Airway remodeling: the clinical significance

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Summary

While structural changes have been observed in asthmatic airways for more than a century, the importance of airway remodeling did not come into view as a key player in asthma until recently. Its significance can be derived from the fact that supramaximal doses of histamine are not capable of causing full airway constriction in healthy subjects, indicating that more structural changes must be at play. Initially it was believed that airway remodelling was caused by chronic inflammation and repeated allergen exposure resulting in a continuous process of damage and repair. Evidence is now pointing to inflammation and airway remodelling acting in parallel, perhaps mutually reinforcing each other.
The primary focus in airway remodelling research has been on airway smooth muscle changes. It is well established that airway smooth muscle mass is increased in asthma, primarily through hyperplasia, although some studies also show evidence of hypertrophy. Recent results show that airway smooths muscle proliferation in asthma, particularly severe asthma, presumably in balance with cell apoptosis, which may indicate that airway remodelling is in fact potentially reversible. It is not yet known whether airway smooth muscle force or velocity of shortening is altered in asthma, either intrinsically or modulated by the inflammatory environment of the asthmatic airway. Some *in vitro* and animal studies do suggest that inflammatory mediators can result in increased force generation and proliferation of airway smooth muscle.
Airway remodelling could influence airway responsiveness in a number of other ways too. An increased airway wall thickness provides a greater load for the airway smooth muscle to contract against, but also requires less airway smooth muscle shortening to achieve the same reduction in airway diameter. Recent studies have shown the bronchodilating and bronchoprotective effects of airway diameter oscillations that occur in healthy subjects during breathing. These airway diameter oscillations that are likely reduced because of airway remodelling, may abrogate the beneficial effects of breathing in asthma.
There are no known drugs that have been proven to reverse airway remodelling, although this may be partly due to the lack of accurate non-invasive tools available to measure the degree of airway remodelling. Bronchial thermoplasty, which relies on radiofrequency ablation of airway smooth muscle, has been shown to result in some improvement in quality of life in severe asthmatic patients, but minimal improvement in lung function. Identification of the dominant site of airway hyperresponsiveness may increase the effectiveness of such techniques.

KEY WORDS: bronchial asthma; airway remodelling; airway hyperresponsiveness; bronchothermoplasty.

Introduction

The link between exaggerated airway narrowing and the symptoms of asthma has been made for several centuries. The mechanistic basis for the marked bronchospasm of an acute attack of asthma has remained obscure but is generally attributed to the presence of an underlying hyperresponsiveness of the airways that can be detected by challenges with a variety of bronchoconstrictive agonists. An excessive responsiveness of the airways to stimulation with histamine was recognized in 1946 (1). However, it was not addressed as a potential consequence of altered airway smooth muscle function for several decades. Indeed there was a great deal of research that examined the possibility that control of the bronchial smooth muscle by the autonomic nervous system lay at the root of the problem. However research on the regulation of bronchomotor tone gave way to a focus on inflammatory mediators by the 1980s. From the 1990s there was renewed interest in the structural changes in the airways with the development of animal models demonstrating the plausibility of proliferation of airway smooth muscle in response to allergen challenges. Indeed the changes in muscle mass and other forms of remodeling have dominated the discourse about asthma pathogenesis. In addition the function of airway smooth muscle and its regulation have received intensive scrutiny. However if remains a great deal of uncertainty about the causes of the acute and often relatively refractory asthma attack, airway hyperresponsiveness and persistent loss of lung func-

tion associated with severe forms of asthma. In this review we will examine some of the pathophysiological processes that may help us understand these features of disease.

