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SERIES "AIRWAY REMODELLING: FROM BASIC SCIENCE TO CLINICAL PRACTICE"
Edited by L-P. Boulet and P.J. Sterk
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Allergen-induced airway remodelling

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ABSTRACT: Airway remodelling is associated with chronic asthma but it remains unclear whether it results from airway inflammation in response to allergens or immune-mediated events such as viral infections. Although the acute inflammation associated with asthma has been modelled extensively both *in vitro* and *in vivo*, the structural changes occurring in the lung have only recently been investigated. These *in vitro*, *in vivo* and *in silico* systems have been designed to examine the pathways leading to allergen-induced airway remodelling and have enabled investigators to draw conclusions about the participation of key cells and molecules in the development of allergen-induced airway remodelling. However, fundamental questions remain regarding the genesis of remodelling as well as the relationship between functional symptoms and pathological changes that occur. In this review the key questions relating allergen exposure to development of remodelling are discussed, as well as the steps that are being undertaken to investigate them.

KEYWORDS: Airway function, airway remodelling, asthma, eosinophils, inflammation

Airway remodelling is a feature of asthma. It is a collective term that can be defined as the presence of persistent changes to normal airway structure involving changes in the composition, organisation and function of structural cells, as well as enhanced turnover of extracellular matrix components (fig. 1). Structural changes include subepithelial fibrosis, which contributes to the thickening of airway walls due to the deposition of extracellular matrix proteins such as collagens, laminin and tenascin. Increased myocyte smooth muscle mass, also indicative of airway remodelling, is thought to be due to an increase in myofibroblast proliferation or recruitment, and further enhances extracellular matrix deposition. Mucous gland hyperplasia leads to excessive mucus secretion and can, in severe cases, lead to occlusion of the airways. Increased bronchial vascularity has also been documented. Thus, airway remodelling results in thickened airway walls in asthma. The physiological consequences of these changes remain uncertain, in part because these changes are not fully reversed by current asthma therapy. However, airway remodelling is postulated to be a determinant of airway hyperresponsiveness (AHR) as well as the accelerated loss of lung function over time that is documented in many asthmatics.

This collective description of airway remodelling was originally generated on the basis of descriptive pathological studies of biopsies either taken *post mortem* from patients who died from asthma, or more recently by fibreoptic bronchoscopy in volunteers with asthma [1]. Such studies have demonstrated changes of remodelling even in mild asthma and in children, and most studies have failed to relate asthma severity to the degree of airway remodelling. While these observational studies are important and have yielded important information regarding tissue structural changes, they cannot impart much about the natural history of remodelling or determine potential therapeutic targets. Important questions that remain unanswered include the following. How is airway remodelling initiated? How is it related to airway inflammation or how does it represent a separate genetic determinant of asthma? Are the changes reversible? How important is it as a therapeutic target? Interventional studies were hampered by the lack of laboratory models but a variety of modelling approaches have now been described

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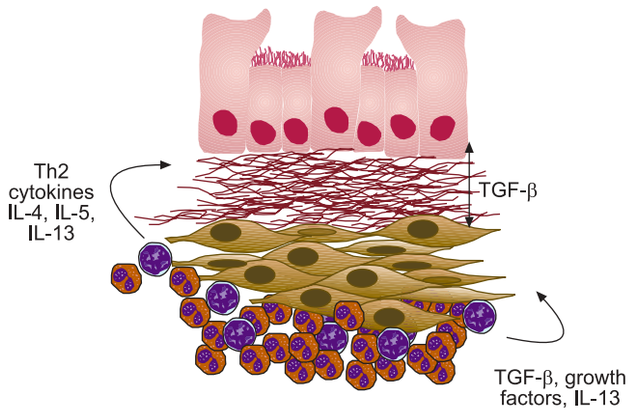

FIGURE 1. Mediators involved in airway remodelling. Th2: T-helper cell type 2; IL: interleukin; TGF: transforming growth factor.

to address mechanistic questions and allow pre-clinical intervention studies.

MODELS TO STUDY AIRWAY REMODELLING

In vitro *cell cultures*

Classical *in vitro* cell-culture systems have been enormously valuable in delineating some of the functions that lung structural cells might play in the development of airway remodelling. These systems have not specifically examined allergen-induced events but rely on the addition of mediators such as T-helper cell (Th) type 2 cytokines to the cells in culture. Cultures of epithelial cells, fibroblasts and smooth muscle cells using immortalised cell lines established that these cells were capable of more than merely maintaining the structural integrity of the lung. Experiments have determined that these cells are able to respond to, and in many cases secrete, a range of inflammatory mediators, including cytokines and chemokines, as well as profibrotic factors such as transforming growth factor (TGF)-β. However, in order to try and obtain information more relevant to the lung, primary cells have also been isolated from the lung. Normal lung fibroblasts, epithelial cells, smooth muscle cells and vascular endothelial cells can be isolated from surgical specimens or *post mortem*, and are now available commercially. In addition, it is possible to obtain these cells from allergic as well as nonallergic patients [2], enabling comparisons to be made. *In vitro* cultures of these cells have enabled researchers to examine the effects of inflammatory or profibrotic mediators on a wide range of functions, including proliferation, mediator secretion, extracellular matrix metabolism, protease secretion and, in some cases, migration. The culmination of these experiments is the recognition that lung resident cells are integral to the development and maintenance of lung inflammation and remodelling.

Epithelial cells

Primary lung epithelial cells have been isolated from cadavers and are available commercially. When grown on plastic in submerged culture, epithelial cells have been shown to secrete a range of inflammatory mediators and have the capacity to present antigen. Therefore, it has been postulated that epithelial cells make an active contribution to the generation of allergic airway inflammation after allergen challenge. However, investigators have more recently developed more sophisticated air–liquid interphase culture (ALI) techniques to allow their differentiation into mucus and ciliated cells. The apical secretions from normal human tracheobronchial epithelial cells cultured in this system were found to contain mucin-like materials as well as lysozyme, lactoferrin and secretory leukocyte protease inhibitor [3]. Others have also shown that normal human bronchial epithelial cells grown on microporous inserts at ALI express a well-differentiated mucociliary phenotype compared with cells grown on plastic in submerged cultures, which tend to be poorly differentiated. Furthermore, responses to the Th2-type cytokines interleukin (IL)-4 and IL-13 are highly dependent on the culture technique; cells cultured on plastic exhibited significant concentration-dependent increases in granulocyte-macrophage colony-stimulating factor and TGF-β_2 secretion, whereas cells grown at ALI showed no statistically significant response [4]. These results suggest that functions such as cytokine secretion may be critically dependent on the cell-culture technique employed and that results from *in vitro* cultures must be interpreted with caution.

