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## Insights into asthmatic airway remodelling through murine models

SABA AL HEIALY, TOBY K. MCGOVERN AND JAMES G. MARTIN

*Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada*

### ABSTRACT

Asthma is a chronic disorder of the airways associated in many instances with structural changes of the airways, termed airway remodelling. Irritant and allergen-induced murine models have been used to further understand the mechanisms of airway remodelling. The infiltration of the airways by inflammatory cells, such as T lymphocytes, mast cells, eosinophils, neutrophils and macrophages after repeated allergen challenges may be important effectors in the initiation and perpetuation of airway remodelling through the release of inflammatory mediators and growth factors. Interleukins-4 and -13 have been widely studied in experimental models, and have been shown to play a significant role in airway remodelling. Recently, a role for Th17 cells has been established. Other mediators involved in this process are ligands of the epidermal growth factor receptor, matrix metalloproteases and cysteinyl leukotrienes. A better understanding of the mechanisms leading to airway remodelling in allergic diseases may lead to the identification of novel therapeutic strategies but validation in human subjects is required for potential targets.

**Key words:** airway smooth muscle, cysteinyl leukotriene, epidermal growth factor, goblet cell, inflammatory cell, interleukins.

### INTRODUCTION

Animal models of asthma have burgeoned in the past 20 years. Most of the modelling has focused on airway inflammation and airway hyperresponsiveness (AHR), two defining features of asthma. Airway

remodelling, a key element of asthma that has been noted for almost 100 years and that has been well quantified through careful morphometric studies since the 60s,<sup>1</sup> has been less extensively explored. All of the tissues of the airway wall are involved in asthma and of particular note are the epithelial changes with enhanced turnover and goblet cell differentiation, increases in airway smooth muscle (ASM) mass and changes in matrix protein deposition. Each of these features has the potential to contribute to asthma pathophysiology through alterations in barrier function, lack of endogenous bronchodilators, mucous secretions, excessive airway narrowing and alterations in tissue mechanical properties. Increase in ASM mass is arguably of greatest importance to asthma pathogenesis through its effects on AHR and excessive airway narrowing.<sup>2</sup> In this review we focus on mechanisms of airway remodelling and, where possible, discuss the insights obtained through current small animal models. Figure 1 provides an overview of potential mechanisms involved in airway remodelling such as relevant cytokines, growth factors as well as relevant inflammatory cells.

A number of animal species have been used as models to explore airway structure–function relations by reproducing the pathobiological changes in the airways so as to mimic human asthma. Equine, feline and primate models have also been described; however, descriptions of these large animals are beyond the scope of this review that draws principally upon murine (mouse and rat) models. We have attempted to highlight our current understanding of the role of various cell types, bioactive molecules and inflammatory mediators thought to play critical roles in the development of allergic asthma incorporating insights gleaned from murine models.

### OVERVIEW OF MURINE MODELS

The first studies of airway remodelling in an animal were performed on the sensitized and repeatedly challenged Brown Norway (BN) rat,<sup>3,4</sup> a rat with an atopic disposition that has many features in common with human allergic asthma and were focused on the

**The Authors:** James G. Martin is a professor of medicine, member of the respiratory division and research scientist at the Meakins-Christie Laboratories of McGill University. Saba Al Heialy and Toby K. McGovern are PhD students in the Division of Experimental Medicine at the Meakins-Christie Laboratories of McGill University.

Correspondence: James G. Martin, 3626 St. Urbain, Montreal H2X 2P2, Quebec, Canada. Email: james.martin@mcgill.ca

