REVIEW

Transforming growth factor β and severe asthma: A perfect storm

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Received 8 May 2014; accepted 21 August 2014
Available online 6 September 2014

Summary
Asthma is a chronic inflammatory airway disease involving complex interplay between resident and infiltrative cells, which in turn are regulated by a wide range of host mediators. Identifying useful biomarkers correlating with clinical symptoms and degree of airway obstruction remain important to effective future asthma treatments. Transforming growth factor β (TGF-β) is a major mediator involved in pro-inflammatory responses and fibrotic tissue remodeling within the asthmatic lung. Its role however, as a therapeutic target remains controversial. The aim of this review is to highlight its role in severe asthma including interactions with adaptive T-helper cells, cytokines and differentiation through regulatory T-cells. Associations between TGF-β and eosinophils will be addressed and the effects of genetic polymorphisms of the TGF-β1 gene explored in the context of asthma. We highlight TGF-β1 as a potential future therapeutic target in severe asthma including its importance in identifying emerging clinical phenotypes in asthmatic subjects who may be suitable for individualized therapy through TGF-β modulation.

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http://dx.doi.org/10.1016/j.rmed.2014.08.008
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Introduction

Asthma is a complex chronic inflammatory lung disease where eosinophils, T-lymphocytes, macrophages, mast cells, neutrophils and epithelial cells play key roles in releasing cellular mediators including cytokines, chemokines and growth factors that all influence disease. Inhaled allergens sensitize dendritic cells to stimulate the proliferation of T helper type 2 (Th2) cells and subsequent release of cytokines that include interleukin IL-4, IL-5 and IL-13 [1]. Sensitized epithelial cells also release profibrotic mediators that include transforming growth factor-β (TGF-β), fibroblast growth factor (Fgf) and endothelin, stimulating fibroblasts and myofibroblasts to release collagen, proteoglycan, and glycoproteins that induce airway thickening [2]. These mediators control the chronic inflammation observed that induces bronchoconstriction and airway remodeling with subsequent structural airway changes. Allergic asthma, representing a subset of asthmatics is characterized by chronic mucosal Th2 dominated inflammation.

TGF-β plays a central role in the complex relationship between the propagation of the inflammatory cascade within the airway and association with suppressor T cell immune function. This review aims to highlight the evidence for the role of TGF-β in severe asthma which while contributing to disease pathogenesis remains less well-studied. Its role in the asthmatic airway has been controversial likely because of its complex signaling pathways and immune interactions that influence several differing asthma phenotypes. As both airway remodeling and poor treatment response are significant in severe asthma, it would appear logical that TGF-β would be relevant in this context and warrants review [3]. Severe asthma is a spectrum of disease with significant morbidity and mortality [4]. The National Heart, Lung, and Blood Institute (NHLBI) Severe Asthma Research Program identifies five clusters of phenotypically distinct individuals with severe asthma by performing cluster analysis. The groups range from early onset atopic asthma with normal lung function to subjects with severe airflow obstruction (Table 1) [5].

Airway remodeling in asthma constitutes subepithelial fibrosis, deposition of extracellular matrix protein, goblet cell hyperplasia, mucus gland and smooth muscle hypertrophy, and epithelial damage [6–8]. Inflammatory pathways regulate all such features [8]. Several key mediators including TGF-β, vascular endothelial growth factor (VEGF), ADAM metalloproteinase domain 33 (ADAM-33), matrix metalloproteinase-9 (MMP-9) and the Th2 cytokine family including IL-5, −13 and −14 have been identified.

TGF-β has been a focal point of considerable investigation as both a mediator and effector molecule in the Th2 driven immune cascade. Produced by numerous cell types including epithelial cells, eosinophils, macrophages, and fibroblasts, it is involved in epithelial transformation, subepithelial fibrosis, airway smooth muscle (ASM) remodeling, microvascular changes, and mucus production. Insight into mechanisms of TGF-β signaling provides key information into the crosstalk between activation pathways, T-regulatory (Treg) signaling, and immune cell interactions hence its importance in the context of asthma.

Table 1 Demographics, clinical characteristics and biomarker subsets classified by cluster analysis. Five clinical phenotypes of asthma identified using unsupervised hierarchical cluster analysis [5]. Clusters contain subjects who meet the American Thoracic Society definition of severe asthma and correlate with phenotypes that can be based on the outlined clinical variables. Definition of abbreviation: FEV1 — Forced expiratory volume in 1 s, FVC — Forced vital capacity, BMI — Body mass index.

