

# Airway Smooth Muscle and Asthma

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## ABSTRACT

The airway smooth muscle is the key determinant of airway narrowing in asthma but its function in the absence of disease is unknown. Evidence for an intrinsic abnormality in the muscle in asthma is only just emerging. The airway smooth muscle is not merely a contractile cell, but also one which determines the composition of, and interacts with the extracellular matrix, and which may participate in inflammatory and allergic reactions and viral infections. The reason for the differences which have been observed in the *in vitro* properties of airway smooth muscle derived from asthmatic individuals may result from an inherent "supercontractility", an increased tendency to proliferate due to the absence of an inhibitory transcription factor C/EBP- $\alpha$ , the influence of an altered extracellular matrix and/or a decrease in release of factors such as PGE<sub>2</sub> which would under normal circumstances inhibit both proliferation and contraction. Although long acting beta agonists and corticosteroids are successful treatments for inflammation and bronchoconstriction, the structural changes which constitute airway remodelling may require additional therapeutic intervention, the nature of which will be determined by thorough investigation of the mechanisms underlying the asthmatic phenotype.

## KEY WORDS

airway remodelling, airway smooth muscle, asthma, extracellular matrix, inflammation

## INTRODUCTION

The association between airway smooth muscle and asthma has been acknowledged for nearly a hundred years, in that excessive contraction of the muscle was known to form the basis of the exaggerated airway narrowing which is an integral part of an asthma "attack". With the recent advances made in understanding the biology of the airway smooth muscle cell, its role as simply a contractile element has expanded into that of a cell which can also modulate the immune response, affect airway remodelling, and contribute significantly to the pathology of airways disease. The airway smooth muscle cell is the target for many therapeutic regimens, and has been shown to respond both *in vivo* and *in vitro* to anti-inflammatory agents such as corticosteroids, and to bronchodilators such as  $\beta_2$ -agonists. Given that increased airway smooth muscle mass is a consistent finding in the asthmatic remodelling process, this cell has become the focus of research for many groups world wide.

It is interesting however to speculate on the function of the airway smooth muscle in the non-diseased airway. It appears to serve little purpose either in terms of playing a role in inhalation or exhalation, as-

sisting the propulsion of mucus, optimising ventilation/perfusion relationships or protecting the peripheral lung from noxious particles/allergens. Mitzner has therefore suggested that the airway smooth muscle is vestigial and likens it to the appendix—assuming importance only in the diseased state.<sup>1</sup>

The importance of the smooth muscle in asthma has recently been emphasised by the investigation of the use of thermoplasty—*i.e.* the delivery of thermal energy via a bronchoscope to target the airway smooth muscle. Initial experiments in dogs revealed that the ensuing reduction in smooth muscle mass resulted in an increase in airway size whether the airway was contracted or relaxed<sup>2</sup> and a reduction in airway hyperresponsiveness.<sup>3</sup> Preliminary studies in non asthmatic humans have established feasibility and safety.<sup>4</sup> Recently, in a study of 16 subjects with mild to moderate asthma<sup>5</sup> thermoplasty was well tolerated and produced improvements in airway hyperresponsiveness which were maintained for 2 years. Whether thermoplasty will become a widely accepted therapeutic intervention for remains to be seen.

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## THE AIRWAY SMOOTH MUSCLE CELL AND CONTRACTION

The nature of the essential abnormality which causes contraction of airway smooth muscle in an asthmatic subject in response to stimuli as diverse as allergens, occupational triggers, cold air etc while these have no effect in a nonasthmatic subject, remains unknown. Attempts to prove that the muscle is “supercontractile” have met with little success, in that the opportunities to conduct contractile studies on asthma-derived muscle are rare and the results of the few studies that do exist vary from demonstrating increased contractility to decreased contractility to decreased relaxation to no clear differences.<sup>6-10</sup>

Several theories proposing a contractile dysfunction have been proposed. The difference in response of asthmatics and nonasthmatics to deep inspiration (DI) following bronchoconstriction has provided a stimulus for much discussion.<sup>11</sup> DI in nonasthmatic patients produces prolonged bronchodilatation, whereas this effect is diminished in patients with asthma. The reasons for this difference are not known but could be the result of stiffer airways in asthmatic patients, or perhaps an increased propensity for the asthmatic airways to renarrow after dilatation.

