

REVIEW

Airway smooth muscle function in asthma

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Introduction

The traditional view of airway smooth muscle in asthma is of a passive partner in airway inflammation, contracting in response to pro-inflammatory mediators and neurotransmitters, and relaxing in response to endogenous and exogenous bronchodilators [1]. Early studies of human airway smooth muscle (HASM) function in asthma therefore tended to concentrate on the contractile aspects in order to try to explain airway hyperresponsiveness, the characteristic of asthma in which the airways show an exaggerated response to a large number of different stimuli acting through disparate pathways [1,2]. A number of studies has addressed the issue of whether this hyperresponsiveness is due to altered properties of airway smooth muscle itself. These studies have failed to consistently demonstrate an alteration in contractile properties, although studies in animal models of asthma, and in airway smooth muscle preparations from asthmatic patients *in vitro*, have suggested that relaxant responses may be impaired. It has become apparent that the airways in asthma undergo marked structural changes and that these may contribute to the underlying hyperresponsiveness [3,4]. Changes in airway smooth muscle thickness may be particularly important in this respect. Most recently, attention has turned to the synthetic properties of airway smooth muscle. Airway smooth muscle is a rich source of many cytokines, chemokines and inflammatory mediators which may modulate airway inflammation in asthma [5]. In this review we will discuss the current state of knowledge of the contractile, proliferative and synthetic functions of airway smooth muscle in asthma (Fig. 1).

Contraction

Asthma is characterized by non-specific bronchial hyperresponsiveness to a variety of direct and indirect stimuli [1,2]. A number of research groups has addressed the

question of whether the contractile properties of airway smooth muscle are altered due to an underlying abnormality in airway smooth muscle itself. Although a number of studies has been performed, relatively few of these have been in patients with clear cut asthma and in those that have, the numbers have been very small. Most studies have tried to correlate bronchial reactivity to spasmogens, such as histamine [6,7], methacholine [8,9] LTD₄ [10] or adenosine [11], in patients *in vivo* with the contractile responses to these agents in isolated bronchial segments in organ baths *in vitro*. These studies have usually been performed on surgical lobectomy or pneumonectomy samples from patients undergoing resections for bronchogenic carcinoma. The results of these studies are fairly consistent, showing no relationship between bronchoconstrictor responsiveness *in vivo* and tension generation *in vitro*. Caution should be shown when extrapolating the findings of these studies to asthma, however. The airway responsiveness to bronchoconstrictor challenge *in vivo* in patients having lung resections for bronchial carcinoma may consist of several components. Factors such as airway geometry, the presence of underlying chronic obstructive pulmonary disease (COPD) and the altered aerosol deposition caused by the carcinoma itself may play a confounding role and mask changes in the underlying contractility of airway smooth muscle. It is also likely that mediators and cytokines responsible for hyperresponsiveness *in vivo* may have been lost by the time the tissue was studied *in vitro*. For these reasons, it is perhaps less surprising that no relation was found in these studies.

Few studies have looked at airway smooth muscle taken exclusively from asthmatic subjects [12–14]. However, there are isolated reports in patients with asthma of increased contractile responses or increased sensitivity to spasmogens *in vitro*. A possible mechanism whereby airway smooth muscle could become hyperresponsive to bronchoconstrictors is through the actions of pro-inflammatory cytokines such as tumour necrosis factor α (TNF α) and interleukin-1 β (IL-1 β). Studies in cultured airway smooth muscle have shown that these cytokines can increase cytosolic calcium responses when cells are stimulated with

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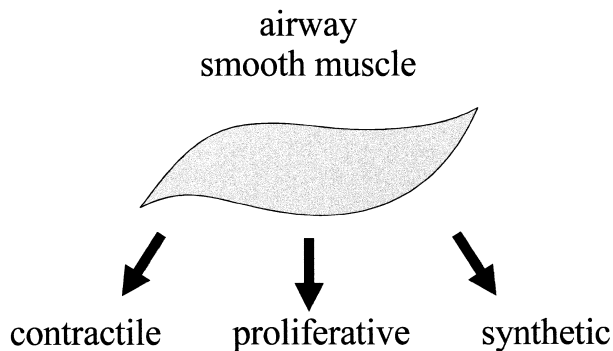


Fig. 1. Functions of airway smooth muscle in asthma.

spasmogens such as carbachol, bradykinin or thrombin [15–17]. Recently, it has been shown that the enhanced signal response coupling after $\text{TNF}\alpha$ treatment may be due to increased expression of $\text{G}_{\text{q}\alpha}$, with a resulting increase in inositol phosphate synthesis [18]. Consistent with this, $\text{TNF}\alpha$ can induce airway hyper-responsiveness when given by inhalation to asthmatic subjects *in vivo* [19].

Relaxation

Although there is scant evidence to support the view that ASM contraction is abnormal, the evidence that β_2 adrenoceptor ($\beta_2\text{AR}$)-linked relaxant mechanisms may be dysfunctional in asthmatic airways is more compelling. Airway smooth muscle relaxation *in vitro* to β_2 AR agonists is impaired in airways taken from patients who died of asthma exacerbations [13,20], in surgical lobectomy samples from patients with stable asthma [12] and in animal models of asthma [21,22]. The attenuated β_2 AR relaxation in asthmatic airways is not due to decreased β_2 AR density or affinity [23,24], suggesting that there is uncoupling of airway smooth muscle β_2 ARs from adenylyl cyclase in asthma.