<table>
<thead>
<tr>
<th>Cluster group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>FEV1 (%)</td>
<td>≥108</td>
<td>68–108</td>
<td>68–108</td>
<td>&lt;68</td>
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<td>Sputum neutrophils (%)</td>
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<td>33</td>
<td>38</td>
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<td>48</td>
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<tr>
<td>Representative patient profile</td>
<td>Younger female patient with childhood onset asthma</td>
<td>Older female patient with childhood onset asthma</td>
<td>Older female patient with high BMI and late onset asthma</td>
<td>Adult patient with childhood onset asthma and atopy</td>
<td>Older female patient with late onset asthma and minimal atopy</td>
</tr>
</tbody>
</table>
Key pathological features of severe asthma share processes occurring in idiopathic pulmonary fibrosis and pulmonary arterial hypertension [9]. These include barrier dysfunction, smooth muscle proliferation and accumulation of lamina reticularis, myofibroblasts and smooth muscle cells that lead to fibrotic airway remodeling [10,11]. Characterization of pathophysiologic sub-phenotypes of severe refractory asthma is beneficial in expanding our understanding of the underlying biologic factors that are potentially 'druggable' therapeutic targets. For instance, sputum eosinophilia identified by cluster analysis is characteristic of later onset asthma (>12 years), severe eosinophilic inflammation and association with nasal polyposis and rhinosinusitis [12–15]. Targeting eosinophilia with the IL-4-blocker dupilumab has demonstrated fewer asthma exacerbations and a reduced level of Th2-associated inflammatory markers [16]. Treatment of severe refractory asthma remains a key clinical challenge and is associated with a high and disproportionate consumption of healthcare resources. Therefore, emerging avenues to improve understanding and therapy is critical for progress in the field [12,17].

Transforming growth factor beta (TGF-β)

The TGF-β superfamily of ligands are multifunctional regulators implicated in various biological processes within the airways including alveolarization, epithelial and endothelial barrier function, immune cell recruitment, platelet aggregation, apoptosis, cell differentiation and proliferation [18]. These regulators consist of more than 33 members including TGF-β, bone morphogenetic proteins (BMP), growth and differentiation factors (GDFs), activins and inhibins. This super-family in humans demonstrates three mammalian isoforms (TGF-β1, 2 and 3) that share 60–80% homology.

TGF-β isoforms have a number of specific and shared roles in the regulation of airway inflammation and the remodeling process [19]. The three isoforms described each have important roles in the regulation of inflammation, cell growth and differentiation [20]. TGF-β bound to latency-associated peptide (LAP) is inactive and therefore understanding its regulation is pivotal to appreciating functional consequences [21,22]. Although each isoform is encoded by differing genes, important properties are shared such as common cell surface receptors and cellular targets [23].

The expression of TGF-β1 is altered in the asthmatic airway and current evidence suggests this is the case for both human and animal settings. Known as the ubiquitous prototype of this family, TGF-β1 is most prevalent in mammalian tissues [24]. Produced as an inactive latent complex made up of LAP and latent TGF-β-binding protein (LTBP), it is targeted to the extracellular matrix (ECM) [25]. Maturation of inactive complexes depends on dissociation of covalent bonds that in turn rely on pH changes enabling binding to cell surface receptors. TGF-β1 activation however requires further binding of α(ν) integrin to a sequence in its pro-domain [23]. Its ability to recruit single transmembrane subunits into a hetero-oligomer enables TGF-β1 to form a functional receptor (Fig. 1). TGF-β1 signal transduction occurs through Smad-dependent or independent pathways [26]. TGF-β1 type I and II receptors both transmembrane serine/threonine kinases (e.g. activin receptor-like kinase 5 (ALK5)) stimulate a cascade of intra-

