

Remodelling of airway smooth muscle in asthma: what sort do you have?

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Summary

Background Shortening of smooth muscle around airways is the basis of symptoms and abnormal lung function in asthma. The airway wall is increased in thickness in small and large airways asthma, in relation to the clinical severity of asthma and the airway smooth muscle layer is the main contributor to this thickening. The relative contributions of more airway smooth muscle cells, bigger cells or more extracellular matrix to the increased thickness of the smooth muscle layer in asthma is not clear and has been examined in only a small number of cases. Studies of the natural history of asthma suggest that the clinical severity of asthma is relatively constant over time, deficits in lung function compared with nonasthmatic subjects occur early in the course of the disease and the decline in lung function with age is increased in asthma.

Hypothesis The observations from studies of the quantitative pathology and the natural history of asthma might be combined in the hypothesis that the severity of asthma is determined early (in its natural history) and is related mainly to increased volume density of airways smooth muscle cells (hyperplasia) and that later deposition of extracellular matrix from larger, hypertrophic smooth muscle cells results in fixed and increasing deficits in lung function.

Speculation The relative contribution of more smooth muscle cells, bigger cells and extracellular matrix will be determined by unbiased stereological measurements in many cases of asthma of varying severity. The outcomes of such studies will be methods of monitoring and of treatment that will be tailored to the sort of smooth muscle modelling that is present in individual cases.

Keywords: asthma, extracellular matrix, hyperplasia, hypertrophy, smooth muscle, stereology

Why is the airway smooth muscle so important in asthma?

Shortening of airway smooth muscle (ASM) is the basis of asthmatic symptoms and disability. The width/area of the ASM layer seen on transverse sections of airways is increased in asthma on average by 50–200% in fatal and 25–55% in non-fatal cases, compared with control cases, and is the major contributor to the increased area of the inner airway wall in asthma [1]. It is probably also the major contributor to the functional effects of airway wall remodelling [2] – either by taking up space and amplifying the effects of normal shortening or by undergoing excessive, abnormal shortening [3].

It is not understood why more smooth muscle is present in the airways of patients with asthma, although the commonly held paradigm suggests that mediators released during chronic inflammation result in ASM proliferation [4, 5]. The area fraction of the ASM layer seen on transverse sections of airways could be increased by the presence of more cells, enlarged cells or increased matrix between the cells. Mediators, such as IL-1b and IL-6, can promote intracellular protein production ('hypertrophy') and DNA

production ('hyperplasia') *in situ* in ASM [6]. Both ASM and myofibroblasts can secrete a number of extracellular matrix (ECM) proteins, depending on their mechanical and chemical environment [7].

Hyperplasia and hypertrophy

Although numerous quantitative studies of transverse airway sections have shown that the area fraction of the ASM layer is increased in cases of asthma, compared with control cases [8–14], these have not used randomized and unbiased sampling techniques and have used measurements obtained in two dimensions to infer three-dimensional structure. They have also not taken into account the contribution of ECM to the increased area of the smooth muscle layer observed in asthma. Ebina et al. [15] used the physical disector technique [16], which involves serial sectioning of tissues, to examine the size and number of ASM cells in cases of asthma with more ASM than control cases [17]. They showed that cases of asthma with increased muscle confined to the central airways had only ASM hyperplasia. Those cases with increased muscle in both the central and peripheral airways had hypertrophy in the central and the peripheral airways, and some hyperplasia in the central airways. A recent study by Woodruff et al. [18] found that hyperplasia and not hypertrophy was responsible for a 50–83% increase in ASM

