SERIES "SIGNALLING AND TRANSCRIPTIONAL REGULATION IN INFLAMMATORY AND IMMUNE CELLS: IMPORTANCE IN LUNG BIOLOGY AND DISEASE"
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The contribution of transforming growth factor-β and epidermal growth factor signalling to airway remodelling in chronic asthma

C. Boxall, S.T. Holgate and D.E. Davies

ABSTRACT: Asthma is increasing in prevalence in the developing world, affecting ~10% of the world's population. It is characterised by chronic lung inflammation and airway remodelling associated with wheezing, shortness of breath, acute bronchial hyperresponsiveness to a variety of innocuous stimuli and a more rapid decline in lung function over time.

Airway remodelling, involving proliferation and differentiation of mesenchymal cells, particularly myofibroblasts and smooth muscle cells, is generally refractory to corticosteroids and makes a major contribution to disease chronicity. Transforming growth factor-β is a potent profibrogenic factor whose expression is increased in the asthmatic airways and is a prime candidate for the initiation and persistence of airway remodelling in asthma.

This review highlights the role of transforming growth factor-β in the asthmatic lung, incorporating biosynthesis, signalling pathways and functional outcome. *In vivo*, however, it is the balance between transforming growth factor-β and other growth factors, such as epidermal growth factor, which will determine the extent of fibrosis in the airways.

A fuller comprehension of the actions of transforming growth factor-β, and its interaction with other signalling pathways, such as the epidermal growth factor receptor signalling cascade, may enable development of therapies that control airway remodelling where there is an unmet clinical need.

KEYWORDS: Asthma, fibroblast, fibrosis, myofibroblast, transforming growth factor-β

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Asthma is a chronic inflammatory disease of the airways characterised by recurrent respiratory symptoms, such as wheezing, breathlessness, chest tightness and coughing. Airway remodelling is the general description for the thickening and restructuring of the airways seen in asthma patients (fig. 1 and 2). The characteristics of airway remodelling include subepithelial fibrosis, myofibroblast hyperplasia, myocyte hyperplasia and hypertrophy, together with epithelial damage, goblet cell metaplasia, oedema [1] and increased vascularity. Mathematical models suggest that the remodelling and thickening observed in the airway of asthmatic subjects will enhance airway narrowing in response to bronchospasm during an asthma attack [2, 3]. Remodelling is also considered to be responsible for the more rapid decline in lung function over time experienced in the asthmatic compared with the nonasthmatic population [4].
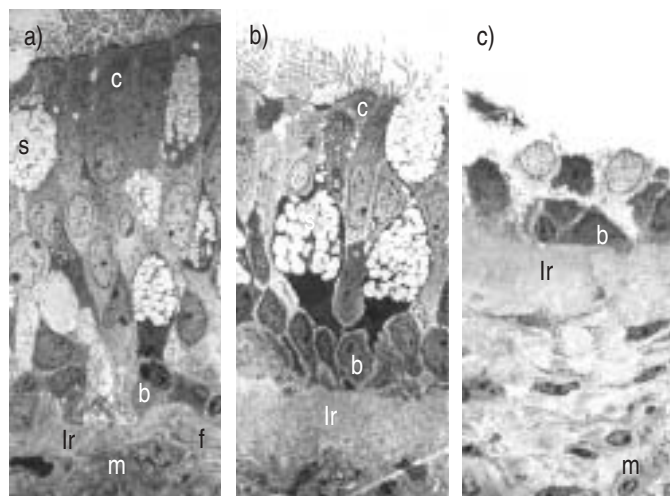

FIGURE 1. Transmission electron micrographs showing a) normal, b) mild and c) severe asthma airway epithelia. The epithelium is frequently damaged in asthmatic airways, revealing denuded areas where the basal cells are exposed (c). b) shows an increase in goblet cells in the epithelial layer. Transmission electron micrographs b) and c) of the asthmatic airway show thickening of the lamina reticularis. b: Basal cell, c: columnar epithelial cells, f: (myo)fibroblast, lr: lamina reticularis, m: mast cell, s: secretory goblet cells. Reprinted with permission from [6].
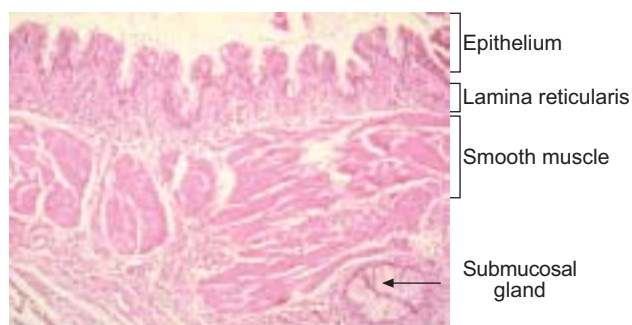

FIGURE 2. An increase in smooth muscle mass and thickening of the lamina reticularis are phenomena frequently observed in asthmatic airways as a result of remodelling. Reprinted with permission from [7].

Furthermore, it has been reported that airway hyperresponsiveness is associated with features of both remodelling and airway inflammation [5].

This review initially discusses the current findings in the field of remodelling in asthma. Recognising the role of transforming growth factor (TGF)-β as a central mediator of tissue fibrosis and structural remodelling, the mode of action and cell type-specific effects of TGF-β will then be discussed, with a view to understanding the advantages and disadvantages behind the application of anti-TGF-β therapies in the clinic. The review will continue by describing how the ultimate balance of growth factors in the lung can alter the delicate equilibrium that determines whether the epithelium has the capacity to repair after insult without triggering uncontrolled fibrosis. This will focus on the relationship between TGF-β and members of the epidermal growth factor (EGF) receptor ligand family, both of which show dysregulation in asthma. These growth factors

are not only connected historically, as TGF-α and TGF-β were originally co-purified as ''transforming growth factor'' on the basis of anchorage-independent growth assays [8], but also demonstrate many archetypal properties as regulators of epithelial and mesenchymal cell behaviour.

AIRWAY REMODELLING IN ASTHMA
Epithelial changes
The lung epithelium is continually subjected to environmental insults, such as air pollution, viruses and bacteria, as well as allergens, such as grass pollen and house dust mite allergens. Some irritants, such as house dust mite allergens, exert protease activity and can degrade the tight junctions of the epithelium [9]. Although shedding of the epithelium is part of airway homeostasis, it has been suggested that the epithelium of asthmatics is more susceptible to environmental insults compared with that of nonasthmatics. Indeed, clumps of attached ciliated epithelial cells (creola bodies) have been observed in bronchoalveolar lavage (BAL) fluid from asthmatics [10]. The extent of epithelial injury observed in asthmatics is controversial, with some reports showing no differences between normal and mild-to-moderate asthmatic biopsies [11]. However, this study was based on morphometry and did not use markers of epithelial repair to discriminate between damage that had occurred *in vivo* and artefactual damage that inevitably arises due to the bronchoscopy procedure [12]. More recent ultrastructural studies have provided further insight into the structure of the asthmatic epithelium, where it has been suggested that reduced desmosomal contact may be an important factor in the epithelial shedding observed in patients with asthma [13]. *In vitro* studies have also identified functional differences in the responses of asthmatic epithelial cells to environmental injury. For example, WARK *et al.* [14] showed that asthmatic cells have a defective innate immune response following infection with rhinovirus (RV-16), while BUCCHIERI *et al.* [15] showed that asthmatic bronchial epithelial cells are more susceptible to apoptosis in response to oxidant stress than normal epithelial cells. Additionally, PUDDICOMBE *et al.* [16] reported that p21waf, a cyclin-dependent kinase inhibitor, is expressed at a higher level in asthmatic epithelium compared with healthy controls. Expression of p21waf was also induced by TGF-β and oxidants, suggesting that these agents may contribute to a slower repair response within the context of airway inflammation and remodelling.

Other characteristic features of the asthmatic epithelium are an increase in the number of mucus-containing goblet cells [17] (fig. 1) and an increase in the size of submucosal glands [18] in asthmatic airways, resulting in an excessive release of mucus. The increased production of mucus can lead to the formation of plugs that can extend to the membranous bronchioles and contribute to airway obstruction, although, interestingly, mucus release into the BAL fluid was not found to be affected either 1 or 24 h after allergen challenge [19]. Mucin glycoproteins (MUC) are the major macromolecular component of mucus and are expressed as two major forms: membrane-tethered mucins and secreted mucins. Immunohistochemical staining for MUC5AC, one of the major gel-forming secreted mucins, has revealed abundant staining in goblet cells situated in the epithelial surface lining and glandular ducts of tissues

from patients with fatal asthma [20]. MUC2 and MUC4 mRNA expression has also been reported to be increased in asthmatic bronchial biopsies [21].

Thickening of the lamina reticularis

The ''true'' basement membrane, comprising the lamina rara and lamina densa, separates the airway epithelium from the mesenchyme. While the lamina rara and lamina densa in airways of asthmatic subjects are not reported to differ from nonasthmatics, the lamina reticularis is altered in asthmatics (fig. 1). This region, which is composed of collagen I, collagen III, collagen V, fibronectin and tenascin, and is situated just below the basement membrane, has an overall thickness of ~3–4 μm in nonasthmatics, while in asthma this is increased two- to three-fold [22, 23]. Although increased collagen deposition in the lamina reticularis is a characteristic of asthma, it may not explain the differences in severity of asthma [24]. Functionally, thickening of the lamina reticularis has been linked to reduced airway distensibility and increased airflow limitation in asthma [25], suggesting that this altered structure has a negative impact on lung function. However, it has been suggested that thickening of the lamina reticularis may actually serve as a protective mechanism by increasing the stiffness of the airways to attenuate the sporadic bronchoconstriction [2, 26].

Increased numbers of structural mesenchymal cells

Fibroblasts and myofibroblasts are the main structural cells of the mesenchyme that mediate the majority of the events contributing to the subepithelial fibrosis in remodelled airways. A number of studies have shown that there is an increase in myofibroblasts [22] in the submucosal layer of asthmatics. BENAYOUN et al. [27] demonstrated that there were significantly greater numbers of fibroblasts in bronchial biopsy samples from severe asthmatic compared with normal subjects. The number of fibroblasts in the submucosa of asthmatic subjects has also been significantly correlated with thickening of the subepithelial lamina reticularis [28].

In addition to an increase in the number of myofibroblasts, a number of reports describe smooth muscle hyperplasia in asthmatic airways. For example, WOODRUFF et al. [29] demonstrated a 50–83% (p<0.005) increase in the amount of smooth muscle in the submucosa of asthmatics. It was reported that tritiated thymidine incorporation was increased in asthmatic airway smooth muscle cells in vitro compared with normal cells, indicating that airway smooth muscle proliferation may be increased in asthma, thus contributing to the hyperplasia [30]. In 2004, JOHNSON et al. [31] suggested that the increased proliferation may be attributable to the altered matrix deposition by asthmatic smooth muscle cells. Specifically, extracellular matrix (ECM) rich in perlecan and collagen I, but with lower amounts of laminin α1 and collagen IV deposited by asthmatic cells, enhanced the proliferation of nonasthmatic smooth muscle in vitro. There are conflicting reports as to whether there is also a difference in the size (hypertrophy) of asthmatic smooth muscle cells. BENAYOUN et al. [27] described hypertrophic smooth muscle cells with an accompanied increase in myosin light-chain kinase expression from severe persistent asthmatic subjects compared with those with milder disease. Conversely, in mildly to moderately severe subjects, WOODRUFF et al. [29] measured no differences in cell size.

DULIN et al. [32] report that smooth muscle isolated from asthmatic tissues exhibits normal sensitivity to constrictor agonists when studied during isometric contraction. The sheer increase in smooth muscle mass was suggested to be responsible for generating more contractile force than that experienced in the nonasthmatic airways. The increased muscle bulk can also reduce the luminal circumference and alter the pattern of mucosal folding, resulting in a reduced number of folds and enhanced airway narrowing.

THE EPITHELIAL-MESENCHYMAL TROPHIC UNIT

In order to help explain some of the structural changes that occur in asthmatic airways, it has been proposed that the epithelium and the underlying fibroblasts communicate through autocrine and paracrine mechanisms, effectively forming the epithelial-mesenchymal trophic unit. The epithelial-mesenchymal trophic unit functions during development and is crucial to the process of branching morphogenesis. It is postulated that reactivation of developmental pathways contributes to the structural alterations of airways in asthma [33]. Thus, it is proposed that, in asthma, epithelial susceptibility to injury and repair of the wounded bronchial epithelium leads to perpetuation of a chronic inflammatory cycle [33]. During exacerbations, activated inflammatory cells interact with the epithelium to produce an array of T-helper cell type-2 cytokines and chemokines. As well as having a protective role, the inflammatory cells can have a detrimental effect by causing further damage to the epithelium through the release of free radicals and proteases.

The epithelium can also communicate with the underlying mesenchyme (fig. 3). TGF-β is released from damaged epithelial cells [34] and can mediate its effects by interacting with TGF-β receptors on fibroblasts. This interaction can promote the transformation of fibroblasts into myofibroblasts. Myofibroblasts are more biosynthetic in nature and can synthesise and release an array of growth factors and cytokines, resulting in remodelling of the airways and continuation of the chronic inflammatory events. This is amplified by release of various factors, such as endothelin-1, a mitogen for smooth muscle, eotaxin, a chemoattractant for eosinophils and vascular endothelial growth factor, an angiogenic factor that promotes the growth of new blood vessels [35, 36].

EGF receptor (EGFR) ligands can also be released from epithelial cells, fibroblasts and smooth muscle cells. EGFR ligands can promote either proliferation or differentiation. It is proposed that ligands of the EGF family are released from the damaged epithelium and cause the proliferation of the underlying mesenchymal cells, as demonstrated in TGF-α transgene mice [38].