Studies in animal models have investigated the mechanisms involved and have suggested that inflammatory cytokines, particularly IL-1 β , are responsible. In rabbit airway smooth muscle, a reduced relaxant sensitivity to isoprenaline and prostaglandin E_2 was observed after sensitization of the muscle with atopic serum [25]. Responses to forskolin, which activates adenylyl cyclase directly, were unchanged, suggesting that the site of the defect in relaxation was located at a site proximal to adenylyl cyclase. The attenuated relaxation to β_2 AR agonists and PGE_2 was inhibited by the muscarinic M_2 antagonist methoctramine, gallamine and pertussis toxin, implicating the involvement of G_i , the inhibitory G protein link to adenylyl cyclase. Western blot studies confirmed increased expression of the $\text{G}_{i\alpha 2}$ and $\text{G}_{i\alpha 3}$ subunits [26]. The effect of atopic serum was partly due to IL-1 β , as the impaired relaxant responses to atopic serum

could be inhibited with an IL-1 antagonist or IL-1 β neutralizing antibody. Consistent with this, enhanced release of IL-1 β was seen after exposure to atopic asthmatic serum [27]. These findings have been replicated in intact tissue from other species. Studies in guinea pig [28] and rat [29] airways have also shown impaired relaxant responses to β_2 adrenoceptor agonists after IL-1 β treatment, and a reduction in β_2 AR mediated cAMP generation was seen in cultured human ASM after IL-1 β [30]. $\text{TNF}\alpha$ had a similar effect on β_2 adrenoceptor mediated cAMP generation in rabbit ASM, although no effect was seen on G_i [31]. More recently, a study with $\text{TNF}\alpha$ in cultured human ASM [18] has shown increased G_i expression, suggesting that there may be species differences.

Collectively, these findings suggest that β_2 AR relaxant responses are impaired in asthmatic humans and experimental animal models of asthma, and that this effect is mediated in part by IL-1 β . We recently extended these studies and studied the mechanisms responsible in cultured human airway smooth muscle cells [32,33]. As β_2 AR mediated relaxation occurs mainly through cAMP pathways, we investigated the effect of IL-1 β on cAMP generation in response to isoprenaline (ISO). We showed that IL-1 β , but not $\text{TNF}\alpha$ or interferon- γ (IFN γ), impaired cAMP generation in response to ISO. We were struck by the similarity between these results and parallel studies we were performing on cyclooxygenase expression by HASM cells. Cyclooxygenase is a key enzyme in prostanoid synthesis which exists in two forms, constitutive COX-1 and inducible COX-2. We have previously shown that IL-1 β , but not $\text{TNF}\alpha$ or IFN γ , induced COX-2 in HASM cells [34]. As induction of COX-2 was associated with a marked increase in PGE_2 and PGI_2 production, and both are coupled to adenylyl cyclase and therefore intracellular cAMP, we postulated that the elevated levels of PGE_2 and PGI_2 would cause heterologous desensitization of adenylyl cyclase, thereby impairing cAMP generation in response to β_2 AR agonists (Fig. 2). Our studies using COX-2 inhibitors and inhibitors of COX-2 transcription and translation showed

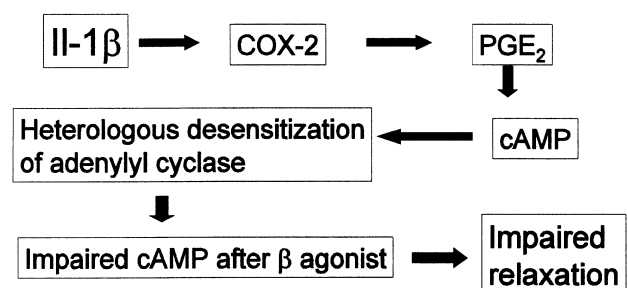


Fig. 2. Mechanism whereby cytokines impair adenylyl cyclase function.

that this was indeed the case. Similar findings were reported in parallel by Shore and colleagues [35] who, in addition, looked at cell stiffness as an index of contractility and found that the changes in cAMP were linked to alterations in cell stiffness. It would therefore appear that the impaired β_2 AR-mediated relaxation of airway smooth muscle in asthma is mediated, at least in part, by cytokine (IL-1 β)-mediated induction of COX-2. We have recently shown that the pro-inflammatory asthma mediator, bradykinin, can produce a similar impairment of β_2 AR-mediated cAMP generation through prostanoid generation via both COX-2 and COX-1 pathways [33,36]. Although there is compelling evidence to suggest that β_2 AR-mediated ASM relaxation is impaired in asthmatic tissue *in vitro*, it is not clear how important this is *in vivo*. It would seem unlikely to be relevant in mild asthma but might be important in chronic severe or acute asthma where the airways often appear to respond poorly to bronchodilators.

Remodelling

Several post-mortem studies from asthmatic airways have shown thickening of the airway smooth muscle layer [37–42]. These studies suggest that the number of smooth muscle cells is increased two to threefold compared with normal airways. Morphometric histopathological studies have suggested that there may be two distinct subtypes of asthmatic patients, one characterized by marked hyperplasia but little hypertrophy with changes confined to the central airways, and a second group where smooth muscle thickening is seen throughout the whole bronchial tree with predominant hypertrophy and a smaller component of hyperplasia [41]. Whether these pathological subdivisions have any clinical significance is unclear. The increased smooth muscle layer is thought to contribute to airway narrowing and bronchial hyperresponsiveness through the geometric effects of airway thickening, and by the thickened muscle developing an exaggerated shortening response to bronchoconstrictor stimuli [3,43].

