. Further studies are required to fully characterise the interactions between RSV and allergen.

Other studies in which mice were sensitised and challenged chronically with ovalbumin over a two month period and which were exposed to environmental tobacco smoke five days/week during the second month exhibited increased airway remodelling compared with those not exposed to tobacco smoke [35]. Changes included potentiation of the increase in smooth muscle thickness, area of α -smooth muscle actin staining, airway hyperreactivity, peribronchial collagen deposition and bronchial epithelial cell mucus expression which was associated with an increase in peribronchial eosinophils, as well as, TGF- β and CTGF immuno-positive cells. Furthermore, in subsequent studies it was shown that these changes as well as changes in IL-5 and IL-13 expression can be inhibited by the TLR-9 ligand, ISS, highlighting the potential importance of Th2 cytokine and TGF- β responses but the inflammatory and remodelling effects were not further delineated [36].

Mold derived mycotoxins including gliotoxin and patulin have been shown to exacerbate ovalbumin-induced inflammation, collagen deposition and area of α -smooth muscle actin staining cells indicative of increased numbers of smooth muscle cells and myofibroblasts [37]. In addition *M. pneumoniae* infection has been suggested to exacerbate ovalbumin-induced airway collagen deposition [38].

Whilst evidence is emerging from animal models demonstrating that a number of environmental and infectious agents are capable of either priming or exacerbating the airway response to a variety of allergens much more work is required to fully understand these relationships.

2.1.2. Neonatal models

The recent recognition that pathophysiologic abnormalities associated with asthma may develop within the first few years of life during development and maturation of the airways and immune system [39,40], and that this may be associated with environmental exposure to allergens, chemical pollutants and infectious agents, such as atypical bacteria, repeated infections with rhinoviruses or severe neonatal RSV infection which may induce or increase susceptibility to development of asthma during childhood through to early adulthood [16,30,41–43], has led to further neonatal animal model development for the assessment of airway remodelling. Studies by Saglani et al. [44] in which mice were challenged intranasally three times weekly with house dust mite extract from day 3 of life up to 12 weeks of age showed goblet cell hyperplasia from 3 weeks of challenge and increased sub-epithelial deposition of reticulin and collagen from 3 and 4 weeks of challenge respectively, and which persisted after cessation of challenge. In addition, development of airway remodelling occurred temporally in parallel with the development of eosinophilic inflammation and airway hyperresponsiveness, suggesting different mechanisms in neonates compared with adult models where inflammation generally precedes the development of airway remodelling and hyperresponsiveness [7,8,24,45]. Furthermore, the parallel changes in this neonatal model are consistent with the findings of studies in children [39,40], providing further confidence in its utilization for investigating pathophysiological mechanisms.

Similarly, neonatal but not adult infection with *C. pneumoniae* enhanced ovalbumin-induced mucus-secreting cell number and airway hyperreactivity but no effects on airway wall remodelling were observed, although an increase in alveolar size was noted in neonatal infected allergen exposed animals [46].

Whilst there is evidence in animals that early neonatal infection with RSV, mouse pneumonia virus or *C. pneumoniae* enhances subsequent peribronchial inflammation and airway hyperresponsiveness in adulthood following ovalbumin sensitisation and challenge there is currently no convincing evidence that airway remodelling is enhanced beyond that observed for allergen alone [47,48]. As with the inflammatory effects, it is likely that the age at which infection occurs is likely to have a significant effect on the remodelling response as indicated above (Section 2.1.1). It is therefore currently unclear whether neonatal viral or bacterial infection that appears to result in increased sensitivity to asthma in children is associated with alterations in the components of airway remodelling and further studies are required to assess this.

2.1.3. Effects of in utero exposures on allergic airway disease

It has been proposed that maternal infections, environmental exposures and dietary supplements may have effects on the unborn foetus and subsequent susceptibility to and severity of allergic airway disease. There is little information on effects on airway remodelling. However of particular interest, it has been shown in mice that *in utero* exposure to methyl donors from the maternal diet alters DNA methylation in the offspring resulting in aberrant gene transcription, as well as, enhanced development and severity of ovalbumin-induced airway responses in terms of airway hyperreactivity, eosinophilia, lavage IL-13 levels and histological assessment [49]. Such studies show not only that maternal exposure but also heritable epigenetic mechanisms are

involved in the susceptibility to and development of allergic airway responses. Further investigations on the effects on airway remodelling are awaited.