REMODELLING IN CHILDHOOD ASTHMA

The chronology of events leading to the onset of disease is currently a subject of much discussion. It is widely accepted that susceptible subjects go on to develop atopic asthma after sensitisation to a particular allergen. The precise initiation of remodelling events in the airways is less certain, although recent studies suggest that remodelling (or pre-modelling) may be an early event in the aetiology of disease linked to the function of the epithelial-mesenchymal trophic unit [39].
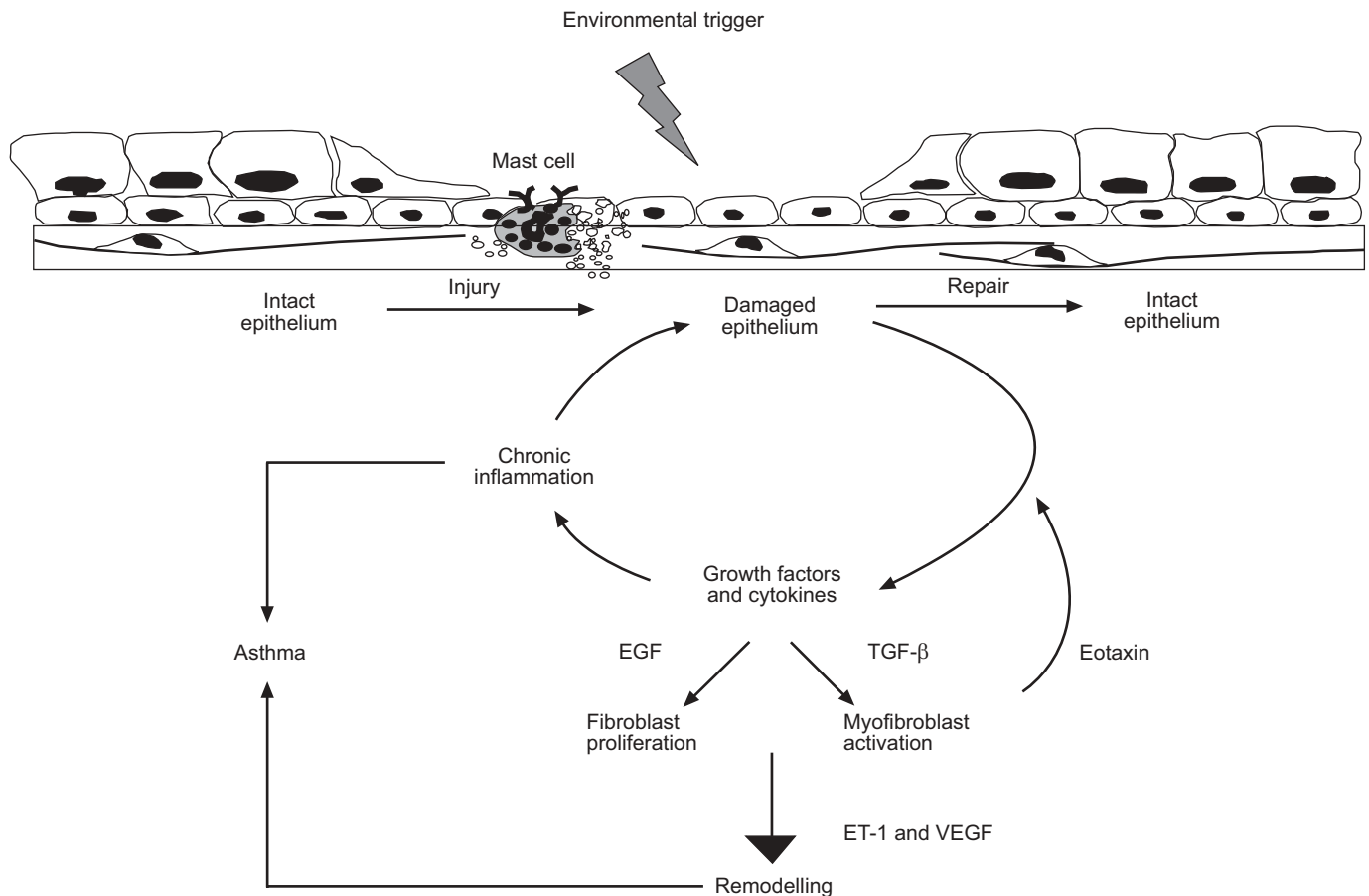
FIGURE 3. The epithelium and the mesenchyme can communicate in asthmatic airways due to the release of soluble growth factors. The epithelium can release pro-inflammatory and profibrogenic growth factors that can mediate remodelling and perpetuate the chronic inflammatory cycle. Activated fibroblasts can in turn release factors that promote inflammation as well as remodelling processes, such as smooth muscle hyperplasia and angiogenesis. It is postulated that the remodelling and inflammation attenuate the normal repair of the damaged epithelium and ultimately lead to the development of asthma. Modified from [37]. EGF: epidermal growth factor; TGF-β: transforming growth factor-β; ET-1: endothelin-1; VEGF: vascular endothelial growth factor.

Indeed, the impact of remodelling may be greater during the childhood years, as children's lungs grow rapidly in the first few years of life.

In addition to the more complex ethical issues surrounding bronchoscopies performed on children, it is often very difficult to differentiate between wheeze induced by viral infections and bronchial asthma in the preschool child. In the studies that have been carried out in paediatric asthma, it appears that a number of phenomena associated with remodelling are present at an early age. For example, COKUGRAS et al. [40] studied biopsy samples from 10 children with moderate asthma and demonstrated thickening and hyalinisation of the sub-basement membrane region in nine patients. Although eosinophils were present in only one sample, 60% had degranulating mast cells in the submucosa. NAJAFI et al. [41] performed BAL on 39 newly diagnosed wheezy children. The study found that children >2 yrs of age had larger amounts of ciliated columnar and goblet cells in the BAL fluid compared with older children, suggesting acute inflammation of the airways early in life. A study comparing the degree of remodelling in asthmatic children prescribed with 1,600 μg·day^{-1} of an inhaled steroid to adult asthmatics

revealed that lamina reticularis thickening is present in children with difficult asthma at a similar level to that observed in biopsies obtained from adult asthmatic subjects [42]. However, there was no association with age, symptom duration, lung function, or concurrent eosinophilic airway inflammation. In a separate study, FEDEROV et al. [39] showed thickening of the lamina reticularis in asthmatic biopsies with increased deposition of collagen III. As in adults, there was an asthma-related increase in epithelial EGFR and this correlated with the thickness of the lamina reticularis, suggesting a link between subepithelial fibrosis and epithelial damage and/or activation [39].

IS REMODELLING POSITIVELY CORRELATED TO DISEASE SEVERITY?

The majority of studies carried out to compare the effect of disease severity suggest that tissue remodelling is more advanced in subjects with severe asthma. HARMANCI et al. [43] demonstrated that bronchial wall thickening was more prominent with increased severity, decreased forced expiratory volume in one second (FEV1) values and the duration of asthma. The submucosa of asthmatic subjects contained more

blood vessels; this also correlated with the severity of the disease [44].

BENAYOUN *et al.* [27] revealed that mucosal eosinophilia, neutrophilia, epithelial damage and subepithelial basement membrane thickness were not related to disease severity. However, a number of observations distinguished patients with severe persistent asthma from patients with milder disease. These included increased numbers of fibroblasts, larger areas of mucous gland and smooth muscle, smooth muscle hypertrophy, increased collagen type III deposition, and higher myosin light-chain kinase expression. CHAKIR *et al.* [45] described an association between disease severity and collagen deposition. In contrast, CHU *et al.* [24] found no identifiable differences in collagen deposition or TGF-β expressing cells in the large airways of mild when compared with severe asthmatics.

REVERSIBILITY OF REMODELLING?
Airway remodelling has been considered to be an irreversible process, although there is evidence to suggest that early use of corticosteroids can delay or even reverse the structural changes [46]. A 5-yr follow-up study comparing subjects treated with corticosteroid early in the course of their disease to a delayed treatment group showed that early use of corticosteroids (budesonide) significantly improved lung function and increased exercise tolerance. Furthermore, the early treatment group used reliever medication less, had fewer exacerbations and, after 5 yrs, 17% were able to stop using budesonide when compared with 3% in the delayed treatment group [47]. Furthermore, in a randomised, double-blind clinical trial of 7,241 patients in 32 countries performed by the START (Steroid Treatment As Regular Therapy) investigators group [48], treatment with budesonide (in addition to their usual asthma medication) in patients who had mild persistent asthma for ≤2 yrs had fewer courses of systematic steroids and more symptom-free days than those on placebo. FEV1 readings, pre- and post-bronchodilator, from patients taking budesonide (400 μg for adults, 200 μg for children aged 5–11 yrs) were significantly increased after 3 yrs. Although the outcomes of the study were beneficial to the patients, it was noted that growth over 3 yrs was suppressed by 1.34 cm in children aged >11 yrs in the treatment group.

A number of studies have directly examined the effect of corticosteroids on remodelling by performing bronchoscopies. A study carried out by SONT *et al.* [49] showed that subjects treated with corticosteroids over a period of 2 yrs experienced a lower rate of mild exacerbations, an improved FEV1 and a reduction in the thickness of the subepithelial layer compared with the control group. In a double-blind study of 28 asthmatic subjects, HOSHINO *et al.* [50] demonstrated that treatment with the inhaled steroid beclomethasone dipropionate significantly reduced the vessel number and vascularity in the airways of steroid treated patients. Further study showed that biopsy samples taken before and after treatment with an inhaled steroid revealed a decrease in inflammatory cells and a reduced amount of epithelial damage after 10 yrs, although bronchial hyperresponsiveness remained the same [51]. The majority of investigations carried out do suggest that early and prolonged use of corticosteroids can provide some improvement in lung function and may minimise some of the remodelling events, although it should be remembered that long-term use of corticosteroids can in itself bring about other adverse effects, such as adrenal suppression and arterial hypertension. Furthermore, the absence of an effect of corticosteroids on bronchial hyperresponsiveness suggests that some of the underlying structural changes and/or disease mechanisms are poorly responsive to the effects of corticosteroids.

INCREASED DEPOSITION OF EXTRACELLULAR MATRIX
The ECM is a complex network of secreted polysaccharides and proteins that creates a microenvironment which has the capacity to influence the behaviour of cells in very distinct, as well as cooperative, ways. Thus, in addition to forming a scaffold on which tissues are organised, ECM molecules, such as proteoglycans, can modify the binding of cytokines to their cell surface receptors, or they can lead to the storage of the soluble factors in the matrix. This is particularly important in the case of TGF-β, which can be sequestered by a number of different binding proteins, including decorin, biglycan, type IV collagen, fibronectin and elastin [52].

In asthma, there is an altered profile of ECM proteins present in the submucosal layer below the lamina reticularis. It is proposed that this is due not only to the increased number of synthetic cells present, but also due to differences in the degradation of the existing proteins. The ''true'' basement membrane in asthma contains less collagen IV than normal, although the submucosa of asthmatic airways contains more collagen I, III and V [28, 31, 45, 53]. Additionally, the orientation and fibre thickness of collagen I appears to be altered in asthmatics [23, 54]. DUBE *et al.* [55] suggested that differences in collagen expression were due to post-translational modifications, as baseline procollagen production was not found to differ between nonasthmatic and asthmatic fibroblasts.

There are a number of reports demonstrating increased levels of ECM proteins in the submucosa of asthmatics. For example, tenascin [56], lumican, biglycan and versican [57] are all present at elevated levels in the submucosa of asthmatic subjects. WESTERGREN-THORSSON [58] reported that subjects with the most hyperresponsive airways produced up to four-fold more total proteoglycan than subjects with less responsive airways. Laminin β_2 and α_1 staining [59] was also higher in biopsies from asthmatic subjects. Elastin fibres are needed to maintain bronchial patency and contribute to the elastic recoil of the lungs. BOUSQUET *et al.* [60] reported that biopsy samples from asthmatics showed altered elastin staining. In addition, VIGNOLA *et al.* [61] showed that the enzyme responsible for elastin degradation, elastase, was increased in sputum samples from asthmatics compared with healthy controls. Apart from elastin degradation, it is proposed that neutrophil elastase can also act as a smooth muscle mitogen [62] and promotes mucin gene expression by cleavage of EGF receptor ligands [63].

Matrix metalloproteinases (MMPs) were first recognised for their ability to degrade many ECM proteins, including collagens, fibronectin, laminin, proteoglycans and elastin. However, it is now clear that MMP activity also causes the release of cryptic information from the ECM by cleaving large insoluble ECM components to liberate bioactive fragments and

growth factors that can have marked effects on cell behaviour. Thus, in combination with proteases, the ECM is a dynamic environment, where small changes in levels of MMPs and other enzymes may alter the equilibrium of a range of bioactive proteins in the submucosal layer.

An altered expression profile of ECM-degrading enzymes has been reported in asthma. For example, asthmatic bronchial fibroblasts produce less MMP-3 (stromolysin) than normal fibroblasts [64]. LALIBERTÉ et al. [65] demonstrated that baseline MMP-2 (gelatinase A) secretion was lower in asthmatic fibroblasts when compared with those from normal airways. The group also showed that asthmatic fibroblasts displayed a decreased capacity to degrade collagen by phagocytosis.

MMP-9 (gelatinase B), a metalloproteinase responsible for cleaving collagen IV and degrading denatured collagen, is reportedly expressed at higher levels in eosinophils isolated from asthmatics compared with nonasthmatics [66]. A significant increase in both the level of circulating MMP-9 [67] and its activity in BAL fluid [68] was also seen in patients after exacerbation compared with patients with asthma. Additionally, MMP-9 staining in the subepithelial basement membrane region was reportedly higher in severe asthmatic biopsies than in control subjects [69]. These findings may account for the decreased expression of collagen IV in the basement membrane of asthmatic subjects.

Tissue-specific inhibitors of metalloproteinases (TIMPs) can inhibit activation of the MMPs by binding an MMP in a 1:1 stoichiometric fashion. LALIBERTE et al. [65] hypothesised that a compromised balance between TIMP-1 and MMP-1 levels may be responsible for the increase in collagen deposition observed in asthmatic airways. Indeed, TIMP-1 mRNA and protein levels were elevated in asthmatic alveolar macrophages isolated from BAL fluid, a finding demonstrated by OHNO et al. [66]. Additionally, CATALDO et al. [70] reported that MMP-1 and TIMP-1 levels were increased at the mRNA level in induced sputum from asthmatic subjects compared with nonasthmatics. Recent studies using computed tomography have linked induced sputum MMP-9/TIMP-1 ratios to thickening of the airway wall in asthmatics [71, 72].

The A Disintegrin And Metalloprotease (ADAM) family of proteins belongs to the metzincin subgroup of zinc-dependent proteases; it encompasses a large number of proteins, 40 to date [73], although only 23 are expressed in humans. ADAM9, -10, -15 and -17 are ubiquitously expressed in human tissue, whilst the majority of the ADAMs display restricted expression in the testis and are involved in spermatogenesis and egg fertilisation.

Approximately half of the ADAM family contains a zinc-binding consensus motif in their metalloprotease domain; this is essential for enzymatic activity. In regard to the processing of EGFR ligands, ADAM9, -10, -12, -17 and -19 have all been reported to play a role in pro-growth factor cleavage. Proteolytic ADAM activity can be inhibited in vivo by TIMPs. For example, TIMP-3 can inhibit ADAM17 [74] and ADAM12 [75], whilst ADAM10 activity can be inhibited by both TIMP-1 and TIMP-3 [74].

Using positional cloning, the 33rd member of the ADAM family has been linked to asthma and bronchial hyperresponsiveness [76]. ADAM33 contains a zinc-binding consensus sequence in its metalloprotease domain [77], which has been shown to be catalytically active in an α_2-macroglobulin complex formation assay [78]. Expression of the purified metalloprotease domain in Drosophila S2 cells also showed that the domain has some functional activity, although ZOU et al. [79] reported cleavage of only four synthetic peptides. While the metalloprotease activity of ADAM33 represents a potential target for therapeutic intervention, the only single nucleotide polymorphism that has been identified that could cause a coding change within the metalloprotease domain has a weak association with bronchial hyperresponsiveness [76]. Furthermore, POWELL et al. [80] have reported that the metalloprotease domain is spliced out over 95% of transcripts in bronchial fibroblasts, suggesting that other domains of ADAM33 play important functional roles. Similarly, in bronchial biopsies, transcripts encoding the metalloprotease domain are of low abundance and there is no clear disease-related difference in ADAM33 expression [81]. These data suggest that simple up- or downregulation of ADAM33 is unlikely to explain its contribution to the development of asthma. A more likely explanation is that polymorphic variation in ADAM33 may have functional effects, possibly by affecting its regulation within the cell. Ongoing work in the field should hopefully reveal its role in asthma pathogenesis. In this context, it is noteworthy that ADAM33 is selectively expressed in mesenchymal cells [76], strongly implicating it in airway remodelling. Consistent with this proposal, a rare allele of the S_2 polymorphism of ADAM33 is significantly associated with excessive decline in FEV1 in asthmatic subjects [82] and chronic obstructive pulmonary disease patients [83]. Furthermore, in a prospective birth cohort study, polymorphisms in ADAM33 predict impaired early-life lung function [84].

TGF-β SIGNALLING AND ITS ROLE IN ASTHMA

In asthma, it has been hypothesised that an enhanced expression of TGF-β in the airways may have far reaching effects in the pathophysiology of the disease. TGF-β released from inflammatory cells, the epithelium and (myo)fibroblasts can promote remodelling by inducing expression of a wide range of ECM components. Although TGF-β is anti-inflammatory (for a recent review see [85]) and can promote cellular migration [86], an excess of TGF-β can inhibit subsequent epithelial cell proliferation that is required during early wound healing. The net result of excessive TGF-β in the airways in asthma may explain many of the morphological changes that occur in airway remodelling (fig. 4).