Figure 1  TGF-β1 activation. Activation of TGF-β1 receptors leads to dimerization [1] and phosphorylation of receptor-activated Smad proteins [2] that act as transcription factors to regulate gene expression. Smad 2 and 3 form complexes with Smad 4 [3] that translocate into the nucleus [4]. In a negative feedback loop, inhibitory Smad 7 induced by Smad 3 blocks TGF-β signaling by binding to the type I receptor.[5].
cytoplasmic intermediates termed Smads which facilitate signaling, and are surrogates to assess levels of pathway activity. Activated TGF-β1 receptors lead to phosphorylation of Smad proteins that in turn are transcription factors regulating gene expression. Smads 2 and 3 form complexes with Co-Smad (Smad 4) and translocate into the nucleus. In a negative feedback loop, inhibitory Smad (Smad 7) induced by Smad 3 blocks TGF-β signaling by type I receptor binding (Fig. 1). Increased TGF-β1 signaling as evidenced by increased phospho-Smad 2 levels, have been reported in the bronchial biopsies of asthmatic subjects [27]. Smad 3 signaling however is required for the recognized allergen-induced airway remodeling and myofibroblast accumulation [28]. TGF-β1 also activates Smad independent pathways such as the mitogen-activated protein kinase (MAPK) pathways: extra cellular signal-regulated kinase (ERK), p38 MAPK and c-Jun-N-terminal kinase (JNK) [29–31].

TGF-β2 isoforms are expressed by eosinophils and are the predominant form in severe allergic asthma where it promotes profibrotic responses affecting airway remodeling in addition to regulating mucin production and impacting on non-asthma related atopy in children [32–35]. Interestingly, TGF-β3 is implicated in the development of a severe phenotype of chronic airway remodeling induced by house dust mite (HDM) [35]. In this setting, increased mucus production, collagen deposition, and a dysregulated cytokine environment are observed. Our understanding of isoform specific effects of TGF-β in the context of asthma is only in its infancy and will need to be considered in future treatment studies.

While TGF-β is secreted by almost all immune cells including fibroblasts, endothelial cells and vascular/ASM cells [36–39], airway epithelia remain the major site of TGF-β1 expression. In asthmatic airways however, TGF-β expression is markedly increased and further augmented by the infiltrative inflammatory groups of cells that serve as an additional reservoir. An example of this is active eosinophil recruitment by chemokine ligand 5 (CCL-5) and CCL-11 that account for 80% of TGF-β expression in asthma [40–42]. A reduced deposition of the TGF-β binding proteoglycan decorin within the asthmatic airway wall further increases its bioavailability in this context [16]. Owing to its abundance in the asthmatic airway and its complex but well understood signaling mechanisms, it appears logical that therapies directed at TGF-β1 be considered in the context of severe refractory asthma.

TGF-β airway remodeling

Airway remodeling is the pathophysiologic modification of normal airway wall structure comprising a complex re-organization of the wall’s molecular and cellular constituents [43]. Including a repair process in response to airway wall injury, the dysregulated ensuing inflammation leads to structural change that results in loss of epithelial integrity, basement membrane thickening, subepithelial fibrosis, mucus gland and goblet-cell hyperplasia, smooth muscle hypertrophy and increased airway vascularity [44–46].

Fibrosis is the end-point of such a process within the ECM that also affects other organs including the liver and kidney. During its induction, TGF-β1 promotes target genes including connective tissue growth factor (CTGF), z-smooth muscle actin, collagen and plasminogen activator inhibitor [43,47]. In mild-to-moderate asthma, such modifications are partially reversible, however in severe chronic asthma, this becomes irreversible. Studies show that airway remodeling is primarily responsible for symptoms associated with decreased pulmonary function [48].

In the context of asthma, TGF-β induces both anti- and pro-apoptotic effects in airway epithelial cells [47]. Anti-apoptotic effects are mediated through the SMAD 2/3 pathway and occur in the absence of chemical or physical stress. Chronic allergic exposure allows TGF-β1 to induce apoptosis through activation of MAPK-signaling [49]. Injury to surface epithelium follow aggravated by a dysregulated repair process causing detachment of these cells and epithelial–mesenchymal transition (EMT) [50]. TGF-β1 also affects sub-epithelial fibrosis by increasing deposition of ECM such as type I and III collagen, fibronectin and proteoglycans. By inducing fibroblastic differentiation to myofibroblasts, increased proliferation follows [51,52]. Fibroblasts maintain crucial roles during the pulmonary fibrotic response which are dependent on activation of TGF-β1 via ECM receptor integrins [53,54]. Additionally, TGF-β1 induces myofibroblast differentiation and collagen production in nasal polyposis re-iterating its important role in airway disease [55].

