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Bronchial smooth muscle cell in asthma: where does it fit?

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ABSTRACT

Asthma is a frequent respiratory condition whose pathophysiology relies on altered interactions between bronchial epithelium, smooth muscle cells (SMC) and immune responses. Those leads to classical hallmarks of asthma: airway hyper-responsiveness, bronchial remodelling and chronic inflammation. Airway smooth muscle biology and pathophysiological implication in asthma are now better understood. Precise deciphering of intracellular signalling pathways regulating smooth muscle contraction highlighted the critical roles played by small GTPases of Rho superfamily. Beyond contractile considerations, active involvement of airway smooth muscle in bronchial remodelling mechanisms is now established. Not only cytokines and growth factors, such as fibroblasts growth factor or transforming growth factor- β , but also extracellular matrix composition have been demonstrated as potent phenotype modifiers for airway SMC. Although basic science knowledge has grown significantly, little of it has translated into improvement in asthma clinical practice. Evaluation of airway smooth muscle function is still limited to its contractile activity. Moreover, it relies on tools, such as spirometry, that give only an overall assessment and not a specific one. Interesting technics such as forced oscillometry or specific imagery (CT and MRI) give new perspectives to evaluate other aspects of airway muscle such as bronchial remodelling. Finally, except for the refinement of conventional bronchodilators, no new drug therapy directly targeting airway smooth muscle proved its efficacy. Bronchial thermoplasty is an innovative and efficient therapeutic strategy but is only restricted to a small proportion of severe asthmatic patients. New diagnostic and therapeutic strategies specifically oriented toward airway smooth muscle are needed to improve global asthma care.

INTRODUCTION

Airway smooth muscle cells (aSMC) derive mainly during embryogenesis from mesenchymal precursors, in parallel with epithelial buds, and are associated with the correct development of the airways tree.¹ Present all along the respiratory tree from the trachea to the bronchioles, it is conserved in vertebrae through evolution.² aSMCs are thought to maintain basal tone in bronchi and homogeneous lung ventilation through modulation of local airflow resistance.³ Though, its real

physiological role after development remains controversial in part due to the difficulties to design experimental procedure to test specific hypothesis (impact on mucus expulsion and cough, ventilation to perfusion matching etc).⁴ However, while the physiological role of aSMC is controversial, their involvement in airways diseases, especially in asthma, is now better understood.

Asthma is a respiratory condition defined by the association of variable respiratory symptoms, such as acute dyspnoea, chest tightness, wheezing and chronic cough, associated with impaired airflow and chronic bronchial inflammation.⁵ It is a frequent disease affecting around 250 million patients worldwide with an increased incidence for the last decade.⁶ Asthma treatment is mainly based on inhaled corticosteroids associated with long-acting and short-acting bronchodilators. The main objective of those treatments is to achieve a complete control of the disease.⁷

Asthma pathophysiological hallmark can principally be divided into three inter-related components: airway inflammation, airway hyper-responsiveness and airway remodelling (figure 1).

Inflammatory pathways involved in asthma course have been extensively studied. Two main pathophysiological pathways are now commonly accepted: eosinophilic (or type-2-driven asthma) and non-eosinophilic.⁸ Considering type-2-driven asthma, key inflammatory cytokines can be highlighted: interleukine (IL)-4 implicated in T-helper 2 polarisation and IgE switching, IL-5 associated with eosinophils production and trafficking and IL-13 that plays a central role in airway remodelling. Some biotherapies targeting those cytokines pathways had proven their efficiency in selected severe asthmatics and are now available in clinical practice.⁹ On the contrary, non-eosinophilic asthma, including paucigranulocytic and neutrophilic asthma, remains poorly understood.

Airway remodelling is defined by an association of structural modifications of bronchial

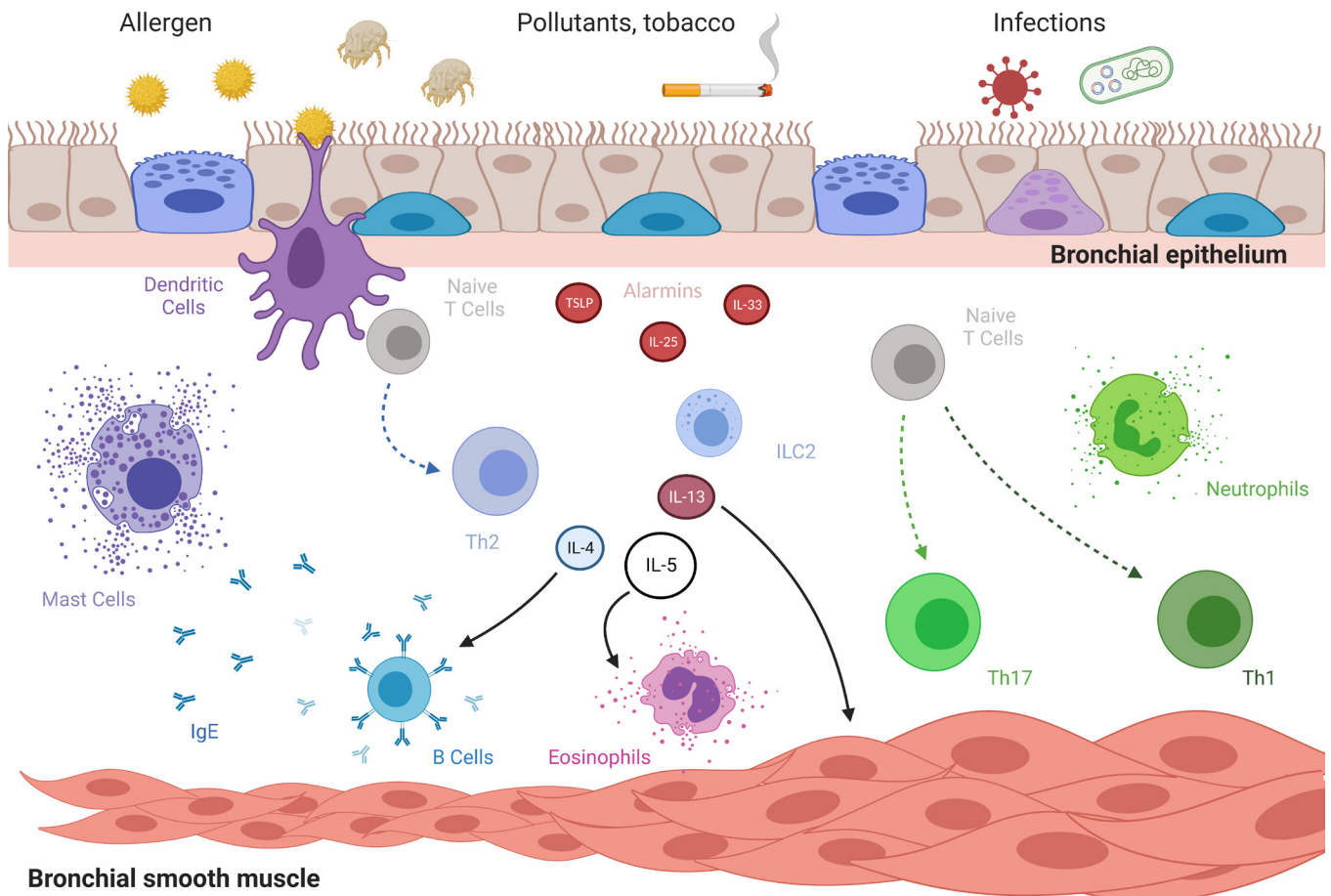


Figure 1 Pathophysiology of asthma. Under stimulation by noxious and/or harmless environment, bronchial epithelium secretes alarmins. Such molecules stimulate innate and adaptive immunity giving rise to infiltration of bronchi by eosinophils or neutrophils or both. Conversely, under sustained inflammation, bronchial remodelling developed with increased basal membrane thickness and hypertrophy and hyperplasia of bronchial smooth muscle. Along with specific bronchial smooth muscle cells acquired hypercontractility, all those mechanisms participate to airway hyper-responsiveness. IgE, immunoglobulin E; IL, interleukine; Th, T-helper cell, TSLP, thymic stromal lymphopoietin. Created with BioRender.com.

wall including aSMC alterations, epithelial dysfunction, reticular membrane thickening and oedema.^{10–11} Such lesions can be irreversible and lead to the progressive loss of respiratory function through time.¹² Airway remodelling unlinked with airway smooth muscle biology is presented elsewhere.¹³

Airways hyper-responsiveness (AHR) is defined by an exaggerated response of airways to harmless or harmful stimuli. This altered response of bronchi to environment depends on bronchial smooth muscle's activity, principal actor of bronchoconstriction. Noteworthy, inflammation, by acting on bronchial smooth muscle, is also closely linked to this phenomenon.

Although recent biotherapies critically improved asthma outcomes in severe asthmatic patients, some inflammatory endotypes, noteworthy non-eosinophilic asthma, remain orphan of efficient treatment.^{14–17} New strategies are evaluated in preclinical setup on non-inflammatory components, notably in aSMC. In this review, we will discuss the biological dysfunctions of the bronchial smooth muscle during asthma, the different techniques of evaluation of these dysfunctions in the

clinic as well as the existing or developing therapeutic strategies to manage them.

