Obstructive lung disease: The Role of Smooth Muscle

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INTRODUCTION

Airway smooth muscle (ASM) plays a major role in airway inflammation, hyperresponsiveness and excessive airway obstruction in various obstructive diseases. It is now widely accepted that ASM cells possess new functions, in addition to their classical role as end-effector cells mediating bronchomotor tone. These functions include the secretion of cytokines and matrix proteins, cytokine-induced expression of cell adhesion molecules, migration, proliferation and cell to cell interactions by direct interaction with immune or structural cells (1-3).

Exaggerated inflammation and remodelling in the airways are a consequence of abnormal injury and repair responses arising from a subject's susceptibility to components of the inhaled environment (4). It has been suggested that the epithelial-mesenchymal trophic unit becomes activated to drive pathologic remodeling and smooth muscle proliferation through complex cytokine interactions (4). This response involves reciprocal activities of several growth factors (FGF, EGF, TGF-b). Once Th-2 inflammatory responses are established, altered function of constitutive airway cells provides an abnormal microenvironment in which to generate inflammation triggered by viruses, pollutants, and allergens [Holgate, 2003 #94].
In this review, the role of ASM in the perpetuation of bronchial hyperresponsiveness (BHR) and chronic local inflammation as well as the structural remodelling process of immature airways will be examined.

Role of ASM in BHR

Airway innervation

The human airways are innervated via afferent and efferent autonomic nerves that regulate many aspects of airway function. Innervation of the lung can be functionally divided into adrenergic, cholinergic, and non-adrenergic non-cholinergic neuronal pathways. The parasympathetic (cholinergic) nervous system is “excitatory” and regulates ASM tone via bronchoconstrictor responses. Acetylcholine, the principal neurotransmitter of this system, is released at both ganglionic synapses and postganglionic neuroeffector junctions and acts by activation of nicotinic and muscarinic cholinoceptors. Four distinct subtypes of muscarinic cholinoceptor, denoted M1, M2, M3 and M4 receptors have been identified (5). At the site of the smooth muscle itself, muscarinic receptor subtypes present are M2 and M3. M2 receptors are coupled to Gi proteins and adenylyl cyclase inhibition and thus to cAMP signaling. M3 receptors are coupled to Gq/11 protein and phosphoinositide hydrolysis and thus to calcium signaling. Muscarinic-induced contraction of ASM is mediated by M3 receptors. M2-mediated inhibition of adenylyl cyclase contributes to the prevention of bronchodilation.

The sympathetic (adrenergic) nervous system represents only a minor component of total human airway innervation without direct sympathetic lung innervation. However, adrenergic receptors that are important in regulating bronchomotor tone are found in many cell types including alveolar, epithelial and ASM with increasing numbers in the smaller airways of the lung periphery.

In addition to cholinergic and adrenergic pathways, nonadrenergic and noncholinergic (NANC) pathways affect human airway functions. The NANC-system has been subdivided into the excitatory (e-NANC) and inhibitory (i-NANC) system. The e-NANC system exhibits bronchoconstrictory effects (main neurotransmitters: tachykinins, e.g. substance P or neurokinin A, and calcitonin gene-related peptide). Tachykinins bind to distinct tachykinin (neurokinin) receptors—NK1, NK2 and NK3. The i-NANC system is a bronchodilatatory pathway, mediating its effects mainly by nitric oxide and vasoactive intestinal peptide (6).
Presence of airway tone control mechanisms at birth

The study of the ontogeny of ASM reactivity has revealed that spontaneous or agonist-induced contraction of ASM can be observed very early in fetal life (7), thus explaining the possible occurrence of bronchospasm within the first days of life (8). We studied the mechanical activity of proximal airways isolated from human lung specimens obtained at autopsy from human neonates (9). Maximal active muscle stress of human neonatal bronchi was induced by carbachol (a cholinergic agonist) and averaged 95 ± 25 mN/mm² ASM surface area. The rank of maximal force induced by the contractile agonists was carbachol > histamine > KCl > neurokinin A, and the rank of the concentration of drug producing one-half of the maximum effect (EC50) was neurokinin A < carbachol < histamine < KCl. The EC50 value for isoproterenol was the lowest, although it generated the smallest mechanical response.