The contraction of airway smooth muscle is dependent on activation of actin and myosin cross-bridges and the phosphorylation of myosin light chain by myosin light chain kinase. The degree to which myosin light chain is phosphorylated can determine the velocity of shortening and maximum contraction and there is a possibility that this may be altered by the coexistence of allergy or sensitisation. Recently Ma *et al.*<sup>12</sup> have attempted to show that a phenomenon that exists in canine isolated airway smooth muscle cells *viz* the presence of supercontractile muscle cells<sup>13</sup> occurs in humans. They found that single cells isolated from asthmatic subjects contracted to a greater extent and more quickly than muscle cells from non asthmatic subjects. Moreover there was increased messenger RNA for myosin light chain kinase in the asthmatic cells, which they hypothesise explains their findings. More studies such as these are needed to confirm that asthmatic muscle is intrinsically hypercontractile.

## THE AIRWAY SMOOTH MUSCLE CELL AND ALLERGY

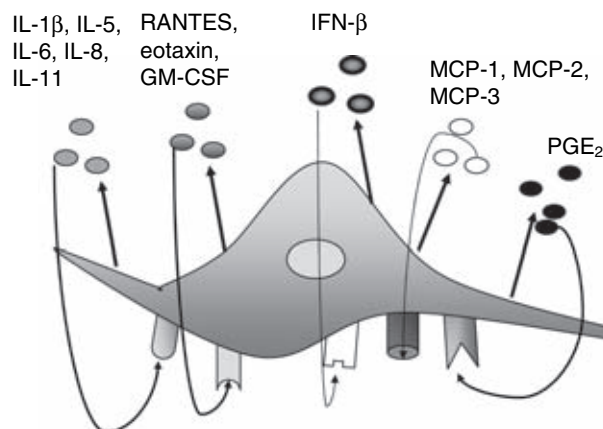
The link between asthma and allergy is well established but the role of the airway smooth muscle cell in allergic events is controversial. Almost 20 years ago it was noted that muscle tissue derived from atopic nonasthmatic subjects exhibited different *in vitro* contractile properties compared with muscle from non atopic controls.<sup>14</sup> Mast cell derived tryptase potentiated contractile responses in tissue derived from

atopic but nonasthmatic subjects but not that obtained from nonatopic subjects, and the mechanisms appeared to be calcium dependent.<sup>15</sup> In addition the incubation of human airway smooth muscle rings in serum from highly atopic asthmatic patients—so-called passive sensitisation—altered contractile and relaxation responses to a wide range of agonists.<sup>16,17</sup> The atopic serum contained high concentrations of IgE. Incubation of bronchial rings in either high concentrations of IgE or atopic serum increased the number of IgE bearing cells which were identified as largely mast cells.<sup>18</sup> In addition a contractile response to anti IgE could be elicited in both these situations. All these findings would lead to a logical conclusion that IgE itself was the agent responsible for the changes in contractile and relaxant properties. Moreover human airway smooth muscle cells have been shown to constitutively express Fc epsilon R1.<sup>19</sup> However Watson *et al.* demonstrated convincingly that IgE was not the factor responsible for the increased responsiveness induced *in vitro* by passive sensitisation.<sup>20</sup> Tunon de Lara has speculated that specific IgG may play a contributory role, as could the presence of inflammatory cell derived proteases and cytokines such as stem cell factor and tryptase.<sup>21</sup> It is of interest that the anti-human IgE antibody (omalizumab) has been demonstrated to have beneficial effects as an add on therapy in several clinical studies but it is unlikely that these can be related to effects on the airway smooth muscle cell.

