Transforming Growth Factor β 1 Function in Airway Remodeling and Hyperresponsiveness The Missing Link?

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Abstract

The pathogenesis of asthma includes a complex interplay among airway inflammation, hyperresponsiveness, and remodeling. Current evidence suggests that airway structural cells, including bronchial smooth muscle cells, myofibroblasts, fibroblasts, and epithelial cells, mediate all three aspects of asthma pathogenesis. Although studies show a connection between airway remodeling and changes in bronchomotor tone, the relationship between the two remains unclear. Transforming growth factor β 1 (TGF- β 1), a growth factor elevated in the airway of patients with asthma, plays a role in airway remodeling and in the shortening of

various airway structural cells. However, the role of TGF- β 1 in mediating airway hyperresponsiveness remains unclear. In this review, we summarize the literature addressing the role of TGF- β 1 in airway remodeling and shortening. Through our review, we aim to further elucidate the role of TGF- β 1 in asthma pathogenesis and the link between airway remodeling and airway hyperresponsiveness in asthma and to define TGF- β 1 as a potential therapeutic target for reducing asthma morbidity and mortality.

Keywords: bronchoconstriction; airway smooth muscle; airway epithelium; cytokines; contraction

Asthma affects ~300 million people worldwide (1) and is a significant source of global morbidity and mortality. Asthma presents a substantial economic and financial burden to industrialized and developing countries (2), with direct health care costs reaching approximately \$50 billion annually in the United States alone (3). The management of asthma includes inhaled corticosteroids to combat inflammation and β 2-agonist bronchodilators to reverse airway smooth muscle (ASM) bronchoconstriction. Approximately 50% of patients with asthma, however, experience inadequate

control with current therapeutics (4). Because of the increasing prevalence and per-patient costs of asthma (5), an urgent need exists to determine therapeutic targets to reduce asthma-related morbidity.

Airway remodeling and airway hyperresponsiveness (AHR) are two defining characteristics of asthma. Airway remodeling, defined as cellular and molecular architecture changes that manifest as structural alterations in the airway wall, may be a major contributor to lung function decline in asthma (6). AHR is governed by the contraction of ASM, the key regulator of bronchomotor tone, and is

defined as exaggerated narrowing of the airway to stimuli (7, 8).

Airway Remodeling and AHR: A Direct Relation?

Airway remodeling and sustained AHR in asthma are closely correlated. The mechanisms by which each exacerbates the other, or whether a direct connection exists between the two, remain highly debated. Although it is suggested that airway inflammation precedes the development of both pathologies (9, 10), airway structural

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alterations may also independently modify AHR (11, 12). It is suggested that frequent asthma exacerbations evoke an airway injury–repair cycle that promotes sustained AHR by altering the structure and function of ASM cells (13, 14). In addition, recent literature suggests an uncoupling of airway inflammation and AHR in asthma (9, 11, 15, 16). Understandably, the link between injury–repair responses and AHR remains unclear.

Transforming growth factor β 1 (TGF- β 1), a cytokine elevated in the airway of patients with asthma (17, 18), contributes to airway structural cell remodeling in asthma (19, 20). In addition to facilitating structural cell remodeling, TGF- β 1 may participate in airway narrowing by increasing ASM contractile protein expression (21). Conversely, the act of ASM shortening alone activates TGF- β 1 signaling. Agonist-induced ASM bronchoconstriction (22) and myofibroblast contraction (23) induce the release of active TGF- β 1, which may augment both airway remodeling and airway narrowing. Furthermore, challenge with the contractile agonist methacholine induces active TGF- β 1 release and airway remodeling without affecting airway eosinophil recruitment, suggesting an uncoupling of airway inflammation from both AHR and remodeling (24). Thus, TGF- β 1 is emerging as a potential target mediating the connection between airway remodeling and AHR in asthma. In this review, we investigate TGF- β 1's role in airway remodeling and AHR to determine the joint position of TGF- β 1 in both arms of asthma pathogenesis, and we examine the targeting of the TGF- β 1 signaling pathway as a therapeutic for asthma-related illness.