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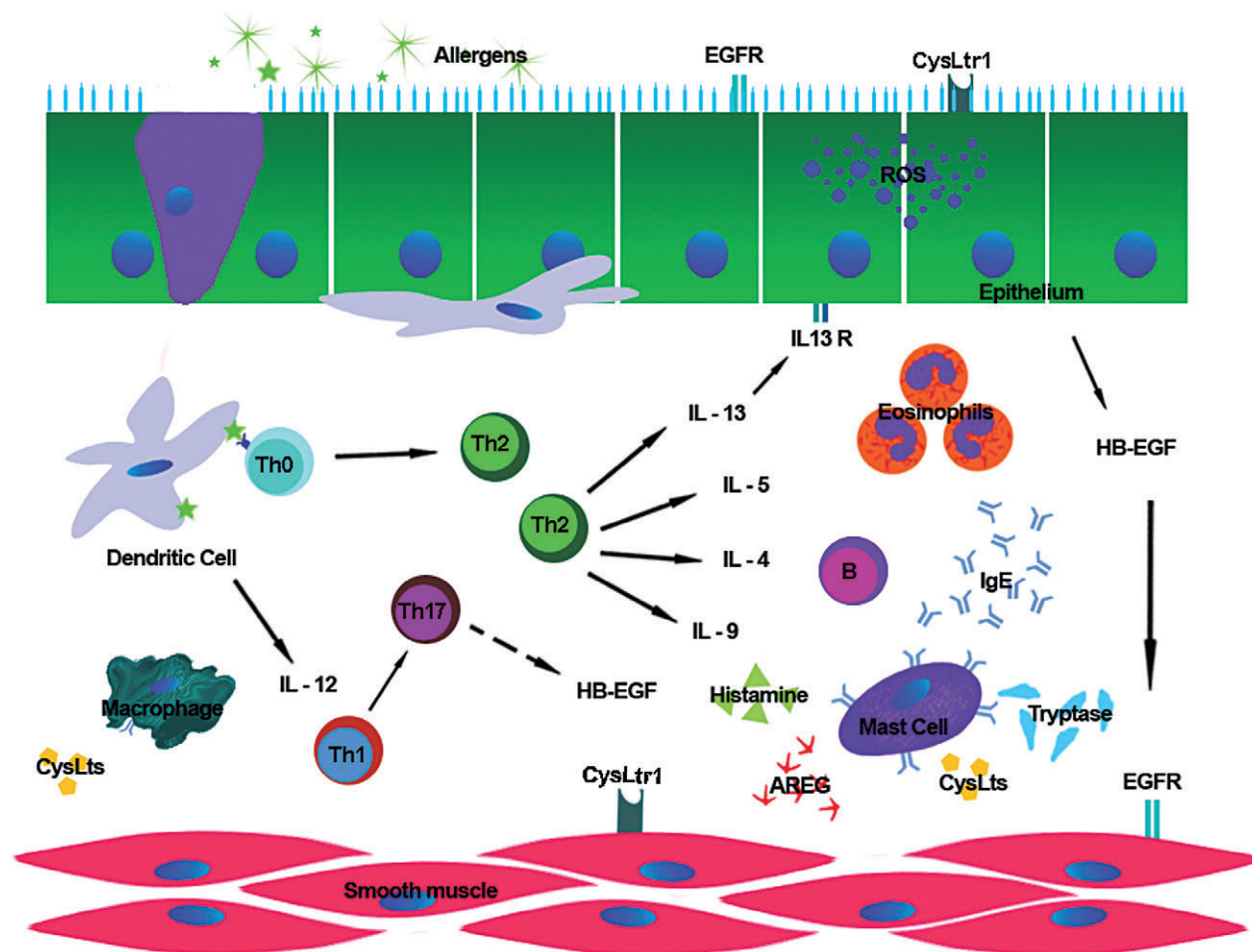


**Figure 1** Schematic of mechanisms involved in airway remodelling in murine models. Many mediators and inflammatory cells are involved in the process of airway remodelling. Following antigen presentation by dendritic cells, T lymphocytes can differentiate into several subsets of effector cells. Through the release of various interleukins (IL) such as IL-4,-5,-9 and -13, Th2 cells may potentially participate in airway remodelling. IL-13 may affect a variety of structural cells, in particular promoting epithelial cell differentiation and together with IL-4 may amplify the Th2 environment and Immunoglobulin E (IgE) synthesis. Th17 cells may release heparin-binding epidermal growth factor (HB-EGF) that binds to the epidermal growth factor receptor (EGFR) expressed on epithelium and smooth muscle cells. Other EGFR ligands include amphiregulin (AREG) that is present in mast cells and may be released by IgE cross-bridging by antigen. Following cross-linking of IgE bound to the FcεRI, the high-affinity IgE receptor, mast cells also synthesize *de novo* cysteinyl leukotrienes (CysLts), which bind to the cysteinyl leukotriene receptor-1 (CysLtr1) as well as releasing preformed histamine and tryptase, which may also be involved in airway remodelling. CysLts are also released by macrophages. Acute exacerbation of asthma induces the release of reactive oxygen species (ROS) by the epithelium, which may participate in airway remodelling. Interleukin-13 receptor (IL-13R); B cells (B); T helper cells (Th).

increase in ASM that was induced. Most current models are based on active sensitization to an allergen such as ovalbumin and varying numbers of subsequent exposures via the airways. Following the success of these early studies, there have been many reports of airway remodelling in mice using ovalbumin sensitization and repeated allergen challenge although the number of challenges required to cause substantial remodelling in the mouse is quite high by comparison with the BN rat. There are increasing numbers of studies of potentially more relevant aeroallergens such as birch pollen extract, house dust mite antigen, *Aspergillus fumigatus* and ragweed.

