

Airway smooth muscle as a target in asthma

S. J. HIRST

Department of Respiratory Medicine and Allergy, The Guy's, King's and St Thomas' School of Medicine, Guy's Hospital, King's College London, London, UK

Summary

Traditionally, the contractile properties of airway smooth muscle have been regarded as its sole contribution to the pathogenesis of asthma. However, our understanding of the role that this structural cell plays in asthma is changing. Airway smooth muscle can undergo hyperplasia and/or hypertrophy leading to structural changes in the airway wall which contribute to the development of persistent airway obstruction and increased non-specific airway hyperresponsiveness in chronic severe asthma. Many studies *in vitro* have characterized airway smooth muscle proliferation induced by various pro-inflammatory mediators, growth factors and components of the extracellular matrix, but the mediator(s) responsible for the observed increase in airway wall smooth muscle content *in vivo* remain to be determined. In addition to geometric obstruction by increased airway wall thickening, proliferating airway smooth muscle cells undergo phenotypic modulation from a contractile to synthetic-proliferative state where additional functions of airway smooth muscle such as cytokine/chemokine and extracellular matrix secretion may become more apparent. This may be especially relevant in the diseased airway where the content of airway smooth muscle as a fraction of the total cells present in the airway wall is already increased. Airway smooth muscle cells may also interact by direct contact with immunocytes such as T lymphocytes through expression of cell adhesion molecules with the result that myocyte DNA synthesis is induced. As additional functions of airway smooth muscle are described, a more contemporary view is emerging that airway smooth cells may adopt an immunoeffector role in chronic asthma by proliferating, secreting cytokines, expressing adhesion molecules and by interacting with various inflammatory cells. This may involve changes in the phenotypic status of airway smooth muscle, and as a result, these cells may play an active role in perpetuating and orchestrating airway inflammation in the remodelled airway. An important phase for future airway smooth muscle research will be to determine the extent that these putative mechanisms exist *in vivo* in the pathogenesis of chronic severe asthma.

Keywords: airway smooth muscle, asthma, remodelling, phenotype, mitogenesis

Introduction

Traditionally, the role of the smooth muscle cell in airway inflammation has been regarded to be passive, contributing to the pathogenesis of asthma solely by its contractile properties. In recent years, however, our understanding of the role that this structural cell plays in the pathogenesis of asthma is changing.

Airway wall remodelling

Patients with asthma develop airways hyperreactivity, where their airways respond to lower concentrations of inhaled spasmogens such as methacholine or histamine. They also exhibit airway hyperresponsiveness, where the airways can narrow to a greater extent. In patients with severe asthma it is often difficult to obtain a plateau of the lung function response to inhaled methacholine or histamine, even following long-term administration of inhaled glucocorticosteroids. This implies a persistent component to the long-term pathological process, which is believed to

Correspondence: Dr S. J. Hirst, Department of Respiratory Medicine and Allergy, The Guy's, King's and St Thomas' School of Medicine, 5th Floor, Thomas Guy House, Guy's Hospital, London SE1 9RT, UK.

involve remodelling or architectural changes within the airway. These changes involve a marked thickening of the airway wall [1], which may be associated with repeated episodes of intense inflammation within the airway wall and are related to asthma severity. There are several components of airway wall thickening: increased airway wall oedema; increased inner wall thickness due to extracellular matrix deposition; increased muscle layer thickness and increased outer wall thickness.

Mathematical models have been used to examine the potential effects of each of these components on airway function. They suggest that any process that results in thickening of the airway wall can lead to the development of persistent bronchial hyperresponsiveness [2]. Although increased airway wall thickness has little effect on airway resistance in the absence of smooth muscle shortening, in the thickened airway it amplifies the effect of normal amounts of smooth muscle shortening leading to a much greater narrowing of the airway and to airflow obstruction [3]. These models also predict that the smooth muscle component of the airway wall thickening process is by far the most important structural abnormality responsible for the development of persistent and excessive airway lumen narrowing (bronchial hyperresponsiveness) and reduced airway compliance in patients with chronic severe asthma [3]. The cellular and molecular mechanisms which lead to these changes are the subject of intense research, and this short, oversimplified review is intended to highlight certain aspects of our current state of knowledge, as well as suggesting possible strategies for future development.