Increasing evidence also shows that epithelial cells play a crucial role in the development of airway remodelling and it has been further suggested that these may act independently of inflammatory events. Epithelial shedding and hypertrophy, particularly of the goblet cells, are indicative of remodelled airways and many investigators have used isolated epithelial cells to try and determine how they contribute to airway fibrosis. One emerging theory regarding the contribution of epithelium proposes that an inability to appropriately repair damage to the epithelium leads to activation of the attenuated myofibroblast sheath (part of the embryological epithelial-mesenchymal trophic unit (EMTU)), leading to the deposition of further myofibroblasts and extracellular matrix (ECM) proteoglycans. Evidence in support of this comes from the fact that asthmatic epithelium has an increased susceptibility to oxidant injury and an inability to repair rapidly. Asthmatic epithelium has a high expression of $p21^{waf}$, an inhibitor of cell cycle progression [5]. This regulator of G1 cyclins can be induced by TGF-β. Epidermal growth factor receptor is also upregulated in the epithelium of patients with chronic asthma, although paradoxically there is no evidence for increased proliferation of the epithelium [6]. It has been suggested that in asthma there is reactivation of the EMTU, which is involved in the development of airway structure during embryological development, thus contributing to the long-term changes seen in the remodelled asthmatic airway. These findings have led to the suggestion that the bronchial epithelium is fundamental to the development of allergic pathology, and that the relationship between the bronchial epithelium, stem cells and the mesenchyme is crucial. It is proposed that epithelial damage and Th2 cytokines cooperate to promote functional disturbance of the EMTU, leading to myofibroblast activation and induction of the inflammation and remodelling that is characteristic of chronic asthma [7]. It is also suggested that this might result from inherited susceptibility genes such as disintegrin and metalloproteinase (ADAM)33 [8], which is a matrix-active enzyme. These theories, however, have not been fully investigated. The ideas stem from *in vitro* investigations with isolated epithelial cells, but interactions between the different

cell populations making up the EMTU have not been studied either *in vitro* or *in vivo*. The future challenge will be to design systems which enable these hypotheses to be tested. One study has used laser capture microdissection to separate the bronchial wall epithelium and smooth muscle in lung sections from mice chronically exposed to allergen. Gene expression of TGF-β_1 and plasminogen activating inhibitor-1 were determined to be significantly upregulated only in the airway epithelium, implicating these cells in the pathogenesis of airway remodelling [9].

Smooth muscle cells

Smooth muscle cells are increasingly regarded as important in the development of lung pathology during asthma. *In vitro* culture of these cells has shown that they are able to produce cytokines and chemokines that promote the recruitment of inflammatory cells and lead to the activation of other lung-resident cells that participate in the development of the remodelled airway. Infiltration of the airway smooth muscle by mast cells has been shown to be the main distinguishing feature between asthma and eosinophilic bronchitis (a condition incorporating cough with eosinophilic airway inflammation in the absence of AHR), and the interaction of these cell types is the subject of intense investigation [10, 11]. Furthermore, increased smooth muscle mass is postulated to contribute to the development of AHR [12]; therefore, understanding the signals that lead to increased proliferation or recruitment of smooth muscle is important.

Another possible contributor to smooth muscle mass is the presence of myofibroblasts. These are a specialised type of fibroblast that constitutes an important component of fibrotic reactions in many tissues and diseases. In asthma, myofibroblasts are increased in the lamina reticularis and lie in close proximity to the smooth muscle bundles of the airway. The *in vivo* origins of myofibroblasts are not well understood but they are thought to derive from either smooth muscle or from fibroblasts. Recent studies have determined that progenitor cells termed fibrocytes are recruited to areas of tissue injury and then differentiate to form fibroblasts [13]. These fibrocytes are circulating cells distinguished by their unique characteristic of expressing the haematopoietic stem cell antigen CD34 in conjunction with collagen I, and are thought to contribute to wound healing and fibrotic reactions *in vivo*. Once recruited to the site of tissue injury or inflammation, fibrocytes are thought to differentiate into myofibroblasts, gaining expression of a smooth muscle actin but losing expression of CD34. Allergen exposure has been shown to induce the accumulation of these fibrocytes in the bronchial mucosa of patients with allergic asthma [14]. These results indicate that circulating fibrocytes might function as precursors of myofibroblasts and thus contribute to the pathogenesis of airway remodelling in asthma, but this has not yet been demonstrated directly.

Mixed cell cultures

The vast majority of *in vitro* studies have been carried out on isolated cells cultured in suspension with recombinant proteins. It is questionable whether these cells provide much information about the potential function of the particular cell type *in vivo*. However, there are a number of models that are being developed that place the cells on a support to allow three-dimensional culture or interaction of cells to model the role of matrix proteins in the three-dimensional milieu of the airways in asthma. These models aim to retain the advantages of isolated tissue assays but also allow continuous investigation of long-term events. These models are needed to facilitate the study of changes in pharmacological properties, of intra- and intercellular processes that characterise AHR. One study attempted to ''engineer'' bronchial mucosa by incorporating human bronchial fibroblasts from either normal or asthmatic donors into collagen gel and then seeding bronchial epithelial cells over this gel before culture in an ALI in the presence or the absence of T-lymphocytes [15]. Histological analysis showed that engineered mucosa with normal bronchial cells exhibited a pseudo-stratified ciliated epithelium with the presence of mucus secretory cells. These features were comparable with those observed in normal bronchial tissues. In contrast, engineered mucosa from asthmatic subjects showed a disorganised tissue structure, particularly with respect to the epithelial cell arrangement. The percentage of IL-5-positive T-cells was significantly higher in engineered bronchial mucosa from asthmatic subjects compared with mucosa from normal volunteers.

Cultures of bronchial biopsy explants have also been developed to try and take into account interactions between different populations of cells. One such bronchial explant system sought to gain insight into which cytokines are involved in perpetuating allergen-induced inflammatory processes in the asthmatic airways [16]. The results showed that bronchial biopsy tissue maintained in culture for 24 h actively transcribes cytokine mRNA without any overt stimulation. It was possible to stimulate the explants with allergen *in vitro* and monitor cytokine responses. Clear differences in the spectrum of cytokine expression between atopic asthmatic and normal control tissue were observed. Stimulation with Der p allergen did not alter the cytokine profile of biopsies from control individuals, but elevated the expression of IL-5 and -13 mRNA and significantly induced the secretion of the protein by the asthmatic airway tissue. Another three-dimensional organ culture system used portions of guinea pig airways and showed that airways became hyperresponsive to a range of different agonists while maintaining their three-dimensional organisation [17]. These models have potential for testing future therapeutic targets.