2.2. COPD

Cigarette smoke inhalation has become the method of choice for modelling the pathologic features of COPD as it is the primary cause of disease in humans and models many of the main disease features. Chronic cigarette smoke inhalation protocols have been shown to result in mucus cell metaplasia, mucus hypersecretion, epithelial cell hypertrophy, small airways remodelling and concomitant airspace enlargement in several species including mice, rats and guinea pigs [50–54]. Early studies by Rubio et al. [50] in which rats were exposed to smoke three times daily for 10 weeks showed increases in wall thickness of small bronchi with an internal perimeter of <1000 μm , consistent with changes reported in human small airways of individuals with COPD [55,56]. However, the nature of the wall thickening in this model was not further investigated in these studies. More recently, similar studies in mice exposed chronically to smoke five days/week for periods up to six months showed an increased area of picosirius red staining of collagen and bronchiolar type I procollagen gene expression in the bronchioles of smoke exposed mice was associated with increased expression of CTGF, PDGF-B and TGF- β in laser capture micro-dissected bronchioles [51]. Whilst CTGF and PDGF-B expression remained high throughout the time-course, TGF- β expression declined over time. In addition, they showed that increased growth factor expression occurred within 2 h, suggesting direct rather than inflammation induced effects of initial smoke exposure. The importance of selective study of the airways to investigate airway remodelling in models of COPD is highlighted by studies comparing extracellular matrix and profibrotic mediator gene expression in airway and parenchymal compartments which showed different patterns of expression related to the airway remodelling and emphysema respectively [52]. It may also potentially explain why gene set enrichment analysis of whole lung tissue expression profiling in another study did not identify gene sets associated with regulation of the extracellular matrix [54].

Viral and bacterial induced exacerbations, as in asthma, are a major cause of accelerated decline in lung function and disease progression in COPD [32]. Researchers have begun to investigate the effects of combined smoke exposure and infection but these have not thus far been related to airway remodelling. However, administration of polyinosinic:polycytidylic acid (poly-IC), a synthetic dsRNA sequence, which is present in viruses and known to trigger activation of pathogen-associated molecular pattern pathways (PAMPs), during the last two weeks of a four week smoke exposure protocol has been shown to lead to airway remodelling and airspace enlargement that was not apparent in animals exposed to smoke alone [57]. It appears therefore that viral components are capable of exacerbating the effects of short-term exposures to cigarette smoke. The effects of infection on chronic smoke exposure-induced airway remodelling are awaited.

3. *Drosophila* as a model for airway disease

With the advent of increasingly affordable approaches to genome-wide analysis of genetic variation, differential gene expression and altered epigenetic marks producing unprecedented amounts of data, there is a considerable need for simpler, higher throughput models of airway disease to investigate the relevance of the large number of potentially important genes, pathways and networks that are being identified. One potential solution to this is the use of the fruit fly, *Drosophila melanogaster* (reviewed in [58]).

Drosophila has a simple airway architecture consisting of epithelial lined interconnecting tubes terminating in blind-ended structures. It has a relatively simple genetic organisation and there are a plethora of genetic variants with systems readily available that allow generation of mutants in which specific genes are overexpressed or silenced in a particular organ or in which expression is temperature sensitive, providing a system for excluding unwanted developmental effects of altered gene expression. Of particular interest it has been shown that previously discovered asthma susceptibility genes [59,60], excluding genes associated with adaptive immunity, all have homologs in the *drosophila* genome that are expressed in the fruit fly airway epithelium [58]. Moreover these include genes known to be involved in airway remodelling, including ADAM33, MMP9, TNF, TLRs and TGF- β 1. Fruit flies are amenable to airway infection or induction of epithelial remodelling/metaplasia through overexpression of a pattern-recognition receptor leading to impaired oxygenation, effects that can be readily observed and quantified [61].

Whilst *drosophila* has some disadvantages including the lack of an adaptive immune system they have major advantages, as indicated above, and are therefore likely to be of considerable value in higher throughput screening of novel gene targets for their potential role in airway remodelling prior to committing to time consuming and expensive studies in mammals.

4. In vitro systems

4.1. Complex culture systems

Although single cell and co-culture cell approaches have provided considerable valuable data on the function and interaction of cells and their potential roles in airway remodelling they fail to model the complex cellular, macromolecular, structural and mechanical forces/properties of the airway. Recently however, taking a tissue engineering approach, Miller et al. [62] have developed a cylindrical model of the human bronchiole wall which incorporates airway epithelial cells, extracellular matrix, fibroblasts and smooth muscle cells constructed around thin walled silicone tube that is subsequently mechanically pulsed to stimulate circumferential alignment of the smooth muscle cells. Finally the silicone tube is removed and the lumen seeded with epithelial cells to allow epithelialisation, air–liquid epithelial interface culture and flow of humidified air promoting cilia formation and mucus production. The future application and adaptation of this model has great potential for the investigation of airway remodelling over extended periods of time *in vitro*. This could include investigating the effects of epithelial exposure to bioactive compounds, allergens, smoke, bacteria, viruses or adaptation of the model with genetically modified cells.