The neural regulation of bronchomotor tone

The airways are supplied by autonomic fibers, predominantly via the vagus. The postganglionic nerves release acetylcholine that may activate bronchial smooth muscle, and also may affect mucus gland secretion and airway blood flow (2). Studies of isolated airways demonstrate a non-cholinergic non-adrenergic negative regulatory nervous system that may cause bronchodilation when airway smooth muscle is contracted (3). A small number of studies have provided evidence of a similar system *in vivo* (4, 5). Nitric oxide seems to be one of the mediators of this relaxant system (6). There appears to be no significant direct sympathetic supply of nerves to the airway smooth muscle despite the abundance of adrenergic receptors that may mediate either contraction or relaxation. Inhibition of beta adrenergic receptors or ganglionic neurotransmission does not affect airway responsiveness to methacholine (7), indicating that the principal pathways for neural regulation are not responsible for the enhanced airway responsiveness that is a defining feature of asthma. However, recently it has been shown that the late asthmatic response, a secondary bronchoconstrictive event which occurs 2-24 hours after the acute response, can be inhibited by anesthesia and anticholinergic drugs (8). Certain inflammatory mediators may accelerate the release of acetylcholine from post-synaptic nerve endings (9, 10). The lack of major efficacy of anti-cholinergic drugs in asthma argues against the generalized importance of such mechanisms. Although airway hyperresponsiveness may not be caused by excessive cholinergic stimulation or defective NANC relaxation, links between innervation and inflammation may have pertinence for some form of hyperalgesia that leads to cough and sputum production or indeed nerves may promote inflammation through concomitant release of neuropeptides (11). The lack of effective antagonists of neurokinins has retarded our understanding of their roles in airway disease.

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Inflammatory mediators and airway hyperresponsiveness

It seemed a plausible hypothesis that an excess of inflammatory mediators could account for airway hyperresponsiveness in asthma by providing a strong stimulus to airway smooth muscle to contract. Although there is abundant evidence that inflammatory mediators trigger airway narrowing in asthma, the exaggerated narrowing seen is unlikely to be explained solely by this mechanism. The evidence for this statement comes from a consideration of bronchial provocation tests. Since one cannot evoke substantial falls in the forced expiratory volume in one second (FEV_1) in the majority of healthy human subjects using high concentrations of histamine (12) then an excess of other contractile agonists in the airways, such as the cysteinyl leukotrienes, should not enhance the methacholine response to the point of revealing the degree of responsiveness associated with asthma. An enhancement of the organ responsiveness is still required for excessive airway narrowing to occur. The search for inflammatory mediators that could alter airway smooth muscle properties has revealed some possible clues. Cytokines associated with asthma such as interleukin-13 and tumor necrosis factor-α enhance the contractility of airway smooth muscle (13-15) so that they may hypothetically contribute to AHR. It should be stressed that the evidence for this phenomenon has been generated in murine models and in cell culture systems. There is little or no direct evidence for this at present in human subjects. Targeting TNF-α has demonstrated little evidence for its involvement as a driver of disease and neutralization of IL-13 showed only a modest effect on lung function in asthmatics (16).

The search for inflammatory mediators that could alter airway smooth muscle properties has revealed some possible clues.

Airway remodeling and airway hyperresponsiveness

Airway remodeling or the structural changes in the airways were described by many investigators spanning over a century (17). The application of the tools of quantitative histology clearly demonstrated the increase in airway smooth muscle mass (18), subepithelial thickening (19) and a range of non-structural changes compared to healthy control subjects. Repeated cycles of tissue injury and repair from chronic inflammation are believed to be responsible for these structural changes, although the presence of some of the changes in childhood asthma may suggest that inflammation and remodeling occur in parallel (20, 21). Increase in airway smooth muscle mass has now been repeatedly reported (Figure 1), in particular as a function of asthma severity (22-25). It is also associated with fixed airflow limitation in both children and adults (26, 27).

Increased airway wall thickness in remodeled airways may provide a greater load for the smooth muscle to overcome, but with the thicker airway wall the muscle needs to shorten less to achieve the same level of airway constriction.