Matrix metalloproteinases (MMPs) are key enzymes responsible for ECM remodeling, cell migration, and airway epithelial repair [56]. Cytokines including TGF-β1 play pivotal roles in MMP-9 production and secretion [57]. The quantity of submucosal neutrophils and macrophages, but not eosinophils, is significantly higher in asthmatics and in addition MMP-9 staining of the subepithelial basement membrane detected [58]. Neutrophils from allergic asthmatics produce and release MMP-9 upon direct allergen exposure [59]. In tandem with up-regulation of TGF-β1 gene expression by neutrophil elastase (NE) [39], activated neutrophils release reactive oxygen species (ROS) that exacerbate the airway remodeling process by promoting goblet cell hyperplasia [60].

As described previously, TGF-β is rendered latent by binding to LAP, which is in turn is covalently bound to a fibrillarin protein LTBP. Inflammatory ECM degradation releases latent TGF-β–LAP complexes that dissociate and increase active TGF-β bioavailability within the airway [61]. LAP complexes also act as substrates for MMP-2, 9, 13 and –14 [61]. MMPs alter transcription of ECM proteins responsible for collagen fibril laydown [62,63], and function as proteases that lyse ECM components with release of bioactive byproducts. In turn pro-inflammatory IL-13 stimulates collagen type I production by airway fibroblasts in an MMP and TGF-β1 dependent manner in asthma [64].

TGF-β also stimulates IL-6 which acts on ASM cells to induce release of eotaxin and VEGF concurrently mediating smooth muscle driven mast cell activation [65–67]. Despite our depth of knowledge of epithelial repair mechanisms, key questions relating to how activated cells organize into a matrix that functions as a regulator of the repair process still remain unanswered [56].

By far, the most crucial advance of our understanding of TGF-β1 function within the airway is its regulatory role in EMT. Pulmonary alveolar epithelium, primary human
bronchial epithelia and airway epithelial cells isolated from asthmatic lungs have all demonstrated the ability to undergo EMT upon TGF-β1 stimulation. This process can be inhibited with use of TGF-β1 inhibitors such as Smad 7 [68,69]. In addition, TGF-β1 influences EMT degradation by delicately balancing MMP and tissue-inhibitor of metalloproteinase (TIMP) activity during airway remodeling [57,70].

Sub-epithelial basement membrane (BM) thickness correlates with asthma severity [71,72]. Severe asthma is characterized by an increased ASM mass extending from the trachea to the smallest bronchioles and alveolar ducts [73–76]. Increases in ASM mass are a key feature of the airway remodeling process that includes MAPK mediated thickening of the BM. It is predominantly induced by migration of MMPs toward the epithelium to form new matrix bundles [77]. TGF-β mediated proliferation of collagen, fibronectin, serum and platelet-derived growth factor all play a role and require signaling through RGD (Arg-Gly-Asp) binding integrins [78]. This leads to stiffer airways and greater fixed airflow obstruction characteristic seen in severe asthma [79].

TGF-β1 immunosuppressive effects and regulatory inflammatory tolerance

In its role as a pleiotropic and multifunctional growth factor, TGF-β1 is a master regulator of immune responses resulting in fibrosis. As a potent anti-inflammatory, its chemo-attractive properties cause accumulation of macrophages and granulocytes in local inflammatory sites [80]. Additionally, it promotes development and differentiation of Th17 and forkhead box P3 (FoxP3) regulatory T-cells while inducing IL-9-producing T-helper cells [24,81,82].

Regulatory T cells (Treg) are a subpopulation of CD4+ T cells that maintain immunological self-tolerance and control the development of autoimmune disease [83]. Treg cells express the high affinity interleukin-2 (IL-2) receptor (CD25) and the transcription factor, Foxp3 that play critical roles in the function and development of Tregs [84]. Two subtypes of Foxp3+ CD25+ CD4+ Tregs are described: i) natural Treg (nTreg) from the thymus and ii) acquired/induced Treg (iTreg) that develop peripherally in response to TGF-β1 stimulation [84]. Foxp3+ CD25+ CD4+ Tregs regulate allergy, auto-immunity, transplant rejection, tumor immunity, and microbial responses [83]. Immunosuppressive Treg effects are mediated by IL-2R (CD25) and indirectly through antigen presenting cells (APCs) which in turn are mediated by cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [85,86]. Although Foxp3+ CD4+ Tregs have suppressive capabilities, CD4 Foxp3 expression alone does not always indicate suppressive consequences. Transient Foxp3 expression in subsets of TGF-β1-induced iTregs do not functionally equate to nTreg [87,88]. Emerging evidence provide insight into the regulatory behavior of Tregs in the context of immune function, stimulators in certain conditions whilst suppressive in others largely dependent on the context, location, conditions and target cells in question [89,90].