A biologically simpler system incorporating extracellular matrix, epithelial and endothelial cells but technologically more complex system termed a 'lung on a chip' has been developed to model the epithelial–capillary interface including air–liquid interface and cyclic mechanical strain to model breathing [63]. This system has been used to model the effects of epithelial cell stimulation with TNF α or IL-8 which lead to endothelial cell activation and production of ICAM-1 with subsequent adhesion of neutrophils flowing through the vascular channel with diapedesis and transmigration across the capillary–epithelial barrier. In addition, they showed that epithelial exposure to *E. coli* in this system resulted in recruitment of neutrophils from the vascular channel and phagocytosis of the bacteria. Such models could also be adapted to investigate epithelial responses to smoke, allergen and viruses involved in development of airway remodelling.

4.2. Lung slices

The culture of precision cut sections from resected lung provides a culture system with the native lung structure but is limited by the time that the slices can be maintained in culture. Use of lung slices has enabled analysis of small airway function by myography and video microscopy linked to image analysis [64]. Such methods can be utilized to examine functional differences associated with size of airway, comparisons between normal and diseased airways [65,66] and for comparisons between human and animal airways for development and validation of models [67] and have the potential for further development for the analysis of relationship between lung function and airway remodelling [66].

Interestingly, studies of ovalbumin sensitised and challenged mice showed increased responsiveness to methacholine *in vivo* together with increases in goblet cells and subepithelial collagen and α -smooth muscle actin (SMA) but analysis of lung slices from these mice showed no change in maximal or peak velocity of airway narrowing in response to methacholine [68]. One possible explanation suggested by the authors was that the increased collagen deposition may be protective against airway narrowing although other potential explanations exist. In contrast, increased sensitivity to contractile agonists (carbachol and 5-HT) was observed in similar studies of rats exposed to tobacco smoke where increased smooth muscle actin but no significant increase in collagen deposition was observed [66]. Clearly further studies are required to understand the relationships between *in vitro* and *in vivo* measurements of airway responsiveness but once these are defined lung slices will form a useful tool in the assessment of relationships between airway remodelling and function.

5. Imaging technology and its application to the assessment of airway remodelling

Most current approaches to the assessment of airway remodelling and their associated molecular mechanisms require tissue or fluid sampling in humans or in animal models, termination at defined time-points. High-resolution imaging could provide the ability to monitor disease progression or response to treatment longitudinally.

In recent years there have been significant advances in the imaging of cell and molecular processes, as well as, the structural components of airways non-invasively *ex vivo* and *in vivo*. These include high-resolution computed tomography (HRCT), positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) and optical imaging of fluorescence and bioluminescence. Whilst HRCT, MRI, PET and SPECT provide resolution in the 0.1–10 mm range, near infrared fluorescence (NIRF) optical imaging provides resolution down to 1–100 μ m resolution at microscopic and macroscopic levels, respectively. Bioluminescence imaging provides intermediate resolution of several millimetres. Optical imaging techniques therefore provide several advantages in terms of imaging the airways and their structure including their high sensitivity, resolution and the lack of a requirement for ionizing radiation in the imaging process, although lower resolution imaging modalities can still be useful in combination with optical imaging for examining airway remodelling.

Optical imaging approaches enable the visualisation of cell and molecular processes. Bioluminescence imaging generally involves the use of cells or animals engineered to express the luciferase gene constitutively or under the control of specific gene promoters thus providing the means to monitor gene expression or track cell migration and/or proliferation non-invasively over time. Applications include the use of transgenic mice harbouring reporter genes under the control of specific promoters. An example of particular relevance here is the type I procollagen alpha 2 chain promoter (pro-

Col1a2) fused to luciferase and β -galactosidase reporter genes which has been used in the study of lung fibrosis [69,70] and has potential for application to non-invasive imaging of airway ECM remodelling. They may also be used to image localisation and expression of genes delivered via the airways [71] and cell signalling pathways [72].

Fluorescence imaging currently provides the highest resolution and utilizes three types of reporters, on specific, targeted or activatable compounds. Non-specific fluorescent dyes can be used to examine blood flow or perfusion [73,74] and could also be applied to examine airways *ex vivo*, although detection is limited to several millimetres. Fluorescent probes targeted to adhesion molecules have allowed visualisation of endothelial cell activation and leukocyte transmigration [75,76]. Protease activatable probes, cleaved by cathepsins and MMPs, provide scope for non-invasive imaging of protease activity in the airways. These techniques have been applied to the study of myocardial infarction and atherosclerotic plaques [77–79] but have not thus far been utilized in the study of airway remodelling.