Biology of aSMC and role in asthma pathophysiology aSMC contraction dysfunction in asthma

aSMC is essential in the development and sustainment of AHR. Ex vivo studies demonstrated that aSMC harvested from asthmatic subjects displayed increased maximum capacity and shortening velocity compared with controls.¹⁸ Moreover, aSMCs isolated from asthmatic patients presented a stronger contraction in response to histamine compared with controls.¹⁹ Contractile capacity of bronchial SMC principally depends on the phosphorylation of the 20 kDa myosin light chain (MLC₂₀), which leads to the activation of the contractile apparatus (figure 2). The phosphorylation level of MLC₂₀ is regulated by two enzymes: the myosin light chain kinase (MLCK) and the myosin light chain phosphatase (MLCP). Two distinct signalling pathways regulate the activity of these enzymes: the Ca²⁺ and the Ca²⁺ sensitisation pathway.

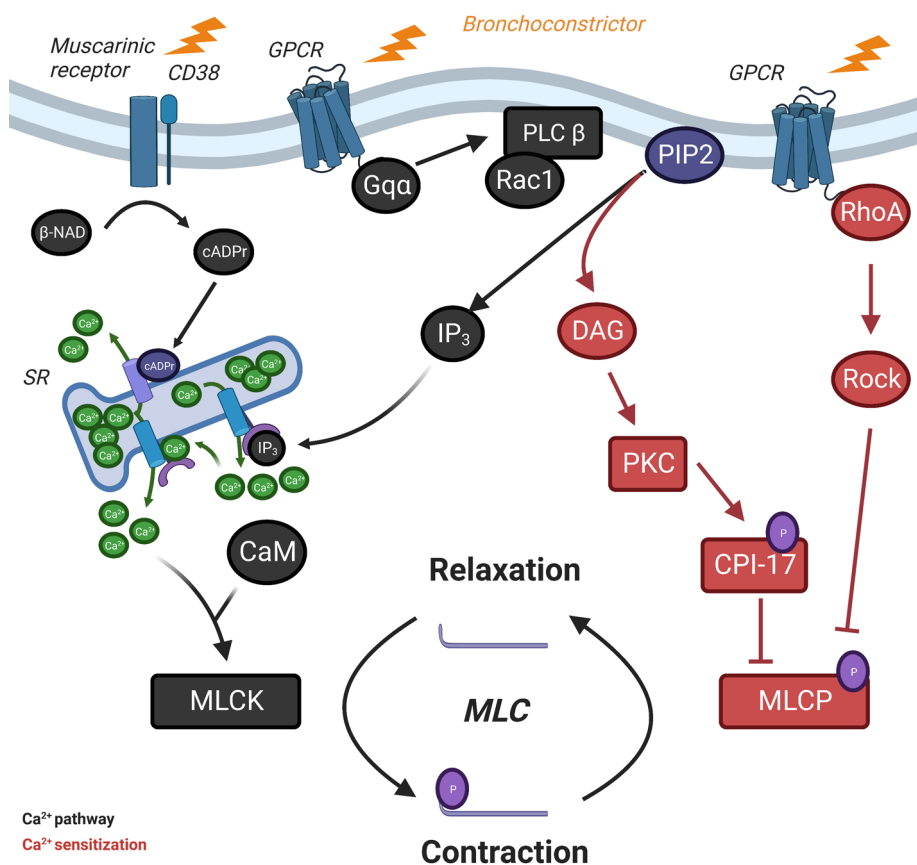


Figure 2 Intracellular regulation of airway smooth muscle cells contraction. Smooth muscle cells contraction and relaxation cycle depends on phosphorylation and dephosphorylation of MLC, respectively. Increase of intracellular Ca^{2+} concentration in response to bronchoconstrictor stimuli leads to the CaM-dependent activation of MLCK, which phosphorylates MLC: it is the Ca^{2+} pathway. In parallel, activation of RhoA–Rock pathways and CPI-17 deactivates MLCP, which prevents MLC from dephosphorylation: it is the Ca^{2+} sensitisation. β-NAD, beta-nicotinamide adenine dinucleotide; cADPr, cyclic adenosine diphosphate ribose; CaM; calmodulin; CD, cluster of differentiation; DAG, diacylglycerol; GPCR, G protein-coupled receptors; IP₃, inositol triphosphate; MLC, myosin light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; PKC, protein kinase C; PLC, phospholipase C; PIP₂, phosphatidylinositol-4,5-bisphosphate; Rac1, Rac family small GTPase 1; ROCK, Rho kinase; RhoA, Rho family small GTPase A; SR, sarcoplasmic reticulum.

Considering the Ca^{2+} pathway, the phosphorylation of MLC₂₀ by MLCK leads to cross bridges with actin that conducts the aSMC contraction cycle. Interestingly, overexpression of MLCK in aSMC has been observed during asthma associated with its overcontractility.^{18–20} MLCK activity is principally controlled by the rise in cytosolic Ca^{2+} concentration coming from extracellular calcium influx through ion channels and sarcoplasmic reticulum (SR) calcium stores.²¹ SR stores release is principally triggered by the inositol 1,4,5-trisphosphate (IP₃) produced by activated phospholipase C (PLC) after the binding contractile agonists to G protein-coupled receptors or to M3 muscarinic receptors.²²

Recently, this signalling pathway has been complemented by studies demonstrating the involvement of the monomeric GTPase Rac1 in aSMC contraction. Rac1 protein is activated in murine and human aSMC by bronchoconstrictors such as methacholine leading to its association with the pleckstrin homology domain of PLC β2 to potentiate the production of IP₃ required for aSMC contraction.²³ The relevance of this signalling pathway in

aSMC was highlighted by demonstrating that Rac1 was overactivated in aSMC from asthmatic patients as well as in aSMC from mice developing allergic asthma. In this experimental model, deletion of the Rac1 gene specifically in SMCs or pharmacological inhibition of Rac1 activity prevents AHR. These results identify the Rac1 protein as a new therapeutic target in respiratory pathologies associated with AHR.²³

Independently of IP₃ production, cyclic adenosine diphosphate ribose (cADPr) could activate the ryanodine receptors channel (RyR) on the SR leading to the liberation of Ca^{2+} from the internal SR stores.^{24–25} Such metabolite is produced from beta-nicotinamide adenine dinucleotide next to the stimulation of the muscarinic receptor M3. Moreover, Ca^{2+} liberation is increased by Ca^{2+} -induced Ca^{2+} release through RyRs, resulting in Ca^{2+} wave propagation and the simultaneous aSMC twitching.²⁶ In asthma, overexpression of CD38 induced by proinflammatory cytokines such as IL-1β, IL-13 and tumor necrosis factor-α leads to increased RyR activation by cADPr.^{24–27}



Calcium uptake and sustainment of ATP synthesis by mitochondria are essential to smooth muscle contraction.²⁸ Calcium uptake is mediated by the mitochondrial Ca^{2+} uniporter while its release mainly depends on $\text{Na}^{2+}/\text{Ca}^{2+}$ or $\text{H}^{+}/\text{Ca}^{2+}$ exchanger.²⁹ In aSMC from asthmatic patients, mitochondrial dysfunction can be observed. Downregulation of the expression of sarcoendoplasmic Ca^{2+} ATPases 2 is associated with the dysregulation of Ca^{2+} homeostasis in asthma.³⁰

The Ca^{2+} sensitisation pathway leads to a maximal contraction independently of the intracellular Ca^{2+} concentration by the regulation of the MLC_{20} phosphorylation state by MLCP. On the one hand, activation of protein kinase C by diacylglycerol leads to the phosphorylation of protein kinase C-potentiated phosphatase inhibitor protein of 17 kDa (CPI-17), which binds to the catalytic subunit of MLCP, inhibiting its phosphatase activity.³¹ On the other hand, activated RhoA interacts with its downstream effector Rho kinase (ROCK), which inactivates MLCP by phosphorylating its myosin-binding subunit.³² Moreover, ROCK can also regulate the activity of MLCP by the phosphorylation of CPI-17.^{31, 33} Interestingly, expression and activity of CPI-17 are increased in aSMC from rats model of allergic asthma.³⁴ In parallel, several studies have shown an increase of RhoA expression in aSMC in animal models.³⁵ Inhibition of RhoA activity prevents and reverses AHR induced in allergic asthma models of guinea pigs.³⁶ Conversely, inflammatory cytokines can significantly influence Ca^{2+} sensitisation pathway. IL17A, a cytokine secreted by Th17 cells, is able to induce an upregulation of RhoA protein in human aSMC.³⁷ Conversely, it can also increase aSMC contraction by the activation of RhoA–ROCK2 through NF- κ B pathway.³⁸ Targeting IL-17 pathway with neutralising antibody decreased the expression of NF- κ B, ROCK-I and ROCK-II in lung parenchyma in a mouse model of asthma compared with controls.³⁹ Interestingly, it has been recently proven in mouse that combination of anti-IL17 antibodies along with ROCK inhibitor (Y-27632) significantly improved respiratory resistance, bronchial remodelling and inflammation.⁴⁰ Similar findings were observed with IL-13, which induces an increase in RhoA expression by aSMC.⁴¹ Those results suggest a potential role of Ca^{2+} sensitisation pathway dysfunction associated with asthma AHR.

Aside inflammatory cytokines, small molecules can also interact with the contractile apparatus such as nitric oxide (NO). In physiological conditions, NO is a potent bronchodilator through the production of cGMP by activated cytosolic guanylate cyclase leading to decreased intracellular Ca^{2+} .⁴² Bronchial NO mainly derived from epithelial cells on the one hand and inhibitory non-adrenergic non-cholinergic nerve terminals.^{43, 44} Inhibition of NO synthesis by L-NG-Nitro arginine methyl ester in vitro and in vivo in guinea pigs enhances the airway hyperresponsiveness in response to histamine.⁴⁵ Though, its precise role in the context of asthma remains controversial.⁴⁶ Indeed, inflammatory environment, especially type

2 driven, is a potent activator of inducible NO synthase of type 2 in epithelial cells.^{47, 48} Exhaled NO measured in asthmatic patients is correlated with AHR.^{49, 50} Association of NO with worsened AHR, despite its physiological bronchodilator effect, can be partly explained by collateral damages linked with peroxynitrite production and side effects on vessels with increased permeability and bronchial oedema.

