

REVIEW

Transforming growth factor- β 1 in asthmatic airway smooth muscle enlargement: is fibroblast growth factor-2 required?

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Summary

Enlargement of airway smooth muscle (ASM) tissue around the bronchi/bronchioles is a histopathological signature of asthmatic airway remodelling and has been suggested to play a critical role in the increased lung resistance and airway hyperresponsiveness seen in asthmatic patients. The pleiotropic cytokine, TGF- β 1, is believed to contribute to several aspects of asthmatic airway remodelling and is known to influence the growth of many cell types. Increased TGF- β 1 expression/signalling and ASM growth have been shown to occur concurrently in animal models of asthma. Abundant studies further substantiate this association by showing that therapeutic strategies that reduce or prevent TGF- β 1 overexpression/signalling lead to a parallel decrease or prevention of ASM enlargement. Finally, recent findings have supported a direct link of causality between TGF- β 1 overexpression/signalling and the overgrowth of ASM tissue. To follow-up on these *in vivo* studies, many investigators have pursued detailed investigation of ASM in cell culture conditions, assessing the direct role of TGF- β 1 on cellular proliferation and/or hypertrophy. Inconsistencies among the *in vitro* studies suggest that the effect of TGF- β 1 on ASM cell proliferation/hypertrophy is contextual. A hypothesis focusing on fibroblast growth factor-2 is presented at the end of this review, which could potentially reconcile the apparent discrepancy between the conflicting *in vitro* findings with the consistent *in vivo* finding that TGF- β 1 is required for ASM enlargement in asthma.

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Introduction

Asthma is a prevalent condition touching people of every age that can severely affect the quality of life, and sometimes progress to a life-threatening condition. The diagnosis of asthma is based on recurrent paroxysms of symptoms such as respiratory distress, wheezing and coughing, as well as on the degree of reversibility of airflow limitation induced by bronchodilator inhalation. In addition, airway hyperresponsiveness, which is defined as an excessive airway narrowing in response to a wide variety of contractile stimuli, is a characteristic feature. The treatment modalities are usually directed to the attenuation of symptoms and focus on preventing or reversing the airway narrowing by the use of bronchodilators (β_2 adrenoceptor agonists) and on reducing the overall inflammatory process by using broad-spectrum anti-inflammatory medications (corticosteroids). The common symptoms among asthmatic individuals, together with the usually favourable responsiveness to

mainstay therapies (β_2 adrenoceptor agonists and corticosteroids), render asthma readily identifiable clinically. For this reason, asthma is often erroneously considered as one disease rather than a syndrome. However, the triggering agents (allergen, virus, bacteria, fungus, air pollution, occupational triggers, aspirin and exercise) leading to asthma inception and progression are variable from one asthmatic patient to the other; as are the age of onset and the degree of responsiveness to mainstay and alternative therapies [1, 2]. Hence, the causes and the underlying pathogenesis leading to these common symptomatic manifestations are heterogeneous among asthmatic individuals. For scientists trying to understand the pathogenesis of asthma, these facts confirm that we are dealing with a syndrome rather than a single disease [3].

Despite differing aetiologies among asthmatics, the syndrome is characterized by common structural alterations in the airways. Histological assessment of asthmatic airway walls reveals the presence of epithelial metaplasia,

increased vascularity, enlargement and hyperplasia of submucosal glands and thickening of several airway wall layers including the lamina reticularis, the lamina propria, the adventitia and the airway smooth muscle (ASM) layer [4]. These remodelling features are thought to contribute to the symptoms, the accelerated decline in lung function [5] and a degree of irreversibility of airway obstruction found in some asthmatics [6–9]. The molecular mechanisms leading to the pathognomonic features of asthmatic airway remodelling are currently under intense investigation. ASM enlargement, in particular, has received a lot of attention because of its potential adverse influence on airway resistance and airway responsiveness [10]. Excessive growth of ASM has also been observed in different animal species used as models of human asthma, including mice [11], rats [12], guinea-pigs [13], cats [14], monkeys [15, 16] and horses [17].

Although the underlying causes of asthma may differ between asthmatic individuals and between different animal models of the syndrome, the similar histological alterations, including ASM enlargement, point to the plausibility of a common causative mechanisms at the molecular level. Recently gained insights suggest that TGF- β 1, or other members of the TGF- β superfamily, may represent cornerstone molecules required for ASM tissue enlargement in asthma. The biological activity of TGF- β 1 is known to be regulated by mechanisms other than simply its level of expression [18]. Despite controversies surrounding the increased expression of TGF- β 1 and its cellular sources in asthmatic lungs [18], augmentation of its intracellular signalling has been confirmed on several occasions in lung specimens from both human asthmatics [19–21] and animal [11, 22, 23] models of asthma, which provide an evidence of its increased level of biological activity in asthma. *In vitro* studies also support a direct role for TGF- β 1 in ASM cell proliferation, provided that ASM cells are stimulated previously with fibroblast growth factor (FGF)-2 [24]. The expression of the latter has been consistently shown to be increased in asthmatic airways [25], and appears to be released early on, following a bronchoprovocative challenge with an allergen [26]. The pro-mitogenic effect of FGF-2 on ASM cells is also well-established [25]. The present review seeks to update the current state of knowledge regarding the possibility that ASM enlargement is the histopathological signature of a molecular synergism between FGF-2 and TGF- β 1 signalling.

Canonical receptor and intracellular signalling pathway borrowed by transforming growth factor- β 1 to transduce its biological effect from the plasmalemma to the nucleus

TGF- β 1 is an intercellular signalling molecule produced constitutively and/or inducibly by basically all cells present in the lungs (see [18], for a review). TGF- β 1 is

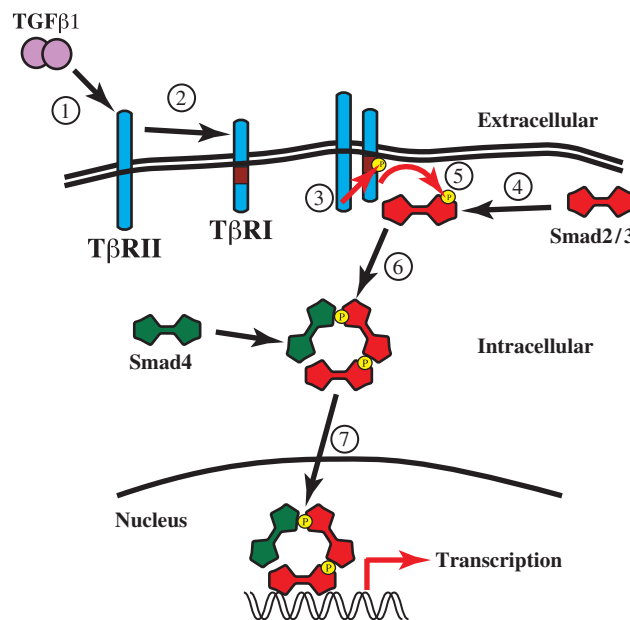


Fig. 1. The canonical receptor for TGF- β 1 and the canonical signalling pathway borrowed by the activated receptor to transduce its biological signal from the plasmalemma to the nucleus. The sequence of events are numbered appropriately: (1) TGF- β 1 binds to T β R-II; (2) the TGF- β 1-T β R-II complex recruits T β R-I; (3) bringing T β R-II and T β R-I in close vicinity, which allows T β R-II to activate T β R-I by phosphorylation; (4) the phosphorylated T β R-I acts a docking site for the Smad2/3, which are recruited to T β R-I; (5) the recruited Smad2/3 gets activated by phosphorylation by T β R-I; (6) the phosphorylated Smad2/3 then oligomerize with Smad4; and (7) the whole complex translocates to the nucleus to modulate gene expression.