Most mechanistic studies of airways smooth muscle proliferation have used cell culture systems, as these allow controlled experimental manipulation [44,45]. A number of growth factors has been identified in airway smooth muscle taken from a number of species (Table 1). Those which are mitogenic in human airway smooth muscle include platelet-derived growth factor [52], fibroblast growth factor [52], epidermal growth factor [47,48], histamine [58] and thrombin [17,48]. Other substances, such as endothelin-1 [47,60] and LTD₄ [66], are not mitogenic on their own but potentiate the response to other mitogens. Growth factors can be divided broadly into the products of inflammatory cells, which are likely to come into increased contact with airway smooth muscle in the inflammatory airway milieu of

asthma, blood derived factors, which are likely to gain increased access to airway smooth muscle through microvascular leakage, and structural cell products which may modify proliferation in a paracrine or autocrine manner. Although a number of mitogens for airway smooth muscle has been identified in cultured airway smooth muscle *in vitro*, it is still not clear which are the most important in human asthma *in vivo*.

Inhibition of the signal transduction pathways involved in proliferation represents a possible target for new therapeutic strategies. A number of signalling pathways has been implicated in the proliferative response; detailed consideration of all of these is outside the scope of this review and has been covered elsewhere [75]. They include the tyrosine kinase cascades associated with classical growth factors, like platelet-derived growth factor, fibroblast growth factor, epidermal growth factor and insulin like growth factors. One group of enzymes which may be particularly important is the extracellular signal regulated kinases (ERKs). ERKs are cytosolic serine/threonine kinases of the mitogen activated protein kinase (MAPK) super family which transduce growth-promoting signals to the nucleus [75]. ERKs are activated by platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF)-1 and 5-HT in bovine ASM [76], and thrombin [56] and 5 hydroxytryptamine endothelin (ET)-1 [56,57] in rat ASM, and studies using dominant negative mutants have shown that inhibition of MEK-1, which is required for ERK activation, inhibits PDGF-induced DNA synthesis in bovine ASM [78]. PI₃ kinase, a heterochimeric lipid kinase, may also be involved in the proliferative process by activating protein kinase B (PKB) and p70 ribosomal S6 kinase. PI₃ kinase is activated by EGF [79] in human ASM, and by PDGF [53] and thrombin [80] in bovine ASM. Protein kinase C (PKC) may also be important in ASM proliferation. PKC can be activated either by PLC, PLA₂ or PLD pathways and upon activation, can translocate to the cell nucleus where it can phosphorylate transcription factors and regulate gene expression and cell division [81]. PKC exists in a family of calcium-dependent, calcium-independent and atypical isoforms [81]. Studies in rabbit ASM have shown that PKC inhibitors inhibit proliferation [82], and a recent study in human ASM suggests that PKC- ζ , an atypical isoform, may be involved in PDGF induced human ASM proliferation [83].

Agents which bind to receptors linked to inhibitory pathways may also have a therapeutic role. Elevation of cyclic AMP by agents binding to adenylyl cyclase coupled receptors, such as β_2 adrenoceptor agonists [47,84], vasoactive intestinal peptide [58] and prostaglandin E₂ [85], inhibits proliferation. The effect of β_2 AR agonists can be potentiated by phosphodiesterase inhibitors, which prevent cAMP breakdown, and type III cAMP selective PDE

Table 1 Promoters of ASM mitogenesis

Factor	Source	Species	(Ref)
Growth factor/cytokine			
EGF	P	Human	47,48
	Ep	Bovine	49
bFGF	ECM, M θ , SMC, Ep	Bovine	50
		Human	48,51
PDGF	P, M θ , SMC, Ep	Rabbit	52
		Human	52
		Bovine	53
IGF-1	P, M θ , SMC, Ep	Bovine	50
		Rabbit	54
IL-1 β	T, M θ , SMC, Ep	Guinea-pig	55
IL-6	T, M θ , SMC, Ep	Guinea-pig	55
Thrombin	P1	Human	17,48
		Rat	56
TGF- β 1	T, M θ , ECM, P, Ep	Bovine	49
Inflammatory mediators			
Histamine	M	Canine	57
		Human	58
Endothelin-1	P1, M θ , P, Ep	Rat	56
		Rabbit	59
		Human	47,60
		Bovine	61
		Ovine	62
Serotonin	P, P1	Bovine	63
Substance P	Neuronal, Ep	Rabbit	64
LTD ₄		Rabbit	65
		Human	66
Thromboxane A ₂		Rabbit	65
Enzymes			
Tryptase	M	Canine	67
β -Hexosaminidase	M	Bovine	68
Lysosomal hydrolase	M	Canine	69
Miscellaneous			
α -adrenoceptor agonists		Rabbit	70
Mechanical stretch		Canine	71–73
Lysophosphatidic acid		Human	74

EGF: epidermal growth factor; bFGF: basic fibroblast growth factor; PDGF: platelet-derived growth factor; IGF-1: insulin-like growth factor-1; IL: interleukin; TGF- β 1: transforming growth factor- β 1; M: mast cells; P: platelet; M θ : macrophage/monocyte; Ep: epithelial cells; P1: plasma; SMC: smooth muscle cells; ECM: extracellular matrix; T: T-lymphocytes.

inhibitors also have antiproliferative effects [86]. The mechanism of the cAMP effect is complex and may involve inhibition of Raf-1 activity [75], PI₃ kinase [80], PKC ζ [83], or cyclin D protein expression [87]. Several miscellaneous agents, such as corticosteroids [48,84], heparin [88], TGF β ₁ [49,89], interleukin 4 [51] and dehydroepiandrosterone [90], have also been shown to have antiproliferative effects, although most agents are either partially effective or preferentially inhibit the response to selective mitogens. Elevations in cyclic GMP

through the use of NO donors or natriuretic peptides represents another potential antiproliferative pathway which is being explored [91].