Autonomous innervation of aSMCs is another modulator of AHR. TRPA1 channels are located at sensory nerves, predominantly on C fibres and is also expressed in non-neuronal cells including airway inflammatory cells, SMC, epithelial cells and fibroblasts.⁵¹ Activation of TRPA1 by environmental irritants such as cigarette smoke or air pollution leads to the activation of bronchopulmonary C fibres in an experimental model and is implicated in cough.⁵² Inhibition or knockout of TRPA1 channels leads to an inhibition of neuropeptide release and airway hyperreactivity in an ovalbumin-challenged mice model of asthma.⁵³ Whether such pathway is implicated in human asthma pathophysiology remains to be demonstrated.

aSMC beyond contraction: bronchial remodeling

Airway remodelling presentation may differ between groups of moderate-to-severe asthmatic patients in terms of aSMC mass and basement membrane thickening.⁵⁴ Interestingly, airway remodelling can appear early in asthma, even in children.^{55, 56} Significant bronchial remodelling in childhood, characterised by reticular basement thickening and increased aSMC mass, is associated with severe disease.⁵⁷ Moreover, airway remodelling is also associated with persistent obstruction in severe asthmatic children.⁵⁸ Those elements are in favour of an early role played by airway remodelling in asthma natural course.

Physiologically, aSMC presents low proliferation capacities and a contractile phenotype characterised by the expression of sm- α -actin, smooth muscle γ -actin, smooth muscle myosin heavy chain, calponin, h-caldesmon, SM22, smoothelin and metavinculin.⁵⁹ Though, in inflammatory environment, aSMC have the ability to switch their phenotype with an increase of their proliferation and migration capacity leading to hyperplasia.⁶⁰ Mitochondria biogenesis is also affected. In proliferative aSMC from asthmatic patients, increased mitochondrial mass and activity can be observed associated with altered calcium homeostasis.⁶¹

Phenotype switch can be induced in vitro by several growth factors and cytokines presented in table 1. Analysis of induced sputum in asthmatics showed that many of these molecules are indeed oversecreted.⁶² Bronchial inflammation associated with remodelling can be driven by the environment through the ability of bronchial epithelium to secrete alarmins, namely thymic stromal lymphopoietin (TSLP), IL-25 and IL-33, in response to harm.¹¹ It has been demonstrated that human aSMC expressed receptor to TSLP and IL-25 and that their

Table 1 Mitogenic factors of aSMC

Factors	Cellular source	Effects	Ref
Growth factors			
PDGF	Platelets, monocytes/macrophages, ASMC, epithelium	Pro-proliferative	123–126
FGF	Extracellular matrix, monocytes/macrophages, ASMC	Pro-proliferative	126–129
EGF	Epithelium, platelets	Pro-proliferative	130
Cytokines			
TGF- β	ASMC, T-lymphocytes, epithelium	Pro-proliferative	128 131–133
TNF- α	ASMC, epithelium, T cells, monocytes/macrophages	Pro-proliferative, antiproliferative	126 134–136
IL-1 β	T cells, monocytes/macrophages, ASMC, epithelium	Pro-proliferative	137 138
IL-6	T cells, monocytes/macrophages, ASMC, epithelium	Pro-proliferative	135 138
IFN- γ	T cells, NK cells	Antiproliferative	139
IL-4	T-cell, mast cells	Antiproliferative	140 141
Inflammatory mediators			
Histamine	Mast cells, basophils	Pro-proliferative	142 143
Thromboxane A2	Mast cells, monocytes/macrophages	Pro-proliferative	144 145
Sphingosine 1-phosphate	Plasma, platelets	Pro-proliferative	146
Enzymes and diverse			
Tryptase	Mast cells	Pro-proliferative	143 147
Thrombin	Plasma	Pro-proliferative	148
Elastase	Neutrophils	Pro-proliferative	149
Reactive oxygen species	Monocytes–macrophages, neutrophils, eosinophils, mast cells	Pro-proliferative	150

aSMC, airway smooth muscle cells; EGF, epidermal growth factor; FGF, fibroblast growth factor; IFN, interferon; IL, interleukine; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α .

stimulation lead to proinflammatory and synthetic phenotypes.^{63 64} Conversely, it has been shown by air–liquid interface coculture that injured epithelial cells stimulate aSMC proliferation through the production of proinflammatory molecules (IL-6, IL-8, monocyte chemoattractant protein-1) and matrix metalloproteinase-9.⁶⁵ Mechanical stimulation of epithelial cells is another path leading to phenotype switch of aSMC. A significant proliferation of aSMC could be induced in vitro by compression of epithelial cells.⁶⁶ Combination of inflammatory cytokines, such as type 2 cytokines, leads to complex modification of aSMC phenotype. For example, whereas IL-13 alone have an antiproliferative effect on cultured aSMC, it also increases the expression of CysLT1 receptor enhancing leukotriene induced proliferation.^{67 68} Interestingly, stimulated aSMC are also able to synthesise proinflammatory factors such as the platelet-derived growth factor (PDGF), fibroblast growth factor, IL-1 β , transforming growth factor- β , IL-5, IL-6, IL-8, IL-17, which further amplifies phenotype switch.³

Composition of the extracellular matrix (ECM) itself also influences the phenotype and functions of aSMC.⁶⁹ Laminin reduces the aSMC proliferation and increases the expression of contractile proteins such as α -actin and smooth muscle myosin heavy chain.⁷⁰ On the contrary, fibronectin promotes the aSMC proliferation

but decreases the expression of contractile proteins.⁷⁰ During asthma, the synthesis of laminin is reduced while the fibronectin synthesis is increased promoting the switch of aSMC to a proliferative phenotype.⁷¹ In response, aSMC participate to the deposition of the ECM through increased MMP-9 and MMP-12 expressions as it has been shown in bronchial biopsies from severe asthmatic patients.⁷²

At a cell signalling level, aSMC proliferation is principally under the control of extracellular signal-regulated kinase (ERK) and PI3K pathways by increased expression of cyclin D1. ERK protein, a member of the MAPK family, is a central regulator of cell cycle entry and G1 progression essential to aSMC proliferation.⁷³ In parallel, Akt1, an effector of PI3K, inhibits the constitutively active glycogen synthase kinase 3 and an activator mTOR and p70 S6 kinase which are important for transcriptional activation and protein translation leading to aSMC proliferation and hypertrophy.⁷⁴ PI3K can also activate Rac1 and Cdc42 in order to promote the cell proliferation thanks to cyclin D1.⁷⁵ In addition, Rac1 forms part of the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase complex and also participates in the reactive oxygen species production which is involved in the aSMC mitogenesis.⁷⁶ We recently demonstrated that Rac1 was essential to the increased proliferation capacities of aSMC



from asthmatic patients in comparison to controls in basal condition and after mitogenic stimulation.⁷⁷ We identified the signal transducer and activator of transcription 3 as the main effectors involved in such Rac1-dependent mechanism. Interestingly, inhibition of Rac1 activity in a mouse model of asthma prevented aSMC hyperplasia. aSMC hyperplasia also results from the migration of aSMC or progenitors in response to mitogen factors such as the PDGF, the vascular endothelial growth factor, the transforming growth factor β (TGF- β) or IL-1 β . The P38 MAPK, PI3K and ERK pathways are involved in migration as their inhibition leads to decreased aSMC migration due to reduced phosphorylation of heat shock protein 27 implicated in the F-actin polymerization necessary for cell motility.⁷⁸ Moreover, the migration of aSMC in response to PDGF is significantly impacted by the inhibition of PI3K, ERK or ROCK pathway.^{79 80}

Aside its proliferative capacity, activated aSMC also demonstrate improved capacity to interact with immune cells through increased expression of their surface molecules such as VCAM-1, ICAM-1, CD44 and LFA-1.⁸¹ Noteworthy, aSMC display closed interaction with mast cells and are able to synthesize powerful mastocyte chemotactic agents such as the stem cell factor but also TGF- β 1 and tumour suppressor in lung cancer-1.⁸² In parallel, aSMC express both CD44 and CD51 which are involved in the mast cell adhesion.⁸³ Interaction of mast cells with aSMC promotes the degranulation and cytokines production by mast cells ultimately leading to aSMC contraction and proliferation.⁸⁴ aSMC can also interact with other immune cell types such as T cells via CD44, which induces DNA synthesis of aSMC and promotes its proliferation.⁸⁵

As presented, aSMC has important impacts on asthma pathophysiology not solely due to their contractile activity but also to their ability to interact with other cell types leading to complex remodelling activity. Such a broad involvement makes the clinical evaluation of its action at a patient level complex, as it is discussed in the next section.

Bronchial smooth muscle in asthma clinical practice

In routine practice, evaluation of aSMC's function cannot be fulfilled directly. Indeed, conventional pulmonary function tests, such as spirometry and plethysmography only give access to an overall sight of their implication in airway obstruction. New tools currently developed to further assess new facets of its function are described below.