secreted in an inactive form and needs to be separated from its non-covalently associated latency-associated peptide (LAP) before binding its cognate cell-surface receptor. It is by its ability to recruit single transmembrane subunits into a hetero-oligomer that TGF- β 1 promotes the formation of a functional receptor. The functional complex is composed of two type I receptors (T β R-I, which is also known as ALK-5, standing for activin receptor-like kinase 5) and two type II receptors (T β R-II). The kinetics of binding, together with the ensuing activation of the canonical intracellular cascade that relays the message from the plasma membrane to the nucleus occur as follows and is illustrated in Fig. 1. TGF- β 1 initially binds to T β R-II, which possesses a serine/threonine kinase in its cytoplasmic domain that is constitutively active. The formed complex subsequently recruits T β R-I, which also possesses a serine/threonine kinase in its cytoplasmic domain. However, in contrast to T β R-II, the serine/threonine kinase activity of T β R-I is not constitutively active but rather activated by T β R-II via the phosphorylation of several threonine and serine residues in its intracellular GS juxtamembrane domain. This phosphorylated GS domain then serves as a docking site for intracellular

proteins, which would subsequently carry the message to the nucleus after their activation by the receptor. These intracellular intermediates are called Smads. There are eight Smad proteins, which are simply called Smad1 to Smad8. However, T β R-1 can only activate Smad2 and Smad3. The other Smads are involved in the signalling of other members of the TGF- β superfamily (Smad1, 5 and 8) or act as inhibitors of Smad signalling (Smad6 and 7). Following their activation by T β R-1, the active phospho-Smads (pSmad2 and pSmad3) homo- or hetero-oligomerize with each other and with at least one Smad4. Finally, the Smad complex translocates to the nucleus where it binds the Smad binding element-containing promoters or interacts with other transcriptional partners to regulate gene expression. For these reasons, Smad2 or 3 phosphorylation or nuclear localization of Smad2, 3 or 4 are often used as surrogates for assessing the levels of TGF- β 1 signalling activity. For a more extensive review on the intracellular signalling pathways borrowed by TGF- β 1 to transduce its biological messages from the plasmalemma to the nucleus, see Shi and Massague's review [27].

***In vivo* association between transforming growth factor- β 1 and increased airway smooth muscle mass in asthma**

Studies reporting concurrent increases in TGF- β 1 and ASM tissue in different animal models of asthma abound [23, 28–46]. ASM tissue enlargement occurring in conjunction with an increased TGF- β 1 expression/signalling has been shown in murine models of: (1) chronic airway allergic inflammation induced by sensitization and repetitive challenges with ovalbumin (OVA) for an extended period of time [23, 28–30, 32–34, 36, 38, 39, 41, 42, 44–46]; (2) allergic airway inflammation induced by house dust mite (HDM) sensitization and repetitive challenges [31, 40]; (3) endotoxin-induced airway inflammation caused by repetitive inhalations of lipopolysaccharide (LPS) [35]; and (4) a model of occupational asthma induced by repetitive sensitizations and challenges with toluene diisocyanate [37]. This positive association between ASM overgrowth and an increased TGF- β 1 expression has also been shown in a rat model of allergic airway inflammation induced by sensitization and repetitive challenges with OVA [43].

The association between TGF- β 1 and ASM enlargement was further confirmed by comparing different strains of mice. Hirota et al. [47] used immunochemistry to measure and compare the number of airway cell nuclei staining positive for phosphorylated Smad2 (pSmad2), which is a surrogate of active TGF- β signalling, in BALB/c and C57BL/6 mice subjected to a brief or a chronic protocol of allergic sensitization/challenge with OVA. Only BALB/c mice showed an increased pSmad2 expression following a 2-week recovery after either a brief or a chronic protocol of allergen exposure, which occurred in conjunction with

an increased ASM tissue content. The pSmad2 staining was apparent in ASM, among other cells. On the other hand, the C57BL/6 mice showed no sign of increased pSmad2 and no ASM remodelling.

More convincingly, strategies that have been successfully used to reduce the TGF- β 1 expression/signalling in different models of asthma were shown to be equipotent in decreasing ASM enlargement (Table 1). The particular molecular mechanisms scrutinized in each of the studies presented in Table 1 are not necessarily related to one another. However, they are a good reflection of the array of mechanisms currently explored to shed light on the pathogenesis of asthma.

Among the arsenal of pharmacological compounds tested in experimental animal models of asthma, fluticasone [23], dexamethasone (DEX) [39, 44], Astragaloside IV [42] (a traditional Chinese medicinal herb isolated from the root of *Astragalus membranaceus* Bunge), immunostimulatory sequences of DNA [33, 34] and a bi-specific antibody (Ab) directed against CD300a and targeting CCR3⁺ cells [38] were shown to be effective in reducing both the TGF- β 1 expression/signalling and the area of ASM in chronic mouse models of allergic (OVA) airway inflammation. Surprisingly, fluticasone also increased the lung tissue expression of the inhibitory Smad, Smad7, providing a possible mechanism by which corticosteroids alleviate the biological effect of TGF- β 1 [23]. In a mouse model of occupational asthma, peroxisome proliferator-activated receptor (PPAR)- γ agonists, rosiglitazone and pioglitazone, as well as intratracheal administration of an adenovirus gene vector carrying the PPAR γ 2 cDNA, also prevented ASM tissue enlargement in conjunction with a decreased induction of TGF- β 1 [37]. In a rat model of allergic (OVA) airway inflammation and remodelling, prophylactic treatments with valsartan (an angiotensin II receptor antagonist) concomitantly reduced the increased expression of TGF- β 1 in the whole-lung lavages and the increased mass of ASM [43].

Genetic manipulations in mice also substantiate the positive association between TGF- β 1 and ASM enlargement. Different single gene deletions, which prevent or reduce the TGF- β 1 overexpression in congenic compared with wild-type animals subjected to the same protocol for induction of asthma were shown to be equipotent in preventing or reducing ASM enlargement. This has been shown in: (1) IL-5-deficient [32], IL-18-deficient [41], phosphatidylinositol 3-kinase (PI3K) γ -deficient [45] and osteopontin-deficient mice [46] subjected to allergic (OVA) airway inflammation; (2) LPS binding protein (LBP)-deficient mice in a model of endotoxin-induced asthma [35]; and (3) IL-4-deficient mice suffering from allergic airway inflammation induced by HDM exposure [40]. In the latter study, a transient transduction of IL-4 in IL-4-knock-out (KO) animals, via intratracheal instillation of an IL-4 cDNA-containing adenovirus before the

Table 1. Different strategies reducing TGF- β 1 expression in different models of asthma caused a parallel decrease in ASM mass