More than 30 members of the TGF-β superfamily have been identified [87]. The superfamily is divided into four main families: 1) TGF-β family; 2) bone morphogenetic (BMP) family; 3) inhibin/activin family; and 4) the Müllerian-inhibiting substance family. There are three TGF-β isoforms identified in mammals, namely TGF-β_1, -β_2 and -β_3. It should be noted that, for much of the early work on TGF-β, it would not have been possible to distinguish which isoform of TGF-β was responsible for the observed effect, hence these studies refer simply to TGF-β. Although TGF-β was first isolated and characterised from human platelets [88], it is produced by most
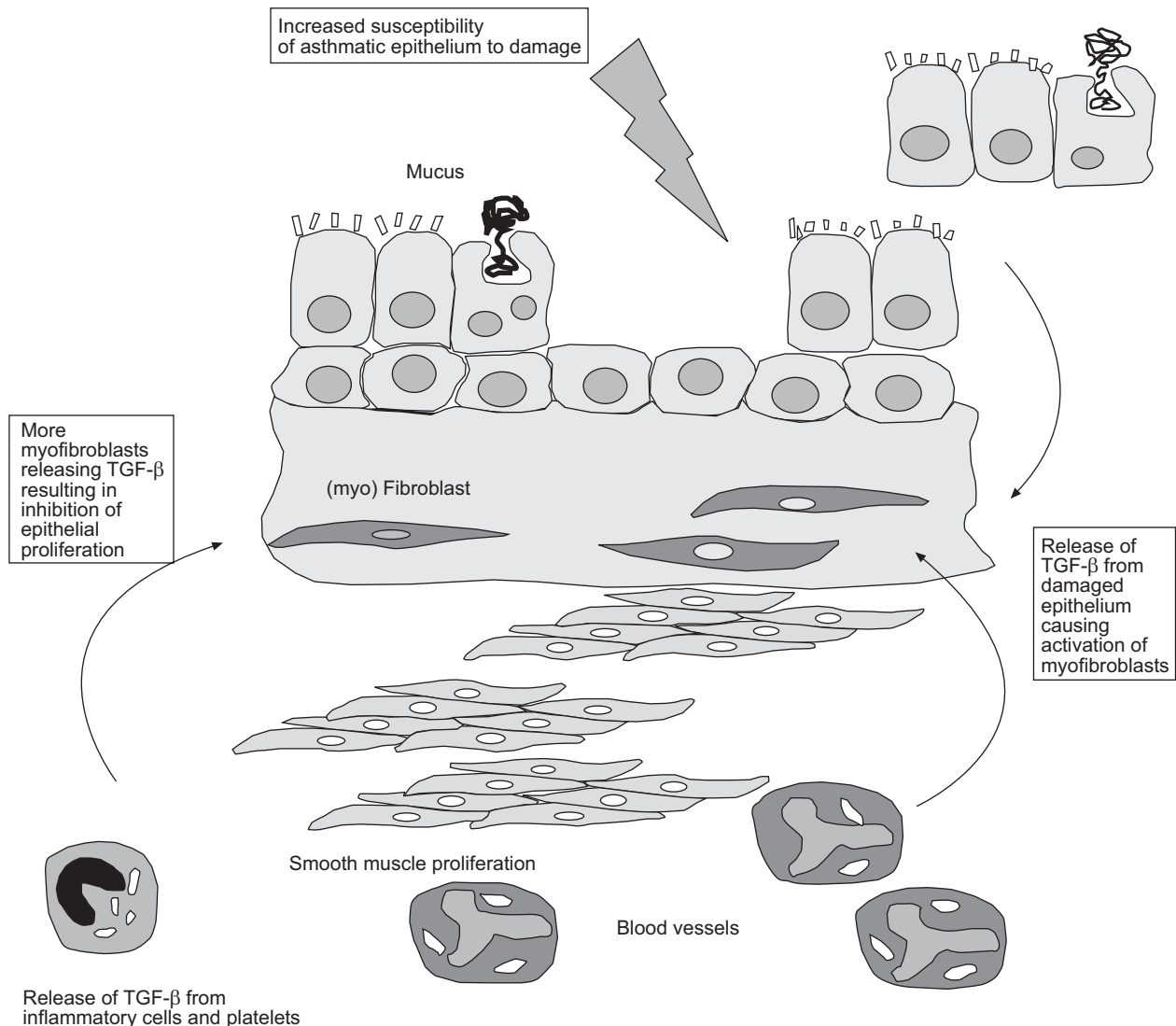
Increased susceptibility
of asthmatic epithelium to damage

Mucus

More
myofibroblasts
releasing TGF-β
resulting in
inhibition of
epithelial
proliferation

(myo) Fibroblast

Release of
TGF-β from
damaged
epithelium
causing
activation of
myofibroblasts

Smooth muscle proliferation

Blood vessels

Release of TGF-β from
inflammatory cells and platelets

FIGURE 4. Studies have revealed that there is an increased expression and release of transforming growth factor (TGF)-β in asthmatic airways. TGF-β is released from the epithelium, fibroblasts, smooth muscle cells and inflammatory cells, and is proposed to have a major role in the fibrosis associated with airway remodelling.

cell types, including fibroblasts, smooth muscle, epithelial and inflammatory cells.

TGF-β is encoded as a biologically inactive large precursor peptide termed pre-pro-TGF-β. Endopeptidases catalyse the cleavage of the precursor molecule, yielding the mature 25 kDa protein. The mature TGF-β dimerises and associates noncovalently with latency associated peptide, the precursor cleavage product, to form small latent TGF-β. TGF-β is secreted from the cell as either small latent TGF-β or, more often, as large latent TGF-β (small latent TGF-β bound to latent TGF-β binding protein). TGF-β can then be rapidly sequestered by a number of different binding proteins, including decorin, biglycan, type IV collagen, fibronectin and elastin [52]. The binding protein also has the ability to bind to sites in the ECM [89].

The mechanism by which latent TGF-β is activated *in vivo* remains elusive, although a number of molecules have been implicated *in vitro*. TGF-β can be activated by thrombospondin, which binds to latency-associated peptide and induces a

conformational change [90, 91]. Plasmin, low-dose radiation, low pH, reactive oxygen species [92] and nitric oxide nitrosylation [93] have all been suggested to play a role in TGF-β activation.

TRANSFORMING GROWTH FACTOR-β RECEPTORS

There are six types of TGF-β receptor (RI–VI). Most of the information in the literature concerns RI, RII and RIII. TGF-β RIV [94] and RVI [95] bind TGF-β, but are not known to propagate downstream signalling. TGF-β RV is a serine/threonine kinase like RI and RII, and further investigation may reveal its importance in controlling downstream signalling events [96]. Betaglycan and endoglin comprise the two types of TGF-β RIII. Endoglin is expressed only on endothelial and myeloid cells. Betaglycan is expressed on most cell types and has a variable molecular weight due to the heparan sulphate and glycosaminoglycan chains attached to it. The receptor is responsible for binding TGF-β and presenting the ligand to the type II receptor. This enables the local concentration of ligand

to be increased and may also have a role in stabilising TGF-β in the optimal conformation for binding to the type II receptors. TGF-β$_2$ has a weak affinity for the type II receptor alone and can only signal *via* interaction with betaglycan [97] where the short cytoplasmic tail of the receptor acts to enhance TGF-β$_2$ signalling. In contrast, TGF-β$_1$ can signal directly through TGF-β RII.

The type II receptor is a 70 kDa constitutively active serine/threonine kinase. Upon ligand binding, the receptor recruits and phosphorylates the type I receptor in the GS domain and juxtamembrane regions. Whilst the type II receptor can bind TGF-β$_1$ independently of betaglycan, recruitment of the type I receptor is still required for downstream signalling. The type I receptor is a 55 kDa protein, responsible for downstream signalling *via* a cascade of Smad proteins. There are seven type I receptors. ALK-1 (mainly associated with endothelial cells) and ALK-5 are type I receptors for TGF-β [98], ALK-4 is the receptor for activin and ALK-2, -3 and -6 are receptors for the BMPs [99, 100]. TGF-β receptor-associated protein 1 binds to the inactive type I receptor but is released when the receptor is activated by ligand binding [101]. The activated receptor complex is a heteromeric complex consisting of two type II and two type I receptors (fig. 5).

DOWNSTREAM SIGNALLING

TGF-β mediates intracellular signalling *via* the ''Smad'' proteins. Mad was the first member of the Smad family to be identified in Drosophila. Its discovery led to the identification of Mad homologues in *Caenorhabditis elegans*; these were named Sma-2, -3 and -4 because of their small size. Smad (Smad/Mad-related) proteins were later identified as TGF-β signalling molecules in vertebrates (fig. 6). Receptor Smads, *i.e.* Smad-2 and -3, reside in the cytoplasm in their inactivated state. Smad-2 exists as a monomer due to a structural element in the MH1 domain that inhibits protein–protein interactions in the basal state. Smad-3 has multiple oligomeric states and Smad-4 most likely exists as a trimer [102]. The Smads are serine phosphorylated by the type I receptor after ligand-dependent activation. The interaction of the Smad anchor for activation protein [103] with the receptor allows interaction with Smad-2 and Smad-3. Smad-2 signalling is dependent
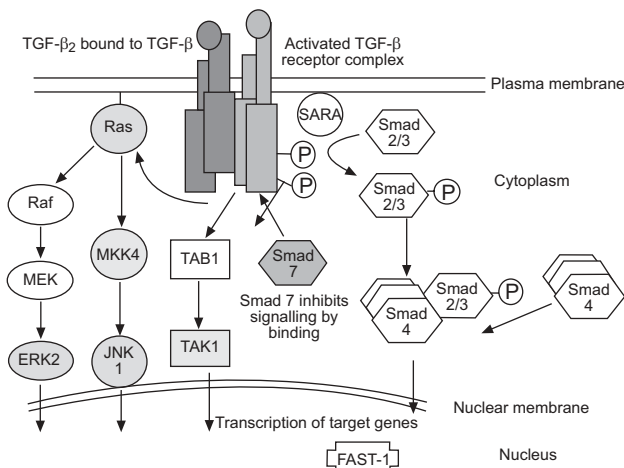

FIGURE 5. Scheme showing steps involved in forming the activated transforming growth factor (TGF)-β receptor complex. Betaglycan (TGF-β RIII) binds TGF-β$_2$, enhancing the affinity of the growth factor for TGF- βRII. Once bound, TGF-β RII recruits and phosphorylates TGF-β RI, thus promoting downstream signalling. TRAP-1: tumour necrosis factor-associated protein 1.

FIGURE 6. Transforming growth factor (TGF)-β can signal *via* Smads or a mitogen-activated protein MAP kinase-like pathway, resulting in transcription of target genes. Nuclear transcription of target genes occurs either by Smads binding directly to Smad-binding elements of target genes or by functional co-operation with transcription factors, such as fork-head activin signal transducer 1 (FAST-1). Phosphorylation changes are only indicated on the Smad pathway for ease of interpretation. MEK: MAP kinase kinase; ERK2: extracellular-regulated protein kinase 2; MKK4: MAP kinase kinase 4; JNK1: Jun N-terminal kinase 1; TAK1: TGF-β-activated kinase 1; TAB1: TAK1-binding protein; SARA: Smad anchor for activation; Smad: TGF-β signalling molecules.

upon the Smad anchor for activation but the Smad 3/Smad anchor for activation interaction is not required for downstream signalling [104]. The phosphorylated receptor Smads bind Smad-4 (a co-Smad), and the complex translocates to the nucleus. There is evidence to suggest that TGF-β receptor-associated protein 1 interacts with Smad-4 (fig. 6) and may act as a chaperone protein [105].

The Smads can bind directly to the Smad-binding element of various genes, such as junB, c-jun and immunoglobulin (Ig) A [106, 107]; they can also mediate gene transcription via functional co-operation with other transcription factors, such as FAST-1 [108]. TGF-β-activated Smad-2 and -3 can also bind to co-repressors, *e.g.* cAMP response element-binding (CREB)-binding protein and p300, which can in turn interact with transcription factors to modify gene transcription [109]. HOCEVAR *et al.* [110] described Smad-4 independent activation of TGF-β with resultant induction of fibronectin synthesis. The mechanism involves TGF-β activation of mitogen-activating protein kinase (MAPK) kinase 4, which activates Jun N-terminal kinase 1; this in turn phosphorylates c-jun, promoting the formation of the c-jun-ATF 2 heterodimer. These heterodimers can bind to cAMP-response elements in the fibronectin promoter and thus promote fibronectin synthesis.

There are other possible pathways by which TGF-β receptors can transmit signals. Protein farnesyltransferase-α [111] and FK506 binding protein-12 (FKBP-12) [112] have both been shown to bind to the TGF-β receptors, although FKBP-12 might act as a negative regulator of TGF-β receptor endocytosis [113]. It is also possible that a MAPK-like pathway is involved in the propagation of signals from the membrane to the nucleus, *via* TAB1 and TAK1 (MAPK kinases) [114, 115].
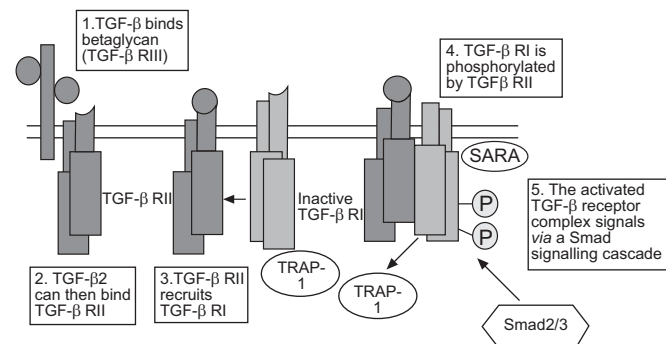
REGULATION OF TGF-β SIGNALLING

After ligand binding, a process of endocytosis typically downregulates growth factor receptors. In fibroblasts, hetero-meric TGF-β receptors are internalised whereas homomeric receptors are recycled back to the membrane [116, 117]. The diversity of TGF-β signalling is controlled in part by the residues phosphorylated on the receptor in response to ligand stimulation. SAITOH *et al.* [118] observed that serine 172 and threonine 176 of TGF-β RI are dispensable for ECM protein production but essential to epithelial growth inhibition mediated by TGF-β. LUO and LODISH [119] demonstrated that autophosphorylation of serine 213 on TGF-β RII is essential for activation of the TGF-β RII kinase, activation of TGF-β RI and TGF-β-induced growth inhibition.

There are two mammalian antagonistic Smads, Smad-6 and Smad-7, which can inhibit BMP signalling; TGF-β signalling can be inhibited by Smad-7. Smad-6 inhibits BMP/Smad-1 signalling by competing with Smad-4, forming a Smad-1/Smad-6 inactive complex [120]. Smad-7 acts as an antagonist of TGF-β RI [121] and is induced by TGF-β. Conversely, EGF can induce the mRNA levels of Smad-7, providing evidence of cross-talk between the pathways [122]. Serine-threonine kinase receptor-associated protein (STRAP) stabilises the association between the type I receptor and Smad-7, preventing the receptors association with Smad-2 or -3 and thus preventing signalling [123].

Another form of negative regulation of Smads is by the interaction with MAPK. The linker region between the MH1 and MH2 domain of the receptor Smads can be phosphory-lated, which prevents the Smad-2/Smad-4 complex from entering the nucleus and eliciting gene transcription. There are also a number of inhibitory transcription factors, such as Evi-1 [124], c-ski [125] and SnoN [126], which can prevent activated Smads from binding to Smad-binding elements on DNA. Transforming growth- interacting factor is an example of a co-repressor that can compete with p300 for Smad-2 association, preventing TGF-β-mediated signalling [127].

TGF-β AND THE EPITHELIUM

TGF-β1 regulates the migratory phase of epithelial repair. BOLAND *et al.* [128] reported that 1–10 ng·mL^{-1} TGF-β$_1$ inhibited the proliferation of primary epithelial tracheal cells but enhanced cell migration. TGF-β also induced cell spreading, reduced the number of cell–cell contacts and increased cell-substratum anchorage, thus favouring a migratory phenotype. Furthermore, HOWAT *et al.* [129] showed that TGF-β$_1$, but not TGF-β$_2$, could progressively increase the migration of damaged bronchial epithelial monolayers at concentrations down to 250 pg·mL^{-1}.

TGF-β$_1$ can inhibit cell proliferation of epithelial cells by a number of different mechanisms involving the downregulation of c-myc [130], and the upregulation of the cyclin-dependent kinase inhibitors, p15INK4B, p21waf1 and p27kip1 [131]. p21waf1 is over-expressed in asthmatic epithelium, which is strongly suggestive of cell stress and growth arrest [16].