TGF-β1 suppresses immune responses through inhibition of inflammatory cell function and promotion of Treg production. TGF-β functions as an inducer for Treg generation [91]. It converts naive CD25− T cells to regulatory CD25+ cells, and additionally regulates Treg suppressive function [92–94]. Antigens may induce TGF-β−producing Treg (Th3), which in turn mediates immunosuppression [95]. Blocking TGF-β in vivo abolishes tolerance and impairs CD25+ Foxp3+ Treg induction; however the precise role of TGF-β in inducing mucosal Th3 cells is unclear. Evidence underpinning molecular mechanisms that drive Treg proliferation, activation, survival and targeting will in the future likely provide potential therapeutic targets in severe asthma.

By regulation of the homeostasis of lymphocytes and Tregs, which in turn inhibit Th1 and Th2 responses, inflammation can be restricted. TGF-β1 prevents house-dust mite-induced allergic reactions in murine lungs by generating CD4+ and CD25+ Treg cells [96]. Administration of CD4+ and CD25+ Treg cells in an experimental setting reduces established lung eosinophilia, Th2 infiltration and promotes expression of IL-5, IL-13 and TGF-β1 in airway specimens [97]. TGF-β1 directly delivered into the trachea suppresses allergen-induced inflammation suggestive of a potential therapeutic role [98]. The evidence underlying the molecular mechanisms driving immunosuppressive Treg cell proliferation, activation, survival and subsequent targeting however remain to be elucidated.

Pro-inflammatory role of TGF-β1

TGF-β1 also plays a key role in inflammation acting as a potent chemotactic factor and activator for a variety of inflammatory cells. Both inflammatory and structural cells produce TGF-β1 contributing to the increased levels observed in BAL fluid obtained from asthmatic airways [20,99,100]. Increased TGF-β1 is associated with higher macrophage concentrations following allergen challenge and significantly amplified inflammation through induction of Th17 cells [42,80,101].

TGF-β1 airway concentrations have been associated with airflow limitation in a cross-sectional sample of children with severe asthma [102]. Increased expression of airway TGF-β1 is coupled with an increase in BAL macrophages and concentrations of lipid peroxidation biomarkers 8-isoprostanes and malondialdehyde. Oxidative stress may thus mediate TGF-β1 effects and promote airway remodeling in children with severe asthma. Nuclear factor-like 2 (Nrf2) is another factor responsible for regulating glutatione, antioxidant responses and reducing ASM proliferation in severe asthma [103]. This pathway is also disrupted in severe asthma due to chronic oxidative stress [104]. TGF-β also plays an important role here, suppressing Nrf2 activity in ASM cells and consequently promoting a dysregulated antioxidant response [103]. What remains lacking is a definitive link between elevated airway TGF-β1 and progressive loss of pulmonary function from an oxidative perspective.

Increased expression and activation of airway TGF-β1 coupled with an increased airway oxidant burden underlies the physiological alterations accompanying severe asthma in children. Traffic-related air pollutants and cigarette smoke cause oxidant mediated airway inflammation and
are also associated with asthma in children [105,106]. Children with the TGF-β –509 TT genotype are at higher risk of asthma occurrence if they live near a freeway or were exposed to tobacco smoke in utero [107]. In an experimental model of rat tracheal explants, two-to-threefold increases in TGF-β1 expression was observed when the explants were exposed to urban air particles [108] and cigarette smoke [109]. Cigarette smoke exposure is known to enhance antigen-induced mast cell activation via TGF-β1/Smad signaling pathways in a mouse model of allergic asthma [110]. Kang et al. (2007) demonstrated that cigarette smoking is a potent stimulator of IL-18 release from pulmonary macrophages [111]. In turn, activated macrophages and airway epithelial cells release IL-18 that contributes to the proinflammatory cascade in asthma [112].

For instance, it enhances remodeling through production of IFN-γ, IL-13 and TGF-β1 in an asthma mouse model [113]. In further animal work, chronic exposure to house dust mite demonstrated elevated IL-4, IL-13 and TGF-β1 accompanied by goblet cell hyperplasia, subepithelial fibrosis and increased ASM surface area [114]. Taken together this current evidence suggests that inflammatory and allergenic mechanisms both influence the TGF-β pathway, and insight into the effect of cigarette smoking on the induction of TGF-β1 release from other cells remains to be explored.