## THE AIRWAY SMOOTH MUSCLE CELL AND INFLAMMATION

Airway smooth muscle cells are a key component of the inflammatory milieu, and have the ability to synthesise a vast repertoire of cytokines, chemokines and other inflammatory mediators, such as RANTES, eotaxin, IL-1 $\beta$ , IL-5, IL-6, IL-8, IL-11, MCP-1, MCP-2, MCP-3, GM-CSF, IFN- $\beta$ , leukaemia inhibitory factor, and prostanoids such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>).<sup>22</sup> Many of these factors are produced in response to stimulation with proinflammatory cytokines such as TNF- $\alpha$ , INF- $\gamma$ , and IL-1 $\beta$ , and since these factors are found to be elevated in the airways of asthmatic patients, *in vitro* stimulation is likely to reflect the inflammatory cascade present within the asthmatic airway. In addition many cytokine and chemokine receptors are present upon the surface of these cells, suggesting that both autocrine and paracrine signalling control airway smooth muscle function *in vivo* (see Fig. 1).

Antigen presentation is function which is generally considered to be restricted to so called professional antigen presenting (APC) cells (macrophages, dendritic cells and B lymphocytes) of the immune system. Given that airway smooth muscle cells can express MHC class II,<sup>23</sup> and the co-stimulatory molecules CD80 and CD86,<sup>24</sup> it is surprising that they ap-



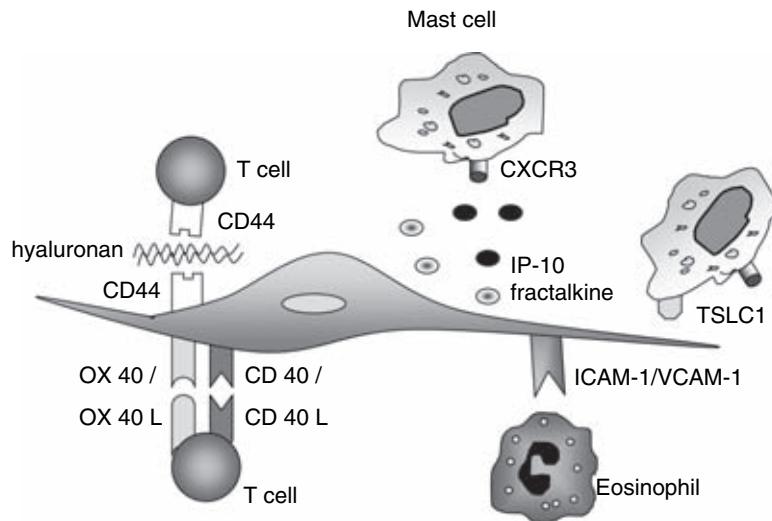
**Fig. 1** A diagrammatic representation of the potential autocrine feedback loops which exist within human smooth muscle cells. For example, eotaxin which is produced both constitutively and in response to a diverse range of stimuli such as mast cell adhesion, cigarette smoke, and other cytokines such as IL-4 and 13 can potentially activate CCR3 upon smooth muscle cells to induce the release of other cytokines and/or their receptors and to mediate chemotaxis of surrounding smooth muscle cells.

pear unable to act as antigen presenting cells. However as alveolar macrophages from only asthmatic subjects are able to function as APC,<sup>25</sup> it remains to be determined if the same is true of asthmatic muscle cells. Airway smooth muscle cells do however possess the ability to function as a cell of the innate immune system since they express, and respond to ligation of, several Toll-like receptors.<sup>26</sup>

Eosinophils bind to smooth muscle cells, adhesion is modulated by TNF- $\alpha$  and is also mediated through ICAM-1 and VCAM-1.<sup>27</sup> Mast cells are found to localise within smooth muscle bundles *in vivo*, however, the mechanism by which mast cells are recruited and adhere to smooth muscle cells is not known. It is hypothesised that the elevated cytokine and chemokine production observed in asthma, in combination with smooth muscle derived stem cell factor (SCF)<sup>28</sup> could provide a mechanism by which mast cell numbers within smooth muscle bundles are elevated in asthmatic subjects. Recently Brightling *et al.* have reported that the chemokine CXCL10 (IP-10) is expressed preferentially in the muscle of asthmatic patients and *ex vivo* asthma derived smooth muscle cells. Together with the fact that the chemokine receptor CXCR3 was preferentially expressed on mast cells located in the muscle, this constitutes a mechanism for the attraction of the mast cells to the muscle.<sup>29</sup> The chemokine fractalkine or CX3CL1 which is also produced by airway smooth muscle cells also may contribute to mast cell recruitment, especially when the mast cell are stimulated with vasoactive intestinal polypeptide (VIP).<sup>30</sup> Mast cell adhesion to smooth muscle cells has recently been shown to be