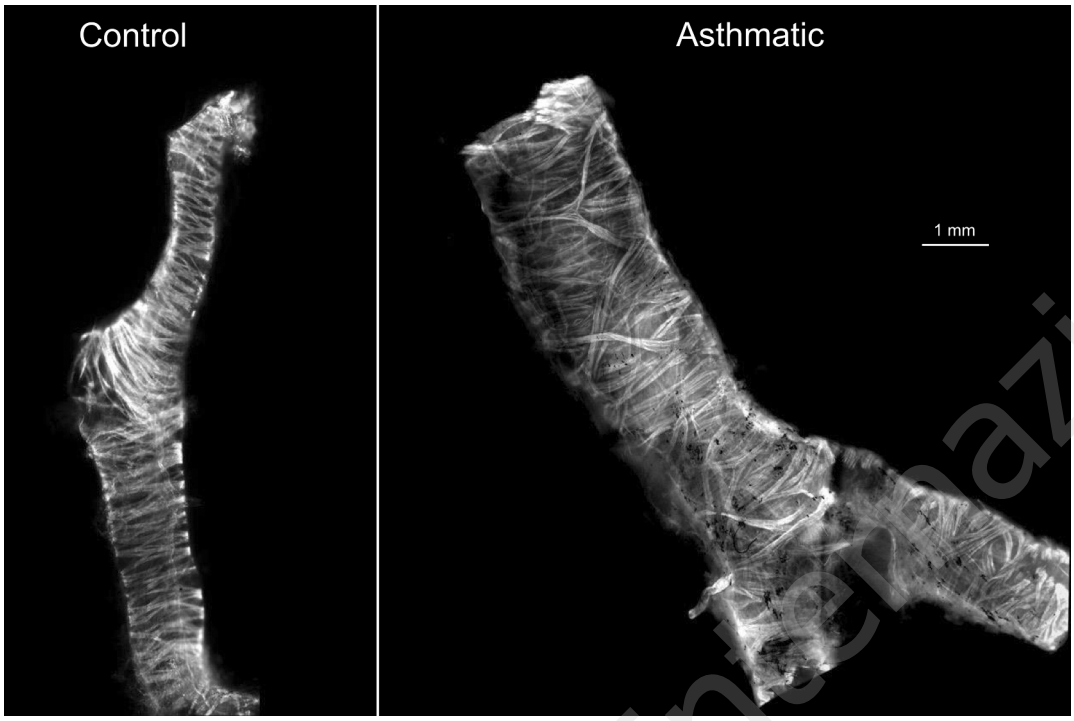

Figure 1 - Airway smooth muscle in (severe) asthma and a non-asthmatic control. Airway smooth muscle fluorescently labelled with TRITC-phalloidin in airways dissected from human lungs procured through the International Institute for Advancement of Medicine. The spiral arrangement of the airway smooth muscle is evident in both, although it appears somewhat less ordered in the severe asthma specimen.

Isolated airway smooth muscle, similar to other forms of smooth muscle, has the capacity to shorten to very short lengths. Such degrees of shortening would lead to complete closure of the airways (28). How is it then that healthy subjects do not experience marked airway closure even when challenged with high concentrations of methacholine? The answer proposed is that the force of contraction of airway smooth muscle is not sufficient to overcome the elastic resistance to deformation of the parenchyma to which the outer aspect of the airway wall is attached via alveolar attachments, as well as the intrinsic stiffness of the airway wall as it undergoes constriction (29). The effect of airway remodeling on this force balance is not fully understood. Increased airway wall thickness in remodeled airways may provide a greater load for the smooth muscle to overcome, but with the thicker airway wall the muscle needs to shorten less to achieve the same level of airway constriction (30). While the airway smooth muscle mass is increased, it is unclear whether this is associated with increases in the contractile force generated by the muscle (31-35).

Recent research has indicated that the airway diameter is not determined by a static force balance but rather a dynamic one, influenced by the constant lung volume changes that occur during normal breathing (36, 37). In fact, under static lung inflation airways do constrict to the point of airway closure (38, 39). For over half a century it has been known that deep inspirations in particular are a potent bronchodilator in healthy subjects, but not in most asthmatics (40, 41). A single deep inspiration is capable of reversing bronchoconstriction (40), while inhibition of deep inspirations results in hyperresponsiveness (42). In asthmatics deep inspiration does not lead to bronchodilation, and may even cause increased bronchoconstriction, particularly in severe asthmatics (43, 44). Tidal breathing and increased lung volume also seem to reduce bronchoconstriction from metacholine challenge in healthy subjects (45-47). *In vitro* studies have shown that airway smooth muscle contraction is partially inhibited when subjected to oscillatory stretches equivalent to those occurring *in vivo* during breathing (48). The mechanism of the “softening” of airway smooth muscle in response to stretching is still a matter of active investigation. The degree of stretch that results from a pressure applied by the expanding lung parenchyma on the airway will depend upon the stiffness of the airway wall. Fibrosis of the airway such as is described in asthma may diminish the effectiveness of breathing by reducing the length changes of the muscle caused by any given transmural pressure developed across the bronchial wall. This can be further compounded by a change in the elasticity of the parenchyma, which is characteristic of COPD, but may also occur in (severe) asthma (49).