In vivo *models*

Animal models

Mouse models of allergic inflammation have been useful in defining roles for particular inflammatory mediators or cells. Historically, these have generally involved systemic sensitisation with a protein in conjunction with an adjuvant, followed some weeks later by local inhalation challenge *via* the airway with the same protein allergen [18]. Even with the variety of results obtained due to using different protocols and strains of mice, these models have yielded a substantial amount of information regarding pathophysiological processes after allergen challenge *in vivo*, and the use of knockouts, transgenics and administration of antibodies or pharmacological inhibitors has led to the identification of multiple pre-clinical targets. More recently, investigators have developed longer-term challenge protocols (often involving several months of

challenge) in order to try and replicate the more chronic aspects of asthma seen in patients. Generally these ''chronic'' models demonstrate considerably less eosinophilic inflammation in the lungs, but lungs show evidence of remodelling with increases in collagen deposition, smooth muscle mass and goblet cell hyperplasia [19, 20]. Interestingly, continued use of an environmentally relevant allergen, house dust mite, has been shown to elicit a chronic inflammatory response together with structural remodelling, even in naïve, unsensitised mice [21]. Consequently, studies are now emerging in which the relationship of individual cell types and specific mediators with the development of remodelling events is being investigated. IL-13 is thought to be a critical mediator in airway remodelling since IL-13 knockout mice, or treatment with neutralising antibodies, ameliorates many of the symptoms associated with chronic allergen challenge [22–24]. Lack of IL-5 is also beneficial in reducing airway remodelling [25], perhaps due to effects on eosinophils, which play an important role in its development [26].

Transgenic technology has also been used to develop model systems to study airway remodelling *in vivo*. Interestingly, lung-specific expression of many of the Th2-type cytokines results in the development of airway remodelling spontaneously, without the need for any allergen challenge. In particular, transgenic expression of either IL-13 or -9 driven by a lung-specific promoter results in excessive mucus secretion and increased ECM deposition in the airways [27, 28]. However, it could be argued that, particularly for IL-13, the observed pathology is more comparable to that seen in chronic obstructive pulmonary disease rather than chronic asthma. In addition, although these transgenic models allow observation of the consequences of high levels of a particular mediator, they may overemphasise the contribution of that particular molecule; it is probable that the inflammatory mediators associated with asthma function as a tightly controlled network to elicit the characteristic pathological changes.

One major criticism of mouse models of airway remodelling has been that the murine airways are relatively simple compared with those of humans. Other models have emerged that use larger animals that are arguably more anatomically similar to humans. A recent sheep model takes advantage of the fact that ovine lung innervation and blood supply is nearer to that of humans than mice [29]. SNIBSON *et al*. [29] have described a model of airway inflammation and remodelling in sheep induced by chronic inhalation of house dust mite by segmental allergen challenge. These sheep developed an immune response to house dust mite, as characterised by increased allergen-specific immunoglobulin (Ig)E. After a 6-month challenge protocol, lung tissue showed evidence of goblet cell hyperplasia, collagen deposition and smooth muscle hyperplasia, but in only three out of the seven sheep studied.

Chronic allergen challenge models have also been established using nonhuman primates, which are arguably closer still in anatomy to humans [30]. Perhaps more importantly, the physiological state of both the respiratory and immune systems are considerably different at birth in mice compared with humans or primates, with development occurring in a matter of weeks in mice and years in humans. Primate models generally involve either challenge with Ascaris in naturally sensitised monkeys or sensitisation and challenge with house dust mite antigen [31, 32]. In the latter model, long-term challenge with house dust mite resulted in eosinophilic inflammation in conjunction with mucus metaplasia. Thickening of the basement membrane was reported, although there was no description of changes in fibrotic mediator production or analysis of ECM components [32, 33]. This protocol also induces hypertrophy of the airway smooth muscle, but these changes were not related to AHR or inflammation [34].

Human models

One of the advantages of studying asthma is that it is possible to induce acute changes in patients by controlled allergen challenge in the clinic. These inhalational challenge protocols have been used for many years to study the mechanisms of asthma in affected individuals. Sensitised atopic asthmatics show an early asthmatic response within 15 min of segmental allergen challenge that is characterised by airway narrowing measured by reduced forced expiratory volume in one second (FEV_1). This phenomenon is thought to occur largely as a result of mast cell degranulation after IgE binding. Around half of these individuals will then experience a later fall in FEV_1 $\sim$ 3–8 h after challenge, with an accompanying increase in AHR which might persist for days or weeks [35]. This late asthmatic reaction has been the subject of much investigation. It is characterised by an influx of eosinophils, Th2 cells and basophils and has been used as a model of airway inflammation in many drug-development programmes. Interestingly, it has been more recently demonstrated that this protocol also leads to activation of the EMTU and upregulation of several molecules and mediators that are indicative of the remodelling process. PHIPPS *et al*. [36] examined endobronchial biopsies obtained before and 24 h after inhalational challenge of mild asthmatics for evidence of ECM deposition in the reticular basement membrane (RBM). Allergen challenge was shown to increase the number, of fibroblasts that were synthesising collagen as well as the thickness of tenascin in the RBM. Increases in the number of epithelial cells positive for the TGF-β signalling molecule, phospho-Smad2, were also seen after allergen challenge. Thus, allergen challenge in patients with mild asthma induces activation of epithelial cells and fibroblasts in the EMTU, as well as increasing production of ECM proteins within the RBM. The authors concluded that airway remodelling in asthma may, in part, result from repeated acute activation of the EMTU by allergen exposure [36]. How remodelling is activated in nonatopic asthma and the role of viral infections (persistent or transient) remain important unanswered questions.