Airway obstruction in asthma

Airway obstruction is necessary to asthma diagnosis along with relevant and consistent respiratory symptoms. Pulmonary function tests in asthma, particularly forced spirometry, are standardised and aim to prove such obstruction.⁸⁶ Evolution of obstruction through time is critical considering asthma care. Data from birth cohorts showed that children with low forced expiratory volume

in one second (FEV1)/forced vital capacity (FVC) ratio had a steeper slope of evolution of FEV1/FVC ratio through time until adulthood that increases the risk of developing asthma.⁸⁷ In parallel, patients self-reporting asthma experienced a faster decline of FEV1 through time than healthy volunteers in a 15-year prospective study performed in Denmark.⁸⁸

Bronchodilators response tests are available to assess the role of bronchial smooth muscle contraction in obstruction for an individual patient.⁸⁶ However, negative bronchodilator test does not imply that aSMC are not relevant in asthma symptoms. Indeed, a fixed obstruction (FEV1/FVC ratio under lower limit of normal values and/or FEV1 under 80% of predicted value after bronchodilators) can appear in about 20% of never ever-smoking adult asthmatic patients after 10 years of follow-up.⁸⁹ Such persistent airway obstruction had been linked with increased airway smooth muscle area in a population of severe asthmatics under standardised high dose anti-inflammatory treatment.⁹⁰ Though, airway remodelling is not only dependent on aSMC. It has been shown recently by cluster analysis of pathological examination of bronchial biopsies from asthmatic patients and healthy individuals that bronchial remodelling could be classified into several groups depending on the component involved (bronchial smooth muscle, basal membrane).⁵⁴

Considering small airways impairment in asthma, available explorations are currently imperfect. A study highlighted that mid-expiratory and instantaneous flows (FEF25-75 and FEF75) did not significantly add informations to FEV1 and FEV1 to FVC ratio.⁹¹ Use of impulse oscillometry and nitrogen breath washout technics identified around 1/3 of asthmatic patients displaying markers of small airways dysfunction.⁹² Though, data clearly linking pathology with oscillometric data are still lacking. Conversely, specific imagery approaches are currently assessed. Non-invasive evaluation of obstruction by hyperpolarized ³HE MRI showed that ventilation defects due to obstruction often persisted in time and location under stable or provoked (methacholine) conditions in a small series of patients.⁹³ Interestingly, markers of ventilation heterogeneity linked to small airways involvement inversely correlated with variation of asthma control score under inhaled corticosteroid treatment of asthmatic patients.⁹⁴ Nevertheless, MRI lacks availability needed for clinical practice.

Airway hyper-responsiveness in asthma

AHR is an important argument in favour of asthma diagnosis that can be sought by direct and indirect provocation bronchial tests.^{95 96} Methacholine and histamine tests are the principal direct provocation tests available. They aim at triggering direct bronchial smooth muscle contraction through inhalation of determined cumulative doses of stimulant. However, this test explores only selected pathways of bronchial smooth muscle contraction to the exclusion of the others described in the previous sections. In addition, its overall sensitivity is around 60%–90% and

Table 2 Effect of approved biotherapies on pulmonary function tests in allergic and eosinophilic severe asthma (phase III trials)

Phenotype Endotype	Molecule Type	Target	Main inclusion criteria	Effect on respiratory function	Ref
Allergic	Omalizumab Humanised monoclonal antibody	IgE	Severe asthma with high dose ICS Positive SPT to aeroallergen Serum total IgE 30–700 IU/mL	Improved morning PEF +2.8% predicted FEV1 in comparison with placebo	15 106
Eosinophilic	Mepolizumab Humanised monoclonal antibody	IL-5	Severe asthma with high dosage ICS. Peripheral blood eosinophils count $\geq 150/\text{mm}^3$ at screening or $\geq 300/\text{mm}^3$ during the previous year. + Maintenance treatment with systemic corticosteroids (5–35 mg of prednisone or equivalent) ⁴	Slight improvement of pre-BD and post-BD FEV1 (+98 mL, +138 mL, respectively) in comparison with placebo Statistically non-significant improvement of pre-BD and post-BD FEV1 in the corticosteroid weaning trial	14 151
Eosinophilic	Reslizumab Humanised monoclonal antibody	IL-5	Inadequately controlled asthma despite at least medium dosage ICS. Peripheral blood eosinophils count $\geq 400/\text{mm}^3$	Statistically significant improvement of pre-BD FEV1 in comparison with placebo (+0.11 L LS mean)	152
Eosinophilic	Benralizumab Humanised monoclonal antibody	IL-5 receptor	Severe asthma with high dosage ICS. Baseline peripheral blood eosinophils count $\geq 300/\text{mm}^3$	Statistically significant improvement of pre-BD FEV1 in comparison with placebo in SIROCCO and CALIMA studies (+159 mL and +116 mL, respectively, LS mean)	16 153 154
Type-2 inflammation	Dupilumab Fully human monoclonal antibody	IL-4 receptor α	Uncontrolled asthma despite medium to high dosage ICS and up to two controller	Statistically significant improvement of pre-BD FEV1 in comparison with placebo at 12 wks (+130 mL LS mean) and 24 wks (+ 220 mL LS mean).	17 108
Eosinophilic and non-eosinophilic	Tezepelumab Fully human monoclonal antibody	TSLP	Uncontrolled asthma despite medium to high dosage ICS	Statistically significant improvement of pre-BD FEV1 in comparison with placebo at 52 wks (+130 mL LS mean)	110

BD, bronchodilators; FEV1, forced expiratory volume in one second; ICS, inhaled corticosteroid; IgE, immunoglobuline E; IL-5, interleukine 5; LS, least squares; PEF, peak expiratory flow; SPT, skin prick test; TSLP, thymic stromal lymphopoietin; wks, weeks.

specificity around 90%.⁹⁷ Its use in clinical practice is then principally reserved to intermediate probability of asthma at diagnosis and is not recommended for follow-up.

Indirect tests mainly include exercise-induced and eucapnic hyperventilation tests. The objective of such tests is to provoke a deshydration of respiratory airways that stimulates the secretion of various cytokines and inflammatory mediators by bronchial epithelium and sub-mucosa triggering hypersensitive aSMC contraction.⁹⁶ Exercise-induced bronchoconstriction is associated with asthma with a good specificity but low sensitivity. In an overall population, exercise-induced bronchoconstriction under standardised exercise correlated with airflow limitation (FEV1) but also with age and sex.⁹⁸ Noteworthy, some patients displaying significant positive indirect AHR tests would not react to direct stimulation of airway smooth muscle contraction.⁹⁹

Unmet needs in aSMC functional evaluation in asthma

Diagnostic tools determining the exact role played by bronchial smooth muscle in asthma at an individual level

need to be developed. Indeed, spirometry isn't sufficient to precisely incriminate the responsible agent (inflammation, bronchial smooth muscle, infection triggers, combination of those). Tests specifically exploring bronchial contractility and its determinants could potentially be of interest in order to guide therapy, particularly concerning the use of long-acting bronchodilators. Conversely, it would be interesting to evaluate the proliferative activity of bronchial smooth muscle in order to early detect and prevent bronchial remodelling. Finally, the improvement of physiopathological knowledge could lead to the development of new targeted therapeutic strategies alongside their specific biomarkers.

aSMC as a target in asthma

Conventional therapies: inhaled and oral treatments

Conventional therapies in asthma principally target 2 pathophysiological mechanisms: inflammation and bronchoconstriction. Inhaled bronchodilators (beta-2 agonist and anticholinergic) directly target aSMC by decreasing

its contractility in order to improve airflow and limit chronic and acute symptoms. Though, pharmacological researches mainly focused on the improvement of the length and/or delay of action. Interestingly, a proof-of-concept clinical trial showed in a small cohort of severe asthmatics (31 patients) that gallopamil, a calcium ion channel inhibitor, could reduce the aSMC bronchial area and thickness after 1 year of treatment in comparison with baseline associated with reduced exacerbation after the end of treatment.¹⁰⁰ However, further clinical trials were withdrawn by the pharmaceutical companies.

Even if inhaled corticosteroids target principally inflammatory effectors, it also affects aSMC contractility and proliferation. Glucocorticoids reduce the expression of α -smooth actin and the short isoform of MLCK by aSMC in response to TGF- β which dampens its contractility.¹⁰¹

It also decreases the expression and phosphorylation of CPI-17 by aSMC in a rat model leading to lower MLC phosphorylation and improved AHR.¹⁰² Furthermore, ciclesonide effectively reduced key bronchial remodeling features, such as goblet cell hyperplasia or immune reactive aSMC, in a rat model of asthma.¹⁰³ Considering bronchial remodelling, high doses of inhaled steroids also improved the submucosal hypervascularity but also the basement membrane thickness in small clinical studies.^{104 105}

Biotherapies and aSMC

No biotherapy directly targeting aSMC is currently under clinical development to our knowledge. However, available biotherapies that target inflammation can have implication in aSMC (table 2).