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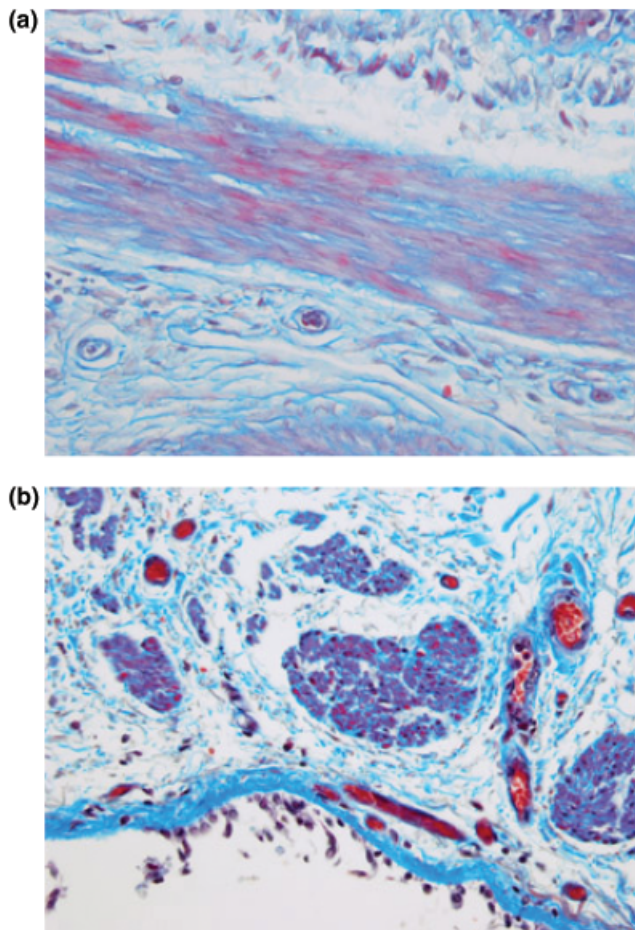


Fig. 1. Photomicrograph of airway smooth muscle (ASM) (pink) and extracellular matrix (ECM) (blue) seen in (a) longitudinal section and (b) transverse section in the same airway from a patient with asthma. The volume fraction of smooth muscle and the number and average size of ASM cells may be estimated from measurement of the relative amounts of ASM and ECM.

in the submucosa of central airways of cases of mild to moderately severe asthma. Neither of these stereological studies assessed changes in the ECM.

The increased proliferative response of 'asthmatic' ASM *in vitro* [19] may be because of an increased sensitivity to growth factors [20]. Enlargement of ASM cells may occur in response to increased loads [21] or cell cycle arrest [5], the latter in response to substances such as transforming growth factor beta (TGF- β), TNF- α , IFN- γ and IL-4 [22]. ASM cells cultured from asthmatic airway biopsies show an increased ability to proliferate [19]. In contrast, studies of proliferation *in situ*, which used markers such as Ki67 or proliferating cell nuclear antigen (PCNA), have not shown increased ASM proliferation in asthma [23–26]. This does not appear to be an artefact of the measurement, as PCNA is able to label actively proliferating cells *in situ* in horses with heaves [27]. The apparently conflicting findings of increased ASM cells *in vitro* without *in situ* evidence of proliferation may be explained by an inability of current techniques to detect proliferation *in situ* because it occurs very slowly, only intermittently or was completed before or at the onset of asthma.

Extracellular matrix deposition in ASM

Asthma is associated with the deposition of a number of ECM proteins within the airway wall including abnormal collagens, laminin, tenascin and fibronectin [28, 29]. These changes predominantly manifest as an increased thickness of the reticular basement membrane, which has been related to airway wall thickness [30] as well as to the amount of ASM in central airways [31]. Increased thickness of the reticular basement membrane has also been related to the number of myofibroblasts in the subepithelial layer [32] and is present in asthmatic children as young as 6 years of age [33]. The ASM layer seen on low power within the airway wall (Fig. 1) is comprised of ASM cells and ECM [34] and both are increased in asthma [35]. Fibroblasts and their secretion of ECM proteins may be influenced by a number of inflammatory and mechanical stimuli. It has been suggested that IL-4 and TGF- β may cause differentiation of fibroblasts to myofibroblasts while IL-4 also induces synthesis of collagen III [36]. Importantly, allergen challenge induces activation of epithelial cells [37] and activation of fibroblasts [36, 37]. Mechanical strain may also alter the production of ECM proteins by fibroblasts [38].