MODELLING REMODELLING

An emerging area of research aims to negate the use of animal or human models. Mathematical modelling using basic fluid dynamics to model airway resistance has been applied to examine the effects of airway smooth muscle shortening and airway wall thickening on changes in pulmonary resistance [37]. Studies using this technique have determined that increased muscle mass is likely to be the most important abnormality responsible for the increased resistance observed in asthma [38]. Others have used similar systems in order to

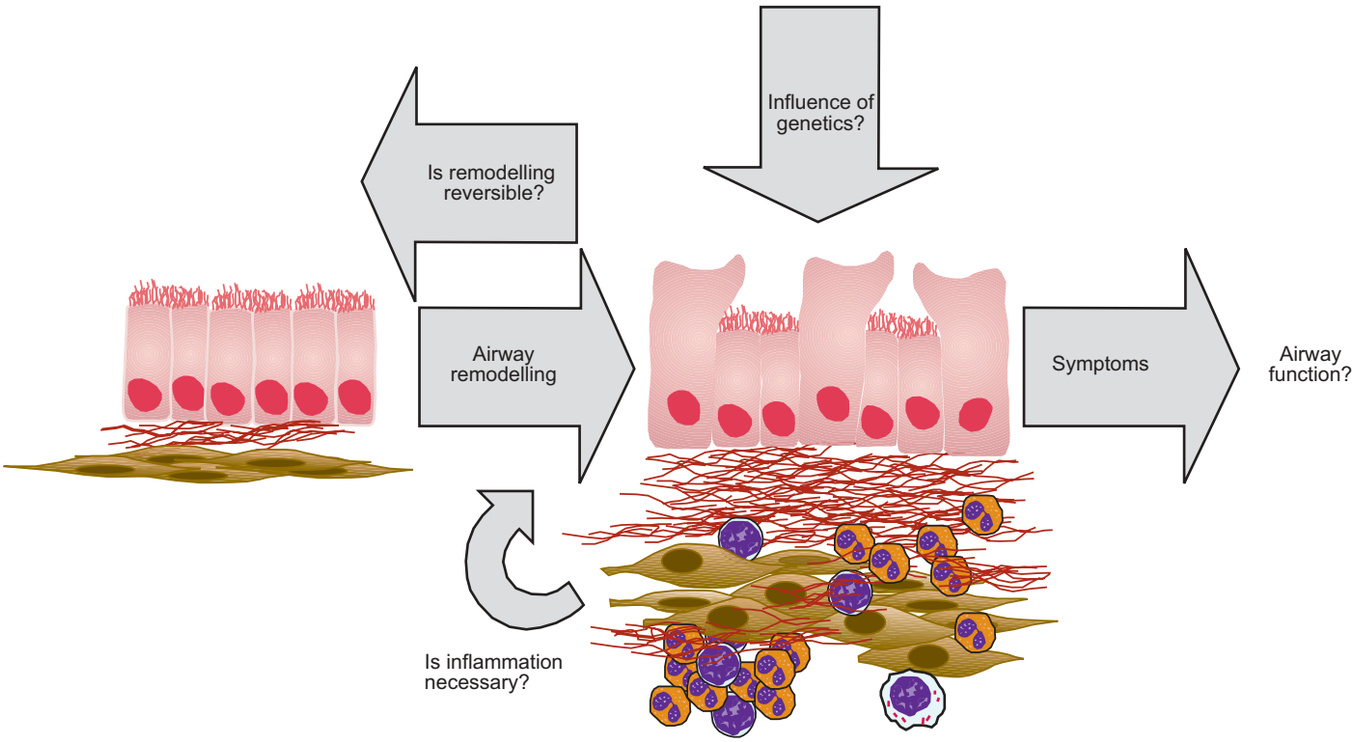

FIGURE 2. Critical questions in airway remodelling today.

predict the consequences of phenomena, such as airway narrowing [39] or changes to the integrity of the mucosa [40].

Computer or *in silico* modelling of the airways have also been investigated in order to make correlations between structure and function. Structural models of the airways have been generated to take into account airway dimensions and connectivity, and have then been applied to the study of lung mechanics and gas exchange. Until recently these models did not take into account spatial arrangement of the airway structure. However, the rapid advancement of three-dimensional imaging of the lung has strengthened clinical diagnosis of various lung diseases and has improved the development of three-dimensional lung models to include vascular branching [41]. Structure–function relationships of the tracheobronchial tree have been studied by three-dimensional modelling of airway–parenchymal interactions to try to generate simulation models of the airways during disease. Computer-generated three-dimensional displays of human lungs based on raw data from magnetic resonance imaging have been described. Transverse slices of lung were stacked, compiled and used as a source to generate images of control and asthmatic lungs [42]. In generating a model of the asthmatic lung the following features of asthma were considered: bronchoconstriction of the smooth muscle; inflammation of the airway wall; thickening of the mucus layer; and that the combination of these three effects results in a reduction in the diameter of the airway lumen. The authors point out, however, that the relative magnitudes of these components may be anticipated to vary between different human subjects. Importantly, the authors did not consider the effects of increased matrix deposition, *i.e.* the effects of remodelling have not yet been simulated by computer. However, the mathematical descriptions of the changes in human lungs produced by asthma have been developed and reflect the heterogeneity and severity of asthma. The physical manifestation of asthma was formulated in the large and central airways and the small airways of the tracheobronchial compartment. Severity of asthma was simulated by reducing airway diameters by 20–40% due to bronchoconstriction, inflammation and mucus. The resultant ''asthma model'' was used to calculate the effects of disease on the deposition of inhaled particles. For the diverse conditions simulated (*e.g.* particle sizes, ventilatory parameters, disease locations, asthma severities) airway congestions consistently produced notable increases in particle deposition at the affected site [42]. In the future similar *in silico* models could be developed in an attempt to gain more information regarding the relationship between airway structure and function.

TODAY'S QUESTIONS

There has been a significant increase in the interest and amount of investigation into the mechanisms behind airway remodelling in patients and the development of more relevant *in vivo* and *in vitro* models to study these mechanisms. However, there remain several critical questions (summarised in figure 2), as follows. 1) What is the relationship between airway function and airway remodelling: is airway remodelling the cause of asthma symptoms and morbidity or merely a byproduct of airway inflammation? 2) Is inflammation necessary for the development of airway remodelling; are they parallel or sequential events? 3) Is airway remodelling reversible? 4) Is airway remodelling genetically determined?