TGF-β, FIBROBLAST AND MYOFIBROBLASTS

Fibroblasts comprise the major structural cell in the mesench-yme in its resting state. Quiescent fibroblasts are involved in

the baseline secretion of proteins, such as collagen, contribut-ing to the normal turnover of ECM in the airways. Upon insult to the tissue, fibroblasts become activated and capable of migrating to the site of injury. In order to carry out this function, the fibroblasts secrete collagen and fibronectin fibrils to enable orientation of the cell, thus creating both a migratory meshwork as well as lines of stress and mechanical tension.

Myofibroblasts are generally viewed as morphological inter-mediates of fibroblasts and smooth muscle cells. They are the predominant cell type found in wound granulation tissue, but they play a major role in fibroproliferative diseases when unregulated. Myofibroblasts are more biosynthetic in nature than the fibroblasts and are capable, due to activation of myosin light chain kinase and Rho kinase, of both rapid contraction and a more sustained contraction, respectively. *In vivo*, the myofibroblast is a transient cell type and is normally activated upon connective tissue injury. A combination of TGF-β$_1$ and extra domain A fibronectin [132–134] induces the transformation of proto-myofibroblasts into myofibroblasts (fig. 7). TGF-β induces α-smooth muscle actin (αSMA) and smooth muscle protein 22-α (SM22α) *via* interactions with the TGF-β controlling element and two serum response element regions in the αSMA promoter of fibroblasts [135]. THANNICKAL *et al.* [136] reported that expression of a stable myofibroblast phenotype is dependent upon both TGF-β- and adhesion-dependent signals. Inhibition of TGF-β$_1$-induced phosphoryla-tion of focal adhesion kinase on tyrosine 397, a cell adhesion-dependent event, resulted in inhibition of αSMA expression. TGF-β$_1$ was found to increase integrins α$_4$, α$_5$ and β$_1$ as well as fibronectin in human lung fibroblasts. The authors proposed that TGF-β-induced fibroblast differentiation occurs *via* a Smad-mediated, adhesion-independent signalling path-way followed by a delayed adhesion-dependent pathway involving focal adhesions that are essential for maintenance of the phenotype. Studies of wound healing suggest that myofibroblast disappearance occurs *via* apoptosis [137]. TGF-β promotes the transformation from fibroblast to myofibroblast
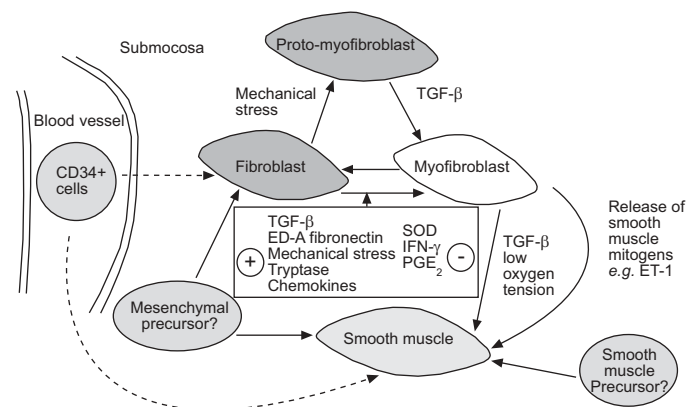
FIGURE 7. Although the established dogma describes fibroblasts transform-ing into myofibroblasts under the influence of transforming growth factor (TGF)-β, there are reports of other precursor cells and a number of other inducers of the myofibroblast phenotype. There is also some evidence to suggest that myofibro-blasts may act as smooth muscle precursors. ED-A: extra domain A; SOD: superoxide dismutase; IFN-γ: interferon-γ; PGE$_2$: prostaglandin E2; ET-1: endothelin-1.

as well as enhancing the survival of myofibroblasts by inhibiting interleukin (IL)-1β-induced apoptosis [138].

The cytokines IL-4 and -13 have been reported to induce the myofibroblastic phenotype in a time- and dose-dependent manner [139, 140]. However, RICHTER et al. [35] demonstrated that IL-4 and -13 were relatively ineffective in promoting myofibroblast transformation directly, although transformation was effective through IL-4 or -13-mediated TGF-$β_2$ release from epithelial cells [35]. In vivo, the myofibroblast is only present transiently. VOZENIN-BROTONS et al. [141] reported that addition of superoxide dismutase to porcine dermal myofibroblasts reduced the levels of αSMA and collagen production, an event not associated with an increase in cell death. Interferon-γ (IFN-γ) has been implicated in preventing the generation of myofibroblasts and can also moderately inhibit the production of αSMA in TGF-β-induced myofibroblasts [142, 143]. Prostaglandin E$_2$, signalling via E prostanoid receptor 2, has also been reported to prevent the TGF-β-induced fibroblast undergoing myofibroblast transition in primary foetal and adult lung fibroblasts [144].

The accepted view is that myofibroblasts are derived from fibroblasts. A recent study, however, has identified another potential source of myofibroblasts. SCHMIDT et al. [145] suggest that bronchial myofibroblasts can originate from fibrocytes, blood-borne cells that can home to sites of tissue damage (fig. 7). These cells are CD34+, express both collagen I and αSMA, and localise to areas of collagen deposition below the epithelium. By tracking labelled circulating fibrocytes in a mouse model of allergic asthma, the authors showed that fibrocytes are recruited into the bronchial tissue following allergen exposure and differentiate into myofibroblasts.

The potential involvement of fibrocytes in airway remodelling in asthma has been further highlighted in a study by LARSEN et al. [146] who found activated and mobile fibroblasts in BAL fluid of subjects with mild asthma.

In addition to induction of myofibroblast differentiation and deposition of ECM proteins, TGF-β also has a role in fibroblast proliferation. TGF-β has been reported to have different effects on the proliferation of fibroblasts DUBE et al. [55] reported that TGF-$β_1$ had no significant effect on bronchial fibroblast proliferation. KAY et al. [147] reported that corneal stromal fibroblasts produce fibroblast growth factor-2 in response to TGF-β, and this is suggested to be a direct stimulator for TGF-β-mediated cell proliferation. It was further reported by THANNICKAL et al. [148] that TGF-$β_1$ (2 ng·mL^{-1}) upregulated fibroblast growth factor R-1 and -2 in a time-dependent manner, thus contributing to the proliferative response. MCANULTY et al. [149] also demonstrated that TGF-β (5 pg·mL^{-1}) could stimulate fibroblast proliferation.

TGF-β-treated fibroblasts also have indirect effects on inflammation by releasing a number of chemoattractants. For example, release of eotaxin in response to TGF-β and IL-13 can act as a chemoattractant for eosinophils [150]. TGF-β-induced CCL-2 (monocyte chemoattractant protein-1) acts as a chemoattractant for monocytes [151]. TGF-β is also reported to induce fibroblast-like synoviocytes from rheumatoid arthritis and osteoarthritis patients to induce CXCL-8 (IL-8), a neutrophil attractant, and CCL-3 (macrophage inflammatory

protein 1-α) mRNA which can recruit monocytes, macrophages and T-cells [152].

RELATIONSHIP BETWEEN TGF-β AND AIRWAY SMOOTH MUSCLE

Smooth muscle is primarily under the control of the autonomic nervous system and can develop an isometric force per cross-sectional area equal to skeletal muscle, although the speed of contraction is much slower than skeletal muscle. Airway smooth muscle plays a major role in the pathophysiology of a number of airway diseases, including asthma. As described previously, the smooth muscle layer in asthma is significantly increased in size due to hyperplasia and possibly hypertrophy. In addition to its role as regulator of bronchomotor tone, smooth muscle can also secrete cytokines and growth factors, thus contributing to the inflammation and remodelling of the airways.

It has been proposed that TGF-$β_1$ and TGF-$β_2$ are responsible for the conversion of myofibroblasts into smooth muscle cells (fig. 7). Microarray analysis of foetal lung fibroblasts have shown that TGF-β can induce expression of a number of smooth muscle-specific genes, including smooth muscle myosin heavy chain, basic calponin and smoothelin [153]. However, expression of some of these genes appears to be further regulated at the translational level [154]. Furthermore, there is evidence that rabbit bladder myofibroblasts are able to transform into smooth muscle cells after treatment with TGF-β for 21 days [155]. The smooth muscle transition has also been reported to occur independently of TGF-β. For example, a study by JONES and JACOBSON [156] identified interstitial rat lung fibroblasts as the source of perivascular smooth muscle cells in response to low oxygen levels.

It is possible that the role of TGF-β is to induce the expression of smooth muscle mitogens, such as endothelin-1 [35], and thus play a more indirect role in smooth muscle differentiation. Alternatively, YEH et al. [157] demonstrated that adult peripheral blood CD34+ cells can transdifferentiate into smooth muscle cells in vivo, and that this is augmented significantly by local tissue injury.

ROLE OF TGF-β AND EXTRACELLULAR MATRIX FORMATION

Activated smooth muscle cells and fibroblasts can release ECM proteins in response to TGF-β. For example, TGF-β induces α$_1$ collagen I mRNA and α$_2$ collagen protein in human lung fibroblasts [158]. EICKELBERG et al. [159] showed that the percentage of collagen deposited in the ECM was higher in TGF-$β_1$-treated human lung fibroblasts compared with controls. TGF-$β_1$ has also been shown to stabilise tropoelastin mRNA in human foetal lung fibroblasts [160].

TGF-β also has indirect effects through the upregulation of inhibitors of ECM proteases, such as plasminogen activator inhibitor-1 (PAI-1) [161], TIMP-1 [162] and TIMP-3, [163], and the downregulation of degrading ECM proteases, such as interstitial collagenase [164].

INVOLVEMENT OF TGF-β IN ASTHMA

Increased TGF-β immunoreactivity is found in mucosal bronchial biopsies from asthmatic subjects compared with

controls [165, 166]. Indeed, a significant correlation has been found between the number of epithelial or submucosal cells expressing TGF-β in asthma and the thickness of the basement membrane and fibroblast number. Elevated phosphorylated Smad-2 levels have been demonstrated in bronchial biopsies obtained from asthmatic subjects [167], indicating that TGF-β signalling downstream from the receptor is also enhanced in asthma.

CHU et al. [168] reported that unstimulated bronchial epithelial cells express five times more TGF-β_2 than TGF-β_1. Although no differences in TGF-β_1 expression were observed between normal and asthmatic bronchial epithelial cells, there were significantly higher amounts of TGF-β_2 protein in asthmatic compared with normal epithelial cells. TGF-β_2 was shown to induce MUC5AC mRNA and protein *in vitro*, although interestingly TGF-β_1 did not induce mucin expression.

TGF-β_1 is released in higher amounts in basal and allergen-challenged sites in the BAL fluid of asthmatic patients [169] than in controls [170]. Although TGF-β_1 levels are raised in the plasma of nonatopic asthmatics, this is not seen in atopic asthma [171]. TGF-β_1 levels are also increased in eosinophils from severe asthmatics at the mRNA level [172, 173]. Recently, BALZAR et al. [174] reported that there were more TGF-β_2 positive cells in endobronchial tissue biopsies from eosinophilic severe asthmatics. Furthermore, the tissue eosinophils from eosinophilic severe asthmatics constantly co-localised with the TGF-β_2-positive cells. Alveolar macrophages from asthmatic subjects also release greater amounts of TGF-β compared with control subjects [165].

TGF-β has been implicated as a key mediator in asthma, responsible for a number of remodelling events. Four polymorphisms of TGF-β have been identified. A single polymorphism (C/T at position -509) in the TGF-β_1 promoter has been linked to elevated TGF-β_1 plasma levels [175] and IgE levels [176]. A later study by SILVERMAN et al. [177] has revealed that the T allele of C-509T is associated with the diagnosis of asthma and may increase transcription by altering TGF-β promoter-reporter activity by interacting with the transcription factor Yin Yang 1. PULLEYN et al. [178] demonstrated that there is a greater relative frequency of homozygosity for this allele in severe asthmatics compared with a control group. Conversely, BUCKOVA [179] showed that the polymorphism was not linked to elevated IgE levels in the Czech population.

A mouse model has been described where BALB/C mice were intratracheally instilled with either TGF-β_1 or buffered saline [180]. An increase in collagen I and III mRNA was seen in the airways after 1 week and an increase in total collagen was detected after a month following TGF-β treatment. There was also a detectable increase in airway hyperreactivity in the growth factor-treated mice, thus highlighting the importance of TGF-β in the pathogenesis of remodelling.

TGF-β AS A THERAPEUTIC TARGET
Due to the physical nature of TGF-β, mature growth factor is bound in close proximity to the cell, attached to binding proteins within the ECM. As a result, increased production of TGF-β will affect only those cells in the nearby vicinity.

Experiments on adult skin revealed that TGF-β mRNA is induced in response to wounding, whereas no upregulation was detected in human foetal skin under similar conditions. This explains why foetal wounds heal without the deposition of scar tissue. SHAH et al. [181] demonstrated that treating dermal wounds in adult rats with a neutralising TGF-β antibody prevented the formation of scar tissue. In a later study, SHAH et al. [182] showed that administration of anti-TGF-β_1 or anti-TGF-β_2 antibodies to wounds could increase wound-healing time and reduce scarring, as can addition of TGF-β3 directly to wounds. Anti-TGF-β_2 antibodies have also been utilised in the field of conjunctival scarring and significantly improved glaucoma filtration surgery outcome in animal models [183].

A number of small molecule inhibitors of the type I TGF-β receptor have been identified. For example, SB-431542 inhibits TGF-β_1-induced Smad-3 translocation to the nucleus, as well as inhibiting TGF-β_1-induced fibronectin mRNA and collagen Iα_1 induction in renal epithelial carcinoma A498 cells [184]. More recently another type I TGF-β receptor, SB-505124, has been shown to display three to five times the potency of SB-431542 [185] against ALK5, as well as concentration-dependently inhibiting the activity of ALK4 and ALK7.

A novel small compound, SMP-534, has been shown to reduce ECM production induced by TGF-β in normal rat kidney fibroblasts [186]. SMP-534 selectively inhibited TGF-β-induced p38 MAPK activation, but did not inhibit EGF-induced extracellular regulated protein kinase-2 (ERK) activation. Encouragingly, in a rat anti-Thy-1 nephritis model, oral administration of SMP-534 dose-dependently lowered hydroxyproline levels in the cortex. SMP-534 or a similar drug may have a therapeutic benefit in the asthmatic lung, acting to reduce TGF-β-mediated fibrosis associated with airway remodelling without suppressing EGF-mediated restitution of the injured epithelium.

Inhibition of TGF-β signalling may be a beneficial target in asthma. In theory it would have far reaching effects in reducing remodelling by lifting the inhibition on epithelial proliferation, preventing myofibroblast transformation, repressing the proliferative effect on myofibroblasts and inhibiting the excessive production of ECM components. A potentially negative effect would be the alleviation of immunosuppression and thus the possible accentuation of inflammation. Epithelial cells release TGF-β in order to initiate cellular migration in the process of epithelial repair. Therefore, an inhibition of this process may be detrimental to airway remodelling.

MODULATION OF TGF-β AND DOWNSTREAM EFFECTS
NOGAMI et al. [187] reported that TGF-β_1 decreased the number of β-adrenoceptors on human tracheal smooth muscle cells. TGF-β treatment partially suppressed the β_2-agonist stimulated increase in cAMP, an observation that may explain the defective relaxation to β_2-agonists shown by some asthmatics. ISHIKAWA et al. [188] showed that IFN-γ could suppress this TGF-β response. Interestingly, mucosal adenoviral IFN-γ gene transfer can effectively attenuate established allergen-induced airway inflammation and airway hyperresponsiveness in ovalbumin-sensitised mice [189], although human trials have not been so successful [190].

Glucocorticosteroids are the mainstay therapy for the majority of mild to severe, persistent asthmatic subjects. Glucocorticoids exert an anti-inflammatory effect, but can also have an anti-fibrotic action (for a review see SHUKLA et al. [191]) by decreasing collagen synthesis. Dexamethasone, for example, has been shown to block TGF-β-induced collagen synthesis in rats [192]. In a murine model of prolonged allergen challenge [193], budesonide was shown to regulate TGF-β signalling by reducing phospho-Smad-2 expression and increasing Smad-7 in lung tissue.