Airway smooth muscle remodeling may not be limited to an increase in its mass. Molecular remodeling may also potentially occur, affecting the velocity of airway smooth muscle contraction. Mathematical modeling has shown that an increase in velocity of shortening of the airway smooth muscle could explain the difference in response to deep inspirations between asthmatics and controls (50). The airway smooth muscle contrac-

tile apparatus, like skeletal muscle, consists of myosin and actin filaments, with protruding myosin heads responsible for the movement of these filaments relative to each other. The 2 dominant isoforms of myosin, SM-A and SM-B, have been shown to have distinctive velocities, in motility studies, with SM-B being twice as fast as SM-A. In asthmatics the ratio of SM-B/SM-A mRNA is increased (51), but this has yet to be followed by measurements at the protein level. Alternatively a change in the ratio of myosin light chain kinase and myosin light chain phosphatase, which regulate myosin activity through phosphorylation of the myosin light chain, could result in increased shortening velocity (52).

In addition to molecular remodeling that may result in enhanced contractile properties there may be an alteration in the phenotype of the muscle. Airway smooth muscle may adopt a secretory phenotype resulting in the secretion of a variety of chemoattractants for inflammatory cells such as interleukin-8 (CXCL8; a neutrophil chemoattractant), RANTES (CCL5; a lymphocyte chemoattractant) and eotaxin (CCL11; an eosinophil chemoattractant) (53). These cells may therefore attract immune effector cells into the airways and more specifically into the muscle bundles that may then alter their properties or trigger proliferation. Proliferation of smooth muscle may be caused by contact with activated T lymphocytes (54). T cells are present in significant numbers within airway smooth muscle bundles and increase in numbers correlate with increase in severity of disease (55). There is also an increase in mast cells within the airway smooth muscle cells (56), postulated to be caused by the CXCR3/CXCL10 chemoattractant pathway (57).

Proliferation of airway smooth muscle

Morphometric measurements of airway smooth muscle mass have demonstrated a consistent increase in muscle mass in asthmatic subjects (18, 22-25). The increase in mass is linked to disease severity and not to the duration of disease (58). This suggests that host susceptibility to airway remodeling may determine the outcome of environmental factors that induce asthma, such as repeated exposure to sensitizing substances. To date no studies have identified muscle-specific genes associated with asthma but it would not be surprising to discover that such associations were present. Attempts at determining the mechanisms of the increase in muscle mass have been made by quantifying the number of nuclei per unit area of the muscle. Most studies have found a proportionate increase in nuclei to area, leading to the conclusion that proliferation of muscle or hyperplasia has occurred (59, 60). Only one study of a small number of subjects with severe asthma using a marker of cellular proliferation, proliferating cell nuclear antigen, has identified an excess rate of proliferation of muscle (61). The authors have concluded that muscle remodeling is not only through proliferation of muscle cells but is a dynamic phenomenon. If confirmed, and this study requires confirmation, then it would imply that there is a balance between cellular apoptosis and proliferation that sets a new balance of increased muscle mass in these subjects. It also implies that muscle remodeling is not necessarily an irreversible phenomenon.

An important issue in remodeling is whether it is fixed or a new dynamic equilibrium based on cell proliferation and cell death.

Some studies have also found evidence of increased mass that is attributable to hypertrophy (24, 62). The pattern of hypertrophy, whether associated with small or large airways also seems to vary. Some subjects demonstrate hypertrophy in large airways but not in small, whereas others have no evidence for hypertrophy. The significance of these changes (Figure 2) for airway function is unknown, although the contractility of hypertrophic muscle is likely to be impaired compared to normal muscle or muscle that has undergone an increase through hyperplasia. Understanding the implications of these different patterns of growth of muscle will require appropriate measurements of the mechanical properties of these muscles and perhaps also the elucidation of the mechanisms of the changes.