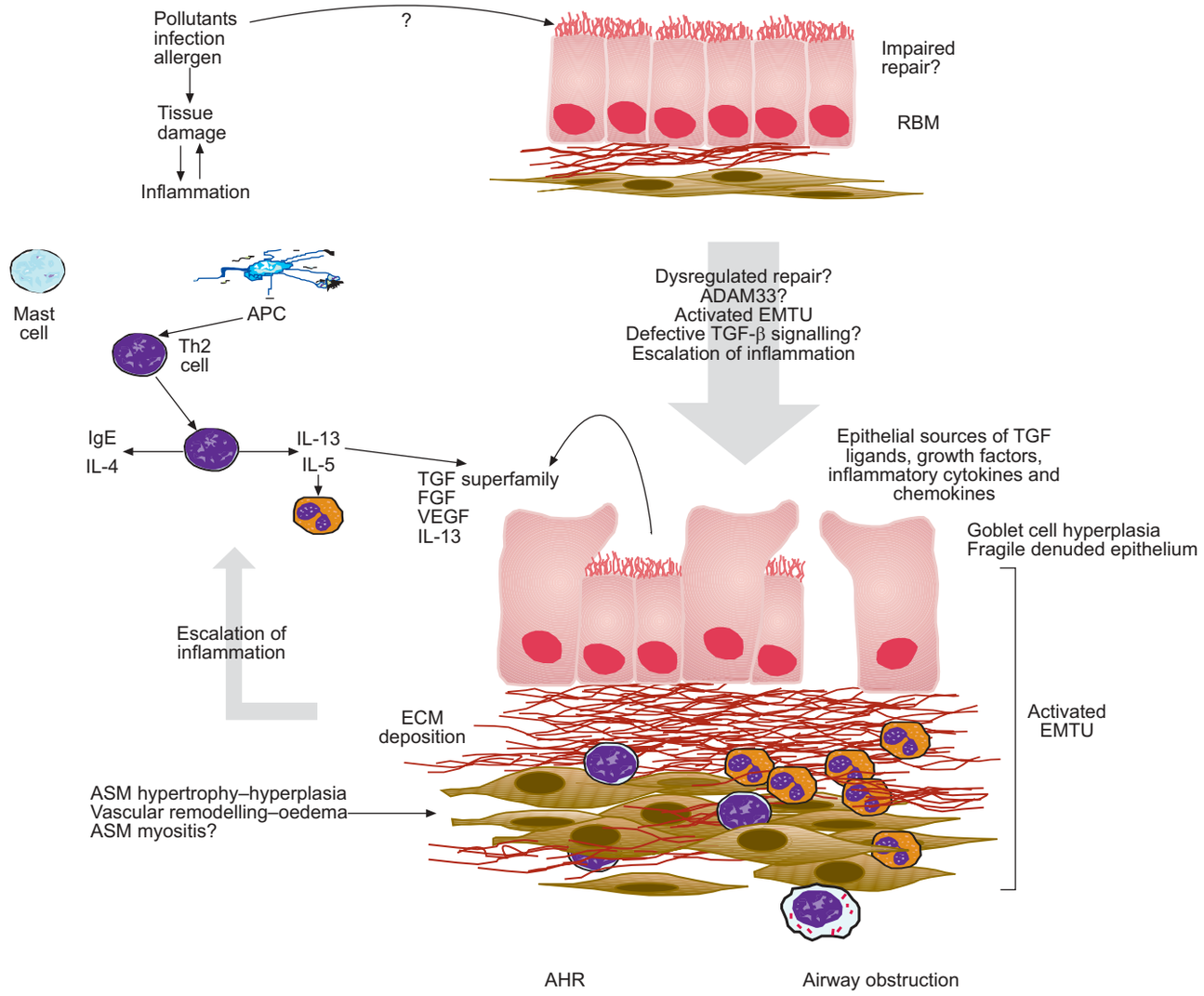

FIGURE 3. Summary of current concepts in the pathogenesis of airway remodelling in allergen-induced asthma. Exposure to environmental insults can lead to airway damage, and individuals with a genetic predisposition to atopy and/or dysregulated or impaired airway repair may go on to develop sustained inflammation and tissue remodelling. Increased activation of the epithelium leads to signalling to inflammatory cells and also activation of the underlying mesenchymal cells (activated epithelial-mesenchymal trophic unit (EMTU)). Both structural and inflammatory cell sources of growth factors, such as those of the transforming growth factor (TGF)-superfamily as well as vascular endothelial growth factor (VEGF) and interleukin (IL)-13, are thought to be important. Progressive structural changes include increased numbers and size of fibroblasts and airway smooth muscle and vascular remodelling together with excessive and dysregulated extracellular matrix (ECM) deposition, the balance of which may lead to a phenotype that is characterised by increases in airway hyperresponsiveness (AHR) or airway obstruction. In turn the pro-inflammatory environment generated by chronic structural cell activation will sustain and propagate the inflammatory response to ongoing environmental insults. RBM: reticular basement membrane; APC: antigen-presenting cell; ADAM: disintegrin and metalloproteinase; Th2: T-helper 2; Ig: immunoglobulin; FGF: fibroblast growth factor; ASM: airway smooth muscle.

What is the relationship between airway function and airway remodelling?

Perhaps one of the most urgent questions relating to the development of airway remodelling is that of the functional consequences of the remodelling for the patient. The attempts to make correlations between the development of tissue structural changes and changes in lung function parameters have been controversial and remain the subject of intense discussion and investigation. Changes in airway function include hypersensitivity (a leftward shift in curves for bronchoconstrictor dose–response), hyperreactivity (increased slope of these curves) and a greater maximum degree of induced bronchoconstriction. Longitudinal studies, however, imply that AHR should be considered as an independent risk factor for development of asthma rather than as an outcome itself [43]. Multiple studies have determined that changes in airway function are accompanied by inflammatory infiltrates composed of eosinophils, T-cells, monocytes and neutrophils and cytokines characteristic of a classical Th2 response. This implies that AHR is at least partially dependent on inflammatory events [44–46], but the exact contribution of inflammation to airway dysfunction remains ill-defined [47]. The observation that AHR persists in patients despite prolonged treatment with anti-inflammatory corticosteroids [48–50] implies that other

mechanisms account for a major component of airway dysfunction.