Models	Location of TGF- β 1	Assessment of ASM	Associations	References
WT and IL-5 ^{-/-} C57BL/6 mice subjected to a chronic protocol of allergic (OVA) airway inflammation	Total TGF- β 1 in lung homogenates or TGF- β ⁺ cells in peribronchial region	1. Histologic assessment of ASM thickness by measuring the transverse diameter of the ASM layer (μ m) 2. Area of α -SMA immunostaining per μ m circumference of bronchiole	$\uparrow$ TGF- β 1 was associated with $\uparrow$ ASM. $\downarrow$ TGF- β 1 in IL-5 ^{-/-} mice was associated with $\downarrow$ thickness of the ASM layer.	[32]
WT and LBP ^{-/-} C57BL/6 mice subjected to inhaled endotoxin	Total and active TGF- β 1 in whole-lung lavages	Proliferation by BrdU incorporation in the submucosal compartment	$\uparrow$ active TGF- β 1 was associated with $\uparrow$ proliferation. $\downarrow$ active TGF- β 1 in LBP ^{-/-} mice was associated with $\downarrow$ proliferation.	[35]
BALB/c mice subjected to a chronic protocol of allergic (OVA) airway inflammation treated or not a posteriori with ISS	1. Total TGF- β 1 in whole-lung lavages 2. in lung homogenates 3. TGF- β ⁺ cells in peribronchial region	Peribronchial area of α -SMA immunostaining per μ m circumference of bronchiole (μ m ² / μ m)	$\uparrow$ TGF- β 1 was associated with $\uparrow$ ASM. Faster $\downarrow$ in TGF- β 1 post-allergen exposure by ISS treatment was associated with a faster reversal of $\uparrow$ ASM area.	[34]
BALB/c mice subjected to a chronic protocol of allergic (OVA) airway inflammation treated or not with ISS	Total TGF- β 1 in whole-lung lavages and in lung homogenates	1. Histologic assessment of ASM thickness by measuring the transverse diameter of the ASM layer (μ m) 2. Area of α -SMA immunostaining per μ m length of BM	$\uparrow$ TGF- β 1 expression was associated with $\uparrow$ ASM. $\downarrow$ TGF- β 1 expression induced by ISS was associated with $\downarrow$ ASM.	[33]
WT and transgenic BALB/c mice overexpressing either GATA-3 or T-bet in their lymphocytes subjected to a chronic protocol of allergic (OVA) airway inflammation	Active form of TGF- β 1 in lung homogenates	Percent of α -SMA immunostaining in the 20 μ m region beneath the epithelium	$\uparrow$ TGF- β 1 in GATA-3 transgenic animals was associated with $\uparrow$ ASM mass. Lack of $\uparrow$ TGF- β 1 in either WT or T-bet transgenic animals were associated with the lack of $\uparrow$ ASM.	[36]
BALB/c mice sensitized and challenged with toluene diisocyanate treated or not with PPAR- γ agonists or with an adenovirus carrying PPAR- γ 2 cDNA	Total TGF- β 1 in whole-lung lavages and in lung homogenates	Area of α -SMA immunostaining per μ m length of BM	$\uparrow$ TGF- β 1 was associated with $\uparrow$ ASM. $\downarrow$ TGF- β 1 by PPAR- γ agonists or the PPAR- γ 2-containing adenovirus was associated with $\downarrow$ ASM area.	[37]
BALB/c mice subjected to a chronic protocol of allergic (OVA) airway inflammation treated or not with DEX	Total TGF- β 1 in lung homogenates or TGF- β ⁺ cells in peribronchial region	1. Histologic assessment of ASM thickness by measuring the transverse diameter of the ASM layer (μ m) 2. Peribronchial area of α -SMA immunostaining per μ m circumference of bronchiole (μ m ² / μ m)	$\uparrow$ TGF- β 1 was associated with $\uparrow$ ASM. 1. $\downarrow$ TGF- β 1 by DEX was associated with an insignificant $\downarrow$ in ASM area. 2. $\downarrow$ TGF- β 1 by DEX was associated with $\downarrow$ ASM area.	[39]
BALB/c mice subjected to a chronic protocol of allergic (OVA) airway inflammation treated or not with a bispecific antibody directed against CCR3 and CD300a (LC1)	Total TGF- β 1 in whole-lung lavages	Histologic assessment of ASM thickness by measuring the transverse diameter of the ASM layer (μ m)	$\uparrow$ TGF- β 1 expression was associated with $\uparrow$ ASM. $\downarrow$ TGF- β 1 expression induced by LC1 was associated with $\downarrow$ ASM.	[38]
WT and IL-4 ^{-/-} BALB/c mice were subjected chronically to HDM. A group of IL-4 ^{-/-} were transiently reconstituted with IL-4 by an airway gene transfer approach	Total TGF- β expression in whole-lung lavage	Percent of area immunostained+ for α -SMA in the 20 μ m band immediately beneath the epithelium	$\uparrow$ TGF- β 1 expression was associated with $\uparrow$ ASM. $\downarrow$ TGF- β 1 expression in IL-4 ^{-/-} animals was associated with $\downarrow$ ASM.	[40]

Table 1. continued

Models	Location of TGF- β 1	Assessment of ASM	Associations	References
BALB/c mice exposed to a chronic protocol of allergic (OVA) airway inflammation treated or not with fluticasone	Total TGF- β 1 in whole-lung lavages and active TGF- β 1 signalling by measuring phosphorylation of Smad2/3	Area of α -SMA immunostaining per μ m length of BM	Transduction of IL-4 ^{-/-} animals with IL-4-containing adenovirus restored both TGF- β 1 overexpression and ASM enlargement. ↑ TGF- β 1 expression and signalling were associated with ↑ ASM. ↓ TGF- β 1 expression and signalling induced by fluticasone were associated with ↓ ASM.	[23]
BALB/c mice subjected to a chronic protocol of allergic (OVA) airway inflammation treated or not with astragaloside IV	Number of cells expressing TGF- β 1 per bronchus	Area of α -SMA immunostaining per μ m length of BM	↑ TGF- β 1 expression was associated with ↑ ASM. ↓ TGF- β 1 expression induced by astragaloside IV was associated with ↓ ASM.	[42]
WT and IL-18 ^{-/-} C57BL/6 mice subjected to a chronic protocol of allergic (OVA) airway inflammation	Total TGF- β 1 expression in whole-lung lavages	Thickness of ASM layer following α -SMA immunostaining	↑ TGF- β 1 expression was associated with ↑ ASM. ↓ TGF- β 1 expression in IL-18 ^{-/-} animals was associated with ↓ ASM.	[41]
Wistar rats subjected to a chronic protocol of allergic (OVA) airway inflammation pre-treated or not with valsartan before every challenge	TGF- β 1 expression in whole-lung lavages	Area immunostained+ for α -SMA/ μ m BM (μ m ² / μ m)	↑ TGF- β 1 expression was associated with ↑ ASM. ↓ TGF- β 1 expression by valsartan treatment was associated with ↓ ASM.	[43]
WT and PI3K γ ^{-/-} C57BL/6 mice subjected to a chronic protocol of allergic (OVA) airway inflammation	Number of peribronchial cells+ for TGF- β 1 and pSMAD2/3, as well as percent of epithelial cells+ for pSmad2/3	Area of α -SMA immunostaining per μ m length of BM	↑ TGF- β 1 expression and signalling were associated with ↑ ASM. ↓ TGF- β 1 expression and signalling in PI3K γ ^{-/-} animals were associated with ↓ ASM.	[45]
WT C57BL/6 and OPN ^{-/-} B6.Cg-Spp1tm1Blh/J mice subjected to a chronic protocol of allergic (OVA) airway inflammation	Total TGF- β expression in whole-lung lavages	Area (μ m ²) of peribronchial α -SMA immunostaining per μ m of BM	↑ TGF- β 1 expression was associated with ↑ ASM. ↓ TGF- β 1 expression in OPN ^{-/-} animals was associated with ↓ ASM.	[46]
BALB/c mice subjected to a chronic protocol of allergic (OVA) airway inflammation with or without coexposure to ETS treated or not with DEX	Number of peribronchial TGF- β 1 ⁺ cells/bronchus	1. Histologic assessment of ASM thickness by measuring the transverse diameter of the ASM layer (μ m) 2. Peribronchial area+ for α -SMA immunostaining per BM length (μ m ² / μ m)	OVA sensitization/challenge ↑ TGF- β 1 expression, which was associated with ↑ ASM. ETS coexposure augmented TGF- β 1 overexpression, which was associated with further ↑ ASM. DEX ↓ TGF- β 1 overexpression in sensitized/challenged animals with or without the ETS coexposure, which was associated with commensurate ↓ ASM.	[44]

ASM, airway smooth muscle; BM, basement membrane; DEX, dexamethasone; ETS, environmental tobacco smoke; HDM, house dust mite; ISS, immunostimulatory sequences of DNA; LBP, LPS-binding protein; OPN, osteopontin; PI3K, phosphatidylinositol 3-kinase; PPAR- γ , peroxisome proliferator-activated receptor γ ; pSmad, phosphorylated Smad; WT, wildtype. See text for further details.

initiation of HDM exposure, concurrently recreated TGF- β 1 overexpression and ASM enlargement [40].