Current guidelines for randomized controlled trial recruitment include recruitment of patients “free” of co-morbid disease that demonstrate good inhaler technique, and an asthma diagnosis. Cigarette smoking for example typically results in patients being excluded from consideration. Consequently, there is exclusion of current and ex-smokers in addition to patients with at least a ten-pack year smoking history. Given the foundation of knowledge on the effects of cigarette smoking on murine models of asthma [109,110], there is an inherent bias in current trial recruitment preventing the extrapolation of understanding in vivo TGF-β mediated effects in human asthmatic smokers [115].

Th2 cytokines IL-5 and IL-13 have been intimately linked to airway TGF-β1. IL-5 receptor deficient mice have undetectable airway eosinophils and reduced TGF-β1 concentrations concomitant with decreased fibrotic change in a chronic exposure model [116]. Conversely, IL-5 overexpression leads to increased eosinophils, TGF-β1, higher collagen content and more fibrotic consequences. Inhibiting TGF-β1 signaling specifically in T-cell subsets has led to enhanced airway inflammation, hypersensitivity and increased Th2 cytokine production hence its potential therapeutic role has been controversial [117]. IL-13 induces TGF-β1 expression through synergistic mechanisms on airway processes including remodeling and fibrosis [64,118], IL-13 deficient mice have decreased signal transduction, activation of transcription-6 (STAT6) phosphorylation and limited TGF-β1 activity following allergenic stimulation resulting in attenuated bronchial hyperresponsiveness, ASM hyperplasia, and immunoglobulin-E (IgE) synthesis [34,119].

An increased quantity and size of goblet cells and mucosal glands are characteristic of asthma-related airway obstruction. TGF-β2 importantly regulates the transcription and translation of mucin in bronchial epithelial cells [34] and anti-TGF-β treatment in animal models of asthma illustrate a decreased goblet cell burden [120].

Expression of VEGF promotes angiogenesis and permeability. Changes to airway microvasculature accompany airway remodeling and result in VEGF release. TGF-β1 promotes further microvascular congestion through up-regulation of pro-angiogenic factors such as VEGF in the severe asthmatic airway [121]. Interestingly, VEGF inhibition has been shown to attenuate peribronchial fibrosis through effects on the phosphoinositide 3-kinase(PI3K)/Akt pathway [122].

**TGF-β and eosinophil crosstalk**

Asthma severity is related to a relationship between TGF-β expression and the presence of submucosal eosinophils [40–42,123]. Minshall et al. examined bronchial biopsies of severe asthmatics and demonstrated that 65% of TGF-β1 mRNA-positive cells were eosinophils and that 75% of eosinophils were positive for TGF-β1 mRNA, findings reflective of earlier work performed by others [40,42,124]. The study interestingly also reported strong correlations between mRNA-positive TGF-β1 cells beneath the basement membrane and degree of eosinophilia within the same compartment. The extent of TGF-β1 expression is significantly higher in those with severe compared to mild asthma. TGF-β1 is therefore upregulated in severe asthma and a strong correlation exists between TGF-β1 expression, eosinophil burden and asthma severity.

In the upper airway, eosinophils remain a major source of TGF-β in patients with allergic disease. Linear relationships are evident between the degree of eosinophilia and TGF-β1 expression in patients with rhinosinusitis [47]. Similar associations are observed with atopic dermatitis where Th2-predominant infiltration similar to that in asthma is the major driver of the inflammatory state [125]. Persistent eosinophilia relates to asthma disease severity and correlates with fixed airflow obstruction, frequent exacerbations, hospitalizations and increased intubation rates [15,126,127]. Targeting the eosinophil itself has been effective and studies of the anti-IL-5 agent, mepolizumab illustrates significant reduction in exacerbation rates in corticosteroid dependent patients. In addition, improved symptom control and quality of life is noted [128–130]. Flood-Page et al. attempted treatment of patients with mild atopic asthma with mepolizumab resulting in decreased tissue eosinophilia, lung TGF-β1 expression, reduced remodeling and ECM deposition [41]. Such observations were further reinforced when IL-5 knockout mice chronically exposed to allergens showed reductions in the pulmonary expression of TGF-β1 [131].

