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Molecules in focus

Asthma: Pharmacological degradation of the airway smooth muscle layer

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ABSTRACT

Asthma: A disease characterised by excessive and variable airway narrowing, and pathologies of inflammation and remodelling, particularly thickening of the airway smooth muscle (ASM). Treatment approaches dilate narrowed airways and reduce inflammation; however, remodelling seems largely neglected. This review considers the evolution of remodelling in asthma and whether conventional hypotheses that inflammation causes ASM thickening has mislead the medical community into thinking that anti-inflammatories will remedy this ASM defect. There is instead reasonable evidence that ASM thickening occurs independently of inflammation, such that therapies should employ strategies to directly modify ASM growth. Lessons have been learned from the use of untargeted bronchial thermoplasty and there should also be consideration of pharmacological therapies to ablate ASM. We discuss several new approaches to target ASM remodelling in asthma. A major current obstacle is our inability to image the ASM layer and assess treatment response. In this regard, polarisation-sensitive optical coherence tomography offers future promise.

1. Introduction

Asthma is a prevalent obstructive airway disease that is identified based on a phenotype of 'variable' airflow limitation. Underlying pathologies are airway remodelling (structural modification to the airway wall) and inflammation, each of which contributes to disease severity. Increased thickness of the layer of airway smooth muscle (ASM) is the major contributor to airway remodelling in asthma. The temporal relationship between airway remodelling and inflammation is in dispute. According to the conventional hypothesis (Fig. 1a), inflammation drives remodelling in asthma such that individuals who are prone to inflammatory disease are more likely to develop downstream structural abnormalities. An alternative point of view challenging this paradigm is that airway remodelling and inflammation are separately acquired and independently contribute to asthma (Fig. 1b). With respect to asthma management, the debate surrounding the origin of airway remodelling is not trivial and highlights the limitations of current treatments and the need for new therapies directed at ASM. If part of the intention of anti-inflammatory treatment is to attenuate and/or reverse airway remodelling, this may be ineffective if remodelling is acquired independently of inflammation. This review considers the evidence supporting the possibility that remodelling of ASM develops independently of inflammation and discusses strategies to directly target ASM remodelling, beginning with bronchial thermoplasty (BT) and exploring possible future pharmacological therapies.

2. Why should we directly target airway smooth muscle remodelling?

Airway remodelling is a primary feature of asthma that includes thickening of most airway wall components and in particular the ASM layer (James and Wenzel, 2007). Expansion of the ASM layer in asthma is due to a greater number of, and larger, ASM cells and extracellular matrix that increases in proportion to contractile elements (Araujo et al., 2008; James et al., 2012). The thickness of the ASM layer is increased in both large and small airways from subjects with asthma and to a greater extent in more severe disease (Carroll et al., 1993). Biological (Noble et al., 2013) and mathematical (Donovan, 2016) models demonstrate increased airway narrowing due to the mechanical advantage (high circumferential tension) generated by greater muscle bulk.

Reversing or preventing contraction of ASM and anti-inflammatory

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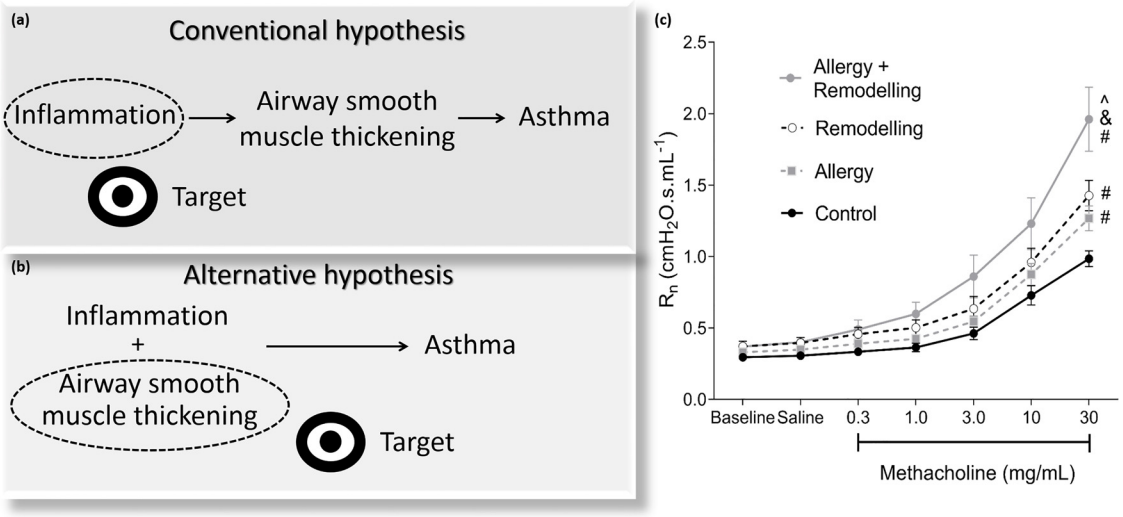