TGF- β 1 Signaling

TGF- β 1 is a growth factor and cytokine involved extensively in the pathogenesis of asthma and other airway diseases. TGF- β 1 belongs to a family of growth factors that includes TGF- β 2, TGF- β 3, and the bone morphogenic protein kinases. TGF- β 1 is synthesized in a latent form that is secreted into the extracellular space or held near the extracellular matrix by integrins (25) (Figure 1). Before binding to its receptor, TGF- β 1 must be cleaved into its active form. Latent TGF- β 1 can be activated by integrins, reactive oxygen species (ROS), mechanical stress, and other mechanisms, as shown in Table 1.

Canonical TGF- β 1 signaling begins when activated TGF- β 1 binds to the serine-threonine kinase TGF- β type II receptor (T β R-II) (Figure 1). Subsequently, T β R-II recruits and phosphorylates TGF- β type I receptor (T β R-I) at serine/threonine residues. Activated T β R-I then phosphorylates Smad2 and Smad3 (Smad2/3) transcription factors. After association with Smad4, the phosphorylated Smad complex translocates to the nucleus to promote the transcription of target genes (26, 27). The Smad complex binds to DNA directly or indirectly via interaction with other proteins, recruiting coactivators to mediate the transcription of genes involved in a variety of pathways (28). In addition, TGF- β 1 signals through a variety of diverse, Smad-independent pathways that modulate $[Ca^{2+}]^i$ levels (29, 30), ras homolog gene family, member A (RhoA)/rho-associated protein kinase (ROCK) (31), MAPK, and ERK pathways (32, 33). Both Smad-independent and Smad-dependent pathways may mediate airway remodeling and AHR.

TGF- β 1 and Airway Disease

TGF- β 1 promotes the pathogenesis of several airway diseases involving inflammation and remodeling, including chronic obstructive pulmonary disease, parenchymal lung diseases, and asthma. Active TGF- β 1 is found to be elevated in the bronchoalveolar lavage fluid (17) and in bronchial biopsy samples (18) taken from subjects with asthma. In addition, TGF- β 1 expression is elevated in the bronchial epithelium (34), submucosa, subepithelial connective tissue, subepithelium (35), and ASM (35) of subjects with asthma (18). Although it is unclear which structural cell produces the majority of TGF- β 1 in the airway, epithelial-derived TGF- β 1 is suggested to be an important contributor to asthma pathogenesis (36). However, much of the elevation of TGF- β 1 in the airway of subjects with asthma may result from the secretion of TGF- β 1 by invading inflammatory cells in asthma (37–39), with eosinophils accounting for the largest source of TGF- β 1 in the airway (37).

TGF- β 1 itself plays a significant and complex role in the airway immune response, mediating a variety of proinflammatory or immunosuppressive effects. TGF- β 1 stimulates the recruitment of inflammatory cells to the airway and promotes the secretion of additional

growth factors and inflammatory cytokines (40, 41). Conversely, TGF- β 1 can suppress both lymphocyte proliferation (42, 43) and the production of certain cytokines (44). Although the detailed role of TGF- β 1 in airway inflammation is not covered in this review, the participation of TGF- β 1 in all aspects of asthma pathogenesis (inflammation, remodeling, and AHR) elevates the potential benefit of targeting the TGF- β 1 signaling pathway to reduce asthma morbidity and mortality.

Airway Remodeling and Proliferation

TGF- β 1 contributes to airway remodeling in asthma by mediating the differentiation, proliferation, apoptosis, and size of structural cells in the airway (19).

These reversible or partially irreversible modifications alter the composition of the airway wall, contributing to luminal diameter narrowing in asthma (13, 45). Furthermore, studies suggest that alterations in airway structural cell size or accumulation can influence AHR (46, 47). In this section, we identify mechanisms underlying the fibrosis, differentiation, proliferation, and apoptosis involved in TGF- β 1-induced airway structural cell remodeling.

Fibrosis. TGF- β 1 promotes airway structural cell fibrosis in asthma through various mechanisms. TGF- β 1 mediates the expression of genes implicated in fibrosis, including collagen (48), proteases (49), and fibronectin (50), through a Smad-dependent mechanism. In addition, TGF- β 1 may mediate a subset of its fibrotic effects through the production of ROS generated by the mitochondrial electron transport chain (51) and the ROS-producing enzyme nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) (52).