On the other hand, strategies that magnify the increased expression of TGF- β 1 induced by OVA sensitization and repetitive challenges have been shown to amplify ASM growth. For example, transgenic mice overexpressing GATA-3 in T lymphocytes and submitted to a chronic protocol of allergic (OVA) asthma, demonstrated an increased expression of TGF- β 1 compared with wild-type animals, which correlated with the augmentation of ASM mass [36]. Similarly, coexposure to environmental tobacco smoke (ETS) during the allergen (OVA) challenge period in sensitized mice further increased both the TGF- β 1 expression and ASM mass [30, 44]. In the same studies, chronic ETS exposure alone did not increase either the number of peribronchial TGF- β 1-positive cells or the mass of ASM [30, 44].

***In vivo* association between transforming growth factor- β 1 and increased airway smooth muscle mass in human asthma**

ASM enlargement in human asthmatics has been reported extensively, and although more controversial [18], the current bulk of evidence suggests that TGF- β 1, or at least its active signalling [19–21], is elevated in human asthmatic airways. However, in contrast to the abundance of studies documenting concurrent increases in the TGF- β 1 expression/signalling and ASM mass in animal models of asthma, human studies measuring both the expression levels of TGF- β 1 in any airway compartment and the amount ASM tissue are scarce. Sumi et al. [48] have recently measured the TGF- β 1 expression and the ASM area in bronchial biopsies obtained from healthy subjects and individuals diagnosed previously with occupational asthma. Despite being away from the triggering occupational exposure for an average of 14 years, the number of positive cells for TGF- β 1 was up-regulated in occupational asthmatics compared with controls. However, even though the ratio of the ASM area to the total biopsy area was almost double in the past occupational asthmatic patients (the ratio of ASM area to total biopsy area in past occupational asthmatics was 0.19 compared with 0.11 in specimens from control subjects), the increase was not statistically significant. Taken together, the relationship between the TGF- β 1 expression/signalling and ASM enlargement in human is tenuous and needs to be assessed more thoroughly.

Limitations

Some of the aforementioned studies assessed ASM remodelling by measuring the amount of α -smooth muscle actin (α -SMA) immunostaining in peribronchial regions. Because α -SMA is also a well-known marker of

myofibroblasts, and because of the close biological association between TGF- β 1 and myofibroblasts, the parallel modulation of TGF- β 1 expression and α -SMA immunostaining in the peribronchial region may not represent a true estimate of ASM mass but rather a variation in the number of peribronchial myofibroblasts. In fact, Miller et al. [39] reported a significant reduction in the peribronchial area staining positively for α -SMA in a mouse model of allergic (OVA) airway inflammation treated with DEX (a corticosteroid). However, true ASM layer thickening still occurred in the treated mice (the reduced ASM thickness induced by DEX treatment in OVA sensitized/challenged mice was insignificant compared with sham-treated animals) despite the efficacy of DEX in reducing the increased expression of TGF- β 1 in whole-lung lavages and tissues, and reducing the increased amount of cells immunostaining for TGF- β 1 around the airways [39]. This study suggested that the decreased TGF- β 1 expression induced by DEX treatment is associated with a diminution in peribronchial myofibroblast accumulation but not a decrease in true ASM enlargement. This result is in contrast to a more recent study by the same group. Using the same mouse model and the same treatment (DEX), they showed a significant decrease in ASM mass occurring in conjunction with a decrease in the TGF- β 1 expression and α -SMA immunostaining [44]. The difference between the two studies could be related to the duration of the concomitant repetitive challenges and DEX treatment (3 months vs. 1 month, in Miller vs. Song et al.' studies, respectively).

All of the studies cited previously also need to be viewed with caution with regard to the association between the TGF- β 1 expression and the amount of ASM because they simply correlate two phenomena occurring in asthma, namely the increased expression/signalling of TGF- β 1 and the ASM enlargement. However, they do not support a cause and effect relationship between them. The studies described in the following section provide the proof of causality in this relationship, and thus represent pivotal advancements in the understanding of ASM cell hyperplasia occurring in asthma (Table 2).

Studies supporting a causal link between the increased transforming growth factor- β 1 expression/signalling and the increased airway smooth muscle mass

Several transgenic mice have been generated to investigate the *in vivo* role played by a particular cytokine on the altered airway structure and function observed in animal models of asthma. Transgene constructs containing the Clara cell 10KDa protein (CC10) promoter are the most utilized to target the transgene of interest exclusively to the airways. Mice overexpressing a transgene in an airway-restricted fashion using this CC10 promoter approach have already been used successfully to investigate the

Table 2. Different strategies blocking the biological activity of TGF- β 1 reduced ASM mass in different animal models of asthma.

Models	Location of TGF- β 1	Assessment of ASM	Effects	References
BALB/c mice subjected to a prolonged protocol of allergic (OVA) airway inflammation therapeutically treated or not with a pan-anti-TGF- β neutralizing Ab	Immunohistologic assessment of active TGF- β 1 signalling by measuring pSmad2 expression in tissue lung sections	1. Histologic assessment of total ASM cells by measuring the number of ASM-like cells in peribronchiolar regions (cells/30 μ m of BM). 2. Immunohistologic assessment of proliferating ASM cells by measuring the number of PCNA ⁺ , ASM-like cells in peribronchiolar regions (+ cells/30 μ m of BM)	$\uparrow$ TGF- β 1 signalling was associated with $\uparrow$ ASM. TGF- β Ab abrogated pSmad2 expression in ASM, which occurred in conjunction with a $\downarrow$ in ASM	[11]
Brown-Norway rats subjected to a protocol of allergic (OVA) airway inflammation prophylactically or therapeutically treated or not with SD-208, a pharmacological inhibitor targeting ALK5	No direct assessment of TGF- β 1 expression or active signalling was performed. Immunohistologic assessment of Smad2/3 expression	1. Immunohistologic assessment of proliferating ASM cells by measuring the number of BrdU ⁺ cells in peribronchial regions staining+ for α -SMA (percent of BrdU ⁺ cells/total ASM cell nuclei). 2. Immunohistologic assessment of ASM thickness by measuring the transverse diameter of the region staining+ for α -SMA (μ m)	Allergic protocol caused parallel increases in ASM expression of Smad2/3, ASM proliferation and ASM thickness. Prophylactic or therapeutic treatment with SD-208 decreased ASM expression of Smad2/3 and ASM proliferation, but insignificantly decreased ASM layer thickening	[55]
WT and SMAD3 ^{-/-} C57BL/6 mice subjected to a prolonged protocol of allergic (OVA) airway inflammation	Peribronchial cells immunostained+ for TGF- β 1, pSmad2 and pSmad3 (+ cells/bronchus)	Histologic assessment of ASM thickness by measuring the transverse diameter of the ASM layer (μ m)	$\uparrow$ TGF- β 1 expression and signalling were associated with $\uparrow$ ASM. $\uparrow$ TGF- β 1 and pSMAD2 were not affected in SMAD3 ^{-/-} animals but ASM thickening was attenuated	[56]