In human samples, WEN et al. [194] reported a significant inhibition of TGF-β_1 and TGF-β_2 production and a reduced expression of autoinduced TGF-β_1 and TGF-β_2 mRNA in response to glucocorticoid treatment in human foetal lung fibroblasts. Furthermore, flunisolide can decrease TGF-β and fibronectin release from mild-to-moderate asthmatic sputum cells cultured for 24 h [195].

Connective tissue growth factor (CTGF) is a potential target to combat the remodelling seen in asthma. CTGF is considered to be an immediate early gene for TGF-β [196]. CTGF antisense constructs and neutralising antibodies have been reported to block the effect of TGF-β on fibroblast collagen production and proliferation [197–199]. CTGF is also profibrogenic per se, for example, it can stimulate collagen I, fibronectin and α_5 integrin expression in NRK cells [200], as well as inducing lysyl oxidase and collagen in human gingival cells [201]. Recently, it has been reported that the N-terminal domain of CTGF mediates myofibroblast differentiation and collagen synthesis, while the C-terminal domain of CTGF regulates fibroblast proliferation [202].

In 2003, BURGESS et al. [203] reported that the fold induction of CTGF in response to TGF-β was greatest in the airway smooth muscle cells isolated from asthmatic subjects, and that the induction occurred earlier, in comparison to nonasthmatic airway cells. A study by KUCICH et al. [204] suggested the possible use of protein kinase C inhibitors in suppressing CTGF levels. Protein kinase C was found to phosphorylate Smad-3 directly, inhibiting its ability to bind DNA and enhance transcription.

FU et al. [205] demonstrated that both the natural ligand (15-deoxyprostaglandin J [2]) and a synthetic ligand (GW7845) for the peroxisome proliferator-activated receptor gamma (PPAR-γ) could significantly inhibit TGF-β-induced CTGF production in a dose-dependent manner in human airway smooth muscle cells. It was further reported that PPAR-γ could physically interact with Smad3, highlighting a potential mechanism of action for the ligand. More recent studies have shown that PPAR-γ ligands can prevent TGF-β_1-induced fibronectin in mesangial cells [206] and skin fibroblasts [207].

Ligands for PPAR-γ represent important anti-fibrotic targets, which potentially could be manipulated in asthma. For example, in a murine asthma model, ciglitazone, a synthetic PPAR-γ agonist used in the treatment of diabetes, inhibited a number of remodelling events [208]. Specifically, ciglitazone nebulisation reduced mucus gland hyperplasia and airway occlusion caused by the mucus hypersecretion. Increases in collagen levels and basement membrane thickness were suppressed and TGF-β levels were decreased by 54%.

Additionally, ciglitazone has been shown to decrease both eosinophil numbers and T-helper cell type-2 cytokine (IL-4, IL-5 and IL-13) levels in murine airways [209]. Natural and synthetic ligands of PPAR-γ can also inhibit serum-induced smooth muscle proliferation more effectively than dexamethasone, as well as inhibiting the release of GM-CSF, a survival factor for leukocytes [210].

Interestingly, PPAR-γ expression is increased in the submucosa, smooth muscle layer and epithelium of bronchial tissue in asthmatics [211]. Indeed, TGF-β can indirectly activate cytosolic phospholipase A2alpha (cPLA2-α), via p38 and ERK activation, resulting in activation of PPAR-γ activation [212] in human liver epithelial cells. HAN et al. [212] demonstrated that overexpression of cPLA2-α or PPAR-γ could block TGF-β_1-induced Smad transcriptional activity. Both Smad-7 and PPAR-γ therefore act as negative feedback pathways and further elucidation of the pathways in asthma may yield exciting new targets to combat remodelling.

A novel approach to combating the TGF-β-induced fibrotic response is the use of the human pregnancy hormone, relaxin. Relaxin has been shown to inhibit the TGF-β-induced expression of both collagen and fibronectin in human lung fibroblasts, as well as inducing levels of MMP-1 [213]. The hormone also stimulated wound healing of bronchial epithelial cells in vitro [214] and, in a murine model, relaxin restored bleomycin-induced collagen accumulation back to normal levels [213].

A QUESTION OF BALANCE

This review has appraised the actions of TGF-β in the airways and other tissues. However, it would be a naive to suppose that TGF-β is the only fibrotic mediator in the airways or even that in the milieu of growth factors present in the lung that it was solely responsible for fibrosis associated with remodelled asthmatic airways. The actions of TGF-β are controlled not only by the expression levels of this growth factor in the lung and the ability of cells to respond to this signal, but also on the balance of fibrotic versus anti-fibrotic factors produced as a result of wound healing.

TGF-β can interact with a large number of different growth factors and cytokines in the lungs, each affiliation mediating a different outcome. Attempting to unscramble the interconnecting pathways of only two growth factors among the thousands of potential couplings that exist in the plethora of growth factors in the lung is a daunting task. The current authors have focussed on the interactions between TGF-β and EGF chosen because of their much published and often opposing actions in proliferation and differentiation.

EPIDERMAL GROWTH FACTOR RECEPTOR

EGFR (ErbB1), a member of the ErbB family, is expressed on the majority of cell types, including fibroblasts and epithelial cells of the respiratory tract. The receptors have a restricted expression pattern in polarised lung epithelial cells, with ErbB receptors expressed basolaterally and, thus, are exposed only after epithelial damage. Immediately following a mechanical injury, ErbB ligands can activate EGFR [215] or ErbB2 [216] at the edge of the wound, thus hastening the restoration of epithelial integrity. Mammalian ligands for the EGFR include

EGF, TGF-α, amphiregulin, betacellulin, heparin-binding epidermal growth factor-like growth factor (HB-EGF), epiregulin and epigen [217]. A number of EGFR ligands are present in all cell types, although the expression profile of the individual ligands varies. For example, HB-EGF, amphiregulin and EGF have a widespread tissue distribution pattern, although TGF-α is expressed mainly in epithelial cells, macrophages and eosinophils. Immunolocalisation studies have revealed that EGF is primarily expressed in bronchial glands [218, 219], although there is weak expression in the bronchial epithelium and smooth muscle [220]. Interestingly, stronger EGF immunoreactivity has been demonstrated in the bronchial epithelium, glands, smooth muscle [220] and submucosa [165] of asthmatic subjects compared with nonasthmatics. TGF-α, amphiregulin, betacellulin and HB-EGF expression has also been observed in airway epithelium, but does not differ in asthma [221].

FUNCTIONS OF EGFR LIGANDS

Proliferation is the most well studied function of EGFR signalling. All of the EGFR ligands are capable of promoting mitogenesis, although their potency is dependent on cell type. For example, amphiregulin induces proliferation of epithelial cells, whereas HB-EGF is a potent smooth muscle mitogen. EGFR ligands can also promote a migratory phenotype in cells, a response clearly illustrated by EGF. For example, EGF can enhance the repair of the mechanically damaged monolayers of bronchial epithelial cells [215]. EGF-dependent cell motility is regulated by phospholipase c-α activation [222, 223] and MAPK activation [224]. EGF can stimulate the disassembly of focal adhesions and alter components of the adherens [225] and desmosomal junctions, thus decreasing cell–cell contact and contributing to cell motility [226]. Therefore, TGF-β and EGF can have opposing roles in terms of proliferation, but similar roles in terms of migration.

The TGF-β and EGF signalling pathways are not discrete pathways as they share many downstream signalling molecules. TGF-β and EGF can both mediate distinct cellular events via activation of ERK1 and ERK2 and, as a result, TGF-β can act synergistically, antagonistically or independently to EGF signalling. There are a number of reported examples of crosstalk between the two pathways at the level of receptor–ligand interactions. A study by THOMPSON et al. [227] using normal rat kidney fibroblasts indicated that TGF-β was capable of increasing expression of EGFR. Conversely, DANIELPOUR et al. [228] showed that stimulation by EGF in the same cell type could induce the secretion of TGF-β_1. TGF-β can also regulate expression of other members of the EGF family. For example, a study by BENNETT et al. [229] showed that TGF-β treatment reduces the level of expression of amphiregulin in the human lung adenocarcinoma cell line A549.

TGF-β and EGF are known to mediate a number of different cellular events, including growth, migration and branching morphogenesis of the lung. MISHIMA et al. [230] demonstrated that TGF-β could antagonise EGF-induced attachment of cells onto a fibronectin matrix, migration over corneal stroma, and proliferation of corneal epithelial cells. TGF-β was also found to inhibit the EGF-induced filapodia extension associated with migrating human bronchial epithelial cells [231]. Both TGF-β and EGF can control lung branching in the developing foetus. TGF-β causes growth arrest of the developing airways

[232–235], whereas EGF has been reported to cause significant elongation of the bronchial tubes [236]. In combination, TGF-β promotes cell arrest, allowing EGF to induce branching in the outgrowing terminal buds.

These examples show how the action of TGF-β can influence EGF-signalling pathways and vice versa in a number of ways. Regulation of the pathways occurs at a number of different levels and the signalling outcome is dictated by the relative balance between the TGF-β and EGF signalling pathways. No signalling-mediated event can occur in isolation in the cell, but the TGF-β–EGF axis is distinct in its degree of overlap, especially when the antagonistic nature of the two growth factors is taken into consideration. The balance between EGF-driven proliferation and TGF-β-mediated differentiation of fibroblasts is therefore of potential importance in the pathogenesis of airway remodelling in asthmatics.

EPIDERMAL GROWTH FACTORS AND ASTHMA

EGFR, ErbB2 and ErbB3 receptors are expressed in the epithelium of the upper and lower airways, as well as in primary cultures of human bronchial epithelial cells and human bronchial epithelial derived cell lines, such as H292 and 16HBE 14o- [217, 219]. In asthma, it has been observed that the expression of EGFR is increased on the remaining epithelial cells around the site of damage, and on the apical side of the exposed basal cells [219]. EGFR expression levels are increased in asthma and correlate to disease severity [215]. This increase in EGFR expression is a typical response to tissue injury; however, in asthma there is no evidence that this is coupled with an increased proliferative response [16]. Furthermore, EGFR expression is insensitive to the effects of corticosteroids [215, 237].

Increased expression of EGFR ligands has been reported in asthmatic airways. AMISHIMA et al. [220] reported that EGF was expressed at higher levels in the bronchial epithelium and glands of asthmatic subjects compared with normals. Additionally, TGF-α release was enhanced in asthmatic epithelial cells in response to pro-inflammatory cytokines, in particular tumour necrosis factor-α and combinations of IL-4, IL-13 and allergen [238].

VARGAFTIG and SINGER [239] demonstrated that AG1478, an EGFR antagonist, could inhibit bronchial hyperresponsiveness, inflammation and lung remodelling induced by ovalbumin in a murine model. EGF and TGF-α have been implicated in induction of MUC5AC expression, a mucin released from goblet cells, via activation of the EGFR/Ras/Raf/ERK-signalling pathway [240]. TAKEYAMA et al. [241] demonstrated a positive correlation between EGFR immunoreactivity and the area of MUC5AC-positive staining. Increased release of EGFR ligands into the luminal space, such as EGF, coupled with increased EGFR expression, may account for the increase in mucus secretion by asthmatic epithelial cells.

The inflammatory cell profile in severe asthma is characterised by high numbers of circulating neutrophils. HAMILTON et al. [242] demonstrated that damage to the epithelium has the potential to contribute to neutrophilic inflammation through enhanced production of CXCL-8 (IL-8) via EGFR-dependent mechanisms, which again are insensitive to corticosteroids.

MODULATION OF EGFR EXPRESSION IN ASTHMA

β_2-agonists can stimulate cAMP release, causing activation of protein kinase A and subsequent serine phosphorylation of the EGFR [243]. Phosphorylation of serine residues on the EGFR deactivates the receptor, thus preventing signalling downstream from the receptor. Additionally, KIMURA and OGIHARA [244] showed that increasing concentrations of the β_2-agonist metaproterenol markedly reduced the proliferative effects of TGF-α in adult rat hepatocytes. These observations provide an example where a pre-existing therapy could indeed be counter-productive and prevent epithelial repair in the airways of asthmatics.

Studies have revealed that corticosteroids do not alter either EGFR expression [215] or activation [237]. A study by KIBE et al. [245] reported that corticosteroid treatment inhibited eotaxin expression and eosinophil accumulation, but had no affect on airway hyperresponsiveness, MUC5AC overexpression, or goblet cell hyperplasia induced by IL-13 in a murine model. Corticosteroids have also been shown to have no effect on the expression of p21waf expression, a cyclin-dependent kinase inhibitor.

EGF/EGFR AS A NOVEL THERAPEUTIC TARGET IN CHRONIC ASTHMA

EGFR can regulate mucin production in the airways [246]. A treatment whereby secretion is reduced would be beneficial to the asthma sufferer, although complete inhibition of mucus secretion may indeed be counter-productive. Controlled mucus secretion plays a protective role in the innate immune system and helps to eliminate airborne allergens via ciliary action. Inhibitors of EGFR would potentially also suppress EGFR-mediated signals needed to initiate repair of the damaged epithelium, evident in asthmatic airways. Therefore, targeted downstream interventions may be appropriate.

A number of studies have concentrated on the effect of blocking nonreceptor tyrosine kinases in inflammatory cells (as reviewed by WONG and LEONG [247]). Binding of allergen to immunoceptors causes stimulation of nonreceptor tyrosine kinases, such as Lyn, Lck, Syk and Fyn. Use of both nonselective and selective tyrosine kinase inhibitors can induce a number of beneficial effects. For example, the Jak-3-specific inhibitor WHI-P97 was reported to suppress leukotriene synthesis, airway hyperresponsiveness and recruitment of eosinophils after ovalbumin challenge in a mouse model [248]. However, in some cases, inhibition of tyrosine kinases can be counter-productive, e.g. Lck is also required for downregulating TCR expression by increasing the internalisation and degradation of receptors [249].

CONCLUDING COMMENTS

Transforming growth factor-β is a potent fibrogenic growth factor, which is overexpressed in the asthmatic lung. The growth factor appears to play an essential role in mediating the fibrosis associated with the remodelled airways of asthmatics. In addition to transforming growth factor-β, abnormal epidermal growth factor receptor function may account for the excess mucus production in asthma. However, it is likely that the ultimate response in vivo will not depend solely on the balance between pro-fibrogenic and anti-fibrogenic growth factors.

Although complete ablation could cause a whole host of new problems, as is shown in knockout mice, restoration of the balance between different growth factors may be of therapeutic benefit, for example, through the use of neutralising antibodies. Hopefully, the fuller comprehension of how signalling pathways interact in the asthmatic lung can be manipulated to provide novel therapeutic targets to combat the remodelling and maybe attenuate the chronic inflammation, so intimately linked to the altered structure of the lungs.

REFERENCES

1 Persson CC. The role of microvascular permeability in the pathogenesis of asthma. Eur J Respir Dis Suppl 1986; 144: 190–216.

2 Pare PD, Roberts CR, Bai TR, Wiggs BJ. The functional consequences of airway remodeling in asthma. Monaldi Arch Chest Dis 1997; 52: 589–596.

3 Lambert RK. Role of bronchial basement membrane in airway collapse. J Appl Physiol 1991; 71: 666–673.

4 Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998; 339: 1194–1200.

5 Laprise C, Laviolette M, Boutet M, Boulet LP. Asymptomatic airway hyperresponsiveness: relationships with airway inflammation and remodelling. Eur Respir J 1999; 14: 63–73.

6 Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. Allergy Clin Immunol 2000; 105: 193–204.

7 Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodelling in asthma: new insights. J Allergy Clin Immunol 2003; 111: 215–225.

8 Anzano MA, Roberts AB, Meyers CA, et al. Synergistic interaction of two classes of transforming growth factors from murine sarcoma cells. Cancer Res 1982; 42: 4776–4778.

9 Wan H, Winton HL, Soeller C, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest 1999; 104: 123–133.

10 Gleich GJ. The pathology of asthma: with emphasis on the role of the eosinophil. N Engl Reg Allergy Proc 1986; 7: 421–424.

11 Ordonez C, Ferrando R, Hyde DM, Wong HH, Fahy JV. Epithelial desquamation in asthma: artifact or pathology?. Am J Respir Crit Care Med 2000; 162: 2324–2329.