TGF- β 1 may also mediate pulmonary fibrosis through the induction of endothelin-1 (53), a vasoconstrictor peptide induced by Smad-independent (54) or Smad-dependent (55) mechanisms after TGF- β 1 exposure. Interestingly, investigations have demonstrated a role for TGF- β 1-induced endothelin-1 in AHR pathogenesis (56), suggesting another mechanism by which dysregulation of TGF- β 1 signaling may contribute to airway bronchoconstriction. In addition to endothelin-1 induction, TGF- β 1 may induce fibrosis by activating caspases that mediate cell death and apoptosis. In one study, investigators found that Bax and Bid,

Figure 1. The tentative role of TGF- β 1 signaling in airway remodeling and airway hyperresponsiveness. TGF- β 1 is synthesized as a latent precursor molecule that is secreted into the extracellular space or held in the extracellular matrix by integrins. After activation by integrins, proteases, and other mechanisms, activated TGF- β 1 binds to T β R-I/II. T β R-I kinase activity induces Smad2/3 phosphorylation, and phosphorylated Smad2/3 associates with Smad4 and translocates to the nucleus to mediate gene expression. In addition, studies show that TGF- β 1 can signal through numerous Smad-independent pathways. Both Smad-dependent and Smad-independent pathways may work to affect facets of airway remodeling and hyperresponsiveness in airway structural cells. ROCK, rho-associated protein kinase; ROS, reactive oxygen species; T β R-I/II, transforming growth factor β receptor I/II; TGF- β 1, transforming growth factor β 1.

two apoptotic regulatory proteins suggested to mediate fibrotic responses (57, 58), may be activated by TGF- β 1 and may facilitate bleomycin-induced pulmonary fibrosis in mice (59). In this study, TGF- β 1-mediated increases in inflammation, lung collagen content, and lung cell apoptosis were decreased significantly in both Bax- and Bid-null mice versus wild-type control mice. In addition, TGF- β 1-induced matrix metalloproteinase-12 expression was inhibited in Bax- and Bid-null mice, suggesting a role for matrix metalloproteinase-12 in the Bid- and Bax-mediated induction of fibrotic responses by TGF- β 1.

Activation of TGF- β 1 by integrins is pivotal in mediating the fibrotic response. The α V β 8 integrin in epithelial cells activates TGF- β 1 through matrix metalloproteinase-14 and proteolytic cleavage of latent TGF- β 1 into active TGF- β 1 (60). Activation of TGF- β 1 can also occur through the integrins α V β 3, α V β 5, and α V β 6 by way of cellular contraction and the cellular cytoskeleton, as listed in Table 1. The α v β 6 integrin, whose expression is increased in injured epithelia (61), is constitutively bound to TGF- β 1 and can be induced by lysophosphatidic acid or thrombin-mediated changes in the cytoskeleton (62). Furthermore, activation

of α v β 6 may modulate AHR by altering the expression of mast-cell proteases that can contribute to or inhibit airway narrowing (63). In addition, proteases can cleave latent TGF- β 1 into its active form, inducing the synthesis of collagen in an autocrine fashion (64). Other mechanisms of TGF- β 1 activation, such as ROS, mechanical stretch, contraction, and even extreme temperature changes, may also be pivotal to contributing to TGF- β 1-induced fibrotic responses in asthma (Table 1).

Differentiation. An increase in the number of structural cells with a contractile-like phenotype may contribute to increased and sustained AHR in subjects with asthma.