Airway smooth muscle thickening

Although thickening of the bronchial smooth muscle layers is a well-documented feature of the airways of patients with persistent severe asthma, little is known of the cellular and molecular mechanisms which contribute to this increase in muscle content of the airway wall. The thickening of the airway smooth muscle layer may be the result of various processes including hyperplasia (i.e. an increase in cell number) and/or hypertrophy (i.e. an increase in cell size), particularly in the lower airways [4]. It is also possible that airway smooth muscle thickening may involve increased cell survival, where the normal attritional removal of cells is slowed resulting in a net increase in smooth muscle mass. Alternatively, myobundle extracellular matrix deposition may also give rise to an apparent increase in airway wall smooth muscle content [5]. All of these processes may operate to varying degrees in asthma, but their relative importance to the increase in airway wall smooth muscle content *in vivo* is unknown. Research efforts so far have focused on the contribution made by proliferative mechanisms. However, the importance of cellular proliferation to

the observed changes in airway wall smooth muscle content *in vivo*, and the contribution of additional mechanisms requires further investigation.

Airway smooth muscle mitogens and their intracellular signalling pathways

Which mediators are responsible for the increase in smooth muscle content of the airways in patients with chronic severe asthma? The major problem in addressing this question and in looking for therapeutic targets, is the continued lack of availability of smooth muscle from asthmatic airways and the lack of suitable animal models that can mimic the structural changes seen in asthmatic airways. Consequently, most of our knowledge in evaluating possible therapeutic targets and target molecules has been obtained from *in vitro* cell culture-based model systems.

Many studies *in vitro* have characterized airway smooth muscle proliferation induced by pro-inflammatory mediators and growth factors [6,7]. Other trophic factors such as altered mechanical stress and reactive oxygen species have also been identified. Table 1 lists some of the candidate mediators that have been proposed to act as smooth muscle mitogens *in vivo* based on cell culture studies where the proliferation of human and other species of airway smooth muscle have been recorded [6]. These include various polypeptide growth factors and cytokines, classical inflammatory mediators, inflammatory cell-derived proteases and components of the extracellular matrix. Recent unpublished (Hirst) studies have shown that several components of the extracellular matrix such as laminin and fibronectin (which are thought to be increased or modified during asthma) exert a differential proliferative response on human airway smooth muscle cells in response to classical smooth muscle mitogens such as platelet-derived growth factor (PDGF) and α -thrombin. The importance of these and other mechanisms remains unclear.

What is clear, however, is that airway smooth muscle is potentially a target for the action of a variety of structurally unrelated molecules derived from a wide variety of cell types that are present within the inflamed airway wall. Targeting of any one mediator in isolation is unlikely to be effective in reducing the smooth muscle content seen in the disease situation.

With this in mind, many investigators have sought to understand the intracellular signalling pathways that mediate proliferation of airway smooth muscle in response to a wide range of receptor-mediated mitogens. To date, the pathways that are associated with accelerated growth and division of smooth muscle appear largely to involve receptors with intrinsic tyrosine kinase activity (e.g. PDGF, EGF, bFGF) and those coupled directly to heterotrimeric GTP-binding proteins (e.g. α -thrombin, endothelin-1). In airway

Table 1. Promoters and inhibitors of airway smooth mitogenesis *in vitro*

	Factor	Cellular source	Effect on mitogenesis	Species
Growth factors/cytokines	EGF	Ep, P,	+	human
	bFGF	ECM, M θ , SMC	+	human, bovine
	PDGF	P, M θ , SMC, Ep	+	human, rabbit
	IGF-1	P, M θ , SMC	+	bovine, rabbit
	IL-1 β	T cell, M θ , SMC, Ep	+	guinea-pig
	IL-6	T cell, M θ , SMC, Ep	+	guinea-pig
	TNF α	T cell, M θ , SMC, Ep	$\pm$	human
	TGF β	M θ , P, ECM, T cell	$\pm$	human, bovine
Inflammatory mediators	Histamine	M	$\pm$	human, canine
	Endothelin-1	P, M θ , Ep, Pl	+	human, rabbit, ovine
	5-HT	P, Pl	+	rabbit, bovine
	Substance P	neuronal, Ep	+	rabbit
	LTD $_4$	M θ , SMC, Ep	+	human, rabbit
	VIP	neuronal	—	human
	PGE $_2$	Ep, SMC	—	human, guinea-pig
Enzymes	Tryptase	M	+	canine
	β -Hexosaminidase	M	+	bovine
	Lysosomal hydrolases	M	+	canine
	Thrombin	Pl	+	human, rabbit, bovine
Extracellular matrix	Laminin	ECM, SMC, BMf	—	human
	Fibronectin	ECM, SMC, BMf	+	human
	Heparin	M, ECM	—	human, canine, bovine
	Collagen I	ECM, SMC, BMf	+	human