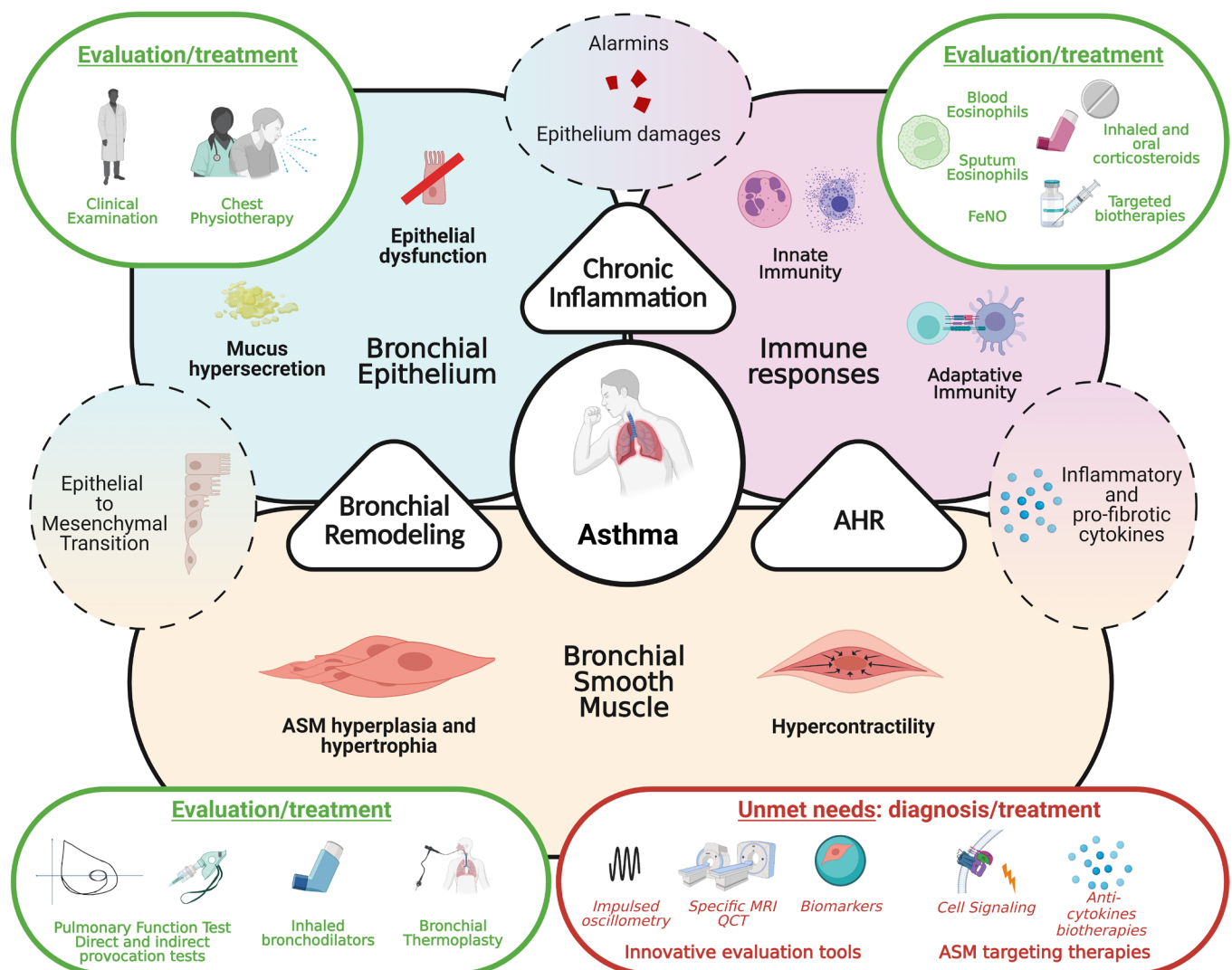


Figure 3 Bronchial smooth muscle cells in asthma from pathophysiological consideration to evaluation and treatments. Asthma development relies on three main pathophysiological processes, bronchial remodelling, chronic inflammation and airway hyper-responsiveness, consequences of alteration of bronchial epithelium and ASM and development of inadequate immune responses. Whereas anti-inflammatory therapeutic strategies got significantly improved in the last years, little progress has been made considering ASM. Innovative precise evaluation tools of ASM function along with specific targeting strategies need to be developed. AHR, airway hyper-responsiveness; ASM, airway smooth muscle; FeNO, exhaled fraction of azote monoxide.

Omalizumab is a humanised monoclonal antibody targeting immunoglobulin (Ig) E to treat severe allergic asthmatic patients. Phase III study demonstrated that omalizumab significantly improves asthma exacerbation rate compared with placebo.^{15 106} It also showed that omalizumab could slightly improve morning peak expiratory flow and FEV1. Monoclonal antibodies targeting IL-5 pathway namely mepolizumab, reslizumab and benralizumab are now approved to be used to treat severe eosinophilic asthma.¹⁰⁷ Considering improvement of prebronchodilator and postbronchodilator FEV1, biotherapies targeting IL-5 reached statistical significance overall, though clinical significance remains questionable (about +100 mL vs placebo). Conversely, dupilumab, a fully human monoclonal antibody targeting IL-4 receptor α also demonstrated a significant reduction of exacerbation rate and efficient oral corticosteroid tapering in patients suffering of uncontrolled moderate to severe asthma.^{17 108} Interestingly, a significant improvement of FEV1 under treatment with dupilumab was observed at 24 weeks of treatment with mean difference above 200 mL.¹⁰⁸ In a real-life asthma cohort, dupilumab also improved FEV1 by 10% (predicted values) after 1 year of treatment.¹⁰⁹ Recently, a treatment with tezepelumab, a human monoclonal antibody targeting the TSLP, lead to a 130 mL increase of pre bronchodilator FEV1 in comparison with the control group.¹¹⁰ Such improvements, to be confirmed in the long term, tends to underline the importance of the interactions between inflammation and bronchial smooth muscle and the interest in simultaneously targeting multiple actors.

Few trials specifically studied the effect of biotherapies on pulmonary functions. Benralizumab failed to significantly improve prebronchodilator FEV1 and hyperinflation in SOLANA trial.¹¹¹ To note, this trial aimed at assessing benralizumab efficiency at short-term (84 days) and potentially lack interesting effects on obstruction through prolonged treatment. Small-sized clinical studies have shown that mepolizumab could possibly improve small airway function evaluated by multiple-breath nitrogen washout test and forced oscillometry after a few months of treatments.¹¹²

Bronchial thermoplasty

In order to specifically target bronchial wall including aSMC, an interventional endoscopic technic was developed: bronchial thermoplasty. Its objective is to lower the airway wall thickness by direct thermic energy application. In AIR-2 clinical trial, bronchial thermoplasty significantly improved quality of life with a trend in favour of better asthma control through lower exacerbation rate in comparison with the sham group.¹¹³ Good long-term tolerance has been shown after 5 years of follow-up.¹¹⁴ Considering bronchial remodelling, in a small prospective case series (n=11), bronchial thermoplasty slightly decreased the airway wall thickness and air-trapping 2 years after the procedure, even if no significant improvement of airway lumen could be observed.¹¹⁵ However,

no significant effect had been observed on small airways evaluated by oscillometry 6 months after the procedure, despite significantly improved clinical markers.¹¹⁶ Interestingly, a proof-of-concept pilot study found that bronchial thermoplasty could nevertheless improve dynamic hyperinflation in selected patients.¹¹⁷ Though, such results need to be confirmed in larger and control clinical studies.

Data about bronchial thermoplasty effects on airways physiology and asthma pathophysiology are now available. aSMC area (α -SMA staining) significantly decreased in short term (6 weeks) after bronchial thermoplasty consistent with lower smooth muscle mass.¹¹⁸ The TASMA randomised clinical study confirmed such data by including a parallel delayed bronchial thermoplasty group of severe asthmatic patients as controls.¹¹⁹ Nevertheless, modification of aSMC mass did not correlate with improvement of asthma control and related quality of life scores (Asthma Control Questionnaire, Asthma Quality of Life Questionnaire). In parallel, it was recently reported that bronchial thermoplasty induced a decrease of mucus production assessed by MUC5AC epithelial expression at 12 months post procedure.¹²⁰ Bronchial thermoplasty can also modify cell cross-talk between the different components of airway wall. It has been reported that it blocked the production and secretion of heat-shock protein-60 by the epithelium that triggered in part remodelling in asthma by fibroblasts.¹²¹

Unmet needs in targeted treatment of aSMC in asthma

Few innovative strategies targeting specifically aSMC are available. Although bronchial thermoplasty has shown interesting results, this technique remains complex and reserved to a limited number of patients. Besides, in an era of precision medicine, tools that predict response to a treatment strategy in an individual setting are essential. Few studies are available concerning such biomarkers to predict efficacy of bronchial thermoplasty. Fixed or reversible obstruction status was not significantly associated with clinical response to bronchial thermoplasty.¹²² Small molecules that directly target key signalling pathways implicated in aSMC contraction and proliferation could be an interesting therapeutic opportunity. For example, inhibition of Rho and Rac activation could potentially reduce airway hyper-responsiveness and remodelling. Nevertheless, those have long been considered as undruggable due to their ubiquitous expression and critical biological roles that could lead to serious side effects. The identification of tissue and condition specific regulators of Rho superfamily activation, such as guanine exchange factors, could be a promising way to overcome such limit.

CONCLUSION

Asthma represents a broad-spectrum disease involving at different levels epithelial dysfunction, sustained bronchial inflammation and bronchial smooth muscle

dysfunction. Knowledge about bronchial smooth muscle role in asthma pathophysiology had been considerably improved (figure 3). Mechanisms of bronchial contraction are better understood and new intracellular pathways had been discovered. However, aSMC should not be only considered as simple contraction actors. Indeed, implication of aSMC airway remodelling and their secretion capacities are far more important than previously expected.

In routine practice, aSMC functions are mainly assessed through pulmonary function tests and provocation tests. Innovative tools such as forced oscillometry and dedicated imaging are used in clinical research but did not reach clinical practice yet. In parallel, improvement of basic sciences comprehension of airway smooth muscle biology doesn't lead to real targeted strategies for now. On the one hand, bronchodilators developed for the last decades mainly targeted the same pathways (adrenergic and muscarinic receptors) and on the other hand, bronchial thermoplasty still lacks predictors of success even though promising results have been described. Development of tools to better assess aSMC activity in clinical practice along with new targeted treatment are mandatory to identify patients for whom SMC dysfunction is preponderant and need to be specifically treated.