Fig. 1. (a) Conventional *vs* (b) alternative hypothesis on how inflammation and airway smooth muscle thickening interact in the development of asthma. (c) Empirical data from mice where allergy and remodelling were induced seperately. Airway resistance (R_n) to methacholine was enhanced by allergy and remodelling alone, and was most severe when the abnormalities were combined. Data are from mice which was originally published in https://doi.org/10.1042/CS20171386, (Wang et al., 2018).

therapies are the mainstays of asthma therapy for the majority of patients with asthma (GINA). Although anti-inflammatory therapies (predominantly inhaled corticosteroids) reduce airway inflammation and airway wall thickness (based on CT scan), it is unknown if current therapies are effective in reversing ASM remodelling. The 'case for the defence' of inflammation-leads-to-remodelling (validating the use of corticosteroids to reduce remodelling) is supported by decades of animal studies where induction of an inflammatory phenotype produces structural alterations to the airway wall consistent with airway remodelling present in patients with asthma (Nials and Uddin, 2008). The extent of airway remodelling is however dependent on the duration and dose of exposure (Woo et al., 2018) and studies recapitulating a 'real world exposure' show less pronounced changes (Temelkovski et al., 1998).

Histological studies from patients with asthma consistently report the co-occurrence of inflammation and ASM remodelling (Benayoun et al., 2003; Elliot et al., 2015; James and Wenzel, 2007). These studies are invariably cross-sectional in nature. Longitudinal studies of patients with asthma showing inflammation preceding ASM remodelling are non-existent. There are studies that suggest that ASM remodelling and inflammation may be independent. Firstly, the thickness of the ASM layer in asthma is related to the clinical severity of asthma but not related to the duration of asthma, patient age or age of asthma onset (James et al., 2009) and, in general, the severity of asthma and the treatment required to control symptoms remain stable over many years (Phelan et al., 2002). Assuming that remodelling is not readily reversible, the severity of asthma should steadily increase over time if persistent inflammation caused ongoing ASM remodelling. Secondly, increased thickness of the ASM layer is observed early in children with asthma (Regamey et al., 2008) and even before a diagnosis of asthma (O'Reilly et al., 2013). A systemic review of 39 studies examining inflammation and remodelling in asthma concluded, *"The relationship between inflammation and remodelling in children cannot be determined. Failure to demonstrate eosinophilic inflammation in the absence of remodelling is contrary to the hypothesis that inflammation causes these changes"* (Castro-Rodriguez et al., 2018). Finally, airflow limitation is detectable from the earliest stages of postnatal life in individuals who go on to develop asthma (Owens et al., 2017). If function is tightly coupled with structure, such observations implicate a developmental origin of airway remodelling; there is evidence that fetal growth abnormalities increase risk of asthma (Källén et al., 2013), potentially as a result of ASM thickening (Wang and Noble, 2020).

Therefore, there is at least 'reasonable doubt' as to whether inflammation is the stimulus driving ASM remodelling and it is important to appreciate that just because inflammation and remodelling are frequently observed together, this does not necessarily imply a cause and effect relationship. In another histological study (Elliot et al., 2018), airway wall dimensions were compared between subjects with asthma who were grouped based upon the presence (or absence) of granulocytic inflammation. The results of the study indicated that while outer wall thickening occurred only in the presence of inflammation, ASM thickening was independent of inflammation (Elliot et al., 2018). We therefore offer an alternative hypothesis for the development of airflow limitation in asthma (Fig. 1b) where inflammation and remodelling are derived from separate susceptibilities that independently contribute to increase the severity of disease. This idea was well demonstrated through use of a mouse model (Wang et al., 2018) where ASM was increased in thickness by overexpression of a growth factor (Remodelling) and allergic disease simulated by exposure to ovalbumin (Allergy). Both exposures separately increased airway narrowing capacity (Fig. 1c) but the response was most severe when the exposures were combined. These observations are analogous to those of patients with asthma who show independent effects of allergy (skin prick responses to allergen) and non-allergic ASM responsiveness (to inhaled ASM agonists such as methacholine) on the airway response to inhaled allergen (Cockcroft et al., 2005).