ASM, airway smooth muscle; Ab, antibody; ALK5, activin receptor-like kinase 5; BM, basement membrane; BrdU, 5-bromo-2'-deoxyuridine; PCNA, proliferating cell nuclear antigen.

respective roles of IL-4, IL-5, IL-13, IL-6 and IL-11 [49–51]. However, the effect of TGF- β 1 in the CC10-promoter transgenic mice has required further molecular engineering, as the targeted overexpression of TGF- β 1 in the lung in a constitutive manner induced fetal lethality [52]. This problem has been overcome by using an externally regulate-able, triple transgenic system described earlier [53]. Although being able to ablate leakage of the inducible transgene, this system allows the TGF- β 1 to be induced at any given time in a lung-specific fashion by adding an agent (doxycycline) to the animal's diet. Using this innovative system, Lee et al. [54] studied the effects of TGF- β 1 overexpression in the lungs of adult mice. Interestingly, TGF- β 1 overexpression reproduced many of the remodelling features pathognomonic of human asthma, including ASM tissue enlargement. This result was the first to suggest that the overexpression of TGF- β 1 alone in mouse lungs, as it might occur in asthmatic subjects following an allergic challenge, is sufficient to cause ASM growth.

One of the most convincing proofs of the concept that ASM cells are an *in vivo* target of TGF- β is the fact that

ASM cells from airway sections derived from mice sensitized and chronically challenged with OVA strongly express pSmad2 [11]. The same group of investigators have also shown that treatment of these mice with TGF- β Abs prevented the phosphorylation of Smad2 in ASM tissue [11]. More importantly, this reduced TGF- β signalling resulted in a reduced number of total and proliferating ASM cells [11]. These results clearly indicate that ASM cells are targeted by the increased expression and/or activation of TGF- β in the lungs of sensitized and challenged mice and that active TGF- β signalling may be involved in ASM cell proliferation *in vivo*.

In addition to active TGF- β signalling in ASM [11], Leung et al. [55] showed an increased expression of Smad2/3 in ASM tissue of Brown Norway rats sensitized and exposed to allergen. This result indicates that an asthmatic state may prime the ASM tissue to respond in an excessive manner to TGF- β . This altered expression of Smad2/3 was reversed with an oral administration of SD-208, a pharmacological inhibitor that targets ALK-5 (also known as T β R-1), and suggests that TGF- β is involved in the up-regulation of its own signalling intermediates that

ensures its signal transduction from the cell membrane to the nucleus. Of greater importance, both preventive and curative treatments with SD-208 successfully abrogated ASM cell enlargement in these asthmatic rats.

Another piece of evidence came from mice deficient for Smad3, a signalling intermediate responsible for transducing some of the biological activity of TGF- β from the cell-surface TGF- β receptor to the nucleus. Le et al. [56] demonstrated that Smad3^{-/-} mice do not develop ASM thickening in response to sensitization and repetitive OVA challenges for 5 weeks despite showing a normal increase in the number of peribronchial cells immunostained for TGF- β 1 and an increased expression of pSmad2. This study clearly indicates that interfering with specific signalling pathways downstream of the TGF- β receptor activation is sufficient to prevent ASM enlargement in this mouse model of allergic airway inflammation.

The only exception to the mounting evidence implicating this pathway is the recent study by Fattouh et al. [57], showing that TGF- β blockade by a pan-anti-TGF- β neutralizing Ab has no effect on ASM enlargement induced by chronic exposure to HDM. They also confirmed their finding by showing that Smad3^{-/-} animals were not protected from the development of increased ASM mass following HDM exposure, which is in direct contrast to Le et al. [56] using a murine model of allergic (OVA) airway inflammation. The authors deserve credit for publishing negative results, but obvious issues can be raised. First, the Ab was administered intraperitoneally (i.p.), in contrast to the other group using the intranasal route to deliver anti-TGF- β Ab locally into the lung [58]. Therefore, the concentration obtained in the lung may not have been sufficient to block the biological effect of TGF- β . Second, the onset of Ab treatment might have been too late, because it was started at day 4 following HDM exposure. Although it is to be mentioned that therapeutic treatment (i.e., after the onset of established airway inflammation) with anti-TGF- β neutralizing Ab administered i.p. at a dose 10-fold lower was shown previously to be effective in preventing ASM enlargement in a murine model of airway inflammation induced by sensitization and a prolonged challenge with OVA [11]. One of the main differences in these contrasting studies may be related to the time course of antigen delivery in relation to Ab treatment. In McMillan et al.' study [11], the Ab was injected 30 min before every OVA challenge; whereas in Fattouh et al.' study [57], the Ab was injected every other day in mice continuously exposed to the antigen (administration of antigen was performed intranasally once daily). It is possible that the Ab was not able to block the biological action of TGF- β for the full 48 h between every Ab injection. Alternatively, the difference may be related to the allergen used (OA vs. HDM) and as suggested by Fattouh et al. [57], TGF- β may simply not be involved in ASM enlargement in this more germane murine model of human asthma.

Overall, these results indicate that the role of TGF- β 1 in ASM enlargement is contextual. Apart from its apparent lack of involvement in the increased mass of ASM observed in a murine model of allergic airway inflammation induced by chronic HDM exposure [57], TGF- β 1 appears to play a significant role in ASM enlargement observed in several other *in vivo* contexts. The cellular mechanisms (hyperplasia, hypertrophy, reduced rate of apoptosis) and the downstream molecular mechanisms governing TGF- β 1-induced ASM enlargement *in vivo* remain unknown. The following section will describe the *in vitro* studies that have aimed to define the mechanisms by which TGF- β 1 could foster ASM cell mitogenesis and/or hypertrophy.

***In vitro* effect of transforming growth factor- β 1 on airway smooth muscle cells**

The *in vitro* effect of TGF- β 1 on ASM growth has been studied in cells from two species, bovine and human. For the sake of clarity, the following sections discuss separately the results obtained using these two species.

Bovine airway smooth muscle cells

Using bovine ASM cells, Black et al. [59] initially reported a bimodal, time-dependent effect of TGF- β 1 on ASM cell mitogenesis. Although TGF- β 1 inhibited DNA synthesis between 8 and 24 h post-stimulation, it increased DNA synthesis and cell counts at later time-points. Increased DNA synthesis in bovine tracheal ASM cells between 24 and 48 h post-TGF- β 1 stimulation was also observed by Okona-Mensah et al. [60]. This delayed proliferative response to TGF- β 1 was consistent with the one observed in other mesenchymal cells [61–63]. In these latter studies, the delayed effect was explained by an autocrine loop of growth factors, which were secreted following TGF- β 1 stimulation. However, Chen and Khalil [64] have demonstrated recently that neutralizing Abs against FGF-2, platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and insulin-like growth factor (IGF)-1 had no effect on bovine ASM cell mitogenesis induced by TGF- β 1. They also demonstrated that an earlier assessment of DNA synthesis following TGF- β 1 stimulation (i.e., between 20 and 24 h post-stimulation) was sufficient to detect the pro-mitogenic effect of TGF- β 1, which was somewhat contrasting to the results presented by Black et al. [59]. The only obvious difference that can potentially explain the different results in these two studies is related to the length of the radiolabelled thymidine pulse at the end of the 24 h stimulation. Owing to the bimodal, time-dependent effect of TGF- β 1 on bovine ASM mitogenesis, it is possible that the 4 h pulse of thymidine in Chen and Khalil's study [64] captured mainly the pro-mitogenic effect of TGF- β 1 (hence an overall increase in DNA

synthesis would occur in this 4 h time frame post-TGF- β 1 stimulation), whereas the 16 h pulse of thymidine used in Black *et al.*' study [59] would capture mainly the anti-mitogenic effect of TGF- β 1 (hence the decrease in DNA synthesis occurring early in this time frame in the presence of radiolabeled thymidine would not be overcome by the increased DNA synthesis occurring later in this same time frame). This would also suggest that the switch of TGF- β 1 from an anti- to a pro-mitogenic factor occurs somewhere in the 8–24 h period post-stimulation.