Accumulation of mesenchymal cells

An increased number of activated myofibroblasts is observed beneath the epithelium and basement membrane [32] in cases of asthma, which have been related to asthma severity in some studies [39] but not others [40]. There are a number of mechanisms that might result in increased numbers of myofibroblasts within the inner airway wall. The motile appearance of myofibroblasts [41] has led to the hypothesis that stimulation and proliferation of subepithelial myofibroblasts result in the formation of new smooth muscle cells that migrate to the smooth muscle layer [42]. This is supported by the increased numbers of activated motile fibroblasts observed in some cases of asthma [43]. In mice it has been shown that undifferentiated bone marrow-derived cells may accumulate in the smooth muscle layer [44]. This study raises the possibility of a source of activated fibroblasts that is outside the airway wall. Finally, apoptosis is another mechanism that may lead to differences in the number of ASM cells between asthmatic and non-asthmatic subjects and even between asthmatic subjects. For example, it has been shown that, in rats exposed to allergen, the increase in ASM results from a decrease in the rate of apoptosis, not an increase in proliferation [45].

Remodelling of ASM and the natural history of asthma

Population studies have shown that adult asthmatics have an increased rate of decline of lung function with age [46, 47]. This has been attributed to the progressive development of fixed airflow obstruction [48] similar to that observed in smokers who develop chronic obstructive pulmonary disease (COPD) [49]. This progressive and irreversible decline is thought to be caused by fibrosis of the airway wall in COPD

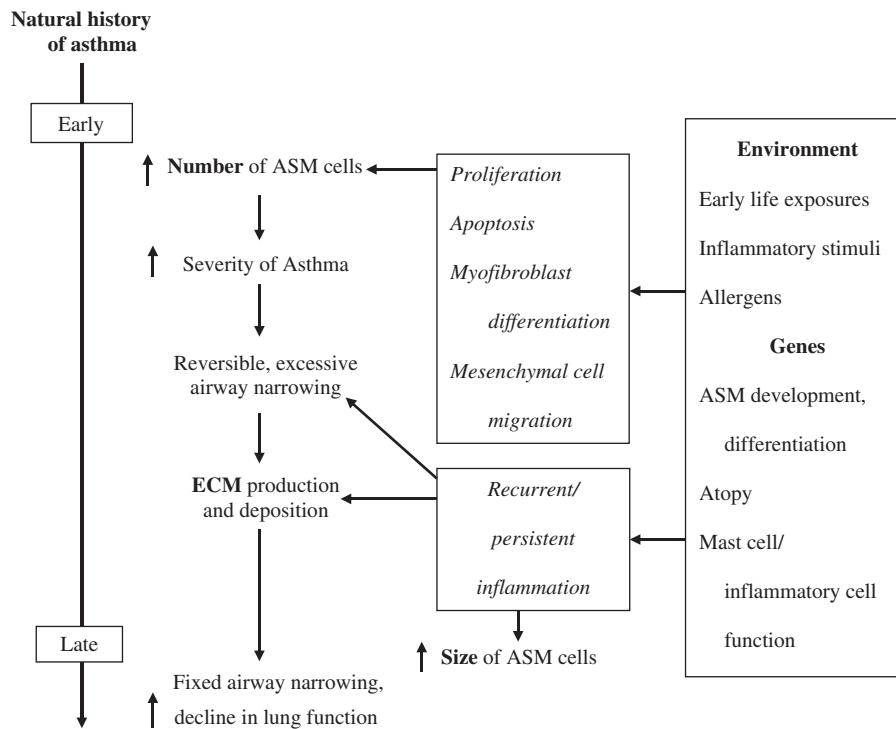


Fig. 2. Schematic of hypothetical relationships between airway smooth muscle (ASM) cell number and size, extracellular matrix (ECM), asthma severity and airway narrowing during the natural history of asthma.