Table 1. Mechanisms Governing TGF- β 1 Activation

Activation Mechanism	Subtype	Reference
ROS	NOX4-mediated Asbestos-generated CD4 ⁺ CD25 T-cell activation-generated Artificially produced	(90, 141–143) (144) (116) (145)
Integrins	α V β 3 α V β 5 α V β 6 α V β 8	(146, 147) (146, 148) (149, 150) (60)
Proteases	Plasmin Thrombin/thrombospondin-1 MMPs	(151, 152) (152–154) (155–158)
Mechanical stretch/ contraction	Mechanical stretch Contraction	(159) (23)
pH	N/A	(151, 160)
Temperature	N/A	(160–162)
Coculture	N/A	(163–166)
Plasmin	N/A	(151, 164, 167)
Deglycosylation	N/A	(168)
M6P/IGF2R	N/A	(169, 170)

Definition of abbreviations: IGF2R, insulin-like growth factor II receptor; M6P, mannose-6-phosphate; MMPs, matrix metalloproteinases; N/A, not applicable; NOX4, nicotinamide adenine dinucleotide phosphate oxidase 4; ROS, reactive oxygen species; TGF- β 1, transforming growth factor β 1.

TGF- β 1 promotes the differentiation of epithelial cells, fibroblasts, and ASM cells in asthma into a more contractile phenotype through both canonical (65) and noncanonical (66) signaling pathways. After airway injury, injured epithelia undergo a process termed epithelial-to-mesenchymal transition (EMT). The transition of epithelial cells to a mesenchymal phenotype may possibly play a role in the development of airway remodeling and increased airway contractility. Although several cytokines are implicated in EMT, this process is driven largely by the Smad-dependent and Smad-independent actions of TGF- β 1 (67–69). In addition, collagen and cadherin, two profibrotic proteins induced by TGF- β 1, have been shown to promote EMT (70, 71). Furthermore, p38 MAPK activation, oxidative stress, endothelin-1, Wnt signaling, and cytokine signaling modulate TGF- β 1-induced EMT (68, 72–76).

Evidence demonstrates that TGF- β 1 also promotes the differentiation of fibroblasts into myofibroblasts through a variety of mechanisms (77, 78). Interestingly, it has been demonstrated that increased substrate stiffness enhances the differentiation of bronchial fibroblasts into myofibroblasts (79), suggesting the potential of a feed-forward cycle governing

TGF- β 1-induced structural changes, differentiation, and airway contractility.

Proliferation and apoptosis. The role of TGF- β 1 in structural proliferation is complex and may differ depending on cell type or the presence of additional growth factors. TGF- β 1 induces the proliferation of goblet cells and fibroblasts but induces apoptosis in epithelial cells (19, 80). In human ASM (HASM) cells, TGF- β 1 both induces proliferation (81) and inhibits mitogen-induced proliferation (82) through different pathways. In addition, TGF- β 1 induces greater proliferation in HASM cells from patients with severe asthma as compared with those from patients with nonsevere asthma (83), suggesting that the effects of TGF- β 1 on structural cell proliferation may depend on disease state.

Studies suggest that TGF- β 1-induced proliferation may be attributed to the stimulation of MAPK phosphorylation (84) and/or ROS production via NOX4 activation (85). TGF- β 1 may also inhibit proliferation by affecting the release of other growth factors such as vascular endothelial growth factor (VEGF) (86), or by mediating store-operated calcium (Ca^{2+}) entry (87). Furthermore, TGF- β 1 may affect proliferation alone or in conjunction with other growth factors. Evidence also suggests that TGF- β 1 can cross-talk with the muscarinic (M2)

receptor to further augment proliferation in HASM (88). TGF- β 1-induced proliferation may also be Ca^{2+} dependent, as suggested by studies in rat ASM cells (87).

In addition to increasing cell number, TGF- β 1 augments HASM cell size in a process termed hypertrophy. Studies show that preventing TGF- β 1 overexpression or signaling reduces and/or leads to a parallel decline in HASM hypertrophy (89). It is suggested that TGF- β 1 may mediate its effects on HASM hypertrophy through the activation of NOX4 (81).

In contrast to its effects on proliferation and cell size, TGF- β 1 promotes cell death under certain circumstances. Evidence shows that TGF- β 1-induced NOX4 plays a major role in epithelial cell death, a consequence that may contribute to the development of pulmonary fibrosis (90). In addition, TGF- β 1 may stimulate apoptosis-mediated pulmonary fibrosis through its interaction with the Bax pathway and stimulation of matrix metalloproteinases (91). TGF- β 1 has also been shown to inhibit apoptosis in fibroblasts through the activation of phosphoinositide 3-kinase (PI3K)/Akt and p38 MAPK (92–94).