mediated in part by tumour suppressor in lung cancer-1 (TSLC1), which is preferentially expressed upon the surface of human lung mast cells, but not by ICAM-1, VCAM-1, CD18, and the alpha4 and beta1 integrins.<sup>31</sup> Adhesion of Mast cells to smooth muscle cells induces the release of eotaxin which is dependent upon p38 MAPK.<sup>32</sup> Mast cells located within the airway bundles of asthmatic subjects produce IL-4 and IL-13.<sup>33</sup> IL-4 and IL-13 are T helper 2 cytokines that are thought to contribute to the pathogenesis of asthma, and, furthermore, have been shown to have direct effects upon airway smooth muscle cells, such as the induction of eotaxin<sup>34</sup> and the reduction of beta-adrenergic responsiveness by IL-13.<sup>35</sup> In addition, *in vivo* degranulation of mast cells correlates to asthma severity, and the increased degranulation observed in cartilaginous versus membranous bronchioles suggests an inhaled stimulus is activating these cells.<sup>36</sup>

Smooth muscle cells express several cell surface receptors capable of activating and binding other immune cells. ICAM-1 and vascular cell adhesion molecule (VCAM) -1 are constitutively expressed upon the cell surface and are inducible by pro-inflammatory cytokines such as TNF- $\alpha$ .<sup>37</sup> ICAM-1, in addition to being the cellular receptor for the major groups of rhinoviruses, and VCAM-1 can be used by activated T cells to mediate adhesion. CD44, which is the cellular receptor for hyaluronan, is also constitutively expressed upon the surface of smooth muscle cells and T cells. It is hypothesized that hyaluronan which is bound to CD44 upon the surface of smooth muscle cells can act as a binding site for T cell CD44.<sup>37</sup> Of



**Fig. 2** A cartoon representing the potential interaction of human airway smooth muscle cells and inflammatory cells. The possibility exists for many inflammatory cells to interact with smooth muscle cells, which is usually mediated via adhesion molecules such as ICAM and VCAM, however components of the extracellular matrix such as hyaluronan may also act as a bridge to mediate binding between smooth muscle cells and inflammatory cells.