12 Ordonez CL, Fahy JV. Epithelial desquamation in asthma. Am J Respir Crit Care Med 2001; 164: 1997.

13 Shahana S, Bjornsson E, Ludviksdottir D, et al. Ultrastructure of bronchial biopsies from patients with allergic and non-allergic asthma. Respir Med 2005; 99: 429–443.

14 Wark PA, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med 2005; 201: 937–947.

15 Bucchieri F, Puddicombe SM, Lordan JL, et al. Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. Am J Respir Cell Mol Biol 2002; 27: 179–185.

16 Puddicombe SM, Torres-Lozano C, Richter A, *et al.* Increased expression of p21(waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. *Am J Respir Cell Mol Biol* 2003; 28: 61–68.

17 Ordonez CL, Khashayar R, Wong HH, *et al.* Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001; 163: 517–523.

18 Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* 2002; 57: 677–682.

19 Hays SR, Woodruff PG, Khashayar R, *et al.* Allergen challenge causes inflammation but not goblet cell degranulation in asthmatic subjects. *J Allergy Clin Immunol* 2001; 108: 784–790.

20 Groneberg DA, Eynott PR, Lim S, *et al.* Expression of respiratory mucins in fatal status asthmaticus and mild asthma. *Histopathology* 2002; 40: 367–373.

21 Fahy JV. Goblet cell and mucin gene abnormalities in asthma. *Chest* 2002; 122: Suppl. 6, 320S–326S.

22 Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990; 3: 507–511.

23 Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; 1: 520–524.

24 Chu HW, Halliday JL, Martin RJ, Leung DY, Szefler SJ, Wenzel SE. Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. *Am J Respir Crit Care Med* 1998; 158: 1936–1944.

25 Ward C, Johns DP, Bish R, *et al.* Reduced airway distensibility, fixed airflow limitation, and airway wall remodeling in asthma. *Am J Respir Crit Care Med* 2001; 164: 1718–1721.

26 Wiggs BR, Hrousis CA, Drazen JM, Kamm RD. On the mechanism of mucosal folding in normal and asthmatic airways. *J Appl Physiol* 1997; 83: 1814–1821.

27 Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003; 167: 1360–1368.

28 Hoshino M, Nakamura Y, Sim JJ. Expression of growth factors and remodelling of the airway wall in bronchial asthma. *Thorax* 1998; 53: 21–27.

29 Woodruff PG, Dolganov GM, Ferrando RE, *et al.* Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. *Am J Respir Crit Care Med* 2004; 169: 1001–1006.

30 Johnson PR, Roth M, Tamm M, *et al.* Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001; 164: 474–477.

31 Johnson PR, Burgess JK, Underwood PA, *et al.* Extracellular matrix proteins modulate asthmatic airway smooth muscle cell proliferation via an autocrine mechanism. *J Allergy Clin Immunol* 2004; 113: 690–696.

32 Dulin NO, Fernandes DJ, Dowell M, *et al.* What evidence implicates airway smooth muscle in the cause of BHR? *Clin Rev Allergy Immunol* 2003; 24: 73–84.

33 Holgate ST. Epithelial damage and response. *Clin Exp Allergy* 2000; 30: Suppl. 1, 37–41.

34 Zhang S, Smartt H, Holgate ST, Roche WR. Growth factors secreted by bronchial epithelial cells control myofibroblast proliferation: an *in vitro* co-culture model of airway remodeling in asthma. *Lab Invest* 1999; 79: 395–405.

35 Richter A, Puddicombe SM, Lordan JL, *et al.* The contribution of interleukin (IL)-4 and IL-13 to the epithelial-mesenchymal trophic unit in asthma. *Am J Respir Cell Mol Biol* 2001; 25: 385–391.

36 Vignola AM, La Grutta S, Chiappara G, Benkeder A, Bellia V, Bonsignore G. Cellular network in airways inflammation and remodelling. *Paediatr Respir Rev* 2002; 3: 41–46.

37 Hamilton LM, Davies DE, Wilson SJ, Kimber I, Dearman RJ, Holgate ST. The bronchial epithelium in asthma – much more than a passive barrier. *Monaldi Arch Chest Dis* 2001; 56: 48–54.

38 Korfhagen TR, Swantz RJ, Wert SE, *et al.* Respiratory epithelial cell expression of human transforming growth factor-alpha induces lung fibrosis in transgenic mice. *J Clin Invest* 1994; 93: 1691–1699.

39 Fedorov IA, Wilson SJ, Davies DE, Holgate ST. Epithelial stress and structural remodelling in childhood asthma. *Thorax* 2005; 60: 389–394.

40 Cokugras H, Akcakaya N, Seckin, Camcioglu Y, Sarimurat N, Aksoy F. Ultrastructural examination of bronchial biopsy specimens from children with moderate asthma. *Thorax* 2001; 56: 25–29.

41 Najafi N, Demanet C, Dab I, De Waele M, Malfroot A. Differential cytology of bronchoalveolar lavage fluid in asthmatic children. *Pediatr Pulmonol* 2003; 35: 302–308.

42 Payne DN, Rogers AV, Adelroth E, *et al.* Early thickening of the reticular basement membrane in children with difficult asthma. *Am J Respir Crit Care Med* 2003; 167: 78–82.

43 Harmanci E, Kebapci M, Metintas M, Ozkan R. High-resolution computed tomography findings are correlated with disease severity in asthma. *Respiration* 2002; 69: 420–426.

44 Salvato G. Quantitative and morphological analysis of the vascular bed in bronchial biopsy specimens from asthmatic and non-asthmatic subjects. *Thorax* 2001; 56: 902–906.

45 Chakir J, Shannon J, Molet S, *et al.* Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003; 111: 1293–1298.

46 Selroos O, Pietinalho A, Lofroos AB, Riska H. Effect of early vs late intervention with inhaled corticosteroids in asthma. *Chest* 1995; 108: 1228–1234.

47 Selroos O, Lofroos AB, Pietinalho A, Riska H. Asthma control and steroid doses 5 years after early or delayed introduction of inhaled corticosteroids in asthma: a real-life study. *Respir Med* 2004; 98: 254–262.

48 Pauwels RA, Pedersen S, Busse WW, *et al.* Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. *Lancet* 2003; 361: 1071–1076.

49 Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. *Am J Respir Crit Care Med* 1999; 159: 1043–1051.

50 Hoshino M, Takahashi M, Takai Y, Sim J, Aoike N. Inhaled corticosteroids decrease vascularity of the bronchial mucosa in patients with asthma. *Clin Exp Allergy* 2001; 31: 722–730.

51 Lundgren R, Soderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. *Eur Respir J* 1988; 1: 883–889.

52 Miyazono K, Ichijo H, Heldin CH. Transforming growth factor-beta: latent forms, binding proteins and receptors. *Growth Factors* 1993; 8: 11–22.

53 Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. *Clin Exp Allergy* 1997; 27: 363–371.

54 Brinckmann J, Notbohm H, Tronnier M, *et al.* Overhydroxylation of lysyl residues is the initial step for altered collagen cross-links and fibril architecture in fibrotic skin. *J Invest Dermatol* 1999; 113: 617–621.

55 Dube J, Chakir J, Laviolette M, *et al. In vitro* procollagen synthesis and proliferative phenotype of bronchial fibroblasts from normal and asthmatic subjects. *Lab Invest* 1998; 78: 297–307.

56 Laitinen A, Altraja A, Kampe M, Linden M, Virtanen I, Laitinen LA. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med* 1997; 156: 951–958.

57 Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig M. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. *Am J Respir Crit Care Med* 1999; 160: 725–729.

58 Westergren-Thorsson G, Chakir J, Lafreniere-Allard MJ, Boulet LP, Tremblay GM. Correlation between airway responsiveness and proteoglycan production by bronchial fibroblasts from normal and asthmatic subjects. *Int J Biochem Cell Biol* 2002; 34: 1256–1267.

59 Altraja A, Laitinen A, Virtanen I, *et al.* Expression of laminins in the airways in various types of asthmatic patients: a morphometric study. *Am J Respir Cell Mol Biol* 1996; 15: 482–488.

60 Bousquet J, Lacoste JY, Chanez P, Vic P, Godard P, Michel FB. Bronchial elastic fibers in normal subjects and asthmatic patients. *Am J Respir Crit Care Med* 1996; 153: 1648–1654.

61 Vignola AM, Bonanno A, Mirabella A, *et al.* Increased levels of elastase and alpha1-antitrypsin in sputum of asthmatic patients. *Am J Respir Crit Care Med* 1998; 157: 505–511.

62 Huang CD, Chen HH, Wang CH, *et al.* Human neutrophil-derived elastase induces airway smooth muscle cell proliferation. *Life Sci* 2004; 74: 2479–2492.

63 Kohri K, Ueki IF, Nadel JA. Neutrophil elastase induces mucin production by ligand-dependent epidermal growth factor receptor activation. *Am J Physiol Lung Cell Mol Physiol* 2002; 283: L531–L540.

64 Tremblay GM, Chakir J, Boulet LP, Jordana M, Richards CD, Gauldie J. Bronchial myofibroblasts and tissue remodelling in asthma. *Can Respir J* 1998; 5: 59–61.

65 Laliberte R, Rouabhia M, Bosse M, Chakir J. Decreased capacity of asthmatic bronchial fibroblasts to degrade collagen. *Matrix Biol* 2001; 19: 743–753.

66 Ohno I, Ohtani H, Nitta Y, *et al.* Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1997; 16: 212–219.

67 Oshita Y, Koga T, Kamimura T, Matsuo K, Rikimaru T, Aizawa H. Increased circulating 92 kDa matrix metalloproteinase (MMP-9) activity in exacerbations of asthma. *Thorax* 2003; 58: 757–760.

68 Mattos W, Lim S, Russell R, Jatakanon A, Chung KF, Barnes PJ. Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. *Chest* 2002; 122: 1543–1552.

69 Wenzel SE, Balzar S, Cundall M, Chu HW. Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. *J Allergy Clin Immunol* 2003; 111: 1345–1352.

70 Cataldo DD, Gueders M, Munaut C, *et al.* Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases mRNA transcripts in the bronchial secretions of asthmatics. *Lab Invest* 2004; 84: 418–424.

71 Vignola AM, Paganin F, Capieu L, *et al.* Airway remodelling assessed by sputum and high-resolution computed tomography in asthma and COPD. *Eur Respir J* 2004; 24: 910–917.

72 Matsumoto H, Niimi A, Takemura M, *et al.* Relationship of airway wall thickening to an imbalance between matrix metalloproteinase-9 and its inhibitor in asthma. *Thorax* 2005; 60: 277–281.

73 Seals DF, Courtneidge SA. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev* 2003; 17: 7–30.

74 Amour A, Knight CG, Webster A, *et al.* The *in vitro* activity of ADAM-10 is inhibited by TIMP-1 and TIMP-3. *FEBS Lett* 2000; 473: 275–279.

75 Loechel F, Fox JW, Murphy G, Albrechtsen R, Wewer UM. ADAM12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. *Biochem Biophys Res Commun* 2000; 278: 511–515.

76 Van Eerdewegh P, Little RD, Dupuis J, *et al.* Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418: 426–430.

77 Yoshinaka T, Nishii K, Yamada K, *et al.* Identification and characterization of novel mouse and human ADAM33s with potential metalloprotease activity. *Gene* 2002; 282: 227–236.

78 Garlisi CG, Zou J, Devito KE, *et al.* Human ADAM33: protein maturation and localization. *Biochem Biophys Res Commun* 2003; 301: 35–43.

79 Zou J, Zhu F, Liu J, *et al.* Catalytic activity of human ADAM33. *J Biol Chem* 2004; 279: 9818–9830.

80 Powell RM, Wicks J, Holloway JW, Holgate ST, Davies DE. The splicing and fate of ADAM33 transcripts in primary human airways fibroblasts. *Am J Respir Cell Mol Biol* 2004; 31: 13–21.

81   Haitchi HM, Powell RM, Shaw TJ, *et al.* ADAM33 expression in asthmatic airways and human embryonic lungs. *Am J Respir Crit Care Med* 2005; 171: 958–965.

82   Jongepier H, Boezen HM, Dijkstra A, *et al.* Polymorphisms of the ADAM33 gene are associated with accelerated lung function decline in asthma. *Clin Exp Allergy* 2004; 34: 757–760.

83   van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Schouten JP, Boezen HM. A desintegrin and metalloprotease 33 polymorphisms and lung function decline in the population. *Am J Respir Crit Care Med* 2005; 172: 329–333.

84   Simpson A, Maniatis N, Jury F, *et al.* Polymorphisms in a disintegrin and metalloprotease 33 predict impaired early-life lung function. *Am J Respir Crit Care Med* 2005; 172: 55–60.

85   Schmidt-Weber CB, Blaser K. Regulation and role of transforming growth factor-beta in immune tolerance induction and inflammation. *Curr Opin Immunol* 2004; 16: 709–716.

86   Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. *J Exp Med* 1987; 165: 251–256.

87   Miyazono K, Kusanagi K, Inoue H. Divergence and convergence of TGF-beta/BMP signaling. *J Cell Physiol* 2001; 187: 265–276.

88   Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB. Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. *J Biol Chem* 1983; 258: 7155–7160.

89   Saharinen J, Hyytiainen M, Taipale J, Keski-Oja J. Latent transforming growth factor-beta binding proteins (LTBPs) – structural extracellular matrix proteins for targeting TGF-beta action. *Cytokine Growth Factor Rev* 1999; 10: 99–117.

90   Schultz-Cherry S, Murphy-Ullrich JE. Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. *J Cell Biol* 1993; 122: 923–932.

91   Schultz–Cherry S, Chen H, Mosher DF, *et al.* Regulation of transforming growth factor–beta activation by discrete sequences of thrombospondin 1. *J Biol Chem* 1995; 270: 7304–7310.

92   Khalil N. TGF–beta: from latent to active. *Microbes Infect* 1999; 1: 1255–1263.

93   Vodovotz Y, Chesler L, Chong H, *et al.* Regulation of transforming growth factor beta1 by nitric oxide. *Cancer Res* 1999; 59: 2142–2149.

94   Cheifetz S, Ling N, Guillemin R, Massague J. A surface component on GH3 pituitary cells that recognizes transforming growth factor-beta, activin, and inhibin. *J Biol Chem* 1988; 263: 17225–17228.

95   Segarini PR, Ziman JM, Kane CJ, Dasch JR. Two novel patterns of transforming growth factor beta (TGF-beta) binding to cell surface proteins are dependent upon the binding of TGF-beta 1 and indicate a mechanism of positive cooperativity. *J Biol Chem* 1992; 267: 1048–1053.

96   O'Grady P, Kuo MD, Baldassare JJ, Huang SS, Huang JS. Purification of a new type high molecular weight receptor (type V receptor) of transforming growth factor beta (TGF-beta) from bovine liver. Identification of the type V TGF-beta receptor in cultured cells. *J Biol Chem* 1991; 266: 8583–8589.

97   Blobe GC, Liu X, Fang SJ, How T, Lodish HF. A novel mechanism for regulating transforming growth factor beta (TGF-beta) signaling. Functional modulation of type III TGF-beta receptor expression through interaction with the PDZ domain protein, GIPC. *J Biol Chem* 2001; 276: 39608–39617.

98   Oh SP, Seki T, Goss KA, *et al.* Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. *Proc Natl Acad Sci USA* 2000; 97: 2626–2631.

99   ten Dijke P, Yamashita H, Ichijo H, *et al.* Characterization of type I receptors for transforming growth factor-beta and activin. *Science* 1994; 264: 101–104.

100   Macias-Silva M, Hoodless PA, Tang SJ, Buchwald M, Wrana JL. Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2. *J Biol Chem* 1998; 273: 25628–25636.

101   Charng MJ, Zhang D, Kinnunen P, Schneider MD. A novel protein distinguishes between quiescent and activated forms of the type I transforming growth factor beta receptor. *J Biol Chem* 1998; 273: 9365–9368.

102   Jayaraman L, Massague J. Distinct oligomeric states of SMAD proteins in the transforming growth factor-beta pathway. *J Biol Chem* 2000; 275: 40710–40717.

103   Tsukazaki T, Chiang TA, Davison AF, Attisano L, Wrana JL. SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. *Cell* 1998; 95: 779–791.