M, mast cell; P, platelet; M θ , macrophage/monocyte; Ep, epithelial cell; Pl, plasma; SMC, smooth muscle cell; BMf, bronchial myofibroblast; ECM, extracellular matrix; T, T lymphocyte. +, stimulant; —, inhibitor; $\pm$, both stimulant and inhibitor, depending on conditions. Adapted from Hirst [6].

smooth muscle, a major intracellular pathway which mediates cell cycle progression and proliferation involves members of the mitogen-activated protein (MAP) kinase superfamily. Activation of this extracellular signal-regulated kinase (ERK) pathway occurs through various adaptor proteins and the Ras/Raf complex [8] (Fig. 1). Recent studies of bovine tracheal smooth muscle suggest that activation of the phosphoinositide (PI) 3' kinase pathway and subsequent activation of ribosomal S6 kinase through the signalling intermediate protein, protein kinase B, may also be important for transducing the mitogenic signal [9]. Both the ERK and PI 3' kinase pathways may offer potential targets for intervention in the smooth muscle remodelling process. Nevertheless, further studies are required to determine the relative importance of each of these pathways, as well as activation of other mechanisms such as the protein kinase C pathway, in promoting or modulating the mitogenic signal to the nucleus. The activation of these pathways in airway

smooth muscle mitogenesis is reviewed in detail elsewhere [10].

Phenotype instability of airway smooth muscle

The contribution made by proliferation of airway smooth muscle may not be limited merely to geometric obstruction to airflow by airway wall thickening. Indeed, when airway smooth muscle cells proliferate they undergo phenotypic modulation from a contractile to a synthetic-proliferative phenotype (Table 2) [6], concomitant with decreased smooth muscle- α -actin and smooth muscle-myosin heavy chain, and increased γ -actin and non-muscle myosin content [11]. This process may result in intrinsic changes in the biochemical and pathophysiological function of the airway smooth muscle.

Several cell culture-based reports suggest that one additional function of airway smooth muscle may include the