Multiphoton microscopy (MPM) has been used for *ex vivo* imaging of intrinsic fluorescence and second-harmonic generation associated with elastin and collagen respectively in the airways [80]. This approach has also been applied to examine the effects of epithelial injury on remodelling of the tracheal ECM microstructure and in combination with other techniques to assess the effects of changes in collagen and elastin on tissue mechanics [81]. Furthermore, a modified approach has been developed for *in vivo* imaging of arteries subject to movement caused by cardiac and respiratory activity [82], whether similar strategies can be applied to the lung is still to be determined.

Airway wall thickness can be measured in humans by high resolution computed tomography (HRCT) and is increased in asthma and COPD (reviewed in [83–85]). The changes likely reflect a combination of events including oedema and cell infiltration, as well as, structural changes including ECM deposition and increased ASM. However in one study, individuals were pre-treated with oral steroids and β_2 -agonists in order to minimise reversible changes, and in this study wall thickness measured by HRCT was shown to correlate with subepithelial ECM deposition, assessed by biopsy, and airflow limitation [86]. Respiratory-gated micro-CT has been successfully undertaken in a chronic model of ovalbumin sensitisation and challenge in 3rd–7th generation mouse airways [87]. They measured a peribronchial density index which was found to increase in challenged compared with control mouse airways, which correlated with the Penh ratio and with histological parameters, including peribronchial space, wall area and bronchial muscle area. Whilst such techniques are feasible in small animals there are limitations to its repeated use including the time for which the animal must be mechanically ventilated and radiation dose, both of which may cause lung injury.

More recently bronchoscopic optical coherence tomography (OCT) and anatomical OCT have been developed for thoracic imaging. Advantages of OCT include higher resolution and lack of associated radiation risks of HRCT. Studies have demonstrated that airway measurements determined by OCT, correlate with those obtained by HRCT and that OCT is a more sensitive technique for detecting small airway changes [88,89]. In addition, the increase in resolution allows subsurface imaging of the microstructure of the airway wall [90,91]. OCT has been used to examine changes in the airway wall in an *in vivo* animal model of airway injury [91]. Furthermore, anatomical OCT, has been used for the *ex vivo* examination of airway structure-function relationships in response to multiple stimuli in the same airway [92]. This recently developed imaging modality is improving our knowledge of the interrelationship between different structural components of the airway wall and airway narrowing. Its application to airway remodelling

will likely provide further novel insights into the role of structural changes in altered airway function.

Fluorescence molecular tomography (FMT) adds three-dimensional deep tissue capacity to optical imaging. Protease activatable probes have been used to study inflammatory processes, visually and quantitatively, in ovalbumin and lipopolysaccharide induced models of airway inflammation, as well as their response to treatment with dexamethasone [93–95]. The study of Korideck and Peterson showed good correlation between FMT and bronchoalveolar lavage based measures of inflammation [95] with the added advantage of FMT allowing non-invasive sequential study of the same animal over time. Furthermore Cortez-Retamozo et al. used intravital microscopy and near infrared fluorescence (NIRF) fiberoptic bronchoscopy to visualize inflammation-mediated protease activity with single cell resolution in third-order branches of mouse airways [93]. Similar approaches have been used to study angiogenesis using $\alpha v \beta 3$ integrin targeted fluorescent quantum dots to detect angiogenically activated endothelium in tumours [96]. Matrix metalloproteinase activity has been examined during aortic aneurysm formation and was able to detect an increase in MMP activity prior to development of the aneurysm [97]. In addition, optical imaging of fluorescein labelled angiotensin peptides has been used to image increased binding to angiotensin receptors associated with myofibroblasts in myocardial infarcts and coronary arteries [98]. There is therefore potential for the application such techniques to the study of airway remodelling.

6. Summary

Over the last 10–15 years there have been great advances in the development of vertebrate, invertebrate and *in vitro* models to investigate airway remodelling (Table 1). In addition, imaging techniques have developed rapidly in recent years and it is now possible with a combination of techniques to image structural components, cells and proteins of the airway wall as well as analysis of biological processes, cell and receptor activation non-invasively and longitudinally (Table 2). The integration of such techniques with models of airway remodelling should provide previously unattainable information on the importance of remodelling in airway function as well as analysis of drug efficacy. In addition, several of these techniques can be applied in studies of both humans and animals providing scope for improved correlation and validation of animal models to disease activity in patients. It is also hoped that modelling approaches that more closely correlate with disease activity will provide readouts from potential therapeutic agents that more accurately predict efficacy in the clinic.

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