

OPINIONS IN ALLERGY

Intrinsic asthma: is it intrinsic to the smooth muscle?

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Summary

The traditional view of the pathophysiology of asthma is that its characteristic features are secondary to a major allergic or immunological dysfunction. However, this does not explain intrinsic asthma, which can occur in the absence of atopy. An alternative view is that an abnormality in the airway smooth muscle cell, which is capable of producing inflammatory, immunological and growth factors as well as molecules, which facilitate interaction with inflammatory cells, is the primary or instigating event. Evidence is rapidly accumulating that the smooth muscle is abnormal, in that it proliferates faster, produces more chemokines and cytokines as well as a different profile of extracellular matrix proteins than its non-asthmatic counterpart. These abnormalities may arise from altered calcium homeostasis leading to increased mitochondrial biogenesis and/or decreases in the levels of key transcription factors such as CCAAT enhancer binding protein- α . Conditions under which smooth muscle is ablated, such as bronchial thermoplasty, may help us to understand more about the contribution of an airway smooth muscle dysfunction to asthma aetiology.

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Is an abnormality of airway smooth muscle sufficient to explain intrinsic asthma?

Whether one regards asthma as a syndrome or collection of diseases related to altered immunity, or as a manifestation of an allergic diathesis, what is readily accepted is the fact that the primary event in an asthmatic 'attack' is airway narrowing. Contraction of airway smooth muscle plays a major role in this narrowing but it is also likely that obstruction with excessive mucus contributes. The very diversity of the stimuli that can lead to contraction and subsequent narrowing raises the strong possibility that an innate or intrinsic abnormality of the muscle could exist in asthma. If this were to be true, then the mechanism whereby provoking factors such as allergens, cold air, occupational irritants or exercise cause airway narrowing could be readily understood. It is now known that the airway smooth muscle cell does more than contract and relax. It can produce growth factors, cytokines, chemokines and molecules, which participate in interactions with inflammatory cells [1]. Thus an abnormal muscle cell may be the instigator of, rather than a bystander in inflammatory or allergic events. As such, it would not be necessary to invoke a major primary allergic or immuno-

logical aetiology for asthma, which could arise from smooth muscle abnormalities occurring in the absence of atopy.

So what evidence do we have that the smooth muscle is abnormal in the asthmatic?

Certainly the fact that it is different has been acknowledged since the early part of the 20th century when Huber and Kessler [2] reported that the bulk of the muscle was increased in the airways of patients who had died of asthma. Over the subsequent decades, the role of inflammation in the aetiology of asthma broadened such that it became an abnormality of T cell function with the muscle shrinking back into obscurity. In an attempt to revive it, researchers studied the contractile and relaxant properties of the smooth muscle in isolated tissue systems, adding in various factors such as products of inflammatory cells or serum from allergic asthmatics to reproduce *in vitro* the milieu that was supposedly so influential *in vivo*. Indeed, some of these experiments did result in seemingly relevant changes in contractile and relaxant properties of isolated airway smooth muscle [3]. Recent evidence has

also emerged to support the concept of hypercontractility, based on the study of single smooth muscle cells in an isolated system. Ma et al. [4] reported increased shortening capacity and velocity in asthma-derived cells, which was associated with higher levels of mRNA for smooth muscle myosin light chain kinase (MLCK). Consistent with this finding, was a study which examined single smooth muscle cells embedded in a collagen gel revealing greater contraction of asthmatic cells, although MLCK levels were not increased [5].

The next step in the unravelling process was to address the issue of why the muscle bulk was increased, and this could have been the result of an increase in individual muscle size – hypertrophy – or an increase in the number of cells – hyperplasia [6]. Along with the advent of the capacity to culture human airway smooth muscle *in vitro* came a flurry of studies, which revealed that numerous putative inflammatory mediators and endogenous growth factors could cause airway smooth muscle cells to proliferate. In these studies evidence also started to emerge that non-inflammatory, non-allergic factors such as stretch could also cause proliferation [7]. Moreover, it became apparent that the muscle cell was capable not only of contracting, relaxing and proliferating but also of producing myriad cytokines chemokines and growth factors. Furthermore, these cells express adhesion molecules and integrins, which brings into focus a whole new series of capabilities such as cell-to-cell contact with inflammatory cells [1] (Fig. 1). Eventually the opportunity arose to study human airway smooth muscle cultured from biopsies obtained from asthmatic volunteers, and this led to some interesting findings. In culture, airway smooth muscle cells from asthmatic volunteers proliferated at one and half to two times the rate of that of non-asthmatic muscle.