The above conjecture in no way disproves the conventional hypothesis nor dismisses the essential role of inflammation in asthma morbidity. Nonetheless, the idea of reducing ASM thickness through a pathway other than alleviating inflammation should at the very least be considered. The best demonstration of how directly reducing ASM thickness can impact patient welfare is the symptomatic response of many patients treated with BT.

3. Lessons learned from bronchial thermoplasty

Bronchial thermoplasty is a specialist treatment for asthma whereby ablative thermal energy is delivered to large airways that are accessible by bronchoscopy (Castro et al., 2010a). An immediate conceptual advantage of BT is that the procedure is not reliant on the mechanism producing ASM thickening; whether this underlying cause is inflammation-dependent or independent, BT directly targets the

structural abnormality. Bronchial thermoplasty is also the first therapy available to patients that is designed to specifically reverse ASM remodelling. The area of ASM on bronchial biopsies, as a proportion of total tissue area (an index of muscle thickness or bulk) has been shown to be reduced by up to 75 % at the site of treatment with BT (Pretolani et al., 2014).

Does BT work? The most objective answer to this question is reflected in a Cochrane Review (Torrego et al., 2014) which states *"further research should provide better understanding of the mechanisms of action of bronchial thermoplasty, as well as its effect in different asthma phenotypes or in patients with worse lung function"*. There is certainly good evidence from clinical trials showing that BT reduces exacerbations, hospitalisations attributed to asthma and improves overall asthma control (Castro et al., 2010b). From the perspective of a treating physician, and certainly the patient, improvement in such indices is evidence of a successful therapy. However, since sham controls have not regularly been considered, and in view of established placebo effects associated with BT (Cox et al., 2007), there is persisting scepticism regarding its mode of action. Perhaps the greatest foundation for clinical caution and the reluctance for widespread use of BT, is the failure to regularly demonstrate improved lung function after BT, particularly using conventional measures such as FEV_1 (Castro et al., 2010b; Cox et al., 2007).

It is encouraging that evidence of functional improvement after BT is now beginning to emerge. A mathematical model of human lung function before and after BT has advanced our understanding of the mechanism of BT and suggested how treatment response might be optimised (Donovan et al., 2018). In the model, treated airways are the same as per clinical practice, with a realistic reduction in ASM thickness of 75 %, albeit at the top end of what is observed clinically (Donovan et al., 2019b). Model outputs show that BT reduces lung ventilation heterogeneity and airway resistance following maximal ASM stimulation. Since measuring lung function with FEV_1 does not provide information on spatial distribution of flow and maximal ASM stimulation is not safe in patients with severe asthma, the functional effects of BT may be underestimated. Outside the context of the clinical laboratory, ASM contraction to an environmental trigger is likely to exceed that which would be deemed safe by respiratory scientists. Reduction in this level of ASM stimulation by the use of BT may be reflected in the reduced rate of hospitalisation observed in clinical trials.

More recent studies in patients with asthma have reported improvement after BT using different outcome measures. Airway lumen volume, assessed by CT, is increased after BT (Langton et al., 2019b) which agrees well with modelling predictions (Langton et al., 2019a). Plethysmographically-determined airway resistance is decreased after BT and correlates with improvement in asthma control (Langton et al., 2020). These observations also suggest that the benefits of BT may be underestimated using FEV_1 as a treatment outcome. Bronchial thermoplasty is not without side effects, particularly transient worsening of asthma symptoms, and not all patients treated with BT achieve a symptomatic response (Pavord et al., 2007). Therefore there is a need for improved selection of patients undergoing BT, possibly based upon patient-specific airway structure (Donovan et al., 2019a). Alternatively, treatments that can achieve the same benefit without 'injuring' the airway wall may be a better option in the future.