The clinical consequences of airway remodelling remain uncertain but contributions to AHR and fixed airflow obstruction have been suggested. Asthmatic subjects show an accelerated decline in lung function compared with nonasthmatics, and this is more marked in those asthmatics that smoke [51, 52]. Research has been hampered by the fact that there are no noninvasive methods of assessing airway remodelling at present, and human airway research is limited to mainly morphological and *in vitro* experimental work that is constrained further by ethical limitations. Nevertheless, recent bronchoscopic studies have enabled hypotheses to be formed regarding the role of particular mediators and cells in the development of structural changes following allergen challenge [36, 43, 53]. In addition, high-resolution computed tomography (HRCT) shows promise in evaluating structure–function relationships in asthma [54]. HRCT reveals that an increase in the ratio of bronchial wall thickness to bronchial diameter exists in the large airways in asthma [55]. Interestingly, wall thickness as assessed by HRCT correlates with subepithelial fibrosis, as assessed by biopsy [56]. In addition, HCRT wall thickness is associated with increased airflow limitation and correlates with asthma duration, as well as traditional indices of asthma severity and control [43]. In these computational models the increase in reactivity and maximal response caused by airway thickening is related to one or a combination of mechanisms. Increase in the airway wall thickness internal to the smooth muscle layer can amplify the airway narrowing for a given degree of airway smooth muscle shortening. Increase in the thickness of the adventitial layer has the potential to uncouple the airway smooth muscle layer from the surrounding parenchyma, allowing the smooth muscle to shorten more before being balanced by parenchymal tethering. Increase in the smooth muscle layer could result in greater force development and therefore shortening against the loads that normally attenuate airway muscle shortening [57]. Paradoxically, a recent study determined that airway reactivity negatively correlated with airway wall thickness. Airway sensitivity was related to the degree of airway inflammation as determined by sputum eosinophilia, but not to any index of airway wall thickness. It was concluded that airway wall thickening was perhaps protective against excessive airway narrowing in asthma [58]. This apparent contradiction of accepted dogma might be explained by the fact that the computational studies undertaken are primarily based on altered geometry and do not fully take into account the potential effect of airway wall thickening on the mechanical properties of the airway. It has been argued that there are several potential explanations to this apparent paradox, all relating to the stiffness of the airways. Deposition of ECM could separate the smooth muscle layer from the parenchyma, thus limiting the ability of the smooth muscle to narrow the airway lumen. Similarly, thickening of the airway wall between the smooth muscle layer and the epithelium might stiffen the airway. In addition, although the smooth muscle layer is increased during chronic asthma, it has been argued that it changes from a mainly contractile phenotype to a secretory phenotype [59]. Therefore, the apparent paradoxical relationship between airway geometry and AHR described by NIIMI *et al.* [58] may simply be a function of the mechanical properties of the material deposited within the layers of the airway wall. However, these results suggest that the present author's ideas regarding the relationship between airway remodelling and airway function are simplistic; the mechanical properties of the material thickening the airway wall influence the ultimate change in physiological response, not just the amount [57]. It is clear that some of the changes characteristic of airway remodelling occur in other chronic lung diseases and are not specific to asthma.

Investigating the relationship between airway remodelling and changes in airway function in humans is difficult, since endobronchial biopsies do not always show the true extent of changes to the airway wall and indeed serial biopsies from the same patient are largely impractical and unethical. At present, there is a lack of reliable noninvasive predictive markers and insufficient data from longitudinal studies to define the natural history of remodelling from childhood wheezing into adulthood. The development of such noninvasive systems is urgently needed in order to further the analysis of the relationship between airway remodelling and changes in lung function. In the absence of suitable human studies, animal models can attempt to dissect the relationship between lung structural changes and lung function after allergen challenge. The majority of mouse models report some change in lung function after prolonged challenge [25, 60–63], which correlates with airway inflammation and airway remodelling. However, the relationship between airway inflammation, airway remodelling and changes in airway function is still ambiguous. Several of these studies argue that airway structural changes can be uncoupled from AHR. One elegant study compared brief or chronic exposure of mice to allergen and determined that airway dysfunction and remodelling persisted beyond the resolution of immune-mediated inflammatory events [62]. Airway responses to methacholine were measured and increases in the maximal inducible bronchoconstriction persisted for $\geqslant$8 weeks after cessation of allergen challenge. These functional changes were seen in conjunction with increases in contractile tissue in the airway wall. In contrast, observed increases in airway reactivity (rate of increase in respiratory resistance for a given dose of methacholine) only persisted beyond resolution of allergen-induced inflammation in chronically challenged mice. The authors concluded that sustained airway hyperreactivity was not associated with ongoing Th2 inflammatory markers, such as eosinophilia or IL-13, but that sustained dysfunction occurred as a consequence of airway remodelling rather than these immune-mediated events. One can speculate that the initial development of airway dysfunction is dependent on acute inflammatory responses to allergen challenge, such as recruitment of eosinophils and Th2 cells and production of Th2 cytokines, but that sustained dysfunction is dependent on structural changes to the airways, such as changes in the smooth muscle layer and increased deposition of ECM. Subsequent studies using knockout mice revealed that IL-4 and -13, but not IL-5, are critical for the development of sustained airway remodelling and AHR [64]. However, it could be argued that since these mice are completely devoid of the individual cytokines for the duration of the entire protocol, the observed effect may reflect differences in the initial development of remodelling or AHR rather than in the

persistence. Studies with neutralising antibodies during different phases of the protocol would determine this.

Intervention studies in mouse models of chronic allergen challenge have revealed some interesting correlations between airway inflammation, development of airway dysfunction and airway remodelling. Several of these models have shown that it is possible to uncouple airway function from either inflammation or airway remodelling. Mice genetically deficient in eosinophils are protected from development of remodelling but, surprisingly, not from changes in airway function [26]. These mice develop robust AHR to acute and chronic allergen challenge but fail to develop airway fibrosis or airway smooth muscle hypertrophy after chronic challenge. Another study has shown that airway inflammation and AHR is preserved in mice that lack mast cells, but that allergen-induced subepithelial fibrosis was partially attenuated [65]. Therapeutic administration of anti-TGF-β antibody after the onset of eosinophilic inflammation and AHR had no effect on these parameters but did prevent the development of airway remodelling [66]. These studies show that it is possible to affect one parameter of chronic allergen challenge without affecting others, and will permit the testing of potential therapeutic treatment regimens on outcomes of the various aspects of chronic allergen challenge. In the future it will be important to consider the success of these therapeutic regimes on facets of chronic allergen challenge, such as airway remodelling rather than just eosinophil recruitment. In addition, it will be important to test potential therapeutic treatments using therapeutic rather than prophylactic regimens.

These developments in animal models will increasingly allow dissections of potential components contributing to airway remodelling and their impact in airway physiology. However, relating these findings to chronic asthma in humans remains a challenge. The use of monoclonal antibody therapy in asthma and allergen challenge models may give some insights. For example, FLOOD-PAGE *et al.* [53] reported reduced subepithelial tenascin staining after anti-IL-5 treatment of asthmatics, but this was not associated with any change in AHR, lung function or asthma symptoms. Chronic intervention and observational studies are required but are difficult to both fund and execute. Of importance here is the question of which parameters of lung function relate to asthma outcomes and can these be related to remodelling? For example, tailoring treatment to AHR [49] or sputum eosinophilia [67] reduced asthma exacerbation rates, suggesting that these are important determinants of morbidity, but it will also be important to determine whether remodelling is related to the development of fixed airflow obstruction, the rate of change of airway calibre or the response to acute challenge (allergen or infection).