Black *et al.* [59] also demonstrated that TGF- β 1 can induce bovine ASM cell hypertrophy. This result was subsequently confirmed by others in human ASM cells [65, 66]. Whether the increase in ASM mass in asthmatic airways is due to hyperplasia [67–69] or hypertrophy [70] is an ongoing debate as both phenotypes have been observed. Ebina *et al.* [71] have suggested the existence of two types of asthma based on the stereological assessment of ASM tissue in human lungs. Type 1 asthma was characterized by hyperplasia of ASM cells in the larger airways of the respiratory tract, whereas type 2 asthma was predominantly characterized by ASM hypertrophy in the smaller, peripheral airways of the respiratory tract with moderate hyperplasia observed in the major bronchi. Interestingly, the hyperplastic or hypertrophic response to TGF- β 1 was shown to be animal specific in Black *et al.*' study [59]. They reported a strong inverse correlation ($r = -0.97$) between changes in cell size and cell number after TGF- β 1 stimulation; i.e., whereas cellular hypertrophy was observed in ASM cells derived from some animals, the predominant effect of TGF- β 1 on ASM cells from other animals was cellular hyperplasia. These results suggest that the individual response of asthmatics to TGF- β 1 stimulation may determine whether they develop type 1 (ASM hyperplasia) or type 2 (predominantly ASM hypertrophy) asthma.

TGF- β 1 was also shown to synergize with other mediators to induce bovine ASM cell proliferation. For instance, the non a peptide bradykinin, which has no mitogenic effect when administered alone, potentiates the weak mitogenic effect of TGF- β 1 in ASM cells by acting on its B₂ receptor [72]. Similarly, receptor tyrosine kinase (RTK) ligands, such as EGF [60], were shown to synergize with TGF- β 1 to induce bovine ASM cell mitogenesis.

Human airway smooth muscle cells

The first data concerning the effect of TGF- β 1 on the proliferation of human ASM cells came a little bit later. In two initial studies [73, 74], TGF- β 1 had no effect on its own, but inhibited the growth-promoting effect of EGF and thrombin. The lack of mitogenic effect of TGF- β 1 (when administered alone) on human ASM cells was subsequently confirmed repetitively [24, 66, 75, 76]. However, these studies are contradicted by an equivalent

number of studies. These discrepancies heralded another controversial issue concerning the effect of TGF- β 1 in asthma [18]. In fact, in some workers' hands, TGF- β 1 was shown to induce human ASM cell mitogenesis/proliferation when administered alone [77–79]. Consistent with bovine ASM cells, the mitogenic effect of TGF- β 1 on human ASM cell was delayed compared with other growth factors. The earliest time recorded for the pro-mitogenic effect of TGF- β 1 on human ASM cells was reported in the study of Ediger and Toews [77], where increased DNA synthesis was shown to occur during a 2 h pulse of radiolabelled thymidine beginning 22 h post-stimulation.

TGF- β 1 was also shown to enhance the growth-promoting effect of different ASM mitogens [24, 77, 78]. For example, Cohen *et al.* [80] demonstrated that TGF- β 1 enhanced the mitogenic effect of a growth medium containing FBS (5%), FGF-2, EGF and insulin. They also demonstrated that the growth-enhancing effect of TGF- β 1 was dependent on an autocrine loop of IGF-binding protein (IGFBP)-3, which potentially explains the delayed mitogenic effect. However, the mechanism by which IGFBP-3 enhances ASM proliferation has yet to be determined. It has been proposed that IGFBP-3 can exert its pro-mitogenic effect by either modulating the interaction of IGFs with their cognate cell-surface receptors or by an intrinsic, IGF-independent action via binding to IGFBP-3-associating proteins (potential IGFBP-3 receptors) found on the ASM cell sarcolemma [80]. It is worth mentioning that human ASM cells secrete impressive amounts of IGFBP-3 protein in response to TGF- β 1 stimulation. Cohen *et al.* [80] reported that IGFBP-3 levels reached over 1 μ g/mL in the conditioned medium of human ASM cells following 3-day stimulation with 1 ng/mL of TGF- β 1 [80]. This tremendous increase of IGFBP-3 was corroborated by Jarai *et al.* [81] using gene chip array technology. Among ~12 500 genes analysed, IGFBP-3 was the most up-regulated (54.1-fold over baseline) following a 24 h stimulation of human ASM cells with 10 ng/mL of TGF- β 1 [81]. But importantly, the supplemented medium, containing serum, FGF-2, EGF and insulin, was required for the proliferative effect of TGF- β 1 in human ASM [80], suggesting that one or more of these factors is(are) capable of conferring to TGF- β 1, or IGFBP-3, a mitogenic effect on human ASM cells [80].

As observed with bradykinin [72] and EGF [60] in bovine ASM, TGF- β 1 was shown to synergize with other mediators to enhance human ASM cell mitogenesis. For example, lysophosphatidic acid (LPA), a lipid ligand for the G protein-coupled receptors (GPCR) LPA1, 2 and 3 (also called EDG2, 4 and 7, respectively), was shown to amplify the mitogenic effect of TGF- β 1 on human ASM cells [77]. TGF- β 1 can also potentiate ASM cell proliferation via indirect mechanisms. We demonstrated previously that leukotriene (LT)D₄, a lipid mediator involved in the pathogenesis of asthma, was without any effect in

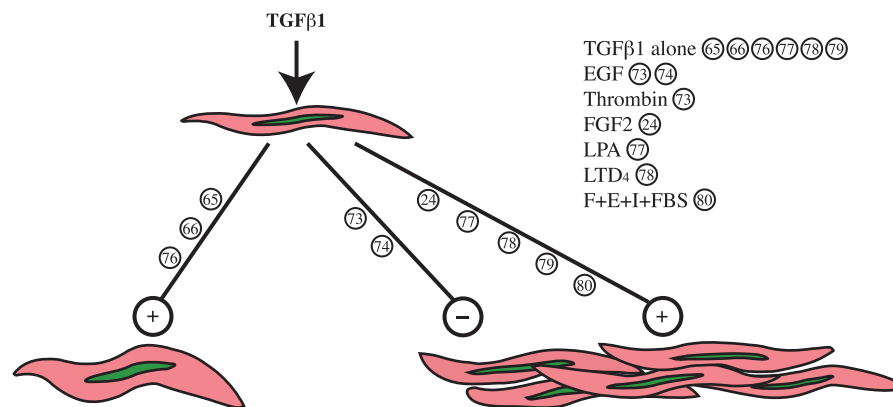


Fig. 2. The context-dependent effects of TGF- β 1 on human airway smooth muscle (ASM) cell proliferation and hypertrophy *in vitro*. + and - signs at the end of the arrows designate positive and inhibiting effects, respectively. Encircled numbers running along the arrows and beside the different mitogens refer to the references demonstrating the pointed effect for any given mitogen. As illustrated in the figure, TGF- β 1 has been shown to induce both cell hypertrophy and proliferation. Although, its effect on proliferation when administered alone is weak and controversial. Its influence on the proliferation induced by other mitogens is also hard to predict and sometimes opposite. Whereas it synergizes with some mitogens, such as FGF-2 and lysophosphatidic acid (LPA), it antagonizes the action of other mitogens, such as epidermal growth factor (EGF) and thrombin. TGF- β 1 also renders ASM permissive to the proliferative effect of leukotriene D₄ (LTD₄). Studies showing no effect of TGF- β 1 on changes in either proliferation or cell size are not depicted. F + E + I + FBS, FGF-2 + EGF + insulin + fetal bovine serum.