Many studies have shown the plausibility of smooth muscle growth through the actions of various mediators present in the inflamed airways (63). An array of substances from histamine to chemokines such as IL-8 and classical growth factor receptors such as the epidermal growth factor receptor (EGFR) has been implicated in the growth of smooth muscle. Exploration of airway smooth muscle growth has been largely dependent on models of asthma. Both rat and mouse models have been developed based on allergic sensitization and subsequent repeated exposures to the allergen. Anti-leukotrienes and inhibitors of the epidermal growth factor receptor have been demonstrated to inhibit smooth muscle growth (64, 65). Not surprisingly compounds that are effective in inhibiting some component of the allergic response also inhibit smooth muscle growth. Thus it has not been clearly established to what extent the inflammatory process is necessary for smooth muscle growth. Mechanical stress may release EGFR ligands (66) and lead to remodeling without inflammation, raising the possibility that the very process of bronchoconstriction could lead to remodeling. Anti-cholinergic drugs also inhibit remodeling but the mechanism may go beyond inhibition of airway narrowing (67, 68). Less is known about the process of hypertrophy. *In vitro* studies suggest that transforming growth factor-β may cause upregulation of smooth muscle contractile proteins (69). This mediator has many actions and is likely present in asthma. The consequences of hypertrophy of airway smooth muscle require further exploration.

An important issue in remodeling is whether it is fixed or a new dynamic equilibrium based on cell proliferation and cell death. Normal tissue remodeling is a function of cellular turnover and although smooth muscle is more slowly proliferating than epithelium, for example there is evidence of some proliferation of cells in normal animals, even large mammals such as the horse (70). There is less information available for human subjects but one study has suggested that at least in severe asthma airway smooth muscle may be in a state of dynamic remodeling (61) and therefore amenable to therapy.