While all of these models have provided valuable insights into the processes leading to the induction of asthma through allergen exposure, they do not address the significant proportion of individuals afflicted by irritant-induced asthma in which asthma is triggered by mechanisms that are less obviously related to adaptive immunity.

Irritant-induced asthma is a type of occupational asthma that may include up to 20% of reported asthma cases.<sup>5</sup> It is caused by either acute or repeated exposure to gas, fumes, vapors or inhaled particles. Irritant-induced asthma is similar to allergen-induced asthma in its clinical manifestations, airway

inflammation, structural changes in the airway and decline of basal lung function. Symptoms of irritant-induced asthma characteristically persist for at least 3 months and average 3 years from initial exposure.<sup>6</sup> The prognosis for individuals suffering from irritant-induced asthma is generally accepted as poor.<sup>7</sup> Several murine models have been developed to further elucidate the mechanisms of the airway damage induced.<sup>8–10</sup> Exposure to chlorine gas, one of the well-documented causes of irritant-induced asthma, has attracted recent attention because of the ever-present danger of accidental spills and because of its use in bombings, where it has been combined with conventional explosives. Five-year cumulative data between 1988–1992 from the American Association of Poison Controls Centers' National Data Collection System reported 27 788 exposures to chlorine in the USA.<sup>5</sup> Since then, several studies have characterized the effects of chlorine on the lungs of mice and rats and have linked damage to oxidative processes. Exposure of mice and rats to levels of chlorine comparable with those a human might encounter in the event of a chemical spill have demonstrated increases in macrophages, eosinophils, neutrophils and lymphocytes in the bronchoalveolar lavage fluid (BALF) as well as shed epithelial cells.<sup>11,12</sup> Changes in airway mechanics and responsiveness are also altered following chlorine with concomitant changes in airway wall structure.<sup>11</sup> Most studies have addressed the acute effects of chlorine-induced airway dysfunction, and responses to long-term, low-dose exposures to chlorine or other irritant gases are relatively under-explored. Airway injury from chlorine may have much in common with other oxidants such as ozone that may be encountered in lower concentrations but for much longer periods in association with air pollution.

Diesel exhaust particles (DEP) constitute another significant and frequently encountered airway irritant that evokes airway inflammation, enhanced production of pro-inflammatory cytokines and chemokines, production of reactive oxygen species and altered lung function.<sup>13</sup> While DEP are principally comprised of elemental carbon, they also contain polycyclic aromatic hydrocarbons, quinones, oxides of nitrogen and naphthalene, several of which are known respiratory tract irritants and result in oxidative stress when inhaled. Additionally, DEP contain particles less than 1 µm in diameter that are readily respirable.<sup>14</sup> While DEP themselves appear to produce an asthma-like condition in murine models by direct effects, exposure to DEP has proven also to have adjuvant effects, enhancing allergic asthma and increasing specific IgE against various allergens including ovalbumin and Japanese cedar pollen.<sup>15</sup> Models addressing these effects and their long-term consequences for airway structure may be of great relevance to the human population as epidemiological studies have shown that exposure to DEP may greatly exacerbate asthma in persons with asthma or in atopic individuals.

Mouse models of asthma have provided opportunities to study the importance of specific mediators in remodelling by knocking-out or overexpressing genes of interest. Many of the early models provided interesting data for consideration but interpretation is

complicated because the overexpression constructs often led to the production of pharmacological levels of cytokines and their ectopic expression, usually in the airway epithelium, may also have led to anomalous effects. Conditional gene knock-out and overexpression models will help to provide more realistic conditions for better modelling. Despite considerable effort there are still major gaps in our understanding of the links between the cells activated in asthma and the mediators responsible for remodelling.

## CELLULAR MECHANISMS OF REMODELLING

### Mast cells

Mast cells are the primary responders in allergic reactions and are activated in response to a variety of stimuli. Following their activation by cross-linking of the high-affinity IgE receptor (FcεRI), mast cells release leukotrienes, histamine, tryptase, protease and various cytokines.<sup>16</sup> Through the release of these inflammatory mediators, mast cells not only play a major role in immediate hypersensitivity and late-phase responses, but may also contribute to airway remodelling. It has been reported that asthmatic patients exhibit a higher number of mast cells localized among smooth muscle bundles, and this positively correlates with the severity of the disease.<sup>17</sup> Numerous studies have shown that mast cells induce human ASM proliferation through the release of tryptase, activin A, tumour necrosis factor-α (TNF-α), platelet derived growth factor and transforming growth factor-β (TGF-β).<sup>17–19</sup> Human mast cells also secrete amphiregulin, an epidermal growth factor (EGF) ligand that upregulates mucin gene expression in airway epithelial cells leading to their differentiation into goblet cells.<sup>20</sup> It is also possible that amphiregulin might promote ASM growth if released by the mast cells distributed throughout the muscle bundles. Moreover, cross-talk between immune and structural cells has been suggested where human ASM cells may mediate the survival of mast cells via secretion of stem cell factor and IL-6.<sup>21</sup>