Table 3. Cell culture studies investigating the potential role of TGF- β 1 as a direct inducer of ASM tissue enlargement seen in asthmatic airways

Non-mitogenic	Anti-mitogenic	Pro-mitogenic	Synergized with mitogens	Hypertrophic
Cohen et al. [73]	Cohen et al. [73]	<i>Black et al. [59]</i>	Ediger and Toews [77]	<i>Black et al. [59]</i>
Goldsmith et al. [66]	Krymskaya et al. [74]	<i>Okona-Mensah et al. [60]</i>	Cohen et al. [80]	McKay et al. [65]
Bosse et al. [24]	<i>Okona-Mensah et al. [60]</i>	Ediger and Toews [77]	Espinosa et al. [78]	Goldsmith et al. [66]
<i>Gosens et al. [72]</i>		Espinosa et al. [78]	Bosse et al. [24]	Goldsmith et al. [76]
Goldsmith et al. [76]		<i>Chen and Khalil [64]</i>	<i>Gosens et al. [72]</i>	
Shin et al. [75]		Xie et al. [79]		

Text in italics signifies that the cited studies were performed using bovine cells. All of the other studies were performed using human cells. ASM, airway smooth muscle.

ASM cell proliferation when administered alone. However, pre-treatment of human ASM cells with TGF- β 1 was shown to confer a significant mitogenic effect to LTD₄ [78]. The operational mechanism mediating this proliferative synergism involved the up-regulation of CysLT1 (the high-affinity receptor for LTD₄) by TGF- β 1 [78]. The *in vitro* studies documenting an effect of TGF- β 1 on human ASM cell proliferation or hypertrophy are depicted schematically in Fig. 2.

Inconsistencies among the *in vitro* effect of transforming growth factor- β 1 on airway smooth muscle cells

Taken together, studies published in the last decade concerning the effect of TGF- β 1 on ASM cell proliferation *in vitro* have yielded different, and sometimes, contradictory results (summarized in Table 3). Some data report an anti-mitogenic activity, whereas other studies support the opposite view, reporting a mitogenic activity for this cytokine, acting by itself or in synergy with other mitogens. The effect of TGF- β 1 on ASM cell proliferation

in vitro may be influenced by a plethora of factors, such as: (1) the cell origin (the species, the generation in the airway tree where the cells have been obtained; e.g. trachea vs. bronchi and the individual characteristics of the donor such as gender, age, race, existing lung diseases, smoking status and genetic background); (2) the peculiarities of culture conditions (the medium *per se*, whether the cells were growth-arrested or not and for how long, the presence of serum or BSA and their respective concentrations, the level of cell confluency and the matrix on which the cells were seeded); (3) the conditions of stimulation (concentration of TGF- β 1, the time spent after stimulation to assess proliferation, co-stimulation with other factors and their respective concentrations); and (4) the way to assess ASM cell proliferation (DNA synthesis vs. cell numbers). Taking all of these variables into account, the studies performed so far describing the mitogenic effect of TGF- β 1 on ASM cell proliferation are seldom comparable. Hence, the results may not be conflicting after all. However, if all these inconsistencies are real and could be explained by cells or methodological

peculiarities used by different studies, one can only argue that the effect of TGF- β 1 on ASM cell proliferation *in vitro* is context-dependent. The only trends that are worthy of mention in the two species investigated so far are the following: In bovines, TGF- β 1 appears to exert a weak pro-mitogenic effect in confluent, tracheal ASM cells; and in humans, even if the picture is less clear, the weight of evidence suggests that TGF- β 1 has no effect on its own, but synergizes with other mediators to induce ASM cell proliferation. Unfortunately, based on the current literature, no definitive conclusions can be drawn at the moment. In addition, a recent study has shown that TGF- β 1 can induce a delayed proliferation of human ASM cells irrespective of the level of cell confluency and the presence or absence of serum [79].

Hypothesis

The bulk of *in vivo* evidence (Table 1), including recent convincing findings (Table 2), suggest that TGF- β 1 and the intracellular intermediates used to transduce its biological signal from the cell membrane to the nucleus are required for ASM enlargement in asthma. To reiterate, a neutralizing antibody against the three isoforms of TGF- β was shown to reverse ASM enlargement that occurred in a mouse model of allergic (OVA) airway inflammation [11]. Also, blocking Smad signalling using an ALK-5 pharmacological inhibitor [55] or a KO approach by deleting the Smad3 gene [56] as shown to protect against the development of ASM enlargement in different animal models of allergic airway inflammation. Finally, the overexpression of TGF- β 1 alone in the airways of transgenic mice was shown to be sufficient to induce ASM enlargement [54]. These results are in great contrast to the inconsistent results obtained *in vitro* regarding the effect of TGF- β 1 on monolayer cultures of ASM cells (Table 3). Altogether, these results suggest that one or several factor(s) found *in vivo* renders the ASM cells permissive to the mitogenic effect of TGF- β 1, at least in pathological conditions. The presence or the absence of this(these) factor(s) in cell culture conditions can be context-dependent and ultimately determines the mitogenic effect of TGF- β 1 on ASM cells. One of the factors that could potentially turn on the mitogenic effect of TGF- β 1 for ASM cells is FGF-2 [24]. We have shown previously that TGF- β 1 had no effect on human ASM cell proliferation when administered alone, independent of the concentration used. However, TGF- β 1 increased ASM cell proliferation in a concentration-dependent manner following a 24 h pre-treatment with FGF-2, reaching a value up to six times higher than when FGF-2 was used alone at 4 days post-TGF- β 1 stimulation [24]. The proliferation of ASM cells following this sequential FGF-2 and TGF- β 1 stimulation was nearly threefold greater than the more potent ASM cell mitogen PDGF-BB [24]. FGF-2 is also a well-known mitogen for

ASM cells and has been consistently and repetitively shown to be up-regulated in asthmatic airways (reviewed in Bosse and Rola-Pleszczynski [25]). In addition, its overexpression in bronchoalveolar lavage fluid (BALF) of human asthmatics was shown to occur at an early time-point (10 min post-challenge) [26] compared with TGF- β 1 for which the peak expression occurred 24 h following segmental allergenic bronchoprovocation [82]. This sequential FGF-2-TGF- β 1 stimulation is thus likely to occur in this order *in vivo* following an allergenic bronchial challenge and likely contributes to ASM overgrowth. Hence, we hypothesize that extracellular signals from FGF-2 are a prerequisite for TGF- β 1 to induce human ASM cell proliferation.