Contraction, Cell Shortening, and Cytoskeletal Motors

HASM cells serve as the pivotal cell-regulating bronchomotor tone. HASM excitation-contraction (E-C) coupling occurs when a contractile agonist binds to its G-protein-coupled receptor on the HASM cell membrane, as shown in Figure 2. Agonist binding induces the production of diacylglycerol and inositol-1,4,5-trisphosphate from phosphatidylinositol 4,5-bisphosphate. After phosphatidylinositol 4,5-bisphosphate cleavage, inositol-1,4,5-trisphosphate enters the cytosol and binds to the inositol trisphosphate receptor on the sarcoplasmic reticulum. The subsequent release of Ca^{2+} into the intracellular space by the inositol trisphosphate receptor and ryanodine receptor channels induces myosin light chain kinase (MLCK) activation (95, 96). Activated MLCK phosphorylates myosin light chain 20 (MLC), evoking a 10-fold increase in actomyosin ATPase activity and subsequent contraction (95–97). In addition, HASM contraction and AHR are mediated by the actions of ROCK.

Although studies show an effect of TGF- β 1 on HASM contraction, the mechanisms underlying TGF- β 1-induced

Figure 2. Potential mechanisms governing TGF- β 1-induced HASM contraction. Agonist binding to a GPCR in airway smooth muscle induces both a Ca^{2+} -dependent pathway through $G_{q/11}$ activation and a RhoA/ROCK-mediated Ca^{2+} -sensitization pathway through $G_{12/13}$ activation. In the Ca^{2+} -dependent pathway, IP_3 induces Ca^{2+} release from the sarcoplasmic reticulum stores. Increased $[\text{Ca}^{2+}]$ activates Ca^{2+} /CaMKII, which activates MLCK. MLCK phosphorylates MLC20, leading to actin-myosin cross-bridge cycling and contraction. In addition, RhoA/ROCK activation can suppress MLCP in the Ca^{2+} -sensitization pathway, resulting in increased contractility. After TGF- β 1 binding to T β R-I/II, TGF- β 1 can signal through Smad-dependent or Smad-independent signaling pathways that may affect airway smooth muscle contractile function. α -SMA, α smooth muscle actin; $[\text{Ca}^{2+}]$, intracellular calcium; Ca^{2+} /CaMKII, calcium/calmodulin-dependent kinase II; GPCR, G protein-coupled receptor; HASM, human airway smooth muscle; IP_3 , inositol-1,4,5-trisphosphate; MAPK/ERK, mitogen-activated protein kinases/extracellular signal-regulated kinases; MLC20, 20-kD myosin light chain 20; MLCK, myosin light chain kinase; MLCP, myosin light-chain phosphatase; RhoA, ras homolog gene family, member A; SM22- α , smooth muscle protein 22- α .

HASM contraction remain unknown (Figure 2). Evidence suggests that TGF- β 1 affects HASM shortening by Smad-mediated contractile protein expression and ROS production. In addition, numerous studies demonstrate a role for TGF- β 1 in ROCK activation (98–101). Although the role of TGF- β 1 in mediating classic E-C coupling remains unclear, TGF- β 1 modulates Ca^{2+} homeostasis in a variety of cell types. TGF- β 1 treatment induces an immediate and transient Ca^{2+} release in human osteoblasts (102). In human pulmonary fibroblasts, both acute and overnight TGF- β 1 treatment evokes Ca^{2+}

transients (103). To further determine the potential role of TGF- β 1 in E-C coupling, future studies investigating the role of TGF- β 1 in HASM cell Ca^{2+} mobilization will be essential.

Contractile protein expression. TGF- β 1 increases contractile protein expression in HASM, fibroblasts, and myofibroblasts. TGF- β 1 induces the expression of SM22, calponin, α -SMA, MLCK, and Ca^{2+} pathway proteins in HASM cells, subsequently mediating enhancements in cell stiffness, actin-cytoskeleton reorganization, and contractility (87, 104, 105). In addition, mast cell-derived

TGF- β 1 may modulate the phenotype of HASM cells, promoting their differentiation into a more contractile phenotype and subsequently enhancing agonist-induced contraction (106).