Due to their significance in human disease, mast cells have been targeted for study in mouse models of chronic asthma. Mast cells are required for the development of some of the features of airway remodelling including goblet cell hyperplasia, increase in lung collagen, increase in MUC5AC and MUC5B expression, infiltration of eosinophils and lymphocytes. These features are all significantly reduced in mast cell-deficient mice. In mice whose mast cells lack FcγR, and therefore cannot be activated by either antigen- and IgE-dependent aggregation of FcεRI or the binding of antigen-IgG1 immune complexes to FcγRIII, certain features were still found but to a lesser extent than wild-type animals.<sup>22</sup> Beyond the mast cells themselves, factors that influence their development have also been explored in murine models. One such factor, IL-9, has been reported to cause airway remodelling. Produced by Th2 and Th9 cells, this

cytokine is involved in mast cell growth and differentiation, promoting the expression of mast cell proteases and upregulation of FcεR1α. Neutralizing IL-9 in a chronic mouse model of asthma reduced features of airway remodelling: goblet cell hyperplasia, mucus secretion, subepithelial collagen deposition, ASM proliferation and release of pro-fibrotic factors (TGF-β, vascular endothelial growth factor and fibroblast growth factor (FGF)-2).<sup>23</sup>

### Eosinophils and neutrophils

The inflammatory response in the airways of allergen-driven asthma is characterized by the presence of Th2 lymphocytes, activated mast cells and eosinophils. Similar to mast cells, eosinophils may also play an integral role in airway remodelling, particularly through the release of a variety of cytokines and growth factors that influence the growth and differentiation of surrounding structural cells.

IL-5 is involved in the differentiation, migration and survival of eosinophils. Chronically allergen-challenged IL-5-deficient mice show a reduction in peribronchial fibrosis, smooth muscle layer thickness and number of peribronchial TGF-β positive cells as compared to wild type animals<sup>24</sup> suggesting that eosinophils contribute to the process of airway remodelling. Moreover, IL-5 and eotaxin overexpressing transgenic mice show an increase in airway remodelling following ovalbumin sensitization and challenge.<sup>25</sup> A significant reduction in subepithelial collagen deposition and ASM mass following ovalbumin sensitization and challenge was observed in dbpGATA eosinophil-deficient mice compared with wild-type controls.<sup>26</sup> C-C chemokine receptor 3 (CCR3), a member of the 7 transmembrane-spanning G protein-coupled receptors, is primarily expressed on eosinophils and involved in eosinophil infiltration. A novel CCR3 antagonist, Ki19003, significantly inhibited eosinophilia as well as subepithelial and peribronchial fibrosis in the ovalbumin challenged mouse.<sup>27</sup> Notable, these studies all employed ovalbumin-challenged models. Data from a chronic model of house dust mite-induced airway remodelling suggest that eosinophils may not be required for airway remodelling as the use of anti-CCR3 and two strains of eosinophil-deficient mice had no effect on the inflammatory response, Th2 infiltration or the development of features of remodelling such as subepithelial collagen deposition and increase in ASM mass compared with wild-type controls.<sup>28</sup>

It has also been suggested that IL-13 may play a critical role in the accumulation of intraepithelial eosinophils and features of airway remodelling. IL-13-deficient mice exhibited a reduction in the number of eosinophils and inflammatory cells as well as a reduction in subepithelial fibrosis, epithelial hypertrophy and mucous cell hyperplasia in a chronic asthma model.<sup>29</sup>

Eosinophils are a major source of TGF-β, which has the potential to induce peribronchial fibrosis. TGF-β neutralization significantly reduces peribronchial fibrosis, ASM thickness and mucus production.

Chronically challenged mice that are deficient in the downstream signalling molecule Smad 3 also show a reduction in airway remodelling, supporting the role for eosinophil expression of TGF-β in airway remodelling.<sup>30</sup> TGF-β has been shown to cause ASM hypertrophy,<sup>31</sup> of potential importance in the increase in ASM mass.