Airway smooth muscle may itself be a source of endogenous antiproliferation factors. PGE₂ produced endogenously, either from phospholipase A₂ and constitutive COX-1 or inducible COX-2 [91,92], may put a brake on proliferation and may explain why some cytokines, such as interleukin 1 β which induce COX-2, are only proliferative in the presence of cyclooxygenase inhibitors.

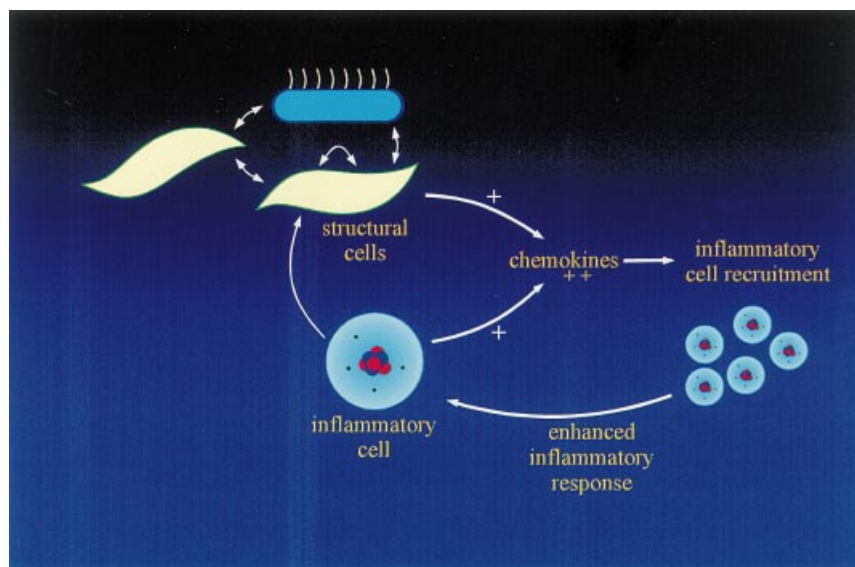


Fig. 3. Structural cells such as airway smooth muscle amplify the chemokine signal produced by inflammatory cells, thereby increasing inflammatory cell recruitment and airway inflammation.

Interactions between smooth muscle cells and the extracellular matrix may also be important. The extracellular matrix consists of fibronectin, glycosaminoglycans, laminin, type IV collagen, tenascin, entactin, integrins and metalloproteinases (MMPs). We have recently shown that autocrine production of MMP-2 is required for human ASM proliferation [94]. ASM-derived MMPs may also play a role in addition to remodelling. Currently the focus of much research in the airways, MMPs are upregulated by antigen challenge, and their inhibition following antigen in a murine model of asthma caused a fall in airway inflammatory cell accumulation and hyperresponsiveness [95].

Synthetic functions

One of the most exciting recent developments in understanding airway smooth muscle function has been the recognition that airway smooth muscle is a rich source of a number of biologically active cytokines, chemokines and mediators, suggesting that rather than being a passive partner in the inflammatory process, airway smooth muscle has the ability to produce factors which regulate the development and progression of airway inflammation [5]. Airway smooth muscle cells are capable of producing a number of growth factors which can themselves potentiate proliferation in an autocrine manner. These include insulin-like growth factor 2 [54], thromboxanes [34] and platelet-derived growth factor [55]. Airway smooth muscle expresses adhesion molecules for inflammatory cells, including CD40 [96], ICAM-1 and VCAM-1 [97], and can produce a number of chemokines, including IL-6 [98], IL-8 [99,100], IL-11 [98], RANTES [101] and eotaxin [102], in response to cytokines such as IL-1 β , TNF α or, in the case of IL-8, mediators such

as bradykinin [99]. Production of these chemokines would be expected to amplify the chemokine signal generated by inflammatory cells in the airways in asthma, thereby potentiating the attraction of eosinophils, neutrophils and T-lymphocytes into the airway milieu (Fig. 3). Production of GM-CSF by ASM cells [103] may also enhance eosinophil survival at the site of airway inflammation. Airway smooth muscle can also produce endogenous prostanoids, such as PGE₂ and PGI₂, which may also modulate the inflammatory process [34].

It is clear that smooth muscle cells in other sites in the body exhibit functional heterogeneity, and it has been suggested that the same may be true in the airway. Studies from animal airway smooth muscle cells which have been serum deprived for prolonged periods show differentiation of these cells into two distinct populations, an elongated hypercontractile cell which stains more strongly for smooth muscle contractile proteins, and a shorter less contractile phenotype [104–106]. It is not known if airway smooth muscle cells in normal and asthmatic airways *in vivo* exhibit similar heterogeneity, and if the phenotype differs between normal and asthmatic airway; this is an area requiring further study.

Conclusions

Airway smooth muscle has a number of properties and functions which contribute to asthma pathogenesis. It undergoes changes in contractile and relaxant properties in response to asthmatic mediators and cytokines. Considerable ASM remodelling occurs in response to the growth factors which are present in asthmatic airways. ASM also functions as a rich source of biologically active cytokines,

chemokines and mediators which can act to alter contractility and the proliferative response in an autocrine manner. The production of chemokines may provide a mechanism whereby signals generated by inflammatory cells are amplified to produce an exaggerated inflammatory response.