**Genetic TGF-β modifiers**

Gene polymorphisms in the TGF-β1 promoter region are associated with the development of asthma [9,132]. Several single nucleotide polymorphisms (SNPs) are associated with peripheral blood eosinophilia in asthmatic patients [133]. Lerodiakonou et al. (2013) identified asthmatic carriers of the TGF-β1 SNP rs6957-G to have significantly higher submucosal eosinophils and macrophages. In the same study, the SNPs rs1800469-T and rs1800470-C were associated with less FEV1 decline and rs4803455-A with accelerated FEV1...
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decline. Also of interest, three other SNPs that associated with increased airflow obstruction (rs6957, rs1800469, and rs1800470) were also linked to less rapid decline in non-smokers. Such data provide evidence of critical interactions between TGF-β1 SNPs and effects of smoking on asthma severity. Other disease associations link the polymorphisms with survival in non-small cell lung cancer [134], breast cancer [135], and risk of radiation pneumonitis [136].

A recent meta-analysis suggested that the −509/T polymorphism in TGF-β1 gene as a risk factor for asthma susceptibility and phenotype [137,138]. Polymorphism T869C has also been directly associated to asthma severity [139−142]. Furthermore, Freimuth et al. importantly concluded that differential inheritance of genetic loci, TGFbms, alters biological responses to reduced TGF-β1 signaling in an experimental asthma model [143]. TGF-β antagonists for treatment of lung diseases might therefore give diverse outcomes among patient cohorts, dependent on genetic variation. Recent work has also shown that patients with TGF-β receptor II mutations were strongly predisposed to the development of multiple immunologic phenotypes, including asthma, food allergies, eczema, allergic rhinitis, and eosinophilic gastrointestinal disease [144]. In Loey’s–Deitz syndrome (LDS), a condition with naturally occurring mutations in this gene, almost half of all patients had been diagnosed with asthma and increased eosinophil counts, elevated IgE and increased Th2 cytokines IL-5 and IL-13. T-cells sequestered from such patients showed increased Smad 2 and 3 phosphorylation in response to TGF-β1 that in turn enhanced its signaling. Angiotensin-II receptor blockade (e.g. losartan) interestingly mitigates TGF-β signaling in lymphocytes of LDS patients suggesting that this or other related treatment approaches hold promise in the prevention of major cardiovascular complications and perhaps the development of asthma if administered at an early stage [145,146]. Future work should re-visit the previously demonstrated interaction between blocking the renin-angiotensin system and effects on TGF-β1 signaling in asthma.

TGF-β targeted therapy

Current therapeutic guidelines for the treatment of asthma are ineffective at targeting any reversal of the airway structural remodeling process. Corticosteroids however have been shown to exhibit an inhibitory effect on TGF-β1 expression in a murine model of asthma hence illustrating some albeit minimal potential in affecting this irreversible process [147]. In humans however, a two-week course of some albeit minimal potential in affecting this irreversible expression in a murine model of asthma hence illustrating the structural remodeling process. Corticosteroids however are ineffective at targeting any reversal of the airway remodeling process, recent evidence identifies novel mechanistic effects of glucocorticoids on TGF-β signaling cross talk [148]. Glucocorticoids recruited the accessory TGF-β receptor, Tgfbr3, and Smad-1 to shift TGF-β signaling from the Tgfbr1/Smad2/3 axis to an Acvrl1/Smad1 axis in lung fibroblasts. Such findings illustrate the importance of glucocorticoids on the array of TGF-β mediated responses in inflammatory airway diseases.

In-vitro and -vivo evidence suggests a role for activin A, a promising target in regulating the inflammatory fibrotic remodeling process within the airway [149,150]. It is a member of the TGF-β superfamily through which inhibition, by follistatin, decreases mucus hypersecretion, sub-epithelial collagen deposition and thickening of the sub-epithelial smooth muscle, indicating a potential therapeutic role in the prevention of airway remodeling [151].

Tiotropium bromide reduced levels of TGF-β1 in BAL of OVA-sensitized mice. In addition, it abrogated airway hyper-responsiveness to serotonin and inhibited Th2 cytokine production [152]. Such inhibitory effects on acute versus chronic asthma are dependent on differential expression of muscarinic receptor subtypes [153]. Evidence by Schaafsma et al. highlights that simvastatin may inhibit TGF-β1-induced fibronectin expression in airway fibroblasts [154] which in turn attenuates fibroblast-to-myofibroblast transition in asthmatic settings [155].