Smad3, but not Smad2, plays a role in the TGF- β 1-induced contraction of various cell types. Studies suggest that TGF- β 1 induces the contraction of human lung fibroblasts through a Smad-dependent mechanism (107, 108). TGF- β 1 may also increase contractile protein expression through PI3K. The induction of α -SMA, calponin, and myosin heavy chain in HASM by TGF- β 1 decreases after

administration of the PI3K pan-inhibitor LY294002 or the PI3K- δ -specific inhibitor IC87114 (109, 110).

RhoA/ROCK signaling pathways. Ca^{2+} sensitization and MLC phosphorylation in ASM E-C coupling occurs in part via the G-protein-coupled receptor-mediated activation of the RhoA/ROCK signaling pathway (Figure 2). TGF- β 1 may induce ASM contraction through the activation of the RhoA/ROCK signaling pathways. Evidence suggests that induction of the RhoA/ROCK pathway after TGF- β 1 treatment mediates the phosphorylation of MLC (111). In this study, inhibition of ROCK with the soluble inhibitor Y-27632, as well as inhibition of RhoA with the C3 exoenzyme, decreased TGF- β 1-induced ROCK activation. Interestingly, activation of RhoA by TGF- β 1 may occur as early as 1–2 hours and last as long as 6 hours, suggesting a mechanism by which TGF- β 1 may induce contraction and AHR on both an acute and chronic level (99, 111).

TGF- β 1 may also induce ASM contraction through cross-talk of the TGF- β 1 and the M2 receptor signaling pathways in HASM as shown in Figure 2. TGF- β 1-mediated induction of α -SMA and calponin expression, as well as the induction of α -SMA stress fibers, was increased by treatment with methacholine (88). These effects occurred through a nongenomic mechanism involving the phosphorylation of GSK-3 β and 4E-binding protein 1, suggesting that TGF- β 1 and methacholine may work together to acutely augment HASM contraction (20).

ROS production and NOX4. TGF- β 1 may induce ASM shortening by inducing the expression of NOX4, the major ROS-generating enzyme in HASM cells (112). Unlike the majority of NOX enzymes, NOX4 is a constitutively active enzyme that may be regulated predominantly by its expression levels (113). Similar to TGF- β 1 levels, NOX4 expression is increased in the ASM and fibroblasts derived from subjects with asthma (114). Interestingly, NOX4 may also regulate TGF- β 1 and Smad signaling in a feed-forward manner (52). Studies suggest that NOX4-mediated ROS production may mediate TGF- β 1 signaling or may activate latent TGF- β 1 in an autocrine fashion (115, 116). In addition, increased cellular ROS may influence E-C coupling by acting on Ca^{2+} signaling pathways or by modifying the phosphorylation of kinases in the

contractile pathway (117). Indeed, the inhibition of NOX4 decreases agonist-induced contractility in HASM (114), suggesting a role for NOX4-mediated ROS in TGF- β 1-induced AHR.

Synthetic function. TGF- β 1 mediates many of its actions by stimulating the release of additional growth factors and inflammatory mediators from airway structural cells. In HASM cells, TGF- β 1 stimulates the expression of COX-2 and the release of PGE-2 and IL-8 (118), mediators demonstrated to affect HASM contractile or fibrotic responses (119–121). In addition, TGF- β 1-induced mediator release from the airway epithelium may affect contractile or fibrotic function in HASM cells. Studies placing TGF- β 1-stimulated airway epithelial cells in coculture with HASM cells, and studies that treated HASM cells with TGF- β 1-stimulated epithelial cell culture media, suggest that TGF- β 1 induces epithelial cell mediator release that may further alter HASM function, contractility, or proliferation (122).