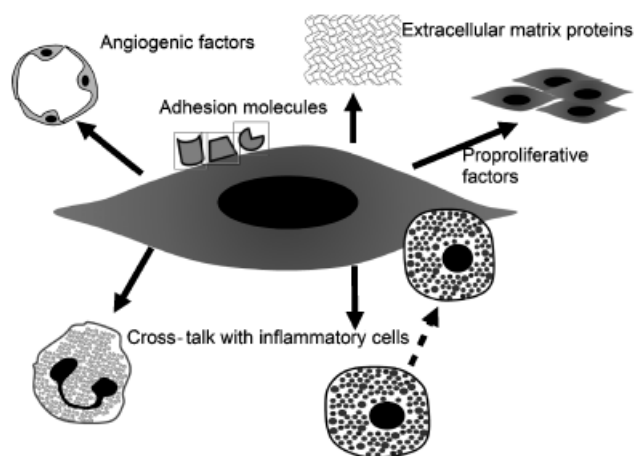


Fig. 1. The airway smooth muscle cell, as well as contracting and relaxing, produces myriad inflammatory and growth factors as well as extracellular matrix proteins and adhesion molecules, which enable interactions with inflammatory cells. Asthma may arise from an intrinsic defect in the smooth muscle cell, which could then be the primary driver of inflammation and remodelling.

This fact has been confirmed from cultures of multiple patients and also from multiple research groups. Johnson et al. [8] first observed this abnormal proliferation in a small group of asthmatics and this was subsequently confirmed in larger numbers and also by Triantafyllidis et al. [9]. Woodruff et al. [10] also noted that increased cell number and not an increase in cell size contributed to the increased muscle bulk in the airway wall in an *ex vivo* study of bronchial biopsies. In contrast, others have reported an increase in airway smooth muscle cell size as opposed to cell number [11] and an increase in fibroblast and not airway smooth muscle cell numbers in asthma-derived biopsies [12]. It is likely that some of these differences arise from difficulties in differentiating mesenchymal cells from each other, as specific markers for myofibroblasts, muscle cells and fibroblasts are lacking [13]. Moreover, using indices of cell proliferation such as immunohistochemical detection of cyclin D1 to observe cell proliferation [11] may be problematic, in that the result is a 'snapshot' of what is likely to be an intermittent process.

So what about the mechanisms underlying this increased proliferation? Roth et al. [14], building on the finding of Johnson et al. [8] observed that the antiproliferative transcription factor C/EBP α (C/EBP α) was absent from cells derived from asthmatics and that this was specific for asthma and not apparent in cells from patients with COPD or other chronic airway diseases. However this may reflect the fact that these comparisons were made in samples taken at the same level in the airways of COPD and asthma patients – i.e. quite proximal. Changes in the smooth muscle in chronic obstructive pulmonary disease (COPD) may be more likely to occur in the smaller airways, which we have not examined for an abnormality in C/EBP α . Subsequently it became apparent that the translation of the mRNA for C/EBP α was impaired [15]. Triantafyllidis et al. [9] recently provided compelling evidence that the increase in proliferation was associated with an elevated mitochondrial biogenesis with an increase in mitochondrial number and in the levels of mitochondrial specific transcription factors. Are these two mechanisms mutually exclusive or compatible? Roth and Black [16] suggest the latter, in that many mitochondrial genes have a C/EBP binding site, and it is known that the number of mitochondria is directly correlated with differentiation status of the cell.