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References

1. Thomson NC. Neurogenic and myogenic mechanisms of non-specific bronchial hyperresponsiveness. *Eur J Respir Dis* 1983; 64:206–11.
2. Holgate ST, Beasley R, Twentyman OP. The pathogenesis and significance of bronchial hyperresponsiveness in airways disease. *Clin Sci* 1987; 73:561–72.
3. Knox AJ. Airway remodelling in asthma: role of airway smooth muscle. *Clin Sci* 1994; 86:647–52.
4. Hirst SJ, Twort CHC. The proliferative response of airway smooth muscle. *Clin Exp Allergy* 1992; 22:907–15.
5. Johnson SR, Knox AJ. Synthetic functions of airway smooth muscle in asthma. *Trends Pharmacol Sci* 1997; 18:288–92.
6. Vincenc CS, Black JL, Yan K, Armour CL, Donnelly PD, Woolcock AJ. Comparison of in vivo and in vitro responses to histamine in human airways. *Am Rev Respir Dis* 1983; 128:875–9.
7. Roberts JA, Rodger IW, Thomson NC. Airway responsiveness to histamine in man: effect of atropine on in vivo and in vitro comparison. *Thorax* 1985; 40:261–7.
8. Roberts JA, Raeburn D, Rodger IW, Thomson NC. Comparison of in vivo airway responsiveness and in vitro smooth muscle sensitivity to methacholine in man. *Thorax* 1984; 39:837–43.
9. Armour CL, Black JL, Berend N, Woolcock AJ. The relationship between bronchial hyperresponsiveness to methacholine and airway smooth muscle structure and reactivity. *Respir Physiol* 1984; 58:223–33.
10. Roberts JA, Rodger IW, Thomson NC. In vivo and in vitro human airway responsiveness to leukotriene D₄ in patients without asthma. *J Allergy Clin Immunol* 1987; 80:688–94.
11. Bjorck T, Gustafsson LE, Dahlen SE. Isolated bronchi from asthmatics are hyperresponsive to adenosine, which acts indirectly by liberation of leukotrienes and histamine. *Am Rev Respir Dis* 1992; 145:1087–91.
12. De Jongste JC, Mons H, Bonta IL, Kerrebijn KF. Human asthmatic airway responses in vitro: a case report. *Eur J Respir Dis* 1987; 70:23–9.
13. Goldie RG, Spina D, Henry PJ, Lulich KM, Paterson JW. In vitro responsiveness of human asthmatic bronchus to carbachol, histamine, β -adrenoceptor agonists and theophylline. *Br J Pharmacol* 1986; 22:669–76.
14. Schellenberg RR, Foster A. In vitro responses of human asthmatic airway and pulmonary vascular smooth muscle. *Int Arch Allergy Appl Immunol* 1984; 75:237–41.
15. Amrani Y, Panettieri RA Jr, Frossard N, Bronner C. Activation of the TNF α -p55 receptor induces myocyte proliferation and modulates agonist-evoked calcium transients in cultured human tracheal smooth muscle cells. *Am J Respir Cell Mol Biol* 1996; 15:55–63.
16. Amrani Y, Krymskaya V, Maki C, Panettieri RA Jr. Mechanisms underlying TNF- α effects on agonist-mediated calcium homeostasis in human airway smooth muscle cells. *Am J Physiol* 1997; 273:L1020–8.
17. Panettieri RA Jr, Hall IP, Maki CS, Murray RK. Alpha thrombin increases cytosolic calcium and induces human airway smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 1995; 13:205–16.
18. Hotta K, Emala CW, Hirshman CA. TNF- α upregulates G_{i α} and G_{q α} protein expression and function in human airway smooth muscle cells. *Am J Physiol* 1999; 276: L405–11.
19. Thomas PS, Yates DH, Barnes PJ. Tumour necrosis factor- α increases airway hyperresponsiveness and sputum neutrophilia in normal subjects. *Am J Respir Crit Care Med* 1995; 152:76–80.
20. Bai TR. Abnormalities in airway smooth muscle in fatal asthma: a comparison between trachea and bronchus. *Am Rev Respir Dis* 1991; 143:441–3.
21. Barnes PJ, Dollery CT, MacDermot J. Increased pulmonary alpha-adrenergic and reduced beta-adrenergic receptors in experimental asthma. *Nature* 1980; 285:569–71.
22. Emala C, Black C, Curry C, Levine MA, Hirshman CA. Impaired β -adrenergic receptor activation of adenylyl cyclase in airway smooth muscle in the Basenji-Grayhound dog model of airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 1993; 8:668–75.
23. Sharma RK, Jeffery PK. Airway β -adrenoceptor number in cystic fibrosis and asthma. *Clin Sci* 1990; 78:409–17.
24. Spina D, Rigby PJ, Paterson JW, Goldie RG. Autoradiographic localisation of β -adrenoceptors in asthmatic human lung. *Am Rev Respir Dis* 1989; 140:1410–5.
25. Hakonarson H, Herrick DJ, Grunstein MM. Mechanism of impaired beta adrenoceptor responsiveness in atopic sensitised airway smooth muscle. *Am J Physiol* 1995; 13:L645–52.
26. Hakonarson H, Herrick DJ, Gonzalez Serrano P, Grunstein MM. Mechanism of cytokine-induced modulation of beta-adrenoceptor responsiveness in airway smooth muscle. *J Clin Invest* 1996; 97:2593–600.
27. Hakonarson H, Herrick DJ, Gonzalez Serrano P, Grunstein MM. Autocrine role of Hakonarson interleukin 1 β in altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 1997; 99:117–24.
28. Wills-Karp M, Uchida Y, Lee JY, Jinot J, Hirata A, Hirata F. Organ culture with proinflammatory cytokine reproduces impairment of the β -adrenoceptor-mediated relaxation in tracheas of a guinea pig antigen model. *Am J Respir Cell Mol Biol* 1993; 8:153–9.