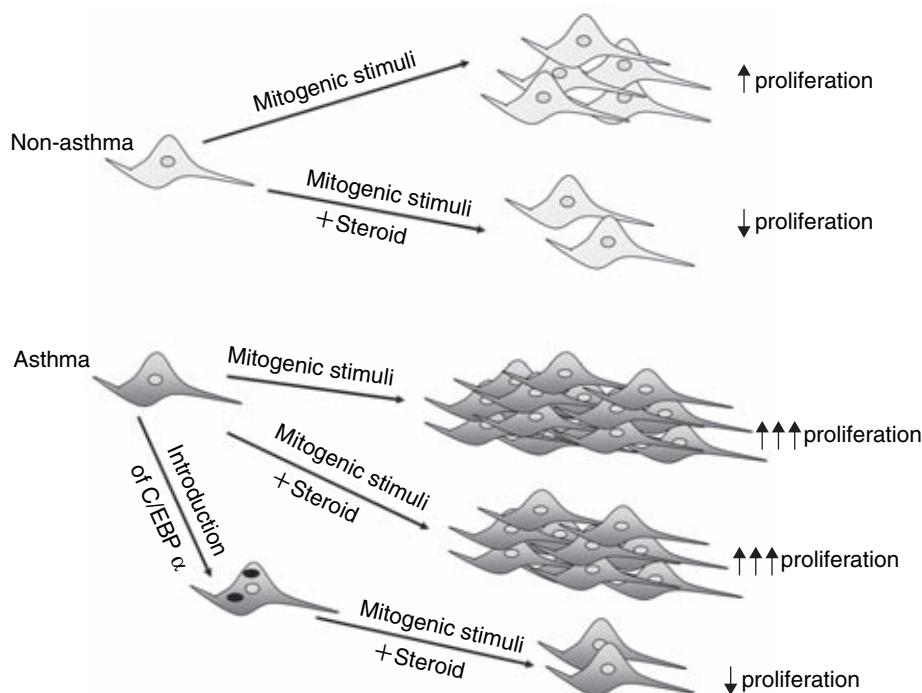
particular importance in the context of asthma, adhesion of T cells to smooth muscle cells induces smooth muscle proliferation.<sup>37</sup> CD40, a member of the TNF receptor family, is expressed upon the surface of airway smooth muscle cells. CD40 binds to a specific ligand (CD40 L), which is expressed upon the surface of T cells. Direct activation of smooth muscle CD40 increased intracellular calcium and IL-6 protein secretion,<sup>38</sup> further indicating the possible importance of T cell airway smooth muscle interaction. Further evidence for the possible interactions between smooth muscle cells and T cells was provided in a recent report by Burgess *et al.*. Smooth muscle cells express OX40 ligand, and furthermore, activation of OX40 ligand increased the IL-6 release from airway smooth muscle cells. As OX40 is expressed upon the surface of T cells activated through T cell receptor stimulation, this report provides further evidence for the possible interaction between T cells and smooth muscle cells in orchestrating the immune response.<sup>39</sup> The potential interactions of smooth muscle cells and inflammatory cells are shown in Figure 2.

### **AIRWAY REMODELLING AND ASM PROLIFERATION**

Airway remodelling is now recognised as a characteristic feature of severe persistent asthma and includes increased smooth muscle mass, increased thickening of the basement membrane, increased new vessel formation and epithelial and mucous cell dysplasia. Initial observations indicated that the increase in

smooth muscle was due to cellular hyperplasia, since a 3 fold increase in nuclei was found.<sup>40</sup> A more recent report which examined the three dimensional structure of the airways in asthma found that both hyperplasia and hypertrophy occur in the large and small airways respectively.<sup>41</sup> The milieu of cytokines, chemokines and matrix proteins produced by the smooth muscle are thought to provide the conditions necessary for the increased smooth muscle mass seen in the asthmatic lung. The ability of cytokines to stimulate proliferation of airway smooth muscle cells may to some extent depend upon both the autocrine and paracrine production of PGE<sub>2</sub>, which inhibits DNA synthesis.<sup>42</sup> Autocrine production of PGE<sub>2</sub> is reduced in asthmatic smooth muscle cells,<sup>43</sup> providing a potential mechanism by which accelerated reproduction of smooth muscle cells may occur. Production of other autocrine mediators such as INF- $\beta$  also have been shown to reduce proliferation,<sup>44</sup> highlighting the importance of the effect of autocrine inflammatory mediators upon the function of the airway smooth muscle cell.

In addition, intrinsic differences in the smooth muscle cells between asthmatics and non-asthmatics now have been discovered.<sup>45,46</sup> *In vitro* airway smooth muscle cells from asthmatic subjects proliferate at a faster rate than non-asthmatic cells,<sup>45</sup> produce greater amounts of proinflammatory cytokines, both constitutively and induced, and respond differently to common respiratory drugs. In addition the transcription factor CCAAT/enhancer binding protein  $\alpha$  (C/



**Fig. 3** Diagrammatic representation of the effects of steroids upon mitogen-induced proliferation of human airway smooth muscle cells. In non-asthmatic cells mitogen-induced proliferation is inhibited by steroids, however in asthmatic cells the inhibitory effects of steroids upon proliferation is only observed upon the reintroduction of C/EBP $\alpha$ .  $\uparrow$  represents an increase in proliferation, and  $\downarrow$  represents a decrease in proliferation.