4. Pharmacological targeting of airway smooth muscle

If a localised reduction in ASM thickness improves respiratory health in patients with asthma, a pharmacological approach could be even more effective if it is able to treat a greater proportion of the bronchial tree (Fig. 2a). More extensive treatment of the bronchial tree is facilitated by oral administration of an appropriate agent or *via* aerosol delivery (with appropriate targeting of peripheral lung regions where needed). The simulated effect of more widespread (*i.e.*, beyond the central airways) treatment of ASM (Donovan et al., 2020) has shown that a 20–30 % reduction in ASM thickness throughout the bronchial tree was as potent as a larger reduction (75 %) confined to the large airways (Fig. 2b). Pharmacological ablation of the ASM layer is therefore worth pursing as a therapy for the treatment of patients with asthma.

A rationale for a pharmacological-based degradation of ASM thickness is suggested by observations that ASM cells reside in a state of ongoing proliferation (Fayon et al., 2015; Hirst et al., 2004) and apoptosis (Freyer et al., 2001; Hirst et al., 2004), biological processes that are potentially sensitive to chemical agents. Primary ASM cells from human subjects proliferate in an age-dependent manner, with a doubling of the population in 37 h in neonates compared with 52 h in adults, occurring concomitantly with increased mitochondrial biogenesis and intracellular calcium concentration (Fayon et al., 2015). Proliferation of ASM cells is further demonstrated *in situ* (histological sections) and is similar between small and large airways (James et al., 2018). Whether rate of proliferation is increased in asthmatic subjects compared with non-asthmatic subjects is a contentious issue, with evidence for (Ramos-Barbón et al., 2010) and against (Ijpma et al., 2017; James et al., 2018) increased proliferation in asthma. Similarly, ASM cells exhibit apoptosis *in situ*, at a rate not discernibly different between subjects with and without asthma (Benayoun et al., 2003; Trian et al., 2007). A pharmacological intervention that shifts the balance between proliferation (low) and apoptosis (high) should reduce the thickness of the ASM layer in asthmatic patients. In the following sections, we examine agents that may be effective in reducing ASM thickness including gallopamil, fevipiprant, macrolides, omalizumab and rosigilitazone.

The first drug trialled to specifically reduce ASM thickness was gallopamil (D-600), an L-type channel blocker, administered in a large double-blinded, randomised, placebo-controlled study in patients with severe asthma (Girodet et al., 2015). Gallopamil treatment was associated with an ~18 % reduction in the thickness of the ASM layer (Girodet et al., 2015), inhibition of mitochondrial biogenesis and reduced ASM cell proliferation (Trian et al., 2007). Thickness of the ASM was reduced after 12 months of treatment with gallopamil and the clinical benefit (reduced exacerbations of asthma) was observed 3 months after therapy ceased. Concerns regarding the study centred on the method of ASM assessment (biopsy and subsequent normalisation to CT) and the asynchrony between reduced ASM thickness and exacerbations (Sumino et al., 2015).

Fevipiprant, a prostaglandin D_2 type 2 receptor antagonist, has been shown to improve asthma symptoms, lung function, airway eosinophilia, and epithelial integrity in a randomized, placebo-controlled trial (Gonem et al., 2016). Saunders et al. suggested that lung function improvement after fevipiprant was mediated by effects on the ASM, since they found a 13 % reduction in ASM mass in biopsy samples (Saunders et al., 2019). A cellular-level mathematical model, informed by observations in cell culture, suggested that the mechanism through which fevipiprant acted was increased apoptosis and reduced recruitment of myofibroblasts to the ASM layer (Saunders et al., 2019). More work using animal models of allergic disease is needed to confirm the predicted mechanisms of fevipiprant.

An established pharmacological therapy for patients with asthma is the macrolide azithromycin, a drug with a broad range of actions including anti-microbial, anti-viral and anti-inflammatory properties (Gibson et al., 2017). Azithromycin has been demonstrated in clinical trials to reduce exacerbations and improve quality of life, although not improve lung function (Brusselle et al., 2013). The mechanism through which azithromycin improves the welfare of asthmatic patients is unclear, but appears independent of anti-bacterial activity (Gibson et al., 2017). In ASM cell cultures, azithromycin is anti-proliferative and induces autophagy leading to cell death (Stamatiou et al., 2010, 2009). Administering azithromycin to naïve (Fig. 2c) or allergic mice decreases ASM thickness, affecting both proximal and distal airways (Donovan et al., 2020; Kang et al., 2016). The reduction of ASM thickness (29 %)