29. Koto Mak JCW, Haddad EB, Xu WB, Salmon M, Barnes PJ, Chung KF. Mechanisms of impaired beta adrenoceptor induced airway relaxation by interleukin-1 β in the rat. *J Clin Invest* 1996; 98:1780–7.
30. Shore SA, Laporte J, Hall IP, Hardy E, Panettieri RA Jr. Effect of IL-1 β on responses of cultured human airway smooth muscle cells to bronchodilator agonists. *Am J Respir Cell Mol Biol* 1997; 16:702–12.
31. Emala CW, Kuhl J, Hungerford CL, Hirshman CA. TNF- α inhibits isoproterenol-stimulated adenylyl cyclase activity in cultured airway smooth muscle cells. *Am J Physiol* 1997; 272:L644–50.
32. Pang L, Holland E, Knox AJ. Role of cyclooxygenase-2 induction in interleukin 1 β induced attenuation of cultured human airway smooth muscle cell cyclic AMP generation in response to isoprenaline. *Br J Pharmacol* 1998; 125:1320–8.
33. Pang LH, Holland E, Knox AJ. Impaired cAMP production in human airway smooth muscle cells by bradykinin: role of cyclooxygenase products. *Am J Physiol* 1998; 275:L322–9.
34. Pang LH, Knox AJ. Effect of interleukin-1 β , tumour necrosis factor- α and interferon- γ on the induction of cyclooxygenase-2 in cultured human airway smooth muscle cells. *Br J Pharmacol* 1997; 121:579–87.
35. Laporte JD, Moore PE, Panettieri RA, Moeller W, Heyder J, Shore SA. Prostanoids mediate IL-1 β -adrenergic hyporesponsiveness in human airway smooth muscle cells. *Am J Physiol* 1998; 1040:L491–9.
36. Pang LH, Knox AJ. PGE₂ release by bradykinin in human airway smooth muscle cells: involvement of cyclooxygenase-2 induction. *Am J Physiol* 1997; 273:L1132–40.
37. Dunnill MS, Massarella GR, Anderson JA. A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis and in emphysema. *Thorax* 1969; 24:176–9.
38. Hossain S. Quantitative measurement of bronchial muscle in men with asthma. *Am Rev Respir Dis* 1973; 107:99–109.
39. Heard BE, Hossain S. Hyperplasia of bronchial muscle in asthma. *J Pathol* 1973; 110:319–32.
40. Ebina M, Yaegashi H, Chibo R, Takahashi T, Motomiya M, Tanemura M. Hyperactive site in the airway tree of asthmatic patients revealed by thickening of bronchial muscles. *Am Rev Respir Dis* 1990; 141:1327–32.
41. Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of ASM underlying bronchial asthma. *Am Rev Respir Dis* 1993; 148:720–6.
42. Saetta M, Di Stefano A, Rosina C, Thiene G, Fabbri LM. Quantitative structural analysis of peripheral airways and arteries in sudden fatal asthma. *Am Rev Respir Dis* 1991; 143:138–43.
43. James AL, Pare PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989; 139:242–6.
44. Hall IP, Kotlikoff M. Use of cultured airway myocytes for study of airway smooth muscle. *Am J Physiol* 1995; 268:L1–11.
45. Hirst SJ. Airway smooth muscle cell culture: application to studies of airway wall remodelling and phototype plasticity in asthma. *Eur Respir J* 1996; 9:808–20.
46. Stewart AG, Tomlinson PR, Wilson J. Airway wall remodeling in asthma: a novel target for the development of anti-asthma drugs. *Trends Pharmacol Sci* 1993; 14:275–8.
47. Tomlinson PR, Wilson JW, Stewart AG. Inhibition by salbutamol of the proliferation of human airway smooth muscle grown in culture. *Br J Pharmacol* 1994; 111:641–7.
48. Stewart AG, Fernandes D, Tomlinson PR. The effect of glucocorticoids on proliferation of human cultured airway smooth muscle. *Br J Pharmacol* 1995; 116:3219–26.
49. Black PN, Young PG, Skinner PJ. Response of airway smooth muscle cells to TGF-beta 1: effects on growth and synthesis of glycosaminoglycans. *Am J Physiol* 1996; 271:L910–7.
50. Kelleher MD, Schneider SD, Naureckas ET. Responsiveness of bovine tracheal smooth muscle cells to various mitogens. *Am J Respir Crit Care Med* 1994; 149:A304.
51. Hawker KM, Johnson PRA, Hughes JM, Black JL. Interleukin-4 inhibits mitogen induced proliferation of human airway smooth muscle cells in culture. *Am J Physiol* 1998; 275:L469–77.
52. Hirst SJ, Barnes PJ, Twort CHC. Quantifying proliferation of human and rabbit airway smooth muscle cells in response to serum and PDGF. *Am J Respir Cell Mol Biol* 1992; 7:574–81.
53. Scott PH, Belham CM, Al-Hafidh J et al. A regulatory role for cAMP on phosphatidylinositol 3-kinase/p70 ribosomal S6 kinase mediated DNA synthesis in PDGF stimulated bovine ASMC. *Biochem J* 1996; 318:965–71.
54. Noveral JP, Bhala A, Hintz RL, Grunstein MM, Cohen P. Insulin like growth factor axis in airway smooth muscle cells. *Am J Physiol* 1994; 267:L761–5.
55. De S, Zelazny ET, Souhrada JF, Souhrada M. Interleukin 1 β and interleukin 6 induce hyperplasia and hypertrophy of cultured guinea pig airway smooth muscle cells. *J Appl Physiol* 1995; 78:1555–63.
56. Shapiro PS, Evans JN, Davis RJ, Posada JA. The seven-transmembrane-spanning receptors for endothelin and thrombin cause proliferation of airway smooth muscle cells and activation of the extracellular regulated kinase and c-Jun NH₂-terminal kinase groups of mitogen-activated protein kinases. *J Biol Chem* 1996; 271:5750–4.
57. Panettieri RA, Yadvish PA, Kelly AM, Rubinstein NA, Kotlikoff MI. Histamine stimulates proliferation of airway smooth muscle and induces c-fos expression. *Am J Physiol* 1990; 259:L365–71.
58. Maruno K, Absood A, Said SI. Vasoactive intestinal peptide inhibits basal and histamine stimulated proliferation of HASMC. *Am J Physiol* 1995; 262:L1047–51.
59. Noveral JP, Rosenberg SM, Anbar RA, Pawlowski NA, Grunstein MM. Role of endothelin-1 in regulating proliferation of cultured rabbit airway smooth muscle cells. *Am J Physiol* 1992; 263:L317–24.
60. Panettieri RA Jr, Goldie RG, Rigby PJ, Eszterhas AJ, Hay DW. Endothelin-1 induced potentiation of human airway smooth muscle proliferation: an ETA receptor-mediated phenomenon. *Br J Pharmacol* 1996; 118:191–7.
61. Malarkey K, Chilvers ER, Lawson MF, Plevin R. Stimulation of endothelin-1 of mitogen-activated protein kinases and