104   Goto D, Nakajima H, Mori Y, Kurasawa K, Kitamura N, Iwamoto I. Interaction between Smad anchor for receptor activation and Smad3 is not essential for TGF-beta/Smad3-mediated signaling. *Biochem Biophys Res Commun* 2001; 281: 1100–1105.

105   Wurthner JU, Frank DB, Felici A, *et al.* Transforming growth factor-beta receptor-associated protein 1 is a Smad4 chaperone. *J Biol Chem* 2001; 276: 19495–19502.

106   Jonk LJ, Itoh S, Heldin CH, ten Dijke P, Kruijer W. Identification and functional characterization of a Smad binding element (SBE) in the JunB promoter that acts as a transforming growth factor-beta, activin, and bone morphogenetic protein-inducible enhancer. *J Biol Chem* 1998; 273: 21145–21152.

107   Wong C, Rougier-Chapman EM, Frederick JP, *et al.* Smad3-Smad4 and AP-1 complexes synergize in transcriptional activation of the c-Jun promoter by transforming growth factor beta. *Mol Cell Biol* 1999; 19: 1821–1830.

108   Zhou S, Zawel L, Lengauer C, Kinzler KW, Vogelstein B. Characterization of human FAST-1, a TGF beta and activin signal transducer. *Mol Cell* 1998; 2: 121–127.

109   Feng XH, Zhang Y, Wu RY, Derynck R. The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for smad3 in TGF-beta-induced transcriptional activation. *Genes Dev* 1998; 12: 2153–2163.

110   Hocevar BA, Brown TL, Howe PH. TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. *EMBO J* 1999; 18: 1345–1356.

111 Kawabata M, Imamura T, Miyazono K, Engel ME, Moses HL. Interaction of the transforming growth factor-beta type I receptor with farnesyl-protein transferase-alpha. *J Biol Chem* 1995; 270: 29628–29631.

112 Wang T, Li BY, Danielson PD, *et al.* The immunophilin FKBP12 functions as a common inhibitor of the TGF beta family type I receptors. *Cell* 1996; 86: 435–444.

113 Yao D, Dore JJ Jr, Leof EB. FKBP12 is a negative regulator of transforming growth factor-beta receptor internalization. *J Biol Chem* 2000; 275: 13149–13154.

114 Shibuya H, Yamaguchi K, Shirakabe K, *et al.* TAB1: an activator of the TAK1 MAPKKK in TGF-beta signal transduction. *Science* 1996; 272: 1179–1182.

115 Sakurai H, Miyoshi H, Mizukami J, Sugita T. Phosphorylation-dependent activation of TAK1 mitogen-activated protein kinase kinase kinase by TAB1. *FEBS Lett* 2000; 474: 141–145.

116 Anders RA, Leof EB. Chimeric granulocyte/macrophage colony-stimulating factor/transforming growth factor-beta (TGF-beta) receptors define a model system for investigating the role of homomeric and heteromeric receptors in TGF-beta signaling. *J Biol Chem* 1996; 271: 21758–21766.

117 Anders RA, Arline SL, Dore JJ, Leof EB. Distinct endocytic responses of heteromeric and homomeric transforming growth factor beta receptors. *Mol Biol Cell* 1997; 8: 2133–2143.

118 Saitoh M, Nishitoh H, Amagasa T, Miyazono K, Takagi M, Ichijo H. Identification of important regions in the cytoplasmic juxtamembrane domain of type I receptor that separate signaling pathways of transforming growth factor-beta. *J Biol Chem* 1996; 271: 2769–2775.

119 Luo K, Lodish HF. Positive and negative regulation of type II TGF-beta receptor signal transduction by auto-phosphorylation on multiple serine residues. *EMBO J* 1997; 16: 1970–1981.

120 Hata A, Lagna G, Massague J, Hemmati-Brivanlou A. Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. *Genes Dev* 1998; 12: 186–197.

121 Hayashi H, Abdollah S, Qiu Y, *et al.* The MAD-related protein Smad7 associates with the TGFbeta receptor and functions as an antagonist of TGFbeta signaling. *Cell* 1997; 89: 1165–1173.

122 Afrakhte M, Moren A, Jossan S, *et al.* Induction of inhibitory Smad6 and Smad7 mRNA by TGF-beta family members. *Biochem Biophys Res Commun* 1998; 249: 505–511.

123 Datta PK, Moses HL. STRAP and Smad7 synergize in the inhibition of transforming growth factor beta signaling. *Mol Cell Biol* 2000; 20: 3157–3167.

124 Kurokawa M, Mitani K, Irie K, *et al.* The oncoprotein Evi-1 represses TGF-beta signalling by inhibiting Smad3. *Nature* 1998; 394: 92–96.

125 Akiyoshi S, Inoue H, Hanai J, *et al.* c-Ski acts as a transcriptional co-repressor in transforming growth factor-beta signaling through interaction with smads. *J Biol Chem* 1999; 274: 35269–35277.

126 Stroschein SL, Wang W, Zhou S, Zhou Q, Luo K. Negative feedback regulation of TGF-beta signaling by the SnoN oncoprotein. *Science* 1999; 286: 771–774.

127 Wotton D, Lo RS, Lee S, Massague J. A Smad transcriptional corepressor. *Cell* 1999; 97: 29–39.

128 Boland S, Boisvieux-Ulrich E, Houcine O, *et al.* TGF beta 1 promotes actin cytoskeleton reorganization and migratory phenotype in epithelial tracheal cells in primary culture. *J Cell Sci* 1996; 109: 2207–2219.

129 Howat WJ, Holgate ST, Lackie PM. TGF-beta isoform release and activation during *in vitro* bronchial epithelial wound repair. *Am J Physiol Lung Cell Mol Physiol* 2002; 282: L115–L123.

130 Moses HL, Pietenpol JA, Munger K, Murphy CS, Yang EY. TGF beta regulation of epithelial cell proliferation: role of tumor suppressor genes. *Princess Takamatsu Symp* 1991; 22: 183–195.

131 Robson CN, Gnanapragasam V, Byrne RL, Collins AT, Neal DE. Transforming growth factor-beta1 up-regulates p15, p21 and p27 and blocks cell cycling in G1 in human prostate epithelium. *J Endocrinol* 1999; 160: 257–266.

132 Serini G, Bochaton-Piallat ML, Ropraz P, *et al.* The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *J Cell Biol* 1998; 142: 873–881.

133 Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 1993; 122: 103–111.

134 Ronnov-Jessen L, Petersen OW. Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. *Lab Invest* 1993; 68: 696–707.

135 Hautmann MB, Adam PJ, Owens GK. Similarities and differences in smooth muscle alpha-actin induction by TGF-beta in smooth muscle *versus* non-smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1999; 19: 2049–2058.

136 Thannickal VJ, Lee DY, White ES, *et al.* Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling *via* focal adhesion kinase. *J Biol Chem* 2003; 278: 12384–12389.

137 Desmouliere A. Factors influencing myofibroblast differentiation during wound healing and fibrosis. *Cell Biol Int* 1995; 19: 471–476.

138 Zhang HY, Phan SH. Inhibition of myofibroblast apoptosis by transforming growth factor beta(1). *Am J Respir Cell Mol Biol* 1999; 21: 658–665.

139 Hashimoto S, Gon Y, Takeshita I, Maruoka S, Horie T. IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH$_2$-terminal kinase-dependent pathway. *J Allergy Clin Immunol* 2001; 107: 1001–1008.

140 Mattey DL. Interleukin-4 induces myofibroblast differentiation is synovial fibroblasts. *Biochem Soc Trans* 1997; 25: 290S.

141 Vozenin-Brotons MC, Sivan V, Gault N, *et al.* Antifibrotic action of Cu/Zn SOD is mediated by TGF-beta1 repression and phenotypic reversion of myofibroblasts. *Free Radic Biol Med* 2001; 30: 30–42.

142 Schmitt-Graff A, Desmouliere A, Gabbiani G. Heterogeneity of myofibroblast phenotypic features: an

example of fibroblastic cell plasticity. *Virchows Arch* 1994; 425: 3–24.

143 Tanaka K, Sano K, Yuba K, *et al.* Inhibition of induction of myofibroblasts by interferon gamma in a human fibroblast cell line. *Int Immunopharmacol* 2003; 3: 1273–1280.

144 Kolodsick JE, Peters-Golden M, Larios J, Toews GB, Thannickal VJ, Moore BB. Prostaglandin E2 inhibits fibroblast to myofibroblast transition *via* E. prostanoid receptor 2 signaling and cyclic adenosine monophosphate elevation. *Am J Respir Cell Mol Biol* 2003; 29: 537–544.

145 Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J Immunol* 2003; 171: 380–389.

146 Larsen K, Tufvesson E, Malmstrom J, *et al.* Presence of activated mobile fibroblasts in bronchoalveolar lavage from patients with mild asthma. *Am J Respir Crit Care Med* 2004; 170: 1049–1056.

147 Kay EP, Lee MS, Seong GJ, Lee YG. TGF-beta s stimulate cell proliferation via an autocrine production of FGF-2 in corneal stromal fibroblasts. *Curr Eye Res* 1998; 17: 286–293.

148 Thannickal VJ, Aldweib KD, Rajan T, Fanburg BL. Upregulated expression of fibroblast growth factor (FGF) receptors by transforming growth factor-beta1 (TGF-beta1) mediates enhanced mitogenic responses to FGFs in cultured human lung fibroblasts. *Biochem Biophys Res Commun* 1998; 251: 437–441.

149 McAnulty RJ, Hernandez-Rodriguez NA, Mutsaers SE, Coker RK, Laurent GJ. Indomethacin suppresses the antiproliferative effects of transforming growth factor-beta isoforms on fibroblast cell cultures. *Biochem J* 1997; 321: 639–643.

150 Wenzel SE, Trudeau JB, Barnes S, *et al.* TGF-beta and IL-13 synergistically increase eotaxin-1 production in human airway fibroblasts. *J Immunol* 2002; 169: 4613–4619.

151 Lin CS, Lin G, Wang Z, Maddah SA, Lue TF. Upregulation of monocyte chemoattractant protein 1 and effects of transforming growth factor-beta 1 in Peyronie's disease. *Biochem Biophys Res Commun* 2002; 295: 1014–1019.

152 Cheon H, Yu SJ, Yoo DH, Chae IJ, Song GG, Sohn J. Increased expression of pro-inflammatory cytokines and metalloproteinase-1 by TGF-beta1 in synovial fibroblasts from rheumatoid arthritis and normal individuals. *Clin Exp Immunol* 2002; 127: 547–552.

153 Chambers RC, Leoni P, Kaminski N, Laurent GJ, Heller RA. Global expression profiling of fibroblast responses to transforming growth factor-beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. *Am J Pathol* 2003; 162: 533–546.

154 Halayko AJ, Kartha S, Stelmack GL, *et al.* Phophatidylinositol-3 kinase/mammalian target of rapamycin/p70S6K regulates contractile protein accumulation in airway myocyte differentiation. *Am J Respir Cell Mol Biol* 2004; 31: 266–275.

155 Roelofs M, Faggian L, Pampinella F, *et al.* Transforming growth factor beta1 involvement in the conversion of fibroblasts to smooth muscle cells in the rabbit bladder serosa. *Histochem J* 1998; 30: 393–404.

156 Jones RC, Jacobson M. Angiogenesis in the hypertensive lung: response to ambient oxygen tension. *Cell Tissue Res* 2000; 300: 263–284.

157 Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells *in vivo*. *Circulation* 2003; 108: 2070–2073.

158 Fine A, Goldstein RH. Regulation of type I collagen mRNA translation by TGF-beta. *Reg Immunol* 1993; 5: 218–224.

159 Eickelberg O, Kohler E, Reichenberger F, *et al.* Extracellular matrix deposition by primary human lung fibroblasts in response to TGF-beta1 and TGF-beta3. *Am J Physiol* 1999; 276: Suppl. 5 Pt 1, L814–L824.

160 Kucich U, Rosenbloom JC, Abrams WR, Rosenbloom J. Transforming growth factor-beta stabilizes elastin mRNA by a pathway requiring active Smads, protein kinase C-delta, and p38. *Am J Respir Cell Mol Biol* 2002; 26: 183–188.

161 Gerwin BI, Keski-Oja J, Seddon M, Lechner JF, Harris CC. TGF-beta 1 modulation of urokinase and PAI-1 expression in human bronchial epithelial cells. *Am J Physiol* 1990; 259: Suppl. 4 Pt 1, L262–L269.

162 Kordula T, Guttgemann I, Rose-John S, *et al.* Synthesis of tissue inhibitor of metalloproteinase-1 (TIMP-1) in human hepatoma cells (HepG2). Up-regulation by interleukin-6 and transforming growth factor beta 1. *FEBS Lett* 1992; 313: 143–147.

163 Leco KJ, Khokha R, Pavloff N, Hawkes SP, Edwards DR. Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues. *J Biol Chem* 1994; 269: 9352–9360.

164 Bullard KM, Cass DL, Banda MJ, Adzick NS. Transforming growth factor beta-1 decreases interstitial collagenase in healing human fetal skin. *J Pediatr Surg* 1997; 32: 1023–1027.

165 Vignola AM, Chanez P, Chiappara G, *et al.* Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997; 156: 591–599.

166 Kokturk N, Tatlicioglu T, Memis L, Akyurek N, Akyol G. Expression of transforming growth factor beta1 in bronchial biopsies in asthma and COPD. *J Asthma* 2003; 40: 887–893.

167 Sagara H, Okada T, Okumura K, *et al.* Activation of TGF-beta/Smad2 signaling is associated with airway remodeling in asthma. *J Allergy Clin Immunol* 2002; 110: 249–254.

168 Chu HW, Balzar S, Seedorf GJ, *et al.* Transforming growth factor-beta2 induces bronchial epithelial mucin expression in asthma. *Am J Pathol* 2004; 165: 1097–1106.

169 Batra V, Musani AI, Hastie AT, *et al.* Bronchoalveolar lavage fluid concentrations of transforming growth factor (TGF)-beta1, TGF-beta2, interleukin (IL)-4 and IL-13 after segmental allergen challenge and their effects on alpha-smooth muscle actin and collagen III synthesis by primary human lung fibroblasts. *Clin Exp Allergy* 2004; 34: 437–444.

170 Redington AE, Madden J, Frew AJ, *et al.* Transforming growth factor-beta 1 in asthma. Measurement in broncho-alveolar lavage fluid. *Am J Respir Crit Care Med* 1997; 156: 642–647.

171 Joseph J, Benedict S, Badrinath P, *et al.* Elevation of plasma transforming growth factor beta1 levels in stable nonatopic asthma. *Ann Allergy Asthma Immunol* 2003; 91: 472–476.

172 Minshall EM, Leung DY, Martin RJ, *et al.* Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997; 17: 326–333.

173 Ohno I, Lea RG, Flanders KC, *et al.* Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor beta 1 gene (TGF beta 1). *J Clin Invest* 1992; 89: 1662–1668.

174 Balzar S, Chu HW, Silkoff P, *et al.* Increased TGF-beta2 in severe asthma with eosinophilia. *J Allergy Clin Immunol* 2005; 115: 110–117.

175 Grainger DJ, Heathcote K, Chiano M, *et al.* Genetic control of the circulating concentration of transforming growth factor type beta1. *Hum Mol Genet* 1999; 8: 93–97.

176 Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998; 158: 1958–1962.

177 Silverman ES, Palmer LJ, Subramaniam V, *et al.* Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. *Am J Respir Crit Care Med* 2004; 169: 214–219.

178 Pulleyn LJ, Newton R, Adcock IM, Barnes PJ. TGFbeta1 allele association with asthma severity. *Hum Genet* 2001; 109: 623–627.

179 Buckova D, Izakovicova HL, Benes P, Znojil V, Vacha J. TGF-beta1 gene polymorphisms. *Allergy* 2001; 56: 1236–1237.

180 Kenyon NJ, Ward RW, McGrew G, Last JA. TGF-beta1 causes airway fibrosis and increased collagen I and III mRNA in mice. *Thorax* 2003; 58: 772–777.