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REFERENCES

- Badri KR, Zhou Y, Schuger L. Embryological origin of airway smooth muscle. *Proc Am Thorac Soc* 2008;5:4–10.
- Cieri RL. Pulmonary smooth muscle in vertebrates: a comparative review of structure and function. *Integr Comp Biol* 2019;59:10–28.
- Berger P, Marthan R, Tunon de Lara J-M. [The pathophysiological role of smooth muscle cells in bronchial inflammation]. *Rev Mal Respir* 2002;19:778–94.
- Mitzner W. Airway smooth muscle: the appendix of the lung. *Am J Respir Crit Care Med* 2004;169:787–90.
- Reddel HK, Bateman ED, Becker A, et al. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015;46:622–39.
- D'Amato G, Vitale C, Molino A, et al. Asthma-related deaths. *Multidiscip Respir Med* 2016;11:37.
- Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343–73.
- Carr TF, Zeki AA, Kraft M. Eosinophilic and Noneosinophilic asthma. *Am J Respir Crit Care Med* 2018;197:22–37.
- Agache I, Beltran J, Akdis C, et al. Efficacy and safety of treatment with biologicals (benralizumab, dupilumab, mepolizumab, omalizumab and reslizumab) for severe eosinophilic asthma. A systematic review for the EAACI Guidelines - recommendations on the use of biologicals in severe asthma. *Allergy* 2020;75:1023–42.
- Pascual RM, Peters SP. Airway remodeling contributes to the progressive loss of lung function in asthma: an overview. *J Allergy Clin Immunol* 2005;116:quiz 487:477–86.
- Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med* 2012;18:684–92.
- Bumbacea D, Campbell D, Nguyen L, et al. Parameters associated with persistent airflow obstruction in chronic severe asthma. *Eur Respir J* 2004;24:122–8.
- Hough KP, Curtiss ML, Blain TJ, et al. Airway remodeling in asthma. *Front Med* 2020;7:191.
- Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;371:1198–207.
- Holgate ST, Chuchalin AG, Hebert J. Omalizumab 011 International study G. efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004;34:632–8.
- FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016;388:2128–41.
- Castro M, Corren J, Pavord ID, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med* 2018;378:2486–96.
- Ma X, Cheng Z, Kong H, et al. Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1181–9.
- Matsumoto H, Moir LM, Oliver BGG, et al. Comparison of gel contraction mediated by airway smooth muscle cells from patients with and without asthma. *Thorax* 2007;62:848–54.
- Benayoun L, Druilhe A, Dombret M-C, et al. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003;167:1360–8.
- Murray RK, Fleischmann BK, Kotlikoff MI. Receptor-activated Ca influx in human airway smooth muscle: use of Ca imaging and perforated patch-clamp techniques. *Am J Physiol* 1993;264:C485–90.
- Sanderson MJ, Delmotte P, Bai Y, et al. Regulation of airway smooth muscle cell contractility by Ca²⁺ signaling and sensitivity. *Proc Am Thorac Soc* 2008;5:23–31.
- André-Grégoire G, Dilasser F, Chesné J, et al. Targeting of Rac1 prevents bronchoconstriction and airway hyperresponsiveness. *J Allergy Clin Immunol* 2018;142:e823:824–33.
- Deshpande DA, Walseth TF, Panettieri RA, et al. CD38/Cyclic ADP-ribose-mediated Ca²⁺ signaling contributes to airway smooth muscle hyperresponsiveness. *FASEB J* 2003;17:452–4.
- Deshpande DA, White TA, Guedes AGP, et al. Altered airway responsiveness in CD38-deficient mice. *Am J Respir Cell Mol Biol* 2005;32:149–56.
- Perez JF, Sanderson MJ. The frequency of calcium oscillations induced by 5-HT, ACh, and KCl determine the contraction of smooth muscle cells of intrapulmonary bronchioles. *J Gen Physiol* 2005;125:535–53.
- Deshpande DA, Dogan S, Walseth TF, et al. Modulation of calcium signaling by interleukin-13 in human airway smooth muscle: role of CD38/cyclic adenosine diphosphate ribose pathway. *Am J Respir Cell Mol Biol* 2004;31:36–42.
- Tarasov AI, Griffiths EJ, Rutter GA. Regulation of ATP production by mitochondrial Ca(2+). *Cell Calcium* 2012;52:28–35.
- Delmotte P, Sieck GC. Interaction between endoplasmic/sarcoplasmic reticulum stress (ER/SR stress), mitochondrial signaling and Ca(2+) regulation in airway smooth muscle (ASM). *Can J Physiol Pharmacol* 2015;93:97–110.
- Mahn K, Hirst SJ, Ying S, et al. Diminished sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA) expression contributes to airway remodelling in bronchial asthma. *Proc Natl Acad Sci U S A* 2009;106:10775–80.
- Sakai H, Hirano T, Takeyama H, et al. Acetylcholine-Induced phosphorylation of CPI-17 in rat bronchial smooth muscle: the roles of Rho-kinase and protein kinase C. *Can J Physiol Pharmacol* 2005;83:375–81.