BHR decreases with age

Immature rabbits have greater maximal airway narrowing and greater maximal fold increases in airway resistance during bronchoconstriction than mature animals (10). They also demonstrate greater peak velocity of shortening and greater maximal airway narrowing (11), suggesting lower elastic load limiting ASM shortening in the immature rabbit (11). Impairment of ASM relaxation also likely contributes to increased BHR (12, 13). Medium light chain phosphorylation in guinea pig intact tracheal strips correlates with ontogenetic changes in shortening velocity and changes in myosin light chain kinase content (13). In human neonatal airways, when compared with results obtained under identical experimental conditions in the adult lung, except for carbachol and isoproterenol, general trends were an increase in force generation with age and little changes in EC50 values. There was a decrease in carbachol-induced force with age, whereas the opposite was observed with isoproterenol (9).

Increased relaxation to b2-agonists in immature airways

We, and others, have shown that the b2-agonist-induced relaxation in rat isolated tracheae preconstricted by carbachol is greater in immature animals (14). This effect is associated with a lower expression of postjunctional M2R in the smooth muscle itself (14). This receptor is involved in the cross-talk between muscarinic receptors and b2-adrenoceptors (5), via the Gai protein-
coupled inhibition of adenylate cyclase. Quantitative real-time PCR did not reveal significant changes in M2R mRNA according to age, suggesting a posttranscriptional mechanism.

**In conclusion**, until they are fully mature, such hyperresponsive airways are highly susceptible to damage (15). For example, a brief period of mechanical ventilation (≤ 4 h duration) in rat pups increases airway reactivity 48 h after such exposure in the presence as well as absence of hyperoxic exposure (16). Alterations in the response to agonists with the maturational process may have implications in the pharmacologic modulation of bronchial obstruction during immaturity.

**Role of smooth muscle in airway remodelling**

Airway remodelling is a complex process that involves all of the component tissues of the airway from the epithelium to the adventitia. It is induced by factors synthesised and secreted both by inflammatory cells and by structural cells, the latter frequently under the influence of the former (17). Not surprisingly, due to the small size of the biopsy sample, most paediatric clinical studies on airway remodelling have investigated only the most superficial components of the airways (18, 19), unlike in adults.

Structural changes, such as epithelial metaplasia, airway fibrosis and ASM hyperplasia, have been successfully modelled in animals (17). Using a mouse model, we showed that after relatively brief allergenic sensitization (i.e. a short 30-day active sensitization period using systemic ovalbumin), immature mice develop more remodelling and bronchial hyperresponsiveness than adult mice. Immature 8 week-old Balb/c mice demonstrated airway remodelling which was not present in adults: a significant fourfold increase in reticular basement membrane thickness, twofold increase in bronchial smooth muscle surface area and threefold increase in perivascular fibrosis were observed in immature vs. adult mice. There was however no significant difference regarding epithelium integrity, bronchial fibrosis and vascular smooth muscle surface area.

While information concerning the genesis of inflammation is abundant, the precise factors responsible for cellular hyperplasia, hypertrophy and altered matrix deposition are far from resolved (17). Moreover, very little is known regarding the relation between inflammatory mediators and/or cytokines and immature ASM.
Proliferation of ASM cells is increased in adult patients with asthma and provides evidence for an intrinsic abnormality in the ASM cell in this disease (20, 21). ASM in adult asthmatic patients is characterized by an altered calcium homeostasis which increases mitochondrial biogenesis that, in turn, enhances cell proliferation leading to airway remodelling (22).

We have shown that stimulated non-asthmatic infant ASM cells may contribute to airway remodelling to a greater extent than adult ASM cells (23). DNA synthesis in 10% fetal calf serum of infant ASMC was significantly enhanced (> 5-fold increase vs. ITS control medium) compared to adults (2-fold increase). PDGF-AA induced a response of lesser magnitude: 2.6-fold increase in infants vs. 1.5-fold in adults ($p < 0.05$). Unlike in asthma, this was not related to specific intrinsic cellular abnormalities.

**Synthesis of inflammatory mediators and cytokines**

Very little is known regarding the secretion of inflammatory mediators and cytokines by immature ASM cells. We thus evaluated the secretion of leukemia inhibitory factor (LIF) (an IL-6 family neurotrophic cytokine) by ASM cells (24). TNFα-stimulated immature ASMC produce more LIF mRNA and protein than adult ASMC. Moreover, human recombinant LIF increased contractility to acetylcholine by 50% in immature, isolated rat tracheae. The secretion of LIF by stimulated immature human ASM cells potentially contributes to neuroimmune airway inflammation and subsequent remodelling.

**Conclusion**

1. BHR is exhibited very early in life.
2. BHR can be further enhanced by transient or long-term noxious stimuli.
3. Immature ASM has the potential to actively participate in airway remodelling, possibly at a greater degree than mature ASM.
4. More attention should be paid to the role of ASM in the clinical assessment of airway remodelling in paediatric chronic pulmonary obstructive diseases.
References