Is inflammation necessary for the development of airway remodelling?

Although it is generally held that airway remodelling occurs as a consequence of chronic inflammation induced by repeated allergen challenge, emerging theories challenge this concept. The idea that reactivation of the EMTU is a key feature of induction of airway remodelling has led to the proposal that inflammation and remodelling are parallel rather than sequential events (fig. 3). Communication between the epithelium and the underlying epithelial fibroblast sheet is reminiscent of the processes that drive branching morphogenesis in the foetus, where the epithelium and the mesenchyme function as a trophic unit. HOLGATE *et al*. [68] have proposed that the EMTU becomes reactivated during chronic asthma to drive pathological remodelling. This theory may explain the potentially conflicting findings linking inflammatory cells with markers of remodelling, some of which may be consequent upon inflammation while others are not. In addition, the hypothesis might answer some of the controversies regarding inflammation during asthma, such as why the prolonged use of corticosteroids has little or no effect on the natural history of asthma, even if treatment is instigated in early childhood.

The question as to whether remodelling of the airway wall might precede asthma has been investigated by studies of childhood asthma. Data from longitudinal studies indicate that wheezing in early childhood is a marker for subsequent reduced pulmonary function and asthma. The question is whether or not these children have smaller airway diameters that were genetically or pre-natally determined; whether this would predispose them to wheeze in association with insults such as viral infections; and whether atopy subsequently manifests as wheezing and bronchial asthma. Alternatively, pre-clinical bronchial asthma may cause abnormal airway development or remodelling, which leads to reduced airway diameter and then renders pre-school children more prone to wheezing with viral infections as well as after allergen exposure [69]. An early paper compared biopsies from 12-yr-old females with bronchial asthma with *post mortem* samples from lungs taken from similar aged children who died of severe acute asthma. In the living patients there was evidence of airway remodelling, including goblet cell metaplasia and mucus plugging, together with thickening of the bronchial epithelial basement membrane [70]. Furthermore, smooth muscle hypertrophy and inflammatory cell infiltration were also apparent. The children who had died of asthma showed more pronounced peribronchiolar eosinophil infiltration in conjunction with focal loss of the bronchial epithelium. Therefore, it seems that there is evidence of lung structural changes in children; this is reinforced by a retrospective study of bronchial biopsies from children with respiratory symptoms that subsequently developed bronchial asthma [71]. The histological features of asthma, including eosinophilia and thickening of the lamina reticularis underneath the bronchial basement membrane, were present even in pre-school children prior to the diagnosis of asthma. Even though the precise relationship between airway inflammation and airway remodelling is unclear, there is evidence that airway remodelling occurs in early childhood and that in some cases it pre-dates onset of symptoms. Other studies report that loss of lung function is determined mainly in childhood, but these physiological findings have not been correlated with histological evidence of airway remodelling [72, 73]. Perhaps more worryingly, biopsy studies of schoolchildren who remained relatively asymptomatic due to high doses of corticosteroids showed thickening of the lamina reticularis of the basement membrane compared with nonasthmatic children [74]. There is also evidence for early bronchial smooth muscle hypertrophy in biopsies from schoolchildren [75, 76]. In contrast, a recent study has looked for even earlier evidence of remodelling in pre-school children [77]. Examination of the bronchial mucosa

of infants aged <2 yrs demonstrates that the eosinophilic inflammation and RBM thickening that are characteristic of asthma in older children and adults are not present in infants with recurrent wheeze and/or cough or in the presence of reversible airflow obstruction. Moreover, there was no correlation with atopy. Further study is required to determine the relationship between airway inflammation and remodelling in children in order to identify whether early intervention will impact on lung function in later life.

This relationship has not been investigated at all in animal models. All of the studies described thus far have been performed on adult mice and the question of influence of early life events on development of airway remodelling with or without inflammation has not been addressed. Furthermore, the question as to whether remodelling precedes airway inflammation or AHR has also not been addressed, probably due to the lack of suitable models. Current models of allergic inflammation and remodelling, including those described previously, require systemic sensitisation with allergen to induce changes to airway pathology [20]. Transgenic models in which various Th2 cytokines have been ectopically expressed specifically in the lung have determined that airway remodelling does occur without immune sensitisation and allergen challenge, but the remodelling occurred in conjunction with airway inflammation, most obviously eosinophilia [27, 28]. Therefore, it is difficult to determine which came first, the inflammation or the structural changes. Whether similar results would be obtained if the mice were developed in a strain lacking CD4 T-cells remains to be seen. Models which address these specific questions are urgently required, particularly due to the ethical limitations of performing allergen challenge studies or even biopsy studies in children and infants.

Is airway remodelling reversible?

One of the most critical questions regarding chronic changes to the lung is that of reversibility. It is not clear how effective current asthma therapies are in reducing remodelling. This is critical since it has become obvious that remodelling events occur early in disease development and it is likely that by the time patients present to their physician and are diagnosed they already display markers of airway remodelling processes that are similar to those observed in patients with long-standing disease [78]. Thus, the true extent of reversibility of changes in airway wall remodelling is unclear, as is the relationship between these airway wall changes and ongoing exposure to allergen. Although studies have reported an improvement in parameters of inflammation and AHR in children undergoing allergen avoidance at high altitude [79], airway remodelling was not included in this study, probably for ethical reasons. One study has reported that airway remodelling is reversible after cessation of exposure to isothiocyanates in sensitised individuals [80]. Reversibility is relatively easy to examine in animal models and investigators have approached this by inducing airway inflammation, AHR and remodelling by chronic allergen challenge and then examining the same parameters at different time points after cessation of allergen exposure. LEIGH *et al*. [62] looked at lungs up to 8 weeks after final allergen exposure and determined that chronic challenge elicited aspects of sustained airway dysfunction and remodelling that persisted beyond the resolution of acute inflammatory events. Similarly, KUMAR *et al.* [81] observed that persistence of airway wall eosinophilia and mucous cell hyperplasia was dependent on continued allergen exposure, whereas subepithelial fibrosis and epithelial hypertrophy were not. The current authors determined that peribronchiolar fibrosis and airway smooth muscle changes persisted for $\geqslant$4 weeks following chronic allergen challenge, but inflammation and AHR resolved [82]. A recent report has described a different model in which allergic inflammation and remodelling are elicited after chronic intranasal instillation of house dust mite extract without prior sensitisation [21]. This protocol resulted in classical Th2-type eosinophilic inflammation concomitant with AHR to methacholine and lung structural changes assessed by goblet cell hyperplasia, collagen deposition and peribronchiolar accumulation of contractile tissue. Interestingly, cessation of house dust mite exposure led to resolution of eosinophilic inflammation within 2 weeks but no resolution of remodelling changes and only partial resolution of AHR [21]. The question of reversibility has not been investigated as yet in larger animal models.