While eosinophils have been largely considered as the most important inflammatory cell type in the pathophysiology of asthma, it should be noted that over 50% of individuals with asthma present with non-eosinophilic inflammation.<sup>32</sup> Most cases of non-eosinophilic asthma are associated with an increase in neutrophils. Increased numbers of neutrophils have been noted in patients with status asthmaticus and had sudden death within 24 hours of exacerbation.<sup>33</sup> Asthmatic patients express FcεRI, in contrast to non-asthmatics, which activates neutrophils. This leads to the release IL-8, a potent neutrophil chemoattractant that further exacerbates inflammation. Activated neutrophils release several reactive oxygen species, including myeloperoxidase and NADPH (nicotinamide adenine dinucleotide phosphate) oxidase.<sup>34</sup> This oxidant induced damage may lead to airway remodelling by promoting goblet cell hyperplasia.

Furthermore, with respect to murine asthma models, sensitization protocols have recently shown discrepancies in the nature of the resulting airway inflammation, which can be skewed towards a Th2 or Th17 dominant response. An intraperitoneal sensitization protocol using low-endotoxin ovalbumin yielded a Th2, eosinophil driven response whereas mice administered low-endotoxin ovalbumin intranasally developed a Th17, neutrophil driven response.<sup>35</sup> These data support a causal role for neutrophils in severe asthma, driven by a Th17 immune response.

### T cells

The accumulation of activated CD4+ T cells, with a Th2 phenotype, in the airways following allergen challenge is believed to play an important role in the initiation and perpetuation of inflammation. CD4+ Th2 cells exert their effects through an array of cytokines that induce the changes in the airways associated with asthma. Several studies have reported that monoclonal antibody depletion of CD4+ T cells in a mouse model inhibits AHR and eosinophilic infiltration.<sup>36</sup> Depletion of CD4+ T cells followed by repeated ovalbumin challenges in BALB/c mice resulted in a marked decrease of AHR and certain features of airway remodelling including subepithelial fibrosis, epithelial thickening and mucus cell hyperplasia and metaplasia.<sup>37</sup> Human *in vitro* studies have indicated that activated T lymphocytes may adhere to human ASM cells via integrins such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 and CD44 and, in doing so, induce ASM cell proliferation.<sup>38</sup> Observations consistent with this mechanism of ASM growth have been made in BN rats, where activated CD4+ T cells are found in direct contact with ASM cells *in vivo* and induce ASM cell proliferation *in vitro*.<sup>39</sup>

To date, the nature of the T cells responsible for proliferation has not been determined. However, recent findings show that the novel Th17 cell subset may be involved in airway remodelling. Using a C57BL/6 mouse model of prolonged allergen exposure, Wang *et al.* showed an increase in Th17 cell infiltration, which correlated with the severity of airway remodelling. Treatment with anti-IL-17 induced a marked decrease in ASM mass, mucus production, peribronchial collagen deposition and expression of heparin-binding epidermal growth factor (HB-EGF). These features, with the exception of peribronchial collagen deposition, were also decreased in the presence of a specific inhibitor of the epidermal growth factor receptor (EGFR) and a HB-EGF monoclonal antibody suggesting a relationship between the Th17 subset and EGFR pathway.<sup>40</sup>

In contrast to the above findings, Doherty *et al.* have suggested that CD4+ T cells are not required for airway remodelling during on-going allergen challenges after the development of acute eosinophilic lung inflammation in a murine model.<sup>41</sup> Following depletion of CD4+ T cells using an antibody for 4 weeks during chronic exposure to ovalbumin failed to reduce mucus metaplasia, peribronchial subepithelial fibrosis and smooth muscle mass despite significantly reduced peribronchial inflammation, BAL eosinophilia, and lung CD11c+, CD8+ and Siglec-F+CD11c- cells. These conflicting data may highlight the importance of timing in the context of interventions. The latter study administered the depleting antibody during allergen challenges, whereas the former depleted the CD4+ T cell population before the challenges, suggesting that once the sensitization process is fully developed the CD4+ T cell may not be essential for remodelling. Whereas CD4+ T cells may be involved in airway remodelling, depletion of CD8+ T cells during repeated ovalbumin challenges in BN rats enhances ASM mass and number of epithelial cells compared with OVA-challenged animals suggesting a protective role for CD8+ T cells.<sup>42</sup>