181 Shah M, Foreman DM, Ferguson MW. Control of scarring in adult wounds by neutralising antibody to transforming growth factor beta. *Lancet* 1992; 339: 213–214.

182 Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *J Cell Sci* 1995; 108: 985–1002.

183 Cordeiro MF, Reichel MB, Gay JA, D'Esposita F, Alexander RA, Khaw PT. Transforming growth factor-beta1, -beta2, and -beta3 *in vivo*: effects on normal and mitomycin C-modulated conjunctival scarring. *Invest Ophthalmol Vis Sci* 1999; 40: 1975–1982.

184 Laping NJ, Grygielko E, Mathur A, *et al.* Inhibition of transforming growth factor (TGF)-beta1-induced extra-cellular matrix with a novel inhibitor of the TGF-beta type I receptor kinase activity: SB-431542. *Mol Pharmacol* 2002; 62: 58–64.

185 DaCosta BS, Major C, Laping NJ, Roberts AB. SB-505124 is a selective inhibitor of transforming growth factor-beta type I receptors ALK4, ALK5, and ALK7. *Mol Pharmacol* 2004; 65: 744–752.

186 Sugaru E, Sakai M, Horigome K, *et al.* SMP-534 inhibits TGF-beta-induced ECM production in fibroblast cells and reduces mesangial matrix accumulation in experimental glomerulonephritis. *Am J Physiol Renal Physiol* 2005; 289: F998–F1004.

187 Nogami M, Romberger DJ, Rennard SI, Toews ML. TGF-beta 1 modulates beta-adrenergic receptor number and function in cultured human tracheal smooth muscle cells. *Am J Physiol* 1994; 266: L187–L191.

188 Ishikawa T, Kume H, Kondo M, Ito Y, Yamaki K, Shimokata K. Inhibitory effects of interferon-gamma on the heterologous desensitization of beta-adrenoceptors by transforming growth factor-beta 1 in tracheal smooth muscle. *Clin Exp Allergy* 2003; 33: 808–815.

189 Behera AK, Kumar M, Lockey RF, Mohapatra SS. Adenovirus-mediated interferon gamma gene therapy for allergic asthma: involvement of interleukin 12 and STAT4 signaling. *Hum Gene Ther* 2002; 13: 1697–1709.

190 Boguniewicz M, Martin RJ, Martin D, *et al.* The effects of nebulized recombinant interferon-gamma in asthmatic airways. *J Allergy Clin Immunol* 1995; 95: 133–135.

191 Shukla A, Meisler N, Cutroneo KR. Perspective article: transforming growth factor-beta: crossroad of glucocorti-coid and bleomycin regulation of collagen synthesis in lung fibroblasts. *Wound Repair Regen* 1999; 7: 133–140.

192 Meisler N, Keefer KA, Ehrlich HP, Yager DR, Myers-Parrelli J, Cutroneo KR. Dexamethasone abrogates the fibrogenic effect of transforming growth factor-beta in rat granuloma and granulation tissue fibroblasts. *J Invest Dermatol* 1997; 108: 285–289.

193 McMillan SJ, Xanthou G, Lloyd CM. Therapeutic administration of Budesonide ameliorates allergen-induced airway remodelling. *Clin Exp Allergy* 2005; 35: 388–396.

194 Wen FQ, Kohyama T, Skold CM, *et al.* Glucocorticoids modulate TGF-beta production. *Inflammation* 2002; 26: 279–290.

195 Profita M, Gagliardo R, Di Giorgi R, *et al. In vitro* effects of flunisolide on MMP-9, TIMP-1, fibronectin, TGF-beta1 release and apoptosis in sputum cells freshly isolated from mild to moderate asthmatics. *Allergy* 2004; 59: 927–932.

196 Igarashi A, Okochi H, Bradham DM, Grotendorst GR. Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. *Mol Biol Cell* 1993; 4: 637–645.

197 Duncan MR, Frazier KS, Abramson S, *et al.* Connective tissue growth factor mediates transforming growth factor beta-induced collagen synthesis: down-regulation by cAMP. *FASEB J* 1999; 13: 1774–1786.

198 Ricupero DA, Rishikof DC, Kuang PP, Poliks CF, Goldstein RH. Regulation of connective tissue growth factor expression by prostaglandin E(2). *Am J Physiol* 1999; 277: L1165–L1171.

199 Kothapalli D, Frazier KS, Welply A, Segarini PR, Grotendorst GR. Transforming growth factor beta induces anchorage-independent growth of NRK fibro-blasts via a connective tissue growth factor-dependent signaling pathway. *Cell Growth Differ* 1997; 8: 61–68.

200 Frazier K, Williams S, Kothapalli D, Klapper H, Grotendorst GR. Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by

connective tissue growth factor. *J Invest Dermatol* 1996; 107: 404–411.

201 Hong HH, Uzel MI, Duan C, Sheff MC, Trackman PC. Regulation of lysyl oxidase, collagen, and connective tissue growth factor by TGF-beta1 and detection in human gingiva. *Lab Invest* 1999; 79: 1655–1667.

202 Grotendorst GR, Duncan MR. Individual domains of connective tissue growth factor regulate fibroblast proliferation and myofibroblast differentiation. *FASEB J* 2005; 19: 729–738.

203 Burgess JK, Johnson PR, Ge Q, *et al.* Expression of connective tissue growth factor in asthmatic airway smooth muscle cells. *Am J Respir Crit Care Med* 2003; 167: 71–77.

204 Kucich U, Rosenbloom JC, Herrick DJ, *et al.* Signaling events required for transforming growth factor-beta stimulation of connective tissue growth factor expression by cultured human lung fibroblasts. *Arch Biochem Biophys* 2001; 395: 103–112.

205 Fu M, Zhang J, Zhu X, *et al.* Peroxisome proliferator-activated receptor gamma inhibits transforming growth factor beta-induced connective tissue growth factor expression in human aortic smooth muscle cells by interfering with Smad3. *J Biol Chem* 2001; 276: 45888–45894.

206 Guo B, Koya D, Isono M, Sugimoto T, Kashiwagi A, Haneda M. Peroxisome proliferator-activated receptor-gamma ligands inhibit TGF-beta 1-induced fibronectin expression in glomerular mesangial cells. *Diabetes* 2004; 53: 200–208.

207 Ghosh AK, Bhattacharyya S, Lakos G, Chen SJ, Mori Y, Varga J. Disruption of transforming growth factor beta signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor gamma. *Arthritis Rheum* 2004; 50: 1305–1318.

208 Honda K, Marquillies P, Capron M, Dombrowicz D. Peroxisome proliferator–activated receptor gamma is expressed in airways and inhibits features of airway remodeling in a mouse asthma model. *J Allergy Clin Immunol* 2004; 113: 882–888.

209 Woerly G, Honda K, Loyens M, *et al.* Peroxisome proliferator-activated receptors alpha and gamma down-regulate allergic inflammation and eosinophil activation. *J Exp Med* 2003; 198: 411–421.

210 Patel HJ, Belvisi MG, Bishop-Bailey D, Yacoub MH, Mitchell JA. Activation of peroxisome proliferator-activated receptors in human airway smooth muscle cells has a superior anti-inflammatory profile to corticosteroids: relevance for chronic obstructive pulmonary disease therapy. *J Immunol* 2003; 170: 2663–2669.

211 Benayoun L, Letuve S, Druilhe A, *et al.* Regulation of peroxisome proliferator-activated receptor gamma expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *Am J Respir Crit Care Med* 2001; 164: 1487–1494.

212 Han C, Demetris AJ, Liu Y, Shelhamer JH, Wu T. Transforming growth factor-beta (TGF-beta) activates cytosolic phospholipase A2alpha (cPLA2alpha)-mediated prostaglandin E2 (PGE)2/EP1 and peroxisome proliferator-activated receptor-gamma (PPAR-gamma)/ Smad signaling pathways in human liver cancer cells. A novel mechanism for subversion of TGF-beta-induced mitoinhibition. *J Biol Chem* 2004; 279: 44344–44354.

213 Unemori EN, Pickford LB, Salles AL, *et al.* Relaxin induces an extracellular matrix-degrading phenotype in human lung fibroblasts *in vitro* and inhibits lung fibrosis in a murine model *in vivo*. *J Clin Invest* 1996; 98: 2739–2745.

214 Wyatt TA, Sisson JH, Forget MA, Bennett RG, Hamel FG, Spurzem JR. Relaxin stimulates bronchial epithelial cell PKA activation, migration, and ciliary beating. *Exp Biol Med (Maywood)* 2002; 227: 1047–1053.

215 Puddicombe SM, Polosa R, Richter A, *et al.* Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *FASEB J* 2000; 14: 1362–1374.

216 Vermeer PD, Einwalter LA, Moninger TO, *et al.* Segregation of receptor and ligand regulates activation of epithelial growth factor receptor. *Nature* 2003; 422: 322–326.

217 Strachan L, Murison JG, Prestidge RL, Sleeman MA, Watson JD, Kumble KD. Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. *J Biol Chem* 2001; 276: 18265–18271.

218 Aida S, Tamai S, Sekiguchi S, Shimizu N. Distribution of epidermal growth factor and epidermal growth factor receptor in human lung: immunohistochemical and immunoelectron-microscopic studies. *Respiration* 1994; 61: 161–166.

219 Polosa R, Prosperini G, Leir SH, Holgate ST, Lackie PM, Davies DE. Expression of c-erbB receptors and ligands in human bronchial mucosa. *Am J Respir Cell Mol Biol* 1999; 20: 914–923.

220 Amishima M, Munakata M, Nasuhara Y, *et al.* Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. *Am J Respir Crit Care Med* 1998; 157: 1907–1912.

221 Polosa R, Puddicombe SM, Krishna MT, *et al.* Expression of c-erbB receptors and ligands in the bronchial epithelium of asthmatic subjects. *J Allergy Clin Immunol* 2002; 109: 75–81.

222 Chen P, Xie H, Sekar MC, Gupta K, Wells A. Epidermal growth factor receptor-mediated cell motility: phospholipase C activity is required, but mitogen-activated protein kinase activity is not sufficient for induced cell movement. *J Cell Biol* 1994; 127: 847–857.

223 Chen P, Gupta K, Wells A. Cell movement elicited by epidermal growth factor receptor requires kinase and autophosphorylation but is separable from mitogenesis. *J Cell Biol* 1994; 124: 547–555.

224 Xie H, Pallero MA, Gupta K, *et al.* EGF receptor regulation of cell motility: EGF induces disassembly of focal adhesions independently of the motility-associated PLCgamma signaling pathway. *J Cell Sci* 1998; 111: 615–624.

225 Shibamoto S, Hayakawa M, Takeuchi K, *et al.* Tyrosine phosphorylation of beta-catenin and plakoglobin enhanced by hepatocyte growth factor and epidermal growth factor in human carcinoma cells. *Cell Adhes Commun* 1994; 1: 295–305.

226 Manske M, Bade EG. Growth factor-induced cell migration: biology and methods of analysis. *Int Rev Cytol* 1994; 155: 49–96.

227 Thompson KL, Assoian R, Rosner MR. Transforming growth factor-beta increases transcription of the genes encoding the epidermal growth factor receptor and fibronectin in normal rat kidney fibroblasts. *J Biol Chem* 1988; 263: 19519–19524.

228 Danielpour D, Kim KY, Winokur TS, Sporn MB. Differential regulation of the expression of transforming growth factor-beta s 1 and 2 by retinoic acid, epidermal growth factor, and dexamethasone in NRK-49F and A549 cells. *J Cell Physiol* 1991; 148: 235–244.

229 Bennett KL, Plowman GD, Buckley SD, Skonier J, Purchio AF. Regulation of amphiregulin mRNA by TGF-beta in the human lung adenocarcinoma cell line A549. *Growth Factors* 1992; 7: 207–213.

230 Mishima H, Nakamura M, Murakami J, Nishida T, Otori T. Transforming growth factor-beta modulates effects of epidermal growth factor on corneal epithelial cells. *Curr Eye Res* 1992; 11: 691–696.

231 Beckmann JD, Stewart A, Kai M, Keeton TP. Controls of EGF-induced morphological transformation of human bronchial epithelial cells. *J Cell Physiol* 2001; 189: 171–178.

232 Serra R, Pelton RW, Moses HL. TGF beta 1 inhibits branching morphogenesis and N-myc expression in lung bud organ cultures. *Development* 1994; 120: 2153–2161.

233 Zhao J, Bu D, Lee M, Slavkin HC, Hall FL, Warburton D. Abrogation of transforming growth factor-beta type II receptor stimulates embryonic mouse lung branching morphogenesis in culture. *Dev Biol* 1996; 180: 242–257.

234 Zhao J, Sime PJ, Bringas P Jr, Gauldie J, Warburton D. Epithelium-specific adenoviral transfer of a dominant-negative mutant TGF-beta type II receptor stimulates embryonic lung branching morphogenesis in culture and potentiates EGF and PDGF-AA. *Mech Dev* 1998; 72: 89–100.

235 Zhao J, Lee M, Smith S, Warburton D. Abrogation of Smad3 and Smad2 or of Smad4 gene expression positively regulates murine embryonic lung branching morphogenesis in culture. *Dev Biol* 1998; 194: 182–195.

236 Warburton D, Seth R, Shum L, *et al.* Epigenetic role of epidermal growth factor expression and signalling in embryonic mouse lung morphogenesis. *Dev Biol* 1992; 149: 123–133.

237 Hamilton LM, Puddicombe SM, Thornber MD, *et al.* The effect of corticosteroids and β-agonists on protein tyrosine phosphorylation in bronchial epithelial cells. *Am J Resp Crit Care Med* 2003; 167: A395.

238 Lordan JL, Bucchieri F, Richter A, *et al.* Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. *J Immunol* 2002; 169: 407–414.

239 Vargaftig BB, Singer M. Leukotrienes mediate part of Ova-induced lung effects in mice via EGFR. *Am J Physiol Lung Cell Mol Physiol* 2003; 285: L808–L818.

240 Perrais M, Pigny P, Copin MC, Aubert JP, Van SI. Induction of MUC2 and MUC5AC mucins by factors of the epidermal growth factor (EGF) family is mediated by EGF receptor/Ras/Raf/extracellular signal-regulated kinase cascade and Sp1. *J Biol Chem* 2002; 277: 32258–32267.

241 Takeyama K, Fahy JV, Nadel JA. Relationship of epidermal growth factor receptors to goblet cell production in human bronchi. *Am J Respir Crit Care Med* 2001; 163: 511–516.

242 Hamilton LM, Torres-Lozano C, Puddicombe SM, *et al.* The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma. *Clin Exp Allergy* 2003; 33: 233–240.

243 Barbier AJ, Poppleton HM, Yigzaw Y, *et al.* Transmodulation of epidermal growth factor receptor function by cyclic AMP-dependent protein kinase. *J Biol Chem* 1999; 274: 14067–14073.

244 Kimura M, Ogihara M. Stimulation by transforming growth factor-alpha of DNA synthesis and proliferation of adult rat hepatocytes in primary cultures: modulation by alpha- and beta-adrenoceptor agonists. *J Pharmacol Exp Ther* 1999; 291: 171–180.

245 Kibe A, Inoue H, Fukuyama S, *et al.* Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. *Am J Respir Crit Care Med* 2003; 167: 50–56.

246 Takeyama K, Dabbagh K, Lee HM, *et al.* Epidermal growth factor system regulates mucin production in airways. *Proc Natl Acad Sci USA* 1999; 96: 3081–3086.

247 Wong WS, Leong KP. Tyrosine kinase inhibitors: a new approach for asthma. *Biochim Biophys Acta* 2004; 1697: 53–69.

248 Malaviya R, Chen CL, Navara C, *et al.* Treatment of allergic asthma by targeting janus kinase 3-dependent leukotriene synthesis in mast cells with 4-(3′, 5′-dibromo-4′-hydroxyphenyl)amino-6,7-dimethoxyquinazoline (WHI-P97). *J Pharmacol Exp Ther* 2000; 295: 912–926.

249 D'Oro U, Vacchio MS, Weissman AM, Ashwell JD. Activation of the Lck tyrosine kinase targets cell surface T cell antigen receptors for lysosomal degradation. *Immunity* 1997; 7: 619–628.