In addition, there are now confirmed reports that the levels of pro-inflammatory cytokines and chemokines released from asthma-derived airway smooth muscle cells are increased. Among these are candidates such as eotaxin [17] and IP-10 [18], which could be responsible for attracting inflammatory cells into the airway and to the muscle itself – eosinophils and mast cells, respectively, and the latter would be consistent with the finding that mast cells seem to be specifically attracted to the muscle in

asthmatic lungs. Thus the interesting possibility is raised that, rather than changes in smooth muscle occurring as a result of airway inflammation, the converse could be true – i.e. that the inflammation so closely aligned with asthma could result from an abnormal release of pro-inflammatory mediators from an innately abnormal muscle cell.

The events, which occur in the airway wall in asthma, if persistent, result in structural changes, which are described as remodelling. In addition to the increase in the bulk of the smooth muscle, these include an increase in vascularization or angiogenesis, an increase in the deposition of extracellular matrix proteins leading to thickening of the airway wall, an increase in the thickness of the basement membrane and mucus gland hyperplasia. Here again the muscle could be responsible for all these effects. It produces a number of pro-angiogenic factors, including vascular endothelial growth factor and conditioned medium from asthma-derived muscle cells in culture contain greater levels, which result in increased tube formation *in vitro* [19]. Muscle cells also release extracellular matrix proteins, which lead to increased fibrosis and basement membrane thickening. Whether the muscle cell has the potential to influence mucus gland morphology and function has not been the subject of investigation. Adding weight to the notion that remodelling can be almost solely the result of changes in smooth muscle function are the findings that the profile of extracellular matrix proteins released from the asthmatic cell differ from their non-asthmatic counterparts, and moreover that the changes lead to a more proliferative and secretory phenotype. Specifically, muscle cells seeded onto a matrix derived from asthmatic muscle cells assume a more 'asthmatic' phenotype in that they proliferate at a more rapid rate [20]. However, if the events, which stimulate smooth muscle proliferation (either of an allergic or non-allergic origin) do not occur in the non-asthmatic individual *in vivo*, it is unlikely that matrix/smooth muscle cell interactions have clinical relevance when asthma, and thus the intrinsic abnormality, is not present. Nevertheless, these findings highlight the potential importance of the interplay between muscle cells and the extracellular matrix they produce in the asthmatic airway.

If there is no muscle, is there still asthma?

One of the most interesting issues pertaining to the smooth muscle in asthma comes from results of studies using bronchial thermoplasty to ablate the muscle from the airways of asthma patients. Although this rather radical treatment is thus far in its infancy, the results of these early studies would suggest that improvements in objective, as well as subjective parameters are sustained for up to 1 year [21]. Not all aspects of the sequelae of thermoplasty are, however, supportive of the concept of an abnormal smooth muscle cell as the pivotal mechanism

underlying asthma. For instance, there was no improvement in airway hyperresponsiveness in these patients. It is difficult to interpret this result in the light of the fact that airway hyperresponsiveness is, after all, a manifestation of contraction of the smooth muscle to an inhaled contractile agonist. It may be that bronchial thermoplasty, which targets the smooth muscle in airways > 3 mm in diameter, fails to reach the more distal airways, which contribute significantly to airway hyperresponsiveness. Because bronchial thermoplasty is not without significant side-effects, at least in the early weeks following treatment, it may be that its greatest value lies in what it tells us about the role of the muscle cell in asthma pathogenesis. What is not known as yet, and, in terms of mechanistic insights, is critically important, is whether there is residual inflammation in the airways of patients who have undergone bronchial thermoplasty. If the muscle plays a leading role in the development of asthma and contributes significantly to inflammation by virtue of its ability to release inflammatory mediators, growth and pro-angiogenic factors, then ablating the muscle should result in a marked decrease of inflammation in the airways. It is surprising that there are no data documenting the pathology of biopsies of airways in these patients at follow-up. If there is little or no inflammation, then this might suggest that the muscle is a prime contributor to inflammation, again lending support to the notion that the abnormality in asthma is intrinsic to the smooth muscle.

Martinez [22] has suggested that inflammation and remodelling occur as separate, parallel events and that remodelling is not necessarily the result of chronic persistent inflammation. An alternative hypothesis is that the initiating process is an intrinsic smooth muscle abnormality, on which are overlaid the allergic and inflammatory events that have hitherto been regarded as primary (Fig. 1).

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