## Macrophages

Alveolar macrophages (AM) are a major source of cytokines, chemokines and arachidonic acid metabolites and initiate and resolve airway inflammation. Depletion of AM in rats resulted in an increase in IgE levels, T and B cell influx to the lungs and AHR.<sup>43</sup> Transfer of AM from Sprague-Dawley rats to AM-depleted BN rats 24 h before ovalbumin challenge eliminated AHR. Interestingly, the levels of TNF- $\alpha$  decreased and IL-13 increased, suggesting that AM reduce AHR through the alteration of the cytokine milieu.<sup>44</sup> In other studies, BALB/c mice were sensitized and challenged with ovalbumin to induce features of airway remodelling. In these animals, cytokine expression by AM did not increase. However, after an acute exacerbation using a single dose of ovalbumin, AM expressed higher levels of pro-inflammatory cytokines such as (IL) 1 $\beta$ , IL-6, CXCL-1 and TNF- $\alpha$ . In parallel, AM from an acutely challenged animal induced a high expression of Th2

cytokines by CD4+ T cells following co-culture compared with AM from chronically challenged or naïve animals.<sup>45</sup> These results suggest an important role for AM in acute exacerbations but not in chronic asthma. In a mouse model of chronic ovalbumin allergen-induced airway remodelling, peribronchial macrophages express basic FGF (bFGF) and TGF- $\beta$ . *In vitro*, bFGF induces the expression of TGF- $\beta$  in peribronchial macrophages.<sup>46</sup> However, little is known on the role of macrophages in airway remodelling and further studies are needed.

## MECHANISMS OF REMODELLING; MEDIATORS

### IL-4/IL-13

IL-13 and IL-4 are Th2 cytokines that bind to the alpha chain of the IL-4 receptor and have been extensively studied for their contribution to the asthma phenotype. IL-13 is a 12-kD protein produced by a variety of cells such as basophils, dendritic cells, ASM cells, mast cells and predominantly Th2 cells. It plays a major role in IgE production and adhesion molecule expression. Over expression of IL-13 in the lungs of transgenic mice results in mononuclear inflammation, subepithelial fibrosis, mucus cell metaplasia, eotaxin production and an increase in airway resistance and AHR to methacholine.<sup>47</sup> However, this model seems to be more appropriately considered a facsimile of emphysema. In the mouse sensitized to house dust mite and repeatedly challenged with the allergen administration of anti-IL-13 monoclonal antibody inhibits cellular inflammation, peribronchial collagen deposition, epithelial goblet cell differentiation and AHR.<sup>48</sup> These studies provide strong evidence that IL-13 plays a significant role in airway remodelling. IL-13 may also mediate airway remodelling in an indirect manner potentially through its role in influencing metalloproteinase expression, another set of molecules associated with airway remodelling.

Human studies have shown that IL-4, which is produced by Th2 lymphocytes, mast cells and basophils, promotes airway remodelling *in vitro*.<sup>49</sup> Fibroblasts incubated with IL-4 have an increase in procollagen 1 transcription and increase in TIMP2/MMP-2 ratio. In the mouse, overexpressing IL-4 in the lungs leads to hypertrophy of epithelial cells of the trachea, bronchi and bronchioles that may be explained in part by the accumulation of mucus glycoprotein. Interestingly, independent of an increase in airway responsiveness, there was an increase numbers of macrophages, lymphocytes, eosinophils and neutrophils.<sup>50</sup> Both IL-4 and IL-13, but not IL-5, deficient mice are protected from the development of sustained AHR and aspects of airway remodelling. This protection extends to increases in smooth muscle, subepithelial airway collagen deposition and goblet cell numbers following ovalbumin challenges. These findings make them critical cytokines for further characterization in the context of allergen-induced asthma.<sup>51</sup>

## EGFR

The EGFR is a tyrosine kinase receptor that plays an important role in cell proliferation, differentiation, motility and survival. EGFR ligands include EGF, HB-EGF, epiregulin, betacellulin, amphiregulin and TGF- $\alpha$ . Ligands are synthesized as Type 1 transmembrane precursor proteins, which are cleaved by proteases to release the active form. Upon ligation, EGFR undergoes a conformational change that allows autophosphorylation of the tyrosine residues located within the cytoplasmic tail. Phosphotyrosines allow the recruitment of signalling molecules that result in some of the features of airway remodelling such as goblet cell proliferation and ASM hyperplasia. Therefore, many studies have focused on the therapeutic effect of EGFR antagonists.<sup>52</sup>