Role of epithelium in TGF- β 1-induced HASM contraction. Given the proximity of the airway epithelium to HASM, soluble mediators released by airway epithelial cells may mediate HASM contraction and relaxation. After an airway injury or assault, bronchial epithelial cells release various soluble mediators, interleukins, arachidonic acid metabolites, and prostaglandins such as prostaglandin F $_{2\alpha}$, which evoke bronchoconstriction (123, 124). Epithelial cell-derived soluble mediators, including cytokines, chemokines, TGF- β 1, and other growth factors, recruit inflammatory cells that induce further damage to the airways (125–127). This influx of cytokines and increased inflammation damages airway epithelium, increasing TGF- β 1 and soluble mediator release and potentially inducing airway hypercontractility. Conversely, airway epithelial cells can also release soluble mediators such as γ -aminobutyric acid or nitric oxide that induce relaxation of HASM (128, 129).

Whether airway epithelium-derived soluble mediators directly affect HASM function remains unclear. Soluble mediators released from airway epithelial cells may be altered by interaction with the subepithelium before reaching HASM (130). Epithelial cell-to-cell contacts, however, may become compromised in the airways of subjects with asthma, allowing epithelial

cell mediators to reach the underlying structural cells. Several studies show that many adhesion proteins necessary for epithelial cell-to-cell contacts are down-regulated in the airway epithelium of patients with asthma (131–133). Because of this uncertainty, determining the circumstances under which airway epithelium and subepithelium modify HASM contraction and relaxation responses will be essential for future studies investigating the effect of the surrounding milieu on HASM contraction and AHR.

TGF- β 1 as a Therapeutic Target in the Airway

Current therapies targeting TGF- β 1 signaling show promising applications in chemotherapy, fibrosis, and tumor vaccines. These pharmaceutical agents target the TGF- β 1 signaling pathway at the level of the ligand, receptor, or intracellular signaling (134).

Because of the multiple roles of TGF- β 1 in airway function, elevated TGF- β 1 in the airway may abrogate the ability of current asthma therapeutics to be effective. Evidence suggests that persistently elevated TGF- β 1 expression may play a role in the failure of corticosteroids to decrease collagen deposition in the airway (135). Furthermore, TGF- β 1 and glucocorticoids may synergize to augment fibroblast-mediated collagen gel contraction (136).

Therapeutic reduction of TGF- β 1 levels in the airway should be considered carefully, because evidence suggests adverse consequences of reducing TGF- β 1 expression. In an experimental asthma model, TGF- β 1 reduction exasperated asthma pathology by increasing eosinophilic inflammation (137), supporting a role for TGF- β 1 in suppressing allergic airway inflammation (138). Furthermore, other studies suggest that TGF- β 1 decreases AHR and fibrosis in the airway. Increased AHR in anti-TGF- β 1 antibody treated ovalbumin-challenged mice has been attributed to a decrease in Smad2 phosphorylation (139), suggesting the importance of TGF- β 1 signaling in reducing AHR responses. In addition, at least one study has shown that TGF- β 1 expression is inversely correlated with collagen III deposition and total basement membrane thickness in the airway (140), suggesting a role for TGF- β 1 in reducing airway fibrosis. Because of the complex and sometimes contradicting roles of TGF- β 1

in the airway, asthma therapeutics targeting the TGF- β 1 signaling pathway may need to be cell-specific and closely regulated.

Conclusions

TGF- β 1 plays diverse roles in mediating airway structural cell proliferation, differentiation, and contractile protein expression. In many airway diseases, these aspects of structural cell function play a substantial role in disease pathology.

TGF- β 1 expression is not only up-regulated in airway structural cells in airway disease, but is also increased in the airway after immune cell activation. Because of the multiple roles of TGF- β 1 in the airway and occasional conflicting literature on TGF- β 1 action, more research is required to elucidate the role of TGF- β 1 in structural airway cells. Because TGF- β 1 can substantially affect cellular differentiation and proliferation, special attention should be paid to normalizing the density, growth

conditions, and cell-cycle state of the cells used in future TGF- β 1 function experiments. Further determination of TGF- β 1 action in specific structural cell types, as well as the development of methods to specifically target TGF- β 1 in these cell types, will be necessary for the future development of effective TGF- β 1 therapeutics that exhibit minimal side effects. ■

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