Targeting TGF-β represents a major challenge as a target for severe asthma therapy. The complexity of its signaling network has thus far hindered the pursuit of specific drug candidates targeting TGF-β. Neutralizing antibodies that block TGF-β activity through phospho-Smad 2 appear at least in preliminary animal work to prevent lung fibrosis in asthma [156,157]. SD-208 has been trialed as a TGF-βRI kinase inhibitor in a chronic rat allergic asthma model [158]. Effects include inhibiting all TGF-β isoforms, bronchial and ASM cell proliferation and goblet cell hyperplasia all induced by exposure to allergens.

The effects of TGF-β1 on airway remodeling are variable and largely depend on the local environment and model used for investigation [47]. The therapeutic treatment of mice with anti-TGF-β antibodies significantly reduced pulmonary peri-bronchiolar ECM deposition, ASM proliferation, and mucus production without affecting established airway inflammation and Th2 cytokine production. These data suggest therefore that inflammation and remodeling may be uncoupled during a prolonged allergic challenge [120]. Chronic exposure to HDM demonstrated elevated IL-4, IL-13 and TGF-β1 levels accompanied by goblet cell hyperplasia, subepithelial fibrosis and increased ASM surface area [114]. Difficulties however arise as contrasting evidence has shown that anti-TGF-β treatment in the context of continuous or intermittent HDM exposure had no effect on the development of HDM-induced airway remodeling. TGF-β neutralization in fact exacerbated eosinophilic infiltrates and led to increased airway hyper-responsiveness in some cases [159]. These findings may in part be explained by the differential expression of TGF-β isoforms in response to duration of HDM exposure [35]. Taken together, this work suggests that we remain unclear as to the true benefits of targeting the TGF-β pathway and need to be fairly specific during the process to ensure beneficial as opposed to adverse effects.

Anti-TGF-β1 antibodies have been demonstrated to inhibit monocyte/macrophage recruitment and reduce eosinophil and lymphocyte numbers within the airway [19]. Treatment with mepacrine, a synthetic anti-malarial drug reduces TGF-β1 and subsequently sub-epithelial airway fibrosis [160]. The cytokine IFN-γ has been shown to downregulate TGF-β2 through Smad-7, however their therapeutic consequences in the asthmatic airway remain undetermined [161].
An integrated approach to target factors regulating inflammation, cell migration, and tissue remodeling in asthmatic airways has important pathophysiological and clinical consequences that must be recognized in the context of emerging asthma therapeutics that target Th2-driven inflammation[162]. Use of antisense oligonucleotide therapy against TGF-ß has been applied in animal models in an attempt to reduce postoperative scarring in ocular glaucoma [163]. Similarly, adenoviral delivery to over-express Smad-2 within the lung results surprisingly in an increased sub-epithelial collagen deposition and ASM hyperplasia. These effects lead to thickening of the ASM layer following HDM challenge [164] (Fig. 2). In view of contrasting outcomes to anti-TGF-ß approaches particularly in the context of allergy and asthma, future work focused on the complex signaling network involved will be necessary before we can progress to valid clinical study.

**Conclusion and future directions**

Suboptimal patient selection for certain asthmatic clinical studies may at least partially account for the contrasting pool of evidence with regards differences in TGF-ß1 immunohistochemical staining between asthmatics and healthy controls [32,165,166]. This is further complicated by our emerging understanding of the various clinical phenotypes within the asthma spectrum. Airway TGF-ß1 release is inducible and transient however does require an inflammatory or antigenic challenge [47]. Effective modulation of TGF-ß is therefore dependent on proliferative factors that induce its transient release in asthma subsequently regulating ASM thickness [167–169]. Contrasting effects of TGF-ß may be due to differences in cell origin (airway versus alveolar, transformed versus primary and human versus rodent), differences in matrix or integrin expression, or concentration-dependent responses [56]. Latent TGF-ß activation and isoform differentiation further complicates the asthmatic setting and adds to the complexity of its role in the diseased airway. Stored in large quantities within the airway ECM, it plays major roles in regulating inflammation and its consequences.

While down regulating the inflammatory process through the induction of immunosuppressive Tregs, it also initiates structural remodeling. A delicate balance between such phenomena defines the precise role for TGF-ß in the clinicopathophysiologic phenotypes observed in severe asthma. TGF-ß modulation is potentially useful in immunosuppression while pure global inhibition may have anti-fibrotic effects. Development of targeted TGF-ß therapy will likely need to be individualized specifically to the phenotypic spectrum of patients with severe asthma where immuno-suppression may be desirable if it is to become a successful component in future asthma care.

**Conflicts of interest**

None of the authors have any conflicts of interest to disclose with respect to this manuscript.
References