These mouse models all allude to the fact that although the inflammatory aspects of allergen challenge are reversible, the lung structural changes are not. However, all of these studies have relied on cessation of allergen challenge to determine reversibility but complete allergen avoidance is not a viable option for many asthmatics. Therefore, it is important to consider whether airway remodelling might be reversible with therapy. Such studies have been undertaken in patients but, unfortunately, the majority of the available evidence regarding regression of airway remodelling in response to classical asthma treatment is contradictory. Reversal of subepithelial fibrosis in allergic asthmatics after prolonged therapy with high-dose corticosteroids has been reported [49, 83, 84] but this is not a universal finding [48, 85, 86]. Taken together, these studies indicate that airway inflammation, basement membrane thickening and AHR are interrelated and can be improved by corticosteroids but that this occurs over different time scales and with a different dose–response relationship [87]. Unfortunately, it does not seem that corticosteroids are totally protective against long-term airway damage; proof of their effectiveness needs to be collected after further investigation and other therapeutic strategies explored. A recent study with a nonbactericidal dose of a macrolide antibiotic promoted reversal of airflow limitation in chronic lung allograft rejection [88] indicating that, ultimately, it might be possible to reverse airway remodelling.

Again, mouse models have been used to investigate the effect of common asthma therapies on the development of airway remodelling. Corticosteroids have been used at the onset of allergen challenge and fluticasone was shown to be effective in preventing increases in airway laminin but, interestingly, had no effect on AHR [89]. In contrast, the current authors have shown that administration of budesonide is effective in resolving established airway eosinophilia and AHR and prevents development of airway remodelling [90]. Therapy directed at leukotrienes has also shown that airway remodelling can be prevented [91]. However, none of these studies has used a treatment regimen designed to reverse established airway remodelling. In all of the studies, therapeutic agents were administered at the start of allergen challenge [89, 91] or

at a time of established inflammation, but before remodelling was observed [90]. In the future it will be important to use these models to determine the efficacy of standard asthma treatments in reducing established airway remodelling.

Is airway remodelling genetically determined?

Asthma is a complex inflammatory disorder in which development is thought to be affected by both genetic and environmental considerations. It is commonly held that asthma is caused by multiple interacting genes, some having protective effects and others contributing to disease pathogenesis, but with each gene having its own tendency to work with, and be influenced by, the environment. Therefore, the complex nature of the disease coupled with the substantial locus heterogeneity and environmental influence has made it difficult to uncover the genetic factors that underlie the disorder [92]. Linkage analysis uses polymorphic markers throughout the genome to determine whether certain markers segregate in the affected family members, indicating that they might be located near one or more genes that cause the disease under study. Numerous studies have examined the association between asthma or its related traits and genetic variations in candidate genes [92]. Most of these studies have utilised common clinical manifestations of the disease, such as AHR, elevated IgE and atopy, in order to look for evidence of genetic linkage. However, there is accumulating evidence that airway remodelling is also genetically linked. Genetic mapping studies have revealed that chromosome 5q31-q33 is of particular interest since it contains the cytokine gene cluster including IL-4, -5, -9 and -13, as well as other growth factors and receptors implicated in asthma pathology [93]. It is of interest that transgenic models developed using tissue-specific promoters to drive lung expression of IL-9 and -13 in particular result in airway remodelling as well as the other common features of the allergic syndrome [27, 28]. Linkage disequilibrium studies identified multiple polymorphisms in the ADAM33 gene that are associated with asthma [94]. Haplotypes comprised of polymorphisms within the ADAM33 gene were significantly associated with asthma in the case–control samples, as well as in family based association tests. ADAM33 is a member of the ADAM subgroup of the zinc-dependent metalloproteinase superfamily and is a very plausible candidate gene for tissue remodelling in asthma. The gene has been found to be expressed on fibroblasts and smooth muscle cells. In addition, polymorphic variations in ADAM33 predict impaired early life functions [95] and single nucleotide polymorphisms in ADAM33 have been linked with accelerated decline in lung function in the general population [96]. Although its precise function is not fully understood, it has similarities to other ADAM genes that possess proteolytic activity and this suggests that ADAM 33 may play a role in airway remodelling. The development of a mutant mouse either overexpressing ADAM33 or genetically deficient in ADAM33 is eagerly awaited and might yield some clues as to the role of this molecule in airway remodelling.

The contribution of genes and environment to the development of airway remodelling highlights a major drawback of almost all animal models. Most strains of mice are inbred and are housed in clean environments that may be beneficial when considering the role of a particular pathway, cell or molecule in isolation. However, it is important to consider that innate tissue responses, as well as primary immune responses, are strain-dependent. Differences in the strain of mouse used for particular experiments have resulted in some contradictory results in the past [97] and are an important factor when comparing similar studies. One study has investigated the effect of mouse strain on the development of airway remodelling by assessing the responses of commonly used strains of mice to airway allergen challenge in the absence of systemic sensitisation [98]. Using this protocol, persistent AHR, eosinophilic inflammation, collagen deposition and airway wall thickening were all found to be strain-dependent. Changes were minimal in the commonly used Balb/c strain and absent in C57BL/6 and C3H/HeJ mice. Interestingly, the strain that gave consistent remodelling responses to airway intranasal challenge (rather than inhalational) was the A/J mouse, which has been shown to exhibit marked methacholine-induced AHR that is independent of allergen sensitisation and challenge [99]. Quantitative locus analysis of the AHR revealed that this naïve AHR is conferred by a major locus on A/J chromosome 2 and is an interacting locus on chromosome 6 [100]. Interestingly, chromosome 2 also contains the mouse orthologue of ADAM33.

CONCLUSIONS

Airway remodelling is a potentially important consequence of asthma. Although there has been considerable effort to design *in vitro*, *in vivo* and *in silico* systems to determine the cells and molecules responsible for the changes in tissue pathology, it is clear that there are still many unanswered questions. Further effort is needed to determine the relationship between changes in pathology and physiology before treatment regimens to prevent or resolve established remodelling can be created. It is clear that an integrated approach is needed to coordinate laboratory models with clinical studies to determine some of the answers to the questions discussed previously.

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