Exposure of rats to ovalbumin has been shown to trigger mucus gene expression by epithelial cells that appears to be mediated by the EGFR. Indeed the EGFR is upregulated by such an exposure.<sup>53</sup> The role of the EGFR, albeit best studied in epithelial remodelling, goes beyond this tissue and repeated ovalbumin challenges in BN rats result in ASM hyperplasia, epithelial cell and goblet cell proliferation with changes that are prevented by AG1478, an EGFR tyrosine kinase inhibitor. Montelukast, a cysteinyl leukotriene 1 (cys LT1) receptor antagonist also resulted in the inhibition of airway remodelling following cysteinyl leukotriene D<sub>4</sub> administration suggesting that aspects of airway remodelling are mediated by cysLTs and cys-LT1 receptor with downstream effects on EGFR.<sup>54</sup> Other studies have also shown that intratracheal administration of AG1478 dose-dependently inhibited eosinophil infiltration, hyperresponsiveness, mucus and MUC5AC production and collagen deposition in a mouse model of asthma.<sup>55</sup> Pretreatment with gefinitib, a compound similar in its actions to AG1478, significantly reduced inflammatory cell counts, released cytokine concentration (IL-4 and IL-13) in BALF and eosinophil recruitment in the lungs lending further support to the idea that EGFR is critically involved in airway remodelling processes.<sup>56</sup>

Recently it has been shown that administration of AG1478 or anti-HB-EGF significantly inhibited the increase in goblet cells and ASM mass following antigen challenge or Th17 adoptive transfer in C57BL/6 mice. *In vitro*, Th17 induced epithelial cell expression of HB-EGF following co-culture of the two types of cells. These observations indicate a role for IL-17 secreted by Th17 cells in the overexpression of HB-EGF that may contribute to mucus production and ASM proliferation.<sup>40</sup> The cells of origin of HB-EGF were not determined although the epithelium was suggested as a possible source. Human asthmatic airways show that ASM may be a significant source of HB-EGF.<sup>57</sup>

Expression of TGF- $\alpha$  induces fibrosis and pleural thickening in addition to an increase in Egr-1, a transcription factor. The lack of Egr-1 exacerbates airway remodelling and hyperresponsiveness following challenge with the bronchoconstrictor methacholine. The absence of Egr-1 also induces ASM hyperplasia.<sup>58</sup>

Intratracheal installation of TNF- $\alpha$  in rats induces the expression of EGFR in airway epithelial cells. Co-stimulation of EGFR by its ligands, TGF- $\alpha$  and EGF, increase the number of goblet cells, mucous glycoconjugates and MUC5AC gene expression. Interestingly, TNF- $\alpha$ , EGF or TGF- $\alpha$  alone had little if any effect. OVA-sensitization and challenge also results in an upregulation of EGFR expression and goblet cell production in airway epithelium. BIBX1522 (EGFR inhibitor) administration prevented goblet cell production both in TNF- $\alpha$ -EGFR stimulated rats and in asthma model.<sup>53</sup> Taken together, these studies suggest a pivotal role for EGFR and its ligands in the induction of some of the major features of airway remodelling.

## Matrix metalloproteases (MMP)

Matrix metalloproteases are members of the metzincin superfamily and are a group of zinc-dependant proteases that are responsible for the degradation of constituents of the extracellular matrix. MMP are grouped according to their substrate specificity as gelatinases (MMP-1,8,13), collagenases (MMP-2,9), stromelysins (MMP-3,10,11) and metalloelastase (MMP-12). Produced as transmembrane proenzymes by different types of cells, their function is influenced by interruption of the catalytic domain blockade and cleavage of the pro-peptide, which results in the release of the catalytically active enzyme. Once activated, MMP can be inhibited non-specifically by  $\alpha$ -2 macroglobulin or specifically by tissue inhibitor of metalloproteinases (TIMP). There are four types of TIMPS (TIMP 1–4) that bind with high affinity to the catalytic site of active MMP in 1:1 ratio. MMP-9 appears to be the predominant MMP in the airway of asthmatics.<sup>59</sup>

In the context of allergenic-asthma, repeated ovalbumin challenges in the mouse results in a significant increase in MMP-9 expression in airway epithelium and an increase in MMP-9 and TIMP-1 expression in peribronchial inflammatory cells.<sup>60</sup> MMP-9-deficient mice have less peribronchial fibrosis and total lung collagen following repeated intranasal ovalbumin challenges than do wild-type mice. However, there do not appear to be any significant differences in other features of airway remodelling such as smooth muscle growth and mucus expression.<sup>61</sup>

A Disintegrin And Metalloproteinase (ADAM) are also members of the metzincin superfamily and have also been studied for their role in asthma. They differ from MMP due to the presence of a disintegrin-like domain that promotes adhesion of ADAM to cell surface integrins. This adhesion plays a role in cell-cell and cell-matrix interactions. Many studies using murine models of allergen-induced chronic airway inflammation have shown increases in ADAM such as ADAM33, 8 and 17.<sup>62,63</sup> However, their role in airway remodelling remains relatively unknown. Numerous human studies have shown an association between ADAM33 and asthma.<sup>64–67</sup> In relation to airway remodelling, it has been shown that soluble ADAM33 may promote angiogenesis in asthmatic patients.<sup>68</sup> Jie *et al.* show that ADAM33 expression, which is

dependent on IL-4 and IL-13 expression, is increased in the lung of ovalbumin-sensitized and challenged rats.<sup>62</sup>