- DNA synthesis in bovine tracheal smooth muscle cells. *Br J Pharmacol* 1995; 116:2267–73.
62. Carratu P, Scuri M, Styblo JL, Wanner A, Glassberg MK. ET-1 induces mitogenesis in ovine airway smooth muscle cells via ETA and ETB receptors. *Am J Physiol* 1997; 272:L1021–4.
 63. Hershenson MB, Chao TS, Abe MK et al. Histamine antagonizes serotonin and growth factor-induced mitogen activated protein kinase activation in bovine tracheal smooth muscle cells. *J Biol Chem* 1995; 270:19908–13.
 64. Noveral JP, Grunstein MM. Tachykinin regulation of airway smooth muscle cell proliferation. *Am J Respir Crit Care Med* 1995; 141:A48.
 65. Noveral JP, Grunstein MM. Role and mechanism of thromboxane-induced proliferation of cultured airway smooth muscle cells. *Am J Physiol* 1992; 263:L555–61.
 66. Panettieri RA, Tan EM, Ciocca V, Luttmann MA, Leonard TB, Hay DW. Effects of LTD4 on human airway smooth muscle cell proliferation, matrix expression and contraction. In vitro: differential sensitivity to cysteinyl leukotriene receptor antagonists. *Am J Respir Cell Mol Biol* 1998; 19:453–61.
 67. Brown JK, Tyler CL, Jones CA, Ruoss SJ, Hartmann T, Caughey GH. Tryptase, the dominant secretory granular protein in human mast cells, is a potent mitogen for cultured dog tracheal smooth muscle cells. *Am J Respir Cell Mol Biol* 1995; 13:227–36.
 68. Lew DB, Nebigil C, Malik KU. Dual regulation of cyclic AMP of β hexosaminidase-induced mitogenesis in bovine tracheal myocytes. *Am J Respir Cell Mol Biol* 1992; 7:614–9.
 69. Lew DB, Ratazzi MC. Mitogenic effect of lysosomal hydrolase on bovine tracheal myocytes in culture. *J Clin Invest* 1991; 88:1969–75.
 70. Noveral JP, Grunstein MM. Adrenergic receptor mediated regulation of cultured rabbit airway smooth muscle cell proliferation. *Am J Physiol* 1994; 267:L291–9.
 71. Smith PG, Janiga KE, Bruce MC. Strain increases airway smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 1994; 10:85–90.
 72. Smith PG, Garcia R, Kogerman L. Strain reorganizes focal adhesions and cytoskeleton in cultured airway smooth muscle cells. *Exp Cell Res* 1997; 232:127–36.
 73. Smith PG, Garcia R, Kogerman L. Mechanical strain increases protein tyrosine phosphorylation in airway smooth muscle cells. *Exp Cell Res* 1998; 239:353–60.
 74. Cerutis DR, Nogami M, Anderson JL et al. Lysophosphatidic acid and EGF stimulate mitogenesis in human airway smooth muscle cells. *Am J Physiol* 1997; 273:L10–5.
 75. Hershenson MB, Naureckas ET, Li J. Mitogen activated signalling in cultured airway smooth muscle cells. *Can J Physiol Pharmacol* 1997; 75:898–910.
 76. Kelleher MD, Abe MK, Chao TS et al. Role of MAP kinase activation in bovine tracheal smooth muscle mitogenesis. *Am J Physiol* 1995; 268:L894–901.
 77. Whelchel A, Evans J, Posada J. Inhibition of ERK activation attenuates endothelin stimulated airway smooth muscle proliferation. *Am J Respir Cell Mol Biol* 1997; 16:589–96.
 78. Karpova AY, Abe MK, Li J et al. MEK1 is required for PDGF-induced ERK activation and DNA synthesis in tracheal myocytes. *Am J Physiol* 1997; 272:L558–65.
 79. Krymskaya VP, Hoffman R, Eszterkas A, Kane S, Ciocca V, Panettieri RA. EGF activates Erb-2 and stimulates phosphatidylinositol 3 kinase in human airway smooth muscle cells. *Am J Physiol* 1999; 276:L246–55.
 80. Walter TR, Moore SM, Lawson MF, Panetteieri RA, Chilvers ER. Platelet derived growth factor-BB and thrombin activate phosphatidylinositol 3-kinase and protein kinase B. Role in mediating airway smooth muscle proliferation. *Mol Pharmacol* 1998; 54:1007–15.
 81. Nishizuka Y. Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 1992; 258:607–13.
 82. Hirst SJ, Webb BLJ, Gienbycz MA, Barnes PJ, Twort CHC. Inhibition of fetal calf serum-stimulated proliferation of rabbit cultured tracheal smooth muscle cells by selective inhibitors of protein kinase C. *Am J Respir Cell Mol Biol* 1995; 12:149–61.
 83. Carlin S, Yang KF, Donnelly R, Black JL. Protein kinase C isoforms in human airway smooth muscle cells: activation of PKC ζ during proliferation. *Am J Physiol* 1999; 276:L506–12.
 84. Young PG, Skinner SJ, Black PN. Effects of glucocorticoids and beta-adrenoceptor agonists on the proliferation of airway smooth muscle. *Eur J Pharmacol* 1995; 273:137–43.
 85. Johnson PRA, Armour CL, Carey D, Blake JL. Heparin and PGE₂ inhibit DNA synthesis in human airway smooth muscle cells in culture. *Am J Physiol* 1995; 269:L514–9.
 86. Billington CK, Joseph SK, Swan C, Scott MGH, Johnson TM, Hall IP. Modulation of human airway smooth muscle proliferation by type 3 phosphodiesterase inhibition. *Am J Physiol* 1999; 276:L412–9.
 87. Musa NL, Ramakrishnam M, Li J et al. Forskolin inhibits cyclin D₁ expression in cultured airway smooth muscle cells. *Am J Respir Cell Mol Biol* 1999; 20:352–8.
 88. Kilfeather SA, Tagoe S, Perez AC, Okona-Mensa K, Matin R, Page CP. Inhibition of serum-induced proliferation of bovine tracheal smooth muscle cells in culture by heparin and related glycosaminoglycans. *Br J Pharmacol* 1995; 114:1442–6.
 89. Cohen MD, Ciocca V, Panettieri RA Jr. TGF-beta 1 modulates human airway smooth muscle cell proliferation induced by mitogens. *Am J Respir Cell Mol Biol* 1997; 16:85–90.
 90. Dashtaki R, Whorton AR, Murphy TM, Chitano P, Reed W, Kennedy TP. Dehydroepiandrosterone and analogs inhibit DNA binding of AP-1 and airway smooth muscle proliferation. *J Pharmacol Exp Ther* 1998; 285:876–83.
 91. Hamad AM, Johnson S, Knox AJ. Antiproliferative effects of nitric oxide and atrial natriuretic peptides in cultured human airways smooth muscle cells. *Am J Physiol* 1999; 277:910–8.
 92. Belvisi MG, Saunders M, Yacoub M, Mitchell JA. Expression of cyclooxygenase-2 in human airway smooth muscle is associated with profound reduction in cell growth. *Br J Pharmacol* 1998; 125:1102–8.