We believe that this FGF-2-TGF- β 1 synergy has already been reported in the literature, but without identifying the incriminated factor added to the smooth muscle medium responsible for the mitogenic effect of TGF- β 1. As aforementioned, Cohen et al. [80] demonstrated that TGF- β 1 was able to induce human ASM cell proliferation, but this effect was absent if the growth factors (FGF-2, EGF and insulin) and the serum (5%) were omitted from the culture medium. In our view, the essential factor was not in the serum, as suggested by the authors, but was the FGF-2 added to the culture medium. Without any effort to identify this factor, Cohen et al. [80] pursued their study and demonstrated convincingly that the IGFBP-3 induced by TGF- β 1 was responsible for its mitogenic effect, offering another perspective of what might be the mechanism explaining the mitogenic synergism between FGF-2 and TGF- β 1. However, considering the fact that TGF- β 1 alone was sufficient to induce IGFBP-3 in the absence of added serum or growth factors [80], but was unable to support ASM cell proliferation by itself [24], we can only anticipate that IGFBP-3 is one of the factors induced by TGF- β 1 that mediates the proliferative synergism with FGF-2.

Even if TGF- β 1 is not up-regulated in asthma, FGF-2-TGF- β 1 synergism is likely to occur and causes ASM cell hyperplasia. As reviewed elsewhere [18], TGF- β 1 is constitutively expressed in normal and asthmatic human airways [83-85]. TGF- β 1 mRNA is expressed in different cells of healthy human lungs, including bronchiolar epithelial, endothelial and mesenchymal cells, as well as in alveolar macrophages [85]. Hence, asthmatic or not, the airways clearly express TGF- β 1. Based on our results, TGF- β 1 cannot trigger ASM cell proliferation in the absence of FGF-2. However, following the release of FGF-2 by an allergen challenge, ASM cells may become permissive to the proliferative effect of TGF- β 1. Consequently, the constitutive expression of TGF- β 1 may become sufficient to cause ASM cell hyperplasia. In addition, TGF- β 1 must be activated by post-translational modifications before binding to its cognate cell surface receptor and mediating its biological effect. Hence, even if its expression is not altered in asthma, its activation may

occur in the face of asthmatic inflammatory reactions, and this will unmask its mitogenic action on FGF-2-primed ASM cells.

The synergy reported *in vitro* between FGF-2 and TGF- β 1 may also reproduce what is really occurring *in vivo* with other factors that act via similar receptors or signalling pathways. Activin A, another activator of Smad2/3/4, is up-regulated in human asthma [86–88] and in animal models of asthma [22, 56, 89]. Activin is also a potent inducer of human ASM cell proliferation [87]. Immuno-histological studies have co-localized Activin A with mast cell tryptase in asthmatics [87], suggesting that mast cells are an important source of Activin A in asthma. Accordingly, the increased expression of Activin A in a murine model of allergic asthma was severely impaired in mast cell-deficient mice (W/W^v) following allergic bronchoprovocation. Activin A secretion by mast cells, following Fc ϵ RI cross-linking *in vitro*, further corroborates these results [87]. Interestingly, mast cells are interspersed in the ASM cell bundles of asthmatics and are well-known modulators of ASM cell functions [90]. Hence, considering the proximity of mast cells and ASM cells in asthmatic lungs, it is conceivable that mast-cell-derived Activin A could be the main TGF- β superfamily member that synergizes with FGF-2 (or other FGF family members) to induce ASM cell hyperplasia. The biology and the overall effect of Activin in asthma pathogenesis seem to be extremely complex though. Both anti-allergic [88] and pro-allergic [89] type of inflammation have been shown for Activin in experimental models of asthma. Just as it is the case for TGF- β [18], its overall effect on asthma progression, as well as on the manifestation of specific symptoms and the overall well-being of asthmatic patients will be hard to predict at the moment. The detrimental effect of Activin on ASM enlargement could be either exacerbated or counterbalanced by its contribution to other facets of asthmatic airway remodelling, by its immunoregulatory functions or by its direct influence on ASM contractility.

In the same vein, TGF- β 2 is also up-regulated in human asthmatic airways [91, 92]. Because the TGF- β 2 isoform acts on the same receptors as TGF- β 1 and also signals via Smad2/3/4, it is an additional likely candidate to synergize with FGF-2, or other FGF family members, for inducing ASM cell hyperplasia in asthma.

On the other hand, no member of the FGF family, apart from FGF-2, has been reported to be up-regulated in asthmatic tissues. However, FGF-1 is overexpressed in other chronic inflammatory lung diseases [93, 94] and was shown to be capable of inducing ASM cell proliferation *in vitro* [94]. In addition, IL-1 β , which is structurally related to the family of FGF [95], is known to be up-regulated in asthma. Interestingly, and as observed for FGF-2 [24, 96], IL-1 β synergizes with PDGF in other mesenchymal cells to induce cellular proliferation

through its ability to increase the expression of the PDGF receptor (PDGFR) α [97–99]. Hence, as observed for FGF-2, both FGF-1 and IL-1 β are likely candidates to prime ASM cells to proliferate in response to TGF- β family members that signal via Smad2/3/4.

It is also noteworthy to mention that each of the individual interactions between members of FGF and TGF- β family, which may occur *in vivo*, are not mutually exclusive. Instead, the extracellular cues from one family of growth factors may be cumulative and synergize with the combined signals generated from simultaneous extracellular cues of several members of another family of growth factors. ASM enlargement is a complex phenotype. It would be surprising that only a single synergism between two growth factors would explain this structural alteration in asthma. It is more likely that a concerted action of two families of growth factors lead to ASM overgrowth, where every member of one family potentiates the mitogenic action of every member of the other family.

Conclusion

Despite being heterogeneous in terms of inception, pathogenesis and progression, the asthma syndrome is characterized by common molecular patterns and pathogenomic remodelling of the airways. Increased TGF- β 1 expression/signalling and increased peribronchial mass of ASM tissue are two such phenomena. Strikingly, these phenomena appears to occur concurrently in different animal models of asthma, suggesting that they are causally related. Several studies also substantiate this association by showing that the lack of ASM growth caused by a therapeutic or prophylactic treatment coincides with a decreased expression/signalling of TGF- β 1 (Table 1). Lately, and more importantly, a few studies have contributed to the proof of concept that TGF- β 1 is required [11, 55, 56] and sufficient [54] to trigger ASM enlargement. However, these conclusions are derived from studies using animals, which were exposed to different asthma triggers to model the human asthmatic syndrome. The association between TGF- β 1 expression/signalling and ASM mass remains to be investigated more closely in human, as does the therapeutic utility of an anti-TGF- β 1 medication to prevent or reverse ASM remodelling in asthma.

On the other hand, *in vitro* investigations looking at the role of TGF- β 1 on the growth of ASM have yielded inconsistent results. The only conclusive statement that can be drawn from these studies is that the effect of TGF- β 1 on the growth of a monolayer of ASM cells is contextual. All together, these results suggest that a factor (or factors), which displays increased biological activity in asthmatic states, renders ASM cells permissive to the mitogenic effect of TGF- β 1 *in vivo*. One such factor is FGF-2, which has been shown to confer to TGF- β 1 a powerful proliferative effect on human ASM cells. In

addition, FGF-2 has been shown to be overexpressed in asthmatic airways and to be further increased following an allergenic bronchoprovocative challenge [25]. Therefore, FGF-2 may be the missing link reconciling the apparent discrepancies among *in vitro* findings documenting the effect of TGF- β 1 on ASM cells with the consistent *in vivo* findings that TGF- β 1 is required for ASM enlargement in asthma.

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