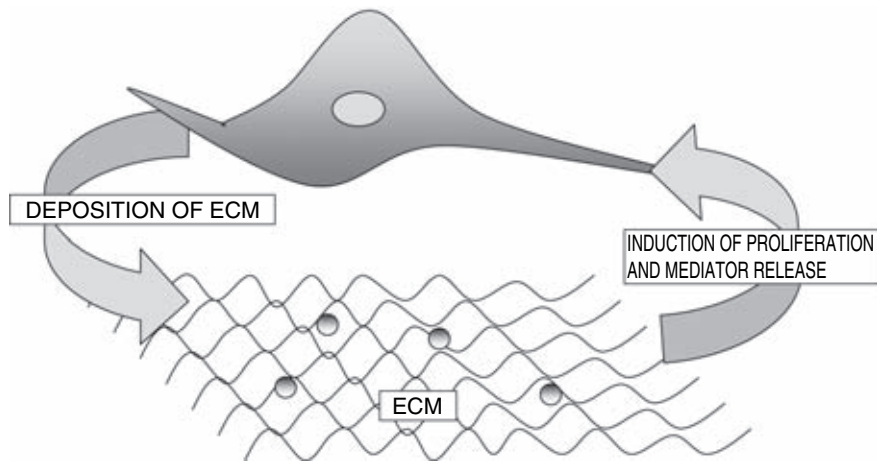
EBP $\alpha$ ) is deficient in airway smooth muscle cells from asthmatic subjects.<sup>46</sup> There are six members of the C/EBP transcription factor family, which not only regulate cytokine expression, but are involved in cell cycle regulation and cellular differentiation.<sup>47</sup> C/EBP $\alpha$  inhibits proliferation of many cell types,<sup>47</sup> and in airway smooth muscle cells derived from non-asthmatic subjects neutralisation of C/EBP $\alpha$  using antisense oligonucleotides reversed the anti proliferative effects of glucocorticoids. Furthermore the reintroduction of C/EBP $\alpha$  into smooth muscle cells derived from asthmatic subjects enabled the anti-proliferative effects of steroids to be observed within these cells. Since C/EBP $\alpha$  is deficient in airway smooth muscle cells from asthmatic patients, this provides a potential mechanism by which increased proliferation occurs (see Fig. 3).

### THE AIRWAY SMOOTH MUSCLE CELL AND THE EXTRACELLULAR MATRIX

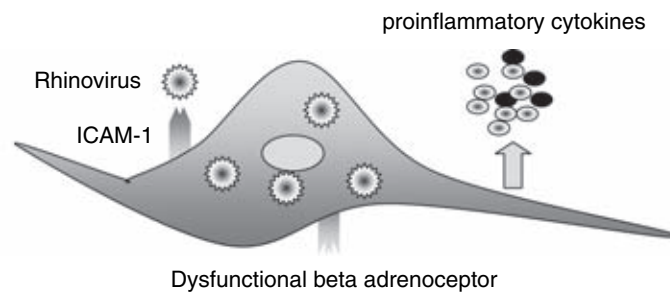
The airway smooth muscle cell is surrounded by an extracellular matrix (ECM) bed. Which consists of matrix proteins, such as collagen I, II, IV and V, fibronectin, decorin, chondroitin sulfate, elastin, perlecan, laminin  $\beta(1)$ ,  $\gamma(1)$ ,  $\beta(2)$ ,  $\alpha(1)$  chains, thrombospondin and versican,<sup>48</sup> matrix degrading enzymes

MMP 1, 2 and 9, as well as tissue inhibitors of metalloproteinases (TIMP)-1<sup>49</sup> and TIMP-2.<sup>50</sup> The interaction between the ECM and the airway smooth muscle cell can regulate both ECM composition and airway smooth muscle cell function. The ECM is very dynamic, with a turnover rate of 10–15% per day. Degradation of the ECM releases inflammatory modulators and growth factors that can feed back on the smooth muscle cell, and in addition the smooth muscle cell can respond to ECM components through integrins. Smooth muscle cell proliferation has been shown to be dependant upon autocrine production of MMP-2,<sup>51</sup> and furthermore smooth muscle proliferation in response to stimulation with PDGF BB or  $\alpha$ -thrombin is inhibited by laminin whereas fibronectin and collagen I increase cell division (see Fig. 4).<sup>52</sup>