### Cysteinyl leukotrienes

Cys-LTs are important lipid mediators involved in asthma and include: LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>. These inflammatory mediators activate two receptors: cys-LT1 and cys-LT2 receptors.<sup>69,70</sup> They are produced by activated eosinophils, mast cells, basophils and macrophages. The use of inhibitors of 5-lipoxygenase and 5-lipoxygenase-activating protein prevent leukotriene formation and result in inhibition of the secretion of airway mucus and of eosinophil infiltration.<sup>71</sup> In ovalbumin-sensitized and challenged BALB/c mice, the use of a cys-LT1 receptor antagonist, montelukast, resulted in a reduction of eosinophilia, mucus plugging, smooth muscle hyperplasia and subepithelial fibrosis. The use of montelukast also resulted in a reduction of IL-4 and IL-13 expression and prevented the formation of Charcot-Leyden-like crystals in macrophages.<sup>72</sup> Pretreatment with the LTD<sub>4</sub> antagonist, MK-571, in ovalbumin-sensitized and challenged BN rats significantly reduced AHR and thickening of the smooth muscle layer of the large airways.<sup>73</sup> Other pharmacological studies confirm these findings in the BN rat.<sup>74</sup> Montelukast, but not dexamethasone, reduced the established airway smooth increase and subepithelial collagen deposition. The authors also reported a reduction in airway eosinophilia and goblet cell metaplasia in the presence of montelukast or dexamethasone in ovalbumin-treated mice. These findings indicate a role for cysLT1 receptor antagonists, but not corticosteroids, in the reversal of established features of airway remodelling.<sup>75</sup>

### Emerging insight from murine models for future therapeutic application

Understanding airway remodelling mechanisms is the first step to designing therapeutic agents that may relieve patients of their symptoms. Recently, interest in mesenchymal stem cells (MSC) has grown as they have been shown to decrease scarring and suppress T cell activity. Intravenous administration of human marrow-derived MSC (hMSC) to a mouse model of chronic asthma localized to the lung and significantly reduced cellular recruitment, number of eosinophils, serum IgE levels and iNOS expression. hMSC also had an effect on the development of features of airway remodelling. Administration of hMSC significantly reduced epithelial lining thickening, extracellular matrix deposition and mucus hyperproduction and these changes may take place through hMSC inducing and release of immunomodulators.<sup>76</sup> One theorized immunomodulator in this process may be TGF- $\beta$ . Studies have shown that IL-4 and/or IL-13 may induce TGF- $\beta$  production through the activation of the STAT6 pathway. TGF- $\beta$  alone or together with

regulatory T cells induce the inhibition of eosinophil recruitment, mucus hyperproduction and Th2 cytokine release (IL-4, IL-5 and IL-13).<sup>77</sup>

Regulatory T cells have also been studied for their potential therapeutic role in asthma. Preliminary evidence for this concept was recently shown by administration of CD4+CD25+ regulatory T cells after the onset of acute inflammation. These cells had the effect of reducing airway inflammation and the development of airway remodelling such as mucus hypersecretion and collagen deposition. However, the reduction in inflammation and airway remodelling did not affect AHR, suggesting that they may not be coupled.<sup>78</sup>

Oxidative stress has been strongly implicated in irritant-induced airway damage. Increases in markers of oxidative stress are identifiable in the lungs of mice and rats following acute chlorine exposure, including peroxynitrite, 4-hydroxynonenol and increased expression of inducible nitric oxide synthase.<sup>9,79</sup> These findings have led to the testing of anti-oxidant rescue treatments that appear to be effective at reducing airway inflammation, airway responsiveness and production of reactive oxygen species even when administered post-chlorine exposure.<sup>12,79</sup>

### CONCLUSIONS

Asthmatic airway remodelling has been well established in murine models and these models have shed light on potential mechanisms of relevance to human asthma. These models are usually driven by allergen in actively sensitized animals. This paradigm does not accommodate other forms of asthma such as irritant-induced asthma for which there is currently a shortage of modelling studies. The extrapolation of mechanisms to human asthma is not warranted at this time given the complexity of human asthma and the possible peculiarities of the murine airway tree compared with the human airways. Unfortunately, mechanistic studies on human airway remodelling suffer from a lack of convenient tools for its evaluation at present time and we will continue to rely on data from murine models for some time.

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