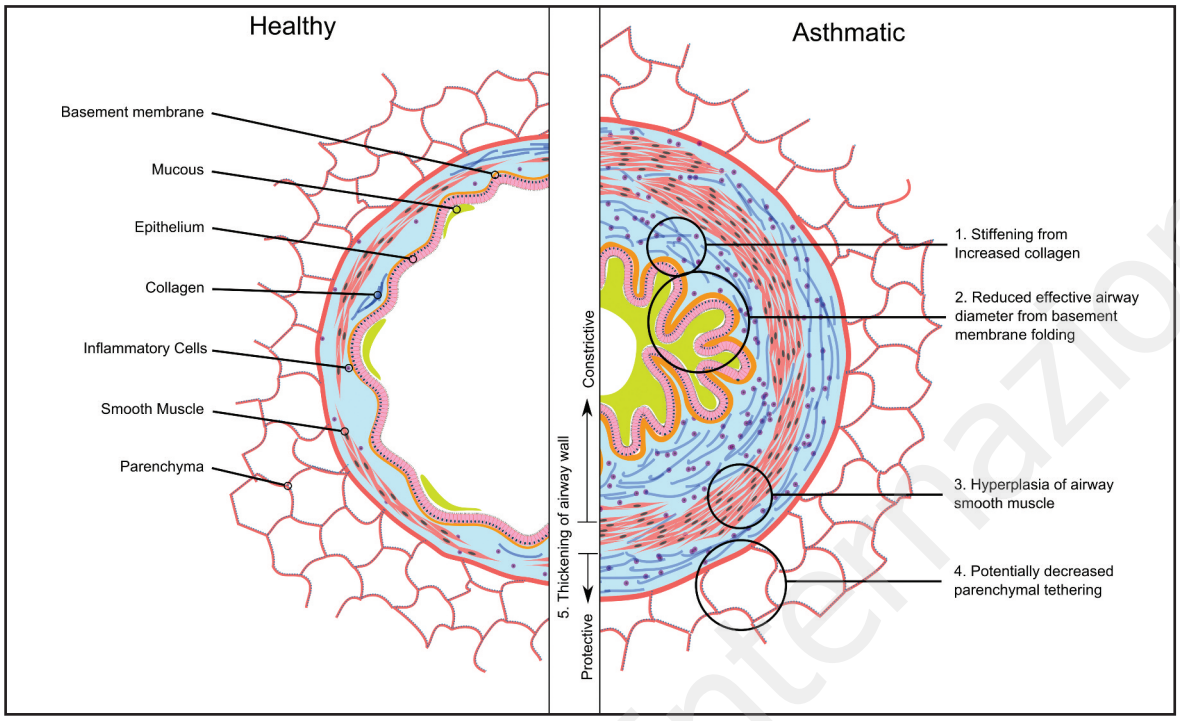

Figure 2 - Schematic drawing of features of airway remodelling influencing AHR. Remodelled and control airway side-by-side comparison. 1) Increased collagen content may lead to stiffening of the airway wall, providing a greater load against which the airway smooth muscle must shorten. 2) Basement membrane folding, caused by the incompressibility of the airway wall, results in a dramatic decrease of the open airway diameter, and trapping of mucous. However, basement membrane folding may also provide an increased load against airway smooth muscle shortening. 3) Airway smooth muscle hyperplasia may increase force generating potential of the airway smooth muscle, but not all cells may be in an optimal contractile state. 4) Parenchymal tethering may be reduced in asthma, but little evidence of this has been found. 5) Airway wall thickening inwards from the airway smooth muscle layer enhances airway constriction, while outwards thickening increases the resistive load to airway constriction.

Therapeutic approaches

To date there are no known drugs proven to reverse airway smooth muscle remodeling, which may in part be due to the lack of methods to non-invasively assess the degree of remodeling. It may be possible that inhibition of airway inflammation has a beneficial effect on remodeling, but it will be necessary to assess muscle remodeling more directly in order to draw conclusions about this. Newer imaging techniques such as optical coherence tomography may provide information of this nature but thus far its usefulness has not been established. CT imaging has proven to be unhelpful in assessing the degree of remodeling in some studies of severe asthma and is a technique that may provide information about airway wall thickness (71). Resolving remodeling from bronchoconstriction may be more difficult to achieve. In severe asthma with fixed airflow limitation and biopsy-proven airway smooth muscle thickening, CT scans did not detect the abnormality (27).

It may be possible that inhibition of airway inflammation has a beneficial effect on remodeling, but it will be necessary to assess muscle remodeling more directly in order to draw conclusions about this.

Bronchothermoplasty is the only current treatment that specifically targets airway smooth muscle with a view to its destruction (72). Radiofrequency ablation is attempted at three bronchoscopic sessions. The effectiveness of the intervention is limited to improvements in symptoms and in rates of exacerbation (73). Substantial change in lung function is not reported. Confirmation of the removal of smooth muscle is supported by human and animal studies (74, 75). It seems reasonable that this postulated mechanism of action does indeed account for clinical outcomes. One could imagine that incomplete ablation could limit improvement since airway narrowing could still occur in inadequately treated areas. Further refinements in this direction seem a promising approach to curing asthma. Many subjects will likely not benefit from thermoplasty if the site of their airway remodeling is distal to the generations of airways targeted by treatment. Identification of the dominant site of airway smooth muscle remodeling would permit appropriate selection of subjects and appropriate targeting of airways.

Conclusion

The search for the culprit in airway hyperresponsiveness has changed focus several times over the years.

Because of our limited knowledge of the precise significance of airway remodeling the therapeutic approaches to limit or reverse it are uncertain.

While the research to date has given us some indication on what is *not* causing airway hyperresponsiveness, the explanation for the observed difference between the apparent behaviour of airway smooth muscle *in vivo* in asthmatic and healthy subjects remains elusive. Airway remodeling certainly plays an important role in AHR, but the jury is still out on its causes and effects. Are injury and repair responsible or are there innate differences in at least some asthmatics, as suggested by findings in childhood asthma? Does airway remodeling provide an element of bronchoprotection through increased airway wall stiffness, or is increasing AHR through increased muscle mass and subepithelial thickness?

Because of our limited knowledge of the precise significance of airway remodeling the therapeutic approaches to limit or reverse it are uncertain. Novel approaches to the ablation of airway smooth muscle through bronchial thermoplasty await more extensive critical studies of efficacy and the optimal choice of subject. Will those with excess smooth muscle prove to be the most benefitted? Perhaps better localization of the precise site of AHR will assist in determining the best approaches to therapy.

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