- 32 Feng J, Ito M, Ichikawa K, *et al.* Inhibitory phosphorylation site for Rho-associated kinase on smooth muscle myosin phosphatase. *J Biol Chem* 1999;274:37385–90.
- 33 Koyama M, Ito M, Feng J, *et al.* Phosphorylation of CPI-17, an inhibitory phosphoprotein of smooth muscle myosin phosphatase, by Rho-kinase. *FEBS Lett* 2000;475:197–200.
- 34 Sakai H, Chiba Y, Hirano T, *et al.* Possible involvement of CPI-17 in augmented bronchial smooth muscle contraction in antigen-induced airway hyper-responsive rats. *Mol Pharmacol* 2005;68:145–51.
- 35 Chiba Y, Ueno A, Shinozaki K, *et al.* Involvement of RhoA-mediated Ca²⁺ sensitization in antigen-induced bronchial smooth muscle hyperresponsiveness in mice. *Respir Res* 2005;6:4.
- 36 Schaafsma D, Bos IST, Zuidhof AB, *et al.* The inhaled Rho kinase inhibitor Y-27632 protects against allergen-induced acute bronchoconstriction, airway hyperresponsiveness, and inflammation. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L214–9.
- 37 Chiba Y, Tanoue G, Suto R, *et al.* Interleukin-17A directly acts on bronchial smooth muscle cells and augments the contractility. *Pharmacol Rep* 2017;69:377–85.
- 38 Kudo M, Melton AC, Chen C, *et al.* IL-17A produced by $\alpha\beta$ T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. *Nat Med* 2012;18:547–54.
- 39 Camargo LdoN, Righetti RF, Aristóteles LRdeCRB, *et al.* Effects of Anti-IL-17 on inflammation, remodeling, and oxidative stress in an experimental model of asthma exacerbated by LPS. *Front Immunol* 2017;8:1835.
- 40 Dos Santos TM, Righetti RF, Camargo LdoN, *et al.* Effect of Anti-IL17 antibody treatment alone and in combination with Rho-kinase inhibitor in a murine model of asthma. *Front Physiol* 2018;9:1183.
- 41 Chiba Y, Nakazawa S, Todoroki M, *et al.* Interleukin-13 augments bronchial smooth muscle contractility with an up-regulation of RhoA protein. *Am J Respir Cell Mol Biol* 2009;40:159–67.
- 42 Carvajal JA, Germain AM, Huidobro-Toro JP, *et al.* Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J Cell Physiol* 2000;184:409–20.
- 43 Ward JK, Barnes PJ, Springall DR, *et al.* Distribution of human i-NANC bronchodilator and nitric oxide-immunoreactive nerves. *Am J Respir Cell Mol Biol* 1995;13:175–84.
- 44 Asano K, Chee CB, Gaston B, *et al.* Constitutive and inducible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells. *Proc Natl Acad Sci U S A* 1994;91:10089–93.
- 45 Nijkamp FP, van der Linde HJ, Folkerts G. Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Role of the epithelium. *Am Rev Respir Dis* 1993;148:727–34.
- 46 Prado CM, Martins MA, Tibério IFLC. Nitric oxide in asthma pathophysiology. *ISRN Allergy* 2011;2011:832560.
- 47 Hamid Q, Springall DR, Riveros-Moreno V, *et al.* Induction of nitric oxide synthase in asthma. *Lancet* 1993;342:1510–3.
- 48 Dweik RA, Sorkness RL, Wenzel S, *et al.* Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med* 2010;181:1033–41.
- 49 Jatakanon A, Lim S, Kharitonov SA, *et al.* Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;53:91–5.
- 50 Dupont LJ, Rochette F, Demedts MG, *et al.* Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naïve patients with mild asthma. *Am J Respir Crit Care Med* 1998;157:894–8.
- 51 Grace MS, Baxter M, Dubuis E, *et al.* Transient receptor potential (TRP) channels in the airway: role in airway disease. *Br J Pharmacol* 2014;171:2593–607.
- 52 Birrell MA, Belvisi MG, Grace M, *et al.* TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 2009;180:1042–7.
- 53 Caceres AI, Brackmann M, Elia MD, *et al.* A sensory neuronal ion channel essential for airway inflammation and hyperactivity in asthma. *Proc Natl Acad Sci U S A* 2009;106:9099–104.
- 54 Siddiqui S, Shikotra A, Richardson M, *et al.* Airway pathological heterogeneity in asthma: visualization of disease microclusters using topological data analysis. *J Allergy Clin Immunol* 2018;142:1457–68.
- 55 Saglani S, Payne DN, Zhu J, *et al.* Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007;176:858–64.
- 56 Regamey N, Ochs M, Hilliard TN, *et al.* Increased airway smooth muscle mass in children with asthma, cystic fibrosis, and non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med* 2008;177:837–43.
- 57 Bossley CJ, Fleming L, Gupta A, *et al.* Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. *J Allergy Clin Immunol* 2012;129:e913:974–82.
- 58 Tillie-Leblond I, de Blic J, Jaubert F, *et al.* Airway remodeling is correlated with obstruction in children with severe asthma. *Allergy* 2008;63:533–41.
- 59 Halayko AJ, Salari H, Ma X, *et al.* Markers of airway smooth muscle cell phenotype. *Am J Physiol* 1996;270:L1040–51.
- 60 Berair R, Saunders R, Brightling CE. Origins of increased airway smooth muscle mass in asthma. *BMC Med* 2013;11:145.
- 61 Trian T, Benard G, Begueret H, *et al.* Bronchial smooth muscle remodeling involves calcium-expression enhanced mitochondrial biogenesis in asthma. *J Exp Med* 2007;204:3173–81.
- 62 Zou H, Fang Q-H, Ma Y-M, *et al.* Analysis of growth factors in serum and induced sputum from patients with asthma. *Exp Ther Med* 2014;8:573–8.
- 63 Shan L, Redhu NS, Saleh A, *et al.* Thymic stromal lymphopoietin receptor-mediated IL-6 and CC/CXC chemokines expression in human airway smooth muscle cells: role of MAPKs (ERK1/2, p38, and JNK) and STAT3 pathways. *J Immunol* 2010;184:7134–43.
- 64 Lajoie-Kadoch S, Joubert P, Létuvé S, *et al.* Tnf-Alpha and IFN-gamma inversely modulate expression of the IL-17E receptor in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L1238–46.
- 65 Malavia NK, Raub CB, Mahon SB, *et al.* Airway epithelium stimulates smooth muscle proliferation. *Am J Respir Cell Mol Biol* 2009;41:297–304.
- 66 Lan B, Mitchell JA, O'Sullivan MJ, *et al.* Airway epithelial compression promotes airway smooth muscle proliferation and contraction. *Am J Physiol Lung Cell Mol Physiol* 2018;315:L645–52.
- 67 Risse P-A, Jo T, Suarez F, *et al.* Interleukin-13 inhibits proliferation and enhances contractility of human airway smooth muscle cells without change in contractile phenotype. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L958–66.
- 68 Espinosa K, Bossé Y, Stankova J, *et al.* CysLT1 receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. *J Allergy Clin Immunol* 2003;111:1032–40.
- 69 Keglowski LF, Borger P. The three A's in asthma - airway smooth muscle, airway remodeling & angiogenesis. *Open Respir Med J* 2015;9:70–80.
- 70 Hirst SJ, Twort CH, Lee TH. Differential effects of extracellular matrix proteins on human airway smooth muscle cell proliferation and phenotype. *Am J Respir Cell Mol Biol* 2000;23:335–44.
- 71 Tran T, McNeill KD, Gerthoffer WT, *et al.* Endogenous laminin is required for human airway smooth muscle cell maturation. *Respir Res* 2006;7:117.
- 72 Araujo BB, Dolhnikoff M, Silva LFF, *et al.* Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 2008;32:61–9.
- 73 Orsini MJ, Krymskaya VP, Eszterhas AJ, *et al.* Mapk superfamily activation in human airway smooth muscle: mitogenesis requires prolonged p42/p44 activation. *Am J Physiol* 1999;277:L479–88.
- 74 Krymskaya VP, Penn RB, Orsini MJ, *et al.* Phosphatidylinositol 3-kinase mediates mitogen-induced human airway smooth muscle cell proliferation. *Am J Physiol* 1999;277:L65–78.
- 75 Schaafsma D, Roscioni SS, Meurs H, *et al.* Monomeric G-proteins as signal transducers in airway physiology and pathophysiology. *Cell Signal* 2008;20:1705–14.
- 76 Page K, Li J, Hodge JA, *et al.* Characterization of a Rac1 signaling pathway to cyclin D(1) expression in airway smooth muscle cells. *J Biol Chem* 1999;274:22065–71.
- 77 Dilasser F, Rose L, Hassoun D, *et al.* Essential role of smooth muscle Rac1 in severe asthma-associated airway remodelling. *Thorax* 2021;76:326–334.
- 78 Hedges JC, Dechert MA, Yamboliev IA, *et al.* A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J Biol Chem* 1999;274:24211–9.
- 79 Carlin SM, Roth M, Black JL. Urokinase potentiates PDGF-induced chemotaxis of human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L1020–6.
- 80 Parameswaran K, Cox G, Radford K, *et al.* Cysteinyl leukotrienes promote human airway smooth muscle migration. *Am J Respir Crit Care Med* 2002;166:738–42.
- 81 Tliiba O, Panettieri RA. Noncontractile functions of airway smooth muscle cells in asthma. *Annu Rev Physiol* 2009;71:509–35.
- 82 Yang W, Kaur D, Okayama Y, *et al.* Human lung mast cells adhere to human airway smooth muscle, in part, via tumor suppressor in lung cancer-1. *J Immunol* 2006;176:1238–43.