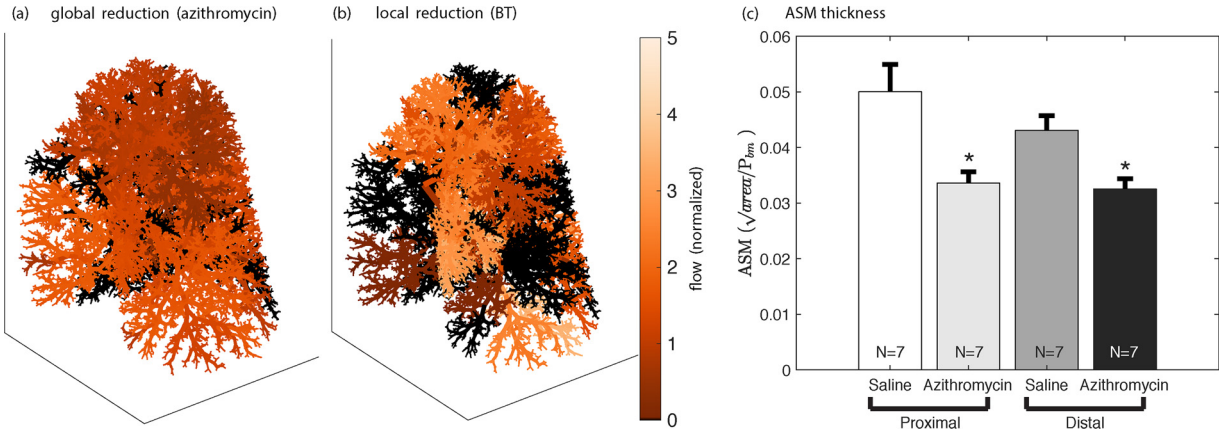

Fig. 2. Mathematically predicted flow patterns in a lung from an asthmatic patient during bronchoconstriction. Lungs were previously 'treated' with an intervention producing a (a) small (30 %) but global reduction in ASM thickness or (b) high (75 %) but local reduction in ASM thickness. Global ASM reduction can potentially be achieved *in vivo* by (c) systemic administration of azithromycin, which in naïve mice reduced ASM thickness (normalised to perimeter of basement membrane perimeter, P_{bm}) at the two different anatomical locations studied. All data originally published in https://doi.org/10.14814/phy2.14451, (Donovan et al., 2020).

in the naïve mouse demonstrated a direct effect of azithromycin on the ASM layer. In allergic mice, reduction of ASM thickness after azithromycin appears to occur through a down-regulation of p38 mitogen-activated protein kinase (MAPK) pathway, specifically *via* c-Jun N-terminal kinase, extracellular signal-regulated kinase (ERK) 1 and p38 proteins (Kang et al., 2016).

Roxithromycin is another macrolide that enhances the bactericidal and phagocytic activities of neutrophils, which in contrast to azithromycin, was associated with a significant improvement in lung function, but not asthma symptoms (Black et al., 2001). Bronchial responsiveness is reduced after roxithromycin and there is a simultaneous reduction in inflammation (Ci et al., 2012). Like azithromycin, roxithromycin inhibits ASM cell proliferation through the ERK pathway (Pei et al., 2016) and induces ASM cell apoptosis by upregulating P27 and the activity of caspase-3 and caspase-9-dependent mitochondrial pathway (Dai et al., 2014). *In vivo* studies examining the effect of roxithromycin on ASM thickness have not yet been undertaken.

It is well established that immunoglobulin E (IgE) plays a central role in the pathophysiology of allergic asthma. Omalizumab is a recombinant humanised IgE monoclonal antibody that is used as an add-on treatment of severe allergic asthma by preventing IgE binding to its target receptors and reducing the allergic cascade (D'Amato et al., 2007). A 48 week prospective study of omalizumab showed reduced airway wall thickness on CT in patients with asthma. Reduced wall thickness may have arisen through reduced inflammatory infiltrates (direct or indirect) (Tajiri et al., 2014), or potentially due to thinning of the ASM layer. A potential effect of omalizumab in reducing ASM thickness has been demonstrated in culture, where incubation of ASM cells with the serum from subjects with allergic asthma induced a hyper-proliferative phenotype, which was reversed upon addition of omalizumab, possibly mediated through the ERK1/2 / MAPK pathway (Roth et al., 2015).