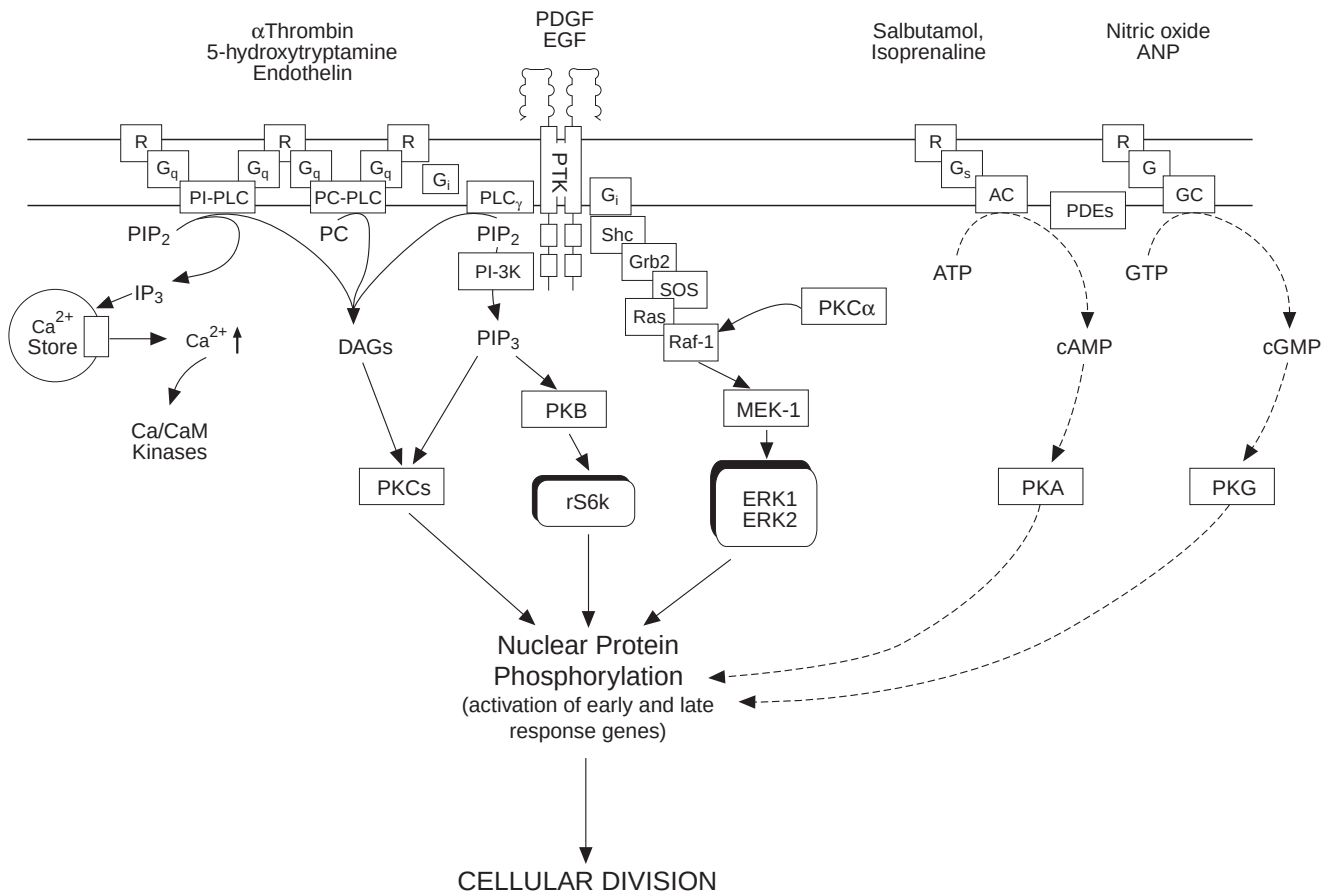


Fig. 1. Putative signalling events controlling airway smooth muscle mitogenesis. Solid lines represent pro-mitogenic signalling pathways; broken lines represent anti-mitogenic pathways. Reviewed in detail in [10].

Table 2. Discriminating ultrastructural and biochemical features of contractile and synthetic-proliferative airway smooth muscle cells in primary culture

	'Contractile'	'Synthetic'
Proliferation	slow	rapid
Myofilaments	present	reduced/absent
Golgi complex/RER	present	increased
Contractile proteins		
sm α -actin	+++	+/-
sm-myosin heavy chain	+++	+/-
Cytoskeletal proteins		
nm-actin isoforms (β/γ)	+/-	+++
nm-myosin heavy chain	+/-	+++
vimentin	+	++

+, expressed; +/-, poorly expressed.

secretion of various pro-inflammatory and bronchoprotective mediators [12]. This may be especially relevant in the diseased lung where the content of airway smooth muscle as a fraction of the total cells in the airway wall is already increased as a result of hyperplastic or hypertrophic changes. Thus airway smooth muscle may exhibit an immuno-effector role through the production of various pro-inflammatory cytokines, which perhaps serve to activate other inflammatory cell types and therefore contribute to the chronicity of the airway wall inflammation and perhaps to the structural remodelling process itself.

Studies of human cultured airway smooth muscle stimulated with pro-inflammatory cytokines have shown increased expression and release of chemokines important for the recruitment and activation of eosinophils and neutrophils. Conditioned supernatants from cytokine-stimulated human airway smooth muscle cells contain eosinophil chemotaxins such as RANTES and eotaxin as well as the neutrophil chemotaxin, interleukin (IL)-8 (Fig. 2). Moreover, human cultured airway smooth muscle cells respond to increasing concentrations of IL-1 β by producing an activity in the