[50] and asthma [51]. In addition to a faster rate of decline in lung function, adults with asthma show a constant deficit in lung function compared with non-asthmatic adults, even at the age of 18 years [46]. This discrepancy tracks back to early childhood [52] and possibly even to infancy [53, 54]. These differences between asthmatics and non-asthmatics are likely to be because of remodelling, although it remains unclear when remodelling of the airways begins. Well-established pathological airway remodelling is observed in children with difficult asthma aged 6–16 years [55], whereas in symptomatic wheezing infants in whom a diagnosis of asthma has not yet been established, these changes are not observed, even in those with atopy [56]. The longitudinal Melbourne study of asthma [52] also showed that, in general, subjects with asthma of different severity tend to stay within their severity groups from childhood until at least middle age. From these longitudinal observations, two questions arise. Firstly, is the remodelling process the same throughout life? The study of Brown et al. [48] suggests that in some cases the development of fixed airflow obstruction is related, in part, to the duration of asthma. Bai et al. [35] found that both ASM cell numbers and ECM deposition within the airway wall were increased in medium and small airways in cases of fatal asthma compared with control cases. However, the amount of ECM within the smooth muscle layer and the ASM itself did not change significantly with age (from 17 to 49 years). Secondly, are there aspects of remodelling that determine asthma severity? It is possible that differences in asthma severity could be because of differences in the number of ASM cells (per volume of tissue or at any given airway size) present within the airway wall. This number may be determined by inherited or perinatal factors, by a balance between cell proliferation

and apoptosis, by the accumulation of undifferentiated mesenchymal cells from the blood or differentiation of myofibroblasts from the subepithelial space (Fig. 2).

To summarize, the area fraction of the ASM layer is increased (in asthma) because of more cells, larger cells and/or ECM deposition. Current data suggest some heterogeneity but the numbers are too small to assess the effects of age, treatment or severity of disease. Asthma severity remains stable during life and is associated with a fixed deficit in lung function from early life and an increased rate of decline of lung function in adult life. ECM deposition is related to the development of fixed airflow obstruction. We might hypothesize (Fig. 2) the following: the severity of asthma is determined very early in the course of the disease and is directly related to the number of ASM cells that are present in the airway wall. The deposition of ECM is a protective mechanism that opposes ASM shortening but results in progressive irreversible airflow obstruction. Hypertrophy of ASM cells is related, not to the duration or severity of asthma, but to variations in airway inflammation, which also contributes to recurrent airway narrowing and the production of abnormal ECM.

Unbiased stereology

Adequate assessment of the three-dimensional structure of ASM within the airway wall requires appropriate sampling and unbiased counting methods. The contribution of ECM also needs to be assessed. Ideally, lung (or lobe) specimens should be obtained and their volume should be measured in inflation before they are sectioned in a randomized, isotropic

plane [57]. Sections thus obtained may then be sampled randomly to assess the volume fractions of ASM, ECM and other tissue components. Alternatively, a sample of an airway chosen from an identifiable site may be extensively and exhaustively sectioned to assess the volume of ASM, ECM and the number of ASM cells and their individual mean volume [15, 58]. In practice, either whole-lung or lobar specimens are not available and exhaustive sectioning is too exhausting for comparisons of numerous airways from large numbers of cases. Another approach is to use blocks of tissue chosen from comparable sites in different cases and cut thick sections (for example, 30 µm) which are examined using thin optical planes of focus to estimate the number and volume of structures in three dimensions – the optical disector [57]. Unbiased counting is achieved using a counting frame and strict rules for the inclusion (or exclusion) of objects of interest [59]. These new approaches are just beginning to be applied to quantitative estimations of airway structure and airway pathology. As both ECM and ASM contribute to what has previously been designated as the 'ASM' layer, the application of unbiased stereology to measurement of ASM in asthma will help future research. Finally, most studies have been undertaken in small groups of asthmatic subjects which have precluded within-study examination of the effects of severity and treatment. Studies using the same techniques applied to larger numbers of subjects are needed [60].

Speculations

Future studies of airway structure will use stereological techniques to unravel the relative contributions of ECM deposition, hypertrophy and hyperplasia (or accumulation of mesenchymal cells) to the increased area fraction of ASM in the airway wall and will relate these to age, duration and severity of asthma. We will see that ECM deposition is largely an adaptation to recurrent excessive ASM shortening associated with abnormal smooth muscle mechanical strain within the airway wall. This reductionist approach will lead to areas of research that establish: (A) simplified and non-invasive methods to detect and monitor ASM cell size and number, and ECM deposition; (B) treatments that are specific to the control of ECM deposition, mesenchymal cell differentiation and ASM proliferation and secretory function; (C) treatments that can reverse the deposition of specific ECM proteins and that can reduce mesenchymal cell numbers (including ASM cells); and (D) strategies (which may be genetically or environmentally based) that will prevent the excessive accumulation of ASM cells in susceptible individuals.

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