Finally, peroxisome proliferator-activated receptor-γ agonists or thiazolidinediones, initially used to treat hyperglycemia and insulin resistance of type 2 diabetes mellitus, have been trialled as treatments for asthma. Effects of rosigilitazone include a modest (15 %) reduction in late phase allergen response (Richards et al., 2010) and reduced bronchoconstriction to methacholine (Sandhu et al., 2012), the latter partially explained by bronchodilatory effects of rosigilitazone (Donovan et al., 2014). Since rosigilitazone also has an anti-proliferative effect on ASM cells in culture (Ward et al., 2004), it is possible that some of the effects *in vivo* are due to thinning of the ASM layer.

5. Future directions and concluding remarks

Evoking a strategy that serves fundamentally to reduce ASM thickness requires sufficient diagnostic guidance. High resolution CT is not able to resolve the ASM layer, providing only a measure of total wall thickness (Hudon et al., 1997). Airway biopsy, while useful in a research population, is not practical for ongoing patient care and management and samples may not be representative of ASM structure elsewhere in the bronchial tree. Therefore, currently there is no reliable method to measure the effects of treatments on the thickness of the ASM *in vivo*.

Optical coherence tomography (OCT) is a non-invasive optical imaging technique that allows identification and quantification of different layers of the airway wall, including mucosa, submucosa and cartilage (Noble et al., 2010). Conventional OCT cannot however resolve the ASM layer. Polarised-sensitive OCT (PS-OCT) (or orientation resolved OCT) is an extension of OCT that uses information on tissue organisation and orientation to delineate the ASM layer (Adams et al., 2016; Li et al., 2018). There is good agreement between PS-OCT and histology when comparing ASM area in cross sections from porcine and canine airways (Adams et al., 2016) and preliminary data (n = 3 per group) shows increases in ASM thickness in asthmatic compared with non-asthmatic subjects (Cho et al., 2016).

Numerous groups are advancing PS-OCT for the assessment of ASM thickness in patients (Adams et al., 2016; Cho et al., 2016; Li et al., 2018). In a research setting, this methodology could be used to explore the uncertainty around the association between remodelling and inflammation. Following translation to the clinic, there are several immediate applications: (1) It is essential to establish how effective current therapies are in reducing ASM thickness, beginning with inhaled corticosteroids and also confirming the results of biopsy studies after BT; (2) It is important to properly phenotype patients in terms of their distribution of ASM thickness in small and large airways which will impact how patients are treated in the future, *i.e.*, patients receiving pharmacological intervention or BT. Is structure-guided treatment feasible in the context of BT or beyond (Donovan et al., 2019a)? and; (3) Clinical trials can begin to test the effects of new therapies specifically designed to reduce ASM thickness, using an approach that can reliably measure ASM thickness, providing further motivation for drug development.

Use of PS-OCT does not reduce the need for preclinical investigations and disease modelling in the drug development pipeline. Isolating the pathway through which an agonist reduces ASM thickness is not straightforward and in the case of agonists like fevipiprant or the macrolides, reduced ASM thickness could be achieved indirectly by

attenuating inflammation. It is recognised however that azithromycin reduced ASM thickness in naïve (presumably free of inflammation) mice (Donovan et al., 2020), a nice demonstration of the utility of the modelling approach. Continued investment in studies using biological and mathematical models is thus important to establish the mechanisms of drug actions.

To conclude, the origin of ASM thickening in the asthmatic airway is an important area of exploration as it impacts strategies designed to reverse airway remodelling. Pharmacological therapies designed to directly reverse ASM thickness are dependent upon a robust method of measurement and in this regard, the field has made significant in-roads. By recognising the independent role of ASM contraction in the pathogenesis of asthma and through application of cell culture and animal models of asthma as testing platforms for ASM-targeting pharmaceutical agents, sophisticated mathematical modelling of airway structure-function, and new technologies to measure ASM thickness, effective therapies to target the ASM in patients with asthma is a realistic future treatment option.

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Authors' contribution

K.C.W.W. and P.B.N. drafted the manuscript. All authors helped critically revised the manuscript. All authors have read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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