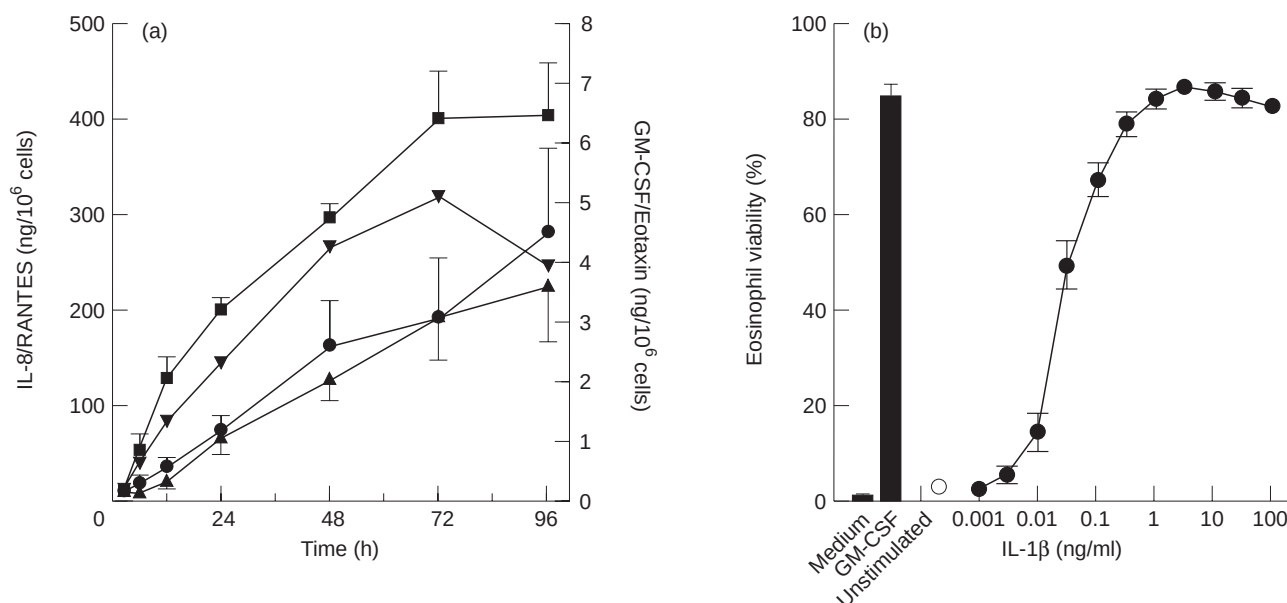


Fig. 2. Production of eosinophil-activating cytokines by airway smooth muscle cells cultured from human airways. (a) Release of the proinflammatory cytokines GM-CSF (●), interleukin (IL)-8 (■), RANTES (▲) and eotaxin (▼) following stimulation by IL-1 β (1 ng/mL). (b) Production of an eosinophil survival enhancing activity by IL-1 β (1 ng/mL). Adapted from Hallsworth *et al.* [13].

supernatant that maintains the survival of human peripheral blood eosinophils *in vitro* (Fig. 2). This activity was identified as GM-CSF [13]. In addition to modulation of inflammatory cell function by cytokine production, airway smooth muscle cells can interact by direct contact with immunocytes such as T lymphocytes through expression of cell adhesion molecules (ICAM-1, VCAM-1, CD44) with the subsequent induction of myocyte DNA synthesis [14], a surrogate for mitogenesis.

Conclusions

The contribution that airway smooth muscle makes to the pathogenesis of asthma may not be limited to contraction; it is multifunctional and airway smooth muscle may exist in multiple phenotypic states. Proliferation of airway smooth muscle can be induced to varying degrees by numerous mediators and a diverse range of stimuli including growth factors and components of the extracellular matrix. Cell culture-based model systems indicate that the proliferation of airway smooth muscle induced by diverse stimuli may involve common intracellular pathways. The current major candidate pathways are the MAP kinase/ERK pathway and the PI-3 kinase pathway [10]. In addition to contraction and proliferation of airway smooth muscle, the smooth muscle may contribute to the pathogenesis of asthma by the production of inflammatory cell cytokines.

Future directions and unresolved issues

For the development of future viable therapeutic targets we have to appreciate that this area of research is still very much in its early stages. There is a need to move away from *in vitro* studies and look more towards the *in vivo* situation and the disease itself. There are a number of issues that remain to be resolved. In particular, we need to understand the importance of altered airway muscle function in the pathogenesis of asthma. Does altered airway smooth muscle function really contribute to the pathogenesis of asthma? Is altered smooth muscle function accompanied by changes in phenotype? If it is, then it may be possible to target the phenotype and therefore control the altered function of the smooth muscle. What are the critical mediators and critical intracellular pathways that lead to airway smooth muscle growth in asthma?

Recently, we have started to address some of these issues by examining structural changes in the airway smooth muscle present in an intact airway preparation of human small bronchioles (<400 μ m internal diameter). Segments of these small bronchioles are maintained in culture for several days in the presence or absence of a smooth muscle mitogen. Then various properties of the smooth muscle including contractile protein expression, contractile function and airway wall morphometry can be studied. Initial airway morphometry studies suggest that there are modest increases in the smooth muscle content [15]. Thus, it appears this *in vitro*

model can mimic some of the changes that are present in the asthmatic airway and may provide interesting data in the future.

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