93. Vlahos R, Stewart AG. Interleukin 1 α and tumour necrosis factor α modulate airway smooth muscle synthesis by induction of cyclooxygenase-2: inhibition by dexamethasone and fluticasone propionate. *Br J Pharmacol* 1999; 126:1315–24.
94. Johnson S, Knox AJ. Autocrine production of matrix metalloproteinase-2 is required for human airway smooth muscle proliferation. *Am J Physiol* 1999; 277:1109–17.
95. Kumagai K, Ohno I, Ohkawara Y et al. Inhibition of matrix metalloproteinases prevents allergen induced airway inflammation in a murine model of asthma. *J Immunol* 1999; 162:4212–9.
96. Lazaar AL, Amrani Y, Hsu J et al. CD40-mediated signal transduction in human airway smooth muscle. *J Immunol* 1998; 161:3120–7.
97. Lazaar AL, Albelda SM, Pilewski JM, Brennan B, Pure E, Panettieri RA. T Lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle DNA synthesis. *J Exp Med* 1994; 180:807–16.
98. Elias JA, Wu Y, Zheng T, Panettieri R. Cytokine and virus-stimulated airway smooth muscle cells produce IL-11 and other IL-6 type cytokines. *Am J Physiol* 1997; 273:L648–55.
99. Pang L, Knox AJ. Bradykinin stimulates IL-8 production in cultured human airway smooth muscle cells: role of cyclooxygenase products. *J Immunol* 1998; 161:2509–15.
100. John M, Hirst SJ, Jose PJ et al. Human airway smooth muscle cells express and release RANTES in response to Th-1 cytokines: regulation by Th-2 cytokines and corticosteroids. *J Immunol* 1997; 158:1841–7.
101. John M, Jose PJ, Lim S et al. Expression and release of interleukin 8 by HASM cells: inhibition by Th-2 cytokines and corticosteroids. *J Immunol* 1998; 161:2509–15.
102. Ghaffar O, Minshall E, Lamkhioed B et al. Eotaxin mRNA expression in mouse and human airway smooth muscle. *Am J Respir Crit Care Med* 1997; 155:A372.
103. Saunders MA, Mitchell JA, Seldon PM et al. Release of granulocyte-macrophage colony stimulating factor by human cultured airway smooth muscle cells: suppression of dexamethasone. *Br J Pharmacol* 1997; 120:545–6.
104. Halayko AJ, Rector E, Stephens NL. Airway smooth muscle cell proliferation: characterization of subpopulations by sensitivity to heparin inhibition. *Am J Physiol* 1998; 274:L17–25.
105. Stephens NL, Li W, Wang Y, Ma X. The contractile apparatus of airway smooth muscle. *Biophysics and Biochemistry. Am J Respir Crit Care Med* 1998; 158:S80–94.
106. Ma X, Wang Y, Stephens NL. Serum deprivation induces a unique hypercontractile phenotype of cultured smooth muscle cells. *Am J Physiol* 1998; 274:C1206–14.