*In vivo* differences are observed in the composition of the ECM in asthma, and *in vitro* airway smooth muscle cells from asthmatic subjects produce greater perlecan and collagen I and decreased collagen IV, laminin  $\alpha 1$ , and chondroitin sulfate in comparison to non-asthmatic cells.<sup>53</sup> In addition the signalling pathways leading to the deposition of extracellular matrix proteins from airway smooth muscle cells may be different in asthmatic and nonasthmatic patients.<sup>54</sup> Interestingly the relationship between the airway smooth



**Fig. 4** Interactions between the extracellular matrix (ECM) and the smooth muscle cells. Complex interactions exist between the ECM and smooth muscle cells, regulating both matrix deposition and smooth muscle proliferation. Many inflammatory mediators are known to bind to components of the ECM which can be released during turnover to act upon the smooth muscle cell or other lung cells.



**Fig. 5** A cartoon illustrating the potential interaction between smooth muscle cells and rhinovirus. Smooth muscle cells constitutively express ICAM-1 which is the cellular receptor for the major group of rhinovirus. Binding of rhinovirus to ICAM-1 induces the release of proinflammatory cytokines, which can then potentially alter the both the contractile response and functional expression of the B2 adrenoceptor on the smooth muscle cell.

muscle cell, matrix protein deposition and cytokines such as vascular endothelial growth factor and connective tissue growth factor may have implications for the development of the angiogenesis which constitutes a component of the remodelling process.

### **THE AIRWAY SMOOTH MUSCLE CELL AND VIRUS INFECTION**

The foremost contributor to morbidity, mortality and health care costs associated with asthma are exacerbations, of which at least 70% are associated with viral infection.

Viruses are pathogens of the lower respiratory tract, however much controversy surrounds the ability for airway smooth muscle to become infected with

virus *in vivo*. Importantly, *in situ* hybridisation has demonstrated that both submucosal<sup>55</sup> and smooth muscle cells,<sup>56</sup> can be infected with viruses *in-vivo*. Rhinovirus is the most commonly encountered viral infection producing exacerbations in adult asthmatics and infection of airway smooth muscle cells has been demonstrated *in vitro*.<sup>57</sup> We as well as others have shown that rhinovirus induced the release of proinflammatory cytokines from airway smooth muscle cells, thereby providing a potential mechanism by which rhinovirus induced exacerbations occur.<sup>58</sup> There is clinical evidence to support a decreased response to beta adrenoceptor agonists during a respiratory virus infection.<sup>59</sup> This has led to the hypothesis that viral infection may cause dysfunction in the beta

adrenoceptor on the smooth muscle and some preliminary evidence exists in support of this,<sup>60</sup> in addition we have preliminary evidence that rhinovirus induces the release of PGE<sub>2</sub> from airway smooth muscle cells, thereby providing a mechanism by which dysfunction in the beta adrenoceptor occurs (see Fig. 5).<sup>61</sup> Other proposed mechanisms of virus-induced exacerbations include downregulation of the M<sub>2</sub> muscarinic on vagal nerve endings<sup>62</sup> and modulation of the endothelin system.<sup>63</sup> Conversely, Billington *et al.*, 2001 suggest that rhinovirus induces sensitisation of the adenylyl cyclase pathway, leading the authors to speculate that the increased production of cAMP is a defensive mechanism designed to promote airway relaxation upon infection with rhinovirus.<sup>64</sup>

The airway smooth muscle cell has many roles in the airway, from maintaining airway tone to regulating ECM composition and the local inflammatory and growth factor milieu. These cells are the therapeutic targets for drugs used to treat asthma and other airway disorders. Since they are intrinsically different in asthmatic patients they are likely to be intimately involved in the remodelling process and the progression of the asthmatic phenotype. Their role in airway disease is only beginning to be understood, and in the future the interaction of allergens and lower respiratory tract infections with the smooth muscle cell may yield valuable insights into the pathogenesis of asthma and other respiratory diseases.

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