- 83 Girodet P-O, Ozier A, Trian T, *et al.* Mast cell adhesion to bronchial smooth muscle in asthma specifically depends on CD51 and CD44 variant 6. *Allergy* 2010;65:1004-12.
- 84 Hamawy MM, Mergenhagen SE, Siraganian RP. Adhesion molecules as regulators of mast-cell and basophil function. *Immunol Today* 1994;15:62-6.
- 85 Lazaar AL, Albelda SM, Pilewski JM, *et al.* T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. *J Exp Med* 1994;180:807-16.
- 86 Graham BL, Steenbruggen I, Miller MR, *et al.* Standardization of spirometry 2019 update. an official American thoracic Society and European respiratory Society technical statement. *Am J Respir Crit Care Med* 2019;200:e70-88.
- 87 Karmaus W, Mukherjee N, Janjanam VD, *et al.* Distinctive lung function trajectories from age 10 to 26 years in men and women and associated early life risk factors - a birth cohort study. *Respir Res* 2019;20:98.
- 88 Lange P, Parner J, Vestbo J, *et al.* A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;339:1194-200.
- 89 Ulrik CS, Backer V. Nonreversible airflow obstruction in life-long nonsmokers with moderate to severe asthma. *Eur Respir J* 1999;14:892-6.
- 90 Ferreira DS, Carvalho-Pinto RM, Gregório MG, *et al.* Airway pathology in severe asthma is related to airflow obstruction but not symptom control. *Allergy* 2018;73:635-43.
- 91 Quanjer PH, Weiner DJ, Pretto JJ, *et al.* Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. *Eur Respir J* 2014;43:1051-8.
- 92 Kjellberg S, Houltz BK, Zetterström O, *et al.* Clinical characteristics of adult asthma associated with small airway dysfunction. *Respir Med* 2016;117:92-102.
- 93 de Lange EE, Altes TA, Patrie JT, *et al.* The variability of regional airflow obstruction within the lungs of patients with asthma: assessment with hyperpolarized helium-3 magnetic resonance imaging. *J Allergy Clin Immunol* 2007;119:1072-8.
- 94 Farah CS, King GG, Brown NJ, *et al.* The role of the small airways in the clinical expression of asthma in adults. *J Allergy Clin Immunol* 2012;129:e381:381-7.
- 95 Coates AL, Wanger J, Cockcroft DW, *et al.* ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J* 2017;49. doi:10.1183/13993003.01526-2016. [Epub ahead of print: 01 05 2017].
- 96 Hallstrand TS, Leuppi JD, Joos G, *et al.* ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. *Eur Respir J* 2018;52. doi:10.1183/13993003.01033-2018. [Epub ahead of print: 15 11 2018].
- 97 Sumino K, Sugar EA, Irvin CG, *et al.* Methacholine challenge test: diagnostic characteristics in asthmatic patients receiving controller medications. *J Allergy Clin Immunol* 2012;130:e66:69-75.
- 98 Satia I, Priel E, Al-Khazraji BK, *et al.* Exercise-induced bronchoconstriction and bronchodilation: investigating the effects of age, sex, airflow limitation and FEV₁. *Eur Respir J* 2021;58:2004026.
- 99 Anderson SD, Charlton B, Weiler JM, *et al.* Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res* 2009;10:4.
- 100 Girodet P-O, Dournes G, Thumerel M, *et al.* Calcium channel blocker reduces airway remodeling in severe asthma. A proof-of-concept study. *Am J Respir Crit Care Med* 2015;191:876-83.
- 101 Goldsmith AM, Hershenson MB, Wolbert MP, *et al.* Regulation of airway smooth muscle alpha-actin expression by glucocorticoids. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L99-106.
- 102 Goto K, Chiba Y, Sakai H, *et al.* Glucocorticoids inhibited airway hyperresponsiveness through downregulation of CPI-17 in bronchial smooth muscle. *Eur J Pharmacol* 2008;591:231-6.
- 103 Leung SY, Eynott P, Nath P, *et al.* Effects of ciclesonide and fluticasone propionate on allergen-induced airway inflammation and remodeling features. *J Allergy Clin Immunol* 2005;115:989-96.
- 104 Chetta A, Zanini A, Foresi A, *et al.* Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. *Am J Respir Crit Care Med* 2003;167:751-7.
- 105 Ward C, Pais M, Bish R, *et al.* Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57:309-16.
- 106 Humbert M, Beasley R, Ayres J, *et al.* Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 2005;60:309-16.
- 107 Holguin F, Cardet JC, Chung KF, *et al.* Management of severe asthma: a European respiratory Society/American thoracic Society guideline. *Eur Respir J* 2020;55. doi:10.1183/13993003.00588-2019. [Epub ahead of print: 02 01 2020].
- 108 Rabe KF, Nair P, Brusselle G, *et al.* Efficacy and safety of Dupilumab in glucocorticoid-dependent severe asthma. *N Engl J Med* 2018;378:2475-85.
- 109 Dupin C, Belhadi D, Guilleminault L, *et al.* Effectiveness and safety of dupilumab for the treatment of severe asthma in a real-life French multi-centre adult cohort. *Clin Exp Allergy* 2020;50:789-98.
- 110 Menzies-Gow A, Corren J, Bourdin A, *et al.* Tezepelumab in adults and adolescents with severe, uncontrolled asthma. *N Engl J Med* 2021;384:1800-9.
- 111 Panettieri RA, Welte T, Shenoy KV, *et al.* Onset of effect, changes in airflow obstruction and lung volume, and health-related quality of life improvements with Benralizumab for patients with severe eosinophilic asthma: phase IIIB randomized, controlled trial (SOLANA). *J Asthma Allergy* 2020;13:115-26.
- 112 Farah CS, Badal T, Reed N, *et al.* Mepolizumab improves small airway function in severe eosinophilic asthma. *Respir Med* 2019;148:49-53.
- 113 Castro M, Rubin AS, Laviolette M, *et al.* Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am J Respir Crit Care Med* 2010;181:116-24.
- 114 Thomson NC, Rubin AS, Niven RM, *et al.* Long-term (5 year) safety of bronchial thermoplasty: asthma intervention research (air) trial. *BMJ Pulm Med* 2011;11:8.
- 115 Konietzke P, Weinheimer O, Wielpütz MO, *et al.* Quantitative CT detects changes in airway dimensions and air-trapping after bronchial thermoplasty for severe asthma. *Eur J Radiol* 2018;107:33-8.
- 116 Langton D, Ing A, Sha J, *et al.* Measuring the effects of bronchial thermoplasty using oscillometry. *Respirology* 2019;24:431-6.
- 117 Guibert N, Guilleminault L, Lepage B. Bronchial thermoplasty in patients with dynamic hyperinflation: results from the proof-of-concept heat trial. *Eur Respir J* 2020.
- 118 Ichikawa T, Panariti A, Audusseau S, *et al.* Effect of bronchial thermoplasty on structural changes and inflammatory mediators in the airways of subjects with severe asthma. *Respir Med* 2019;150:165-72.
- 119 Goosenberg AWM, d'Hooghe JNS, Srikanthan K. Bronchial thermoplasty induced airway smooth muscle reduction and clinical response in severe asthma: the TASMA randomized trial. *Am J Respir Crit Care Med* 2020.
- 120 Haj Salem I, Gras D, Joubert P, *et al.* Persistent reduction of mucin production after bronchial thermoplasty in severe asthma. *Am J Respir Crit Care Med* 2019;199:536-8.
- 121 Sun Q, Fang L, Roth M, *et al.* Bronchial thermoplasty decreases airway remodelling by blocking epithelium-derived heat shock protein-60 secretion and protein arginine methyltransferase-1 in fibroblasts. *Eur Respir J* 2019;54:1900300.
- 122 Langton D, Ing A, Fielding D, *et al.* Bronchodilator responsiveness as a predictor of success for bronchial thermoplasty. *Respirology* 2019;24:63-7.
- 123 Hirst SJ, Barnes PJ, Twort CH. Quantifying proliferation of cultured human and rabbit airway smooth muscle cells in response to serum and platelet-derived growth factor. *Am J Respir Cell Mol Biol* 1992;7:574-81.
- 124 Hirst SJ, Barnes PJ, Twort CH. PDGF isoform-induced proliferation and receptor expression in human cultured airway smooth muscle cells. *Am J Physiol* 1996;270:L415-28.
- 125 Simeone-Penney MC, Severgnini M, Rozo L, *et al.* PDGF-Induced human airway smooth muscle cell proliferation requires STAT3 and the small GTPase Rac1. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L698-704.
- 126 Stamatiou R, Paraskeva E, Gourgouliani K, *et al.* Cytokines and growth factors promote airway smooth muscle cell proliferation. *ISRN Inflamm* 2012;2012:731472.
- 127 Ediger TL, Toews ML. Synergistic stimulation of airway smooth muscle cell mitogenesis. *J Pharmacol Exp Ther* 2000;294:1076-82.
- 128 Bossé Y, Thompson C, Stankova J, *et al.* Fibroblast growth factor 2 and transforming growth factor beta1 synergism in human bronchial smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 2006;34:746-53.
- 129 Zou H, Nie X-hong, Zhang Y, *et al.* Effect of basic fibroblast growth factor on the proliferation, migration and phenotypic modulation of airway smooth muscle cells. *Chin Med J* 2008;121:424-9.
- 130 Krymskaya VP, Hoffman R, Eszterhas A, *et al.* EGF activates ErbB-2 and stimulates phosphatidylinositol 3-kinase in human airway smooth muscle cells. *Am J Physiol* 1999;276:L246-55.

- 131 Chen G, Khalil N. Tgf-Beta1 increases proliferation of airway smooth muscle cells by phosphorylation of MAP kinases. *Respir Res* 2006;7:2.
- 132 Cohen MD, Ciocca V, Panettieri RA. TGF-Beta 1 modulates human airway smooth-muscle cell proliferation induced by mitogens. *Am J Respir Cell Mol Biol* 1997;16:85–90.
- 133 Xie S, Sukkar MB, Issa R, et al. Mechanisms of induction of airway smooth muscle hyperplasia by transforming growth factor-beta. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L245–53.
- 134 Stewart AG, Tomlinson PR, Fernandes DJ, et al. Tumor necrosis factor alpha modulates mitogenic responses of human cultured airway smooth muscle. *Am J Respir Cell Mol Biol* 1995;12:110–9.
- 135 Knobloch J, Yanik SD, Körber S, et al. TNFalpha-Induced airway smooth muscle cell proliferation depends on endothelin receptor signaling, GM-CSF and IL-6. *Biochem Pharmacol* 2016;116:188–99.
- 136 Li X, Zou F, Lu Y, et al. Notch1 contributes to TNF- α -induced proliferation and migration of airway smooth muscle cells through regulation of the Hes1/PTEN axis. *Int Immunopharmacol* 2020;88:106911.
- 137 De S, Zelazny ET, Souhrada JF, et al. Interleukin-1 beta stimulates the proliferation of cultured airway smooth muscle cells via platelet-derived growth factor. *Am J Respir Cell Mol Biol* 1993;9:645–51.
- 138 De S, Zelazny ET, Souhrada JF, et al. Il-1 beta and IL-6 induce hyperplasia and hypertrophy of cultured guinea pig airway smooth muscle cells. *J Appl Physiol* 1995;78:1555–63.
- 139 Amrani Y, Tliba O, Choubey D, et al. IFN-Gamma inhibits human airway smooth muscle cell proliferation by modulating the E2F-1/Rb pathway. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L1063–71.
- 140 Hawker KM, Johnson PR, Hughes JM, et al. Interleukin-4 inhibits mitogen-induced proliferation of human airway smooth muscle cells in culture. *Am J Physiol* 1998;275:L469–77.
- 141 Shim JY, Park SW, Kim DS, et al. The effect of interleukin-4 and amphiregulin on the proliferation of human airway smooth muscle cells and cytokine release. *J Korean Med Sci* 2008;23:857–63.
- 142 Panettieri RA, Yadavish PA, Kelly AM, et al. Histamine stimulates proliferation of airway smooth muscle and induces c-fos expression. *Am J Physiol* 1990;259:L365–71.
- 143 Chhabra J, Li Y-Z, Alkhoury H, et al. Histamine and tryptase modulate asthmatic airway smooth muscle GM-CSF and RANTES release. *Eur Respir J* 2007;29:861–70.
- 144 Noveral JP, Grunstein MM. Role and mechanism of thromboxane-induced proliferation of cultured airway smooth muscle cells. *Am J Physiol* 1992;263:L555–61.
- 145 Capra V, Habib A, Accomazzo MR, et al. Thromboxane prostanoid receptor in human airway smooth muscle cells: a relevant role in proliferation. *Eur J Pharmacol* 2003;474:149–59.
- 146 Ammit AJ, Hastie AT, Edsall LC, et al. Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. *Faseb J* 2001;15:1212–4.
- 147 Berger P, Perng DW, Thabrew H, et al. Tryptase and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells. *J Appl Physiol* 2001;91:1372–9.
- 148 Panettieri RA, Hall IP, Maki CS, et al. Alpha-thrombin increases cytosolic calcium and induces human airway smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 1995;13:205–16.
- 149 Huang C-D, Chen H-H, Wang C-H, et al. Human neutrophil-derived elastase induces airway smooth muscle cell proliferation. *Life Sci* 2004;74:2479–92.
- 150 Brar SS, Kennedy TP, Sturrock AB, et al. NADPH oxidase promotes NF-kappaB activation and proliferation in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L782–95.
- 151 Bel EH, Wenzel SE, Thompson PJ, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;371:1189–97.
- 152 Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 2015;3:355–66.
- 153 Bleecker ER, FitzGerald JM, Chanez P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β_2 -agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 2016;388:2115–27.
- 154 Nair P, Wenzel S, Rabe KF, et al. Oral glucocorticoid-sparing effect of Benralizumab in severe asthma. *N Engl J Med* 2017;376:2448–58.